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Nima Rezaei *Editor*

Tuberculosis

Integrated Studies for a Complex Disease

Springer

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This book series would not have been possible without the continuous encouragement of my family. I dedicate this book series to my daughters, Ariana and Arnika, hoping that integrated science could solve complex problems and make a brighter future for the next generation.

Preface

Tuberculosis (TB) has been a disease of animals and humans since thousands of years ago, but it is still affecting the people, to become the leading cause of death due to a single infectious disease.

A variety of control programs has been launched to eliminate TB. They mainly include national programs to prevent TB, screen for TB, and increase patients' adherence to treatment regimens. However, TB remains to pose its new challenges with regard to effectiveness of control and prevention programs, management, detection and diagnostic techniques, HIV-TB co-infection, drug resistance and drug discovery, childhood TB, genetic susceptibility, environmental factors, epigenetics, vaccine development, social and structural determinants, animal TB, extrapulmonary TB, and research.

Tuberculosis: Integrated Studies for a Complex Disease is a multi-authored comprehensive volume on TB that provides a review of all the above challenges along with recommendations.

Tehran, Iran
April 2022

Nima Rezaei, M.D., Ph.D.

Acknowledgment I would like to express my gratitude to Editorial Assistant of this book series, Dr. Amene Saghazadeh. Without a doubt, the book would not have been completed without her contribution.

Contents

Abbreviations

%T/MIC	Time above the minimum inhibitory concentration
3D	Three dimensional
3HP	Regimen with 12 weekly doses of rifapentine and isoniazid
3HR	Regimen with three months of rifampicin and isoniazid
4R	Regimen with four months of rifampicin
A Mφs	Alveolar macrophages
ABC	ATP-binding cassette
ACF	Active case finding
ACSM	Advocacy communication and social mobilization
ADA	Adenosine deaminase
ADMET	Absorption, distribution, metabolism, excretion, and toxicity
AFB	Acid-fast bacilli
Ag85A	Antigen 85A
Ag85B	Antigen 85B
AIDS	Acquired immunodeficiency syndrome
Ala	Alanine
ALC	Semi-automated line cage
Alr	Alanine racemase
AMDT	Amplified Mycobacterium Tuberculosis Direct Test
AMP	Antimicrobial peptides
APOPO	Anti-Persoonsmijnen Ontmijnende Product Ontwikkeling: "Anti-Personnel Landmines Removal Product Development" in English
Araf	*D*-arabinofuranose
ARMS	Amplification refractory mutation system
ART	Antiretroviral therapy
AS	Ankylosing spondylitis
Asn	Asparagine
Asp	Aspartic acid
ASUN	Asunder spermatogenesis regulator
ATP	Adenosine triphosphate
ATS	American Thoracic Society

ATT	Anti-tuberculosis treatment
AUC	Area under the curve
AuNPs	Gold nanoparticles
B. subtilis	*Bacillus subtilis*
B.C.	Before Christ
BAL	Bronchoalveolar lavage
BCG	Bacillus Calmette–Guérin
BER	Base excision repair
BHL	Swelling of bilateral hilar and mediastinal lymph nodes
BlaC	β-lactamases
BMRC	British Medical Research Council
BSC	Biological Safety Cabinet
BSL	Biosafety level
bTB	Bovine tuberculosis
BTS	British Thoracic Society
BTZ	Benzothiazinone
CABD	Calcium-binding domain
Cas9	CRISPR-associated protein 9
CBP	Ciliary beat frequency
CDC	Centers for Disease Control and Prevention
CDRI	Central Drug Research Institute, Lucknow, India
CECT	Contrast-enhanced computerized tomogram
CF	Cystic fibrosis
CFP-10	Culture filtrate protein 10
CFU	Colony-forming unit
cGMP	Cyclic guanosine monophosphate
CHTB	Childhood tuberculosis
CHW	Community health workers
Cmas	Cyclopropane mycolic acid synthase
C_{max}	Maximum concentration
C_{min}	Minimum concentration
CNS	Central nervous system
COG	Clusters of orthologous groups
COPD	Chronic obstructive pulmonary disease
CPA	Cyclopiazonic acid
CPG	5′—C—phosphate—G—3′
CRC	Colorectal cancer
CrCl	Creatinine clearance
CRISPR	Clustered regularly interspaced short palindromic repeat
CRP	C-reactive protein
CSIR	Council of Scientific and Industrial Research, India
CSs	Corticosteroids
CT	Computed tomography
CTAB	C-etyltrimethylammonium bromide
CXR	Chest X-ray

CYP2C9	Cytochrome P450 family 2 subfamily C member 9
Cys	Cysteine
D	Aspartate
Dam	DNA adenine methyltransferase
DAMPs	Damage-associated molecular patterns
DBT	Direct Benefit Transfer
DBVS	Docking-based virtual screening
Dcm	DNA cytosine methyltransferase
DCs	Dendritic cells
DC-SIGN	Dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin
Ddl	D-alanine:D-alanine ligase
Ddn	Deazaflavin-dependent nitroreductase
DHAP	Dihydroxyacetone phosphate
DHFR	Dihydrofolate reductase
DHX29	DExH-box helicase 29
DIM	Dimycocerosates
DLTLD	Division of Leprosy, Tuberculosis and Lung Disease
DM	Diabetes mellitus
DMARDs	Disease-modifying antirheumatic drugs
DNA	Deoxyribonucleic acid
Ddn	Deazaflavin-dependent nitroreductase
DNMTs	DNA methyltransferases
DosR	Dormancy survival regulator
DOT	Directly observed therapy
DOTS	Directly observed therapy short course
DPA	Decaprenylphosphoryl-β-*D*-arabinose
DPR	Decaprenylphosphoryl-β-*D*-ribose
DPX	Decaprenylphosphoryl-2-keto-β-*D-erythro*-pentofuranose
DR-TB	Drug-resistant tuberculosis
DS	Drug sensitive
DST	Drug susceptibility testing
DS-TB	Drug-susceptible tuberculosis
DTPB	Detect—treat—prevent—build
DUSP4	Dual-specificity MAP kinase phosphatase 4
DwA	Doctors with Africa CUAMM
E. coli	*Escherichia coli*
EBA	Early bactericidal activity
Eis	Enhanced intracellular survival enzyme
ELISPOT	Enzyme-linked Immunospot
EMA	European Medicines Agency
EMB	Ethambutol
EPTB	Extrapulmonary tuberculosis
ERM	Erythromycin resistance rRNA methylase

ESAT-6	Early secretory antigenic target-6
ESR	Erythrocyte sediment rate
ESX-1	ESAT-6 secretion system-1
ETC	Electron transport chain
EUCAST	European community on antimicrobial susceptibility testing
EVs	Extracellular vesicles
Fab	Fragment antigen-binding region
FAD	Flavin adenine dinucleotide
FADH2	Flavin adenine dinucleotide (reduced)
FALCON	Fully automated line cage odor nose-poke holes
FAS-I	Fatty acid synthase type I
FAS-II	Fatty acid synthase type II
FBA-tb	Fructose-1,6-bisphosphate aldolase class II
FBP	Fructose-1, 6-bisphosphate
FcR	Fragment crystallizable region
FCI	Food chain information
FDA	Food and Drug Administration
FNA	Fine needle aspiration
FNAC	Fine needle aspiration cytology
G3P	Glyceraldehyde 3-phosphate
GB	Gall bladder
GC	Glucocorticoid
GCTOFMS	Gas chromatography time-of-flight mass spectrometry
GI	Gastrointestinal tract
Gly	Glycine
GMNPs	Glycan-functionalized magnetic nanoparticles
GR	Glucocorticoid receptor
GRAS	Generally regarded as safe
GUI	Graphical user interface
H_2O_2	Hydrogen peroxide
HAART	Highly active antiretroviral therapy
HATs	Histone acetyltransferases
HBHA	Heparin-binding haemagglutinin
HCWs	Healthcare workers
HDACs	Histone deacetylases
HDMs	Histone demethylases
HFS	Hollow fiber system
HH	Household
HIC	High-income countries
HIF-1α	Hypoxia-inducible factor 1α
His	Histidine
HIV	Human immunodeficiency virus
HIV-TB	Human Immunodeficiency virus-tuberculosis
HLA	Human leukocyte antigen

HMTs	Histone methyltransferases
HNP	Human neutrophil peptide
HOTAIR	HOX transcript antisense RNA
HPLC	High-performance liquid chromatography
HR	Homologous recombination
HRCT	High-resolution computed tomography
HSP65	Heat shock protein 65
HTS	High-throughput screening
HW	Hamazaki-Wesenberg
IBD	Inflammatory bowel disease
IC_{50}	Half maximal inhibitory concentration
IDSA	Infectious Diseases Society of America
IDU	Injection drug use
IEC	Information, education, and communication
IFN	Interferon
IFN-γ	Interferon-gamma
IgG1	Immunoglobulin G1
IGRA	Interferon-gamma release assay
IL-4R	Interleukin-4 receptor
IL-6R	Interleukin-6 receptor
IL-17R	Interleukin-17 receptor
IL	Interleukin
Ile	Isoleucine
IM	Intramuscular
INDELS	Insertions and deletions
INH	Isoniazid
InhA	Enoyl-acyl carrier protein reductase
iNOS	Inducible NO-synthase
INSTIs	Integrase strand transfer inhibitors
IPT	Isoniazid preventive therapy
IRIS	Immune reconstitution inflammatory syndrome
ISCs	Intestinal stem cells
IU	International units
IUATLD	International Union Against Tuberculosis and Lung Diseases
IV	Intravenous
J-ACNES	Japanese Antibacterial Drug Management for Cardiac Sarcoidosis
KatG	Catalase-peroxidase enzyme
LAM	Lipoarabinomannan
LAMP	Loop-mediated isothermal amplification platform
LBP	Laminin-binding protein
LCMS	Liquid chromatography high-resolution mass spectrometry
LED	Light-emitting diodes

Leu	Leucine
LJ	Lowenstein–Jensen
LMICs	Low and middle-income countries
LOD	Limit of detection
LPA	Line probe assay
LTBI	Latent tuberculosis infection
Lys	Lysine
M. bovis	*Mycobacterium bovis*
M. canetti	*Mycobacterium canetti*
M. sm	*Mycobacterium smegmatis*
M. smegmatis	*Mycobacterium smegmatis*
M. tb ΔctpF	*M. tb* H37Rv. defective of the *ctpF* gene
M. tb	*Mycobacterium tuberculosis*
M. w	*M. indicus pranii*
M2	N-monodesmethyl metabolite
mAb	Monoclonal antibody
MAC	*Mycobacterium avium* complex
mAGP	Mycolyl-arabinogalactan-peptidoglycan
MamA	Adenine methyltransferase
MATE	Multidrug and toxic compound extrusion family
MBTC	Mycobacterium tuberculosis complex
Mce	Mammalian cell entry
MDGs	Millennium development goals
MDR	Multidrug resistant
MFS	Major facilitator superfamily
MGIT	*Mycobacterium* growth indicator tube
MHC	Major histocompatibility complex
MH-S	Mouse alveolar macrophages
MIC	Minimum inhibitory concentration
miRNA	MicroRNA
MIRU	Mycobacterial interspersed repetitive units
MKP-1	Mitogen-activated protein kinase phosphatase 1
MKUTA	Mapambano ya Kifua Kikuu na Ukimwi Tanzania
MLS	Macrolide-lincosamide-streptogramin
MM/GBSA	Molecular mechanics/generalized born surface area
MmCO	Multicopper oxidase
MmpL	Mycobacterial membrane protein large
MMPs	Matrix metalloproteinases
MmpS	Mycobacterial small membrane protein
MoA	Mechanism of action
Mo-bis	Molybdopterin guanine dinucleotide
MP	Microparticles
MRI	Magnetic resonance imaging
MSCs	Mesenchymal stem cells
MT	Mantoux test

MTBC	*Mycobacterium tuberculosis* complex
mtDNA	Mitochondrial DNA
Mφs	Macrophages
NAAT	Nucleic acid amplification test
NABL	National Accreditation Board for Testing and Collaboration Laboratories
NACO	National AIDS Control Organization
NAD	Nicotinamide adenine dinucleotide
NAT	N-acetyltransferase
NDH	NADH dehydrogenase
NEMF	Nuclear export mediator factor
NER	Nucleotide excision repair
NFHS	National Family Health Survey
NGOs	Non-Governmental Organizations
NHEJ	Non-homologous end junction
NIAID	National Institute of Allergy and Infectious Diseases
N-MBD	N-terminal metal-binding domains
NMDA	N-methyl-D-aspartic acid
NMR	Nuclear magnetic resonance
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NO	Nitric oxide
NOD	Nucleotide-binding oligomerization domain
NTCP	National tuberculosis control program
NTEP	National tuberculosis elimination program
NTM	Non-tuberculous mycobacteria
NTM-NET	Non-tuberculous Mycobacteria Network European Trials Group
NTP	National tuberculosis program
NYC	New York City
OADC	Oleic acid, albumin, dextrose, and catalase
OPD	Outpatient department
OR	Odds ratio
P. acne	*Propionibacterium acnes*
PABA	Para-aminosalicylic acid
PAL	Practical approach to lung health
PAM	Peptidoglycan-arabinogalactan-mycolic acid
PANTA	Polymyxin B, amphotericin B, nalidixic acid, trimethoprim, azlocillin
PBPs	Penicillin-binding proteins
PBTZ	Piperazine-benzothiazinone
PBVS	Pharmacophore-based virtual screening
PCR	Polymerase chain reaction
PD	Pharmacodynamic
PDE	Phosphodiesterase
PEG	Polyethylene glycol chain

PEP	Phosphoenolpyruvate
PfATP6	Ca^{2+}-ATPase of *Plasmodium falciparum*
PG	Peptidoglycan
PGL	Phenolglycolipid
pIC_{50}	Negative logarithm of IC_{50}
PLF	Phagosome-lysosome fusion
PLHIV	People living with HIV
PMCA	Plasma membrane Ca^{2+}-ATPase
PMF	Proton motive force
PPD	Purified protein derivative
PPE	Proline-proline-glutamate
pre-miRNAs	Precursor microRNAs
pri-miRNAs	Primary microRNAs
PRRs	Pattern recognition receptors
PsA	Psoriatic arthritis
PTB	Pulmonary tuberculosis
PtkA	Protein tyrosine kinase
PTMs	Posttranslational mechanisms
PtpA	Protein tyrosine phosphatase
PTPRC	Protein tyrosine phosphatase, receptor type, C
PWID	People who inject drugs
PWUD	People who use drugs
PY	Patient years
PZA	Pyrazinamide
QDs	Quantum dots
QFT	QuantiFERON
QFT-GIT	QuantiFERON-TB GOLD In-Tube
QFT-Plus	QuantiFERON-TB Gold-Plus
QTc	Corrected QT interval
RA	Rheumatoid arthritis
RCA	Rolling circle amplification
RCTs	Randomized clinical trials
RD	Regions of differences
RFID	Radio frequency identification
RFLP	Restriction fragment length polymorphism
RGM	Rapid growing mycobacteria
RIF	Rifampicin
RNA	Ribonucleic acid
RNAi	RNA interference
RND	Resistance-nodulation cell division family
RNS	Reactive nitrogen species
RNTCP	Revised national tuberculosis control program
ROS	Reactive oxygen species
RR	Relative risk
rRNA	Ribosomal RNA

RR-TB	Rifampicin-resistant tuberculosis
RTX	Repeat-in-Toxin
S. enterica	*Salmonella enterica*
S. roseosporus	*Streptomyces roseosporus*
SABIO	South African Biologics Registry
SAM	S-adenosylmethionine
SAR	Structure-activity relationship
SATB1	Sequence binding protein 1
SCFAs	Short-chain fatty acids
SDGs	Sustainable Development Goals
Ser	Serine
SERCA	Sarco/endoplasmic reticulum Ca^{2+}-ATPase
SGM	Slow-growing mycobacteria
SGRQ	St. George's Respiratory Questionnaire
shRNAs	Short hairpin RNAs
SIR	Standardized incidence ratio
siRNAs	Synthetic small interfering RNAs
SMR	Small multidrug resistance family
SOD	Superoxide dismutase
SodA	Superoxide dismutase A
SodC	Superoxide dismutase C
SPR	Surface plasmon resonance
SQ-109	N-Geranyl-N′-(2-adamantyl)ethane-1,2-diamine
SSA	Strand alignment
SSM	Sputum smear microscopy
SSP	Syringe service program
STPKs	Serine/threonine protein kinases
SUN	The Standardization of Uveitis Nomenclature
TACE	TNF-α converting enzyme
TAT	Turnaround time
TB	Tuberculosis
TB–	Tuberculosis negative
TB+	Tuberculosis positive
TBI	*Mycobacterium tuberculosis* infection
TB-LIMS	Tuberculosis-laboratory information management system
TBM	Tuberculosis meningitis
TCA	Tricarboxylic acid
TDM	Therapeutic drug monitoring
TDR	Totally drug resistant
TDZ	Thioridazine
TGF-β	Transforming growth factor-β
Th1	T helper type 1
Th2	T helper type 2
TIBU	Treatment Information from Basic Unit
TLR	Toll-like receptor

TMD	Transmembrane domain
TMS	Transmembrane segment
TNF-α	Tumor necrosis factor-alpha
TNFi	Tumor necrosis factor-alpha inhibitors
TNFR	Tumor necrosis factor receptor
TPP	Target product profile
TPT	Tuberculosis preventive therapy
tRNA	Transfer ribonucleic acid
Trp	Tryptophan
TSA	Tuberculostearic acid
TSSs	Transcription start sites
TST	Tuberculin skin test
TU	Tuberculin units
Tyr	Tyrosine
TZD	Thiazolidine-2,4-dione
UC	Ulcerative colitis
UHPLC–ESIQTOFMS	Ultra-high-performance liquid chromatography-electrospray, ionization-quadrupole time-of-flight mass spectrometry
UN	United Nations
UNODC	United Nations Office on Drugs and Crime
USG	Ultrasonogram
USP	Universal stress protein
UV	Ultraviolet
VAMNDA	Volume amplified-magnetic nanobead detection assay
VDR	Vitamin D receptors
VEGF	Vascular endothelial growth factor
VNTR	Variable number of tandem repeats
VOC	Volatile organic compound
WGS	Whole-genome sequence
WHA	World Health Assembly
WHO	World Health Organization
WPR	What's the problem represented to be
WRD	WHO-recommended diagnostics
XDR	Extensively drug resistant
ZN	Ziehl–Neelsen stain

Introduction to Tuberculosis: Integrated Studies for a Complex Disease

1

Nima Rezaei, Nastaran-Sadat Hosseini, and Amene Saghazadeh

The diseases we suffer from are a reflection of our lifestyle. Prevent them with regular tests and prevention tips.

World Tuberculosis Day 2022

Summary

Tuberculosis: Integrated Studies for a Complex Disease arose in response to a complex old problem–tuberculosis. The book's outline is presented in this chapter, with the main headings: heterogeneity, diagnosis, treatment, drug resistance, and prevention.

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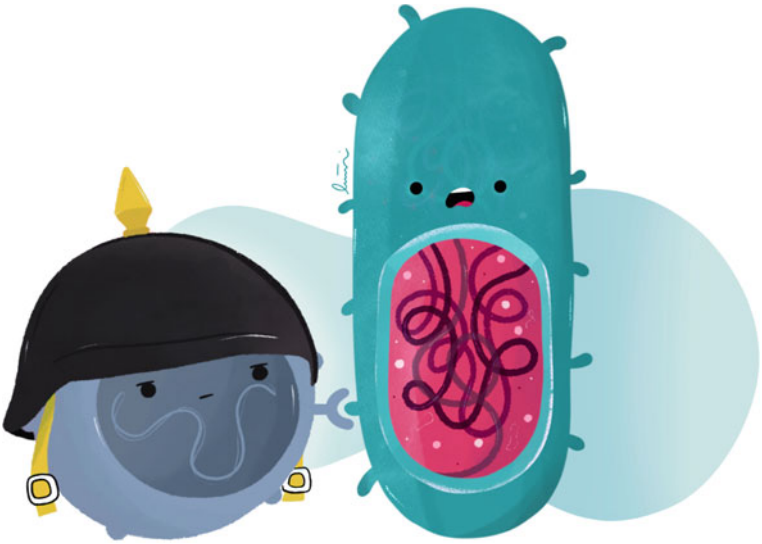
Graphical Abstract



TB or not TB? (Adapted with permission from the Association of Science and Art (ASA), Universal Scientific Education and Research Network (USERN); Made by Nastaran-Sadat Hosseini)

Keywords

Integrated studies · Tuberculosis

1 Introduction

Tuberculosis (TB) is an old disease. It remains a disease with high incidence and mortality, which, along with other respiratory infections, accounts for the first cause of death in the category of communicable, maternal, neonatal, and nutritional disorders (this chapter). Even in the United States, where TB incidence is generally on the decline, the trends are concerning, particularly regarding disparities: the decline in TB cases has been shown to happen only to United States-born individuals, not non-US-born individuals [1]; childhood and adolescent TB cases remain significantly higher in non-white people and those from TB-endemic countries [2].

1.1 Heterogeneity in TB Transmission and Manifestations

Generally, the heterogeneity in TB epidemiology is under the influence of various factors related to the infectious agent, infectious host, susceptible host, environment, and distal determinants (Chap. 2) [3]. Particularly, *Mycobacterium tuberculosis* (*M. tb*)–the organism that is responsible for TB–has different isolates that show heterogeneity in the genotype and phenotype lying in different stochastic processes, growth phase and growth rate, asymmetric cell division and cell aging, and host microenvironment (Chap. 3). With regards to the host, there might be genetic susceptibility. In particular, studies of patients with Mendelian predisposition to severe TB have associated genetic loci with developing TB, including active TB and latent TB infection, and related outcomes [4–6], especially drug-resistant TB [7]. Also, TB heterogeneity is seen with anthropometric measures, age, and sex, with higher TB rates in underweight [8] and older people [9] and in men [10]. The socioeconomic factors have been shown to account for TB heterogeneity as well. Importantly, TB is mostly a disease in the poor. Ecological analyses suggest that the unemployment rate and household crowding are the socioeconomic factors best associated with higher TB rates [11]. Moreover, TB transmission shows a high degree of heterogeneity, as shown in a systematic review of spatial studies of TB [12]. Interestingly, the immune system shows heterogeneity in response to infection with *M. tb*. In particular, macrophages, which comprise the dominant population of cells infected by *M. tb*, display phenotypes and immune functions that vary across individuals [13]. This would, in turn, contribute to the heterogeneity in the immunopathogenesis of TB [14]. Finally, TB patients show different responses to anti-TB therapy [15]. Identifying the sources of heterogeneity for dealing with the problem of TB effectively is imperative [3].

1.2 TB and Related Conditions

1.2.1 Tobacco Use

Meta-analyses indicate a positive, significant association between drug-resistant TB and tobacco smoking (Chap. 4) [16], especially for current smoking, as well as between TB rates and exposure to indoor air pollution, especially for pollutants $PM_{2.5}$ and biomass smoke [17]. Despite this evidence, no consensus has been developed on integrating TB and tobacco control programs, calling a need for TB-tobacco integration [18–20]. For this purpose, understanding the epidemiology of TB and tobacco use in relation to each other is indispensable. There have been launched some joint policies for tackling TB and tobacco use; however, their effectiveness is limited due to challenges at the patient, healthcare provider, and health system levels. Opportunities are available as well, along with recommendations to establish the joint working group at all levels, modify reporting mechanisms, integrate the programs into health and development agendas, and advocate for smoke-free policies (Chap. 4).

1.2.2 Cancer

Following a cancer diagnosis, TB rates increase especially, according to a large cohort study, in patients diagnosed with myelodysplastic syndrome (MDS)/myeloproliferative neoplasm (MPN) [21] (Chap. 5). On the other hand, having TB is associated with an increased risk of developing lung cancer, which is attributed to the ability of *M. tb* in causing cellular transformation in the lung cells, making them vulnerable to lung cancer [22, 23]. Managing TB in patients with cancer is faced with challenges, importantly with regards to anti-TB treatment while the patients are on anti-cancer therapy [22]. Moreover, there have been reports of *M. tb* reactivation following treatment with immune checkpoint inhibitors [24], highlighting the importance of evaluating latent TB infection in patients with cancer. Though some mechanisms have been suggested to underlie the cancer-TB association [25], they remain less understood. Yet, the TB-cancer association poses a complex problem.

2 Diagnosis

Different methods have been developed for TB diagnosis. For a laboratory diagnosis of TB, there are various concerns and topics from laboratory safety, collection of different specimens, importantly blood and sputum, and digestion and decontamination, to microscopy, culture using both solid media and liquid system, tuberculin skin test, interferon-gamma release assays, molecular methods, including signal amplification method and post-amplification analysis, nanodiagnostics, and biosensing techniques for consideration (Chap. 6). Among them, the most commonly used methods are acid-fast staining and culture. According to estimates provided by a pooled analysis, the specificity of acid-fast staining falls between 95 and 98%, while that for culture is 98% [26]. The sensitivity of acid-fast staining is between 20 and 70%, compared to that of 95% for culture. Molecular methods can be used in the step after the positive result is obtained with culture. Importantly, nucleic acid amplification tests help with the direct observation of *M. tb* in tested samples [26]. Molecular methods are still in progress, enabling laboratorians to both establish TB diagnosis and screen for drug-resistance TB-related mutations. Among the recently developed methods are MTBDRplus, loop-mediated isothermal amplification, line probe assay, GeneXpert, whole-genome sequencing, and next-generation sequencing [27]. Machine learning-based approaches have recently been shown to be promising in handling laboratory data and aiding with the differentiation of active TB from latent TB infection [28].

Microfluidics can also apply to TB diagnosis [29]. These devices can diagnose TB from bacterial components and through genotyping (Chap. 7). They offer a lower price and analysis time with acceptable cost-effectiveness and accuracy [30]. They are also easy to use; microfluidic devices can be integrated into standard

laboratory equipment [31]. To exemplify, in a recently published study, the combination of a microfluidic cartridge and FluoroType MTB yielded a 100% sensitivity for TB diagnosis. This cartridge can be integrated into a platform of testing for drug-resistant TB and provide information about drug-resistant isolates, too [32]. Efforts are increasing to integrate microfluidics into TB diagnostic procedures, such as real-time polymerase chain reaction [33].

In addition, immunodiagnostics involves a wide range of blood-based biomarkers, host gene expression profiles, OMICs, etc. (Chap. 8) that can be used as point-of-care tests for TB diagnosis [34]. Cytokine biomarkers, for example, have been shown to diagnose TB, differentiate active TB disease from latent TB infection [35], determine disease severity, and predict and monitor treatment response [36–38]. Gene expression signatures can classify patients according to their immune profiles. This classification is useful for both diagnostic and prognostic purposes and for identifying potential targets for therapy and decision-making about the immune response endotype-based host-directed therapy [39–41]. Multi-OMICs technologies can help develop proteomic, genomic, or transcriptomic diagnostics that can diagnose pulmonary TB and extrapulmonary TB and assess the disease status [42]. Multi-OMICs approaches are promising in the diagnosis of TB in problematic populations [43], e.g., pediatric TB (Chaps. 10–12) and people who live with HIV [44]. They can also contribute to drug discovery for TB by identifying new targets by evaluating drug action and screening by compound activity [45].

Bronchoscopy is used as a diagnostic and therapeutic procedure in the context of TB practice (Chap. 9), especially in cases with endobronchial TB and for those suspected of having pulmonary TB who are smear-negative or sputum-scarce [46]. Different bronchoscopic techniques have been described to provide bronchoalveolar lavage and bronchial washing aspirates in a safe and accurate manner [46]. Even bronchoscopic procedures can apply to pediatric patients [47].

3 Treatment

3.1 Anti-TB Drugs

The treatment of TB mainly involves chemotherapy [48, 49]. Anti-TB medications include first-line (isoniazid, rifampicin, pyrazinamide, and ethambutol) and second-line (streptomycin, injectables (amikacin, kanamycin, and capreomycin), fluoroquinolones, ethionamide/prothionamide, cycloserine/terizidone, para-aminosalicylic acid/Para-aminosalicylic acid sodium, bedaquiline, delamanid, linezolid, clofazimine, augmentin, and thioacetazone) anti-TB drugs. Recommendations on these drugs prescriptions and population-specific advice, i.e., adults, children, TB-HIV-co-infected people, and patients with latent TB infections, are

provided (Chap. 13). Moreover, the exposure to anti-TB medications or their levels correlate with TB outcomes [50]; for example, with regards to the first-line anti-TB drugs, higher exposure to rifampicin is related to a better outcome and lower levels of rifampicin, isoniazid, and pyrazinamide to a weaker outcome. Understanding the pharmacokinetic and pharmacodynamic properties of anti-TB medicines is required for treatment optimization (Chap. 14) [51].

3.2 Immunotherapy

TB and its presentation and outcome are associated with immune responses [52]. CD4+ Th1 cells, CD4+ Th17 cells, CD8+ T cells, $\gamma_9\delta_2$ T cells, mucosa-associated invariant T (MAIT) cells, and antibody responses are known as immune responses potentially contributing to protection against TB reactivation, whereas CD4+ Tregs, exhausted T cells, alternatively activated macrophages, and type 1 interferon-induced polymorphonuclear cells might adversely affect anti-TB immunity [53]. Adjunct immunotherapies have, therefore, been considered for TB [54, 55] to influence the immune system and inhibit *M. tb* replication [53]. Immunotherapy for TB mainly involves cytokines, particularly interferon and interleukin 2, and their inhibitors, especially anti-tumor necrosis factor (Chap. 15) [55]. However, *Mycobacterium vaccae*, RUTI (a vaccine), autologous mesenchymal stem cells (MSC), V5 immunitor, drugs/compounds with immunomodulatory properties, e.g., steroids, Levamisole, Albendazole, and Thalidomide, and vitamin D, have been clinically investigated as well.

3.3 Inhaled Anti-TB Therapy

Inhalation is a method of drug delivery for TB. Different drugs can be used in inhaled TB therapy [56], including old and new drugs (colistin, capreomycin, amikacin/kanamycin/gentamycin, streptomycin, pyrazinoic acid ester and pyrazinoic acid/ester Dry Powder, spectinamides, rifampicin, clofazimine, isoniazid, pretomanid) [57]. Inhaled TB therapy is promising to carry the drug to the lungs directly as well as deliver it to the systemic circulation and maintain drug concentrations optimal while systemic toxicity risk remains low [58–60]. This is very demanding given that conventional drug delivery methods are often unable to hold anti-TB drugs at the optimal concentrations but are also associated with a variety of side effects due to their accumulation in the circulation [61]. Studies are increasing to assess the prospects of inhaled TB therapy keeping in mind the lessons learned from the experience with other pulmonary diseases (Chap. 16) [57, 59]. To investigate the effectiveness of inhaled anti-TB therapy in drug-resistant TB, however, requires clinical studies [57].

3.4 Malnutrition Treatment

Malnutrition is a common problem in patients with TB; therefore, nutrition is significant to control TB [62]. Poor nutrition, decreased appetite, malabsorption, and metabolic changes account for malnutrition in TB. Malnutrition in TB occurs as protein-energy malnutrition and/or essential elements deficiencies [63]. Particularly, patients with TB show deficiencies in micronutrients zinc, vitamin B6, vitamin C, vitamin D, and vitamin E (Chap. 17). Malnutrition is a cause of secondary immunodeficiency resulting in increased individual vulnerability to TB. Studies have associated malnutrition with an increased risk of developing TB, delayed recovery of TB, and death due to TB. Nutrition examination surveys and nutrition-focused programs need to be incorporated in TB prevention and management programs [64, 65]. In particular, micronutrient supplementation is a key for integration into TB management [66].

4 Drug Resistance

Drug resistance is a challenge of modern TB that emerged 30 years ago [67]. It is a threat to the world, from Asian and African countries to European and American countries [68]. Drug-resistant TB is a form of TB where *M. tb* strains undergo changes in cell morphology considerably. Mutations causing these changes are different, so databases have been developed to list them [69]. When standard anti-TB therapy fails, drug resistance testing is done to isolate *M. tb* species and agents to which they are susceptible. This testing can be based on phenotypic and genotypic approaches [70]. Different molecular mechanisms have been linked to drug-resistant TB (Chap. 18) [71], including permeability-associated resistance, acquired resistance by genetic mutations (Chaps. 43 and 47), drug efflux, target mimicry, and drug degradation and modification. Moreover, epigenetic mechanisms have recently been proposed to play a role in anti-TB drug resistance (Chap. 44). These mechanisms might contribute to the host–pathogen connection by affecting the gut-lung microbiota in a way that resistance develops (Chaps. 46 and 47). Molecular and whole-genome sequencing methods are recommended for understanding its pathogenesis and diagnosis along with multiomics integration (Chap. 45). Drug-resistant TB calls for new anti-TB strategies to modulate the resistance, e.g., structural modification in existing drugs to address mutation-associated resistance, targeting efflux pumps associated with drug efflux, and bypassing drug inactivating enzymes. As a result, drug discovery remains an intensely active line of research in TB (Chaps. 21–25), with many drugs are under development for TB therapy, especially for drug-resistant TB (Chap. 19). Individualized treatment is useful to manage drug-resistant TB patients considering the form of TB, existing comorbidities, genetic background, disease severity, nutritional status, demographics, and body composition (Chap. 20) [15].

5 Prevention

According to the World Health Organization (WHO) report, the decline in TB incidence was estimated at about 6% from 2015 to 2018, while the 2020 milestone was set at 20% [72]. That TB elimination is not progressed as it should be because TB prevention is faced with challenges, importantly latent TB infection, drug resistance, missing cases, contact investigation, gender disparities, global migrations, childhood TB, HIV-TB co-infection, TB among people who use drugs, and TB among people with autoimmune diseases who use TNF-targeted therapies (Chaps. 28–32). Moreover, extrapulmonary TB that affects different anatomical sites is associated with difficulties in diagnosis and treatment (Chaps. 33–39). The search for animal TB, which mostly occurs due to *Mycobacterium bovis* in domestic animals, is crucial to the success of TB prevention programs; upon exposure to these animals, individuals might be infected (Chaps. 40–42). TB-related stigma is high, which might prevent people from being interested in knowing about latent TB infection status; stigma reduction is necessary, too (Chaps. 49 and 50) [73]. Preventive efforts are required to tackle TB as a complex global problem that needs attention to identify the targeted population for TB preventive therapy (Chap. 27) and develop TB diagnostics, therapeutics, and vaccines [74, 75].

6 Experience Before US

6.1 Integrated Prevention Services

Integrated prevention services aim to control TB along with comorbid infections, mainly HIV, viral hepatitis, sexually transmitted diseases [76], or non-communicable diseases [77]. These services are, not limited to, ranging from the prevention and treatment of mental health and substance use disorders to treatment of infectious diseases and delivery of preventive services. The inetgartion of TB and HIV care services is, however, poorly implemented [78].

6.2 Integrated Therapy

Accordingly, integrated therapy for TB and comorbid conditions is warranted [79]. For example, patients undergoing an integrated intervention of anti-retroviral therapy (ART)-anti-TB therapy were less likely to die by more than 80% at the end of the study compared to those who sequentially received anti-TB treatment and then ART [80]. Also, an integrated TB treatment-tobacco cessation protocol, called SCIDOTS, has been shown to improve the success of quiting smoking and anti-TB treatment, as compared to the anti-TB treatment (DOTS) merely [81].

6.3 Integrated Research

An integrated approach for TB research can help with the discovery of anti-TB drugs and vaccines. For example, Tuberculosis Database (TBDB) is a database including genomic data and resources to which an integrated approach can apply [82].

6.4 Integrated Understanding of Pathogenesis

6.4.1 Integrated Immune and Endocrine View

TB infection is accompanied by alterations to immune and endocrine processes. In particular, TB is characterized by increased levels of IFNγ, IL6, and IL10 [83]. For the endocrine component, it is related to increased levels of growth hormone, decreased levels of testosterone and dehydroepiandrosterone (DHEA), and an increased cortisol/DHEA ratio. At their intersection, DHEA has been shown to correlate with IFNγ levels. Therefore, TB is considered a immunoendocrine disorder, and understating its pathogenesis calls for an integrated view (Chap. 48).

6.4.2 Integrated Whole-Genome Analysis

Integrtaed studies of whole genome analysis can shed light on TB pathogenesis by establishing relationships between DNA sequences, methylation, and transcription. This leads to the identification of mechnaisms potentially contributing to TB that can serve for targeting options [84].

6.5 Integrated Diagnostic Approach

Integrated diagnostic methods are used to detect *M. tb* with advantages; for example, earlier detection of *M. tb*, *M. tb* detection in smear-negative samples, and drug resistance diagnosis [85–88].

6.6 Integrated Anti-resistance Platform

To attenuate the problem with drug resistance in TB, there have been many efforts for drug discovery. In addition, a bioengineered integrated platform can help with monitoring the response to drugs, i.e., pharmacokinetics modeling. E.g., a three-diemnsional cell culture provides such a platform that integrates a microsphere system and a microfluidic plate and evaluates the effect of drugs on *M. tb* killing [89].

6.7 Integrated Social Epidemiology

By comparing large and small clusteres of TB, an integrated analysis of epidemiological and genomics data could identify various host-related and pathogen-related factors that mediate TB transmission. These include mainly being a non-hispanic black, homelessness, male sex, aged below 65 years, sputum smear positivity, drinking too much acohol, and HIV seropositvity, among which, importantly, being a non-hispanic black and homelessness were the independent risk factors for TB [90]. This calls a need for integration of social factors into the control programs of TB.

6.8 Integrated Contact Tracing

Contact tracing is an important step of TB care. Different strategies have been developed for this purpose; however, detection rates remain low in some areas, indicating that contact tracing has not been effective. Integrated approaches can increase the effectiveness of contact tracing. For example, the performance of a combination of CXR, sputum, and gene Xpert MTB/RIF examination was shown to be suitable [91], yielding a high detection rate in a high-burden area.

6.9 Integrated Forecasting Model

Forecasting the incidence of TB is required to prepare health services and resources. Integrtaed models have shown a good performance, e.g., auto-regressive integrated moving average (ARIMA) model and its seasonal version (SARIMA) [92].

7 Conclusion

As outlined above, in *Tuberculosis: Integrated Studies for a Complex Disease,* TB is highlighted as a disease with complexity in multiple aspects, from the heterogeneity in epidemiology and diagnosis to treatment, drug resistance, and prevention. In parallel, the book includes several nudges to the drug discovery for both tuberculous mycobacteria and non-tuberculous mycobacteria (Chap. 26). In the end, Chap. 51 is an Expert Opinion that includes the opinion of more than 150 authors who contributed to the volume. We have express our opinions on TB 2050. Welcome to *Tuberculosis: Integrated Studies for a Complex Disease*.

Core Messages

- Even in locations where TB incidence is generally declining, the trends are concerning, particularly regarding disparities.
- A variety of concerns exist regarding laboratory TB diagnosis, particularly childhood TB diagnosis in resource-limited settings.
- The treatment of TB mainly involves first-line and second-line medicines; however, adjunct immunotherapy is also promising.
- Drug resistance is a challenge of modern TB, with different molecular mechanisms playing a role.
- That TB elimination is not progressed as it should be because TB prevention is faced with challenges.

References

1. Armstrong LR, Winston CA, Stewart B, Tsang CA, Langer AJ, Navin TR (2019) Changes in tuberculosis epidemiology, United States, 1993–2017. Int J Tuberc Lung Dis 23(7):797–804
2. Cowger TL, Wortham JM, Burton DC (2019) Epidemiology of tuberculosis among children and adolescents in the USA, 2007–17: an analysis of national surveillance data. The Lancet Public Health 4(10):e506–e516. https://doi.org/10.1016/S2468-2667(19)30134-3
3. Trauer JM, Dodd PJ, Gomes MGM, Gomez GB, Houben RMGJ, McBryde ES, Melsew YA, Menzies NA, Arinaminpathy N, Shrestha S (2019) The importance of heterogeneity to the epidemiology of tuberculosis. Clin Infect Dis 69(1):159–166
4. Abel L, Fellay J, Haas DW, Schurr E, Srikrishna G, Urbanowski M, Chaturvedi N, Srinivasan S, Johnson DH, Bishai WR (2018) Genetics of human susceptibility to active and latent tuberculosis: present knowledge and future perspectives. Lancet Infect Dis 18(3):e64–e75
5. Harishankar M, Selvaraj P, Bethunaickan R (2018) Influence of genetic polymorphism towards pulmonary tuberculosis susceptibility. Front Med 5:213
6. Roy RB, Whittaker E, Seddon JA, Kampmann B (2019) Tuberculosis susceptibility and protection in children. Lancet Infect Dis 19(3):e96–e108
7. Consortium CR and the 100,000 Genomes Project (2018) Prediction of susceptibility to first-line tuberculosis drugs by DNA sequencing. N Engl J Med 379 (15):1403–1415
8. Badawi A, Gregg B, Vasileva D (2020) Systematic analysis for the relationship between obesity and tuberculosis. Public Health 186:246–256
9. Ku C-C, Dodd PJ (2019) Forecasting the impact of population ageing on tuberculosis incidence. PLoS ONE 14(9):e0222937. https://doi.org/10.1371/journal.pone.0222937
10. Hertz D, Schneider B (2019) Sex differences in tuberculosis. Springer, pp 225–237
11. Pelissari DM, Rocha MS, Bartholomay P, Sanchez MN, Duarte EC, Arakaki-Sanchez D, Dantas CO, Jacobs MG, Andrade KB, Codenotti SB (2018) Identifying socioeconomic, epidemiological and operational scenarios for tuberculosis control in Brazil: an ecological study. BMJ Open 8(6):e018545
12. Shaweno D, Karmakar M, Alene KA, Ragonnet R, Clements ACA, Trauer JM, Denholm JT, McBryde ES (2018) Methods used in the spatial analysis of tuberculosis epidemiology: a systematic review. BMC Med 16(1):1–18
13. Khan A, Singh VK, Hunter RL, Jagannath C (2019) Macrophage heterogeneity and plasticity in tuberculosis. J Leukoc Biol 106(2):275–282

14. Marakalala MJ, Martinez FO, Plüddemann A, Gordon S (2018) Macrophage heterogeneity in the immunopathogenesis of tuberculosis. Front Microbiol 9:1028
15. Koch A, Cox H, Mizrahi V (2018) Drug-resistant tuberculosis: challenges and opportunities for diagnosis and treatment. Curr Opin Pharmacol 42:7–15
16. Wang M-G, Huang W-W, Wang Y, Zhang Y-X, Zhang M-M, Wu S-Q, Sandford AJ, He J-Q (2018) Association between tobacco smoking and drug-resistant tuberculosis. Infect Drug Resist 11:873
17. Obore N, Kawuki J, Guan J, Papabathini SS, Wang L (2020) Association between indoor air pollution, tobacco smoke and tuberculosis: an updated systematic review and meta-analysis. Pub Health 187:24–35. https://doi.org/10.1016/j.puhe.2020.07.031
18. Hyder MKA, Tripathy JP, Kaur J, Mandal PP, Sharma R, Kumar AMV, Thamarangsi T, Singh RJ (2018) Tuberculosis-tobacco integration in the South-East Asia Region: policy analysis and implementation framework. Int J Tuberc Lung Dis 22(7):807–812
19. Goel S, Siddiqi K, Singh RJ, Lal P, Aghi MB, Gupta P, Elsey H, Bhatt G (2019) Fuelling the tuberculosis epidemic: the role of tobacco control in ending the TB emergency. Indian J Tuberc 66(4):555–560
20. Siddiqi K, Lee ACK (2009) An integrated approach to treat tobacco addiction in countries with high tuberculosis incidence. Tropical Med Int Health 14(4):420–428
21. Ganzel C, Silverman B, Chemtob D, Ben Shoham A, Wiener-Well Y (2019) The risk of tuberculosis in cancer patients is greatest in lymphoma and myelodysplastic syndrome/myeloproliferative neoplasm: a large population-based cohort study. Leuk Lymphoma 60 (3):720–725
22. Ho JCM, Leung CC (2018) Management of co-existent tuberculosis and lung cancer. Lung Cancer 122:83-87
23. Molina-Romero C, Arrieta O, Hernández-Pando R (2020) Tuberculosis and lung cancer. salud pública de méxico 61:286–291
24. Anastasopoulou A, Ziogas DC, Samarkos M, Kirkwood JM, Gogas H (2019) Reactivation of tuberculosis in cancer patients following administration of immune checkpoint inhibitors: current evidence and clinical practice recommendations. J Immunother Cancer 7(1):1–13
25. Kiran D, Basaraba RJ (2021) Lactate metabolism and signaling in tuberculosis and cancer: a comparative review. Front Cell Infect Microbiol 11:37
26. Azadi D, Motallebirad T, Ghaffari K, Shojaei H (2018) Mycobacteriosis and tuberculosis: laboratory diagnosis. Open Microbiol J 12:41
27. Acharya B, Acharya A, Gautam S, Ghimire SP, Mishra G, Parajuli N, Sapkota B (2020) Advances in diagnosis of tuberculosis: an update into molecular diagnosis of Mycobacterium tuberculosis. Mol Biol Rep 47(5):4065–4075
28. Luo Y, Xue Y, Song H, Tang G, Liu W, Bai H, Yuan X, Tong S, Wang F, Cai Y (2022) Machine learning based on routine laboratory indicators promoting the discrimination between active tuberculosis and latent tuberculosis infection. J Infect
29. Cañadas-Ortega M, Gómez-Cruz C, Vaquero JJ, Muñoz-Barrutia A (2022) The contribution of microfluidics to the fight against tuberculosis. Nanotechnol Rev 11(1):40–54
30. Molloy A, Harrison J, McGrath JS, Owen Z, Smith C, Liu X, Li X, Cox JAG (2021) Microfluidics as a novel technique for tuberculosis: from diagnostics to drug discovery. Microorganisms 9(11):2330
31. Homann AR, Niebling L, Zehnle S, Beutler M, Delamotte L, Rothmund M-C, Czurratis D, Beller K-D, Zengerle R, Hoffmann H (2021) A microfluidic cartridge for fast and accurate diagnosis of Mycobacterium tuberculosis infections on standard laboratory equipment. Lab Chip 21(8):1540–1548
32. Beutler M, Homann AR, Mihalic M, Plesnik S, Niebling L, Eckart M, Allerheiligen V, Czurratis D, Maharjan B, Shrestha B (2021) Rapid tuberculosis diagnostics including molecular first-and second-line resistance testing based on a novel microfluidic DNA extraction cartridge. J Mol Diagn 23(5):643–650

33. Ip K-U, Chang J-R, Liu T-H, Dou H-Y, Lee G-B (2018) An integrated microfluidic system for identification of live mycobacterium tuberculosis by real-time polymerase chain reaction. IEEE, pp 1124–1127
34. Halliday A, Masonou T, Tolosa-Wright M, Mandagere V, Lalvani A (2019) Immunodiagnosis of active tuberculosis. Expert Rev Respir Med 13(6):521–532
35. Sudbury EL, Clifford V, Messina NL, Song R, Curtis N (2020) Mycobacterium tuberculosis-specific cytokine biomarkers to differentiate active TB and LTBI: a systematic review. J Infect 81(6):873–881
36. Kumar NP, Moideen K, Banurekha VV, Nair D, Babu S (2019) Plasma proinflammatory cytokines are markers of disease severity and bacterial burden in pulmonary tuberculosis. Oxford University Press US, p ofz257
37. Hong Y, Kim Y, Lee JJ, Lee MG, Lee CY, Kim Y, Heo J, Han S-S, Lee S-J, Kim WJ (2019) Levels of vitamin D-associated cytokines distinguish between active and latent tuberculosis following a tuberculosis outbreak. BMC Infect Dis 19(1):1–8
38. Basingnaa A, Antwi-Baffour S, Nkansah DO, Afutu E, Owusu E (2018) Plasma levels of cytokines (IL-10, IFN-γ and TNF-α) in multidrug resistant tuberculosis and drug responsive tuberculosis patients in Ghana. Diseases 7(1):2
39. DiNardo AR, Gandhi T, Heyckendorf J, Grimm SL, Rajapakshe K, Nishiguchi T, Reimann M, Kirchner HL, Kahari J, Dlamini Q (2022) Gene expression signatures identify biologically and clinically distinct tuberculosis endotypes. Eur Respir J
40. Warsinske H, Vashisht R, Khatri P (2019) Host-response-based gene signatures for tuberculosis diagnosis: a systematic comparison of 16 signatures. PLoS Med 16(4):e1002786
41. Wang Z, Arat S, Magid-Slav M, Brown JR (2018) Meta-analysis of human gene expression in response to Mycobacterium tuberculosis infection reveals potential therapeutic targets. BMC Syst Biol 12(1):1–18
42. Jakhar S, Bitzer AA, Stromberg LR, Mukundan H (2020) Pediatric tuberculosis: the impact of "omics" on diagnostics development. Int J Mol Sci 21(19):6979
43. Walzl G, McNerney R, du Plessis N, Bates M, McHugh TD, Chegou NN, Zumla A (2018) Tuberculosis: advances and challenges in development of new diagnostics and biomarkers. Lancet Infect Dis 18(7):e199–e210
44. Krishnan S, Queiroz ATL, Gupta A, Gupte N, Bisson GP, Kumwenda J, Naidoo K, Mohapi L, Mave V, Mngqibisa R (2021) Integrative multi-omics reveals serum markers of tuberculosis in advanced HIV. Front Immunol 12:2060
45. Goff A, Cantillon D, Muraro Wildner L, Waddell SJ (2020) Multi-omics technologies applied to tuberculosis drug discovery. Appl Sci 10(13):4629
46. Mondoni M, Repossi A, Carlucci P, Centanni S, Sotgiu G (2017) Bronchoscopic techniques in the management of patients with tuberculosis. Int J Infect Dis 64:27–37
47. Goussard P, Gie R (2014) The role of bronchoscopy in the diagnosis and management of pediatric pulmonary tuberculosis. Expert Rev Respir Med 8(1):101–109
48. Sarkar S, Suresh MR (2011) An overview of tuberculosis chemotherapy—a literature review. J Pharm Pharm Sci 14(2):148–161
49. Jnawali HN, Ryoo S (2013) First-and second-line drugs and drug resistance. Tuberc Curr Issues Diagn Manage 20:163–180
50. Sileshi T, Tadesse E, Makonnen E, Aklillu E (2021) The impact of first-line anti-tubercular drugs' pharmacokinetics on treatment outcome: a systematic review. Clin Pharmacol Adv Appl 13:1
51. Motta I, Calcagno A, Bonora S (2018) Pharmacokinetics and pharmacogenetics of anti-tubercular drugs: a tool for treatment optimization? Expert Opin Drug Metab Toxicol 14(1):59–82
52. O'Garra A, Redford PS, McNab FW, Bloom CI, Wilkinson RJ, Berry MPR (2013) The immune response in tuberculosis. Annu Rev Immunol 31:475–527
53. Abate G, Hoft DF (2016) Immunotherapy for tuberculosis: future prospects. ImmunoTargets and therapy 5:37

54. Uhlin M, Andersson J, Zumla A, Maeurer M (2012) Adjunct immunotherapies for tuberculosis. J Infect Dis 205 (suppl_2):S325-S334
55. Wallis RS (2005) Reconsidering adjuvant immunotherapy for tuberculosis. Clin Infect Dis 41 (2):201–208
56. Justo OR, Moraes ÂM (2003) Incorporation of antibiotics in liposomes designed for tuberculosis therapy by inhalation. Drug Delivery 10(3):201–207
57. Braunstein M, Hickey AJ, Ekins S (2019) Why wait? The case for treating tuberculosis with inhaled drugs. Pharm Res 36(12):1–6
58. Misra A, Hickey AJ, Rossi C, Borchard G, Terada H, Makino K, Fourie PB, Colombo P (2011) Inhaled drug therapy for treatment of tuberculosis. Tuberculosis 91(1):71–81
59. Hickey AJ, Durham PG, Dharmadhikari A, Nardell EA (2016) Inhaled drug treatment for tuberculosis: past progress and future prospects. J Control Release 240:127–134
60. Hoppentocht M, Hagedoorn P, Frijlink HW, de Boer AH (2014) Developments and strategies for inhaled antibiotic drugs in tuberculosis therapy: a critical evaluation. Eur J Pharm Biopharm 86(1):23–30
61. Muttil P, Wang C, Hickey AJ (2009) Inhaled drug delivery for tuberculosis therapy. Pharm Res 26(11):2401–2416
62. Gupta KB, Gupta R, Atreja A, Verma M, Vishvkarma S (2009) Tuberculosis and nutrition. Lung India Official Organ Indian Chest Soc 26(1):9
63. Kant S, Gupta H, Ahluwalia S (2015) Significance of nutrition in pulmonary tuberculosis. Crit Rev Food Sci Nutr 55(7):955–963
64. Shaji B, Thomas ETA, Sasidharan PK (2019) Tuberculosis control in India: refocus on nutrition. Indian J Tuberc 66(1):26–29
65. Bennett DE, Courval JM, Onorato I, Agerton T, Gibson JD, Lambert L, McQuillan GM, Lewis B, Navin TR, Castro KG (2008) Prevalence of tuberculosis infection in the United States population: the national health and nutrition examination survey, 1999–2000. Am J Respir Crit Care Med 177(3):348–355
66. Benn CS, Friis H, Wejse C (2008) Should micronutrient supplementation be integrated into the case management of tuberculosis? vol 197. The University of Chicago Press
67. Keshavjee S, Farmer PE (2012) Tuberculosis, drug resistance, and the history of modern medicine. N Engl J Med 367(10):931–936
68. Nachega JB, Chaisson RE (2003) Tuberculosis drug resistance: a global threat. Clin Infect Dis 36 (Supplement_1):S24–S30
69. Sandgren A, Strong M, Muthukrishnan P, Weiner BK, Church GM, Murray MB (2009) Tuberculosis drug resistance mutation database. PLoS Med 6(2):e1000002
70. Schön T, Miotto P, Köser CU, Viveiros M, Böttger E, Cambau E (2017) Mycobacterium tuberculosis drug-resistance testing: challenges, recent developments and perspectives. Clin Microbiol Infect 23(3):154–160
71. Wade MM, Zhang Y (2004) Mechanisms of drug resistance in Mycobacterium tuberculosis. Front Biosci 9:975–994
72. Harding E (2020) WHO global progress report on tuberculosis elimination. Lancet Respir Med 8(1):19
73. Rebeiro PF, Cohen MJ, Ewing HM, Figueiredo MC, Peetluk LS, Andrade KB, Eakin M, Zechmeister EJ, Sterling TR (2020) Knowledge and stigma of latent tuberculosis infection in Brazil: implications for tuberculosis prevention strategies. BMC Publ Health 20(1):1–10
74. Sakamoto H, Lee S, Ishizuka A, Hinoshita E, Hori H, Ishibashi N, Komada K, Norizuki M, Katsuma Y, Akashi H (2019) Challenges and opportunities for eliminating tuberculosis–leveraging political momentum of the UN high-level meeting on tuberculosis. BMC Publ Health 19(1):1–7
75. Chee CBE, Reves R, Zhang Y, Belknap R (2018) Latent tuberculosis infection: opportunities and challenges. Respirology 23(10):893–900
76. Belani H, Chorba T, Fletcher F, Hennessey K, Kroeger K, Lansky A, Leichliter J, Lentine D, Mital S, Needle R (2012) Integrated prevention services for HIV infection, viral hepatitis,

sexually transmitted diseases, and tuberculosis for persons who use drugs illicitly: summary guidance from CDC and the US Department of Health and Human Services. Morb Mortal Wkly Rep 61(5):1–43
77. Hyle EP, Naidoo K, Su AE, El-Sadr WM, Freedberg KA (2014) HIV, tuberculosis, and non-communicable diseases: what is known about the costs, effects, and cost-effectiveness of integrated care? J Acquir Immune Defic Syndr (1999) 67(0 1):S87
78. Ledibane TD, Motlhanke SC, Rose A, Kruger WH, Ledibane NRT, Claassens MM (2015) Antiretroviral treatment among co-infected tuberculosis patients in integrated and non-integrated facilities. Publ Health Action 5(2):112–115
79. Manosuthi W, Wiboonchutikul S, Sungkanuparph S (2016) Integrated therapy for HIV and tuberculosis. AIDS Res Ther 13(1):1–12
80. Padayatchi N, Abdool Karim SS, Naidoo K, Grobler A, Friedland G (2014) Improved survival in multidrug-resistant tuberculosis patients receiving integrated tuberculosis and antiretroviral treatment in the SAPiT Trial. Int J Tuberc Lung Dis 18(2):147–154
81. Awaisu A, Nik Mohamed MH, Mohamad Noordin N, Abd Aziz N, Syed Sulaiman SA, Muttalif AR, Ahmad Mahayiddin A (2011) The SCIDOTS Project: evidence of benefits of an integrated tobacco cessation intervention in tuberculosis care on treatment outcomes. Subst Abuse Treat Prev Policy 6(1):26. https://doi.org/10.1186/1747-597X-6-26
82. Reddy TBK, Riley R, Wymore F, Montgomery P, DeCaprio D, Engels R, Gellesch M, Hubble J, Jen D, Jin H, Koehrsen M, Larson L, Mao M, Nitzberg M, Sisk P, Stolte C, Weiner B, White J, Zachariah ZK, Sherlock G, Galagan JE, Ball CA, Schoolnik GK (2008) TB database: an integrated platform for tuberculosis research. Nucleic Acids Res 37 (suppl_1):D499–D508. https://doi.org/10.1093/nar/gkn652
83. Bottasso O, Bay ML, Besedovsky H, del Rey A (2009) Immunoendocrine alterations during human tuberculosis as an integrated view of disease pathology. NeuroImmunoModulation 16 (2):68–77
84. Gomez-Gonzalez PJ, Andreu N, Phelan JE, de Sessions PF, Glynn JR, Crampin AC, Campino S, Butcher PD, Hibberd ML, Clark TG (2019) An integrated whole genome analysis of Mycobacterium tuberculosis reveals insights into relationship between its genome, transcriptome and methylome. Sci Rep 9(1):1–11
85. Balabanova Y, Drobniewski F, Nikolayevskyy V, Kruuner A, Malomanova N, Simak T, Ilyina N, Zakharova S, Lebedeva N, Alexander HL (2009) An integrated approach to rapid diagnosis of tuberculosis and multidrug resistance using liquid culture and molecular methods in Russia. PLoS ONE 4(9):e7129
86. Moure R, Martín R, Alcaide F (2012) Effectiveness of an integrated real-time PCR method for detection of the Mycobacterium tuberculosis complex in smear-negative extrapulmonary samples in an area of low tuberculosis prevalence. J Clin Microbiol 50(2):513–515
87. Moure R, Muñoz L, Torres M, Santin M, Martín R, Alcaide F (2011) Rapid detection of Mycobacterium tuberculosis complex and rifampin resistance in smear-negative clinical samples by use of an integrated real-time PCR method. J Clin Microbiol 49(3):1137–1139
88. Shankar SU, Kumar AMV, Venkateshmurthy NS, Nair D, Kingsbury R, Velu M, Gupta J, Ahmed J, Hiremath S, Jaiswal RK (2021) Implementation of the new integrated algorithm for diagnosis of drug-resistant tuberculosis in Karnataka State, India: how well are we doing? PLoS ONE 16(1):e0244785
89. Bielecka MK, Tezera LB, Zmijan R, Drobniewski F, Zhang X, Jayasinghe S, Elkington P (2017) A bioengineered three-dimensional cell culture platform integrated with microfluidics to address antimicrobial resistance in tuberculosis. MBio 8(1):e02073-e2016
90. Talarico S, Ijaz K, Zhang X, Mukasa LN, Zhang L, Marrs CF, Cave MD, Bates JH, Yang Z (2011) Identification of factors for tuberculosis transmission via an integrated multidisciplinary approach. Tuberculosis 91(3):244–249. https://doi.org/10.1016/j.tube.2011.01.007
91. Htet KKK, Liabsuetrakul T, Thein S, McNeil EB, Chongsuvivatwong V (2018) Improving detection of tuberculosis among household contacts of index tuberculosis patients by an integrated approach in Myanmar: a cross-sectional study. BMC Infect Dis 18(1):1–8

92. Mao Q, Zhang K, Yan W, Cheng C (2018) Forecasting the incidence of tuberculosis in China using the seasonal auto-regressive integrated moving average (SARIMA) model. J Infect Public Health 11(5):707–712. https://doi.org/10.1016/j.jiph.2018.04.009

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Global Tuberculosis Epidemiology

2

Sobia Faisal

It wasn't raining when Noah built the ark.

Howard Ruff

Summary

Tuberculosis (TB), despite being curable and preventable, remained on the list of top ten mortality causes across the globe. TB-HIV comorbidity and emergence of drug-resistant TB (including MDR and XDR) are superadded problems. Like other infectious diseases, TB has strong ties with socio-economic strata, with a higher incidence among low-income countries compared to their counterpart. TB control came on the list of global priority agenda after being labeled as a global health emergency in 1993. Afterward, implementation of DOTS (Directly Observed Treatment Strategy), Stop TB strategy, and End TB Strategy played a significant role in combating the situation. The global incidence of TB has declined from 172 per 100,000 population (2000) to 132 per 100,000 population (2018). However, the pace of decline is still insufficient to meet global targets and commitments.

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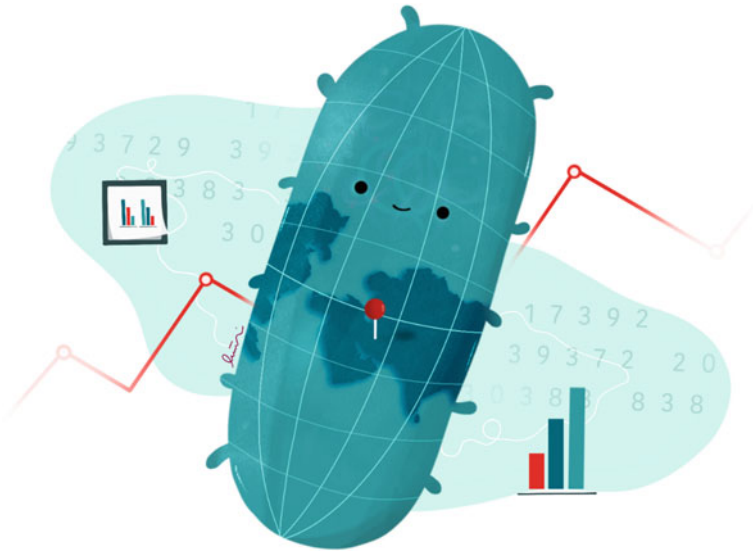
Graphical Abstract



Global tuberculosis epidemiology

Keywords

Childhood TB · Global burden · Incidence · Latent TB · MDR TB · Mortality · TB-HIV co-infection · Tuberculosis

1 Introduction

German microbiologist and physician Robert Koch identified the etiological agent of human tuberculosis (TB) in the 1880s. Despite tremendous success in developing diagnostic tools and therapeutic agents, the highly adaptive nature of *Mycobacterium tuberculosis (M. tb)* kept this bacterium a problem for health. TB is a daunting public health challenge not only because of its mortality but also because of its limited success in preventing transmission. Every minute around 19 people develop TB disease across the globe (incidence is 10 million, range 9–11.1 million) [1]. This disease burden has diverse geographical and demographic patterns.

In 2018, the average global incidence was 132 cases per 100,000 population. Southeast Asia (WHO region) had the maximum share of global TB toll. Around 87% of cases were in 30 countries, amongst which the top eight included India, China, Indonesia, the Philippines, Pakistan, Nigeria, Bangladesh, and South Africa, respectively. Among the estimated incidents, 69% (63–77%) were notified and put on treatment during 2018 across the globe [1].

The gender disparity is also reflected in estimated incidence where men aged 15 and above had 57% of the total caseload while the women of the same age group had 32% of that. Children under the age of 15 bore 11% of the burden of TB [1].

TB has close associations with some common diseases and conditions, including human immunodeficiency virus (HIV) and diabetes. HIV-infected persons account for 8.6% of all TB cases worldwide. In 2018, 64% of the TB patients reported had known HIV status, which is an improvement from prior years, but still a significant number. In 2018, 56% of all TB HIV cases were reported to the national database [1].

The emergence of drug-resistant (DR)-TB has created a menace to the situation. In 2018, around 500,000 new DR-TB cases developed, among which 78% had multidrug-resistant (MDR)-TB. India, China, and the Russian Federation accounted for 27, 14, and 9% of the global DR-TB burden. DR-TB was found in 3.4% of new cases of TB and 18% of cases of TB retreatment. Globally, 32% of the incident DR-TB cases were put on treatment during 2018 [1].

TB is included in the global list of top ten mortality causes and ranks as the top mortality cause from one infectious agent even higher than HIV/AIDS. WHO estimated that TB accounted for 1.2 million (1.1–1.3 million) deaths during 2018 among HIV-negative individuals, with an additional 251,000 deaths among HIV and TB comorbid people [1].

The social and demographic determinants also affect the disease burden in terms of both occurrences as well as transmission. These factors include but are not limited to congested and poor living, limited health education, smoking, alcohol, and under-nutrition [2]. Global statistics of TB, both the incidence and notification, reflect the impact of the existing level of TB control efforts and their potential in reaching “End TB” milestones and targets set in sustainable development goals (SDGs). The global epidemiology of TB also throws light on the areas where there is a need to take novel and bold steps to eradicate the disease.

2 History of Strategies and Commitments to Control the Tuberculosis Epidemic

TB remained gross neglect until 1993 when it was declared a global public health emergency by WHO. Subsequently, the DOTS (Directly Observed Treatment Short-course) strategy was launched and implemented from 1994 to 2005, followed by the development and implementation of the Stop TB Strategy (2006–2015), which showed global commitments and efforts for halting the epidemic [3]. Meanwhile, in 2014, WHOs’ End TB strategy was adopted by World Health

Assembly (WHA). Afterward, ending the TB epidemic was included as a target in SDGs by United Nations (UN) General Assembly in 2015 (Target 3.3) [4].

"End TB Strategy" paved the way for decreasing the TB burden and mortality. The milestones for 2020 include a 35% reduction in TB-related mortality and a 20% reduction in TB incidence as compared to 2015, whereas the targets for 2030 are a reduction of 90% in TB mortality and 80% in TB incidence in comparison to levels in 2015. The UN high-level meeting (2018) reaffirmed the SDG target and End TB Strategy. New global targets were also introduced in the political declaration, including the provision of TB disease treatment to 40 million people and TB preventive therapy for LTBI (latent TB infection) to 30 million people in five years (2018–2022) [1].

3 Global Monitoring of TB

WHO has published annual TB global data since 1997 to provide updated information on the TB epidemic and the achievements in response to the effort made for the cause. The data is collected with the involvement of multiple stakeholders in a systematic manner. Incidence is estimated based on TB prevalence surveys, notifications adjusted by under-diagnosis and under-reporting in high-income countries, national inventory studies, and case notification and detection gaps as per expert opinion in low-income countries [5]. For Global TB Report 2019 (WHO), 202 countries/territories contributed TB data, accounting for more than 99% of the global population [1]. For understanding the epidemiology of the global TB epidemic, the standard definitions of TB related terminologies by WHO are of particular importance:

- latent TB infection (LTBI), LTBI refers to a state where there is a persistent immune response to MTB antigen in the absence of any clinical evidence of TB disease. It also is referred to as "TB infection" depending on the context [6];
- TB case, bacteriologically confirmed (B +ve) TB case is declared when a biological specimen is positive either through culture, sputum smear microscopy (SSM), WHO Recommended Diagnostics (WRD), Gene Xpert MTB/RIF, and others irrespective of notification and registration status; and
- Clinically diagnosed (CD) TB case, if an expert clinician or medical practitioner diagnoses active TB disease without bacteriological confirmation. Basis of clinical diagnosis may include X-ray findings or histological evidence.

3.1 Classification (Anatomical Site)

3.1.1 Pulmonary Tuberculosis

TB lesion exists in lung parenchyma and/or tracheobronchial tree. Pulmonary TB (PTB) may be B +ve or CD. Because of the involvement of lungs in miliary TB, the

disease also comes under PTB. A patient is classified as PTB if both the lung parenchyma and other organs or sites are involved.

3.1.2 Extrapulmonary Tuberculosis

TB involving any organ other than the lung parenchyma and/or tracheobronchial tree is defined as extrapulmonary TB (EPTB). It includes TB involving pleura, lymph nodes, skin, bones and joints, abdomen, genitourinary tract, and meninges. Intra-thoracic lymphadenopathy, including mediastinal and/or hilar lymph nodes and pleural effusion, without evidence of lung parenchymal involvement, also comes under EPTB.

3.2 Classification Based on the History of Previous Tuberculosis Treatment

3.2.1 New Patients

They are patients who have never had anti-tubercular therapy (ATT) or who have received ATT for less than one month.

3.2.2 Previously Treated Patients

These patients had taken ATT for at least one month in the past. Based on their treatment outcome of the most recent course of treatment, these patients are further classified into five groups:

- relapse patients: patients diagnosed again after being declared cured or having finished treatment (being well treated) in the most recent round of TB therapy;
- treatment after failure patients: patients treated for TB in the past and their treatment outcome was a failure during the most recent episode;
- treatment after loss to follow-up TB cases: patients whose most recent treatment outcome was a loss to follow-up;
- other previously treated patients: these are previously treated patients and their last treatment outcome is unknown or undocumented; and
- unknown TB treatment history: patients who do not fit into any of the above categories.

3.3 Classification Based on Drug Sensitivity

Drug susceptibility testing (DST) for anti-tuberculosis drugs on clinical isolates of TB patients differentiate TB into three categories:

DR-TB, DR-TB patients are further classified as per number and types of anti-tuberculosis drugs to which they have developed resistance, e.g.,

i. Rifampicin-resistant TB (RR-TB);
ii. MDR-TB;
iii. Extensive drug-resistant TB (XDR-TB).

3.4 Classification Based on HIV Status

An HIV-positive TB case is defined as any diagnosed TB patient who is either HIV positive at the time of TB diagnosis or has previously been registered/diagnosed for HIV. HIV-negative TB patients have negative HIV results at the time of TB diagnosis. Unknown HIV status refers to diagnosed TB patients whose HIV status is unknown.

3.5 TB Treatment Outcome Definition

Some of the common drug-sensitive (DS) TB treatment outcomes include:

3.5.1 Successful Treatment Outcomes

Cure and completion are both regarded as effective outcomes of TB. Cured refers to any B-+ PTB patient who became negative (on SSM or culture) at the end of treatment and during one of the follow-up examinations. Completed refers to a TB patient who completed treatment without evidence of failure or cure.

3.5.2 Unsuccessful Treatment Outcomes

Unsuccessful treatment outcomes mean treatment failure, death, and loss of follow-up. Treatment failure is assigned to all TB patients who became B-+ during TB treatment at the fifth month or later. Loss to follow-up means after at least one month of treatment, there is an interruption in treatment for two consecutive months or more.

DS-TB and DR-TB are mutually exclusive cohorts (based on registration and management). The outcome of DR is the same as DS-TB as far as terminology is concerned; however, the definitions for DR-TB are as per their management protocols. In DR-TB, too, the sum of cured and completed is considered as a successful treatment.

3.5.3 TB Death

The death (TB mortality) result of TB therapy is ascribed to the death of a TB patient (DSTB and DRTB) before or during TB treatment [7].

3.6 Latent TB Infection (Exposure and Infection with *M. tb*)

LTBI is a condition of persistent immune response to *M. tb* antigen in the absence of any clinical evidence of active TB disease. There are no accurate figures about

the LTBI global burden; however, around one-third of the global population is infected with *M. tb*. Among LTBI cases, around 5–10% might develop the disease later on in their lives, while the vast majority of the LTBI cases never become diseased [8].

Management protocols of LTBI include identifying high-risk people, ruling out active TB disease among them, testing with the available methods for LTBI, and providing an appropriate and effective treatment where needed so that adherence till completion is ensured and adverse effects are monitored and managed. The high-risk groups include people living with HIV (PLHIV), household (HH) members of pulmonary B-+ patients, and clinically high-risk patients (patients on dialysis, patients preparing for an organ transplant, patients with silicosis, etc.) [8].

During the UN high-level meeting, Global commitment for preventive treatment included the provision of LTBI to 30 million people in five years (2018–2022). This included 6 million PLHIV, 4 million under 5 HH contacts of TB patients, and 20 million other eligible HH contacts [1].

Among PLHIV, 1.8 million (in 65 countries) received LTBI treatment in 2018 compared to less than a million in 2017. There was even a great success when we compared from 2005 where 30,000 patients received the same. The global target is 6 million LTBI treatments during 2018–2022 seems achievable with this pace of progress [1].

Around 1.3 million children < five years among the HH contacts of B-+ PTB patients were the eligible candidates for LTBI treatment. However, only 349,487 were given preventive treatment and reported in 109 countries in 2018, which is only 27% in terms of coverage. The number was higher than last year, and since 2015, this number has increased around four-fold [1].

There is around a 30% decrease in uptake of preventive treatment among five years and older age group HH contacts in a year, from 103,344 during 2017 to 79,195 during 2018. This number is quite low compared to the UN high-level meeting [1].

4 The Magnitude of Global TB Disease

4.1 Drug-Sensitive Tuberculosis Incidence

In 2018, around 10 million people developed TB (range 8,990,000–11,100,000) with the incidence rate of 132 per 100,000 (range 118,000–146,000) of population (Fig. 1) [1].

Incident cases in reference to population size give the true picture of the severity of the epidemic and the effectiveness of prevention and control measures. TB incident rate (incident cases per 100,000 population) remained varied across socio-economic strata. In most high-income countries, the incident rate remained under 10 per 100,000 population; however, the same remained 150–400 per 100,000 population in high burden countries. In some countries, including the

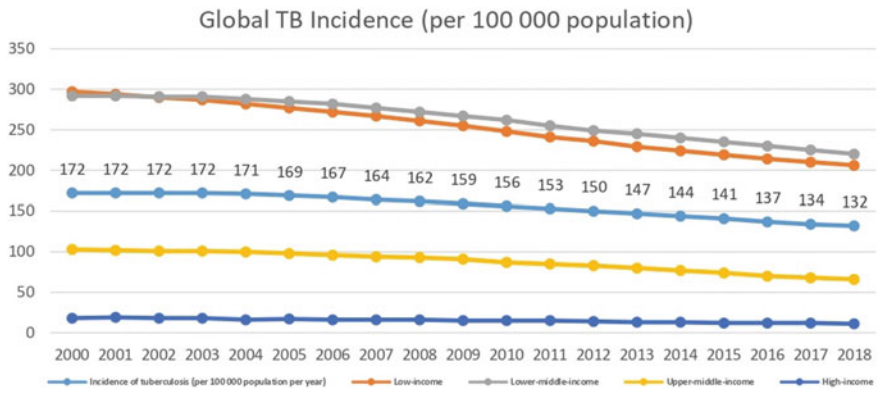


Fig. 1 Global TB incidence. Global Health Observatory data repository 2020. Prepared with data from https://apps.who.int/gho/data/node.main.1315?lang=en

Central African Republic, Lesotho, the Democratic People's Republic of Korea, Namibia, Mozambique, the Philippines, and South Africa, the incident rate was above 500 per 100,000. Among the 30 high TB burden countries, Brazil, China, and the Russian Federation had a very low incidence rate of 45, 61, and 54, respectively [1].

4.1.1 Age and Sex Distribution

All age and sex groups are susceptible to TB; however, men share a greater burden than women. In 2018, men, women, and children shared 57, 32, and 11% of the total caseload [1].

4.1.2 Trends in Line with Global Commitments

Global surveillance data indicate a decline in incidence, both the number and the rate. During 2000–2018, the average rate of decline was 1.6% per annum, increasing to 2% between 2017 and 2018. Still, reaching the End TB Strategy milestones for 2020 seems a challenge with this level of success. Since the end of three years of the "End TB Strategy" (2015–2018), the cumulative decline in TB incidence was 6.3% [1].

The regional comparison shows that the fastest decline is observed among the WHO regions in the European Region (on average, 5% per year), followed by the African Region (3.8% per year). During 2015–2018, the cumulative reductions in incidence were 15, 12, 2.8, 6.6, and 3.8% in European, African, Eastern Mediterranean, Southeast Asian, and the Western Pacific regions. Contrary to these five regions, the estimated incidence is increasing in the Americas region, showing an upward trend [1].

4.2 Drug-Sensitive Tuberculosis Case Notification and Treatment Success

4.2.1 Notification

In 2018, 7,253,116 TB cases were notified across the globe. Of the incident cases (new and relapse), 6,950,750 were notified in 2018, 70% of the total incidents (ten million). 85% of the incident TB cases had PTB. Among these PTB cases, 55% were B-+, and the remaining were CD-TB patients. In countries where WHO-recommended rapid diagnostic (WRD) are universally accessible to all (high-income countries), around 8% of the PTB cases were B-+. However, in low-income countries, where SSM is the available and accessible TB diagnostic tool, B positivity is low; around 50% were B-+ in 2018. The trend of B positivity is declining, which is concordant with an increased notification indicating that efforts to find missed TB cases have led to overdiagnosis without sound diagnostic evidence [1].

4.2.2 Age and Sex Distribution

Incidence as well as case notification increase with age. Globally, male to female ratio as well as childhood TB case notification percentages vary across countries and regions; global M:F is 1.7 [1]. These variations may result from differences in epidemiology or access to standardized healthcare services has played a role.

4.2.3 Pulmonary Tuberculosis Versus Extrapulmonary Tuberculosis

In Global TB Report 2019, around 85% (5.9 million) of notified cases were of PTB, which included both the bacteriological confirmed as well as clinically diagnosed cases [1].

4.2.4 The Trend Is in Line with Global Commitments

Globally, 7 million incident TB cases (new and relapse) were notified in 2018 compared to 6.4 million of same in 2017, showing a 9% increase in the notification. Additionally, 300,000 cases of previously treated TB patients were notified. Incident case notification increased from 2000 to 2009. It remained almost stagnant during the next four years (2009–2012), with a gradual increase afterward (Fig. 2) mainly attributed to high case notification in India and Indonesia [1].

4.3 Childhood Tuberculosis

Over one million children developed TB in 2018. Since childhood TB (CHTB) diagnosis is always a challenge, its notification remained lower compared to incidence. Even the percent notification in all forms is below percent incidence among all cases. In 2018, around 11% of the total TB incident cases were of CHTB, while in the same year, CHTB cases were only 8% among the notified cases [1].

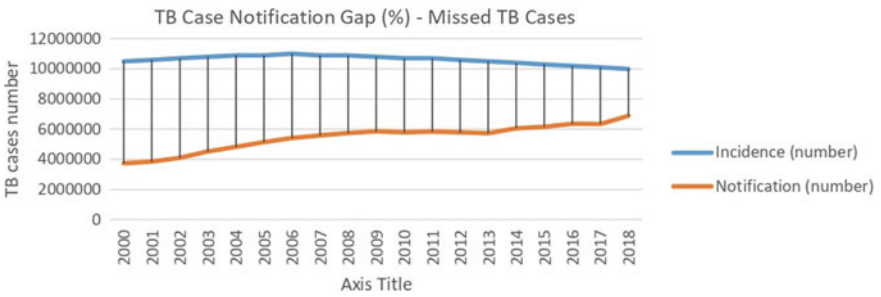


Fig. 2 Missed TB cases. Global Health Observatory data repository 2020. Prepared with data from https://apps.who.int/gho/data/node.main.1315?lang=en

4.3.1 Incidence Estimation

In 2018, incident cases of childhood TB remained at 1.1 million across the globe. Incidence is determined through statistical models involving prevalence surveys and case notification trends with further adjustments. In the case of CHTB, it is difficult to include children in prevalence surveys because of the dependence of surveys on B positivity, while taking sputum samples from children is always a challenge, keeping this segment underestimated [9].

4.3.2 Notification and Treatment

Among the new and relapse cases notified, 8% (around 560,000) were CHTB cases (age 0–14 years) in 2018. [1] Ironically, TB diagnosis remained a challenge for pediatric TB, especially for children under five years of age. Both the household screening and the symptomatic screening miss most cases, while broader approaches are cost-intensive with low yield. The respiratory sample is always difficult to obtain for TB diagnostics among children. Data supporting other types of diagnostic tests are less available. Bacterial confirmation in CHTB is difficult, so children may be undertreated or never treated [10].

Gaps in estimated incidence and notification of pediatric TB are a challenge for TB control. Further, it is estimated that less than 5% of the estimated DR-TB incident children (DR-CHTB incidence: 25,000–32,000) ever receive standardized DR-TB management [9]. TB mortality among children is predominantly with tuberculosis meningitis (TBM) or extensive disease (disseminated TB) [2].

4.4 Drug-Resistant Tuberculosis

In 2018, the estimated incidence of MDR- and RR-TB was 484,000 (range 417,000–556,000), whereas only 186,772 cases (38.6%) of the same were laboratory-confirmed, leaving 297,228 MDR-TB and RR-TB cases undetected. The number of patients who started DR-TB treatment further decreased to 156,071 (including those diagnosed before 2018 and put on treatment this year). This

declining trend in the DR-TB cascade leaves undiagnosed and/or untreated DR-TB patients leading to further transmission of DR-TB [1].

High-income countries have a significantly lower DR-TB burden reflecting that standardized diagnostic and treatment services reduce the gaps in cascade. In high-income, low-prevalence countries, all TB patients receive DST, and their treatment is tailored as per their drug susceptibility leading to a high treatment success rate.

4.5 Access to Drug SensitivityTesting

WHO recommends Gene Xpert, Xpert Ultra, and Line Probe Assay (LPA) to diagnose DR-TB. In resource-deprived settings, SSM, chest X-ray, and clinical diagnosis remain the diagnostic tool for TB; thus, drug sensitivity testing (DST) is usually not performed, or if available, it is only on tertiary care facilities. With the widespread coverage of WRD across high TB burden countries, the number of B-+ cases tested for rifampicin resistance through Xpert has increased from the previous year; still, only 51% (1.8 million) of the total 3.2 million B-+ PTB patients were tested for Rif resistance. Second-line DST is the standard for all MDR/RR patients; however, in 2018, only 59% of all notified RR-TB patients received complete DST [1].

4.6 Gaps in the Drug-Resistant Tuberculosis Care Cascade

In the 2016 cohort, there were 600,000 MDR-TB/RR-TB incident cases, while only 153,119 (25.5%) were notified and 129,689 were enrolled [11], and around 70,000 (56%) of the enrolled patients completed the treatment. This DR-TB cascade, in other words, shows that 12 out of 100 estimated DR-TB incident cases completed their treatment successfully [1].

The cascade of DR-TB, right from the estimated incidence till successful treatment, shows a sharp downward trend at all cascade steps. The burden of DR-TB disease and losses in diagnostic and treatment cascade both are related to standards and access to standardized TB care. Factors contributing to the gaps in the DR-TB treatment cascade include weak political will, complexities and undesired outcomes of the current DR-TB regimen, access to universal healthcare, and an ineffective model of care for DR-TB [12].

4.7 Tuberculosis-Human Immunodeficiency Virus Comorbidity

In 2018, HIV-+ TB incidence was 862,000 (range 776,000–952,000) across the globe while the TB-HIV co-infection incidence rate was 11 (range 10–13) per 100,000 of the population [1]. HIV affects the immune status resulting in the rapid progress of

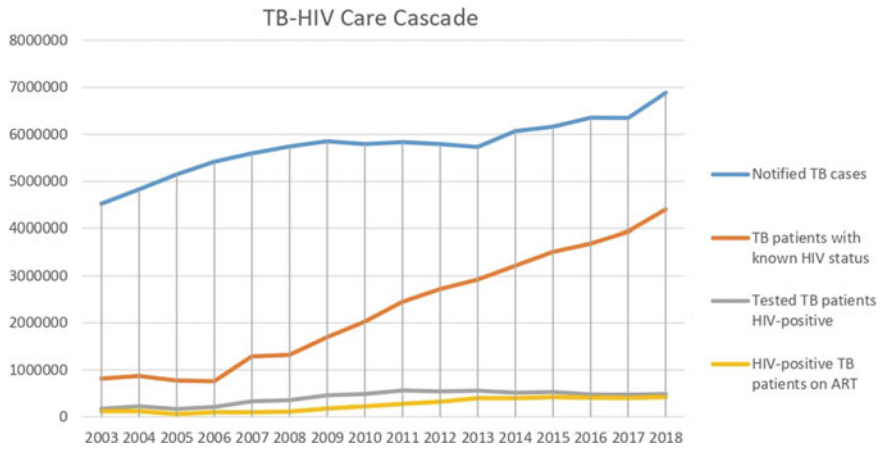


Fig. 3 TB HIV care cascade. Global Health Observatory data repository 2020. Prepared with data from https://apps.who.int/gho/data/node.main.1315?lang=en

innocuous TB infection to TB disease and up to threatening life level [2]. Among the incident TB cases, around 8.6% (range 7.4–10%) were people living with HIV. This TB-HIV co-infection has the highest proportion in countries from WHO African Region. This proportion was even more than 50% in some parts of Southern Africa. Among PLHIV, the risk of TB is 19 times more than the rest of global population [1]. Among the TB patients notified in 2018, around 64% had documented HIV test results (Fig. 3), which is higher than in 2017 (60%). In high-HIV burden countries of the WHO African Region, more notified cases had their HIV status known. Of the total TB-HIV co-infection cases, 56% (477,461) were reported in 2018 [1].

4.8 Treatment Success Rate

TB treatment success rate (cured and completed) varies with the TB treatment status, HIV status, drug susceptibility level, etc. Among the 2017 cohort of new and relapse (incident) cases, 85% successfully completed their TB treatment, and the outcome was declared in 2018. Among the previously treated patients (excluding relapse patients) registered in 2017, treatment success was 61%. Among HIV-positive TB patients registered during the same, 75% were successfully treated. Treatment outcome of DR-TB cases for the cohort of patients registered for DR-TB treatment in 2016 differed based on the type of DR-TB; a favorable outcome in 56% of MDR patients and 39% of XDR cases was achieved (Fig. 4) [1].

The treatment success rate of MDR-/RR-TB among the 2016 cohort was 56%, while unsuccessful outcomes included 15% mortality, 8% treatment failure, 15% lost to follow-up, and 6% had an unknown outcome. Among the XDR-TB cohort notified and registered in 2016, 57 countries/territories reported outcomes. In this group, 39% had successful treatment outcomes, 26% died, 18% had treatment failure, and 18% were lost to follow-up or not evaluated [1].

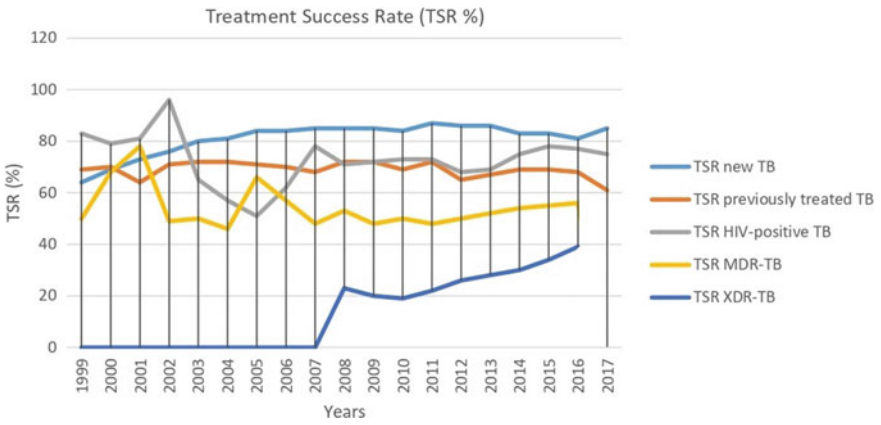


Fig. 4 Treatment success rate. Global Health Observatory data repository 2020. Prepared with data from https://apps.who.int/gho/data/node.main.1315?lang=en

4.9 Estimated Mortality

TB is the leading cause of death from a single infectious agent, even above HIV. In 2018, the estimated death toll from TB was 1.2 million (range 1.1–1.3 million) in HIV-negative patients with additional 251,000 deaths (range 223,000–281,000) among TB-HIV co-infection cases. In 2018, the estimated death rate was 16 (15–17) per 100,000 among HIV-negative while it was 20 (range, 18–21) per 100,000 for both TB deaths among HIV-negative and positive patients [1].

The socio-economic profile of countries seems to be an important player in the TB mortality rate. Many high-income countries have less than one TB death per 100,000 population; on the other hand, the rate is 40 or more than that per 100,000 of the population in other African countries [1].

The estimated death burden among HIV-negative people was distributed among age and sex groups. Men and women of age more than 15 years had 55 and 31% load respectively, while children (age less than 15) had a 14% share in TB mortality. Children had more share in estimated mortality than their estimated share of incident cases (11%). The death toll among HIV-positive patients was distributed: 49% were males, 38% were females, and 13% were children [1].

TB deaths decreased by 27% in the period of 18 years (2000–2018), where in 2000, estimated deaths were 1.7 million, and in 2018, it was 1.2 million among HIV-negative individuals. The same (TB deaths) fell much faster among HIV-positive cases where in 2000 there were 624,000 deaths which reduced to 251,000 deaths in 2018 (60% decrease). The rate of decline in TB mortality was greatest in the WHO European Region, followed by WHO African Region [1].

5 Conclusion

Global commitments and strategies are commendable if implemented in full spirit. Progress in terms of notification is encouraging; however, still, there is a huge gap in incidence and notification more prominently for DR-TB and TB-HIV comorbidity. Financial resources must be mobilized to ensure accessibility of standardized diagnostic and treatment to each and every TB patient.

Core Messages

- TB is public health challenge for the entire globe.
- TB is among the top ten mortality causes globally despite being a preventable and curable disease.
- TB control is part of global commitments.
- The pace of achievements is not enough to meet the requirements and targets for ending TB worldwide.
- The emergence and spread of DR-TB and TB-HIV comorbidity have added misery to menace.

References

1. Global tuberculosis report 2019. https://www.who.int/tb/publications/global_report/en/
2. Khan M, Islam M, Ferdous J, Alam M (2019) An overview on epidemiology of tuberculosis. Mymensingh Med J MMJ 28:259
3. Raviglione M, Director G (2013) Global strategy and targets for tuberculosis prevention, care and control after 2015. World Health Organization, Geneva
4. Organization WWH (2015) Implementing the end TB strategy: the essentials. Rep. 9241509937, World Health Organization
5. Glaziou P, Sismanidis C, Zignol M, Floyd K (2016) Methods used by WHO to estimate the global burden of TB disease. Global TB Programme, WHO, Geneva
6. Organization WH (2020) WHO operational handbook on tuberculosis: module 1: prevention: tuberculosis preventive treatment
7. Organization WH (2013) Definitions and reporting framework for tuberculosis—2013 revision. Rep. 9241505346, World Health Organization
8. Organization WH. https://www.who.int/tb/areas-of-work/preventive-care/ltbi/faqs/en/
9. Furin J (2019) Advances in the diagnosis, treatment, and prevention of tuberculosis in children. Expert Rev Respir Med 13:301–311
10. Reuter A, Hughes J, Furin J (2019) Challenges and controversies in childhood tuberculosis. Lancet 394:967–978
11. Organization WH (2018) Global tuberculosis report 2017. Report no.: 9241565055, World Health Organization
12. Cox V, Cox H, Pai M, Stillo J, Citro B, Brigden G (2019) Health care gaps in the global burden of drug-resistant tuberculosis. Int J Tuberc Lung Dis 23:125–135

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Heterogeneity in Tuberculosis

3

Richa Sinha and Rahul

It is not the strongest of species that survives. It is the one that is the most adaptable to change.

Charles Darwin

Summary

Tuberculosis (TB), an age-old disease, has troubled the human race despite advances in diagnosis and treatment. *Mycobacterium tuberculosis (M. tb),* the causative agent of TB, has gradually developed effective defense mechanisms to evade the host immune system and the toxic effects of medications. Genotypic and phenotypic variations in the bacterium molding the host response to its advantage produce many clinical presentations. While a large proportion of patients develop a latent TB infection, about 10% develop active disease. Nearly 20% remain unaffected and asymptomatic. Heterogeneity in manifestations exists at both the individual and community levels. The host's biological (nutritional status of the patient, immune status, genetic background) and environmental (social, economic, and geographical) factors are responsible for variation in the risk of disease transmission, disease severity, and treatment

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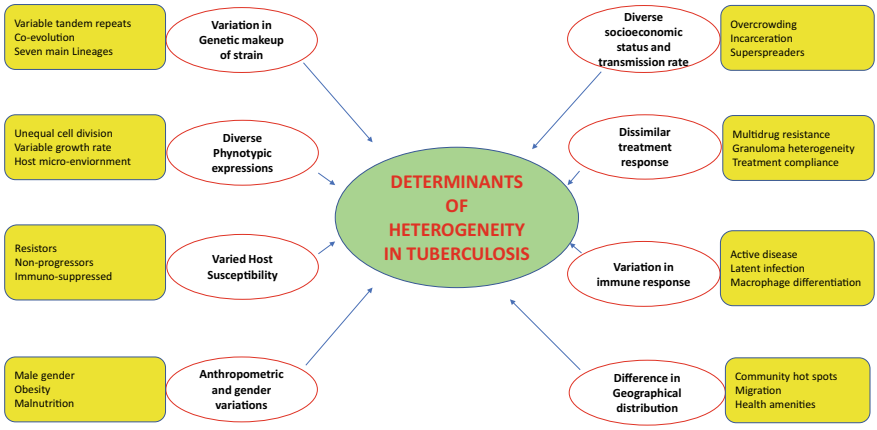
response. Effective disease control entails a good understanding of the disease process and the host–pathogen interaction. This chapter intends to discuss the nuances of heterogeneity in TB.

Graphical Abstract



Determinants of heterogeneity in tuberculosis

Keywords

Adaptation · Heterogeneity · Immunity · Mycobacteria · Transmission

1 Introduction

The beginning quote of the chapter holds for *Mycobacterium tuberculosis (M. tb)*, the causative agent of tuberculosis (TB), which has remained a daunting disease for the human race. Description of an ailment similar to TB is present in the 'Vedas' and the ancient Arabic and Chinese literature. The development of an effective treatment (Streptomycin, Rifampicin, Isoniazid, and Ethambutol) in the mid-twentieth century was a ray of hope. Initial disease control with combination therapy was phenomenal, but with the emergence of the human immunodeficiency virus (HIV) infection and drug resistance (DR), incidence of TB started to rise again [1]. Despite the strategic steps taken by the World Health Organization (WHO) in the last three decades to control disease spread, it continues to over-burden the health infrastructure worldwide. Globally, TB is the leading cause of mortality attributed to infectious diseases. Records of 2018 represent nearly seven million new cases and 1.5 million deaths (including 251 thousand individuals

co-infected with HIV). Several factors contribute to the survival of this deadly infectious agent, despite the global coverage by vaccination and chemotherapy. One of the factors may be heterogeneity in the disease prevalence and healthcare facilities, which also exists across the globe—the data taken from WHO shows that one in three patients has no access to the treatment. Around 0.5 million cases every year are diagnosed as multidrug-resistant (MDR) disease [2].

The clinical spectrum of TB is also varied. It can affect almost all body organs. However, pulmonary tuberculosis (PTB) is the most common form. The invasion by the bacteria broadly results in two types of disease states, active disease and latent TB infection (LTBI). Classical features of the active PTB include persistent cough (> three weeks) despite antibiotic treatment, expectoration, fever, significant weight loss, fatigue, and in severe cases, hemoptysis. LTBI is characterized by the persistence of bacteria in the host with no or minimal symptoms though the patient may positively respond to immunological tests using mycobacterial antigens (hypersensitivity tests). LTBI represents about 90% of all TB infections [3]. Recently, a spectrum of disease manifestations in terms of severity, drug response, immune response, and disease outcomes has been recognizable between these two presentation states. Various host and pathogen factors contribute to the heterogeneity observed in the mycobacterial infection and varied host response.

2 Genotypic and Phenotypic Heterogeneity in *Mycobacterium Tuberculosis* Isolates

The genetic variants of *Mycobacterium* seem to influence the host response. Current knowledge of genetic heterogeneity comes from molecular epidemiological studies (analyses of the global distribution of strains). As evident in animal studies, *M. tb* from East Asia produced a distinct host response compared to other strains (larger areas of pneumonitic patches). The difference in the genetic makeup was possibly responsible for variation in cell wall glycolipids, activation of Toll-like receptors (TLRs), and inflammatory responses, which contribute to the disease progression [4–6]. The gene sequencing techniques have added to the understanding of this genetic heterogeneity. Earlier, detection of repetitive DNA elements was done using PCR-based Spoligotyping and MIRU-VNTR (mycobacterial interspersed repetitive units-variable number of tandem repeats). Spoligotyping analyzes the presence or absence of 43 mercurial spacer sequences in the mycobacterial genome's repeat regions.

On the other hand, MIRU-VNTR typing evaluates the 24 loci containing VNTR of genetic elements, including MIRUs. It identifies phylogenetic lineages better than spoligotyping. Till April 2016, the global genetic database of *M. tb* has identified 19,000 different MIRU-VNTR types and 7,000 variable spoligotypes worldwide [7]. Other methods of genetic sequencing (single nucleotide polymorphism (SNP), large sequence polymorphisms, and whole-genome sequencing) have identified seven main lineages of human-associated strains with the specific

Table 1 Global phylogenetic structure of *M. tb* strains [7, 9, 10]

Lineage	Classification	Geographical distribution	Characteristics
Lineage 1	Ancestral	East Africa 1	A predilection for the central nervous system
Lineage 5		West Africa 1	Less virulent and less risk of progression due to mutation of genes encoding Molybdenum and Vitamin B12 co-factors
Lineage 6		West Africa 2	
Lineage 7		Ethiopia (East Africa)	
Lineage 3	Modern	Asia/Delhi	More commonly affects lymph nodes and the gastrointestinal tract
Lineage 2 (Beijing)		East Asia, Eastern Europe, and Central Asia	Responsible for Central Asian and Eastern European/Russian MDR-TB outbreak
Lineage 4		Europe, America, and Western Africa	No association with any particular organ

geographical association: lineages 1–4 and lineage 7, *M. tuberculosis sensustricto;* and lineages 5, 6, *M. africanum* (Table 1) [8].

Recent studies have demonstrated a difference of not more than 2200 SNPs between a human and an animal strain. Among the strains affecting only humans, this difference is less than 1800 SNPs. This restricted diversity suggests that all the strains originated from a common ancestor (probably *M. carnettii*). Evaluation of the lineages suggests that most of the ancestral lineages affecting humans (lineages 1, 5, 6, and 7) probably have an African origin. The modern lineages show a distribution pattern centered on Asia (lineage 3) and intercontinental regions (lineage 2, Beijing in East Asia; lineage 4, distributed in Europe). The mycobacterial infection has a sympatric distribution among the immune-competent individuals of a particular region. However, an immunodeficient state increases vulnerability to allopatric strains as well [9, 10]. African origin lineage might have followed the human migration out of Africa to other regions some 40–80 thousand years ago. Animal-associated lineages diverged from human strains around ten thousand years ago when the domestication of animals started. The geographical distribution of *M. tb* suggests co-evolution of the pathogen and human host.

An organized and localized pool of immune cells, including macrophages and lymphocytes, called a granuloma, is the hallmark of human TB. It is the host response to persistent stimuli by the pathogenic bacteria. Different structures, mainly cellular components, participate in the formation of TB granulomas. However, it is unclear if the granuloma heterogeneity in a particular host results from the bacterial variation. Evidence from bacterial genetic mapping suggests that a single bacterium triggers most of the granulomas in a host. Unlike other bacilli, *M. tb* is a genetically stable, monomorphic, and slowly dividing organism.

Moreover, the infectious dose is usually low and hence the variation in strains at the time of infection is low [11, 12]. Several studies on macaques and humans have demonstrated little or no genetic changes even after a long disease course. One study noted that less than five SNPs among the bacterial strains were isolated from different granulomas in a single host [13, 14]. Hence, the authors concluded that genetic heterogeneity was less likely to cause the striking phenotypic (difference in appearance or form) heterogeneity in a mycobacterial population.

Though divided asexually encoding the same genes, the bacteria exhibit subtle phenotypic heterogeneity in successive generations. Various driving factors might play a role in the phenotypic variations, including adaptive mutagenesis, amplification of genes, and the effects related to environmental variations. Below, we have discussed changes that help the organism withstand and flourish in the fluctuating environment [15].

2.1 Stochastic Processes

No two bacteria are identical and thus do not exhibit the same phenotype. Noise in gene expression is an important cause for phenotypic heterogeneity. Extrinsic noise resulting from fluctuations in the number of ribosomes and RNA polymerase activity affects gene expression. Intrinsic noise is due to biochemical changes affecting the binding of proteins at the promoter regions. The former often continues to act over the next generation of cells through cellular memory [16]. The bacterium also maintains stability through environmental fluctuations by positive feedback mechanisms at various levels. *Bi-stability* is a prominent feature mediated through the 'mprAB' operon in *Mycobacterium* [17, 18]. Transcriptional noise is another mechanism of metabolic diversification, which facilitates survival in differing substrate concentrations. Recently, a persister lineage of *Mycobacterium* demonstrated a stochastic decreased expression of KatG enzyme (antioxidant) to evade the bactericidal properties of Isoniazid [19].

2.2 Growth Phase and Growth Rate

Not all cells remain in the same division phase at any given point of time in a given population of multiplying cells. It results in marked heterogeneity in terms of cell size, age, and susceptibility to antibacterial agents. Variations in growth rate reflect the microenvironment state (presence of nutrients, synthesis of proteins, presence of toxins, oxygen concentration) and vice versa. *Mycobacterium* is a slow-growing organism and exhibits frequent changes in growth patterns even in optimal conditions. Stressful conditions exacerbate the heterogeneity and are largely responsible for developing a non-growing but metabolically active (NGMA) bacteria. Murine models suggest that macrophage uptake results in slow-growing drug-resistant (DR) bacteria [20, 21].

2.3 Asymmetric Cell Division and Cell Aging

Cell division in bacilli produces two different daughter cells: a relatively large pole but older sibling and a smaller younger sibling. The cellular components are also differentially distributed among the progeny. *Mycobacterium* is no exception. The younger pole sibling has higher ribosomal RNA concentrations and grows faster [22]. A single-cell technique using fluorescent-tagged protease chaperon (ClpB) demonstrated the differential distribution of oxidized proteins, which slowed down the growth and repair system [23]. Other enzymes (AAA^+ proteases) are also involved in antitoxin degradation. Older *Mycobacterium* also tends to accumulate misfolded proteins and overexpress AAA^+ proteases resulting in growth attenuation [24].

Similarly, 88 toxin-antitoxin systems have been described in *M. tb*, most of which result from horizontal gene transfer rather than ancestral inheritance. It results in multi-stable phenotypes and favors mechanisms responsible for its persistence. The triggering factors for such phenotypic changes include nutrient starvation, hypoxia, host immunity, and exposure to toxins or drugs [25].

2.4 Host Microenvironment

It is considered the most important determinant for phenotypic heterogeneity. The differences in innate immune activity (pro-inflammatory and anti-inflammatory mediators) and variable production of cytokines, reactive oxygen substrates, dynamic nutrient availability, and anti-TB drug concentration in different sites affect bacterial growth, damages repairability, and redox modulators. It distinguishes and differentiates each lesion in a given individual [26]. The vascularization of a granuloma also affects bacterial activity. Bacilli from an open cavity can be readily cultured (within six to eight weeks instead of 12–18 weeks from closed cavities) and are more likely to show resistance to treatment than bacilli from a closed one [27].

Diversity in the genetic and phenotypic activity has strong implications on disease persistence, development of DR, and disease progression. Deep learning through computational modeling and single-cell biology can help develop personalized medications and target the subpopulation of the persister.

3 Heterogeneity in Host Genetic Susceptibility to Tuberculosis

As described earlier, a large proportion of the population develops LTBI, termed non-progressors. According to estimates, one-fourth of the world population harbors latent infection, and only 5–15% of the hosts infected with the organism progress and develop clinical disease [28]. Infection with the *M. tb* stimulates a cascade of immune responses. Interestingly, not all hosts become symptomatic or develop the latent disease when infected with *M. tb*. Several epidemiological

studies have shown that, even on prolonged close contact of active TB cases (family members sharing a room, sailors, miners, and students living in confined areas), 5–20% of individuals behave as resilient or resistors [29]. They either do not get infected or rapidly clear the bacteria after a transient infection. The innate immune system muzzles the bacterial infestation without activating the acquired immune system. Therefore, the skin reactivity test (tuberculin skin test, TST) and interferon-gamma release assay (IGRA) remain negative despite persistent heavy exposure of the bacteria. Studies have shown that ability to resist the infection by *Mycobacterium* is more common among siblings than unrelated individuals connoting the role of genes in the susceptibility to infection [30].

Epidemiological studies have demonstrated heterogeneity in TB susceptibility. Amidst a TB storm, 13% of African miners were revealed to be resistors [31]. Similarly, the few healthcare workers treating TB patients have the resilience to the mycobacterial infection [32]. Outcomes of infection with *Mycobacterium* were quite variable, including fatal disease in 50%, chronic disease in 25%, and cure in 25%. Such estimates proved the presence of innate immunity in some individuals [33]. Another report on 251 infants (in 1929) who received contaminated TB vaccine containing virulent *Mycobacterium* behaved differently. While 29% of children succumbed to the illness, 68% developed the mild disease. The dose of inoculum correlated with the outcome in the majority of cases. However, two babies who succumbed to the illness had received the vaccine with minimum contamination, suggesting their high level of inherent susceptibility to TB [34]. The death rate associated with TB among the native population of Canada decreased from 10% in 1890 to 0.2% in 1930, but the disease wiped off half the area's population after just 40 years [35]. This example implies a likely mechanism of natural selection of resistant genes in TB.

Phenotypic heterogeneity of the bacteria and diversity in the human cohort have presented genetic evaluation challenges. Recent developments and advances in genetic epidemiology and functional genetics (epigenetics, microRNA isolation, transcriptomics) have highlighted the role of genes in the mycobacterial susceptibility pattern [36]. Experimental studies on mammals have also documented two types of genetic control on the outcome of infection with mycobacteria. Resistant rabbits in one study survived twice as long as the susceptible rabbits [37]. In another experiment, Werneck-Barroso demonstrated the absence of disease manifestations in 20–40% of rabbits (majority TST negative) exposed to the TB bacteria [38]. Genetic studies on human populations in Africa and North America have shown wide variations in the heritability of TST and IGRA responses to exposure. Less than half of the familial contacts became TST and IGRA positive. In another study on 90 multi-generational families from Columbia, a co-dominant gene was responsible for the variability (65%) in TST positivity [39].

Cobat et al. demonstrated two major loci determining TST reactivity: TST1 on chromosome 11p14 and TST2 on Chromosome 5p15. The latter exhibited association with the intensity of T-cell mediated response to tuberculin injection. A gene (*SLC6A3*) encoding Dopamine transporter seemed to modulate response differences [40]. People who remain TST negative despite exposure to *M. tb* may be innately

resistant to the organism. A report from Ghana showed that TST-negative individuals were less likely to produce Interleukin (IL)-10 than TST-positive individuals (IL-10 haplotype inheritance). IL-10, an anti-inflammatory cytokine, may be associated with suppressing adaptive immune response [41]. Multiple genome-wide association studies (GWAS) have been undertaken to identify the genomic loci responsible for affecting disease development. A study from Iceland demonstrated the correlation between the human leukocyte antigen class II (HLA-II) region, TST response, and resistance to the mycobacterial infection. The HLArs2894257 variant on chromosome 6p21 is associated with the reduced presentation of antigens to the T cells [42, 43]. Another genome-wide transcriptional study from Uganda on HIV-negative individuals suggested an association between the histone deacetylase pathway and TB infection resistance [44, 45]. A number of loci have been identified to affect the outcome: 5p13, 5q23, 11p13, 11p14, 15q11, 20p13, and 20q12. However, inconsistency in the inferences derived from various studies from different regions exists, and therefore gene locus identified in one study could not be validated in another. The limiting factor in the majority of the studies was the small cohort size and variation in non-coding sequences. It thus becomes difficult to explain the development of infectious diseases by SNPs alone [46].

A report from Uganda documented the heritability of IGRA response to *Mycobacterium* ($\sim$ 30%) [47]. Recent studies have focused on the gene mutations responsible for IL-12/IFN-γ activity. Such alterations in these genes may result in atypical TB and other infections and follow Mendelian inheritance. Individuals are susceptible to low pathogenic *Mycobacterium*, called Mendelian susceptibility to mycobacterial disease (MSMD) or atypical familial mycobacteriosis, which correlates with seven autosomal (*IFNGR1, IFNGR2, IL12B, IL12RB1, IRF8, ISG15, STAT1*) and two X-chromosome genes (*CYBB, IKBKG*). Identifying more than 140 mutations in these genes has provided insights into mechanisms responsible for 18 different disease manifestations. However, incomplete penetrance of most mutations results in the heterogeneity of MSMD. It accounts for 3–45% of disseminated TB [48, 49].

Various methods help to identify genetic variants. Transcriptional (unwinding of DNA helix at the RNA synthesis site) studies have identified the histone deacetylase pathway as an important factor in determining resistance against *Mycobacterium* [45]. In studies on animals, 34 transcripts appeared to play a crucial role in determining the course of tubercular infection in advance (active disease or LTBI) [50]. The majority of these transcripts were related to interferon and inflammatory pathways. Suliman et al. described a transcript signature ($RISK_4$) based on blood RNA sequencing to predict the course of PTB in the African population up to two years before the development of symptoms [51]. A recent meta-analysis, which included 16 genetic studies in patients with active TB, described a set of 380 differentially expressed genes. Reports indicate the role of the upregulation of IFN-γ in increasing the susceptibility and severity of disease [52].

Other mechanisms potentially related to increased TB infection susceptibility include epigenetic modifications in monocyte-derived macrophages. These regulatory changes (methylation or acetylation) are responsible for the upregulation or

downregulation of specific genes. They are also responsible for releasing pro-inflammatory cytokines and determining the activity of macrophages against the bacilli [53]. MicroRNAs (non-coding chains of nucleic acids that regulate the degradation of messenger RNAs) play a pivotal role in developing TB. MicroRNA-223 regulates neutrophil-driven inflammation, while microRNA-155 enhances the autophagic response of macrophages to *Mycobacterium* by inducing phagosome maturation. A study showed that their deletion in TB-resistant mice transformed those mice into susceptible hosts [54, 55]. MicroRNA-499 and microRNA-146a are associated with decreased resistance towards mycobacterial infection [56].

Despite the molecular advances, a comprehensive understanding of the genetic basis of susceptibility to TB remains elusive. The majority of studies have limited their evaluation of PTB. The different forms of the disease (active infection, LTBI, secondary forms, primary disease) need to be evaluated simultaneously regarding genetic susceptibility. An integrated approach may serve to develop effective treatment and preventive strategies.

4 Anthropometric Heterogeneity in Tuberculosis

Susceptibility to *Mycobacterium* increases with extremes of the host response. Inadequate response in people with HIV, children under the age of two, and immunocompromised people presents with disseminated disease or extrapulmonary tuberculosis (EPTB), while excessive inflammation correlated with necrotizing granulomas and cavitary pulmonary lesions [57, 58]. The body mass of an individual has an important role in the outcome of TB. Obesity is a state of mild inflammation and has shown protective effects against active TB. Adipocytokines, like leptin, tend to rise with obesity. Leptin can stimulate naïve T-cells proliferation and promote Th1 cytokine response, which plays a pivotal role in containing mycobacterium [59]. A systematic review of six studies by Lönnroth et al. could associate about a 14% reduction in the incidence of TB with a rise in body mass index (BMI) from 18.5 to 30 kg/m^2. The authors noted a log-linear inverse relationship between the infection and BMI [60]. In another study from Hong Kong, Leung et al. [61] reported a three-fold decrease in the risk of developing active TB infection in obese individuals than normal-weight people. This picture was similar to that reported in the USA [62], where the authors documented a five-fold decrease in the risk of progression in obese individuals compared to normal-weight people. A decrease in the risk of new infection among HIV-positive obese patients was also noted in a South African report [63]. Aibana et al., in a prospective cohort study on the risk of 14,000 household contacts, clearly demonstrated a protective effect of high BMI, as high as > 35 kg/m^2, in preventing active disease. However, the protective effect was not evident among children below 12 years, and high BMI was not associated with decreased chances of TB infection [64]. Human adipose tissue rather promotes LTBI by providing a reservoir to the bacilli to survive in a

dormant state. Moreover, obesity is often associated with diabetes, a proven risk factor for TB progression.

Malnutrition (low BMI), on the other hand, is a risk factor for the reactivation of LTBI as well as transmission of TB due to poor cell-mediated immune response. Also, low BMI correlates with increased mortality and DR. Undernutrition is strongly associated with other risk factors of TB, including low socioeconomic status, overcrowding, alcohol consumption, and comorbidities. A BMI < 13 kg/m^2 in males and < 11 kg/m^2 in females is considered lethal among TB patients. BMI of 16 kg/m^2 is associated with a two-fold rise in mortality. Nutritional support with dietary supplements to increase the BMI to 18.5 can decrease the risk of mortality in infected individuals [65].

5 Age- and Sex-Specific Heterogeneity in Tuberculosis

A striking feature of TB, apparent in national surveys across the globe, is that it affects men more than women. The prevalence remains equal in males and females till 10–14 years of age, after which male prevalence starts exceeding that of females [66]. In a report by Martinez et al., the authors documented a male to female ratio of 2.1 in Americans and foreign-born individuals as far as TB incidence above 14 yearsage was concerned. The increased incidence was evident even in the HIV-negative population [67]. In 2018, nearly ten million patients were affected by TB globally. The disease influenced individuals in all age groups. However, males (15–64 years) were affected maximally (57%). Adult females and children (< 15 years) comprised 32 and 11% of all those affected with TB, respectively. These differences between males and females in the adult age group (15–64 years) are possibly attributable to differences in:

- societal roles (males are often the sole bread earners of the family in developing countries and venture out for work leading to more external contact);
- behavioral differences (alcohol intake and smoking, as well as high-risk behavior, are more common among males); and
- living conditions (males often migrate to cities in search of work, stay in slums and work in overcrowded places like mines and factories; males predominantly inhabit prisons).

Such differences increase the risk of exposure and contracting the infection. Moreover, males are more likely to develop advanced clinical disease, as reflected in more hemoptysis and sputum production. Even at the start of symptoms, miliary pattern and cavitary lesions on chest radiographs are more common among males than females [68–70]. However, females experience higher mortality than males in the early years of life. Females younger than 35 are more likely to succumb to the illness. Above 35, the disease becomes several times more fatal in males [66].

This age-related male–female variation might depend upon the effects of hormones, steroids, and immune responses (T-cell differences and variance in antibody production). Also, there are potential effects of pregnancy, co-infection with HIV, and differential individual response to testing [71]. Females have higher levels of IgM antibodies as they can produce a better antibody response to many vaccines. Hence, a woman's immune response can counter a TB infection more efficiently. A Th1 lymphocyte response is associated with protection against TB infection. In women, a shift from Th1 to Th2 activity occurs during pregnancy to suppress the maternal immune system, which would otherwise harm the fetus. Hence, a female capable of preventing TB infection might become susceptible to disease reactivation and even progress to severe disease when pregnancy does occur [72].

Males are more susceptible to post-primary smear-positive PTB, which is responsible for the continuum of spread. On the other hand, females more often present with smear-negative PTB or EPTB, which, in turn, results in decreased notification [73]. Moreover, females show a different reaction to diagnostic tools, a 10% fewer culture positivity on sputum examination and less likelihood of testing positive for TST [74]. It implies an increased chance of missing and underreporting a female case of TB. Therefore, more sensitive methods are necessary to achieve statistical accuracy, especially for women.

The prevalence and incidence of HIV in young females are higher than in males. The risk of transmission of HIV is two to four times higher in a female after intercourse. The co-infection of HIV with TB is devastating. Patients often die with TB due to opportunistic infections rather than due to TB itself, and the mortality is usually due to HIV rather than TB. This explains the low notification rates among co-infected women with HIV [66]. Many questions remain unanswered. The low notification rates in females point out a distinct biological and social behavior. However, the contribution of statistical miscalculation (underreporting of EPTB, a higher chance of social stigma, and lack of awareness among females) needs to be understood.

6 Socioeconomic Heterogeneity in Tuberculosis

There is enough evidence to support the influence of socioeconomic determinants (social, political, and economic circumstances in which the individuals take birth, thrive, work, grow and die) on TB pathogenesis, risk of transmission, host susceptibility, lead time to diagnosis, access to the healthcare system, time to start treatment, compliance, and cure [75]. These factors are responsible for the heterogeneity in transmission and the prevalence of the disease. The economic crisis in the continent (2008–2012) effectively halted the continuous decrease in the incidence rate of TB in European nations. In a Portugal report, Franco et al. documented that urban centers were the areas with the highest prevalence. While unemployment and lack of a socioeconomically qualified population were important factors in one region, immigration from Sub-Saharan Africa (high-TB

incidence) was responsible for the high number of TB cases in another region [76]. As documented in another study from sub-Saharan Africa, high prevalence determinants included low education, unemployment, poverty, smoking, undernutrition, alcohol intake, and incarceration. Malnutrition renders an individual highly susceptible to new infection and reactivation of dormant disease. Financial constraints and poor health facilities at government centers are responsible for delayed or incomplete treatment adding to the transmission, prevalence, morbidity, and mortality rates of TB.

TB is traditionally considered a social stigma. It acts as a deterrent to social support and apt treatment due to a lack of education and awareness in economically backward areas [77]. One-third of the population in South-East Asia lives in an economic crisis. Education is not a priority for low-income families, and the bread earners often contact a large number of dependants. Lack of appropriate education contributes to further poverty. They often live in crowded areas and use biomass fuels for cooking. The small households expose them to smoke, leading to low pulmonary reserve. Migrants from such high burden regions remain at increased risk of contracting TB. The physical health of migrants from a rural area or a developing country is usually better than the residents who stay back but not better than those residing in an urban area or a developed country [78]. In a study from China, individuals who internally migrated to another province with better socioeconomic conditions were less likely to develop TB than those who stayed back. The reason stated by the authors was the selective migration of healthy individuals [79].

Social interventions (social protection, uplifting living standards, and health education) can help improve access, compliance, and completion of treatment. It is likely to bridge the socioeconomic gaps, prevent migration, and positively impact TB epidemiology.

7 Heterogeneity in Tuberculosis Transmission

M. tb is airborne, and therefore spread of infection is possible without direct contact. However, the transmission rate is not as high as respiratory viral infections. As discussed in the previous section, transmission depends on several social and economic factors. It also depends on the characteristics of cough, size of aerosols generated, environmental factors, host susceptibility, and duration of exposure [80]. Melsew et al., in their study (4190 index cases and 18,030 primary contacts), documented that 75% of all the secondary infections in a community were associated with super spreading events (one patient producing three or more infections). Nearly 90% of secondary infections were due to only 20% of the index cases. Some factors affected secondary infections. The co-occurrence of PTB and EPTB was associated with about a 42% reduction in secondary infection rates compared to PTB alone. This may be because patients with disease sites other than pulmonary tend to have a low bacillary load. EPTB alone was associated with nearly zero

transmission. Patients identified through active contact tracing or post-migration follow-up were responsible for lower secondary infections (71% and 46%, respectively) compared to patients with clinical manifestations. Patients identified by symptoms alone remained undiagnosed for a more extended period and posed a constant threat to the community until they became symptomatic. The rate of transmission was low in patients with the disease diagnosed by PCR/histology (70% less) and radiographs (55% less) as compared to those with a culture-positive disease (culture positivity implies an increased viral load) ([81]; Table 2).

8 Heterogeneity in Treatment Responses

Mycobacterium can exhibit several metabolic states at different locations inside the same individual and demonstrate variable tolerance to the medications over a while. It has made prolonged treatment regimens for TB mandatory. The quest for antibiotics that can act across a range of granuloma and target the metabolism of the bacteria has led to the development of new drugs, notably Nitroimidazoles and Bedaquiline [82, 83].

Granuloma heterogeneity is a major determinant of antibiotic response independent of bacterial activity. Not all drugs have equal access inside TB granulomas. Spectrometry studies (MALDI, matrix-assisted laser desorption/ionization) have clarified that while Rifampicin, Isoniazid, and Pyrazinamide aptly penetrate all granulomas, Moxifloxacin (second-line agent) diffuses poorly into the acellular regions (caseum) [84]. The ability of a drug to sterilize the infection correlates with its potential to penetrate the granuloma. Factors that affect the diffusion into the caseum include active transport into immune cells, the binding capacity to transporter proteins and macromolecules, and physiochemical properties (less lipophilic) affecting drug solubility. While Isoniazid and Pyrazinamide distribute equally across walls and necrotic cores but do not accumulate with repeated dosing, Rifampicin preferentially accumulates in the caseum over time in addition to its bactericidal properties. Pyrazinamide is an effective agent against bacteria in an inflammatory environment and acidic pH (inside a phagolysosome of an infected macrophage where pH is around 5). It is relatively ineffective at neutral pH (7.3–7.4 in caseum and 6.4–6.7 in a granuloma center). However, Pyrazinamide, along with Rifampicin, is responsible for the decrease in the duration of treatment to six months compared to the previous strategy (two years therapy) [85].

The most problematic aspect of TB management is the emergence of MDR-TB strains, i.e., strains resistant to at least Rifampicin (R) and Isoniazid (H). In vitro studies have shown that nutrition starvation can affect drug permeability and promote phenotypic resistance to the compounds [86]. Studies on animal models have shown the effect of vascularization on the development of DR. A well-vascularized granuloma (open cavities) promotes bacterial growth in an aerobic microenvironment with neutral pH. Metabolically active bacilli in these granulomas are susceptible to Isoniazid and Rifampicin but more likely to develop resistance and

Table 2 Factors affecting heterogeneity in transmission of TB

Sources	Drivers of heterogeneity	Mechanisms associated with increased transmission	Implications
Infected host	Some patients may be superspreaders	Patients with symptoms or culture-positive pulmonary disease	Less than 20% of individuals are responsible for ~ 90% of the transmission. They can be mainly targeted
Mycobacterium (infectious agent)	Virulent strains or resistant forms of TB (MDR/XDR-TB)	The bacilli are more capable of evading the host's immune system as well as the treatment. Index cases remain infective for a more extended period	Lineage 2 form of mycobacterium was responsible for outbreaks of MDR-TB in Russia and Europe XDR-TB outbreaks have been reported from regions of South Africa. Such cases need a timely diagnosis and apt treatment to curb the spread
Susceptible host	Immunosuppression, HIV infection, malnutrition, smokers	Poor innate immunity or pulmonary reserve to counteract the infection	The emergence of HIV in the early 1980s led to the resurgence of TB in developed countries. Improving nutrition and protecting high-risk people (immunosuppressed) is prudent for controlling the disease
Environmental factors	Closed crowded areas (prisons, less ventilated health centers, mines, slums) and prolonged contacts (family members); higher population density	Poor ventilation and close contact increase the chances of aerosol inhalation	The prevalence of TB is high among the economically deprived population due to overcrowding and poor living conditions. A high-risk environment needs to be targeted (slums, prisons, mines)

(continued)

Table 2 (continued)

Sources	Drivers of heterogeneity	Mechanisms associated with increased transmission	Implications
Other factors	Inadequate health provision and inadequate surveillance increases the risk, migration, poverty	Undiagnosed and untreated cases come in contact with the community for a more extended period spreading the disease. Higher population density increases the chances of transmission	Recently, improvement in living standards has resulted in a decline in infection rates. A strong and equitable policy with planning is the need of the hour

relapse with suboptimal dosing. In a closed cavity lesion with poor circulation, the bacteria stay in an NGMA state due to poor nutrition and an anaerobic environment. Due to the slow growth rate, they also tend to become tolerant to Isoniazid (a cell wall synthesis inhibitor) but remain susceptible to Rifampicin, an RNA polymerase inhibitor. The impeded drug penetration with dynamic oxygen levels in such lesions can augment drug tolerance in due course of time [87]. Hence, a combination of drugs is necessary to sterilize various lesions. Rifampicin and Pyrazinamide are uniquely capable of eradicating drug-tolerant persister subpopulations. Moxifloxacin (a gyrase inhibitor) showed bactericidal properties killing both active and dormant bacilli in murine models but failed to shorten the duration of treatment in human trials [88, 89].

In a nationwide survey, it was noteworthy that one in each of the three patients carried an MDR strain [90], while only one-fifth of all were properly recognizable, diagnosed, and aptly treated. Lack of adequate treatment increases morbidity, mortality, and ongoing transmission. It has also led to the development of extensively drug-resistant (XDR) strains that have resistance to Isoniazid, Rifampicin, Fluoroquinolones, and one of the three injectable drugs (Amikacin, Kanamycin, or Capreomycin). Recently, cases resistant to the latest anti-TB drugs (Delamanid and Bedaquiline) have also been documented [91].

9 Heterogeneity in Immunity and Antibody Responses to Tuberculosis

The immune response to a microbe is complex and highly variable. It involves interplaying the host's virulence factors (toxins, enzymes, immunomodulatory proteins) related to the microorganism and the defense system (macrophages, antigen-presenting cells, lymphocytes, antibodies, and other inflammatory molecules). It may result in four possibilities [92]:

- mild or no symptoms followed by complete eradication of the organism by the host's immune system;
- the immune system gets overwhelmed by the infection, often leading to severe symptoms, complications, and even death of the host;
- microbial proliferation leads to symptoms followed by a persistent state with minimal host cell damage; and
- the organism remains in association with the host without causing obvious damage (commensal).

Each of the above states may coexist in a single host during the infection. TB is an excellent example of such diversity in the immune response. The most common route of transmission is through the respiratory tract. The immune system successfully clears the infection in the majority (90%). The remaining 10% develop clinical symptoms [93]. This is true for non-pulmonary involvement as well. One known contributor to disease susceptibility is malnutrition, well-known to impair immunity. The magnification of disease burden in the areas with high-HIV prevalence also supports the immune system's role against TB.

Seeding the pathogen in the host's lower respiratory tract (alveoli) induces a cascade of events. The first encounter with innate immunity is crucial in deciding the outcome. The plausibility of developing a clinically significant disease is inversely related to the number of activated natural killer (NK) cells, white blood cells, and mucosa-associated T-cells [94, 95]. *Mycobacterium* lacks toxins and typical virulence factors, unlike other bacilli. Instead, it thrives on its ability to evade the host immune system. Lipid in the bacterial membrane and inhibiting proteins impede its recognition by the host's immune cells. The pathogen replicates and is carried to the lymph nodes by migratory dendritic cells [96]. In the case of PTB, the airway epithelial cells and the macrophages are the first to come in contact with the *Mycobacterium*. They recognize the bacilli and possess the ability to eradicate the infection (through phagocytosis and microbicidal agents) if present in adequate numbers. *Mycobacterium*, in turn, has several mechanisms to divert the response of the immune cells, especially the macrophages. It tries to counteract the phagocytic activity of the macrophages through the neutralization of oxidizing molecules and toxic metals. The deficiency of vitamin D in the host appears to result in decreased activity of IFN-γ, which plays a pivotal role in the containment of the *Mycobacterium* [97]. Infection occurs once *Mycobacterium* reaches the lymph nodes, activating the adaptive immune response. It takes two to six weeks for the cellular immune response to manifest and become evident in the form of TST. The TST does not correlate with the protection against the infection [98].

The ESAT-6 (early secreted antigenic target-6), a virulence factor secreted by the *Mycobacterium*, drives macrophage differentiation for its advantages. Initially, it triggers a marked M1 response and later transforms the macrophages from phenotype M1 to M2. M1 phenotype at the start of infection promotes granuloma formation through increased production of pro-inflammatory cytokines (IL-6, IL-12, and TNF-α). It correlates with the activity of IFN-γ, as shown by Mishra et al. [99]. It develops a primary innate granuloma, which has bactericidal

properties and helps to eliminate the invading organism in around 10%. In the rest 90% of cases, *Mycobacterium* gradually shapes the granuloma activity to suit its existence. The same ESAT-6 binds to TLR-2 and induces an immunomodulatory M2 phenotype responsible for the secretion of anti-inflammatory cytokines, e.g., IL-10 [100]. Innate and adaptive immune responses of the host act together in a coordinated fashion to seal the bacilli in a "silent" granuloma, a suitable niche for *Mycobacterium*.

Mass spectrometry studies have also demonstrated anatomical and spatial heterogeneity within the granuloma; a pro-inflammatory environment governs the center of the granuloma while anti-inflammatory mediators act in the periphery. A granuloma is a conglomerate of macrophages, multinucleated giant cells with abundant intracellular lipids in the center and neutrophils, lymphocytes, and dendritic cells in the periphery. Cholesterol forms the major energy source inside a granuloma, resulting in hypoxia and high nitric oxide concentrations [101]. There exists paramount heterogeneity in the morphology of the granuloma at various stages of the disease. This organization allows localized restriction, and the bacteria continue to persist in a non-replicating state or latent state inside the cell but become active in the immunodeficient states. The granuloma's internal milieu (inflammatory markers, hypoxia, lipid metabolism, and bacteria gene expression) changes with alterations in the host's microenvironment. The patient becomes symptomatic and infective when the granuloma drains into the bronchial lumen. We already understand that the granuloma, though it appears to contain the infection, forms an excellent niche for the *Mycobacterium* for long-term survival.

10 Geographical Heterogeneity in Tuberculosis

A third of the world's population is suffering from TB. However, a wide variation in incidence and severity exists among the countries. South-east Asia and Africa share nearly 75% of all the newly diagnosed cases, while developed regions of Europe and America contribute to only 7% of total cases annually. The countries with the highest incidence rate and mortality rate include African nations, Bangladesh, Myanmar, India, and China. India and South Africa alone constitute one-third of global mortality due to TB. The limited access to antiretroviral therapy results in rapid progression to severe TB, complications, and death. The prevalence of TB increases due to HIV but is lower than the increase in incidence due to poor life expectancy in this subgroup. The prevalence rates have followed an inverse relationship with the rate of economic development. Prevalence in America decreased by more than 50% from 1990 to 2004, but it has increased over time in Africa. In 2011, 87% of new TB cases worldwide were treated successfully. The lowest rate of cure was observed in Africa (79%), America (75%), and Europe (72%). Possible reasons for the poor treatment outcomes in Africa were lack of compliance and inadequate resources, while in developed countries of America and Europe was the high prevalence of MDR-TB [102, 103].

Geographical clustering is typical of TB. The main characteristics that define a hyperendemic hotspot are low socioeconomic status, poor hygiene, overcrowding, incarceration, and HIV infection. Through a TB transmission model in the capital city of Brazil, Dowdy et al. demonstrated that hotspots disproportionately increase the chances of community transmission. A city hotspot comprising 6% of the city's population and 16.5% of TB cases was responsible for 35% of the ongoing transmission in the community. Such high incidence clusters correlated with low socioeconomic status. The contribution of a hotspot towards disease transmission was dependent on the hotspot size, hotspot intensity (hotspot-to-hotspot versus community-to-community transmission), and cross-transmission [104]. Targeting such a hotspot for TB control is challenging and resource-intensive but is very useful in limiting community transmission.

11 Conclusion

The biological and social factors are inseparable in the discussion on heterogeneity in TB. Two biological issues that have added to the problem and hindered the abolition of mycobacterial infection are the emergence of HIV and MDR-TB. Studies on genetic sequencing of *M. tb* strains from various geographical stations suggest that the bacilli followed the migration of humans from Africa nearly seventy thousand years ago. Since then, it has remained a principal cause of morbidity and mortality due to infectious diseases [105]. The co-evolution of the organism and the human race is the plausible cause of its successful, long-lasting existence. *Mycobacterium* has adapted to evade the host immune system by conserving its antigenic epitopes [106]. Host immune response and microenvironment continuously propagate subtle but effective adaptations in the pathogen. Diversity in the infection sites, genetic susceptibility, host response, and disease outcomes has obscured recognizing the exact infectious process. The current knowledge on the mycobacterial phenotype comes from laboratory studies. There is a marked difference between the pathogen's in vitro and in vivo behavior. The use of cutting-edge technology to study the host-pathogen interaction and drug effects in the natural environment may shed some light on the knowledge of bacterial heterogeneity, which is still in its infancy.

The heterogeneity of M. tb is diverse, colossal, and deep. Knowledge is growing, but we have miles to go before we sleep.

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Core Messages

- Genetic heterogeneity of *Mycobacterium* is a less likely cause behind the striking phenotypic variation.
- Granuloma heterogeneity determines the antibiotic response and is responsible for developing drug resistance.
- Females of reproductive age group and obese individuals have decreased chances of severe disease.
- Super-spreaders and community hotspots are responsible for 90% of community transmission.

References

1. Sharma SK, Mohan A (2013) Tuberculosis: from an incurable scourge to a curable disease-journey over a millennium. Indian J Med Res 137(3):455–493
2. Floyd K (2019) Global TB Report 2019. Geneva: World Health Organization. License: CCBY-NC-SA3. OIGOhttps://www.who.int/tb/global-report-2019. Published online on 17 October, 2019
3. Andrews JR, Noubary F, Walensky RP, Cerda R, Losina E, Horsburgh CR (2012) Risk of progression to active tuberculosis following reinfection with Mycobacterium tuberculosis. Clin Infect Dis 54(6):784–791
4. Lopez B, Aguilar D, Orozco H, Burger M, Espitia C, Ritacco V, Barrera L, Kremer K, Hernandez-Pando R, Huygen K, van Soolingen D (2003) A marked difference in pathogenesis and immune response induced by different Mycobacterium tuberculosis genotypes. Clin Exp Immunol 133(1):30–37
5. Portevin D, Gagneux S, Comas I, Young D (2011) Human macrophage responses to clinical isolates from the Mycobacterium tuberculosis complex discriminate between ancient and modern lineages. PLoSPathogens 7(3):e1001307
6. Carmona J, Cruz A, Moreira-Teixeira L, Carole S, Sousa J, Osorio NS, Saraiva AL, Svenson S, Kallenius G, Pedrosa J, Rodrigues F, Castro AG, Saraiva M (2013) Mycobacterium tuberculosis strains are differentially recognized by TLRs with an impact on the immune response. PLoSone 8(6):e67277
7. Niemann S, Merker M, Kohli T, Supply P (2016) Impact of genetic diversity on the biology of Mycobacterium tuberculosis complex strains. Microbiol Spectr 4(6). TBTB2-0022-2016
8. Coll F, McNerney R, Guerra-Assunção JA, Glynn JR, Perdigão J, Viveiros M, Portugal I, Pain A, Martin N, Clark TG (2014) A robust SNP barcode for typing Mycobacterium tuberculosis complex strains. Nat Communications 5:4812
9. Merker M, Blin C, Mona S, Duforet-Frebourg N, Lecher S, Willery E, Blum MG, Rüsch-Gerdes S, Mokrousov I, Aleksic E, Allix-Béguec C, Antierens A, Augustynowicz-Kopeć E, Ballif M, Barletta F, Beck HP, Barry CE III, Bonnet M, Borroni E, Campos-Herrero I, Cirillo D, Cox H, Crowe S, Crudu V, Diel R, Drobniewski F, Fauville-Dufaux M, Gagneux S, Ghebremichael S, Hanekom M, Hoffner S, Jiao WW, Kalon S, Kohl TA, Kontsevaya I, Lillebæk T, Maeda S, Nikolayevskyy V, Rasmussen M, Rastogi N, Samper S, Sanchez-Padilla E, Savic B, Shamputa IC, Shen A, Sng LH, Stakenas P, Toit K, Varaine F, Vukovic D, Wahl C, Warren R, Supply P, Niemann S, Wirth T (2015) Evolutionary history and global spread of the Mycobacterium tuberculosis Beijing lineage. Nat Genet 47(3):242–249

10. Gagneux S, DeRiemer K, Van T, Kato-Maeda M, de Jong BC, Narayanan S, Nicol M, Niemann S, Kremer K, Gutierrez MC,Hilty M, Hopewell PC, Small PM (2006) Variable host-pathogen compatibility in Mycobacterium tuberculosis. Proc Natl Acad Sci USA 103 (8):2869–2873
11. Lin PL, Ford CB, Coleman MT, Myers AJ, Gawande R, Ioerger T, Sacchettini J, Fortune SM, Flynn JL (2014) Sterilization of granulomas is common in active and latent tuberculosis despite within-host variability in bacterial killing. Nat Med 20(1):75–79
12. Jacobs AL (1941) Infective dose in pulmonary tuberculosis. Tubercle 22:266–271
13. Ford CB, Lin PL, Chase MR, Shah RR, Iartchouk O, Galagan J, Mohaideen N, Ioerger TR, Sacchettini JC, Lipsitch M (2011) Use of whole genome sequencing to estimate the mutation rate of Mycobacterium tuberculosis during latent infection. Nat Genet 43(5):482–486
14. Bryant JM, Schurch AC, Deutekom HV, Harris SR, Beer JLD, Jager VD, Kremer K, van Hijum SAFT, Siezen RJ, Borgdorff M, Bentley SD, Parkhill J, van Soolingen D (2013) Inferring patient to patient transmission of Mycobacterium tuberculosis from whole genome sequencing data. BMC Infect Dis 13:110
15. Raj A, van Oudenaarden A (2008) Nature, nurture, or chance: stochastic gene expression and its consequences. Cell 135(2):216–226
16. Rosenfeld N, Young JW, Alon U, Swain PS, Elowitz MB (2005) Gene regulation at the single-cell level. Science 307(5717):1962–1965
17. Sureka K, Ghosh B, Dasgupta A, Basu J, Kundu M, Bose I (2008) Positive feedback and noise activate the stringent response regulator rel in mycobacteria. PLoSone 3(3):e1771
18. Ghosh S, Sureka K, Ghosh B, Bose I, Basu J, Kundu M (2011) Phenotypic heterogeneity in mycobacterial stringent response. BMC Syst Biol 5:18
19. Choi SW, Maiga M, Maiga MC, Atudorei V, Sharp ZD, Bishai WR, Timmins GS (2014) Rapid in vivo detection of isoniazid-sensitive Mycobacterium tuberculosis by breath test. Nat Commun 5:4989
20. Manina G, McKinney JD (2013) A single-cell perspective on non-growing but metabolically active (NGMA) bacteria. Curr Top Microbiol Immunol 374:135–161
21. Adams KN, Takaki K, Connolly LE, Wiedenhoft H, Winglee K, Humbert O, Edelstein PH, Cosma CL, Ramakrishnan L (2011) Drug tolerance in replicating mycobacteria mediated by a macrophage-induced efflux mechanism. Cell 145(1):39–53
22. Manina G, Dhar N, McKinney JD (2015) Stress and host immunity amplify Mycobacterium tuberculosis phenotypic heterogeneity and induce non-growing metabolically active forms. Cell Host Microbe 17(1):32–46
23. Fay A, Glickman MS (2014) An essential nonredundant role for mycobacterial DnaK in native protein folding. PLoS Genet 10(7):e1004516
24. Feng J, Kessler DA, Ben-Jacob E, Levine H (2014) Growth feedback as a basis for persisterbistability. Proc Natl Acad Sci USA 111(1):544–549
25. Ramage HR, Connolly LE, Cox JS (2009) Comprehensive functional analysis of Mycobacterium tuberculosis toxin-antitoxin systems: implications for pathogenesis, stress responses, and evolution. PLoS Genet 5(12):e1000767
26. Kim M-J, Wainwright HC, Locketz M, Bekker L-G, Walther GB, Dittrich C, Visser A, Wang W, Hsu F-F, Wiehart U, Tsenova L, Kaplan G, Russell DG (2010) Caseation of human tuberculosis granulomas correlates with elevated host lipid metabolism. EMBO Mol Med 2(7):258–274
27. Vandiviere HM, Loring WE, Melvin I, Willis S (1956) The treated pulmonary lesion and its tubercle bacillus. II. The death and resurrection. Am J Med Sci 232(1):30–37
28. Houben RMGJ, Dodd PJ (2016) The global burden of latent tuberculosis infection: a re-estimation using mathematical modelling. PLoS Med 13(10):e1002152
29. Ringshausen FC, Nienhaus A, Schablon A, Schlösser S, Schultze-Werninghaus G, Rohde G (2010) Predictors of persistently positive Mycobacterium-tuberculosis-specific interferon-gamma responses in the serial testing of health care workers. BMC Infect Dis 10:220

30. Casanova JL, Abel L (2002) Genetic dissection of immunity to mycobacteria: the human model. Annu Rev Immunol 20:581–620
31. Hanifa Y, Grant AD, Lewis J, Corbett EL, Fielding K, Churchyard G (2009) prevalence of latent tuberculosis infection among gold miners in South Africa. Int J Tuberc Lung Dis 13 (1):39–46
32. Dickie HA (1950) Tuberculosis in student nurses and medical students at the University of Wisconsin. Ann Intern Med 33(4):941–959
33. Murray CJ, Styblo K, Rouillon A (1990) Tuberculosis in developing countries: burden, intervention and cost. Bull Int Union Tuber Lung Dis 65(1):6–24
34. Fox GJ, Orlova M, Schurr E (2016) Tuberculosis in newborns: the lessons of the "Lübeck Disaster" (1929–1933). PLoSPathog. 12(1):e1005271
35. Motulsky AG (1960) Metabolic polymorphisms and the role of infectious diseases in human evolution. Hum Biol 32:28–62
36. Orlova M, Schurr E (2017) Human genomics of Mycobacterium tuberculosis infection and disease. Curr Genet Med Rep. 5(3):125–131
37. Lurie MB, Abramson S, Heppleston AG (1952) On the response of genetically resistant and susceptible rabbits to the quantitative inhalation of human type tubercle bacilli and the nature of resistance to tuberculosis. J Exp Med 95(2):119–134
38. Werneck-Barroso E (1999) Innate resistance to tuberculosis: revisiting Max Lurie genetic experiments in rabbits. Int J Tuberc Lung Dis 3(2):166–168
39. Cobat A, Barrera LF, Henao H, Arbeláez P, Abel L, García LF, Schurr E, Alcais A (2012) Tuberculin skin test reactivity is dependent on host genetic background in Colombian tuberculosis household contacts. Clin Infect Dis 54(7):968–971
40. Cobat A, Gallant CJ, Simkin L, Black GF, Stanley K, Hughes J, Doherty TM, Hanekom WA, Eley B, Jaïs JP, Boland-Auge A, van Helden P, Casanova JL, Abel L, Hoal EG, Schurr E, Alcaïs A (2009) Two loci control tuberculin skin test reactivity in an area hyperendemic for tuberculosis. J Exp Med 206(12):2583–2591
41. Thye T, Browne EN, Chinbuah MA, Gyapong J, Osei I, Owusu-Dabo E, Brattig NW, Niemann S, Rüsch-Gerdes S, Horstmann RD, Meyer CG (2009) IL10 haplotype associated with tuberculin skin test response but not with pulmonary TB. PLoSone 4(5):e5420
42. Tian C, Hromatka BS, Kiefer AK, Eriksson N, Noble SM, Tung JY, Hinds DA (2017) Genome-wide association and HLA region fine-mapping studies identify susceptibility loci for multiple common infections. Nat Commun 8(1):599
43. Sveinbjornsson G, Gudbjartsson DF, Halldorsson BV, Kristinsson KG, Gottfredsson M, Barrett JC, Gudmundsson LJ, Blondal K, Gylfason A, Gudjonsson SA, Helgadottir HT, Jonasdottir A, Jonasdottir A, Karason A, Kardum LB, Knežević J, Kristjansson H, Kristjansson M, Love A, Luo Y, Magnusson OT, Sulem P, Kong A, Masson G, Thorsteinsdottir U, Dembic Z, Nejentsev S, Blondal T, Jonsdottir I, Stefansson K et al (2016) HLA class II sequence variants influence tuberculosis risk in populations of European ancestry. Nat Genet 48(3):318–322
44. Stein CM, Zalwango S, Malone LL, Won S, Mayanja-Kizza H, Mugerwa RD, Leontiev DV, Thompson CL, Cartier KC, Elston RC, Iyengar SK, Boom WH, Whalen CC (2008) Genome scan of M. tuberculosis infection and disease in Ugandans. PLoSone 3(12):e4094
45. Seshadri C, Sedaghat N, Campo M, Peterson G, Wells RD, Olson GS, Sherman DR, Stein CM, Mayanja-Kizza H, Shojaie A, Boom WH, Hawn TR (2017) Transcriptional networks are associated with resistance to Mycobacterium tuberculosis infection. PLoS one 12(4):e0175844. Tuberculosis Research Unit (TBRU)
46. Rudko AA, Bragina EY, Puzyrev VP, Freidin MB (2016) The genetics of susceptibility to tuberculosis: progress and challenges. Asian Pac J Trop Dis 6(9):680–684
47. Tao L, Zalwango S, Chervenak K, Thiel B, Malone LL, Qiu F, Mayanja-Kizza H, Boom WH, Stein CM (2013) Genetic and shared environmental influences on interferon-γ production in response to Mycobacterium tuberculosis antigens in a Ugandan population. Am J Trop Med Hyg 89(1):169–73. Tuberculosis Research Unit (TBRU)

48. Boisson-Dupuis S, Bustamante J, El-Baghdadi J, Camcioglu Y, Parvaneh N, El Azbaoui S, Agader A, Hassani A, El Hafidi N, Mrani NA, Jouhadi Z, Ailal F, Najib J, Reisli I, Zamani A, Yosunkaya S, Gulle-Girit S, Yildiran A, Cipe FE, Torun SH, Metin A, Atikan BY, Hatipoglu N, Aydogmus C, Kilic SS, Dogu F, Karaca N, Aksu G, Kutukculer N, Keser-Emiroglu M, Somer A, Tanir G, Aytekin C, Adimi P, Mahdaviani SA, Mamishi S, Bousfiha A, Sanal O, Mansouri D, Casanova JL, Abel L (2015) Inherited and acquired immunodeficiencies underlying tuberculosis in childhood. Immunol Rev 264(1):103–120
49. Qu HQ, Fisher-Hoch SP, McCormick JB (2011) Molecular immunity to mycobacteria: knowledge from the mutation and phenotype spectrum analysis of Mendelian susceptibility to mycobacterial diseases. Int J Infect Dis 15(5):e305–e313
50. Gideon HP, Skinner JA, Baldwin N, Flynn JL, Lin PL (2016) Early whole blood transcriptional signatures are associated with severity of lung inflammation in Cynomolgus macaques with Mycobacterium tuberculosis infection. J Immunol 197(12):4817–4828
51. Suliman S, Thompson EG, Sutherland J, Weiner J III, Ota MOC, Shankar S, Penn-Nicholson A, Thiel B, Erasmus M, Maertzdorf J, Duffy FJ, Hill PC, Hughes EJ, Stanley K, Downing K, Fisher ML, Valvo J, Parida SK, van der Spuy G, Tromp G, Adetifa IMO, Donkor S, Howe R, Mayanja-Kizza H, Boom WH, Dockrell HM, Ottenhoff THM, Hatherill M, Aderem A, Hanekom WA, Scriba TJ, Kaufmann SHE, Zak DE, Walzl G (2018) Four-gene Pan-African blood signature predicts progression to tuberculosis. Am J Respir Crit Care Med 197(9):1198–1208. GC6-74 cohort study team, The ACS cohort study team
52. Blankley S, Graham CM, Levin J, Turner J, Berry MPR, Bloom CI, Xu Z, Pascual V, Banchereau J, Chaussabel D, Breen R, Santis G, Blankenship DM, Lipman M, O'Garra A (2016) A 380-gene meta-signature of active tuberculosis compared with healthy controls. Eur Respir J 47(6):1873–6
53. Esterhuyse MM, Weiner J, Caron E, Loxton AG, Iannaccone M, Wagman C, Saikali P, Stanley K, Wolski WE, Mollenkopf HJ, Schick M, Aebersold R, Linhart H, Walzl G, Kaufmann SHE (2015) Epigenetics and proteomics join transcriptomics in the quest for tuberculosis biomarkers. mBio 6(5):e01187–e01115
54. Dorhoi A, Iannaccone M, Farinacci M, Faé KC, Schreiber J, Moura-Alves P, Nouailles G, Mollenkopf HJ, Oberbeck-Müller D, Jörg S, Heinemann E, Hahnke K, Löwe D, Del Nonno F, Goletti D, Capparelli R, Kaufmann SH (2013) MicroRNA-223 controls susceptibility to tuberculosis by regulating lung neutrophil recruitment. J Clin Invest 123 (1):4836–4848
55. Wang J, Yang K, Zhou L, Minhaowu WuY, Zhu M, Lai X, Chen T, Feng L, Li M, Huang C, Zhong Q, Huang X (2013) MicroRNA-155 promotes autophagy to eliminate intracellular mycobacteria by targeting Rheb. PLoS Pathog 9(10):e1003697
56. Li D, Wang T, Song X, Qucuo M, Yang B, Zhang J, Wang J, Ying B, Tao C, Wang L (2011) Genetic study of two single nucleotide polymorphisms within corresponding microRNAs and susceptibility to tuberculosis in a Chinese Tibetan and Han population. Hum Immunol 72(7):598–602
57. Ehlers S, Schaible UE (2013) The granuloma in tuberculosis: dynamics of a host-pathogen collusion. Front Immunol 3:411
58. Naing C, Mak JW, Maung M, Wong SF, Kassim AI (2013) Meta-analysis: the association between HIV infectionand extrapulmonary tuberculosis. Lung 191(1):27–34
59. Carbone F, La Rocca C, Matarese G (2012) Immunological functions of leptin and adiponectin. Biochimie 94(10):2082–2088
60. Lo¨nnroth K, Williams BG, Cegielski P, Dye C, (2010) A consistent log-linear relationship between tuberculosis incidence and body mass index. Int J Epidemiol 39(1):149–155
61. Leung CC, Lam TH, Chan WM, Yew WW, Ho KS, Leung G, Law WS, Tam CM, Chan CK, Chang KC (2007) Lower risk of tuberculosis in obesity. Arch Intern Med 167(12):1297–1304

62. Cegielski JP, Arab L, Cornoni-Huntley J (2012) Nutritional risk factors for tuberculosis among adults in the United States, 1971–1992. Am J Epidemiol 176(5):409–422
63. Hanrahan CF, Golub JE, Mohapi L, Tshabangu N, Modisenyane T, Chaisson RE, Gray GE, McIntyre JA, Martinson NA (2010) Body mass index and risk of tuberculosis and death. AIDS 24(10):1501–1508
64. Aibana O, Acharya X, Huang C-C, BecerraMC GJT, Chiang SS, Contreras C, Calderon R, Yataco R, Velásquez GE, Tintaya K, Jimenez J, Lecca L, Murray MB (2016) Nutritional status and tuberculosis risk in adult and pediatric household contacts. PLoSone 11(11): e0166333
65. Bhargava A, Chatterjee M, Jain Y, Chatterjee B, Kataria A, Bhargava M, Kataria R, D'Souza R, Jain R, Benedetti A, Pai M, Menzies D (2013) Nutritional status of adult patients with pulmonary tuberculosis in rural central India and its association with mortality. PLoS ONE 8(10):e77979
66. Holmes CB, Hausler H, Nunn P (1998) A review of sex differences in the epidemiology of tuberculosis. Int J Tuberc Lung Dis 2(2):104
67. Martinez AN, Rhee JT, Small PM, Behr MA (2000) Sex differences in the epidemiology of tuberculosis in San Francisco. Int J Tuberc Lung Dis 4(1):26–31
68. Thorson A, Long NH, Larsson OL (2007) Chest X-ray findings in relation to gender and symptoms: a study of patients with smear positive tuberculosis in Vietnam. Scand J Infect Dis 39(1):33–37
69. Vlassoff C, Bonilla E (1994) Gender-related differences in the impact of tropical diseases on women: what do we know? J Biosoc Sci 26:37–53
70. Dean A, Dodd P, Floyd K, Glaziou P, Law I, Auget OT (2019) TB disease burden. In: Cadman H (ed) Global tuberculosis report 2019. Geneva: World Health Organization, Licence: CC BY-NC-SA 3.0 IGO, p 27
71. Caracta C (2003) Gender differences in pulmonary disease. Mt Sinai J Med 70(4):215–224
72. Rook GAW (1998) Steroid hormones and the immune response; sex and gender differences. In: Gender and tuberculosis. An international research workshop. Report from the workshop at the Nordic School of Public Health, Goteborg, 24–26 May, pp 55–75
73. Bothamley G (1998) Sex and gender in the pathogenesis of infectious tuberculosis: a perspectve from immunology, microbiology and human genetics. In: Gender and tuberculosis. An international research workshop. Report from the workshop at the Nordic School of Publice Health, Goteborg, 24–26 May, pp 41–54
74. Kivihya-Ndugga LEA, vanCleeff MRA, Ng'ang'a LW, Meme H, Odhiambo JA, Klaster PR (2005) Sex-specific performance of routine TB diagnosis tests. Int J Tuberc Lung Dis 9 (3):294-300
75. Hargreaves JR, Boccia D, Evans CA, Adato M, Petticrew M, Porter JDH (2011) The social determinants of tuberculosis: from evidence to action. Am J Publ Health 101(4):654–662
76. Franco I, Sousa P, Gomes M, Oliveira A, Gaio AR, Duarte R (2016) Social profile of the highest tuberculosis incidence areas in Portugal. Rev Port Pneumol 22(1):50–56
77. DuarteR LK, CarvalhoC LimaF, CarvalhoACC M-T, Centis R (2018) Tuberculosis, social determinants and comorbidities (including HIV). Pulmonology 24(2):115–119
78. Pescarini JM, Rodrigues LC, Gomes MG, Waldman EA (2017) Migration to middle-income countries and tuberculosis-global policies for global economies. Glob Health 13(1):15
79. Zhang L, Liu S, Zhang G, Wu S (2015) Internal migration and the health of the returned population: a nationally representative study of China. BMC Publ Health 15:719
80. Gardy JL, Johnston JC, Sui SJH, Cook VJ, Shah L, Elizabeth B, Rempel S, Moore R, Zhao Y, Holt R, Varhol R, Birol I, Lem M, Sharma MK, Elwood K, Jones SJM, Brinkman FSL, Brunham RC, Tang P (2011) Whole-genome sequencing and social-network analysis of a tuberculosis outbreak. N Engl J Med 364(8):730–739
81. Melsew YA, Gambhir M, Cheng AC, McBryde ES, Denholm JT, Tay EL, Trauer JM (2019) The role of super-spreading events in Mycobacterium tuberculosis transmission: evidence from contact tracing. BMC Infect Dis 19:244

82. Mukherjee T, Boshoff H (2011) Nitroimidazoles for the treatment of TB: past, present and future. Future Med Chem 3(11):1427–1454
83. Koul A, Dendouga N, Vergauwen K, Molenberghs B, Vranckx L, Willebrords R, Ristic Z, Lill H, Dorange I, Guillemont J, Bald D, Andries K (2007) Diaryquinolines target subunit c of mycobacterial ATP synthase. Nat Chem Biol 3(6):323–332
84. Prideaux B, Via LE, Zimmerman MD, Eum S, Sarathy J, O'Brien P, Chen C, Kaya F, Weiner DM, Chen PY, Song T, Lee M, Shim TS, Cho JS, Kim W, Cho SN, Olivier KN, Barry CE III, Dartois V (2015) The association between sterilizing activity and drug distribution into tuberculosis lesions. Nat Med 21(10):1223–1227
85. Zhang Y, Mitchison D (2003) The curious characteristics of Pyrazinamide: a review. Int J Tuberc Lung Dis 7(1):6–21
86. Keren I, Minami S, Rubin E, Lewis K (2011) Characterization and transcriptome analysis of Mycobacterium tuberculosis persisters. mBio 2(3):e00100–e111
87. Jennewein J, Matuszak J, Walter S, Felmy B, Gendera K, Schatz V, Nowottny M, Liebsch G, Hensel M, Hardt W-D, Gerlach RG, Jantsch J (2015) Low-oxygen tensions found in Salmonella-infected gut tissue boost Salmonella replication in macrophages by impairing antimicrobial activity and augmenting Salmonella virulence. Cell Microbiol 17 (12):1833–1847
88. Nuermberger EL, Yoshimatsu T, Tyagi S, Williams K, Rosenthal I, O'Brien RJ, Vernon AA, Chaisson RE, Bishai WR, Grosset JH (2004) Moxifloxacin-containing regimens of reduced duration produce a stable cure in murine tuberculosis. Am J Respir Crit Care Med 170 (10):1131–1134
89. Jindani A, Harrison TS, Nunn AJ, Phillips PPJ, Churchyard GJ, Charalambous S, Hatherill M, Geldenhuys H, McIlleron HM, Zvada SP, Mungofa S, Shah NA, Zizhou S, Magweta L, Shepherd J, Nyirenda S, van Dijk JH, Clouting HE, Coleman D, Bateson AL, McHugh TD, Butcher PD, Mitchison DA (2014) High-dose rifapentine with Moxifloxacin for pulmonary tuberculosis. N Engl J Med 371(17):1599–1608. RIFAQUIN Trial Team
90. Skrahina A, Hurevich H, Zalutskaya A, Sahalchyk E, Astrauko A, Hoffner S, Rusovich V, Dadu A, de Colombani P, Dara M, van Gemert W, Zignol M (2013) Multidrugresistant tuberculosis in Belarus: the size of the problem and associated risk factors. Bull World Health Organ 91(1):36–45
91. Hoffmann H, Kohl TA, Hofmann-Thiel S, Merker M, Beckert P, Jaton K, Nedialkova L, Sahalchyk E, Rothe T, Keller PM, Niemann S (2016) Delamanid and bedaquiline resistance in Mycobacterium tuberculosis ancestral Beijing genotype causing extensively drugresistant tuberculosis in a Tibetan refugee. Am J Respir Crit Care Med 193(3):337–340
92. Casadevall A, Pirofski LA (2000) Host-pathogen interactions: basic concepts of microbial commensalism, colonization, infection, and disease. Infect Immun 68(12):6511–6518
93. Comstock GW (1982) Epidemiology of tuberculosis. Am Rev Respir Dis 125(3 Pt 2):8–15
94. Cambier CJ, Takaki KK, Larson RP, Hernandez RE, Tobin DM, Urdahl KB, Cosma CL, Ramakrishnan L (2014) Mycobacteria manipulate macrophage recruitment through coordinated use of membrane lipids. Nature 505(7482):218–222
95. Reed MB, Domenech P, Manca C, Su H, Barczak AK, Kreiswirth BN, Kaplan G, Barry CE III (2004) A glycolipid of hypervirulent tuberculosis strains that inhibits the innate immune response. Nature 431:84–87
96. Forrellad MA, Klepp LI, Gioffré A, Sabio y García J, Morbidoni HR, Santangelo M, Cataldi AA, Bigi F (2013) Virulence factors of the Mycobacterium tuberculosis complex. Virulence 4(1):3–66
97. Gou X, Pan L, Tang F, Gao H, Xiao D (2018) The association between vitamin D status and tuberculosis in children: a meta-analysis. Medicine 97(35):e12179
98. McKinney J, Jacobs W, Bloom B (1998) Persisting problems in tuberculosis. In: Krause RM (ed) Emerging Infections. Academic Press, San Diego, pp 51–146

99. Mishra BB, Moura-Alves P, Sonawane A, HacohenN GG, Moita LF, Anes E (2010) Mycobacterium tuberculosis protein ESAT-6 is a potent activator of the NLRP3/ASC inflammasome. Cell Microbiol 12(8):1046–1063
100. Refai A, Gritli S, Barbouche M-R, Essafi M (2018) Mycobacterium tuberculosis virulent factor ESAT-6 drives macrophage differentiation toward the pro-inflammatory M1 phenotype and subsequently switches it to the anti-inflammatory M2 phenotype. Front Cell Infect Microbiol 8:327
101. Gibson S, Harrison J, Cox J (2018) Modelling a silent epidemic: a review of the in vitro models of latent tuberculosis. Pathogens 7(4):88
102. Glaziou P, Sismanidis C, Floyd K, Raviglione M (2015) Global Epidemiology of tuberculosis. Cold Spring Harb Perspect Med 5(2):a017798
103. Corbett EL, Charalambous S, Moloi VM, Fielding K, Grant AD, Dye C, De Cock KM, Hayes RJ, Williams BG, Churchyard GJ (2004) Human immunodeficiency virus and the prevalence of undiagnosed tuberculosis in African gold miners. Am J Respir Crit Care Med 170(6):673–679
104. Dowdy DW, Golub JE, Chaisson RE, Saraceni V (2012) Heterogeneity in tuberculosis transmission and the role of geographic hotspots in propagating epidemics. PNAS 109 (24):9557–9562
105. Comas I, Coscolla M, Luo T, Borrell S, Holt KE, Kato-Maeda M, Parkhill J, Malla B, Berg S, Thwaites G, Yeboah-Manu D, Bothamley G, Mei J, Wei L, Bentley S, Harris SR, Niemann S, Diel R, Aseffa A, Gao Q, Young D, Gagneux S (2013) Out-of-Africa migration and Neolithic coexpansion of Mycobacterium tuberculosis with modern humans. Nat Genet 45(10):1176–1182
106. Comas I, Chakravartti J, Small PM, Galagan J, Niemann S, Kremer K, Ernst JD, Gagneux S (2010) Human T cell epitopes of *Mycobacterium tuberculosis* are evolutionarily hyperconserved. Nat Genet 42(6):498–503

Richa Sinha, an assistant professor in the Department of Microbiology, is determined to excel in academics and research work. She graduated in 2008 from an esteemed university in Ranchi, India. Due to her inclination towards diagnostics and advanced research, she took up Microbiology as a post-graduation subject. Her hard work and dedication during the post-graduation earned her the best outgoing postgraduate student for 2013. Then she joined senior residency in Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India. It was an excellent opportunity for her to learn the intricacies of microbiology. She has a particular interest in molecular diagnostics and infectious diseases. She is a kind lady committed to the improvement of patient care. Richa plans to continue her role as a teacher and researcher and believes in continuous learning.

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4 The Unequivocal Relationship Between Tuberculosis and Tobacco: Integration of Two Maladies

Sonu Goel and Garima Bhatt

Stopping TB requires a government program that functions every day of the year, and that's hard in certain parts of the world. And partly it's because of who tuberculosis affects: It tends to affect the poor and disenfranchised most.

Tom Frieden

Summary

Tuberculosis (TB) and tobacco use are among the leading causes of mortality and morbidity, posing a significant challenge to the health systems across the globe. Smoking tobacco is a significant contributor to one's likelihood of acquiring TB and is associated with poor prognosis among TB patients due to various reasons. This calls for developing and implementing innovative, integrative interventions that address both of these challenges. There are joint global policies for TB and tobacco; however, there are certain impediments broadly at the level of patients, healthcare providers (HCPs), and the health system. Various evidence-based strategies have evolved over a while to overcome these challenges. Utilization of each clinical encounter, religious bodies, and short text messages adapted to cultural context could offer cessation

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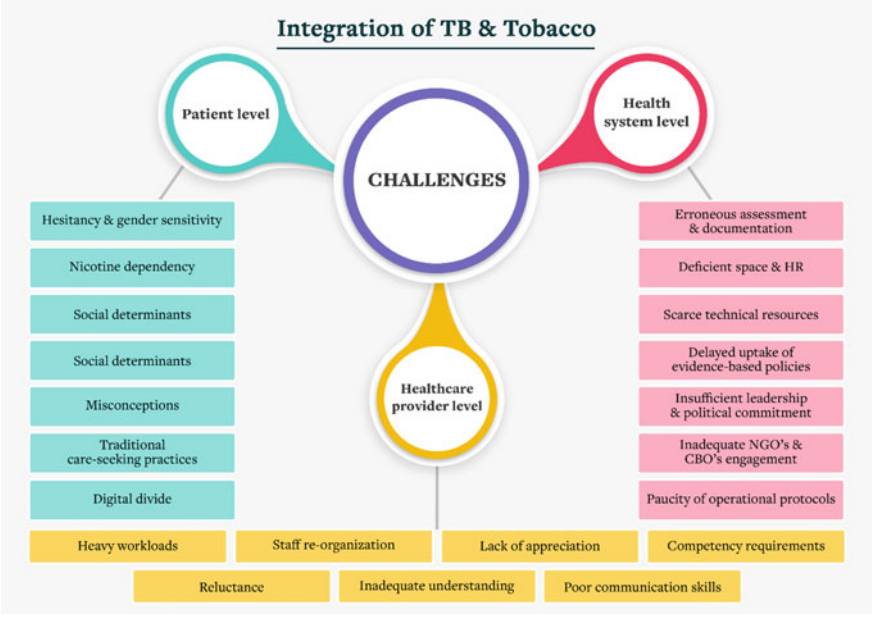
support. Further, integrated capacity building, monitoring and evaluation programs on collaborative initiatives, facilitation of cross-referral at TB-cessation clinics, along with standardized screening and treatment protocols, could address the constraints in implementation. The chapter also highlights certain recommendations for steering TB and tobacco control efforts, such as establishing a joint working group at all levels, modification of reporting mechanisms, advocacy for smoke-free policies, and integration into health and development agendas. Integration may also provide a more regular response point at various levels of the current health system. In a resource-limited setting, linking and utilizing existing programs through a synergistic approach can reduce the burden attributable to both epidemics paving the way to achieve Sustainable Development Goals.

Graphical Abstract



Challenges with the integration of care for tuberculosis (TB) and tobacco

Keywords

Epidemiology · Global policies · Integration · MPOWER · Strategies · SDGs · Tobacco · Tuberculosis

1 Introduction

Globally, tuberculosis (TB) and tobacco consumption are two leading public health challenges that are preventable to a great extent and cause considerable health and economic burden. Smoking is a significant risk factor for the spread of TB. Smoking tobacco more than doubles the risk of developing TB and is associated with increased resistance towards anti-tubercular treatment (ATT), delaying the bacteriological conversion, high relapse rates, high mortality, and varied treatment outcomes among TB patients. This grave situation calls for novel interventions to control the growing confluence of both epidemics (TB and tobacco) [1]. In addition to the 20% incidence of global TB attributed to tobacco smoking, it accounts for about three million new TB cases. Further, projections demonstrate that between 2010 and 2050, tobacco smoking will contribute to a surplus of 18 million TB infections and 40 million deaths from TB [2].

1.1 Epidemiology of Tuberculosis

TB, or white plague, is the captain of all leading causes of death. Globally amongst all infectious diseases, TB remains a major cause of mortality despite being preventable and curable. In 2019, nearly ten million individuals fell sick from TB worldwide. Males (aged $\geq$ 15 years), females, and children accounted for 56%, 32%, and 12%, respectively [3]. Although there is no age predilection for developing TB, studies show that adults are at an increased risk. The majority of cases (44%) were recorded from Southeast Asia, followed by Africa (25%) and closely after by the Western Pacific (18%).

Furthermore, countries such as India, China, Indonesia, Pakistan, Bangladesh, Nigeria, the Philippines, and South Africa contributed to two-thirds of the overall global burden of TB [3]. Also, TB entraps families, communities, and even entire countries into the cycle of poverty, leading to economic devastation. The risk from TB is further accelerated by multi-drug resistant TB (MDR-TB), an emerging public health crisis in almost every country of the world. Individuals suffering from diabetes, malnutrition, those infected with the human immunodeficiency virus (HIV), or people who use tobacco are at an increased risk for TB. Estimates show that, in the absence of adequate control measures to prevent TB transmission, it will lead to 28 million deaths between 2015 and 2030, costing the global economy almost US $1 trillion. Further, TB is likely to be more detrimental in the subsequent 15 years, when the overall cost of the disease would be $984 billion, and nearly one-third of which would fall on Africa [4].

Global TB incidence is declining at around 2% annually, with a cumulative reduction of 9% reported between 2015 and 2019. Also, around 60 million lives were saved between 2000 and 2019 by medical services for TB diagnosis and treatment [3]. One of the targets for the transitioning to Sustainable Development is to end TB by 2030 [5]. The World Health Organization (WHO), the Stop TB

Partnership, and the Global Fund to Fight AIDS, Tuberculosis, and Malaria have collaborated to launch Find. Treat. All. #ENDTB. It facilitates access to TB prevention and care universally by inviting all countries and partners to contribute to the project by concrete commitments [6].

Under the United Nations' Sustainable Development Goals (SDGs) and WHO's End TB Strategy, the heads of state and national governments have concurred to end the TB epidemic by 2030. They focused on minimizing fatalities by 90% and TB incidence by 80%, with no TB-affected households burdened with catastrophic costs [7]. Furthermore, a policy of the political declaration of the 2016 UN High-Level Meeting on HIV comprises an ATT coverage by 90% and high levels of ATT success rates.

According to the Stop TB Global Plan to End TB, 2016–2020, the primary targets are vulnerable and marginalized groups. The End TB Strategy, the Stop TB Global Plan to End TB, the SDGs, and the "triple billion" target for 2023 will all help to meet the goals for a decline in TB incidence and mortality outlined at the End TB Strategy, the Stop TB Global Plan to End TB, and the "triple billion" target for 2023 of the 13th General Programme of Work (GPW) [8].

1.2 Epidemiology of Tobacco Use

Tobacco consumption is a leading public health issue that results in more than eight million deaths per year. Nearly seven million of these deaths are caused by direct tobacco consumption, whereas 1.2 million are caused by second-hand smoke exposure (SHS) among non-smoking individuals. The majority of the globe's 1.3 billion tobacco users (over 80%) reside in low-and middle-income countries (LMICs) that have the largest burden of tobacco-related diseases and deaths. Tobacco use is also a substantial risk factor for many illnesses, including cardiovascular and respiratory diseases, more than 20 different forms of cancer, and many other grave medical conditions [9]. Between 2005 and 2015, the relative ranking of the smoking-attributable disease burden increased from third to second. Further, there were 148.6 million smoking-attributable DALYs worldwide in 2015 [10]. Besides that, the use of smokeless tobacco (SLT) resulted in the loss of 1.7 million DALYs due to mouth, pharynx, and esophagus cancers. South-East Asia carries about 85% of this burden [11]. 10.9 million DALYs are due to second-hand smoke exposure, and 61% of these DALYs were among children [12]. Along with health expenditures and productivity losses, the overall economic cost of smoking tobacco is calculated at around US $1.4 trillion annually, accounting for nearly 1.8% of the global annual gross domestic product (GDP). Furthermore, approximately 40% of this economic burden is borne by developing economies, underlining the massive burden suffered by these countries [13, 14].

Globally, cigarette smoking is perhaps the most popular type of tobacco consumption. Bidis, cigarettes, kreteks, waterpipe smoking, cigarillos, rolling your own tobacco, and other smokeless tobacco products (SLT), for example, snus, snuff, betel quid with tobacco, chewable tobacco, etc. are the other ways in which tobacco

consumption occurs. Exposure to SHS correlates with an increased risk of sudden infant death syndrome (SIDS), complications among pregnant females, and low-birth-weight (LBW) babies [15].

Water pipe tobacco smoking impacts health similar to that of cigarette smoking. It increases the risk of infections, cancers, lung diseases, and other health conditions [16]. However, the health risks of the use of waterpipe tobacco are also poorly understood by users [15]. Also, the use of SLT is extremely addictive and harmful to the consumer's health. SLT constitutes many toxins that cause cancer. Its use raises the risk of head, neck, throat, esophageal cancers, oral cavity (including the mouth, tongue, lip, and gum) cancer, and different dental disorders [17].

The Electronic Nicotine Delivery Systems (ENDS) and Electronic Non-Nicotine Delivery Systems (ENNDS), known as e-cigarettes, do not include tobacco. However, they mostly include nicotine, which may lead to severe pulmonary toxicity [18], damage airway epithelium, and result in small airway constriction [19]. The Heated Tobacco Products (HTPs) do have tobacco and subject its users to lethal emissions, most of which are cancer-causing and detrimental to health [15].

There has been a decrease in total global tobacco consumption during the last two decades, from 1.397 billion in 2000 to 1.337 billion in 2018 [20]. Despite such progress, however, advancement remains behind in achieving the global objective of decreasing tobacco use by 30% by 2025. A 23% decrease will be achieved by 2025 based on ongoing progress. Only 32 nations are currently on their way to meeting the 30% reduction goal [20]. There should be 32 million fewer female tobacco users by 2025. The majority of the achievements have lied in LMICs. The European region has been the slowest to decrease tobacco usage among women. However, the trend is toward reaching a similar degree as observed by around 25% by 2025 in the Western Pacific and European areas. The greatest burden of tobacco consumption exists in WHO's South-East Asian Region (SEAR). However, the trend appears to be towards achieving a degree similar to that observed in Western Pacific and European regions of around 25% by 2025 [20].

2 Interaction Between Tuberculosis and Tobacco

People using tobacco are at an increased risk of developing TB and recurrent TB, along with impairing the response to treatment of the disease. Tobacco use can mask TB-related symptoms, therefore, delaying the diagnosis. Besides, recent studies have shown that SHS raises the risk of contracting the infection, especially in children [21]. Tobacco smoking and TB together induce apoptosis of alveolar macrophages. While cigarette smoke activates these cells to generate a local inflammatory response, nicotine suppresses the antigen presentation and thereby the effective immune response.

Furthermore, chronic exposure to tobacco smoke causes reduced expression of surface antigens. Tobacco smoking undermines the integrity of the respiratory epithelium by increasing its permeability and disrupting mucociliary clearance.

Also, tobacco smoke can increase alveolar macrophage cells and epithelial cells, contributing to pro-inflammatory and immunosuppressive effects. It further activates them to generate reactive oxygen species (ROS) and proteolytic enzymes, thereby creating a cellular mechanism linking inflammation and tissue damage to tobacco smoking. Also, tobacco smoking is known to recapitulate the *Mycobacterium tuberculosis (M. tb)* infection mechanism, wherein the infected macrophages cannot migrate towards other macrophages infected with *M. tb*, which are a major component of the TB granuloma. It further leads to secondary necrosis, resulting in granuloma breakdown and enhanced mycobacterial growth [22–24].

3 Joint Global Policies for Tackling Tuberculosis and Tobacco

Since 2005, the WHO has been considering and exploring collaborative activities to formulate a common approach for both TB as well as tobacco control. The International Union Against Tuberculosis and Lung Disease and the WHO partnered on a collaborative effort as a first step. A systematic review and meta-analysis were conducted to examine the possible relationship between tobacco smoking and TB outcomes. This meta-analysis reported that tobacco smoking significantly impacts TB susceptibility, progression, and ATT outcomes [25].

In 2007, the WHO-Union monograph on TB and tobacco advocated a routine examination of tobacco consumption and tobacco cessation programs for TB patients. Its goal was to improve treatment outcomes by implementing a component of the Stop-TB Strategy called the Practical Approach to Lung Health (PAL). This document encouraged primary HCPs in TB control programs to assess tobacco users as well as provide behavioral and pharmacological interventions for quitting smoking, including nicotine replacement therapy (NRT), non-nicotine medication, referring tobacco smokers to specialists for intensive tobacco cessation treatment (when required and where doable), and providing primary healthcare services in smoke-free settings. This monograph also provided directions to program heads of the national TB control and tobacco control programs to design and execute collaborative activities for tobacco control through the existing framework of the healthcare system and evolving TB control strategies. Further, it also emphasized creating opportunities in the existing healthcare system to offer help to every TB patient who smokes to quit tobacco smoking. It also asserted that every non-smoker TB patient should be sensitized about the harmful impact of SHS [26].

After that, in 2008 and subsequently, in 2010, The Union released guidelines on "Smoking Cessation and Smoke-free Environments for Tuberculosis Patients." These guidelines highlighted the relationship between TB and tobacco smoking. Also, it guided the HCPs to help their TB patients quit tobacco use. It presented the ABC for TB: "Ask, Brief advice, Cessation support" intervention, which premised that TB services could be used to assist their patients in quitting tobacco smoking and the promotion of smoke-free homes for TB patients as well as their families [27].

The World Health Assembly passed a resolution in 2013 about the End TB Strategy that focused on integrated, patient-centered treatment and prevention. It has provided a timely forum for aligning efforts at the same time against two worldwide epidemics, tobacco and TB. The strategy aimed at achieving health under the United Nations SDG Goal 3, calling for an end of TB. Not only is it useful for national TB programs (NTPs) and counterparts in health ministries, but this strategy can aid all stakeholders in TB prevention and treatment [7].

The board of the Global Fund to Fight AIDS, Tuberculosis, and Malaria adopted a financial plan of action on co-infections and co-morbidities (COIMs) in 2015, suggesting that tobacco control could be included in TB and HIV grants [28]. In addition, the Global Plan to End Tuberculosis 2016–2020 highlighted the need to manage co-morbidities as part of integrated, patient-centered care and prevention [29, 30]. "Capability, Opportunity, and Motivation as determinants of behavior" (COM-B) is a concept used by South East Asia, TB and Tobacco Consortium to create a training package for LMICs HCPs to assist their TB patients with quitting tobacco use in routine TB care [31]. Further, the 2017–2021 Regional Response Plan for the integration of TB and tobacco in South East Asia reiterates its member states' commitment to screening tobacco users for TB and providing cost-effective cessation services by TB control programs already available [32].

The secretariat of the Framework Convention on Tobacco Control (FCTC) has collaborated with the United Nations Development Program (UNDP) to incorporate tobacco cessation efforts into funds from the Global Fund to Combat AIDS, TB, and Malaria. Parties should seek connections between tobacco control and HIV and TB services, according to the WHO FCTC [33]. The 2030 Agenda for Sustainable Development calls for work on health and development issues to be integrated. TB and AIDS epidemics must be eradicated by 2030, according to Target 3.3. In order to achieve the 2030 agenda for SDGs, it is necessary to address these co-morbidities through integrated solutions rather than disease-specific responses [34] (Table 1).

4 Challenges to Implementation of Global Policies

Though various policies against TB and tobacco exist, there are certain challenges for implementing the same. These challenges broadly fall into three levels: patient, HCP, and system. In the following section, we will briefly discuss each of these levels (Graphical Abstract).

4.1 Patient-Level

Multiple challenges at the patient level impact outcomes of TB-tobacco integration. These include hesitancy in admitting tobacco use habits (especially females due to religious and socio-cultural leverage), gender sensitivity on the part of patient and HCP/interventionist, nicotine dependency, cravings, withdrawal symptoms, will

Table 1 Few examples of evidence-based integration of tuberculosis and tobacco across the globe and lessons learned

Country	Year	Type of study	Intervention	Outcomes	Conclusion
WHO—South East Asia (SEA) Region					
Indonesia [35]	2012	Cohort study	During each visit at DOTS services, the TB patients were offered five to ten minutes of ABC (Ask, brief advice, cessation support) intervention	In a cohort of registered 750 TB patients, 582 (77.6%) were current smokers, and at the sixth month, 66.8% of these current smokers had quit smoking	The ABC intervention support to TB patients for five to ten minutes during each visit led to higher quit rates and greater awareness about the detrimental impact of secondhand smoke exposure on health
India [36]	2010	Intervention study	During routine interaction for treatment, the TB patients who were tobacco users were given brief cessation advice based on five A's	Among the TB patients undergoing treatment, 46.3% were current tobacco users. Of these patients who were given brief advice, 67.3% has stopped using tobacco, 18.2% had relapsed, while 14.5% were lost to follow-up at the end of the anti-TB medication regimen	The study highlighted the feasibility of introducing a brief advice strategy for tobacco cessation amongst patients with TB, emphasizing close supervision
India [37]	2013	Cluster, randomized controlled trial	The healthcare worker delivered ABC intervention (Ask, brief advice, and cessation support) to TB patients who were tobacco smokers at the time of registration and during his/her sputum reexamination visits	The smoking cessation was reported to be 80.2% in the intervention arm while 57.5% in the comparison arm	The trial suggested that smoking cessation intervention among TB patients effectively promotes smoking cessation and should be recommended as a part of the national TB control program
Bangladesh [27]	2011–2012	Cohort study	The registered TB patients who smoked were given brief advice to quit (five to ten minutes), how to make their homes smoke-free (based upon The Union's ABC guideline), and the removal of smoking aids	615 (20%) were reported to be current tobacco smokers out of 3134 registered TB patients. 82% of these tobacco smokers had quit, and the study highlighted that tobacco smokers with higher nicotine dependence were less likely to quit smoking than those who had lesser dependence on nicotine	The study findings suggested that such a cessation intervention could be useful in helping TB patients quit smoking, especially in resource constraint settings

(continued)

Table 1 (continued)

Country	Year	Type of study	Intervention	Outcomes	Conclusion
WHO—Europe Region					
Armenia [38]	–	Project	A project for integrating tobacco control measures into tuberculosis care was undertaken by the National Institute of Health and Armenia National Tuberculosis Control Center to establish a nationwide healthcare professional partnership between both programs, capacity building for enforcement of smoke-free environment at all facilities, building smoking cessation capacity among TB healthcare providers [38]	–	–
South Africa [39]	Study protocol, 2018	Randomized Controlled trial	The proposed trial will be carried out in three provinces of South Africa consisting of a complex behavioral intervention which includes a motivational interviewing counseling approach, given by lay health workers, along with SMS emphasizing on improving TB outcomes along with the assessment of smoking cessation as secondary outcomes	–	–
WHO—Western Pacific Region					
Malaysia [40]	2008–2009	Quasi-experimental	The TB patients who were current smokers were assigned to either integrated intervention (11 sessions of individualized cognitive NRT) or conventional TB DOTS alone based on the transtheoretical model approach	77.5% of the patients who received the integrated intervention had quit smoking compared to 8.7% of the patients who had only received conventional TB treatment ($p < 0.001$)	The study concluded that an integrated cessation strategy for those who give up tobacco smoking provides beneficial short-term results and potential lung health for TB patients

(continued)

Table 1 (continued)

Country	Year	Type of study	Intervention	Outcomes	Conclusion
China [41]	2010	Prospective study	The health care providers were trained to deliver an integrated tobacco smoking cessation intervention within standard TB services. The TB patients who were current smokers have imparted information on the ill effects of tobacco smoke and smoking	Two hundred forty-four were current tobacco smokers out of enrolled 800 TB patients. Of these, 234 (95.9%) were ready to quit Among these, 156 (66.7%) had reported being abstinent from smoking at the end of the sixth month	The study suggested scaling up the integrated intervention nationwide as most TB patients who were current smokers were willing to quit
China [42]	2017	Prospective longitudinal study	The trained health workers imparted current tobacco smokers (willing to quit) among TB patients with general information on the consequences of tobacco use and specific advice on smoking tobacco and TB. This was reiterated at every visit during the entire TB treatment course	A total of 650 (81.2%) TB patients were tracked among 800 patients registered at baseline. The smoking cessation rates after 5 years were 82.0% (non-smokers), 63.0% (ex-smokers), 49.6% (received smoking cessation intervention), 43.5% (recently quit), and 30.0% (did not receive smoking cessation intervention)	The study reported that the non-smoking rates were higher among those who received cessation intervention at baseline compared to those with those who did not receive the intervention
The Americas—WHO Region					
Brazil [43]	2008 and 2009	Pilot feasibility study	DOTS providers were trained to deliver tobacco cessation counseling. These providers also completed training assessments (pre and post) besides participation in post-program focus group discussions. After three months of completing the program, the TB patients who were tobacco smokers were interviewed	The mean self-efficacy scores of DOTS providers for tobacco cessation counseling enhanced significantly (2–3 before training to 3–4 after training). However, there was no improvement in the provider's knowledge about tobacco cessation (withdrawal, nicotine replacement therapy, pre-contemplation) after training	The findings of the pilot study highlighted the feasibility of DOTS providers in Brazil implementing cessation intervention at TB clinics. Further, the study suggested conducting randomized controlled trials to test the effectiveness of the intervention

power, social environments and determinants, and non-compliance of patients to scheduled counseling sessions or follow-ups and referrals [44]. Further, disadvantaged communities such as refugees, prisoners, injecting drug users, and sex workers face stigma and have challenges using the formal health system facilities [45]. Inadequate information about tobacco smoking as a risk factor for getting tuberculosis, poor prognosis, myths and misconceptions, conventional care-seeking habits, physical and psychological addiction, and low self-efficacy compound the difficulties. There may still be a considerable digital-use divide in current times, reflecting a gap in digital media literacy and digital engagement. Therefore, interventions developed on digital platforms such as mobile health (mHealth) may not help reach the targeted population [46].

4.2 Healthcare Provider Level

The HCPs face many challenges. Heavy workloads, insufficient time per patient to offer behavioral support causing additional time burdens, administrative activities, shifts between colleagues, staff re-organization, high patient numbers, lack of appreciation for their achievements, patients who do not return for follow-up, and lack of privacy are recognized [44]. Furthermore, there is concern about the shortage of adequately skilled and motivated health professionals, the lack of willingness, and the inability of HCPs to provide comprehensive services. Also, lack of information about the interplay between TB and tobacco, inadequate understanding of tobacco cessation, poor communication skills, and reservations about competency requirements accentuate the problem [35].

4.3 Health System Level

Health system capabilities and its preparedness to operationalize integrated services in developing nations pose a challenge. Key examples are inaccurate assessment and documentation of each person's smoking habits as part of routine practice [36], lack of adequate space and human resources within health facilities, privacy issues, failure to provide sufficient technical resources and equipment to support integrated front-line facilities, inappropriately delegated responsibility and decision-making system that allows front-line management and workers to acclimate integration to native conditions. Further, significant obstacles in the successful implementation of integrated services include:

- lack of overarching policies to create standards, guidelines, and capacity building programs;
- sluggish permeation of evidence-based transnational TB and tobacco policy initiatives;
- insufficient stewardship and political engagement;

- a lack of preparedness and assets for initiating national policy measures and evidence-based guidelines;
- subprime coordination between different stakeholders operating in TB and tobacco control programs [47];
- inadequate involvement of civil society organizations (CSOs) and community-based organizations (CBOs) engagement in integrated TB-tobacco activities [37]; and
- lack of specific operational guidance on linking and treatment protocols for HCPs for the proper management of patients with co-morbidities [48].

5 Strategies to Overcome Challenges

Various evidence-based strategies have evolved over a while to overcome the challenges mentioned above. The discussion of these strategies shall also be under the patient, HCP, and system levels (Fig. 1).

5.1 Patient-Level Strategies

Every clinical encounter is an opportunity starting from the point of diagnosis of TB. It is the first teaching opportunity that can be targeted and channeled for offering cessation support if the TB patient reports being a tobacco smoker [44]. The role of religious beliefs in promoting tobacco cessation attempts among patients needs to be harnessed in the integrated TB-tobacco model to break the stigma associated with disease and tobacco use. SMS-based cessation messages for TB patients embedded into the broader education and health promotion messages for TB patients can address the local cultural context as well [46]. Incorporating smoking cessation services in TB treatment facilities frequently visited by specific sub-populations such as refugees, sex workers, prisoners, and injecting drug users who may face stigma and have issues using mainstream health facilities will offer greater coverage for vulnerable populations. Such joint initiatives may create a precedent for other tobacco cessation interventions in specific groups, such as people with substance use disorders (SUD), those who have a serious mental illness (SMI), and people living with HIV/AIDS (PLWHA) [49]. Also, smoke-free homes could promote abstinence by minimizing smoking cues, making it easier for TB patients to avoid smoking [40].

5.2 Healthcare Provider Level Strategies

To deal with long-term implementation and sustainability and keep the HCPs motivated, it is essential to continue their capacity building to carry out effective patient communication to deliver newer cessation treatment algorithms and

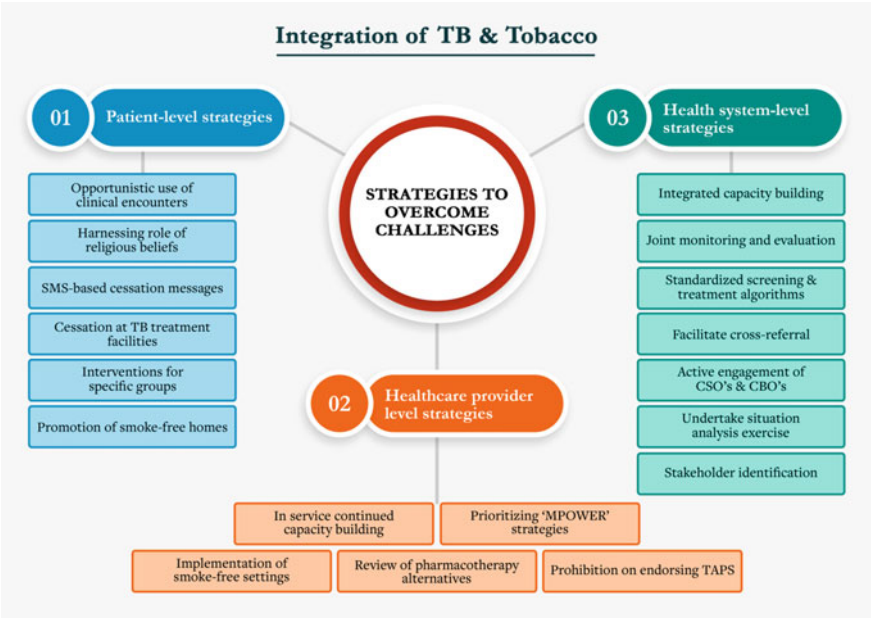


Fig. 1 Integration of care for tuberculosis (TB) and tobacco: strategies to overcome challenges

learning. Their capacity needs to counter social influences on smoking behavior, manage workloads and time, and overcome potential biases (e.g., gender and social desirability) and barriers (socio-cultural) [44]. HCPs in TB can be engaged in interventions by prioritizing 'MPOW' ("Monitor tobacco use, Protect people from tobacco smoke, Offer help to quit tobacco use, Warn about the dangers of tobacco") for individual practitioners and 'E' ("Enforce bans on tobacco advertising, promotion, and sponsorship") for program and organization managers.

Implementation of smoke-free settings must be part of the regular preventive tasks of TB personnel to protect patients, staff, and visitors. Also, smoke-free homes can promote abstinence by minimizing cues to smoking, enabling patients with TB to stop smoking. Assistance and counseling must address the requirements of patients with TB, while physicians must review the pharmacotherapy alternatives to make sure conformity with the ATT regimen. Enforcing institutions and program managers may enact their respective mandates prohibiting the organization and employees from conducting or endorsing tobacco promotions or sponsorships [40].

5.3 Health System-Level Strategies

Integrated capacity-building programs on collaborative initiatives of TB and tobacco control could be established, with reciprocating components in training curricula and resources combined, enabling the capacity building of HCPs of both

programs. Further monitoring and evaluation of both programs can also be integrated. Also, standardized screening and treatment algorithms with implementation guidelines are necessary to facilitate cross-referral at TB-cessation clinics. Active engagement of civil society organizations and CBOs could help garner local expertise and support through their working relationships for implementing cessation interventions at TB clinics [44]. Undertaking a situation analysis exercise could aid in mapping and prioritizing critical areas of primary concerns in TB-tobacco prevention and treatment [45]. Furthermore, the identification of stakeholders in the activities of TB and tobacco help forge synergies, make efficient use of resources, and avoid duplication of effort, especially in the context of resource constraint settings.

6 Recommendations

6.1 Establishment of the Joint Working Group at All Levels

Each country needs to establish a coordination mechanism from national to grass root level between the technical advisory bodies and implementers of TB control and tobacco control programs. A collaborative working forum with both technical advisory bodies must be set up to establish strategic and operational guidance for collective preparation, capacity building, monitoring, and evaluation to develop an organizational framework to ensure the long-term sustainability of such collaborative initiatives (Fig. 2).

6.2 Modification of Reporting Mechanism

The reporting mechanism could also be updated to include indicators for tracking tobacco control activities (e.g., tobacco users amongst TB patients, users provided with advice/pharmacological treatment, users remaining quit after treatment). At the same time, the tobacco control program could promote improved TB case finding among tobacco users.

6.3 Integration into Health and Development Agendas

The "Health-in-All-Policies" strategy should be used, with activities focusing on social determinants, such as tobacco control, which raises the likelihood of obtaining TB. The integrative model might be supported by the incremental adaptation and implementation of evidence-based transnational TB and tobacco policy initiatives, as well as astute leadership and political support, and optimum convergence amongst diverse stakeholders engaged in TB and tobacco control initiatives could support the integrative model [50].

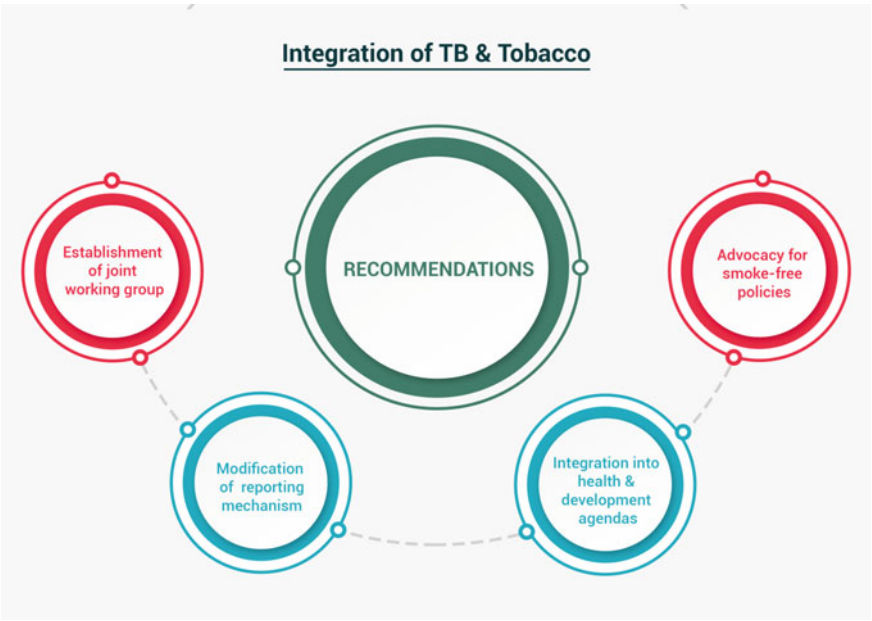


Fig. 2 Integration of care for tuberculosis (TB) and tobacco: recommendations

6.4 Advocacy for Smoke-Free Policies

TB and tobacco control programs must actively advocate for the introduction of smoke-free policies at all healthcare institutions offering TB care services as well as in homes where TB patients reside. NGOs operating in the domain of TB control and tobacco control could collaborate to promote the execution of collaborative measures along with undertaking tracking, analysis of joint activities, and research. With a view to a global coordinated effort to regulate tobacco, this is an opportune time for nations to participate in combating these two formidable epidemics [51].

7 Conclusion

The intersecting epidemiology of TB and tobacco use offers not just a convincing cause for intervention but a chance to resolve a significant cause of preventable mortality and ensure that the advantages of anti-TB prevention and treatment are understandable to TB patients [48]. Integration may also provide a more regular response point at various levels of the current health system. Effective incorporation of tobacco control interventions into TB treatment would provide a prototype framework in which additional risk factors (e.g., opioid abuse) and diseases (e.g., hypertension, diabetes, chronic obstructive pulmonary disease) could be treated to

improve patients' overall health. Collaborative work among TB and tobacco control initiatives will include advantages such as resource sharing, increased outreach to disadvantaged communities, and the potential for rational linkages within health systems that would make patient-centered prevention and management more comprehensive. The guiding frameworks for treating TB should provide guidelines for the treatment of tobacco smoking and all different forms of tobacco being used. It should transcend the conventional domain of promoting tobacco cessation and instead include broader, vitally important overarching aspects of MPOWER. In a resource constraint setting, linking and utilizing existing programs through a synergistic approach can reduce the burden attributable to both epidemics paving the way to achieve SDGs.

Tuberculosis and tobacco are old foes of humanity, supplementing and complementing each other yet giving us opportunity nodes to break the vicious cycle.

Sonu Goel and Garima Bhatt

Core Messages

- A large body of evidence highlights the intersecting epidemiology of tuberculosis and tobacco use.
- The collaborative international initiatives have proposed various models and recommendations to combat the dual burden.
- Multiple challenges at various levels must be addressed to implement these collaborative policies effectively.
- Establishing a collaborative working forum for strategic-operational guidance could support the integrated framework.

References

1. Prasad R, Suryakant Garg R, Singhal S, Dawar R, Agarwal G (2009) A case-control study of tobacco smoking and tuberculosis in India. Ann Thorac Med 2009 4(4):208–10
2. Basu S, Stuckler D, Bitton A, Glantz SA (2011) Projected effects of tobacco smoking on worldwide tuberculosis control: mathematical modelling analysis. BMJ [Internet] 2011 343 (7826):1–21. Available from: /pmc/articles/PMC3186817/?report=abstract
3. World Health Organization. Global Tuberculosis Report 2020 [Internet]. 2020 Available from: https://www.who.int/publications/i/item/global-tuberculosis-report-2020
4. Burki TK (2018) The global cost of tuberculosis. Lancet Respir Med [Internet] 2018. 6(1):13. Available from: http://www.thelancet.com/article/S221326001730468X/fulltext
5. Tuberculosis [Internet]. Available from: https://www.who.int/news-room/fact-sheets/detail/tuberculosis
6. WHO|World Health Organization [Internet]. Available from: https://www.who.int/tb/joint-initiative/en/
7. WHO|The End TB Strategy. WHO [Internet] 2020. Available from: http://www.who.int/tb/strategy/en/

8. WHO|World Health Organization. WHO [Internet] 2018. Available from: http://www.who.int/tb/joint-initiative/en/
9. World Health Organization (WHO). Tobacco [Internet]. Available from: https://www.who.int/health-topics/tobacco#tab=tab_1
10. GBD 2015 Tobacco Collaborators MB, Fullman N, Ng M, Salama JS, Abajobir A, Abate KH et al. (2017) Smoking prevalence and attributable disease burden in 195 countries and territories, 1990–2015: a systematic analysis from the Global Burden of Disease Study 2015. Lancet (London, England) [Internet] 2017 389(10082):1885–906. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28390697
11. Siddiqi K, Shah S, Abbas SM, Vidyasagaran A, Jawad M, Dogar O et al. Global burden of disease due to smokeless tobacco consumption in adults: analysis of data from 113 countries. BMC Med [Internet] 2015 13(1):194. Available from: http://bmcmedicine.biomedcentral.com/articles/https://doi.org/10.1186/s12916-015-0424-2
12. Öberg M, Jaakkola MS, Woodward A, Peruga A, Prüss-Ustün A (2011) Worldwide burden of disease from exposure to second-hand smoke: a retrospective analysis of data from 192 countries. Lancet 377(9760):139–146
13. Tobacco [Internet]. Available from: https://www.who.int/health-topics/tobacco#tab=tab_2
14. Tobacco [Internet]. Available from: https://www.who.int/news-room/fact-sheets/detail/tobacco
15. World Health Organization. Tobacco [Internet]. Available from: https://www.who.int/news-room/fact-sheets/detail/tobacco
16. Qasim H, Alarabi AB, Alzoubi KH, Karim ZA, Alshbool FZ, Khasawneh FT (2019) The effects of hookah/waterpipe smoking on general health and the cardiovascular system [Internet]. Environ Health Prev Med 2019 24(1):1–17. Available from: https://doi.org/10.1186/s12199-019-0811-y
17. WHO EMRO|Page 11 [Internet]. Available from: http://www.emro.who.int/content/Page-44.html
18. Chun LF, Moazed F, Calfee CS, Matthay MA, Gotts JE (2017) Pulmonary toxicity of e-cigarettes [Internet]. Am J Physiol-Lung Cell Mol Physiol 2017 313(2):L193–206. Available from: /pmc/articles/PMC5582932/?report=abstract
19. Unger M, Unger DW (2018) E-cigarettes/electronic nicotine delivery systems: a word of caution on health and new product development [Internet]. J Thorac Dis 2018 10(Suppl 22):S2588–92. Available from: /pmc/articles/PMC6178300/?report=abstract
20. WHO global report on trends in prevalence of tobacco use 2000–2025, third edition [Internet]. Available from: https://www.who.int/publications/i/item/who-global-report-on-trends-in-prevalence-of-tobacco-use-2000-2025-third-edition
21. World Health Organization. Geneva S. Tuberculosis & Tobacco. . 2009.
22. Magdalena Hutahaean L (2013) Effects of smoking habit on the development of tuberculosis disease. IOSR J Nurs Heal Sci [Internet] 2013 2(5):24–29. Available from: www.iosrjournals.org
23. Berg RD, Levitte S, O'Sullivan MP, O'Leary SM, Cambier CJ, Cameron J et al. Lysosomal Disorders Drive Susceptibility to Tuberculosis by Compromising Macrophage Migration. Cell [Internet] 2016 165(1):139–52. Available from: https://pubmed.ncbi.nlm.nih.gov/27015311/
24. Bothamley GH (2005) Smoking and tuberculosis: a chance or causal association? [Internet]. Thorax 2005 60(7):527–528. Available from: www.thoraxjnl.com
25. Brands A, Ottmani SE, Lönnroth K, Blanc LJ, Rahman K, Bettchera DW et al. (2007) Reply to Addressing smoking cessation in tuberculosis control [Internet]. Bull World Health Organ 2007 85(8):647–8. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2636394/
26. WHO/The Union. A WHO/The Union monograph on TB and tobacco control: joining efforts to control two related global epidemics 2007. Available from: https://www.who.int/tobacco/resources/publications/tb_tobac_monograph.pdf
27. Bissell K, Fraser T, Chen-Yuan C, Enarson DA Smoking cessation and smokefree environments for tuberculosis patients second edition 2010. Int Union Against Tuberc Lung Dis

28. Geneva. Thirty-Third Board Meeting Global Fund support for co-infections and co-morbidities GF/B33/11 Board Decision [Internet]. Available from: http://www.theglobalfund.org/Knowledge/Decisions/GF/B32/DP07/
29. The end TB STraTegy global strategy and targets for tuberculosis prevention, care and control after 2015 a. 2014
30. Global Plan to End TB: 2018–2022 GS. The Paradigm Shift 2018–2022. 2019
31. Warsi S, Elsey H, Boeckmann M, Noor M, Khan A, Barua D et al. (2019) Using behaviour change theory to train health workers on tobacco cessation support for tuberculosis patients: a mixed-methods study in Bangladesh, Nepal and Pakistan. BMC Health Serv Res [Internet] 2019 19(1):71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30683087
32. World Health Organization S-EA. South-East Asia Regional Response Plan for Integration of TB and Tobacco 2017–2021 [Internet] 2017. Available from: http://apps.searo.who.int/PDS_DOCS/B5371.pdf
33. United Nations Development Programme (UNDP) Integrating tobacco control into tuberculosis and HIV responses|UNDP [Internet]. Available from: https://www.undp.org/content/undp/en/home/librarypage/hiv-aids/integrating-tobacco-control-into-tuberculosis-and-hiv-responses.html
34. The WHO framework convention on tobacco control an accelerator for sustainable development|UNDP [Internet]. Available from: https://www.undp.org/content/undp/en/home/librarypage/hiv-aids/-the-who-framework-convention-on-tobacco-control-an-accelerator-.html
35. Bam TS, Aditama TY, Chiang CY, Rubaeah R, Suhaemi A (2015) Smoking cessation and smokefree environments for tuberculosis patients in Indonesia-a cohort study. BMC Public Health [Internet] 2015 15(1):604–604 [cited 2020 Aug 11]. Available from: https://europepmc.org/articles/PMC4488952
36. Kaur J, Sachdeva K, Modi B, Jain D, Chauhan L, Dave P et al. (2013) Promoting tobacco cessation by integrating 'brief advice' in tuberculosis control programme. WHO South-East Asia J Public Heal [Internet] 2013 2(1):28 [cited 2020 Aug 11]. Available from: http://www.who-seajph.org/article.asp?issn=2224-3151;year=2013;volume=2;issue=1;spage=28;epage=33;aulast=Kaur
37. Goel S, Kathiresan J, Singh P, Singh R (2017) Effect of a brief smoking cessation intervention on adult tobacco smokers with pulmonary tuberculosis: a cluster randomized controlled trial from North India. Indian J Public Health [Internet] 2017 61(5):47 [cited 2019 Aug 29]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28928319
38. Integrating evidence-based tobacco control services into tuberculosis control in Armenia| Pfizer [Internet] [cited 2020 Aug 2]. Available from: https://www.pfizer.com/node/469826
39. Moriarty AS, Louwagie GM, Mdege ND, Morojele N, Tumbo J, Omole OB et al. (2019) ImPROving TB outcomes by modifying LIFE-style behaviours through a brief motivational intervention followed by short text messages (ProLife): study protocol for a randomised controlled trial. Trials [Internet] 2019 20(1) [cited 2020 Aug 2]. Available from: https://pubmed.ncbi.nlm.nih.gov/31349850/
40. Awaisu A, Haniki Nik Mohamed M, Noordin N, Muttalif A, Aziz N, Syed Sulaiman S et al. (2012) Impact of connecting tuberculosis directly observed therapy short-course with smoking cessation on health-related quality of life. Tob Induc Dis [Internet] 2012 10(1):2 [cited 2020 Aug 2]. Available from: /pmc/articles/PMC3305504/?report=abstract
41. Lin Y, Wang L-X, Qiu L-X, Huang Q, Shu Q, Lin H-X et al. (2015) A smoking cessation intervention among tuberculosis patients in rural China. Public Heal Action [Internet] 2015 5 (3):183–187 [cited 2020 Aug 2]. Available from: https://pubmed.ncbi.nlm.nih.gov/26399289/
42. Lin Y, Dlodlo RA, Shu Q, Lin H, Huang Q, Meng X, Zeng X, Chen Y, Xiao L (2019) Outcomes of a smoking cessation intervention at follow-up after 5 years among tuberculosis patients in China. Tob Induc Dis [Internet] 2019 17:69. Available from: https://doi.org/10.18332/tid/111539

43. Feasibility study of a smoking cessation intervention in directly observed therapy short-course tuberculosis treatment clinics in Rio de Janeiro, Brazil-PubMed [Internet] [cited 2020 Aug 2]. Available from: https://pubmed.ncbi.nlm.nih.gov/23370189/
44. Boeckmann M, Warsi S, Noor M, Dogar O, Mustagfira EH, Firoze F et al. (2019) Health worker and patient views on implementation of smoking cessation in routine tuberculosis care. NPJ Prim Care Respir Med [Internet] 2019 29(1):1–12. Available from: https://doi.org/10.1038/s41533-019-0146-6
45. ENGAGE-TB [Internet] 2015. Available from: www.who.int
46. A handbook on how to implement mTB-Tobacco [Internet]. Available from: https://www.who.int/publications/i/item/a-handbook-on-how-to-implement-mtb-tobacco
47. Abera M, Tesfaye M, Belachew T, Hanlon C (2014) Perceived challenges and opportunities arising from integration of mental health into primary care: a cross-sectional survey of primary health care workers in south-west Ethiopia. BMC Health Serv Res 2014 14
48. Jackson-Morris A, Fujiwara PI, Pevzner E (2015) Clearing the smoke around the TB-HIV syndemic: smoking as a critical issue for TB and HIV treatment and care. Int J Tuberc Lung Dis 19(9):1003–1006
49. Castaldelli-Maia JM, Harutyunyan A, Herbec A, Kessel T, Odukoya O, Kemper KE et al. (2020) Tobacco dependence treatment for special populations: challenges and opportunities. Brazilian J Psychiatry [Internet] 2020 (AHEAD). Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-44462020005015205&lng=en&nrm=iso&tlng=en
50. Goel S, Verma M, Singh R, Bhardwaj A (2018) Integrating tobacco and tuberculosis control programs in India: a win–win situation. Int J Noncommunicable Dis [Internet] 2018 3(5):9 [cited 2019 Aug 29]. Available from: http://www.ijncd.org/text.asp?2018/3/5/9/247254
51. Hyder MKA, Tripathy JP, Kaur J, Mandal PP, Sharma R, Kumar AMV et al. (2018) Tuberculosis-tobacco integration in the South-East Asia Region: Policy analysis and implementation framework. Int J Tuberc Lung Dis [Internet] 2018 22(7):807–812 [cited 2020 Aug 2]. Available from: https://pubmed.ncbi.nlm.nih.gov/29914607/

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Relationship Between Pre-existing Cancer and Tuberculosis

5

Yaşar Barış Turgut, Alican Tahta, and Özgür Tanrıverdi

Science is the most reliable guide for civilization, for life, for success in the world. Searching a guide other than the science is meaning carelessness, ignorance and heresy.

Mustafa Kemal Ataturk

Summary

Tuberculosis (TB) is one of the most serious infectious diseases in the world. Of those latently infected (LTBI), 5–10% will develop an active infection at some point in their lifespan. Screening strategies and management of LTBI have recently been areas of interest to control the global TB epidemic. Having a hematologic malignancy or head and neck cancer, having an immunocompro-

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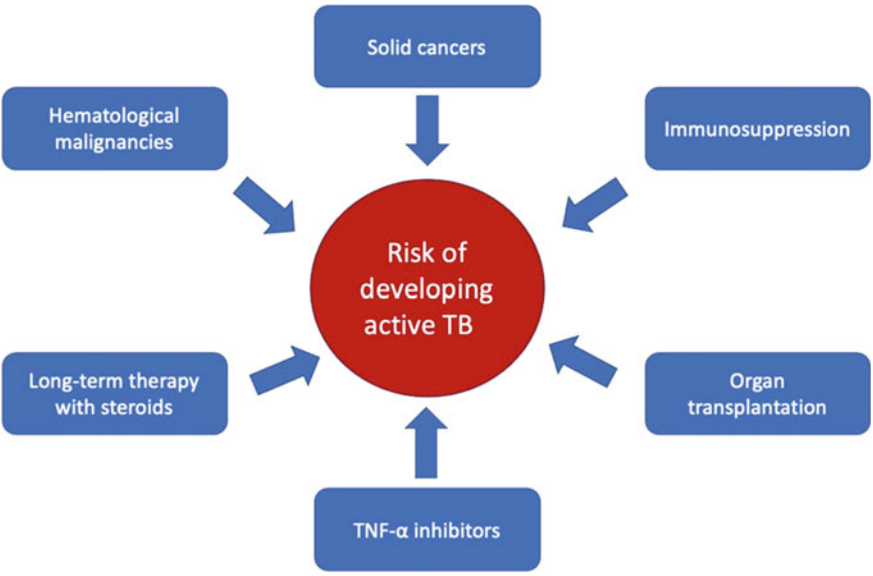
mising condition, undergoing organ transplantation, and long-term treatment with corticosteroids and tumor necrosis factor-alpha (TNF-α) inhibitors are risk factors for the development of active TB. The incidence rate of TB decreases from the time of diagnosis of cancer. Patients with hematological malignancies are more likely to develop TB than those with solid cancers. It is due to having a weakened immune system and treatment with chemotherapeutic agents used for managing the hematological malignancies. Despite advanced radiotherapy options, patients with solid head and neck malignancies are still at an increased risk of developing TB than the general population. Similarly, pediatric cancer patients remain at a higher risk of getting TB than their adult counterparts. Altogether, TB in cancer is an important issue that might contribute to mortality, both directly and indirectly.

Graphical Abstract



Risk factors for developing active tuberculosis (TB)

Keywords

Cancer · Infection · Management · Mortality · Tuberculosis

1 Introduction

Tuberculosis (TB) is one of the major infectious diseases in the world [1]. One-quarter of the world's population has been affected by *Mycobacterium tuberculosis (M. tb)*. Ten million people have active TB infection, and more than one million people die from TB each year [1]. TB is clinically asymptomatic and microbiologically inactive (latent) in most infected people. Of those latently infected (LTBI), 5–10% will develop an active infection at some point in their lifespan [2]. Having a hematologic malignancy or head and neck cancer, having an immunocompromising condition, undergoing organ transplantation, and long-term treatment with corticosteroids and tumor necrosis factor-alpha (TNF-α) inhibitors are risk factors for the development of active TB (Graphical Abstract) [3, 4]. Both screening strategies and management of LTBI have recently been areas of interest to control the global TB epidemic [5]. Also, the World Health Organization (WHO) advised the initiation of screening strategies of high-risk patients in countries having upper-middle- or high-income economies with a TB incidence of < 100 per 100,000 person-years [6]. On the other hand, cancer incidence is increasing steadily. In addition, with the advances in treatment methods, the life span of cancer patients is prolonged [7, 8]. For these reasons, the number of people with malignancies increases, and coping with complications in this population becomes important. Infections remain a leading cause of death and disability in people with cancer worldwide [9]. The focus of this chapter are TB and cancer.

2 Incidence and Risk of Tuberculosis and Cancer

An increasing trend in the incidence of TB has been shown in cancer patients [10, 11]. Lung cancer, gastric cancer, thyroid cancer, hepatocellular carcinoma, breast cancer, colon cancer, pancreatic cancer, and hematological malignancies are frequently associated with TB. Shu et al. found the crude incidence of TB was higher in cancer patients with involvement of respiratory tract (about 900 per 100,000 person-years) than in patients with hematological malignancies (about 500 per 100,000 person-years) [12]. A meta-analysis by Dobler et al. estimated an overall incidence rate ratio (IRR) of TB among adult cancer patients as 2.61 [13]. In particular, it was 3.53 for hematological malignancies and 2.25 for solid cancers [13]. Among solid tumors, the IRR of TB was 6.14 for lung cancer, 2.17 for breast cancer, 2.02 for liver cancer, and 2.00 for colon cancer [13]. For gastric cancer, a highly morbid type of cancer for which the treatment modality of choice is gastrectomy associated with malnutrition [14], the relative risk of TB was not significantly different from other solid neoplasms. The IRR of TB in pediatrics with hematological malignancies or solid neoplasms was also 16.82 [13, 15].

In a large population-based study in South Korea, the SIR (standardized incidence ratio) of TB was 2.22 in cancer patients compared with the overall population [16]. When comparing the SIR of different malignancies, hematological

malignancies have the highest ratio, followed by gastric cancer, pancreatic adenocarcinoma, liver carcinoma, and breast cancer [16]. Notably, pancreatic cancer had the highest SIR (3.17). They presumed that this high SIR value is, in fact, a reflection of the higher probability of advanced or metastatic disease in association with pancreatic cancer compared to other types of cancers [16]. Furthermore, the low SIR value (1.52) of cancer of the thyroid gland was a matter of fact that most patients (99.2%) were early-stage and had low recurrence rates [16, 17].

TB incidence decreases from the time of diagnosis of malignancy [16]. The relative risk of TB was at its highest following the diagnosis of malignancy. Then, it began to decline with a SIR of 3.70 for the first six months; 2.19 for months six–11; 1.75 for months 12–23; and 1.43 after 24 months from diagnosis [16]. The SIRs of TB for patients with cancer of the thyroid gland and the biliary tract are not different in six months after a cancer diagnosis. However, the SIR values for hematological malignancy, gastric cancer, pancreatic adenocarcinoma, and liver carcinoma were still more than one, even two years following diagnosis of cancer [16]. The risk of TB in patients with malignancy was higher than the overall population, and the risk depended on the type of malignancy. Also, the SIR remained high even two years following diagnosis of malignancy in an intermediate TB-incidence country [16]. The high number of TB diagnoses in patients with cancer might lie in the lower immunity these patients have as a result of cancer and cancer treatment, as well as the fact that they receive more frequent examinations and close monitoring than people without cancer [16]. Patients with a history of cancer treatment within the past two years should be included in the high-risk group, like patients with other medical problems, including chronic renal disease and diabetes mellitus [12, 16, 18].

The reported prevalence of TB in cancer patients with hematological malignancies, including chronic lymphocytic leukemia (CLL), Hodgkin's disease, and non-Hodgkin's lymphoma, differs between 1 and 10% in previous studies [19–22]. Different clinical characteristics of the patient, diagnostic approach strategy, and the prevalence of TB in the countries where the studies were performed might account for this heterogeneity [23]. A meta-analytical review reported that the CIR of TB in patients with hematologic malignancies was highest in countries with high-TB incidence (6873/100,000 population) but lower in countries with intermediate- (2686/100,000 population) and low-TB incidence (418/100,000 population) [24]. According to Silva et al. the prevalence of TB in patients with hematological malignancies was only 2.6%, while it was 6.9% in patients with CLL [23]. In previous reports, Hodgkin's disease was closely related to TB [19]; however, in Silva et al.' study, no such correlation existed [23].

Silva et al. found malnourishment and treatment with steroids–which are known to cause impairment of T-cell immunity–as major risk factors for TB [23]. Moreover, studies show that fludarabine and alemtuzumab impair T-cell immunity and cause infections due to T-cell immunodeficiency [25, 26]. Fludarabine is an agent that inhibits the cytokine-induced activation of STAT1 (an important transcription factor in cell-mediated immunity) [27]. The types of infections seen in patients have changed with the addition of fludarabine in CLL treatment. The incidence of diseases associated with T-cell immunodeficiency, such as TB, has increased [28, 29].

Although allogeneic hematopoietic stem cell transplantation (HSCT) has been found to enhance the risk of TB about three times compared to the general population, it has been shown that autologous HSCT does not increase TB risk in a study involving more than 8000 patients with HSCT [30]. Also, no relationship was found between TB and HSCT in a study by Silva et al. in which the majority of HSCT was autologous [23].

3 Mortality of Tuberculosis Associated with Cancer

Mortality directly or indirectly related to TB is an important issue in patients with malignancy. Mortality is a factor of epidemiological interest; the prevention strategies are mainly based on years of lost, and years of lost life is a major factor in determining the cost-effectiveness of the strategy [12]. Shu et al. found the incidence of mortality directly related to TB was 0.83% in the population with cancer; this incidence was 0.28% in the general population, according to the national cause-of-death database [12, 31]. Also, the overall mortality rate during six months after TB diagnosis in patients with cancer was 15.56%, which is higher than the rate during six to 12 months after TB diagnosis (5.0%) [12, 31]. The mortality rate of TB-positive cancer patients was 20.56%, which is higher than that of TB-negative cancer patients (11.84%) at 12 months. They assumed a correlation between TB infection and worse outcomes in cancer patients [12]. Lung cancer is also associated with increased mortality in TB [32, 33]. Shu et al. found the highest mortality rates in TB-positive patients with hematological malignancies, malignancies of the respiratory tract, and head and neck cancers [12]. However, TB-positive female genital and breast cancer patients had lower mortality rates. So, mortality rates in TB-positive cancer patients were associated with not only TB but also cancer types. For hematologic malignancies with TB, the mortality rate was higher than that in solid cancers, with rates between 23 and 90% [10, 23].

4 LTBI Screening in Patients with Cancer

Both solid tumors and hematologic malignancies are immunocompromising conditions. So, on their own and as a result of the associated chemotherapy, they have been shown to increase the chance of TB reactivation by increasing the risk of cancer recurrence [13]. TB reactivation risk has changed over time because of changes in cancer treatment modalities and, therefore, in the extent to which the immune system is impaired. New treatment modalities, including purine analogs, monoclonal antibody therapies, and HSCT, alone or in combination, are more immunosuppressive than old therapies present for half a century. Also, local tissue damage caused by radiotherapy in solid head and neck malignancies has reduced with the technological development of certain radiation therapies [34, 35]. In

addition, the average lifespan of cancer patients is longer than before due to the progress of current drugs [36]. So, the incidence of TB has been recently increased in cancer patients with various malignancies [13, 24, 37].

Patients with cancer, therefore, require LTBI screening as well as treatment. However, there is insufficient evidence to support the use of LTBI screening for these individuals. According to the British National Institute for Health and Care Excellence (NICE), individuals with hematological malignancies, those who have undergone chemotherapy, and those who have undergone a gastrectomy are all at risk of acquiring TB. However, they do not advise screening and management for TB in these patients [38]. Joined guidelines of the American Thoracic Society (ATS) and the Centers for Disease Control and Prevention (CDC) advise treatment of LTBI in patients with leukemia, lymphoma, and certain malignancies (lung, head, or neck cancer) [3]. These guidelines are based on independent studies conducted between the 1950s and 1970s. These studies looked at the cumulative incidence of TB rather than the incidence of TB among cancer patients. Moreover, they had some methodological issues regarding relative risk calculations [19, 39].

Following the initial TB infection, reactivation can occur [2]. Therefore, the estimated cumulative lifetime risk for the development of TB is important to advise screening and treatment of LTBI. There is an increased lifelong risk of TB in chronic diseases, including diabetes mellitus and chronic renal disease, without affecting the overall life expectancy. However, in malignancies, immunosuppressive states are temporary in most cases during chemotherapy [13], and life expectancy might be markedly reduced, therefore lowering the cumulative lifetime risk of developing TB. Based on the findings of a meta-analysis conducted by Dobler et al. there is no need for LTBI screening and management in adult patients with malignancy [13]. As outlined in the WHO guidelines, the effectiveness of LTBI treatment in terms of harms and benefits should be considered on an individual patient basis [6]. This entity's context, accessibility, cost, and implementation are important aspects of screening and treatment for LTBI [6].

Children with cancers have a high risk for the development of TB. They have to be evaluated for screening and treatment of LTBI. In this context, two studies conducted in South Africa suggest an active approach for pediatric patients with malignancy, including:

- screening for TB on the admission, including a detailed history, tuberculin skin test (TST), and physical examination;
- clinical, radiological, and TST screening during immunosuppressive therapy and long-term follow-up; and
- prophylactic management for all exposed to a patient with TB and for positive-TST children in endemic regions [15].

5 Conclusion

Patients with malignancy are at an increased risk of contracting TB than patients without malignancy. Moreover, patients with hematological malignancies are more likely to develop TB than patients with solid cancers. Immunodeficiency and chemotherapeutic agents used in managing hematological malignancies are the causes for this situation. Despite advanced radiotherapy options, patients with solid head and neck malignancies are still at an increased risk of developing TB than the general population. Similarly, pediatric cancer patients remain at a higher risk of getting TB than their adult counterparts. According to recent studies, screening for LTBI is recommended in children with cancer.

Core Messages

- The number of complications in people with malignancies is increasing due to their prolonged life span.
- Cancers, especially hematological ones, are associated with an increased risk of TB.
- There is a correlation between TB and worse outcomes in cancer patients.
- Context, accessibility, cost, and implementation are important in managing LTBI in cancer patients.

References

1. WHO|Global tuberculosis report 2019. In: WHO. http://www.who.int/tb/publications/global_report/en/. Accessed 10 May 2020
2. Bass JB, Farer LS, Hopewell PC et al (1990) Diagnostic standards and classification of tuberculosis. Am Rev Respir Dis 142:725–735
3. Cohn DL, O'Brien RJ, Geiter LJ et al (2000) Targeted tuberculin testing and treatment of latent tuberculosis infection. MMWR Morb Mortal Wkly Rep 49:1–54
4. Menzies D (2014) Canadian tuberculosis standards. 7th Editio. Center for communicable disease and infection control, Public Health Agency of Canada
5. Lönnroth K, Migliori GB, Abubakar I et al (2015) Towards tuberculosis elimination: an action framework for low-incidence countries. Eur Respir J 45:928–952
6. Getahun H, Matteelli A, Abubakar I et al (2015) Management of latent mycobacterium tuberculosis infection: WHO guidelines for low tuberculosis burden countries. Eur Respir J 46:1563–1576
7. Ahmedin Jemal DA, Tiwari RC, Murray T et al (2004) Cancer statistics 2004. CA Cancer J Clin 54:8–29
8. Jung K-W, Won Y-J, Kong H-J et al (2015) Cancer statistics in Korea: Incidence, mortality, survival, and prevalence in 2012. Cancer Res Treat Official J Korean Cancer Assoc 47:127
9. Safdar A, Armstrong D (2001) Infectious morbidity in critically ill patients with cancer. Crit Care Clin 17:531–570

10. De La Rosa GR, Jacobson KL, Rolston KV et al (2004) Mycobacterium tuberculosis at a comprehensive cancer centre: active disease in patients with underlying malignancy during 1990–2000. Clin Microbiol Infect 10:749–752
11. Kamboj M, Sepkowitz KA (2006) The risk of tuberculosis in patients with cancer. Clin Infect Dis 42:1592–1595
12. Shu C-C, Liao K-M, Chen Y-C et al (2019) The burdens of tuberculosis on patients with malignancy: incidence, mortality and relapse. Sci Rep 9:1–7
13. Dobler CC, Cheung K, Nguyen J, Martin A (2017) Risk of tuberculosis in patients with solid cancers and haematological malignancies: a systematic review and meta-analysis. Euro Respir J 50
14. Schölmerich J (2004) Postgastrectomy syndromes-diagnosis and treatment. Best Pract Res Clin Gastroenterol 18:917–933
15. Stefan DC, Kruis AL, Schaaf HS, Wessels G (2008) Tuberculosis in oncology patients. Ann Trop Paediatr 28:111–116
16. Seo GH, Kim MJ, Seo S et al. (2016) Cancer-specific incidence rates of tuberculosis: a 5-year nationwide population-based study in a country with an intermediate tuberculosis burden. Medicine 95
17. Cho BY, Choi HS, Park YJ et al (2013) Changes in the clinicopathological characteristics and outcomes of thyroid cancer in Korea over the past four decades. Thyroid 23:797–804
18. Shen T-C, Lin C-L, Wei C-C et al (2014) Increased risk of tuberculosis in patients with type 1 diabetes mellitus: results from a population-based cohort study in Taiwan. Medicine (Baltimore) 93:e96. https://doi.org/10.1097/MD.0000000000000096
19. Kaplan MH, Armstrong D, Rosen P (1974) Tuberculosis complicating neoplastic disease. A review of 201 cases. Cancer 33:850–858. https://doi.org/10.1002/1097-0142(197403)33:3%3c850::aid-cncr2820330334%3e3.0.co;2-h
20. Karachunskiĭ MA, Pivnik AV, Iuldasheva NE (2002) Tuberculosis in patients with hemoblastoses. Probl Tuberk 24–27
21. Karnak D, Kayacan O, Beder S (2002) Reactivation of pulmonary tuberculosis in malignancy. Tumori 88:251–254
22. Srivastava VM, Krishnaswami H, Srivastava A et al (1996) Infections in haematological malignancies: an autopsy study of 72 cases. Trans R Soc Trop Med Hyg 90:406–408. https://doi.org/10.1016/s0035-9203(96)90524-6
23. Silva FA, Matos JO, de Mello QFC, Nucci M (2005) Risk factors for and attributable mortality from tuberculosis in patients with hematologic malignances. Haematologica 90:1110–1115
24. Cheng MP, Chakra CNA, Yansouni CP et al (2017) Risk of active tuberculosis in patients with cancer: a systematic review and meta-analysis. Clin Infect Dis 64:635–644. https://doi.org/10.1093/cid/ciw838
25. Morrison VA, Rai KR, Peterson BL et al (2001) Impact of therapy with chlorambucil, fludarabine, or fludarabine plus chlorambucil on infections in patients with chronic lymphocytic leukemia: Intergroup Study Cancer and Leukemia Group B 9011. J Clin Oncol 19:3611–3621. https://doi.org/10.1200/JCO.2001.19.16.3611
26. Abad S, Gyan E, Moachon L et al (2003) Tuberculosis due to mycobacterium bovis after alemtuzumab administration. Clin Infect Dis 37:e27-28. https://doi.org/10.1086/375690
27. Frank DA, Mahajan S, Ritz J (1999) Fludarabine-induced immunosuppression is associated with inhibition of STAT1 signaling. Nat Med 5:444–447. https://doi.org/10.1038/7445
28. Sanders C, Perez EA, Lawrence HJ (1992) Opportunistic infections in patients with chronic lymphocytic leukemia following treatment with fludarabine. Am J Hematol 39:314–315. https://doi.org/10.1002/ajh.2830390418
29. Ghosh K, Sivakumaran M, Murphy P, Chapman CS (1995) Pulmonary tuberculosis after fludarabine for chronic lymphocytic leukaemia. Natl Med J India 8:294–295

30. de la Cámara R, Martino R, Granados E et al (2000) Tuberculosis after hematopoietic stem cell transplantation: incidence, clinical characteristics and outcome. Spanish Group on Infectious complications in hematopoietic transplantation. Bone Marrow Transplant 26:291–298. https://doi.org/10.1038/sj.bmt.1702506
31. Bloss E, Chan P-C, Cheng N-W et al (2012) Increasing directly observed therapy related to improved tuberculosis treatment outcomes in Taiwan. Int J Tuberc Lung Dis 16:462–467. https://doi.org/10.5588/ijtld.11.0121
32. Hong S, Mok Y, Jeon C et al (2016) Tuberculosis, smoking and risk for lung cancer incidence and mortality. Int J Cancer 139:2447–2455. https://doi.org/10.1002/ijc.30384
33. Shieh S-H, Probst JC, Sung F-C et al (2012) Decreased survival among lung cancer patients with co-morbid tuberculosis and diabetes. BMC Cancer 12:174. https://doi.org/10.1186/1471-2407-12-174
34. Morrison VA (2014) Immunosuppression associated with novel chemotherapy agents and monoclonal antibodies. Clin Infect Dis 59(Suppl 5):S360-364. https://doi.org/10.1093/cid/ciu592
35. Cognetti DM, Weber RS, Lai SY (2008) Head and neck cancer: an evolving treatment paradigm. Cancer 113:1911–1932. https://doi.org/10.1002/cncr.23654
36. Harris BN, Pipkorn P, Nguyen KNB et al (2019) Association of adjuvant radiation therapy with survival in patients with advanced cutaneous squamous cell carcinoma of the head and neck. JAMA Otolaryngol Head Neck Surg 145:153–158. https://doi.org/10.1001/jamaoto.2018.3650
37. Kuo S-C, Hu Y-W, Liu C-J et al (2013) Association between tuberculosis infections and non-pulmonary malignancies: a nationwide population-based study. Br J Cancer 109:229–234. https://doi.org/10.1038/bjc.2013.220
38. Recommendations|Tuberculosis|Guidance|NICE. https://www.nice.org.uk/guidance/ng33/chapter/Recommendations#latent-tb. Accessed 10 May 2020
39. Feld R, Bodey GP, Gröschel D (1976) Mycobacteriosis in patients with malignant disease. Arch Intern Med 136:67–70

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Laboratory Diagnosis of Tuberculosis

6

Sagar Mali, Anushka V. Devnikar, and Arvind Natarajan

A dread disease in which the struggle between soul and body is so gradual, quiet and solemn and the result so sure that day by day and grain by grain, the mortal part wastes and withers away. A disease… which sometimes moves in giant strides and sometimes at a tardy sluggish pace, but, slow or quick, is ever sure and certain…

Charles Dickens

Summary

The diagnosis of tuberculosis (TB) is multifaceted. It requires an integrated approach that extends to all facets resulting in early diagnosis of TB. In the era of evidence-based medicine, there is an absolute necessity of correctly detecting and promptly treating every case of TB. In this chapter, an attempt has been

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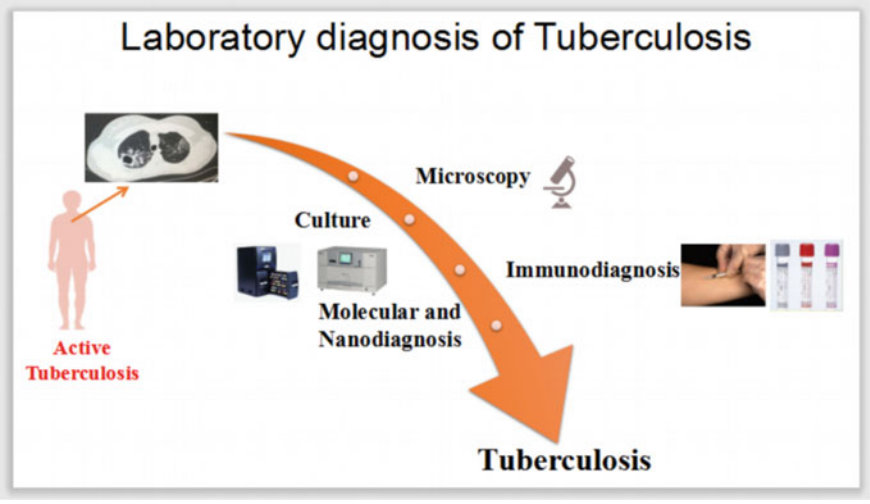
made to describe the advantages and disadvantages of present diagnostic modalities and discuss a few future diagnostic modalities and how their potential has the power to change the entire diagnostic approach of the present day. The technological breakthroughs will increase the accuracy of newer innovations, and large-scale production will make them economical and affordable diagnostic modalities. Considering the increased awareness regarding TB in the general population, an integrated approach by the World Health Organization (WHO), governments, communities, and private sectors, as well as behavioral change like the use of face masks worldwide due to coronavirus pandemic, we are optimistic about reaching the target set by 'The END TB strategy' by 2035.

Graphical Abstract



Tuberculosis diagnosis

Keywords

Biosensor · Culture media · Diagnosis · Immunodiagnosis · Microscopy · Molecular methods · Nanodiagnosis · Nanoparticles · Tuberculosis

1 Introduction

Tuberculosis (TB) is preventable as well as curable. But it has claimed more lives than any other disease (more than HIV/AIDS). The burden of TB is an issue of concern in developing countries. Different aspects of diagnosis related to latent TB infection (LTBI), drug-resistant TB (DR-TB), extrapulmonary TB (EPTB),

HIV-associated TB, and pediatric TB are all challenging and continue to put clinicians and microbiologists in a state of dilemma. These cases may be missed at all points of care. This fact warrants us to systematically fill the gaps which exist from the time of detection of TB till its cure. Early and accurate diagnosis of TB plays a major role in determining the outcome of the disease.

"Find. Treat. All. #END TB" is a joint initiative by the World Health Organization (WHO), the Stop TB Partnership, and the Global Fund. The target is to treat 40 million people with TB between 2018 and 2022. To reach this target, a disparity between estimated incident cases and the number of new cases reported needs to be addressed. This mismatch is due to underreporting and underdiagnosis of TB cases. The majority of disparity is with India (25%), Nigeria (12%), Indonesia (10%), and the Philippines (8%). Efforts should improve the reporting, diagnosis, and treatment of TB. Strict majors and achievable targets backed up by technological breakthroughs will help end the global epidemic of TB by 2035 [1].

2 Laboratory Diagnosis

The conventional diagnostic tools like the culture on solid media, species identification by biochemical tests, and drug susceptibility test on solid media are all time-consuming. These tools have good sensitivity and specificity but fail to provide early results. In recent times, a shift away from traditional practices towards fluorescent microscopy, liquid cultures, and molecular techniques, which provide early results, has been noticed. These newer diagnostic modalities have similar or higher sensitivity and specificity than conventional tools. Before discussing these methods, it is imperative to know about laboratory safety and specimen collection.

2.1 Laboratory Safety

All clinical specimens should be handled carefully to avoid any risk of infection to laboratory personnel. Liquefaction and the concentration of sputum sample can be safely performed on an open bench. Any aerosol-generating procedure needs biological safety cabinet (BSC) class 1 or 2. Handling of *Mycobacterium tuberculosis (M. tb)* or *M. bovis* cultures requires bio-safety level (BSL) 3 cabinets. Laboratory personnel working in the laminar flow BSC or BSL 3 cabinet should wear complete personal protective equipment and respirator [2].

2.2 Specimen Collection

To ensure quality results, a sample reception unit should accept good quality samples in a labeled specimen container. Collection, labeling, transporting, and registering specimens for microscopy, culture, and molecular technique should be as per the respective national TB program.

2.2.1 Sputum

Around three to five milliliters (ml) of well-coughed, non-salivary sputum specimen is considered good quality. Sputum specimens should be strictly collected in ventilated sputum collection rooms or should be collected in an isolated, well-ventilated open area. Sputum samples can be obtained by expectoration or inducing (ultrasonic nebulization). Early morning samples contain the highest concentration of mycobacteria.

It is advisable to collect early morning sputum or urine on three consecutive days for pulmonary TB (PTB) or renal TB patients, respectively, given mycobacteria's intermittent and irregular release. It increases the sensitivity of diagnostic tests. Twenty-four hours of sputum or urine sample collection should be discouraged because of the risk of specimen dilution and overgrowth of contaminant bacteria and fungi. In pediatric PTB cases, fasting, early morning gastric aspirate (five to ten ml) on three consecutive days is recommended [3].

2.2.2 Blood

The use of blood culture for *M. tb* became a common modality after the HIV pandemic. Initially, mycobacteremia was thought to be due to TB-HIV co-infection, but various reports suggested that mycobacteremia (though in low frequency) has been observed in HIV-negative patients as well [4, 5]. Hence blood culture is a necessary tool, especially in the diagnosis of disseminated TB. The yield of *M. tb* from blood can be increased by using a lysis-centrifugation blood culture system or semi-automated liquid culture systems (discussed later in this chapter). Single blood culture for routine diagnostic purposes and two blood culture sets for suspected disseminated TB infection are recommended.

2.2.3 Other Specimens

Specimen collected aseptically that is normally sterile, like CSF, synovial fluid, pleural fluid, etc., can be directly processed bypassing the decontamination step. Biopsy specimen or fine-needle aspiration (FNA) material should be immersed in 7H9 or 7H11 broth and homogenized to make aliquots of suspension. The aliquots are then subjected to different tests. Swabs should not be accepted for culture; if received in the laboratory, the swab tip should be directly placed in 7H9 broth or on solid culture media and incubated for six to eight weeks.

Stool sample (only if AFB positive by smear microscopy) should be processed similar to sputum sample. The isolation of MAC from stool samples is predictive of disseminated disease in HIV infection; hence a stool sample must be processed whenever required [6].

2.3 Digestion and Decontamination

Sputa, stool samples, and other contaminated specimens need digestion decontamination procedure for concentrating mycobacterial cells. The decontamination process inhibits undesirable bacterial overgrowth and digestion of specimen

liquefies mucus, releasing mycobacteria. The standard digestion-decontamination procedure by CDC requires N-acetyl-L-cysteine and 2% NaOH [7]. Other agents used for decontamination and concentration of specimen are:

- Dithiothreitol + 2% NaOH;
- 13% Trisodium phosphate + Benzalkonium chloride;
- 4% NaOH;
- 13% Trisodium phosphate;
- 5% Oxalic acid; and
- 1% Cetylpyridium chloride + 2% NaOH.

After the decontamination-digestion procedure for sputum and liquid specimens (i.e., CSF, synovial fluid, pleural fluid, etc.), centrifugation at a relative centrifugal force of 3000 g for 15 min is optimal to increase recovery of mycobacteria. Sediment formed after centrifugation is divided into aliquots and subjected to different diagnostic tests.

2.4 Microscopy

Mycobacteria have a thick, waxy capsule with high lipid content, which provides them unique property called acid-fastness. The cell wall is rich in mycolic acid, which binds to fuchsin dye and is not destained by acid-alcohol. Hence the staining is called acid-fast staining, and the mycobacteria are called acid-fast bacilli (AFB). For routine microscopy to detect AFB, at least 10,000 AFB per 1 ml sample are required. Smear microscopy of clinical samples is a very useful test in the management of TB because it helps in diagnosis, monitoring progress, and defining cure, acts as an indicator of infectiousness, is feasible in the remote/tribal area, and more importantly, it is inexpensive, reproducible, and reliable [8, 9].

Commonly used stains are:

1. Fluorochrome stain—Auramine O ± Rhodamine
2. Carbolfuchsin stain—
   a. Ziehl Neelsen (hot stain)
   b. Kinyoun (cold stain)

The advantage of fluorochrome staining is that stained smears can be scanned with 25× objectives, reducing the time required to scan each slide. Fluorochrome-stained bacilli glow bright–yellow/orange-red against a dark background, making them readily detectable under low magnification without affecting sensitivity. Fluorochrome stained smear can also be subjected to subsequent staining with carbol fuchsin stain. It has 10% more sensitivity than ZN staining [10]. A halogen or mercury vapor lamp used for fluorescent microscopy has been replaced by light-emitting diodes (LEDs). It costs less than 10% of a mercury vapor lamp and has a life of > 50,000 h. Because it can run on batteries, it has a definite operational advantage in peripheral areas/remote areas [11].

AFB appears bright red following carbol fuchsin stain and depending upon the counterstain used, the background is either blue or green. The reporting of stained smear is based on the number of AFB. Among carbol fuchsin stains, ZN staining is more sensitive than Kinyoun cold staining for AFB detection. ZN staining like fluorochrome staining cannot differentiate between *M. tb* and NTM. It has a low, variable sensitivity ranging between 0 and 40% [12–14].

Smear microscopy is practical, readily available, and has a high predictive value, as well as it is the most rapidly performed test in the diagnosis of TB. The better correlation between positive smear microscopy and positive cultures has made smear microscopy a reliable index of mycobacterial infection [15, 16].

2.5 Culture

For a definitive diagnosis of TB, isolation of *M. tb* from the clinical specimen by culture is considered the gold standard. It requires ten to 100 bacilli per ml of concentrated specimen for culture to confirm the presence of *M. tb* in a clinical specimen. The culture of *M. tb* also provides isolates for determining phenotypic drug susceptibility testing (DST) and species identification. It has a variable sensitivity ranging from 0 to 80% [17–20]. Culture media can be broadly divided into solid and liquid culture media, as shown in Fig. 1.

2.5.1 Solid Culture Media

Non-selective Media

In the nineteenth century, growing mycobacteria on agar culture media was very poor compared to egg-based media. Even isolating mycobacteria on egg-based media was rendered difficult by liquefaction of media due to proteolytic enzymes released by contaminating bacteria. To overcome these difficulties, aniline dyes were added to culture media. Incorporating aniline dyes (malachite green/crystal violate) in varying concentrations has improved mycobacterial recovery by inhibiting the growth of contaminating bacteria in culture (Table 1). Depending on constituents, culture media can be further divided into:

1. Egg-based media
   The most commonly used media is the LJ medium. Other examples are Petragnani medium and the American Thoracic Society (ATS) medium.
2. Agar based media
   These media contain defined salts, organic chemicals, and albumin. Examples are Middlebrook culture 7H10 and 7H11 agar media [21].

Both 7H10 and 7H11 have high contamination rates because of the low quantity of incorporated malachite green. Despite this limitation, using either of these agar media helps in early *M. tb* detection. An experienced microbiologist can

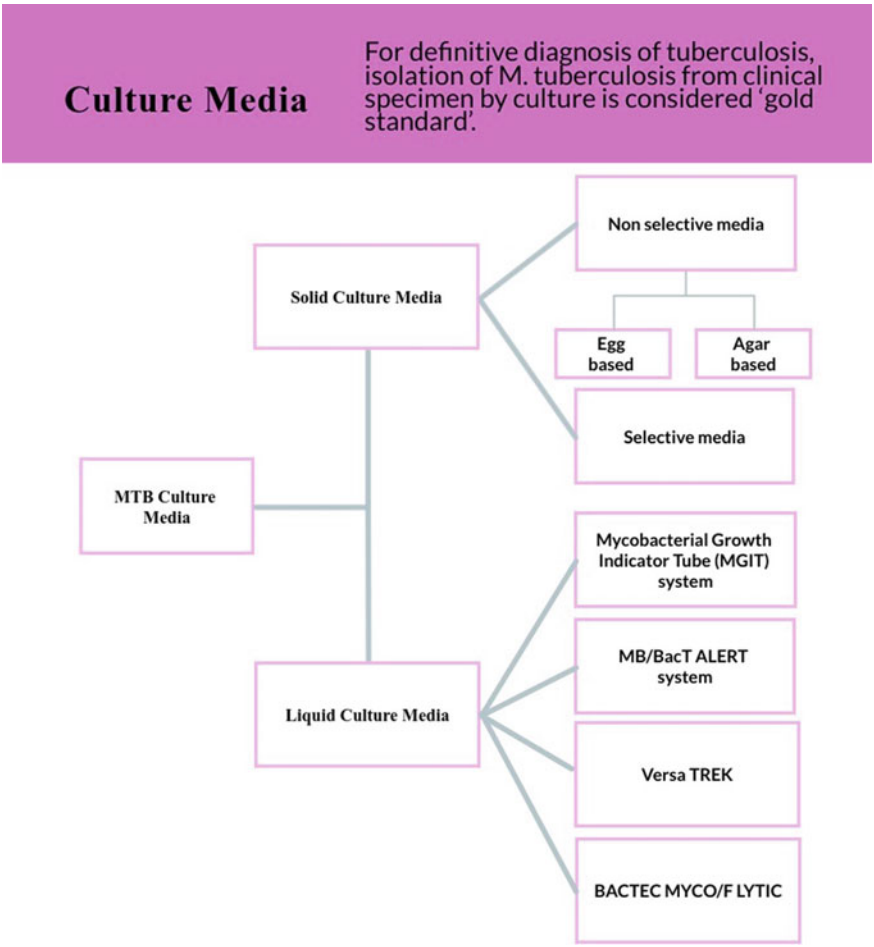


Fig. 1 Broad classification of culture media

Table 1 Non-selective media

Medium	Components	Inhibitory agent
Lowenstein Jensen	Coagulated whole eggs, defined salts, glycerol, potato flour	Malachite green, 0.0025 g/100 ml
Petragnani	Coagulated whole eggs, egg yolks, whole milk, potato, glycerol, potato flour	Malachite green, 0.052 g/100 ml
American Thoracic Society	Coagulated fresh egg yolks, glycerol, potato flour	Malachite green, 0.02 g/100 ml
Middlebrook 7H10	Defined salts, vitamins, cofactors, oleic acid, albumin, catalase, dextrose, glycerol	Malachite green, 0.025 g/100 ml
Middlebrook 7H11	Defined salts, vitamins, cofactors, oleic acid, albumin, catalase, glycerol, 0.1% casein hydrolysate	Malachite green, 0.0025 g/100 ml

presumptively identify *M. tb* within ten days of incubation by examining micro-colonies and observing certain morphological features [22].

The presence of capneic incubation (5–10% CO_2) ensures better growth and larger colonies of mycobacteria on Middlebrook culture media. In contrast, exposure to strong light and storage at 4 °C for > four weeks has a deteriorating effect on mycobacteria due to the formation of formaldehyde [23].

Selective Media

The incorporation of antimicrobial agents inhibits the growth of bacterial and fungal contaminants, thus making media more selective for mycobacterial recovery. Gruft [24], Petran [25], Mitchison [26], and McClatchy [27] suggested modification in non-selective media to make them selective for mycobacterial culture, some of which are listed in Table 2.

2.5.2 Liquid Culture System

Liquid culture or broth system is a type of selective media that is more sensitive than solid media. There are few liquid culture systems built on different principles for mycobacteria's growth and early detection. BACTEC TB 460 (Becton Dickinson, Sparks, MD, USA) was the first semi-automated, radiometric liquid culture system. In this system, mycobacterial growth was detected by quantitatively measuring radioactive $^{14}CO_2$ released by the metabolism of ^{14}C labeled substrate present in the BACTEC 12B media [28]. The disadvantages of this system were:

Table 2 Selective media

Medium	Components	Inhibitory agent
Gruft modification of Lowenstein Jensen	Coagulated whole eggs, defined salts, glycerol, potato flour, RNA 5 mg/100 ml	Malachite green, 0.025 g/100 ml Penicillin, 50 U/ml Nalidixic acid, 35 mg/ml
Lowenstein Jensen (Petran modification)	Coagulated whole eggs, defined salts, glycerol, potato flour	Malachite green, 0.025 g/100 ml Cycloheximide, 400 mcg/ml Lincomycin, 2 mcg/ml Nalidixic acid, 35 mcg/ml
Middlebrook 7H10	Defined salts, vitamins, cofactors, oleic acid, albumin, catalase, glucose, glycerol	Malachite green, 0.0025 g/100 ml Cycloheximide, 360 mcg/ml Lincomycin, 2 mcg/ml Nalidixic acid, 20 mcg/ml
Middlebrook 7H11 (Mitchison media)	Defined salts, vitamins, cofactors, oleic acid, albumin, catalase, glycerol, casein hydrolysate	Carbenicillin, 50 mcg/ml Polymyxin, 200 U/ml Trimethoprim lactate, 20 mcg/ml Amphotericin B, 10 mcg/ml

i. cross-contamination due to the use of needles; and
ii. disposal of radioactive material.

This system is no longer in use and is replaced by other liquid culture systems discussed below.

Mycobacterial Growth Indicator Tube (MGIT) System (BD Diagnostic)

In the MGIT system, all specimens except blood and urine can be processed for culture. It uses a modified Middlebrook 7H9 broth base containing OADC (oleic acid, albumin, dextrose, and catalase) and PANTA antibiotic mixture (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, azlocillin). OADC acts as a growth supplement, and the PANTA mixture inhibits the growth of contaminating bacteria. It is a fluorescence-based technique. A round-bottomed glass tube (MGIT) is fitted with a fluorescent compound embedded in silicone at the bottom. In any uninoculated MGIT, dissolved oxygen in the broth quenches any emission of fluorescent light from silicone. Mycobacterial cells (if present in inoculated media) utilize dissolved oxygen and deplete its concentration. This process unmasks the fluorescence from silicone and can be detected manually using long-wave UV light (Wood's lamp). The MGIT can be placed in MGIT 960 or 320 (non-radiometric, automated) systems, for continuous monitoring and detection of the fluorescence. For optimal performance, 0.5 ml of the specimen is inoculated and incubated at 37 °C.

Rodrigues et al. used BACTEC MGIT 960 TB system and LJ media to test 14,597 specimens. Out of 6143 (42%) positive specimens, MGIT 960 TB system was positive in 6015 (41%) specimens, and LJ media grew *M. tb* in 3526 (24%) specimens. The mean detection time for smear-positive specimens was nine days for MGIT 960 and 38 days for LJ media. The time for smear-negative specimens was 16 and 48 days for MGIT 960 and LJ media, respectively [29].

MB/BacT ALERT System

It is similar to the BacT ALERT blood culture system. This system also uses Middlebrook 7H9 broth. It is used to culture mycobacteria from any specimen except blood. A supplementary growth factor and PANTA antibiotic mixture are added to the culture bottle before use to ensure better growth of mycobacteria and curtail the growth of contaminating bacteria. Each bottle has a gas permeable sensor fitted at the bottom, which changes color from dark green to bright yellow due to the increasing concentration of microbial-generated CO_2. These bottles are continuously monitored for color change in MB/BacT ALERT system. MGIT 960 and MB/BacT ALERT system used for *M. tb* culture are superior to solid media [30].

Versa TREK (Thermo Scientific)

It was previously known as EPS Culture System 2. Each culture bottle contains VersaTREKMyco broth, an antibiotic mixture, a growth supplement, and cellulose sponges (simulating the lungs' alveoli). This bottle is attached to a sensor through a needle for continuous monitoring (every 24 min) of any change in pressure within

the bottle's headspace and rate of oxygen consumption due to the metabolic activity of microorganisms. Early studies have shown that the growth and detection of mycobacteria using the ESP MYCO system from all specimens, including blood, is possible with acceptable results [31, 32].

BACTEC MYCO/F LYTIC (Becton Dickinson, Sparks, MD)
It is a blood culture system. It is designed to culture mycobacteria, fungi, and most aerobic bacteria. The bottle contains a lytic agent that helps to release mycobacteria from WBC. The decrease in oxygen concentration due to the metabolic activity of microorganisms is directly proportional to an increase in the fluorescence and is detected by any BACTEC 900 series system [33]. In a study by Crump et al. the performance of the BACTEC 13A (BD Diagnostic), BACTEC MYCO/F LYTIC (BD Diagnostic), BacT/ALERT MB (bioMérieux), and ISOLATOR 10 lysis-centrifugation (Wampole Laboratories) systems was evaluated. There was no significant change in yields between these systems. However, the mean detection time for MAC was shortest for BacT/ALERT MB, followed by BACTEC MYCO/F LYTIC, BACTEC 13A, and ISOLATOR 10 [34].

2.6 Immunodiagnosis

Immunodiagnostic tests were developed to detect any immune response mounted against *M. tb,* as shown in Table 3.

The commercially available serological tests for detecting antibodies against *M. tb* were developed as point-of-care tests. Unfortunately, the use of these tests to diagnose PTB and EPTB has not been recommended by WHO due to highly inconsistent sensitivity and specificity [35, 36]. The false-positive and false-negative results have led to more patient harm than good due to inaccuracy and diagnostic delay causing increased morbidity and mortality [37].

2.6.1 Tuberculin Skin Test (TST)

For LTBI, there is no gold standard test available, and its global burden is not determined with certainty. Since 5–10% of LTBI cases develop active TB, diagnosis and preventive treatment of LTBI is considered a crucial step by the WHO End TB Strategy [38].

Among the tests available to detect cell-mediated response against *M. tb*, TST is the simplest and low-tech test. In this test, an individual suspected of LTBI is injected intradermally with five tuberculin units (TU), i.e., 0.01 ml of purified

Table 3 Classification of immunodiagnostic tests based on immune response

Immune response	Immunodiagnostic tests
Humoral	ELISA Immunochromatographic tests
Cell-mediated (T-cell)	Tuberculin skin test (TST) IFN-γ release assay (IGRA)

protein derivative (PPD) on the volar aspect of the forearm. The test reaction (due to delayed hypersensitivity) is read after 48–72 h as the diameter of induration (not erythema) seen on the volar aspect of the forearm, measured in millimeters. Though it is a low-cost test and easy to perform, its specificity is reduced by Bacille Calmette-Guérin (BCG) vaccination [39, 40].

2.6.2 Interferon-Gamma Release Assays (IGRAs)

M. bovis BCG lacks a 16-gene sequence that codes for culture filtrate protein 10 (CFP-10) and early secretory antigen target-6 (ESAT-6). During TB infection, cellular immune response produces interferon-gamma (IFN-γ) against these two antigens of *M. tb*. This effector/sensitized memory T cell's response is the basis for T-cell-based IGRAs. Two commercially available IGRAs work on the principle that re-stimulating an individual with LTBI using *M. tb*-specific antigens (CFP-10 and ESAT-6) leads to the secretion of IFN-γ. The IFN-γ is then detected using enzyme-linked immunosorbent assay-based tests like QuantiFERON-TB Gold InTube (QFT-GIT, Cellestis, Australia) or QuantiFERON-TB Gold (QFT-G, Cellestis, Australia). Another IGRA that detects IFN-γ producing peripheral mononuclear cells after stimulation with CFP-10 and ESAT-6 is the enzyme-linked immunospot (ELISPOT)-based T-SPOT.TB (Oxford Immunotec, UK) [41].

2.7 Molecular Methods

Among molecular methods, nucleic acid amplification tests (NAATs) were commercially available to detect *M. tb* complex (MTBC) directly in the mid-to-late 1990s. The recommendations by the CDC on the use of NAATs in the diagnosis of TB are:

i. testing suspected TB patients with NAATs should become the standard of practice;
ii. all TB control programs and clinicians should use NAATs for direct MTBC detection;
iii. at least one respiratory specimen should be tested by NAAT, and all NAAT negative specimens should be tested for inhibitors;
iv. the time between specimen collection and NAAT report should be $\leq$ 48 h; and
v. all laboratories performing NAAT for TB diagnosis should participate in a proficiency testing program.

Early test results of molecular methods used for *M. tb* detection have certain advantages:

i. the positive result helps in:
- early diagnosis and prompt treatment of TB, leading to a decreased period of infectiousness;

- earlier notification to TB public health authorities and also ensuring earlier infection control measures;
- decreased person-to-person transmission; and
- better outcome.

ii. the negative result helps to avoid unnecessary contact tracing and investigations; and
iii. the results can provide substantial savings for the patients, healthcare providers, hospitals, and the public health program.

They have limitations, e.g.,

i. relatively lower sensitivity than culture;
ii. possibility of false-positive results (due to many systemic or sporadic errors) and false-negative results (up to 20% sputum specimen contains inhibitors); and
iii. laboratory costs.

Molecular methods can be divided into

i. signal amplification method;
ii. nucleic acid amplification method; and
iii. post-amplification analysis.

2.7.1 Signal Amplification Method

Nucleic Acid Probe

These were developed and made commercially available by Gen-probe (Hologic, San-Diego, CA). In this method, acridine-ester labeled single-stranded DNA probes were used. These DNA probes were designed to hybridize with mycobacterial ribosomal RNA (rRNA). Ribosomal RNAs are considered an ideal target for identifying mycobacteria because they contain signature sequences and are produced in large quantities by cultured mycobacteria. The DNA probe hybridizes with rRNA during the test and forms a stable DNA-RNA complex. A signal generating step is performed after the inactivation of unhybridized probes. This step produces light which is measured by an instrument. The light generated is directly proportional to the amount of hybridized probe, and the test result is decided based on a pre-determined threshold of generated light. It requires two hours to determine species and is superior to traditional identification methods [42, 43]. The use of these probes on positive broth cultures helps to decrease the time of detection of mycobacteria even further [44, 45].

Nucleic Acid Amplification Methods

FDA approved two tests based on the nucleic acid amplification method for respiratory specimens in the late 1990s. These tests were Amplicor *M. tb* PCR assay by Roche Diagnostics, Indianapolis, IN, and Amplified Mycobacterium tuberculosis Direct test (AMDT). The test was based on transcription-mediated amplification.

These tests performed better on smear-positive specimens, but the sensitivity was lower than culture on a smear-negative respiratory specimen [46–53].

WHO recommended Xpert MTB/RIF assay in 2010, and FDA approved a third nucleic acid amplification assay, i.e., Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA) in the United States in 2012. This assay has revolutionized TB control (by early detection of TB disease and drug resistance). It was introduced as a cartridge-based, near-patient technology with integrated hands-free sputum processing. It was designed based on real-time Hemi-nested PCR to detect *M. tb* and rifampin resistance in a single reaction. The assay amplifies the 81-bp fragment of the *M. tb rpoB* gene and detects mutations associated with rifampin resistance. A limit of detection (LOD) of *M. tb* DNA for this test is 4.5 genomes/reaction (as low as one genome/reaction) or clinical LOD of 131 CFU of *M. tb*/ml (as low as 10 CFU/ml). The test results are available in two hours [54, 55]. Rifampin-resistant strains show resistance to isoniazid in greater than 90% of cases. Hence, detection of rifampin resistance acts as a surrogate marker for multidrug-resistant TB (MDR-TB). This has helped in the early initiation of treatment of MDR-TB [56]. An advanced version of the Xpert MTB/RIF assay, i.e., Xpert MTB/RIF Ultra assay, has improved sensitivity for *M. tb* detection and rifampin resistance, making it useful in paucibacillary TB, as frequently seen in cases of TB-HIV co-infection, in pediatric TB, and those with EPTB [57, 58].

2.7.2 Post-Amplification Analysis

Line Probe Assays (Reverse Hybridization)

In the line probe assay (LPA), using biotinylated PCR primers, target sequences are amplified. The amplified product (amplicon) is applied on nitrocellulose or similar substrate on which multiple probes are immobilized. Amplicon-probe hybridization leads to the formation of lines. The pattern formed is compared to a key for the interpretation of results. The technique is reverse of Southern blotting with the advantage that multiple probes can be assayed simultaneously. The assay does not require any radioisotopes, another advantage of Southern blotting. WHO recommended commercially available LPA, i.e., GenoType MTBDRplus LPA, Hain version 1 (Hain Lifescience, Nehren, Germany) in 2008 [59]. Subsequently, FIND (the Foundation for Innovative New Diagnostics) evaluated Hain version 1, GenoType MTBDRplus version 2 (Hain version 2), and Nipro NTM + MDRTB detection kit 2 (Nipro, Tokyo, Japan) for detecting *M. tb* and resistance to rifampicin and isoniazid in 2015. This study found equivalence among these three commercially available LPAs [60].

DNA Sequencing

DNA sequencing or Sanger's sequencing was widely used in many laboratories to identify mainly slow-growing bacteria such as mycobacteria and Nocardia and fungi [61, 62].

Hypervariable region A of the 16S gene complex has been the most commonly used target to identify and differentiate clinically relevant mycobacteria and isolates that are difficult to characterize [63–65].

A commercially available DNA sequencing system is MicroSeq developed by Applied Biosystems, Inc. (ABI), Foster City, CA. It provides primers to perform PCR. ABI capillary-based sequencing is then performed. The product of sequencing is matched with a genetic database for results. The *rpoB* gene is a popular sequencing target because it provides identification information and information regarding rifampin resistance [66, 67].

TB-Lamp

TB-LAMP, a loop-mediated isothermal amplification platform developed by Eiken Chemical Co. (in Japan), is a commercially available, new, manual molecular method for TB detection [68]. It is a promising diagnostic method requiring minimal instrumentation with a high throughput potential, i.e., 14 samples per test run [69, 70]. The assay requires less than one hour and is read under UV light with the naked eyes. Loop primers speed up target DNA amplification to 10^9–10^{10} times within 15–30 min. Amplified DNA is detected using SYBR green (DNA binding dye), magnesium pyrophosphate, or a non-inhibitory fluorescing agent [71].

Microarray Analysis

It is a great research tool used extensively for research in a few laboratories. It has the potential to examine a large number of DNA sequences in a very short period [72]. The device used for this test is called a gene chip. It is used for mycobacterial detection and genetic determination of drug resistance from a large number of specimens with a single hybridization step. The large expense required for implementing this test is the only major drawback holding its widespread use in diagnosing TB.

Cabibbe et al. evaluated the performance of the VerePLEX Biosystem based on the molecular lab-on-chip technique. It was a single disposable device built based on microfluidic PCR and microarray modules by STMicroelectronics (Geneva, Switzerland) to detect NTM and diagnose MDR-TB. 91 *M. tb* complex (MTBC) isolates and 116 MTBC culture-negative specimens were included to detect mutations in *rpoB, katG,* and *inhA* genes. The results of the VerePLEX Biosystem were compared with Sanger sequencing and GenoType MTBDR*plus* (Hain Lifescience, Nehren, Germany) assay. The VerePLEX Biosystem yielded more than 97.8% of diagnostic accuracy than sequencing and MTBDR*plus*assay [73].

2.8 Nanodiagnosis

Nanodiagnostics or nanotechnology-based diagnostic methods are likely to become mainstream diagnostic modalities. It is a little more than a decade since the research has been happening in this field. Initial results of research have shown great potential in nanotechnology to become a rapid, reliable, reproducible, and

cost-effective tool to be used in the diagnosis of TB and cardiovascular, cancer, and other infectious diseases. We will discuss some nanotechniques which have shown promising results.

2.8.1 Gold Nanoparticles (AuNPs)

Baptista et al. developed a colorimetric method using gold nanoparticles (AuNPs) to detect *M. tb* from a clinical specimen directly. The gold nanoparticle probes (nanoprobes) were designed using a specific thiolated oligonucleotide derived from RNA polymerase β subunit of *M. tb*. The result is based on colorimetric change observed in a solution containing nanoprobes read at peak absorbents wavelength of $\sim$ 526 nm.

The solution with nanoprobes appears red, and the addition of NaCl causes aggregation of nanoprobes making the solution appear purple. If nanoprobes hybridize with DNA, the addition of NaCl fails to cause nanoprobe aggregation, and the solution remains red, indicating the presence of specific target DNA in a clinical specimen.

Initially, target DNA is amplified using PCR. The amplified DNA is then denatured (five minutes at 95 °C). The solution is allowed to cool to room temperature for 30 min. During cooling, DNA hybridizes with the probe. The addition of NaCl to the final solution mixture brings out changes that are measurable using UV light/visible spectroscopy. The lower LOD is 0.75 mcg of total DNA in the testing specimen. The total time required for the study is two hours. It is performed in a single tube, reducing the risk of carryover contamination. It is relatively inexpensive, including first-step PCR of study (< US $0.35 per sample) compared to other molecular assays [74].

Tsai et al. evaluated the use of AuNPs in the diagnosis of TB. They demonstrated the use of the gold nanoparticle colloid system's surface plasmon resonance effect in diagnosing PCR amplified *M. tb* dsDNA sequence. They used the IS6110 target sequence for PCR amplification. The change in color of the solution from red to blue after the addition of NaCl is considered positive for the presence of *M. tb* DNA in the clinical sample. The colorimetric results were analyzed on a simple paper-based platform with the help of a smartphone. It has certain advantages,

i. rapid analysis;
ii. lower reagent consumption;
iii. no need of installing sophisticated analytic equipment; and
iv. LOD ranging from 1.95×10^{-2} to 1.95×10^{1} ng/ml of TB dsDNA sequences.

This method can be a very affordable, user-friendly tool that is very sensitive and specific, not only for TB but also for other infectious diseases [75].

2.8.2 Array Format-Based Surface Plasmon Resonance (SPR)

Hsieh et al. developed a surface plasmon-based assay to detect antibodies against *M. tb* in the patient's serum. An array chip was designed by immobilizing nine different *M. tb* antigens on its surface and subjected to patient serum. The SPR

reflectivity is then measured using the GWC PCR imager system (biosensor). The higher value of reflectivity suggests the presence of specific TB antibodies in the patient's serum. The results of this array were better than the multiple-antigen ELISA assay. Another advantage of this technique is that it is label-free and helps in the real-time detection of antibodies. Since it has high sensitivity (100%) and specificity (85%) for TB antibody detection, it can be further developed as a biosensor array system to detect multiple *M. tb* antibodies [76].

2.8.3 Fluorescent Silica Nanoparticle-Based Indirect Immunofluorescence Microscopy

In the immunofluorescence (IF) technique, the antibodies conjugated to a fluorescent dye (fluorochrome) are used to detect specific target antigens. In indirect IF, primary antibodies are unconjugated, whereas fluorochrome-conjugated secondary antibody is directed against primary antibody for detection.

Qin et al. developed fluorescent silica nanoparticle-based indirect IF microscopy to detect *M. tb*. The primary antibody used in this technique was an unconjugated, specific anti-*M. tb* antibody. An antibody binding protein (protein A) was used to detect the primary antibody. A simple and efficient water-in-oil microemulsion method was used to prepare Tris (2,2-bipyridyl) dichlororuthenium (II) hexahydrate (RuBpy)-doped silica nanoparticles. Protein A was labeled with RuBpy-doped silica nanoparticles. The technique was used for *M. tb* detection in pure culture, mixed bacterial samples, and sputum samples. The RuBpy-doped silica nanoparticles are attributed to increased detection sensitivity in many aspects. The fluorescent silica nanoparticles used as labels yielded amplified signal intensity (five times more) and better photostability than conventional fluorescent dye. The total time required for the assay is within four hours. This study also claims that integrating epifluorescent filter techniques can reduce *M. tb* detection time. It can be further developed into a universal method for detecting a wide variety of bacteria [77].

2.8.4 Magnetic Nanoparticles

Magnetic nanoparticles are used in clinical and molecular diagnostic laboratories because of their unique properties. They can be utilized as contrast material (superparamagnetic iron oxide nanoparticles) in magnetic resonance imaging (MRI) or can be tagged to antibodies for the detection of nucleic acid (mRNA, DNA), bacteria, viruses, and cells [78–81].

Engstrom et al. developed magnetic nanobead-based detection of rifampicin resistance in *M. tb*. They developed nine mutant-specific padlock probes designed to bind to the most common mutation-associated codons of the 81 bp *rpo*B gene. The bound probes were amplified by the rolling circle amplification (RCA) method. Amplified probes were restriction digested, redigested, and again subjected to RCA, known as circle-to-circle amplification, to increase the assay's sensitivity. A dilution of spherical avidin-functionalized magnetic nanoprobes tagged with specific oligonucleotide was added to the solution of RCA products. The reaction was read out using a portable AC susceptometer, and the method was called volume amplified-magnetic nanobead detection assay (VAMNDA). They found that the

method was highly specific and multiplexable. Different padlocks probes can be designed to detect different mutations associated with drug resistance in *M. tb* [82].

Gordillo-Marroquín et al. developed a magnetic nanoparticle-based colorimetric biosensing assay (NCBA) to detect *M. tb* in sputum specimens and compared it with smear microscopy results. The digestion/decontamination of sputum was performed using the NaOH-NALC method. The specimen is divided into two parts. One part was subjected to routine smear microscopy, and another part was treated with glycan-functionalized magnetic nanoparticles (GMNPs) and placed in a magnetic rack. Such treated specimen was then subjected to AFB staining and microscopy. GMNPs bind to the mycobacterial surface through the carbohydrate-binding lectins forming a complex. The advantages of this assay were

- rapidness (< 20 min);
- affordable ($0.10/test);
- room-temperature assay;
- no necessity of power supply;
- simple to implement;
- no refrigeration; and
- improved smear grade from 1 + in sputum smear microscopy to 2 + (in NCBA); hence very useful in paucibacillary TB cases [83].

2.8.5 Quantum Dots Assay

The quantum dots (QDs) technique has become a part of nanotechnology and is used to develop the newer diagnostic tests. It is also known as semiconductor quantum dots or nanocrystals and is a type of fluorophore. It is more photostable than conventional fluorophores and has narrow, symmetrical, and tunable emission spectra. Multiple molecular targets can be detected using QDs in various clinical samples [84]. Liandris et al. developed a novel technique that combined cadmium selenide QDs and magnetic beads to detect surface antigens of mycobacteria. In this method, streptavidin-coated magnetic beads were functionalized with a biotinylated polyvalent antibody (produced in rabbit) against *M. tb* PPD. Cadmium selenide QDs were coated with streptavidin and two monoclonal antibodies against *M. tb* heparin-binding hemagglutinin (murine derived). During the assay, cells were separated using magnetic beads coated with polyclonal and monoclonal antibodies mentioned above. These separated cells were treated with streptavidin-coated QD's, leading to fluorescence detection. Fluorescence was detected using a spectrofluorometer. The LOD was 10^4 CFU/ml with naked eyes and 10^3 CFU/ml with a spectrofluorometer. This assay did not require amplification and can be developed further to detect any other protein target [85].

2.9 Biosensing Techniques

Biosensors or biosensing techniques are used in various fields such as clinical assays, food and agriculture security, disease diagnostics, and environmental monitoring. Certain advantages of using these techniques are:

- need of very little amount of sample;
- high sensitivity and specificity;
- applicable on different specimens such as blood, serum, urine, saliva, etc. [86–96].

A biosensor has two basic components, namely

i. bio-recognition component, it can detect any bio-recognition element such as an enzyme, cell, nucleic acid, antigen–antibody complex, etc.; and
ii. bio-transducer, with the help of different physical or chemical immobilization methods, the bio-recognition element is firmly attached to the bio-transducer, which gives a precise measurement of the bound bio-recognition element [97, 98].

Depending on the method employed for signal transduction, biosensors are divided into electrochemical, optical, mechanical, and magnetic [99]. A summarized list of various biosensors used in different studies is shown in Table 4.

3 Conclusion

Let to remind the words spoken more than 100 years back by Robert Koch,

> The struggle has caught hold along the whole line, and enthusiasm for the lofty aim runs so high that a slackening is no longer to be feared. If the work goes on in this powerful way, then the victory must be won [113].

Unfortunately, his words on the fight against TB hold true to date. TB is affecting humanity in the worst imaginable way. The random efforts by different stakeholders in the struggle against TB are virtually useless. The definitive road to success is the commitment from patients, healthcare workers, clinicians, policymakers, and governments, which will help us win the war against this mortal enemy, *M. tb*.

Core Messages

- The diagnostic modalities for TB are smear microscopy, culture, immunodiagnosis, molecular methods, and nanotechnology.

Table 4 Biosensing techniques

Biosensor transducer	Biosensor technique	Sample analyzed		Limit of detection	Reference
		Sample	Bio-recognition element		
Electro-chemical	Electronic-nose based	Sputum	Volatile substances produced by *M. Tb*	NA	[100]
	Carbon nanotubes	–	ssDNA	NA	[101]
Optical	Fibre-optic	Sputum	Niacin	NA	[102]
	Silicon oxy-nitride planar wave-guide based	Urine	ESAT-6, Antigen 85 complex, LAM	NA	[103]
	Surface plasmon resonance	–	(i) ITS sequence of MTB complex and M. gordonae	30 ng/ml	[104]
		–	(ii) Target sequence in MTB DNA	1 ng/ml	[105]
	Plasmon-induced photoluminescence	–	CPF-10	4.5 pg/ml	[106]
	Breathalyzer	Cough	MTB cells	50–75 CFU/ml	[107]
Mechanical	Quartz crystal microbalance	Exhaled breath	MTB LAM	8.7×10^6cell/ml 8.7×10^5cell/ml	[108]
	Multi-channel series piezoelectric quartz crystal sensor	Sputum	Volatile NH3 and CO_2 produced by MTB	10 CFU/ml	[109]
	Bulk acoustic wave impedance	–	MTB	2×10^3 cell/ml	[110]
	Wireless magneto-elastic sensing system	Sputum	MTB metabolites in culture	10^4 cells/ml	[111]
Magnetic	Magnetic barcode assay	Sputum	ssDNA	10^3 MTB/ml	[112]

- ZN stained smear microscopy is still widely used for AFB detection with a variable sensitivity ranging between 0 and 40%.
- Isolation of *M. tb* from clinical specimens by culture is a gold standard test and has a sensitivity ranging between 0 and 80%.
- Immunodiagnosis for LTBI has low specificity; a nanotechnological advance is a futuristic approach in diagnosing TB.
- Molecular methods are widely used for research and diagnostic purpose, e.g., Xpert MTB/RIF assay, TB-LAMP, microarray, etc.

References

1. World Health Organization (2019) Global tuberculosis report. Geneva
2. Guidelines for the collection, transport, processing, analysis, and reporting of cultures from specific specimen sources. In: Winn W, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, Woods G (2006) Koneman's Color Atlas and Textbook of diagnostic microbiology. 6th edn. Lippincott Williams and Wilkins, New York
3. Clinical and Laboratory Standards Institute (2008) Laboratory detection and identification of mycobacteria; approved guideline—1st edition. In: CLSI document M48-A, Clinical and Laboratory Standards Institute, Wayne, PA
4. Bouza E, Dıaz-Lopez MD, Moreno S, Bernaldo de Quiros JC, Vicente T, Berenguer J (1993) Mycobacterium tuberculosis bacteremia in patients with and without human immunodeficiency virus infection. Arch Intern Med 153(4):496–500
5. Gopinath K, Kumar S, Singh S (2008) Prevalence of mycobacteremia in Indian HIV-infected patients detected by the MB/BacT automated culture system. Eur J Clin Microbiol Infect Dis 27(6):423–431
6. Chin DP, Hopewell PC, Yajko DM, Vittinghoff E, Horsburgh Jr CR, Hadley WK, Stone EN, Nassos PS, Ostroff SM, Jacobson MA (1994) Mycobacterium avium complex in the respiratory or gastrointestinal tract and the risk of M. avium complex bacteremia in patients with human immunodeficiency virus infection. J Infect Dis 169(2):289–295. https://doi.org/10.1093/infdis/169.2.289
7. Kubica GP, Gross WM, Hawkins JE, Sommers HM, Vestal AL, Wayne LG (1975) Laboratory services for mycobacterial diseases. Am Rev Respir Dis 112(6):783–787
8. Shirvastava SR, Shirvastava PS, Ramasamy J (2014) Revised national tuberculosis control program: progress in the diagnostic front. J Mahatma Gandhi Inst Med Sci 19(2):164–165
9. Desikan P (2013) Sputum smear microscopy in tuberculosis: Is it still relevant? Indian J Med Res 137(3):442–444
10. World Health Organization (2006) Handbook for using the International Standards for Tuberculosis Care. Tuberculosis Coalition for Technical Assistance, The Hague
11. Rawat J, Biswas D, Sindhwani G, Masih V (2010) An alternative 1-day smear microscopy protocol for the diagnosis of pulmonary tuberculosis. Respirology 15(7):1127–1130. https://doi.org/10.1111/j.1440-1843.2010.01827.x
12. Liu KT, Su WJ, Perng RP (2007) Clinical utility of polymerase chain reaction for diagnosis of smear-negative pleural tuberculosis. J Chin Med Assoc 70(4):148–151
13. Haldar S, Bose M, Chakrabarti P, Daginawala HF, Harinath BC, Kashyap RS, Kulkarni S, Majumdar A, Prasad HK, Rodrigues C, Srivastava R, Taori GM, Varma-Basil M, Tyagi JS (2011) Improved laboratory diagnosis of tuberculosis-the Indian experience. Tuberculosis 91 (5):414–426. https://doi.org/10.1016/j.tube.2011.06.003

14. Derese Y, Hailu E, Assefa T, Bekele Y, Mihret A, Aseffa A, Hussien J, Ali I, Abebe M (2012) Comparison of PCR with standard culture of fifine needle aspiration samples in the diagnosis of tuberculosis lymphadenitis. J Infect Develop Countries 6(1):53–57
15. Rickman TW, Moyer NP (1980) Increased sensitivity of acid fast smears. J Clin Microbiol 11(6):618–620. https://doi.org/10.1128/jcm.11.6.618-620.1980
16. Lipsky BA, Gates J, Tenover FC, Plorde JJ (1984) Factors affecting the clinical value of microscopy for acid-fast bacilli. Rev Infect Dis 6(2):214–222. https://doi.org/10.1093/clinids/6.2.214
17. Sharma SK, Mohan A (2004) Extrapulmonary tuberculosis. Indian J Med Res 120(4):316–353
18. Padmavathy L, Rao L, Veliath A (2003) Utility of polymerase chain reaction as a diagnostic tool in cutaneous tuberculosis. Indian J Dermatol Venereol Leprol 69:214–216
19. Takahashi T, Tamura M, Asami Y (2008) Novel wide-range quantitative nested real time PCR assay for Mycobacterium tuberculosis DNA: clinical application for diagnosis of tuberculous meningitis. J Clin Microbiol 46(5):1698–1707
20. Abbara A, Davidson RN (2011) Etiology and management of genitourinary tuberculosis. Nat Rev Urol 8(12):678–688
21. Woods GL, Brown-Elliott BA, Desmond EP, Hall GS, Heifets L, Pfyffer GE, Plaunt MR, Ridderhof JC, Wallace Jr RJ, Warren NG, Witebsky GF (2003) Susceptibility testing of Mycobacteria, Nocardia, and other Actinomycetes. In: Approved standard M24-A, vol 23 (no. 18). NCCLS, Wayne, Pa
22. Runyon EH (1970) Identification of mycobacterial pathogens utilizing colony characteristics. Am J Clin Pathol 54:578–586
23. Miliner RA, Stottmeier KD, Kubica GP (1969) Formaldehyde: a photothermal activated toxic substance produced in Middlebrook 7H10 medium. Am Rev Respir Dis 99:603–607
24. Gruft H (1971) Isolation of acid-fast bacilli from contaminated specimens. Health Lab Sci 8:79–82
25. Petran EI, Vera HD (1971) Media for selective isolation of mycobacteria. Health Lab Sci 8:225–230
26. Mitchison DA, Allen BW, Carrol L, Dickinson JM, Aber VR (1972) A selective oleic acid albumin agar medium for tubercle bacilli. J Med Microbiol 5:165–175. https://doi.org/10.1099/00222615-5-2-165
27. McClatchy JK, Waggoner RF, Kanes W, Cernich MS, Bolton TL (1976) Isolation of mycobacteria from clinical specimens by use of selective 7H11 medium. Am J Clin Pathol 65:412–415. https://doi.org/10.1093/ajcp/65.3.412
28. Rodrigues CS, Shanai SV, Almeida D, Sadani MA, Goyal N, Vadher C, Mehta AP (2007) Use of BACTEC 460 TB system in the diagnosis of tuberculosis. Indian J Med Microbiol 25:32–36. https://doi.org/10.4103/0255-0857.31059
29. Rodrigues C, Shenai S, Sadani M, Sukhadia N, Jani M, Ajbani K, Sodha A, Mehta A (2009) Evaluation of bactec MGIT 960 TB systemfor recovery and identification of Mycobacterium tuberculosis complex in a high volume tertiary care centre. Indian J Med Microbiol 27:217–221
30. Gil-Setas A, Torroba L, Fernandez JL, Martinez-Artola V, Olite J (2004) Evaluation of the MB/BacT system compared with Middlebrook 7H11 and Lowenstein-Jensen media for detection and recovery of mycobacteria from clinical specimens. Clin Microbiol Infect 10:224–228. https://doi.org/10.1111/j.1198-743x.2004.00733.x
31. Tholcken CA, Huang S, Woods GL (1997) Evaluation of the ESP culture system II for recovery of mycobacteria from blood specimens collected in isolator tubes. J Clin Microbiol 35:2681–2682
32. Woods GL, Fish G, Plaunt M, Murphy T (1997) Clinical evaluation of Difco ESP culture system II for growth and detection of mycobacteria. J Clin Microbiol 35:121–124
33. Waite RT, Woods GL (1998) Evaluation of BACTEC MYCO/F LYTIC medium for recovery of Mycobacteria and fungi from blood. J Clin Microbiol 36:1176–1179

34. Crump JA, Tanner DC, Mirrett S, McKnight CM, Reller LB (2003) Controlled comparison of BACTEC 13A, MYCO/F LYTIC, BacT/ALERT MB, and ISOLATOR 10 systems for detection of mycobacteremia. J Clin Microbiol 41:1987–1990. https://doi.org/10.1128/JCM.41.5.1987-1990.2003
35. World Health Organization (2011) Policy Statement: commercial serodiagnostic tests for diagnosis of tuberculosis. Geneva
36. Morris K (2011) WHO recommends against inaccurate tuberculosis tests. Lancet 377:113–114
37. Pinto LM, Grenier J, Schumacher SG, Denkinger CM, Steingart KR, Pai M (2012) Immunodiagnosis of tuberculosis: state of the Art. Med Princ Pract 21:4–13. https://doi.org/10.1159/000331583
38. World Health Organization (2018) Latent tuberculosis infection: updated and consolidated guidelines for programmatic management. Geneva
39. Dunn JJ, Starke JR, Revell PA (2016) Laboratory diagnosis of Mycobacterium tuberculosis infection and disease in children. J Clin Microbiol 54:1434–1441. https://doi.org/10.1128/JCM.03043-15
40. Ayub A, Yale SH, Reed KD, Nasser RM, Gilbert SR (2004) Testing for latent tuberculosis. Clin Med Res 2(3):191–194
41. World Health Organization (2011) Use of tuberculosis interferon-gamma release assays (IGRAs) in low- and middle-income countries. Geneva
42. Middleton AM, Chadwick MV, Gaya H (1997) Detection of *Mycobacterium tuberculosis* in mixed broth cultures using DNA probes. Clin Microbiol Infect 3:668–671
43. Scarparo C, Piccoli P, Rigon A, Ruggiero G, Nista D, Piersimoni C (2001) Direct identification of mycobacteria from MB/BacT alert 3D bottles: comparative evaluation of two commercial probe assays. J Clin Microbiol 39:3222–3227. https://doi.org/10.1128/JCM.39.9.3222-3227.2001
44. Metchock B, Diem L (1995) Algorithm for use of nucleic acid probe for Identifying Mycobacterium Tuberculosis from BACTEC12 B Bottles. J Clin Microbiol 33:1934–1937
45. Badak FZ, Goksel S, Sertoz R, Nafile B, Ermertcan S, Cavusoglu C, Bilgic A (1999) Use of nucleic acid probes for identification of Mycobacterium tuberculosis directly from MB/BacT bottles. J Clin Microbiol 37:1602–1605
46. Smith MB, Bergmann JS, Harris SL, Woods GL (1997) Evaluation of the Roche AMPLICOR MTB assay for the detection of Mycobacterium tuberculosis in sputum specimens from prison inmates. Diagn Microbiol Infect Dis 27:113–116. https://doi.org/10.1016/s0732-8893(97)00029-1
47. Ichiyama S, Iinuma Y, Tawada Y, Yamori S, Hasegawa Y, Shimokata K, Nakashima N (1996) Evaluation of the Gen-Probe amplified Mycobacterium tuberculosis direct test and Roche PCR-Microwell plate hybridization method (Amplicor Mycobacterium) for direct detection of mycobacteria. J Clin Microbiol 34:130–133. https://doi.org/10.1128/jcm.34.1.130-133.1996
48. O'Sullivan CE, Miller DR, Schneider PS, Roberts GD (2002) Evaluation of Gen-Probe amplified Mycobacterium tuberculosis direct test by using respiratory and nonrespiratory specimens in a tertiary care center laboratory. J Clin Microbiol 40:1723–1727. https://doi.org/10.1128/JCM.40.5.1723-1727.2002
49. Salfinger M, Hale YM, Driscoll JR (1998) Diagnostic tools in tuberculosis present and future. Respiration 65:163–170
50. Della-Latta P, Whittier S (1998) Comprehensive evaluation of performance, laboratory application, and clinical usefulness of two direct amplification technologies for the detection of Mycobacterium tuberculosis complex. Am J Clin Pathol 110:301–310
51. D'Amato RF, Wallman AA, Hochstein LH, Colaninno PM, Scardamaglia M, Ardila E, Ghouri M, Kim K, Patel RC, Miller A (1995) Rapid diagnosis of pulmonary tuberculosis by using Roche AMPLICOR Mycobacterium tuberculosis PCR test. J Clin Microbiol 33:1832–1834

52. Bodmer T, Gurtner A, Schopfer K, Matter L (1994) Screening of respiratory tract specimens for the presence of Mycobacterium tuberculosis by using the Gen-Probe Amplified Mycobacterium tuberculosis direct test. J Clin Microbiol 32:1483–1487. https://doi.org/10.1128/jcm.32.6.1483-1487.1994
53. Bergmann JS, Yuoh G, Fish G, Woods GL (1999) Clinical evaluation of the enhanced gen-probe amplified Mycobacterium Tuberculosis direct test for rapid diagnosis of tuberculosis in prison inmates. J Clin Microbiol 37:1419–1425. https://doi.org/10.1128/JCM.37.5.1419-1425.1999
54. World Health Organization (2010) Roadmap for rolling out Xpert MTB/RIF for rapid diagnosis of TB and MDR-TB 2010
55. Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, Kop J, Owens MR, Rodgers R, Banada P, Safi H, Blakemore R, Lan NTN, Jones-Lopez EC, Levi M, Burday M, Ayakaka I, Mugerwa RD, McMillan M, Winn-Deen E, Chistel L, Dailey P, Perkins MD, Persing DH, Alland D (2010) Rapid detection of *Mycobacterium tuberculosis* and Rifampin resistance by use of on-demand, near-patient technology. J Clin Microbio 48:229–237. https://doi.org/10.1128/JCM.01463-09
56. Watterson SA, Wilson SM, Yates MD, Drobniewski FA (1998) Comparison of three molecular assays for rapid detection of rifampicin resistance in Mycobacterium tuberculosis. J Clin Microbiol 36:1969–1973. https://doi.org/10.1128/JCM.36.7.1969-1973.1998
57. Chakravorty S, Simmons AM, Rowneki M, Parmar H, Cao Y, Ryan J, Banada PM, Deshpande S, Shenai S, Gall A, Glass J, Krieswirth B, Schumacher SG, Nabeta P, Tukvadze N, Rodrigues C, Skrahina A, Tagliani E, Cirillo DM, Davidow A, Denkinger CM, Persing D, Kwiatkowski R, Jones M, Alland D (2017) The New Xpert MTB/RIF Ultra: Improving detection of Mycobacterium tuberculosis and resistance to Rifampin in an assay suitable for point-of-care testing. mBio 8(4):e00812–e00817. https://doi.org/10.1128/mBio.00812-17
58. Osei Sekyere J, Maphalala N, Malinga LA, Mbelle NM, Maningi NE (2019) A Comparative evaluation of the new genexpert MTB/RIF ultra and other rapid diagnostic assays for detecting tuberculosis in pulmonary and extra pulmonary specimens. Sci Rep 9:16587. https://doi.org/10.1038/s41598-019-53086-5
59. World Health Organization (2008) Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB): policy statement. Geneva.
60. Report for WHO (2015) Non-inferiority evaluation of Nipro NTM+MDRTB and Hain GenoTypeMTBDRplus V2 line probe assays. Geneva
61. Cloud JL, Conville PS, Croft A, Harmsen D, Witebsky FG, Carroll KC (2004) Evaluation of partial 16S ribosomal DNA sequencing for identification of Nocardia species by using the MicroSeq 500 system with an expanded database. J Clin Microbiol 42:578–584. https://doi.org/10.1128/JCM.42.2.578-584.2004
62. Hall L, Doerr KA, Wohlfiel SL, Roberts GD (2003) Evaluation of the MicroSeq system for identification of mycobacteria by 16S ribosomal DNA sequencing and its integration into a routine clinical mycobacteriology laboratory. J Clin Microbiol 41:1447–1453. https://doi.org/10.1128/JCM.41.4.1447-1453.2003
63. Dobner P, Feldmann K, Rifai M, Loscher T, Rinder H (1996) Rapid identification of mycobacterial species by PCR amplification of hypervariable 16S rRNA gene promoter region. J Clin Microbiol 34:866–869
64. Han XY, Pham AS, Tarrand JJ, Sood PK, Luthra R (2002) Rapid and accurate identification of mycobacteria by sequencing hypervariable regions of the 16S ribosomal RNA gene. Am J Clin Pathol 118:796–801. https://doi.org/10.1309/HN44-XQYM-JMAQ-2EDL
65. Springer B, Stockman L, Teschner K, Roberts GD, Bottger EC (1996) Two-laboratory collaborative study on identification of mycobacteria: molecular versus phenotypic methods. J Clin Microbiol 34:296–303. https://doi.org/10.1128/jcm.34.2.296-303.1996

66. Somoskovi A, Song Q, Mester J, Tanner C, Hale YM, Parsons LM, Salfinger M (2003) Use of molecular methods to identify the Mycobacterium tuberculosis complex (MTBC) and other mycobacterial species and to detect rifampin resistance in MTBC isolates following growth detection with the BACTEC MGIT 960 system. J Clin Microbiol 41:2822–2826. https://doi.org/10.1128/JCM.41.7.2822-2826.2003
67. Viader-Salvado JM, Luna-Aguirre CM, Reyes-Ruiz JM, Valdez-Leal R, Bosque-Moncayo MA, Tijerina-Menchaca R, Guerrero-Olazaran M (2003) Frequency of mutations in rpoB and codons 315 and 463 of katG in rifampin- and/or isoniazid-resistant Mycobacterium tuberculosis isolates from northeast Mexico. Microb Drug Resist 9:33–38. https://doi.org/10.1089/107662903764736328
68. Pham TH, Peter J, Mello FCQ, Parraga T, Lan NTN, Nabeta P, Valli E, Caceres T, Dheda K, Hillemann DSE, D, Gray CM, Perkins MD, (2018) Performance of the TB-LAMP diagnostic assay in reference laboratories: Results from a multicentre study. Int J Infect Dis 68:44–49. https://doi.org/10.1016/j.ijid.2018.01.005
69. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, Allen J, Tahirli R, Blakemore R, Rustomjee R, Milovic A, Jones M, O'Brien SM, Persing DH, Ruesch-Gerdes S, Gotuzzo E, Rodrigues C, Alland D, Perkins MD (2010) Rapid molecular detection of tuberculosis and rifampicin resistance. N Engl J Med 363(11):1005–1015
70. Vassall A, van Kampen S, Sohn H, Michael JS, John KR, den Boon S, Davis JL, Whitelaw A, Nicol MP, Gler MT, Khaliqov A, Zamudio C, Perkins MD, Boehme CC, Cobeiens F (2011) Rapid diagnosis of tuberculosis with the Xpert MTB/RIF assay in high burden countries: a cost-effectiveness analysis. PLoS Med 2011 8(11) e1001120. https://doi.org/10.1371/journal.pmed.1001120
71. World Health Organization (2016) The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis: policy guidance. Geneva
72. Gingeras TR, Ghandour G, Wang E, Berno A, Small PM, Drobniewski F, Alland D, Desmond E, Holodniy M, Drenkow J (1998) Simultaneous genotyping and species identification using hybridization pattern recognition analysis of generic Mycobacterium DNA arrays. Genome Res 8(5):435–448. https://doi.org/10.1101/gr.8.5.435
73. Cabibbe AM, Miotto P, Moure R, Alcaide F, Feuerriegel S, Pozzi G, Nikolayevskyy V, Drobniewski F, Niemann S, Reither K, Cirillo DM, TM-REST Consortium, TB-CHILD Consortium (2015) Lab-on-chip-based platform for fast molecular diagnosis of multidrug-resistant tuberculosis. J Clin Microbiol 53(12):3876–3880. https://doi.org/10.1128/JCM.01824-15
74. Baptista PV, Koziol-Montewka M, Paluch-Oles J, Doria G, Franco R (2006) Gold-nanoparticle-probe–based assay for rapid and direct detection of Mycobacterium tuberculosis DNA in clinical samples. Clin Chem 52:1433–1434. https://doi.org/10.1373/clinchem.2005.065391
75. Tsai T-T, Huang C-Y, Chen C-A, Shen S-W, Wang M-C, Cheng C-M, Chen C-F (2017) Diagnosis of tuberculosis using colorimetric gold nanoparticles on a paper-based analytical device. ACS Sensors 2:1345–1354
76. Hsieh S-C, Chang C-C, Lu C-C, Wei C-F, Lin C-S, Lai H-C, Lin C-W (2012) Rapid identification of Mycobacterium tuberculosis infection by a new array format-based surface plasmon resonance method. Nanoscale Res Lett 7:180. https://doi.org/10.1186/1556-276X-7-180
77. Qin D, He X, Wang K, Zhao XJ, Tan W, Chen J (2007) fluorescent nanoparticle-based indirect immunoflfluorescence microscopy for detection of Mycobacterium tuberculosis. J Biomed Biotechnol Article ID 89364. https://doi.org/10.1155/2007/89364
78. Rosi NL, Mirkin CA (2005) Nanostructures in biodiagnostics. Chem Rev 105:1547–1562
79. Kaittanis C, Naser SA, Perez JM (2007) One-step, nanoparticle-mediated bacterial detection with magnetic relaxation. Nano Letters 7:380–383. https://doi.org/10.1021/nl062553z
80. Lee H, Sun E, Ham D, Weissleder R (2008) Chip-NMR biosensor for detection and molecular analysis of cells. Nat Med 14:869–874

81. Perez JM, Josephson L, O'Loughlin T, Hogemann D, Weissleder R (2002) Magnetic relaxation switches capable of sensing molecular interactions. Nat Biotechnol 20:816–820
82. Engström A, de la Torre TZG, Strømme M, Nilsson M, Herthnek D (2013) Detection of rifampicin resistance in Mycobacterium tuberculosis by padlock probes and magnetic nanobead-based readout. PLoS ONE 8:e62015
83. Jaiswal JK, Mattoussi H, Mauro JM, Simon SM (2003) Long-term multiple color imaging of live cells using quantum dot bioconjugates. Nat Biotechnol 21:47–51
84. Gordillo-Marroquín C, Gómez-Velasco A, Sánchez-Pérez HJ, Pryg K, Shinners J, Murray N, Munoz-Jimenez SG, Bencomo-Alerm A, Gomez-Bustamante A, Jonapa-Gomez L, Enriquez-Rios N, Martin M, Romero-Sandoval N, Alocilja EC (2018) magnetic nanoparticle-based biosensing assay quantitatively enhances acid-fast bacilli count in paucibacillary pulmonary tuberculosis. Biosensors (Basel) 8(4):128. https://doi.org/10.3390/bios8040128
85. Liandris E, Gazouli M, Andreadou M, Sechi LA, Rosu V, Ikonomopoulos J (2011) Detection of pathogenic mycobacteria based on functionalized quantum dots coupled with immunomagnetic separation. PLoS ONE 6(5):e20026. https://doi.org/10.1371/journal.pone.0020026
86. Ivnitski D, Abdel-Hamid I, Atanasov P, Wilkins E (1999) Biosensors for detection of pathogenic bacteria. Biosens Bioelectron 14(7):599–624. https://doi.org/10.1016/S0956-5663(99)00039-1
87. Schmid RD, Scheller F (1989) Biosensors—applications in medicine, environmental protection and process control. GBF monographs, 13. Weinheim, Federal Republic of Germany: New York, NY, USA
88. Luong JH, Groom CA, Male KB (1991) The potential role of biosensors in the food and drink industries. Biosens Bioelectron 6(7):547–554. https://doi.org/10.1016/0956-5663(91)80018-s
89. Luong JH, Bouvrette P, Male KB (1997) Developments and applications of biosensors in food analysis. Trends Biotechnol 15(9):369–377. https://doi.org/10.1016/S0167-7799(97)01071-8
90. Feng P (1992) Commercial assay systems for detecting foodborne Salmonella: a review. J Food Prot 55:927–934
91. Deshpande SS, Rocco RM (1994) Biosensors and their potential use in food quality-control. Food Technol 48(6):146–150
92. Alvarez-Icaza M, Bilitewski U (1993) Mass production of biosensors. Anal Chem 65 (11):525A–533A
93. Kim RR, Mulchandani A, Zhou W (1996) Biosensor and chemical sensor technology: process monitoring and control. J Am Chem Soc 118(33):7872. https://doi.org/10.1021/ja9656356
94. Kress-Rogers E (1996) Biosensors and electronic noses for practical applications. In: Kress-Rogers E (ed) Handbook of biosensors and electronic noses: medicine, food, and the environment, 1st ed. CRC Press
95. Morgan CL, Newman DJ, Price CP (1996) Immunosensors: technology and opportunities in laboratory medicine. Clin Chem 42(2):193–209
96. Blum LJ (1997) Bio-and chemi-luminescent sensors. World Sci Publ Co Pte Ltd Bio- and Chemi-Lumines Anal Immobilized Reagents. https://doi.org/10.1142/3317
97. Martinkova P, Kostelnik A, Valek T, Pohanka M (2017) Main streams in the construction of biosensors and their applications. Int J Electrochem Sci 12:7386–7403. https://doi.org/10.20964/2017.08.02
98. Bueno J (2014) Biosensors in antimicrobial drug discovery: since biology until screening platforms. J Microb Biochem Technol S 10–002. https://doi.org/10.4172/1948-5948.S10-002
99. Srivastava SK, van Rijn CJ, Jongsma MA (2016) Biosensor-based detection of tuberculosis. RSC Adv 6(22):17759–17771

100. Pavlou AK, Magan N, Jones JM, Brown J, Klatser P, Turner AP (2004) Detection of Mycobacterium tuberculosis (TB) in vitro and in situ using an electronic nose in combination with a neural network system. Biosens Bioelectron 20(3):538–544. https://doi.org/10.1016/j.bios.2004.03.002
101. Das M, Dhand C, Sumana G, Srivastava AK, Vijayan N, Nagarajan R, Malhotra BD (2011) Zirconia grafted carbon nanotubes based biosensor for M. Tuberculosis detection. Appl Phys Lett 99(14):143702. https://doi.org/10.1063/1.3645618
102. Pariwono AM, Lo T, Lim CS, Wang SX, Chan YW (2007) Rapid tuberculosis detection technique for on-site patient screening. J Biomed Pharm Eng 1:27–33
103. Mukundan H, Kumar S, Price DN, Ray SM, Lee Y, Min S, Eum S, Kubicek-Sutherland J, Resnick JM, Grace WK, Anderson AS, Hwang SH, Cho SN, Via LE, Barry III C, Sakamuri R, Swanson BI Rapid detection of Mycobacterium tuberculosis biomarkers in a sandwich immunoassay format using a waveguide-based optical biosensor. Tuberculosis (Edinb) 92(5):407–416. https://doi.org/10.1016/j.tube.2012.05.009
104. Duman M, Piskin E (2010) Detection of Mycobacterium tuberculosis complex and Mycobacterium gordonae on the same portable surface plasmon resonance sensor. Biosens Bioelectron 26:908–912
105. Prabhakar N, Arora K, Arya SK, Solanki PR, Iwamoto M, Singh H, Malhotra BD (2008) Nucleic acid sensor for M. tuberculosis detection based on surface plasmon resonance. Analyst 133(11):1587–1592. https://doi.org/10.1039/b808225a
106. Lee J, Kim J, Ahmed SR, Zhou H, Kim J-M, Lee J (2014) Plasmon-induced photoluminescence immunoassay for tuberculosis monitoring using gold-nanoparticle-decorated graphene. ACS Appl Mater Interfaces 6(23):21380–21388. https://doi.org/10.1021/am506389m
107. McNerney R, Wondafrash BA, Amena K, Tesfaye A, McCash EM, Murray NJ (2010) Field test of a novel detection device for Mycobacterium tuberculosis antigen in cough. BMC Infect Dis 10:161. https://doi.org/10.1186/1471-2334-10-161
108. Hiatt AL, Cliffel DE (2012) Real-time recognition of Mycobacterium tuberculosis and lipoarabinomannan using the quartz crystal microbalance. Sens Actuators B Chem 174:245–252. https://doi.org/10.1016/j.snb.2012.06.095
109. Ren J, He F, Yi S, Cui X (2008) A new MSPQC for rapid growth and detection of Mycobacterium tuberculosis. Biosens Bioelectron 24(3):403–409. https://doi.org/10.1016/j.bios.2008.04.018
110. He F, Zhao J, Zhang L, Su X (2003) A rapid method for determining Mycobacterium tuberculosis based on a bulk acoustic wave impedance biosensor. Talanta 59(5):935–941. https://doi.org/10.1016/S0039-9140(02)00643-4
111. Pang P, Cai Q, Yao S, Grimes CA (2008) The detection of Mycobacterium tuberculosis in sputum sample based on a wireless magnetoelastic-sensing device. Talanta 76(2):360–364. https://doi.org/10.1016/j.talanta.2008.03.008
112. Liong M, Hoang AN, Chung J, Gural N, Ford CB, Min C, Shah RR, Ahmad R, Fernandez-Suarez M, Fortune SM, Toner M, Lee H, Weissleder R (2013) Magnetic barcode assay for genetic detection of pathogens. Nat Commun 4:1752. https://doi.org/10.1038/ncomms2745
113. Koch R (1967) The current state of the struggle against Tuberculosis. In: Nobel lectures, physiology or medicine 1901–1921, Elsevier Publishing Company, Amsterdam

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The Role of Diagnostic Microdevices in the Fight Against Tuberculosis

7

Marina Cañadas-Ortega, Clara Gómez-Cruz, Juan José Vaquero, and Arrate Muñoz-Barrutia

As any doctor can tell you, the most crucial step toward healing is having the right diagnosis. If the disease is precisely identified, a good resolution is far more likely. Conversely, a bad diagnosis usually means a bad outcome, no matter how skilled the physician.

Andrew Weil

Summary

The high incidence and mortality associated with *Mycobacterium tuberculosis* (*M. tb)* bring forward the necessity of rapid and accurate diagnosis. Microfluidic techniques present characteristics able to cover the gaps between current assays and clinical needs for disease management. Moreover, they bring a cost- and time-effective Point of Care (PoC) diagnosis methodology, with multiples advantages of special importance in countries with low income and resources. The devices can be classified according to the diagnosis method, which can be

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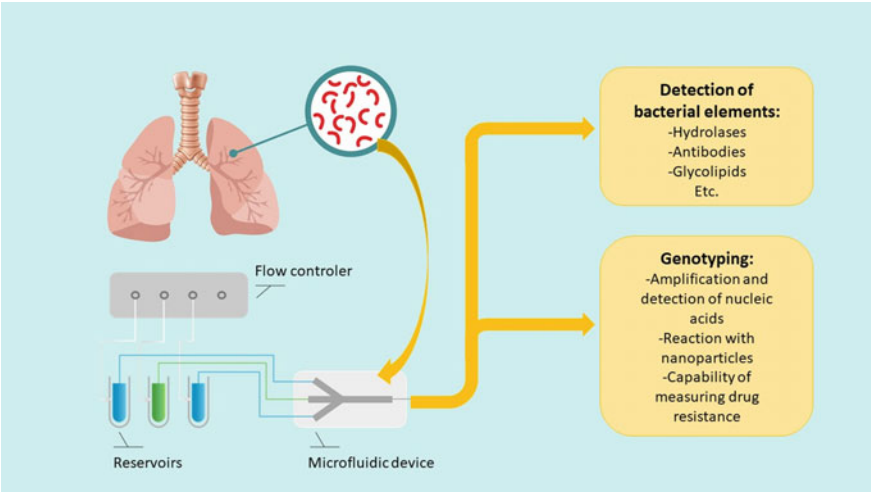
based on the detection of bacterial elements (such as enzymes or antibodies) or on genotyping through amplification of the nucleic acid information. In the latter case, there are devices capable of providing information about the drug resistance of the particular strains, while others provide just a "yes/no" answer, differentiating *M. tb* from other mycobacterial infections. The main goal attempted by all the devices remains to achieve portability and ease of use required for a PoC device, besides the required precision.

Graphical Abstract



The role of microfluidics in tuberculosis (TB) diagnosis. Microfluidic devices can be used to diagnose TB as well as to detect drug-resistant strains. These devices are based on detecting bacterial elements or on genotyping the bacterial nucleic acids.

Keywords

Diagnosis · Immunoassays · Lab-on-a-chip · Microdevices · Tuberculosis

1 Introduction

Tuberculosis (TB) was one of the ten leading causes of death between 2000–2016, making *Mycobacterium tuberculosis (M. tb)* one of the deadliest pathogens in the world. Each year, *M. tb* infects around ten million people and results in over a million deaths worldwide, mainly in developing countries and low-income areas [1]. Most infections remain latent, but around 10% of cases develop an active

infection, where the bacteria multiply and invade tissues. *M. tb* infections primarily affect the lungs, but other organs like the brain, the kidneys, or the spine can also be affected [2].

The lack of fast and reliable methods for diagnosing and treating TB and the development of new drug-resistant (DR) strains of *M. tb* (DR-TB) are some of the most pressing issues in the fight against TB. Regarding TB diagnosis, the main issue is the lack of a reliable, cost-effective diagnostic tool for Point of Care (POC) testing.

Worldwide, the cheapest and most common diagnostic method of pulmonary TB (PTB) consists of sputum smear microscopy, but it has low sensitivity and specificity [3]. Other detection methods like tuberculin skin tests or chest X-rays present a low potential for PoC testing. The detection of antibodies lacks accuracy and cost-effectiveness, while detecting the wall lipopolysaccharide lipoarabinomannan (LAM) has inadequate sensitivity. Molecular genotypic assays based on nucleic acid amplification tests require a laboratory setting with temperature control and complex setups.

Previous work has covered more extensively these detection methodologies [4–6]. This lack of a fast diagnosis test to guide the correct prescription of antibiotics is one factor that has led to the growth of DR-TB. Other factors in the rise of antibiotic resistance are improper self-medication and poor adherence to the long-term anti-TB therapies (ATT), the loose use of these products in the food industry, and incorrect monitoring of treatment efficacy [7]. DR strains can be classified as multidrug-resistant (MDR) or extensively drug-resistant (XDR). MDR strains do not respond to treatment with isoniazid and rifampicin, while XDR ones are resistant to both first-line anti TB drugs and at least one main second-line drug (such as capreomycin, kanamycin, or amikacin) [8].

Treating these 'superbugs' infections requires more complex drug combinations, involving multiple second-line anti-TB drugs and often translating into longer hospital stays. This not only significantly increases the medical costs but also entails enhanced human costs, as these drugs present more side-effects than the first-line core ones [9–11]. These 'superbugs' with reduced drug susceptibility limit the treatment options and force combinations of medicaments to achieve the same result that susceptible strains require a shorter treatment with just two core drugs. The diagnosis of DR-TB requires either the genotyping of DNA regions in centralized laboratories or long phenotypic assays like microscopic observation or culture methods. This leads to a harder diagnosis and worse prescription guidance in areas with low resources. For this reason, developing new diagnostic tools that are accurate, fast, and cheap is critical to accurately detect active and latent TB and the drug susceptibility of the strain at the PoC. This would refine the treatment to be applied and its monitoring over time. To that effect, incorporating microfluidic devices, which can accurately control small fluid volumes [12], can improve the cost, time, and portability of current methods.

These devices take advantage of electrical or mechanical properties in the microscale that can be applied to answer biological demands, such as molecule detection or component mixing or separation. Accurate fluid manipulation of reagents and samples enables complex reactions that can be reproduced inside the microdevices, while the low volumes are required to reduce the use of testing agents, therefore minimizing the costs [13]. Many microdevices are designed as preloaded disposable platforms with reagents stable over time, permitting their shipping and storage for a certain number of months [14]. Most of the microfluidic devices intended to be used for diagnosis are simple, disposable devices with a *sample-in, answer-out* architecture. Following this, the sample is introduced in the chip and automatically processed and evaluated without further human intervention, which allows easy manipulation, not only reducing contamination and contagious risks but going beyond the need of laboratory settings [15].

In this chapter, the advances that have taken place in the last decade in the field of TB diagnosis using microfluidics are reviewed, stating the method of diagnosis employed as well as their state of development.

2 Diagnostic Microdevices for Tuberculosis

The detection and diagnosis of *M. tb* can be performed in different ways, ranging from skin tests to imaging tests, passing through different modalities of molecular tests. During the last decade, this last approach has been the focus of many research works; to this extent, microdevices have turned out to be enormously useful due to the numerous advantages that microfluidic assays present over bulk liquid analyses (i.e., lower reagent volume, portability, incremented number of assays). The technologies presented in this chapter can be distinguished by whether their focus is on detecting bacterial elements or on genotyping. Some of the genotyping technologies require off-chip DNA extraction and amplification, while others manage to perform on-chip amplification or even no amplification step. These platforms also vary in their proximity to the PoC and their development stage: while some could be applied soon, others are just proofs-of-concept, with many improvements ahead. However, this great number of published works gives us an idea of the importance of microfluidic technologies in improving the diagnosis of TB.

2.1 Tuberculosis Diagnosis from Bacterial Elements

2.1.1 Fluorescent Detection of BlaC

An appropriate marker to rapidly diagnose TB is the hydrolase β-lactamase (BlaC) due to its specific enzymatic activity. Some examples are the collaborative works between the Texas A&M Health Science Center and the Stanford University School of Medicine [16, 17]. These groups developed two different microfluidic devices to take advantage of the properties of BlaC for the diagnosis of TB.

In 2015, a polydimethylsiloxane (PDMS) microfluidic system was used to quantify *M. tb* by encapsulating the bacteria with a BlaC fluorogenic probe [18]. First, picolitre drops containing the bacteria and the fluorogenic probe were generated and incubated before fluorescence detection with an inverted microscope. The system gave a detection limit of ten colony-forming units per milliliter (CFU/ml) and was validated using *E. coli* and an attenuated *Mycobacterium bovis* strain (Bacillus Calmette-Guérin, BCG) to prove the specificity to *Mycobacterium*.

The main disadvantages of the system reside in its inability to differentiate between different types of *Mycobacterium* and a limitation in the number of events detected by the droplet generation rate. Although consisting only in a proof-of-concept far from the PoC applications, this platform would allow the quantitative evaluation of therapeutic methods and drug resistance of the strains.

More recently, the same group developed a dual-targeting strategy to achieve fluorescent labeling of single bacilli, to be used within a self-driven PDMS microfluidic chip [19]. This new strategy, based on the fluorescence of the CDG-DNB3[1] molecular probe once the BlaC is activated, allowed automated quantification of individual bacilli and discrimination between live and dead BCG. Moreover, the probe showed specificity for *M. tb* over other non-tuberculosis mycobacteria. The device consisted of four parallel channels flowing from a sample loading area, through a fluid flow delay region and across a detection window (Fig. 1) for detection and counting of fluorescent BCG using publicly available software.[2] The device was mounted on a standard glass microscope for its use with an inverted microscope and could detect up to 100 CFU/ml in a fast, non-invasive and automatic way. The platform can be used for further cell sorting for drug susceptibility tests or culturing, enhancing the assessment of ATT efficacy.

2.1.2 Immunoassays

Immunoassays are a quantitative measure of the analyte present in a sample based on the reaction of the antigen with an antibody [20]. Jing et al. [21] used this antibody-antigen interaction to detect airborne *M. tb* using a two-step microfluidic device (Fig. 2 left). In the first device, the airborne bacterial cells are captured by drowning air through a micropump and enriching it using chaotic flow that causes its adhesion to the channel wall; then, they are lysed and flushed by the lysis buffer into an immunoassay chip. This second chip is preloaded with microspheres coated by polyclonal antibodies, forming an immune reaction column. An inverted fluorescence microscope was used to collect the immune adsorption reaction signal. The whole system has the capacity to analyze low concentrations, about 10^2 cells, in 50 min, differentiating *M. tb* from other airborne bacteria and allowing parallel analysis of multiple samples. The system was validated with different concentrations of BCG, *E. coli* as the negative control, and Ag85B secretory protein of *M. tb* as the positive control.

[1] Dual-targeting fluorogenic probe with a BlaC sensing unit, a fluorescent reporter and an enzymatic signal trapping unit.
[2] FlowJo v10.

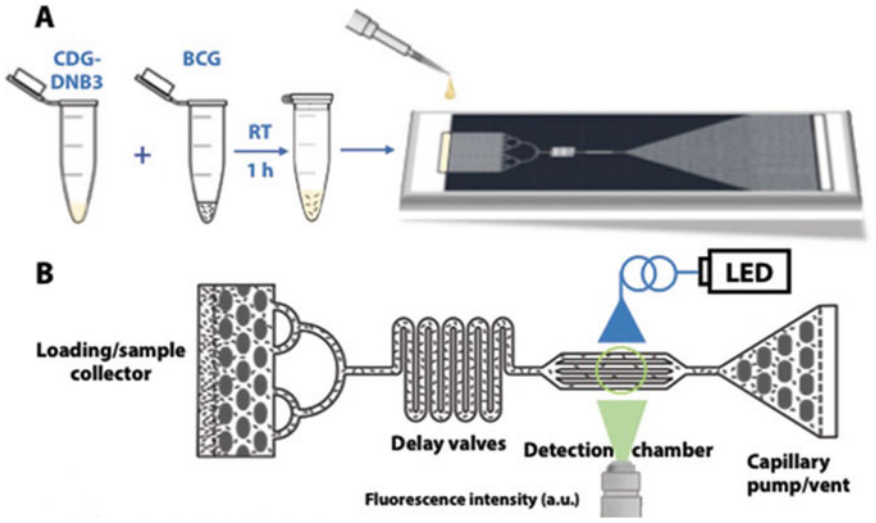


Fig. 1 Schematics of the workflow and image acquisition on the microdevice presented in [19] for labeling of bacteria: **a** staining, incubation, and loading in the device; **b** flow of sample from the collector to the detection chamber. (*Adapted with permission from* [19])

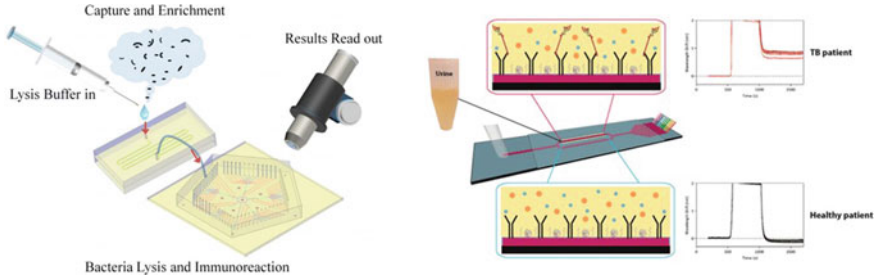


Fig. 2 Representation of the systems based on immunoassays for TB diagnosis: left, an image of the two chips that conform the airborne detection platform presented in [21], (leftmost, airborne bacteria enrichment chip; rightmost, immunoassay chip); right, the layout of the photonic sensor developed for direct detection of LAM glycolipid widely present in the mycobacterial cell wall in urine from [26]. (*Adapted with permission from* [21] *and* [26])

Another potential target for immunoassay detection of *M. tb* is LAM, a glycolipid widely present in the mycobacterium cell wall, with a role in the inactivation of macrophages and excreted in urine [22]. With the proven potential for TB diagnostics [23–25], a nanophotonic platform based on a Mach-Zehnder interferometer (MZI) transducer was developed to directly detect LAM in urine [26]. A disposable cartridge combined the MZI transducer with a spectral filter (Fig. 2 right). After injection onto the MZI sensing arm, the sample is driven through a microfluidic channel. As the sensor surface is functionalized with monoclonal

antibodies against LAM, the binding event changes the refractive index of the MZI transducer, causing a detectable spectral shift once illuminated with a broad-band light source. The system's limit of detection (LoD) for LAM in undiluted and unprocessed urine is 475 pg/ml (27.14 pM). Cases with LAM signals higher than 90 pM are considered TB positive. The device was tested using two different fluid injector mechanisms on 20 real patient samples (ten without and ten with TB; from these ten, five were HIV positive while the other five were negative). The results proved that LAM signal returns to the baseline in healthy patients, while high wavelength shifts are obtained for TB patients, regardless of their co-infection with HIV. Hence, this platform can detect TB infections regardless of HIV co-infection in 15 min, being low-cost, single-use, and not requiring special laboratory infrastructure, being thus very suited for PoC testing of TB.

2.2 TB Diagnosis Through Genotyping

After describing some methods for TB diagnosis based on antigen or bacterial specific compounds detection, it is necessary to focus on the methodology that has drawn a great effort due to the possibilities offered at the PoC. In the last decade, the use of nucleic acid amplification tests (NAATs) for molecular diagnosis has grown due to their high specificity, sensitivity, and rapidity compared to traditional methods [27, 28].

2.2.1 Nucleic Acids Amplification Tests for Diagnosis

In order to detect DNA sequences, sample extraction and amplification of the helix chains are required. Although this is typically done through polymerase chain reaction (PCR), alternative methods are continuously being developed [29] due to the difficulties for PoC implementation entailed by the temperature changes required by PCR. Despite this, several commercialized systems use PCR technology for DNA amplification, as GeneXpert [30, 31] or TrueNAT RT-PCR [32, 33], which have been thoroughly evaluated with clinical samples of varied conditions.

Loop-mediated isothermal amplification (LAMP) has proven to be useful as a replacement of PCR for DNA amplification on-chip to reduce both the time and instrumentation required. In 2014, two different microdevices based on LAMP for *M. tb* diagnostics were developed, one with detection based on turbidity [34], while the second relied on an electrochemical reaction with methylene blue for bacterial identification [35].

In the first microdevice [34], the LAMP reaction is carried out in capillary glass tubes (Fig. 3 left) in an attempt to overcome biocompatibility problems of polymeric materials and fabrication issues. The reaction solution is prepared to contain magnesium-pyrophosphate complexes that cause precipitation. Results can be assessed based on the turbidity of the sample, thus allowing detection with a bare eye. The sensitivity and specificity of microfluidic LAMP in patient samples were higher than 90% and 95%, respectively. The LoD was one pg/ml, accomplished in 15 min. With respect to the second device [35], the LAMP reaction was carried out

Fig. 3 Images of the microdevices that take advantage of loop-mediated isothermal amplification (LAMP) reaction for TB diagnosis through genotyping: left, picture of the glass capillaries for turbidimetric evaluation of the precipitate formed after LAMP reaction in the capillaries; right, indium tin oxide electrodes used for redox detection based on methylene blue reaction with *M. tb* DNA. It contains eight isolated electrochemical microchambers, and an inlet hole and an outlet hole are drilled on each microchamber. (*Adapted with permission from* [34] *and* [35])

on a laser-etched indium tin oxide (ITO) electrode-based chip manufactured in glass and PDMS. The chip includes eight ITO electrodes, a counter electrode, and a base point for reference (Fig. 3 right). The detection reaction relies on the intercalation of methylene blue in the amplified DNA helices, producing a measurable change in the redox current. The system was evaluated using bacterial cultures from clinical sputum specimens, obtaining a sensitivity detection limit of 28 copies/ml of the *M. tb* after the amplification process. The process required 45 min for the test after the DNA extraction had been accomplished. Although presenting important reductions in time, further optimizations in the automatization of the data acquisition and processing and in the selection of the most appropriate primers would be required to achieve on-chip nucleic acid extraction and pretreatment for reliable applications at the PoC.

Another technique that has been commonly used to replace PCR is recombinase polymerase amplification (RPA). Particularly, a *sample-in, answer-out* system took advantage of the isothermal properties of this method to provide quantitative measures of nucleic acids [36]. The platform consists of a cartridge (Fig. 4 left) with the reagents, pipettor, the chip itself, and a portable instrument for temperature control, liquid handling, and optical systems. The chip, made of PDMS (Fig. 4 right), contains a microwell array so that the fluorescent detection reaction is repeated multiple times. All the processes (DNA extraction, processing, etc.) are performed directly from bodily fluids inside the chip. The detection rate was higher when tested in saliva than in serum (91.3 compared to 81.7%). The platform was able to detect *M. tb* when mixed with other *Mycobacteria* but provided no result when non-tuberculous *Mycobacteria* were used as the negative control. The high specificity and the *sample-in, answer-out* design make the system suitable for PoC testing.

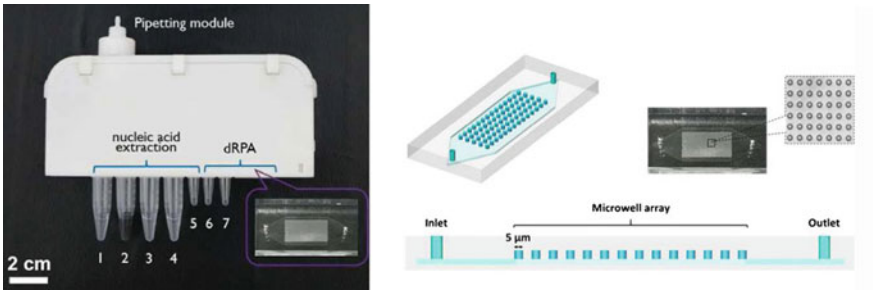


Fig. 4 Pictures of the device developed in [36], composed of cartridge and portable instrument: left, the cartridge containing the reagents, chip, and pipettor for the nucleic acid extraction and amplification using Recombinase Polymerase Amplification (RPA). The reagents, as labeled in the picture, are (1) lysis buffer, (2) magnetic beads within a binding buffer, (3) wash buffer I, (4) wash buffer II, (5) elution buffer, (6) RPA mix, and (7) magnesium acetate solution; right, 3D representation and photographs of the chip for the fluorescent detection reaction, where the microwells patterned in the PDMS are clearly observed. (*Adapted with permission from* [36])

2.2.2 Nanoparticles Applied to Genotyping

Nanoparticles (NPs) are an important tool that, in combination with microfluidics, allow the detection of DNA target sequences–rather than biochemical molecules–in a quicker and more automatized manner. Between the different kinds of NPs existing, gold nanoparticles have been used in a wide variety of biomedical areas as biosensing, cancer therapy, or drug delivery [37]. Baptista et al. proved in 2009 the feasibility of such nanoparticles for identifying *M. tb* complex (MTBC) [38]. They developed in 2013 a microfluidic device for genetic detection of *M. tb* [39], and in 2014, a lab-on-paper platform for molecular diagnostic testing, being *M. tb* one of its potential applications [40].

The microfluidic chip detection technology [39] is based on a colorimetric change of the Au-nanoprobes–coated with specific DNA sequences–from red to blue after $MgCl_2$ salt-induced aggregation. This color variation is mediated by the contact with the complementary DNA strands present in the sample solution, thoroughly proven by the same group [41]. In this way, a blue sample indicates Au-NPs aggregation due to the absence of a complementary target, while a red sample indicates the presence of the MTBC-specific complementary target. This genomic biosensor of DNA-functionalized NPs is integrated into a low-cost PDMS chip in which two optical fibers are inserted to guide the light inside the microchannel (Fig. 5, top left). A 395 base-pair (bp) characteristic fragment of the RNA polymerase subunit was used to validate the device and unequivocally identify the MTBC. In order to check for false positives and negatives, several conditions such as the presence/absence of complementary DNA regions or the use of colloidal or aggregated NPs were used. The LoD of this platform was situated in 90 ng of genetic material to be detected in approximately 30 min. This work was a proof-of-concept, being far away from PoC applications as could be analyzing patient samples, which require extensive preprocessing that is not contemplated

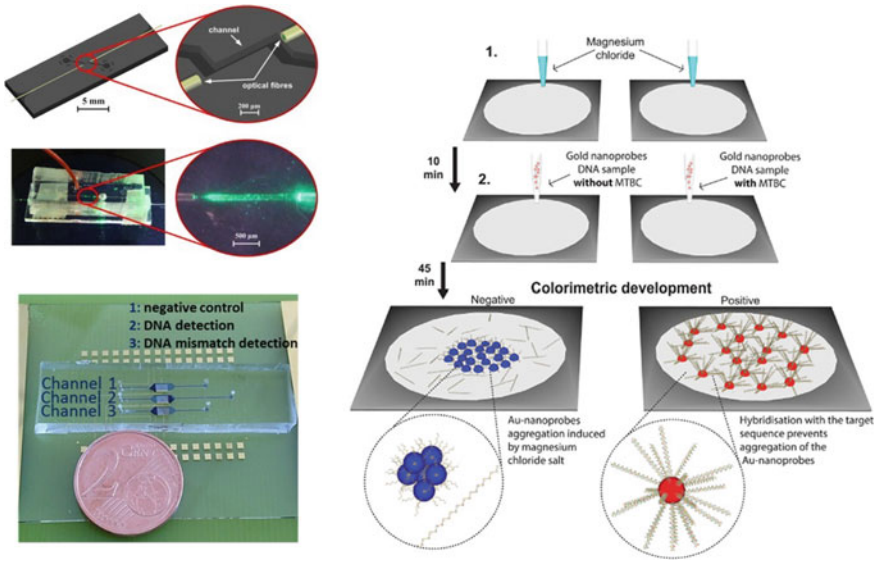


Fig. 5 Devices that combine microfluidics with nanoparticles for genomic diagnosis of TB: top left, microfluidic chip design from [39] and channel filled with gold nanoparticles, with the color changes induced by aggregation of the gold nanoparticles in the presence of salt and detected by the optical fibers. Adapted from Biosensors and Bioelectronics; right, illustration of the lab-on-paper device [40] summarizes the nanoparticles' hybridization and aggregation used in both works. Positive samples get hybridized, preventing aggregation of the gold nanoprobes maintaining the red color. Aggregation of the particles in the absence of the *Mycobacterium tuberculosis* complex induces a detectable change of color from red to blue. From Nanotechnology; bottom left, PDMS microfluidic chip with electrochemical chambers containing gold electrodes coated with carbon nanotubes [46]. The sensors in the chambers get deployed, allowing to capture of DNA strands by hybridization, which produces a measurable change in the redox current. (*Adapted with permission from* [39, 40]*, and* [46])

here. Although the technology developed appears to be promising, the authors have lately concentrated on simplifying the chip regardless of the biological target and on the molecular method for different targets, particularly in chronic myeloid leukemia.

Regarding paper-based analytical devices, in [40], a colorimetric assay with Au-NPs uses wax-printing on paper as a substrate for microfluidic applications. A 384-well plate is printed with wax and impregnated with $MgCl_2$ salt for inducing color change due to aggregation of Au-nanoprobes following the same principle as in the previous device (Fig. 5, right). The time required for the assay, which includes an off-chip PCR amplification step, rounds two and a half hours. Despite the requirement of PCR (which entails trained staff and appropriate level laboratory facilities), the analysis can be done without laboratory access using a simple data analysis tool on a smartphone. Future work is thus required to simplify or even eliminate the amplification step, as well as optimization for direct detection of clinical samples in order to be able to be used at the PoC. Additionally, an extensive characterization of the LoD of the system is mandatory.

In 2017, Tsai et al. [42] combined gold NPs and a lab-on-paper platform for TB detection by taking advantage of the surface plasmon resonance effect. This effect takes place when an incident photon hits a metal surface, creating a plasmon of electron movements in the metal surface. The detection is based on the reflection angle formed by the photon after its incidence on the plasmon; since the plasmon is sensitive to changes in its boundary, adsorption can be easily measured [43]. Similarly, the hybridization event varies the aggregation state, inducing a color change in a label-free environment. This alteration can be determined using a smartphone camera. This work is fairly similar to the previous one; its outcome is produced in less than half the time (60 min compared to 150 min) and provides an LoD of 1.95×10^{-2} ng/ml of required TB DNA.

Gold nanoprobes are the most commonly used NPs; however, other NPs have been studied for TB diagnosis [44, 45]. Although carbon nanotubes are not considered NPs themselves, they lay on the nanometer range and can be integrated into microfluidic platforms. In this case, a PDMS chip could detect label-free DNA using gold electrodes functionalized with multiwalled carbon nanotube/ferrocene coupled to DNA probes [46]. The device consisted of three independent channels over a borosilicate slide that worked as a negative control for DNA detection and mismatch detection. Each channel included an electrochemical chamber and three gold electrodes functioning as counter, reference, and working electrode, respectively (Fig. 5 bottom left). Using a high flow of 150 μl/min, a depletion layer is formed at the sensor's surface, allowing the capture of sample DNA strands by hybridization with the probe. This attachment of the target biomolecule alters the charge transfer, inducing a detectable electrochemical response through a redox reaction. The platform was validated using single-strand oligonucleotide from hepatitis C virus, then applied for direct detection of a wild-type *M. tb* allele in clinical isolated extracted DNA. The LoD was established at 0.7 fM with a total test duration of 90 min. Although DNA extraction is performed off-chip, and improvements in sensitivity could be made by modifying the carbon nanotubes, the amplification step is not strictly required, opening the possibility to PoC applications.

2.2.3 NAATs for Drug Resistance Screening

In order to avoid a long time of cell growth required by *M. tb* to determine its antibiotic susceptibility, a strong interest in sequencing the mutations that produce DR [47] arose in the scientific community. This led to the development of a wide variety of devices that take advantage of microfluidic properties to detect not only the presence of bacteria but also whether the strain was resistant to the most frequently used antibiotics. Most of the devices are intended for the PoC, aiming for a fully integrated *sample-in, answer-out* device that would not require extensive sample preprocessing or the availability of biosafety level 3 (BSL3) laboratories (necessary in handling microbes with lethal potential if inhaled). The sensitivities and specificities of these devices are verified by different sequencing methods or by comparison with traditional drug susceptibility tests (DST). The principles on which these tests are based vary, but most of them focus on three main areas using

microfluidic technology: the development of microarray tests, lab-on-discs, and lab-on-films.

Regarding the microarray tests, Linger et al. [48] detected MDR-TB by integrating a microarray workflow into a microfluidic chamber, converting an open-amplicon microarray test into a closed-amplicon consumable. In this way, the target amplification and microarray hybridization occur simultaneously, combining up to seven processes in a single reaction. A LoD of 100 femtograms (fg) of *M. tb* DNA was achieved. The evaluation was carried out from sputum samples and sediment isolates, being able to diagnose properly and showing more than 99% of concordance with other methodologies. Additionally, the system obtained excellent specificities (higher than 96%) and good sensitivities (75% and 63.6%, respectively) for the detection of rifampin and isoniazid.

Another microarray technology, a TaqMan array card (TAC), was developed by Pholwat et al. [49] to analyze critical regions of ten genes responsible for resistance to nine main anti-TB drugs. Sequence-specific probes were used to detect mutations, and high-resolution melt analysis was used to provide a second layer of detection to cover for missed probes. The system can perform up to 48 different real-time PCRs simultaneously to detect DR mutations in one sample. The accuracy for the different genomic regions varies from 72 to 94% compared to culture-based DSTs. However, PCR requirements in terms of time and price of the reader remain as disadvantages for this platform, as well as the high-quality DNA required by the melt analysis to provide accurate results. Furthermore, the analysis was performed on *M. tb* isolates to sequence and determine DR in isolated strains. Due to this, the authors believe this melt analysis is likely to be limited to pure samples of *M. tb* and fail when presented with clinical specimens or mixed mycobacterial samples.

Lab-on-discs take advantage of centrifugal forces to pump liquids across the microfluidic chambers, eliminating or reducing the needs of complex pumps and tubes, thus making the system more prone for PoC testing [50]. Moreover, in order to fully use this advantage, the changeable temperature regimen required by PCR was substituted by other isothermal techniques. Particularly, Law et al. [51] used an RPA method integrated on a lab-on-a-disc to maintain a constant temperature for the amplification step. RPA advantages rely not only on its working regime at ambient temperature but its rapidness and simplicity in the primer design. Reagents addition from the different chambers (Fig. 6, left) relies on the chamber size and liquid volume, reaching the reaction site at different centrifugation speeds. According to this, shorter and wider channels containing higher volumes will require lower rotational velocity to arrive at the reaction site. When validated with sputum samples (no previous DNA extraction required) with and without the MDR target gene, the LoD was set at 10^2 CFU/ml in 15 min of real-time RPA reaction. The platform can be reutilized after autoclave decontamination and reagent refilling, allowing its reuse as a *sample-in, answer-out* system. More recently, Minero et al. [52] used rolling circle amplification (RCA) as a PCR substitution for the amplification of DNA in a "two-pot" microfluidic disc at 50 ºC. The DNA ligation mixture and the RCA mixture are located in two separate chambers and mixed by spinning after on-disc annealing, initiating the RCA (Fig. 6, right). The LoD

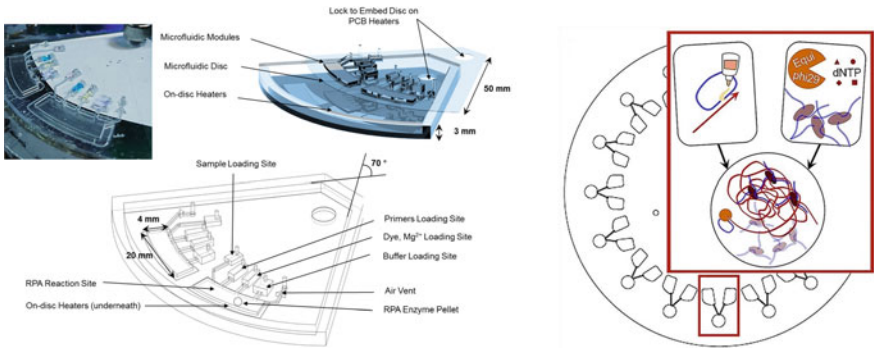


Fig. 6 Representation of the lab-on-disc platforms: left, disc design of [51], based on recombinase polymer amplification; presented as schematics, 3D renders, and photographs. Reagent addition to the reaction site is performed in an orderly manner depending on the spin speed, then mixed by centrifugation; right, "Two-pot" on-disc rolling circle amplification assay [52]. DNA ligation is performed on one chamber, then mixed with RCA components stored in a second independent chamber. (*Adapted with permission from* [51] *and* [52])

obtained was 5 pM after a total assay time of two hours, being able to determine the presence of mutations in the katG gene related to resistance to isoniazid.

Lab-on-films and paper-based microfluidic technologies have strong potential for disease management due to their low price and versatility [53]. In the last two years, the group of Cooney et al. developed first a disposable lab-on-film in which gel elements were printed, forming microfluidic cells in a flexible film substrate [54]. In this device, amplification through asymmetric PCR and hybridization are combined in a single chamber, detecting up to 37 mutations, deletions, or insertions in five main genes related to drug resistance. The processing and detection were automatized through a TruTip workstation (pipette tips with an embedded matrix that isolates nucleic acids) and fluorescence imaging, with a sensitivity of 32 CFU/ml. One year later, this lab-on-film technology was integrated into a bench-top automated system [55], with the addition of a sputum homogenization and cell lysis step using magnetic rotation to increase mixing. This platform is the combination of the previous work of the team, integrating the automated nucleic acid extraction method and the manually operated lab-on-film substrate to obtain an automated system for detection of MDR-TB. The final lab-on-film assembly simplified the amplification and hybridization steps, automated the TruTip process, and provided a detection sensitivity of 43 CFU/ml from raw sputum. Despite its size, its *sample-in, answer-out* system is able to detect DR mutations with a high level of sensitivity in an automated way, being suitable for PoC testing.

Table 1 summarizes all the previous microfluidic devices classified according to the technology used for the detection, as well as their ability to detect antibiotic resistance, sensitivity, or time required to complete the test.

Table 1 Microfluidic devices for diagnosis of *Mycobacterium tuberculosis*

Type of device	Detection methodology	Detects drug resistance?	LoD	Time of assay	POC app.?	Refs.
PDMS microfluidic channel	Fluorescent detection of BlaC	No	10 cfu/ml	Not specified	No	[18]
Microfluidic channel	Fluorescent detection of BlaC	No	100 cfu/ml	20 min	Yes, after testing with patient samples	[19]
PDMS enrichment chip plus immunoassay chip	Immunoassay	No	10^2 cells	50 min	Yes, for airborne detection	[21]
Nanophotonic device in disposable cartridge	Immunoassay	No	475 pg/ml (27.14 pM)	15 min	Yes	[26]
Glass capillary	NAAT-LAMP	No	1 pg/ml	15 min	Yes	[34]
ITO-electrode chip	NAAT-LAMP	No	28 copies/ml	45 min + DNA extraction	Yes, with optimizations on chip DNA extraction	[35]
Cartridge including pipettor and chip	NAAT-RPA	No	$\sim 1.4 \times 10^7$ copies per extraction when tested with 2.9×10^8 cfu/ml	20 min	Yes	[36]
PDMS chip filled with gold NPs	Colorimetric assay	No	90 ng/ml	30 min	No	[39]
Lab-on-paper with gold NPs	Colorimetric assay	No	Not characterized	150 min	Yes, if PCR step is eliminated	[40]
Lab-on-paper with surface plasmon resonance	Colorimetric assay	No	1.95×10^{-2} ng/ml	60 min	Yes	[42]
PDMS chip with gold electrodes	Redox reaction with carbon nanotubes	No	0.7 fM	Around 90 min	Yes	[46]
Microfluidic chamber	Microarray test	Yes	100 fg	Not specified	Yes	[48]
TaqMan array card	Microarray test	Yes	72–94% accuracy	Not specified	No	[49]

(continued)

Table 1 (continued)

Type of device	Detection methodology	Detects drug resistance?	LoD	Time of assay	POC app.?	Refs.
Lab-on-disc	NAAT-RPA	Yes	10^2 cfu/ml	15 min	Yes	[51]
Lab-on-disc	NAAT-RCA	Yes	5 pM	120 min	Yes	[52]
Lab-on-film	Fluorescence imaging	Yes	32 cfu/ml	Not specified	No	[54]
Bench-top automated system using lab-on-film	Fluorescence imaging	Yes	43 cfu/ml	210–630 min	Yes	[55]

ITO, indium tin oxide; LAMP, loop-mediated isothermal AMPlification; LoD, the limit of detection; NAAT, nucleic acids amplification tests; NP, nanoparticles; POC, point of care; PRA, recombinase polymerase amplification

3 Conclusion

Microfluidic devices present numerous advantages compared to more traditional methods in the biomedical field. Nowadays, they constitute a very valuable asset in the basic and translational research field, both for drug testing and development or for clinical diagnosis. In this chapter, we have focused on the potential of microfluidics to provide fast, easy-to-handle options for PoC TB diagnosis. Their reduction in cost, materials, and laboratory requirements can simplify access to fast diagnosis in underdeveloped or resource-constrained countries. An example is the tendency to move towards lab-on-paper chips that can be analyzed using a smartphone, which can be used at the PoC without any medical or scientific equipment. In addition, several new methods are being developed to detect resistant *M. tb* strains, thus allowing for personalized treatments. We expect that incorporating microfluidic devices to the standard methods for diagnosing TB will then suppose an increase in the efficiency of the detection and treatment of the disease, leading to better care for the patients and helping to reduce the burden of TB worldwide.

> *We have to keep searching for novel techniques and use every tool within our grasp to avoid that tuberculosis, which is considered a disease of the past, comes back to be a part of our present. Microfluidics has proven to be a very useful instrument in that particular aspect.*
>
> *Marina Cañadas-Ortega*

Core Messages

- Microfluidic diagnostic tools help bridge the clinical gap for better disease management.
- Cheap, portable, and straightforward microfluidic devices can improve diagnosis in low-income countries.
- Microdevices for TB diagnosis can be based on genotyping or detection of bacterial elements.
- Amplifying nucleic acid information helps determine drug resistance, which improves treatment guidelines.

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References

1. World Health Organization (2019) Global tuberculosis report 2019
2. Jilani TN, Avula A, Zafar Gondal A, Siddiqui AH (2020) Active tuberculosis. In: StatPearls. StatPearls Publishing, Treasure Island (FL)
3. Davies PDO, Pai M (2008) The diagnosis and misdiagnosis of tuberculosis. Int J Tuberc Lung Dis Off J Int Union Tuberc Lung Dis 12:1226–1234
4. Mani V, Wang S, Inci F, De Libero G, Singhal A, Demirci U (2014) Emerging technologies for monitoring drug-resistant tuberculosis at the point-of-care. Adv Drug Deliv Rev 78:105–117. https://doi.org/10.1016/j.addr.2014.05.015
5. Wang S, Inci F, De Libero G, Singhal A, Demirci U (2013) Point-of-care assays for tuberculosis: role of nanotechnology/microfluidics. Biotechnol Adv 31:438–449. https://doi.org/10.1016/j.biotechadv.2013.01.006
6. Dheda K, Ruhwald M, Theron G, Peter J, Yam WC (2013) Point-of-care diagnosis of tuberculosis: past, present and future. Respirol Carlton Vic 18:217–232. https://doi.org/10.1111/resp.12022
7. Ventola CL (2015) The antibiotic resistance crisis: part 1: causes and threats. P T Peer-Rev J Formul Manag 40:277–283
8. Eker B, Ortmann J, Migliori GB, Sotgiu G, Muetterlein R, Centis R, Hoffman H, Kirsten D, Schaberg T, Ruesch-Gerdes S, Lange C (2008) Multidrug- and extensively drug-resistant tuberculosis, Germany. Emerg Infect Dis 14:1700–1706. https://doi.org/10.3201/eid1411.080729
9. Young DB, Perkins MD, Duncan K, Barry CE 3rd (2008) Confronting the scientific obstacles to global control of tuberculosis. J Clin Invest 118:1255–1265. https://doi.org/10.1172/JCI34614
10. Blöndal K (2007) Barriers to reaching the targets for tuberculosis control: multidrug-resistant tuberculosis. Bull World Health Organ 85:387–90; discussion 391–394. https://doi.org/10.2471/06.035345
11. Yang TW, Park HO, Jang HN, Yang JH, Kim SH, Moon SH, Buyn JH, Lee CE, Kim JW, Kang DH (2017) Side effects associated with the treatment of multidrug-resistant tuberculosis at a tuberculosis referral hospital in South Korea: a retrospective study. Medicine (Baltimore) 96:e7482. https://doi.org/10.1097/MD.0000000000007482
12. Ahn CH, Choi J-W (2010) Microfluidic devices and their applications to lab-on-a-chip. In: Bhushan B (ed) Springer handbook of nanotechnology. Springer, Berlin, Heidelberg, pp 503–530
13. Polla DL, Erdman AG, Robbins WP, Markus DT, Diaz-Diaz J, Rizq R, Nam Y, Brickner HT, Wang A, Krulevitch P (2000) Microdevices in medicine. Annu Rev Biomed Eng 2:551–576. https://doi.org/10.1146/annurev.bioeng.2.1.551
14. Hauck TS, Giri S, Gao Y, Chan WCW (2010) Nanotechnology diagnostics for infectious diseases prevalent in developing countries. Adv Drug Deliv Rev 62:438–448. https://doi.org/10.1016/j.addr.2009.11.015
15. Albert-Smet I, Marcos-Vidal A, Vaquero JJ, Desco M, Muñoz-Barrutia A, Ripoll J (2019) Applications of light-sheet microscopy in microdevices. Front Neuroanat 13:1. https://doi.org/10.3389/fnana.2019.00001
16. Xie H, Mire J, Kong Y, Chang M, Hassounah HA, Thornton CN, Sacchettini JC, Cirillo JD, Rao J (2012) Rapid point-of-care detection of the tuberculosis pathogen using a BlaC-specific fluorogenic probe. Nat Chem 4:802–809. https://doi.org/10.1038/nchem.1435
17. Sule P, Tilvawala R, Mustapha T, Hassounah H, Noormohamed A, Kundu S,Graviss EA, Walkup GK, Kong Y, Cirillo JD (2019) Rapid tuberculosis diagnosis using reporter enzyme fluorescence. J Clin Microbiol 57. https://doi.org/10.1128/JCM.01462-19
18. Lyu F, Xu M, Cheng Y, Xie J, Rao J, Tang SK (2015) Quantitative detection of cells expressing BlaC using droplet-based microfluidics for use in the diagnosis of tuberculosis. Biomicrofluidics 9:044120. https://doi.org/10.1063/1.4928879

19. Cheng Y, Xie J, Lee K-H, Gaur RL, Song A, Dai T, Ren H, Wu J, Sun Z, Banaei N, Akin D, Rao J (2018) Rapid and specific labeling of single live *Mycobacterium tuberculosis* with a dual-targeting fluorogenic probe. Sci Transl Med 10. https://doi.org/10.1126/scitranslmed.aar4470
20. Darwish IA (2006) Immunoassay methods and their applications in pharmaceutical analysis: basic methodology and recent advances. Int J Biomed Sci IJBS 2:217–235
21. Jing W, Jiang X, Zhao W, Liu S, Cheng X, Sui G (2014) Microfluidic platform for direct capture and analysis of airborne *Mycobacterium tuberculosis*. Anal Chem 86:5815–5821. https://doi.org/10.1021/ac500578h
22. Whitelaw A, Sturm W (2009) Microbiological testing for *Mycobacterium tuberculosis*. Tuberc Compr Clin Ref 1st Ed 169–178
23. Chan ED, Reves R, Belisle JT, Brennan PJ, Hahn WE (2000) Diagnosis of tuberculosis by a visually detectable immunoassay for lipoarabinomannan. Am J Respir Crit Care Med 161:1713–1719. https://doi.org/10.1164/ajrccm.161.5.9908125
24. Songkhla MN, Tantipong H, Tongsai H, Angkasekwinai N (2019) Lateral flow urine lipoarabinomannan assay for diagnosis of active tuberculosis in adults with human immunodeficiency virus infection: a prospective cohort study. Open Forum Infect Dis 6: ofz132. https://doi.org/10.1093/ofid/ofz132
25. Sigal GB, Pinter A, Lowary TL, Kawasaki M, Li A, Mathew A, Tsionsky RB, Plisova T, Shen K, Katsuragi K, Choudhary A, Honnen WJ, Nahid P, Denkinger CM, Broger T (2018) A novel sensitive immunoassay targeting the 5-methylthio-d-xylofuranose-lipoarabinomannan epitope meets the WHO's performance target for tuberculosis diagnosis. J Clin Microbiol 56. https://doi.org/10.1128/JCM.01338-18
26. Ramirez-Priego P, Martens D, Elamin AA, Soetaert P, Van Roy W, Vos R, Anton B, Bockstaele R, Becker H, Singh M, Bienstman P, Lechuga LM (2018) Label-free and real-time detection of tuberculosis in human urine samples using a nanophotonic point-of-care platform. ACS Sens 3:2079–2086. https://doi.org/10.1021/acssensors.8b00393
27. Peralta G, Barry P, Pascopella L (2016) Use of nucleic acid amplification tests in tuberculosis patients in California, 2010–2013. Open Forum Infect Dis 3:ofw230. https://doi.org/10.1093/ofid/ofw230
28. Eddabra R, Ait Benhassou H (2018) Rapid molecular assays for detection of tuberculosis. Pneumonia Nathan Qld 10:4. https://doi.org/10.1186/s41479-018-0049-2
29. Massung RF (2005) DNA amplification: current technologies and applications. Emerg Infect Dis 11:357–357. https://doi.org/10.3201/eid1102.041049
30. Zeka AN, Tasbakan S, Cavusoglu C (2011) Evaluation of the GeneXpert MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in pulmonary and extrapulmonary specimens. J Clin Microbiol 49:4138–4141. https://doi.org/10.1128/JCM.05434-11
31. Saeed M, Ahmad M, Iram S, Riaz S, Akhtar M, Aslam M (2017) GeneXpert technology. A breakthrough for the diagnosis of tuberculous pericarditis and pleuritis in less than 2 hours. Saudi Med J 38:699–705. https://doi.org/10.15537/smj.2017.7.17694
32. Mangayarkarasi V, Sneka P, Sujith R, Jayaprakash J (2019) Ergonomic diagnostic tool based on chip mini RT-PCR for diagnosis of pulmonary and extra pulmonary tuberculosis. J Pure Appl Microbiol 13:1185–1190. https://doi.org/10.22207/JPAM.13.2.58
33. Nikam C, Kazi M, Nair C, Jaggannath M, Manoj M, Vinaya R, Shetty A, Rodrigues C (2014) Evaluation of the Indian TrueNAT micro RT-PCR device with GeneXpert for case detection of pulmonary tuberculosis. Int J Mycobacteriology 3:205–210. https://doi.org/10.1016/j.ijmyco.2014.04.003
34. Rafati A, Gill P (2014) Microfluidic method for rapid turbidimetric detection of the DNA of *Mycobacterium tuberculosis* using loop-mediated isothermal amplification in capillary tubes. Microchim Acta 182:523–530. https://doi.org/10.1007/s00604-014-1354-y

35. Luo J, Fang X, Ye D, Li H, Chen H, Zhang S, Kong J (2014) A real-time microfluidic multiplex electrochemical loop-mediated isothermal amplification chip for differentiating bacteria. Biosens Bioelectron 60C:84–91. https://doi.org/10.1016/j.bios.2014.03.073
36. Yang H, Chen Z, Cao X, Li Z, Stavrakis S, Choo J, DeMello AJ, Howes PD, He N (2018) A sample-in-digital-answer-out system for rapid detection and quantitation of infectious pathogens in bodily fluids. Anal Bioanal Chem 410. https://doi.org/10.1007/s00216-018-1335-9
37. Versiani A, Andrade L, Martins E, Scalzo S, Geraldo JM, Chaves CR, Ferreira DC, Ladeira M, Guatimosim S, Ladeira L, da Fonseca FG (2016) Gold nanoparticles and their applications in biomedicine. Future Virol 13. https://doi.org/10.2217/fvl-2015-0010
38. Costa P, Amaro A, Botelho A, Inácio J, Baptista PV (2010) Gold nanoprobe assay for the identification of mycobacteria of the *Mycobacterium tuberculosis* complex. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis 16:1464–1469. https://doi.org/10.1111/j.1469-0691.2009.03120.x
39. Bernacka-Wojcik I, Lopes P, Vaz AC, Veigas B, Wojcik PJ, Simões P, Barata D, Fortunato E, Baptista PV, Aguas H, Martins R (2013) Bio-microfluidic platform for gold nanoprobe based DNA detection–application to *Mycobacterium tuberculosis*. Biosens Bioelectron 48:87–93. https://doi.org/10.1016/j.bios.2013.03.079
40. Costa MN, Veigas B, Jacob JM, Santos DS, Gomes J, Baptista JV, Martins R, Inácio J, Fortunato E (2014) A low cost, safe, disposable, rapid and self-sustainable paper-based platform for diagnostic testing: lab-on-paper. Nanotechnology 25:094006. https://doi.org/10.1088/0957-4484/25/9/094006
41. Baptista P, Pereira E, Eaton P, Doria G, Miranda A, Gomes I, Quaresma P, Franco R (2008) Gold nanoparticles for the development of clinical diagnosis methods. Anal Bioanal Chem 391:943–950. https://doi.org/10.1007/s00216-007-1768-z
42. Tsai T-T, Huang C-Y, Chen C-A, Shen SW, Wang MC, Cheng CM, Chen CF (2017) Diagnosis of tuberculosis using colorimetric gold nanoparticles on a paper-based analytical device. ACS Sens 2:1345–1354. https://doi.org/10.1021/acssensors.7b00450
43. Nguyen HH, Park J, Kang S, Kim M (2015) Surface plasmon resonance: a versatile technique for biosensor applications. Sensors 15:10481–10510. https://doi.org/10.3390/s150510481
44. Bhusal N, Shrestha S, Pote N, Alocilja EC (2018) Nanoparticle-based biosensing of tuberculosis, an affordable and practical alternative to current methods. Biosensors 9. https://doi.org/10.3390/bios9010001
45. Gupta AK, Singh A, Singh S (2019) Diagnosis of tuberculosis: nanodiagnostics approaches. NanoBioMed 261–283. https://doi.org/10.1007/978-981-32-9898-9_11
46. Zribi B, Roy E, Pallandre A, Chebil S, Koubaa M, Mejri N, Gomez HM, Sola C, Horri-Youssoufi H, Haghiri-Gosnet AM (2016) A microfluidic electrochemical biosensor based on multiwall carbon nanotube/ferrocene for genomic DNA detection of *Mycobacterium tuberculosis* in clinical isolates. Biomicrofluidics 10:014115. https://doi.org/10.1063/1.4940887
47. Cohen KA, Manson AL, Desjardins CA, Abeel T, Earl AM (2019) Deciphering drug resistance in *Mycobacterium tuberculosis* using whole-genome sequencing: progress, promise, and challenges. Genome Med 11:45. https://doi.org/10.1186/s13073-019-0660-8
48. Linger Y, Knickerbocker C, Sipes D, Golova J, Franke M, Calderon R, Lecca L, Thakore HR, Qu P, Kukhtin A, Murray MB, Cooney C, Chandler D (2018) Genotyping multidrug-resistant *Mycobacterium tuberculosis* from primary sputum and decontaminated sediment with an integrated microfluidic amplification microarray test. J Clin Microbiol 56:e01652-17. https://doi.org/10.1128/JCM.01652-17
49. Pholwat S, Liu J, Stroup S, Gratz J, Banu S, Mazidur Rahman SM, Ferdous SS, Foongladda S, Boonlert D, Ogarkov O, Zhdanova S, Kibiki G, Heysell S, Houpt E (2015) Integrated microfluidic card with TaqMan probes and high-resolution melt analysis to detect tuberculosis drug resistance mutations across 10 genes. mBio 6:e02273. https://doi.org/10.1128/mBio.02273-14

50. Kim T-H, Sunkara V, Park J, Kim CJ, Woo HK, Cho YK (2016) A lab-on-a-disc with reversible and thermally stable diaphragm valves. Lab Chip 16:3741–3749. https://doi.org/10.1039/C6LC00629A
51. Law ILG, Loo JFC, Kwok HC, Yeung HY, Leung CHH, Hui M, Wu SY, Chan SH, Kwan YW, HoHP KSK (2018) Automated real-time detection of drug-resistant *Mycobacterium tuberculosis* on a lab-on-a-disc by recombinase polymerase amplification. Anal Biochem 544:98–107. https://doi.org/10.1016/j.ab.2017.12.031
52. Minero A, Bagnasco M, Fock J, Tian B, Garbarino B, Hansen MF (2020) Automated on-chip analysis of tuberculosis drug-resistance mutation with integrated DNA ligation and amplification. Anal Bioanal Chem 412:2705–2710. https://doi.org/10.1007/s00216-020-02568-x
53. Sher M, Zhuang R, Demirci U, Asghar W (2017) Paper-based analytical devices for clinical diagnosis: recent advances in the fabrication techniques and sensing mechanisms. Expert Rev Mol Diagn 17:351–366. https://doi.org/10.1080/14737159.2017.1285228
54. Kukhtin AC, Sebastian T, Golova J, Perov A, Knickerbocker C, Linger Y, Bueno A, Qu P, Villanueva M, Holmberg RC, Chandler DP, Cooney CG (2019) Lab-on-a-film disposable for genotyping multidrug-resistant *Mycobacterium tuberculosis* from sputum extracts. Lab Chip 19:1217–1225. https://doi.org/10.1039/C8LC01404C
55. Kukhtin AV, Norville R, Bueno A, Qu P, Parrish N, Murray M, Chandler DP, Holmberg RC, Cooney CG (2020) A benchtop automated sputum-to-genotype system using a lab-on-a-film assembly for detection of multidrug-resistant *Mycobacterium tuberculosis*. Anal Chem 92:5311–5318. https://doi.org/10.1021/acs.analchem.9b05853

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Immunodiagnostics of Tuberculosis: Recent Discoveries

8

Shima Mahmoudi, Babak Pourakbari, and Setareh Mamishi

Stopping TB requires a government program that functions every day of the year, and that's hard in certain parts of the world. And partly it's because of who tuberculosis affects: It tends to affect the poor and disenfranchised most.

Tom Frieden

Summary

Tuberculosis (TB) is a serious public health concern worldwide. Although numerous current immunological approaches for TB detection have advanced significantly, there remains a significant restriction due to the lack of extremely sensitive or specific tests to identify all TB patients reliably. Therefore, developing a panel of markers or a biomarker might help achieve global TB control. This chapter aims to evaluate the recent immunodiagnostics of TB and novel biomarker discoveries.

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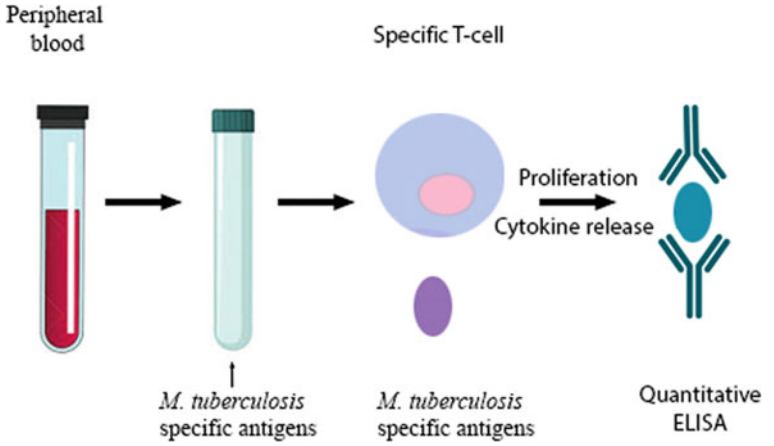
Graphical Abstract



Tuberculosis immunodiagnostics

Keywords

Biomarker · Immunodiagnostics · Tuberculosis

1 Introduction

Biomarker-based tests that can detect tuberculosis (TB) and track the response to anti-TB therapy (ATT) are becoming more popular. Although numerous current immunological approaches for TB detection have advanced significantly, there remains a significant restriction due to the lack of extremely sensitive or specific tests to identify all TB patients reliably. Therefore, developing a panel of markers or a biomarker might help achieve global TB control.

TB is a serious public health concern worldwide. Greater than one billion people are believed to carry latent TB infection (LTBI) [1]. Because LTBI may lead to TB disease in 5–10% of cases, biomarkers for diagnosis of *Mycobacterium tuberculosis (M. tb)*-infected people and the estimation of their risk to progress to TB disease are highly needed for rapid initiation of preventive therapy, as well as for prognostic purposes.

Although management of LTBI is crucial for controlling TB, the current diagnostic methods are not optimal yet [2]. Immunological tests have been introduced as potentially useful approaches for diagnosing TB, particularly LTBI, as screening tests by using easily collected samples including blood, saliva, or urine [2, 3]. Interferon gamma-release assays (IGRAs) and the tuberculin skin tests (TST) are

widely used as immunological tests to diagnose TB. However, TST has several limitations due to the possible technical errors, inadequate positive predictive value, and being influenced by Bacillus Calmette–Guérin (BCG) vaccination. IGRAs were introduced with superior specificity for the diagnosis of TB. They are not affected by *M. bovis*, BCG vaccination, and other environmental mycobacteria. C-Tb test (Statens Serum Institut, Copenhagen, Denmark) has recently been launched as immunological testing for LTBI. It employs early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10) antigens, which are extremely specific skin tests for the diagnosis of TB. A high correlation with the QuantiFERON-TB Gold In-Tube (QFT-GIT) results and better performance than TST in BCG-vaccinated people was reported [4, 5]. It can be used in individuals irrespective of BCG and HIV status [6, 7].

Although the above current diagnostic procedures have greatly improved and play an important role in TB diagnosis, they cannot differentiate LTBI from active TB due to the indirect detection of TB infection after an immune response to the *M. tb* antigen [8, 9]. So, evaluation of different biomarkers to determine TB disease states is highly needed. This chapter aims to briefly evaluate the recent immunodiagnostics of TB and novel biomarker discoveries.

2 Biomarker Discoveries for Tuberculosis

We categorize biomarkers into the following groups:

- *M. tb* components antigen detection;
- anti-*M. tb* antibodies produced by the host;
- *M. tb*-specific cellular immunological responses;
- host gene expression;
- omics approach that includes transcriptomics, proteomics, and metabolomics;
- cell-derived microparticles (MPs); and
- microRNAs.

2.1 *M. tb* Components Antigen Detection

TB antigen detection in different samples, including blood, pulmonary specimen, or urine, using enzyme-linked immunosorbent assay (ELISA) or immunochromatographic (ICT) method has been introduced. The only commercially available biomarker for TB diagnosis in urine is lipoarabinomannan (LAM), which is considered the key component of *M. tb* cell wall [10, 11]; however, several conditions, including proteinuria, hematuria, urinary tract infection, and pneumonia with culture result of *M. avium* and *Streptococcus pneumoniae* could affect the possibility of the false-positive result of urine LAM test [12]. According to the meta-analysis,

the pooled sensitivity of the urine LAM test varied from 13 to 93%, while a better specificity ranging from 87 to 99% [13] was reported. This test is helpful for the detection of TB in HIV-positive cases with low $CD4^+$ T-cell counts [11].

2.2 Host Responses to *M. tb* Antigens

Evaluation of anti-*M. tb* antibodies produced by the host by measuring the level of specific antibodies (mostly IgG) against antigens is another approach. First-generation antibody-based detection tests were designed using the crude mixtures of *M. tb*, including culture filtrate proteins and purified protein derivatives. This generation had low specificities due to the shared antigens between different bacterial species. New antigens with enhanced sensitivity and specificity have been discovered as a result of further attempts to create antibody detection techniques [14, 15].

Until now, a large number of recombinant proteins have been introduced for serodiagnostic purposes. Antibody response to several markers including mycobacterial lipids [16, 17], mammalian cell entry (Mce1A) protein of the *M. tb* cell wall [18, 19], cardiolipin, sulfatide, and mycolic acid [19] has also been introduced as a probable candidate for both TB diagnosis and ATT monitoring; however, this test has low sensitivity and the negative result was observed in nearly 30–40% of TB patients. Moreover, interference with the BCG vaccine might occur due to the presence of similar antigenic epitopes with BCG [20].

Totally, serological tests based on recombinant antigens alone or with the combination of two or three proteins [21] or synthetic peptides derived from antigenic proteins have been introduced [22, 23]. Moreover, multi-epitope or fusion recombinant antigen and the use of a single polyprotein might help the serodiagnosis of TB to increase the chance of *M. tb* antibody detection [21, 24–26]. Several studies have evaluated the use of recombinant proteins of *M. tb* including ESAT-6, CFP-10 and TB7.7, 38 kDa (Rv0934), MPT51 (Rv3803c), Ag85B (Rv1886c), *TbF6 plus DPEP,* an antigenic glycolipid compound*,* 2,3,6-Triacyltrehalose (TAT), malate synthase, α-crystallin, cord factor (trehalose 6,6′-dimycolate) and lipid (DAT) for the serodiagnosis of pulmonary TB (PTB). These assays have indicated variable sensitivity ranging from 14 to 85% and specificity of 53 to 98.7% [27–29].

2.3 Host Cytokine Responses to *M. tb* Antigens–interferon-Gamma

The cellular immune responses that evaluate host cytokines after stimulation with mycobacterial antigens are more consistent than antibody responses. The IGRA tests are based on the production of IFN-γ derived from T cells activated by specific *M. tb* antigens. IGRA has been recommended for use in BCG-vaccinated individuals because of its increased sensitivity and specificity. Moreover, it can be used

during pregnancy for contact investigations, screening of healthcare workers, as well as treatment monitoring [30–32].

IGRAs are available as different tests. International units (IU) per milliliter are used to measure IFN-γ, which is deemed positive if it exceeds the test limit.

Until now, five commercialized IGRA kits, including the T-cell spot of the TB assay (T-SPOT.TB, Oxford Immunotec, Abingdon, UK), QuantiFERON-TB Gold In-Tube (QFT-GIT, Qiagen GmbH, Hilden, Germany), QuantiFERON-TB Gold-Plus (QFT-Plus, Qiagen, MD, United States), LIAISON QuantiFERON-TB Gold Plus (LIAISON QFT-Plus, DiaSorin S.p.A., Italy), and LIOFeron TB/LTBI (LIONEX GmbH, Braunschweig, Germany) are introduced. Moreover, five products are in development—T-Track (R) TB (Lophius Biosciences GmbH), VIDAS TB-IGRA (bioMérieux), Access QuantiFERON-TB (Boditech Med Inc.), ichromaTM IGRATB (Boditech Med Inc.), and IP-10 IGRA ELISA/lateral flow (R-Biopharm) (WHO 2020).

A new TB antigen tube has been added to the QFT-In-Tube test that previously included ESAT-6, CFP-10, and TB7.7 peptides. The first TB antigen tube (TB1) induces CD4 + T-cell responses, whereas the second tube (TB2) stimulates both CD4 + and CD8 + T-cell responses.

As another example of IGRA, the T-SPOT.TB assay uses ESAT-6 and CFP-10 to induce peripheral blood mononuclear cells. Another commercial kit, LIOFeron®TB/LTBI, has been introduced for measuring the T-cell response to specific *M. tb* antigens [33]. Although current methods such as the Diaskintest, C-Tb skin test, EC-Test, T-SPOT.TB, QFT-GIT, QFT-Plus, and LIAISON QFT-Plus have greatly improved the accuracy, sensitivity, and specificity for detection of *M. tb* infection; they are still unable to distinguish LTBI from active TB.

2.4 Other Host Cytokine Responses to *M. tb* Antigens

Numerous research has investigated alternate antigens and host biomarkers for TB diagnosis and the distinction of active TB from LTBI, in addition to ESAT-6 and CFP-10 [34, 35] and host biomarkers other than IFN-γ [36–39].

Multiplex cytokine arrays have been employed on plasma to distinguish between active TB and LTBI. Plasma cytokines and chemokines are introduced as helpful immunological markers for diagnosing and discriminating active TB disease and LTBI and monitoring ATT [36, 37, 40–47]. Although various biomarkers are used in different studies, the most commonly used biomarkers are IFN-γ and IP-10.

3 Host Gene Expression

Several host biomarkers have been identified as possible biomarkers for the diagnosis of TB in whole blood culture supernatants, serum, plasma, saliva, and cerebrospinal fluid [39, 48–51].

Studies have frequently investigated the use of immune-related genes and host transcriptional biosignatures, which are primarily neutrophil-driven type 1 interferon, type 1 interferon-α or interferon-β, *FCGR1B* gene CD64 (also known as FCGR1A), *RAB24, TLR1, TLR4, MMP9, NLRC4*, *IL1B, GBP5*, and *GZMA* as diagnostic candidates for discrimination between TB and LTBI as well as the risk of potential TB development [52–56].

4 Biomarkers Using "Omics" Approach

Not only have "omics" methods, such as genomics, transcriptomics, proteomics, and metabolomics, been used in TB detection, monitoring drug effectiveness, and predicting treatment effects, but they have also been used to better understand the pathogenesis of the disease [29].

In the era of transcriptomics, which is the study of genome-wide gene expression with a focus on protein-coding genes, RNA transcript abundance may be assessed using gene chip microarrays or RNA sequencing.

Metabolic biomarkers, including the small molecule metabolites including fatty acids, lipids, sugars, amino acids, and nucleotides, have been introduced as potential approaches for TB diagnostics [57–59]. Metabolomics, which employs new blood biomarkers such as glutamate, glutamine, sulfoxy methionine, methionine, aspartate, and asparagine, as well as their ratios, has recently emerged as a viable tool for detecting PTB [2].

Several metabolomics studies have been conducted on sputum, blood, breath, and urine samples using various platforms, including nuclear magnetic resonance (NMR) spectroscopy, gas chromatography time-of-flight mass spectrometry (GCTOFMS), liquid chromatography high-resolution mass spectrometry (LCMS), and ultra-high-performance liquid chromatography-electrospray, ionization-quadrupole time-of-flight mass spectrometry (UHPLC–ESIQTOFMS) to find new markers for diagnosis of TB infection and/or monitoring ATT [2, 53, 60–66].

5 Cell-Derived Microparticles

There is increasing interest in cell-derived microparticles (MP)–small, membrane-coated vesicles that may indicate disease activity and evaluate treatment efficacy. MPs may be used as biomarkers for clinical diagnostic testing and disease progression monitoring. They are subcellular components that play a role in cell signaling and intercellular connectivity and supply important knowledge about the immunopathogenesis from LTBI to active TB disease [67]. Cell-derived MPs mediate intercellular connectivity. MPs from *M. tb*-infected cells can activate innate and adaptive responses without direct contact with antigen-presenting cells and T cells [67, 68].

6 microRNA

The use of microRNAs as diagnostic biomarkers for TB diagnosis and monitoring ATT has been described [53, 69–71]. However, further studies on the possible effect of various factors on microRNA expression in TB disease are required to progress the creation of microRNAs-based biomarkers as a diagnostic signature for TB.

7 Conclusion

There is increasing interest in developing novel biomarker-based TB diagnostics that can identify TB disease and monitor the treatment response. Although numerous current immunological approaches for TB detection have advanced significantly, there remains a significant restriction due to the lack of extremely sensitive or specific tests to identify all TB patients reliably. Therefore, developing a panel of markers or a biomarker might help achieve global TB control.

Core Messages

- There are several biomarker-based TB diagnostics.
- Biomarker-based TB diagnostics are not sensitive or specific enough to accurately classify all TB cases.
- Novel biomarkers to identify latent TB infection and active TB disease are highly required.

References

1. World Health Organization (2019) Global tuberculosis report 2018. Geneva
2. Cho Y, Park Y, Sim B, Kim J, Lee H, Cho S-N, Kang YA, Lee S-G (2020) Identification of serum biomarkers for active pulmonary tuberculosis using a targeted metabolomics approach. Sci Rep 10(1):1–11
3. Eribo OA, Leqheka MS, Malherbe ST, McAnda S, Stanley K, van der Spuy GD, Walzl G, Chegou NN (2020) Host urine immunological biomarkers as potential candidates for the diagnosis of tuberculosis. Int J Infect Dis 99:473–481
4. Aggerbeck H, Ruhwald M, Hoff ST, Borregaard B, Hellstrom E, Malahleha M, Siebert M, Gani M, Seopela V, Diacon A (2018) C-Tb skin test to diagnose *Mycobacterium tuberculosis* infection in children and HIV-infected adults: a phase 3 trial. PLoS ONE 13(9):e0204554
5. Ruhwald M, Aggerbeck H, Gallardo RV, Hoff ST, Villate JI, Borregaard B, Martinez JA, Kromann I, Penas A, Anibarro LL (2017) Safety and efficacy of the C-Tb skin test to diagnose *Mycobacterium tuberculosis* infection, compared with an interferon γ release assay and the tuberculin skin test: a phase 3, double-blind, randomised, controlled trial. Lancet Respir Med 5(4):259–268

6. Aggerbeck H, Giemza R, Joshi P, Tingskov PN, Hoff ST, Boyle J, Andersen P, Lewis DJ (2013) Randomised clinical trial investigating the specificity of a novel skin test (C-Tb) for diagnosis of *M. tuberculosis* infection. PloS one 8(5):e64215
7. Hoff ST, Peter JG, Theron G, Pascoe M, Tingskov PN, Aggerbeck H, Kolbus D, Ruhwald M, Andersen P, Dheda K (2016) Sensitivity of C-Tb: a novel RD-1-specific skin test for the diagnosis of tuberculosis infection. Eur Respir J 47(3):919–928
8. Targowski T, Chelstowska S, Plusa T (2014) IGRA as a predictive factor of silent pulmonary changes in individuals following exposure to tuberculosis. Lung 192(6):869–874
9. Mamishi S, Pourakbari B, Marjani M, Mahmoudi S (2014) Diagnosis of latent tuberculosis infection among immunodeficient individuals: review of concordance between interferon-gamma release assays and the tuberculin skin test. Br J Biomed Sci 71(3):115–124. https://doi.org/10.1080/09674845.2014.11669976
10. Bulterys MA, Wagner B, Redard-Jacot M, Suresh A, Pollock NR, Moreau E, Denkinger CM, Drain PK, Broger T (2020) Point-of-care urine LAM tests for tuberculosis diagnosis: a status update. J Clin Med 9(1):111
11. Songkhla MN, Tantipong H, Tongsai S, Angkasekwinai N (2019) Lateral flow urine lipoarabinomannan assay for diagnosis of active tuberculosis in adults with human immunodeficiency virus infection: a prospective cohort study. In: Open forum infectious diseases, vol 4. Oxford University Press US, p ofz132
12. Iskandar A, Nursiloningrum E, Arthamin MZ, Olivianto E, Chandrakusuma MS (2017) The diagnostic value of urine lipoarabinomannan (LAM) antigen in childhood tuberculosis. J clin Diagnostic Res: JCDR 11(3):EC32
13. Minion J, Leung E, Talbot E, Dheda K, Pai M, Menzies D (2011) Diagnosing tuberculosis with urine lipoarabinomannan: systematic review and meta-analysis. Eur Respir J 38 (6):1398–1405
14. Steingart KR, Dendukuri N, Henry M, Schiller I, Nahid P, Hopewell PC, Ramsay A, Pai M, Laal S (2009) Performance of purified antigens for serodiagnosis of pulmonary tuberculosis: a meta-analysis. Clin Vaccine Immunol 16(2):260–276
15. Laal S, Skeiky YA (2005) Immune-based methods. In: Tuberculosis and the tubercle bacillus. American Society of Microbiology, pp 71–83
16. Goodridge A, Cueva C, Lahiff M, Muzanye G, Johnson JL, Nahid P, Riley LW (2012) Anti-phospholipid antibody levels as biomarker for monitoring tuberculosis treatment response. Tuberculosis 92(3):243–247
17. Togun TO, MacLean E, Kampmann B, Pai M (2018) Biomarkers for diagnosis of childhood tuberculosis: a systematic review. PLoS ONE 13(9):e0204029
18. Takenami I, de Oliveira CC, Lima FR, Soares J, Machado A Jr, Riley LW, Arruda S (2016) Immunoglobulin G response to mammalian cell entry 1A (Mce1A) protein as biomarker of active tuberculosis. Tuberculosis 100:82–88
19. Dos Santos DC, Lovero KL, Schmidt CM, Barros ACM, Quintanilha AP, Barbosa AP, Pone MV, Pone SM, Araujo JM, de Paula MC (2020) Serological biomarkers for monitoring response to treatment of pulmonary and extrapulmonary tuberculosis in children and adolescents. Tuberculosis 123:101960
20. Wu X, Yang Y, Zhang J, Li B, Liang Y, Zhang C, Dong M (2010) Comparison of antibody responses to seventeen antigens from *Mycobacterium tuberculosis*. Clin Chim Acta 411(19–20):1520–1528
21. Khurshid S, Afzal M, Khalid R, Akhtar MW, Qazi MH (2017) Potential of multi-component antigens for tuberculosis diagnosis. Biologicals 48:109–113
22. Kashyap RS, Rajan AN, Ramteke SS, Agrawal VS, Kelkar SS, Purohit HJ, Taori GM, Daginawala HF (2007) Diagnosis of tuberculosis in an Indian population by an indirect ELISA protocol based on detection of antigen 85 complex: a prospective cohort study. BMC Infect Dis 7(1):74

23. Goyal B, Kumar K, Gupta D, Agarwal R, Latawa R, Sheikh JA, Verma I (2014) Utility of B-cell epitopes based peptides of RD1 and RD2 antigens for immunodiagnosis of pulmonary tuberculosis. Diagn Microbiol Infect Dis 78(4):391–397
24. Yang Y, Feng J, Zhang J, Zhao W, Liu Y, Liang Y, Bai X, Wang L, Wu X (2015) Immune responses to a recombinant Rv0057-Rv1352 fusion protein of *Mycobacterium tuberculosis*. Ann Clin Lab Sci 45(1):39–48
25. Araujo LS, Moraes RM, Trajman A, Saad MHF (2010) Assessment of the IgA immunoassay diagnostic potential of the *Mycobacterium tuberculosis* MT10. 3-MPT64 fusion protein in tuberculous pleural fluid. Clin Vaccine Immunol 17(12):1963–1969
26. Cheng Z, Zhao JW, Sun ZQ, Song YZ, Sun QW, Zhang XY, Zhang XL, Wang HH, Guo XK, Liu YF (2011) Evaluation of a novel fusion protein antigen for rapid serodiagnosis of tuberculosis. J Clin Lab Anal 25(5):344–349
27. Lagrange PH, Thangaraj SK, Dayal R, Deshpande A, Ganguly NK, Girardi E, Joshi B, Katoch K, Katoch VM, Kumar M (2014) A toolbox for tuberculosis (TB) diagnosis: an Indian multi-centric study (2006–2008); evaluation of serological assays based on PGL-Tb1 and ESAT-6/CFP10 antigens for TB diagnosis. PLoS ONE 9(5):e96367
28. Achkar JM, Ziegenbalg A (2012) Antibody responses to mycobacterial antigens in children with tuberculosis: challenges and potential diagnostic value. Clin Vaccine Immunol 19(12): 1898–1906
29. Yong YK, Tan HY, Saeidi A, Wong WF, Vignesh R, Velu V, Eri R, Larsson M, Shankar EM (2019) Immune biomarkers for diagnosis and treatment monitoring of tuberculosis: current developments and future prospects. Front Microbiol 10:2789
30. Pourakbari B, Mamishi S, Benvari S, Mahmoudi S (2019) Comparison of the QuantiFERON-TB gold plus and QuantiFERON-TB gold in-tube interferon-gamma release assays: a systematic review and meta-analysis. Adv Med Sci 64(2):437–443. https://doi.org/10.1016/j.advms.2019.09.001
31. Pourakbari B, Mamishi S, Benvari S, Sauzullo I, Bedini A, Valentini P, Keicho N, Mahmoudi S (2020) Can interferon-γ release assays be useful for monitoring the response to anti-tuberculosis treatment?: A systematic review and meta-analysis. Arch Immunol Ther Exp 68(1):4
32. Keshavarz Valian S, Mahmoudi S, Pourakbari B, Abdolsalehi MR, Eshaghi H, Mamishi S (2019) Screening of healthcare workers for latent tuberculosis infection in the low tuberculosis burden country: QuantiFERON-TB gold in tube test or tuberculin skin test? Arch Environ Occup Health 74(3):109–114
33. Della Bella C, Spinicci M, Alnwaisri HFM, Bartalesi F, Tapinassi S, Mencarini J, Benagiano M, Grassi A, D'Elios S, Troilo A (2020) LIOFeron® TB/LTBI: a novel and reliable test for LTBI and tuberculosis. Int J Infect Dis 91:177–181
34. Mahmoudi S, Pourakbari B, Mamishi S (2017) Interferon gamma release assay in response to PE35/PPE68 proteins: a promising diagnostic method for diagnosis of latent tuberculosis. Eur Cytokine Netw 28(1):36–40. https://doi.org/10.1684/ecn.2017.0391
35. Pourakbari B, Mamishi S, Marjani M, Rasulinejad M, Mariotti S, Mahmoudi S (2015) Novel T-cell assays for the discrimination of active and latent tuberculosis infection: the diagnostic value of PPE family. Mol Diagn Ther 19(5):309–316. https://doi.org/10.1007/s40291-015-0157-0
36. Mamishi S, Mahmoudi S, Banar M, Hosseinpour Sadeghi R, Marjani M, Pourakbari B (2019) Diagnostic accuracy of interferon (IFN)-gamma inducible protein 10 (IP-10) as a biomarker for the discrimination of active and latent tuberculosis. Mol Biol Rep 46(6):6263–6269. https://doi.org/10.1007/s11033-019-05067-0
37. Mamishi S, Pourakbari B, Sadeghi RH, Marjani M, Mahmoudi S (2019) Diagnostic accuracy of monocyte chemotactic protein (MCP)-2 as biomarker in response to PE35/PPE68 proteins: a promising diagnostic method for the discrimination of active and latent tuberculosis. Protein Pept Lett 26(4):281–286. https://doi.org/10.2174/0929866526666190119165805

38. Mamishi S, Pourakbari B, Shams H, Marjani M, Mahmoudi S (2016) Improving T-cell assays for diagnosis of latent TB infection: confirmation of the potential role of testing Interleukin-2 release in Iranian patients. Allergol Immunopathol 44(4):314–321. https://doi.org/10.1016/j.aller.2015.09.004
39. Manngo PM, Gutschmidt A, Snyders CI, Mutavhatsindi H, Manyelo CM, Makhoba NS, Ahlers P, Hiemstra A, Stanley K, McAnda S (2019) Prospective evaluation of host biomarkers other than interferon gamma in QuantiFERON plus supernatants as candidates for the diagnosis of tuberculosis in symptomatic individuals. J Infect 79(3):228–235
40. Mihret A, Bekele Y, Bobosha K, Kidd M, Aseffa A, Howe R, Walzl G (2013) Plasma cytokines and chemokines differentiate between active disease and non-active tuberculosis infection. J Infect 66(4):357–365
41. Clifford V, Tebruegge M, Zufferey C, Germano S, Forbes B, Cosentino L, Matchett E, McBryde E, Eisen D, Robins-Browne R (2019) Cytokine biomarkers for the diagnosis of tuberculosis infection and disease in adults in a low prevalence setting. Tuberculosis 114:91–102
42. Tebruegge M, Dutta B, Donath S, Ritz N, Forbes B, Camacho-Badilla K, Clifford V, Zufferey C, Robins-Browne R, Hanekom W (2015) Mycobacteria-specific cytokine responses detect tuberculosis infection and distinguish latent from active tuberculosis. Am J Respir Crit Care Med 192(4):485–499
43. Mamishi S, Pourakbari B, Teymuri M, Rubbo P-A, Tuaillon E, Keshtkar A, Mahmoudi S (2014) Diagnostic accuracy of IL-2 for the diagnosis of latent tuberculosis: a systematic review and meta-analysis. Eur J Clin Microbiol Infect Dis 33(12):2111–2119
44. Mamishi S, Pourakbari B, Marjani M, Bahador A, Mahmoudi S (2015) Discriminating between latent and active tuberculosis: the role of interleukin-2 as biomarker. J Infect 70 (4):429–431
45. Wu J, Wang S, Lu C, Shao L, Gao Y, Zhou Z, Huang H, Zhang Y, Zhang W (2017) Multiple cytokine responses in discriminating between active tuberculosis and latent tuberculosis infection. Tuberculosis 102:68–75
46. Chen T, Li Z, Yu L, Li H, Lin J, Guo H, Wang W, Chen L, Zhang X, Wang Y (2016) Profiling the human immune response to *Mycobacterium tuberculosis* by human cytokine array. Tuberculosis 97:108–117
47. Yao X, Liu Y, Liu Y, Liu W, Ye Z, Zheng C, Ge S (2017) Multiplex analysis of plasma cytokines/chemokines showing different immune responses in active TB patients, latent TB infection and healthy participants. Tuberculosis 107:88–94
48. Chegou NN, Sutherland JS, Malherbe S, Crampin AC, Corstjens PL, Geluk A, Mayanja-Kizza H, Loxton AG, van der Spuy G, Stanley K (2016) Diagnostic performance of a seven-marker serum protein biosignature for the diagnosis of active TB disease in African primary healthcare clinic attendees with signs and symptoms suggestive of TB. Thorax 71 (9):785–794
49. Chegou NN, Sutherland JS, Namuganga A-R, Corstjens PL, Geluk A, Gebremichael G, Mendy J, Malherbe S, Stanley K, Van Der Spuy GD (2018) Africa-wide evaluation of host biomarkers in QuantiFERON supernatants for the diagnosis of pulmonary tuberculosis. Sci Rep 8(1):1–12
50. Manyelo CM, Solomons RS, Snyders CI, Manngo PM, Mutavhatsindi H, Kriel B, Stanley K, Walzl G, Chegou NN (2019) Application of cerebrospinal fluid host protein biosignatures in the diagnosis of tuberculous meningitis in children from a high burden setting. Mediat Inflamm
51. Mamishi S, Pourakbari B, Sadeghi RH, Marjani M, Mahmoudi S (2020) Differential gene expression of ASUN, NEMF, PTPRC and DHX29: candidate biomarkers for diagnosis of active and latent tuberculosis. Infect Disord Drug Targets. https://doi.org/10.2174/1871526520666200313144951

52. Maertzdorf J, Repsilber D, Parida SK, Stanley K, Roberts T, Black G, Walzl G, Kaufmann SH (2011) Human gene expression profiles of susceptibility and resistance in tuberculosis. Genes Immun 12(1):15–22
53. Walzl G, McNerney R, du Plessis N, Bates M, McHugh TD, Chegou NN, Zumla A (2018) Tuberculosis: advances and challenges in development of new diagnostics and biomarkers. Lancet Infect Dis 18(7):e199–e210
54. Berry MP, Graham CM, McNab FW, Xu Z, Bloch SA, Oni T, Wilkinson KA, Banchereau R, Skinner J, Wilkinson RJ (2010) An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature 466(7309):973–977
55. Gebremicael G, Kassa D, Alemayehu Y, Gebreegziaxier A, Kassahun Y, van Baarle D, Ottenhoff T, Cliff J, Haks M (2019) Gene expression profiles classifying clinical stages of tuberculosis and monitoring treatment responses in Ethiopian HIV-negative and HIV-positive cohorts. PloS one 14(12):e0226137
56. Lee S-W, Wu LS-H, Huang G-M, Huang K-Y, Lee T-Y, Weng JT-Y (2016) Gene expression profiling identifies candidate biomarkers for active and latent tuberculosis. BMC Bioinf S1: S3. Springer
57. Wang C, Peng J, Kuang Y, Zhang J, Dai L (2017) Metabolomic analysis based on 1H-nuclear magnetic resonance spectroscopy metabolic profiles in tuberculous, malignant and transudative pleural effusion. Mol Med Rep 16(2):1147–1156
58. Haas CT, Roe JK, Pollara G, Mehta M, Noursadeghi M (2016) Diagnostic 'omics' for active tuberculosis. BMC Med 14(1):1–19
59. Lau SK, Lam C-W, Curreem SO, Lee K-C, Lau CC, Chow W-N, Ngan AH, To KK, Chan JF, Hung IF (2015) Identification of specific metabolites in culture supernatant of *Mycobacterium tuberculosis* using metabolomics: exploration of potential biomarkers. Emerg Microbes Infect 4(1):1–10
60. Frediani JK, Jones DP, Tukvadze N, Uppal K, Sanikidze E, Kipiani M, Tran VT, Hebbar G, Walker DI, Kempker RR (2014) Plasma metabolomics in human pulmonary tuberculosis disease: a pilot study. PLoS ONE 9(10):e108854
61. Zhou A, Ni J, Xu Z, Wang Y, Lu S, Sha W, Karakousis PC, Yao Y-F (2013) Application of 1H NMR spectroscopy-based metabolomics to sera of tuberculosis patients. J Proteome Res 12(10):4642–4649
62. Schoeman JC, Du Preez I (2012) A comparison of four sputum pre-extraction preparation methods for identifying and characterising *Mycobacterium tuberculosis* using GCxGC-TOFMS metabolomics. J Microbiol Methods 91(2):301–311
63. Du Preez I, Loots D (2013) New sputum metabolite markers implicating adaptations of the host to *Mycobacterium tuberculosis*, and vice versa. Tuberculosis 93(3):330–337
64. Phillips M, Basa-Dalay V, Bothamley G, Cataneo RN, Lam PK, Natividad MPR, Schmitt P, Wai J (2010) Breath biomarkers of active pulmonary tuberculosis. Tuberculosis 90(2):145–151
65. Kolk A, Van Berkel J, Claassens M, Walters E, Kuijper S, Dallinga J, Van Schooten F (2012) Breath analysis as a potential diagnostic tool for tuberculosis. Int J Tuberc Lung Dis 16 (6):777–782
66. Mahapatra S, Hess AM, Johnson JL, Eisenach KD, DeGroote MA, Gitta P, Joloba ML, Kaplan G, Walzl G, Boom WH (2014) A metabolic biosignature of early response to anti-tuberculosis treatment. BMC Infect Dis 14(1):1–11
67. Moreira JD, Silva HR, de Toledo VdP, Guimarães TM (2020) Microparticles in the pathogenesis of TB: novel perspectives for diagnostic and therapy management of *Mycobacterium tuberculosis* infection. Microb Pathog 104176
68. Angelot F, Seillès E, Biichlé S, Berda Y, Gaugler B, Plumas J, Chaperot L, Dignat-George F, Tiberghien P, Saas P (2009) Endothelial cell-derived microparticles induce plasmacytoid dendritic cell maturation: potential implications in inflammatory diseases. Haematologica 94 (11):1502–1512

69. Alipoor SD, Adcock IM, Tabarsi P, Folkerts G, Mortaz E (2020) MiRNAs in tuberculosis: their decisive role in the fate of TB. Eur J Pharmacol 173529
70. Ndzi EN, Nkenfou CN, Mekue LM, Zentilin L, Tamgue O, Pefura EWY, Kuiaté J-R, Giacca M, Ndjolo A (2019) MicroRNA hsa-miR-29a-3p is a plasma biomarker for the differential diagnosis and monitoring of tuberculosis. Tuberculosis 114:69–76
71. Pedersen JL, Bokil NJ, Saunders BM (2019) Developing new TB biomarkers, are miRNA the answer? Tuberculosis 118:101860

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Role of Bronchoscopy in Diagnostics and Treatment of Tuberculosis

9

Ilya Sivokozov and Atadzhan Ergeshov

Any process in the lung should be considered tuberculosis until proven otherwise.

Robert Hegglin

Summary

Modern bronchoscopy plays a crucial role in confirming pulmonary or endobronchial tuberculosis (TB), providing a safe, rapid, and effective diagnostic modality. Numerous biopsy modalities can be used to confirm specific lesions both in lung parenchyma and bronchial wall. Bronchoalveolar lavage and bronchial washings are the most simple and popular, followed by endobronchial biopsy, brushing, and conventional transbronchial needle aspiration, providing enough material to obtain both cytopathological and microbiological confirmation of diagnosis. New diagnostic modalities, like cryobiopsy and endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), navigation with radial EBUS can be used to improve the diagnostic yield of conventional modalities, especially in relatively small pulmonary lesions and mediastinal adenopathy. Apart from its diagnostic aspect, bronchoscopy is crucial with its curative intent. Local chemotherapy, endoscopic lung volume reduction, and recanalization of fibrostenotic complications are now available to improve the treatment efficacy of advanced pulmonary and endobronchial TB.

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Graphical Abstract

Bronchoscopy: a diagnostic tool for tuberculosis. Adapted with permission from the Association of Science and Art (ASA), Universal Scientific Education and Research Network (USERN); Made by Nastaran-Sadat Hosseini

Keywords

BAL · Biopsy · Bronchoscopy · Diagnostics · EBUS-TBNA · Navigation · rEBUS · Therapy · Tuberculosis

1 Introduction

Bronchoscopy plays a crucial role in differential diagnostics of pulmonary tuberculosis (PTB), mycobacteriosis, lung cancer, sarcoidosis, and many other illnesses which a clinician can confusedly accept as tuberculosis (TB). Modern bronchoscopy also can give a cytopathological, microbiological, and molecular confirmation of disease, thus giving a doctor a definitive diagnosis and drug-sensitivity profile of mycobacteria. In endobronchial tuberculosis (EBTB) cases, especially when it is associated with bronchial obstruction, only bronchoscopy can achieve a fast, effective, and safe cure of infectious lesions in the bronchial tree.

2 Indications for Bronchoscopy in Pulmonary Tuberculosis

Diagnostic bronchoscopy is indicated in patients with:

- suspected with PTB to confirm the final diagnosis (by cytopathology and/or microbiology), when other less invasive diagnostic tests failed or were inconclusive;
- suspected PTB when it needs to provide a differential diagnosis with central/peripheral lung cancer, lymphoma, non-specific bronchiectasis, invasive pulmonary mycosis, etc.;
- confirmed PTB to exclude EBTB;
- suspected EBTB to finally confirm the diagnosis and assess the extent severity of bronchial lesion; and
- confirmed EBTB to provide dynamic assessment during chemotherapy.

Therapeutic bronchoscopy is indicated in patients with:

- confirmed PTB with cavitation with endoscopic lung volume reduction to close the cavities and accelerate sputum conversion;
- active EBTB to perform photodynamic therapy as well as local endoscopic chemotherapy via peribronchial injections of antibiotics or steroids; and
- prominent or critical central airways obstruction due to active EBTB or fibrostenotic lesions–to perform recanalization and restoration of bronchial lumen patency using numerous techniques.

3 Efficacy of Diagnostic Bronchoscopy in Pulmonary Tuberculosis: From Diagnosis to Treatment

There are a lot of diagnostic modalities to obtain a specimen during a bronchoscopic examination. They can vary from ordinary washings of proximal bronchial generations with saline via an instrumental bronchoscope channel to bronchoalveolar lavage (BAL), which gives a much more return volume and reaches terminal bronchioles alveolar surface. If needed, a bronchologist can achieve cytopathology specimens from pulmonary parenchyma, bronchial wall (brush, forceps, needle biopsies), and from mediastinum (using conventional transbronchial needle aspiration or endoscopic ultrasound-guided interventions). All the specimens retrieved can be referred for cytopathological, culture, and polymerase chain reaction (PCR) detection of mycobacteria.

3.1 Washings and Bronchoalveolar Lavage

Two of these techniques are most widely used in routine clinical practice to achieve specimens from deeper parts of the lung. Technically, the procedure of washing and BAL during bronchoscopy is shown in Fig. 1.

The effectiveness of BAL in the diagnosis of PTB varies. According to Baughman et al. [1], cytological examination of BAL fluid can detect mycobacteria in 68% of cases, and microbiological confirmation of TB in BAL fluid reaches 92%. Another study [2] showed diagnostic efficacy of BAL according to culture as high as 87% in the pediatric population (269 children with smear-negative pulmonary TB). Comparable data were shown by Kalawat et al. [3], where cytological detection of mycobacteria in BAL fluid reached 82% and exceeded 90% for culture. At the same time, the efficacy of sputum culture diagnostics reached only 26%.

However, another study [4], performed among patients with suspected tuberculosis and multiple smear-negative sputum microscopy and PCR results, estimated extremely low sensitivity of BAL for mycobacteria detection: neither microscopy nor PCR or culture detected more than 10% of TB cases. Also, BAL fluid

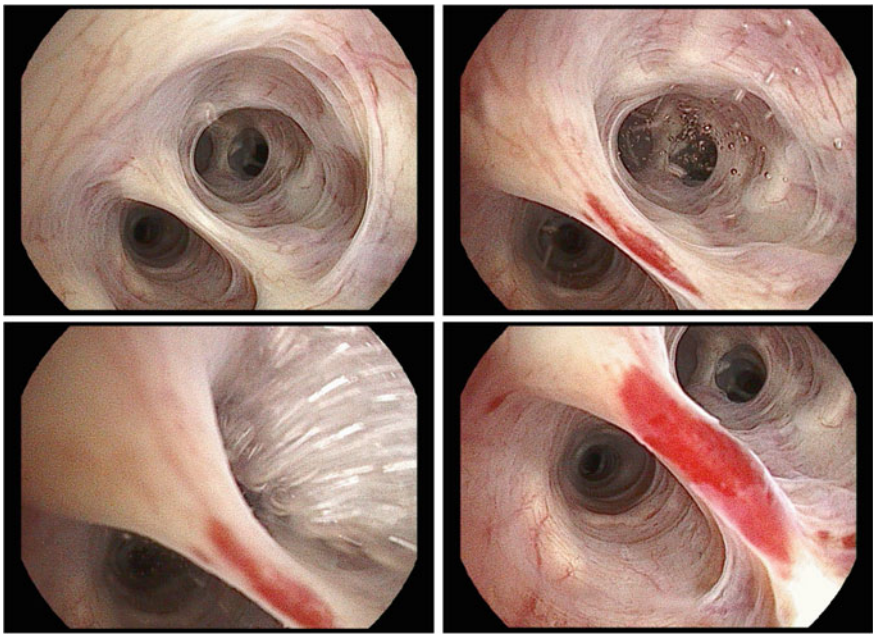

Fig. 1 Endoscopic view of BAL and bronchial washing (Videobronchoscope Pentax EB15 J10): **a** and **b**, instillation of warm saline into subsegmental bronchi of RB_5; **c**, aspiration of saline through the instrumental channel of bronchoscope; **d**, orifices of two subsegmental bronchi of RB_5 immediately after aspiration (note local edema and contact bruising of mucosa on secondary carina)

microscopy in smear-negative patients with TB was sensitive in 47% of cases, expectedly inferior to the PCR, which reached 78%, with total efficacy of TB detection by both microscopy and PCR of 83%.

The smaller volume of specimen retrieved by washing does not allow to perform a cell count analysis; however, volume is sufficient for performing microscopy, PCR detection, and culture for mycobacteria. Among 136 patients with smear-negative PTB [5], microscopy of washings was sensitive to detect only 10% of patients, while PCR provided more than 50% sensitivity, and a combination of two methods gave a final sensitivity of 57%.

The number of studies comparing the effectiveness of BAL versus bronchial washings in the diagnosis of TB is extremely limited. In a study of Shabalina et al. [6] on a limited volume of smear-negative patients with finally confirmed pulmonary TB, the sensitivity of mycobacteria microscopy detection was lower and reached 21% and 25% for BAL and bronchial washing, respectively. At the same time, according to this study, the efficacy of culture confirmation of TB reached 75% and 70% for BAL and washing, respectively.

3.2 Brush-Biopsy

Technically, brush-biopsy is performed during bronchoscopy using a cytological brush either covered by an outer sheath or uncovered (Fig. 2). This biopsy technique can retrieve cytology smears and/or be immersed in saline solution for further PCR or culture detection of mycobacteria. This technique can be used for the peripheral part of the lung or visible endobronchial lesions.

Data regarding the specified efficacy of brush-biopsy in pulmonary TB detection is scarce. According to a recent publication [6], this modality reaches the highest microscopy yield of mycobacteria among all the other biopsy techniques (84%). Another study [7] reported lower efficacy for brushing–60% in smear-negative patients.

3.3 Transbronchial Lung Biopsy with Forceps

Conventional transbronchial biopsy is performed using dedicated endoscopic forceps using the instrument channel of the bronchoscope. A biopsy is done without visual control, and the position of the forceps' tip can be controlled either by fluoroscopy or computer tomography. It is also possible to perform transbronchial lung biopsy with forceps (TBLB) without any radiological guidance, using either radial ultrasound mini-probe or relying on chest computed tomography (CT) scan mapping. Usually, TBLB is performed in patients with pulmonary TB as a part of differential diagnosis, mostly with lung cancer and sarcoidosis. This modality gives a chance to retrieve a cytopathology specimen, less suitable for the culture and PCR testing for infections (Fig. 3).

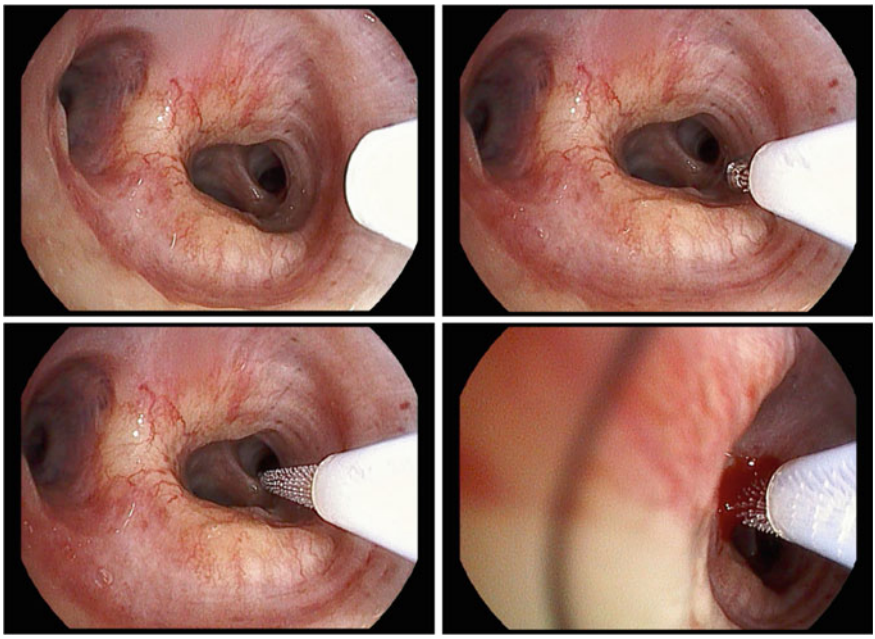

Fig. 2 Endoscopic view of brush-biopsy (Videobronchoscope Pentax EB15 J10): **a** and **b**, the outer sheath of the cytological brush is well seen on the right, with the partial opening of brush out of the sheath; **c**, full opening of brush out of the sheath just before the scarification of bronchial mucosa; **d**, the exact moment of brush-biopsy of bronchial mucosa (note a bit of blood around the instrument)

Data regarding the efficacy and role of TBLB in the diagnosis of PTB are also varying. Our local data [6] showed that cytological and pathological diagnosis for TBLB specimens in smear-negative TB patients was quite high–77% and 80%, respectively. Otherwise, in another study done by the Indian group [8], diagnostic yield for granulomas detection for TBLB was lower and reached 67%. There are some data [9] suggesting that in the "real world," efficacy of TBLB in PTB can be even lower and come fall to 55%. Not surprisingly, some authors now argue the real need in TBLB for suspected TB, raising the question of totally avoiding tissue confirmation for disease [10].

3.4 Transbronchial Cryobiopsy

Endoscopic cryobiopsy potentially can retrieve a larger tissue specimen compared to conventional TBLB. Another unique aspect of this technique is the much better quality of the tissue, escaped from crushing artifacts. Unfortunately, better quality takes its cost–a higher rate of complications (namely bleeding and pneumothorax) and a need for precautions to escape or control them. Usually, transbronchial

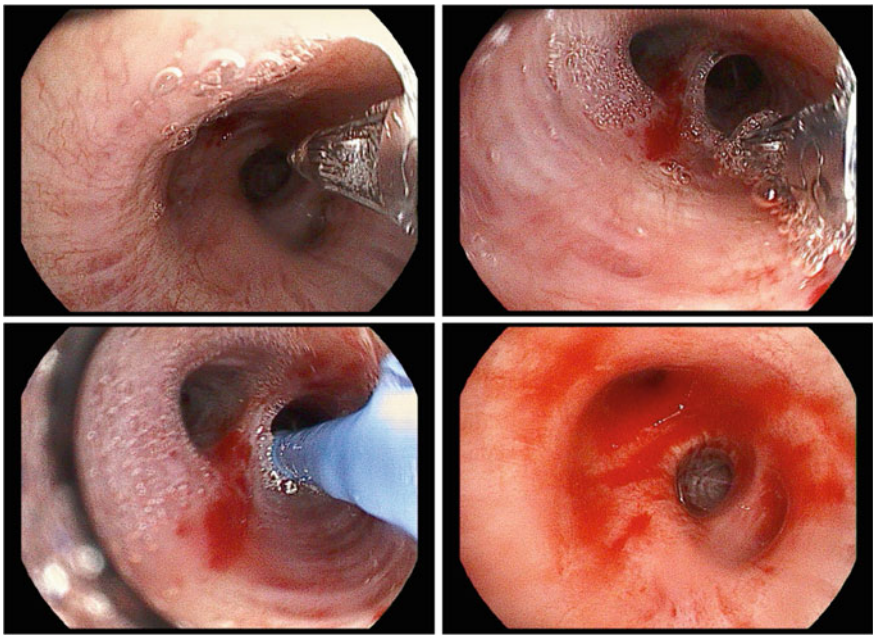

Fig. 3 Endoscopic view of TBLB (Videobronchoscope Pentax EB15 J10): **a** and **b**, the tip of the forceps inside the instrumental channel of the bronchoscope is well seen on the right, during gradual movement of the scope towards target bronchus; **c**, biopsy forceps are deeply inserted into the orifice of middle lobe bronchus, performing a TBLB; **d**, view of target bronchus immediately after the TBLB, forceps withdrawn (note the minimal amount of blood after intervention)

cryobiopsy (TBCB) is done in a combination procedure using a rigid bronchoscope, a Fogarty balloon to control bleeding, and a flexible bronchoscope with a dedicated cryoprobe (Fig. 4). As with TBLB, TB itself is not a common indication for TBCB procedure. It is widely used as an alternative for surgical diagnostics in patients with interstitial lung diseases, mostly fibrotic [11].

There is a lack of publications dedicated to TBCB in pulmonary TB patients. In a very recent study [12], in 54 patients with suspected TB, the efficacy of TBCB in detecting granulomas reached 87%, overriding the efficacy of GeneXpert assay (72.7%). Comparable data were shown in another study [13], where TBCB showed an efficacy of 76%. For both studies, cryobiopsy showed a high safety profile; moderate bleeding was detected in 7.4% of cases.

3.5 Conventional Transbronchial Needle Aspiration of the Mediastinum and Pulmonary Tissue

An endoscopic needle biopsy takes a special place in a long list of bronchoscopic interventions. Apart from all the rest instruments, the needle does not need a

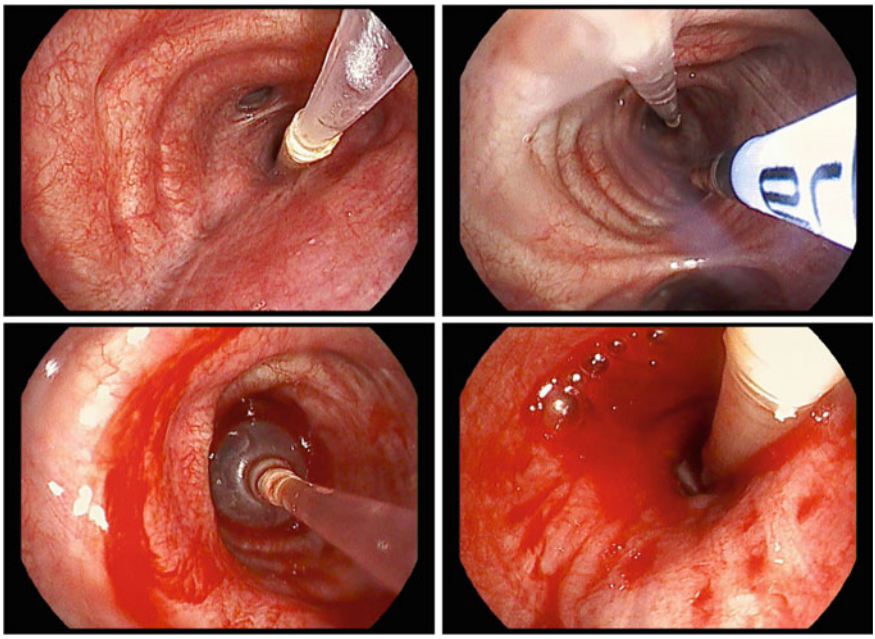

Fig. 4 Endoscopic view of TBCB (Videobronchoscope Pentax EB15 J10, cryostation ErbeCryo II): **a**, Fogarty balloon is placed in the orifice of right lower lobe bronchus, provided parallel to the scope; **b**, 1,9-mm cryoprobe inserted through the instrumental channel of the bronchoscope is well seen on the right, going parallel to Fogarty balloon; **c**, view of target bronchus immediately after TBCB was performed, specimen retrieved, and the balloon inflated in the orifice of bronchus to avoid severe bleeding proximally in the bronchial tree; **d**, view of target bronchus after deflation of Fogarty balloon (note a moderate amount of blood after intervention)

bronchial tree to follow its target; it can simply create its path anywhere and anyway. But as simple as it can penetrate the bronchial wall to reach a lymphatic node or pulmonary parenchyma, it also can pierce the scope's instrumental channel. Partially because of that reasons and partially because of lack of training, nowadays, conventional transbronchial needle aspiration (cTBNA) is not widely used and mostly accumulated in dedicated expert centers.

Among TB patients, cTBNA can be used to sample mediastinal lymphatic nodes in tuberculosis lymphadenitis (TBLA), peribronchial stenoses, and probing pulmonary parenchyma. In most cases (using 21–25G needles), cTBNA retrieves cytology smears, and saline washed through the needle can be sent for mycobacterial culture and PCR detection. Sometimes pathology specimens can be obtained via 19G bore. Rarely, rigid TBNA of 16-18G bores with spring-cut design can be used for true tissue core (Fig. 5).

Data on the efficacy of cTBNA in TBLA [14, 15] has shown that it varies from 56 to 100%, greatly depending on the operator's experience and size of the lymphatic node. In the last decade, cTBNA almost completely was substituted by endoscopic ultrasound-guided interventions. Several publications have been

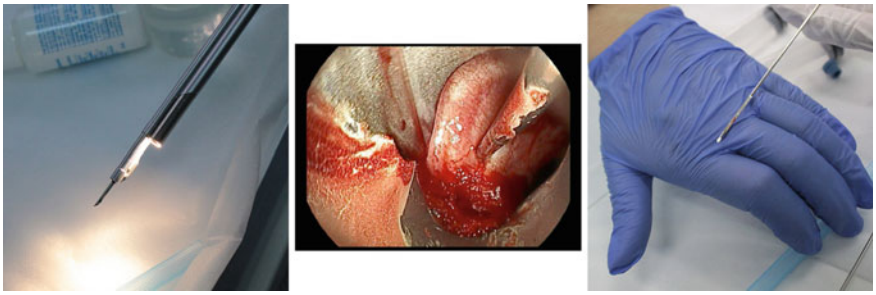

Fig. 5 Rigid TBNA (Videobronchoscope Pentax EB15 J10, Karl Storz rigid bronchoscope, Tru-Cut 18G Sterylab needle): **a**, a rigid needle is loaded in Karl Storz rigid guide and ready to fire; **b**, 18G rigid needle inserted through the rigid barrel is well seen on the right, going parallel to the video bronchoscope piercing huge subcarinal node; **c**, macroscopic view of obtained tissue (note the true core of lymphatic node obtained)

dedicated to the efficacy of cTBNA for pulmonary lesions, mostly solitary pulmonary nodules at radiographic appearance [16, 17]. Currently, cTBNA is used mostly when lung cancer is suspected, and as with mediastinum, these interventions are widely done under ultrasound control.

3.6 Endoscopic Ultrasound-Guided Biopsies of the Mediastinum and Pulmonary Tissue

In recent years, navigational bronchoscopy techniques have become widespread, both for mediastinum and lung parenchyma. Initially proposed for lung cancer staging [18], endobronchial ultrasound-guided TBNA (EBUS-TBNA) (Fig. 6) dramatically changed the approach to the staging in thoracic oncology. Secondly, with spread of technology, diagnostics of mediastinal adenopathy have become easier and less invasive, and now EBUS-TBNA is the first choice in suspected sarcoidosis [19], lung cancer [20], and lymphoma [21, 22]. TB, especially TBLA, also became a target for this new bronchoscopic modality. Several publications were dedicated to this question, including meta-analyses [23–25]. According to publications, EBUS-TBNA was quite sensitive (80%) and specific (100%) in detecting mediastinal TB. New modalities of endosonography, like elastography or tissue harmonic echo, can potentially improve diagnostic yield, providing more precise targeting of a biopsy [26–28]. The safety profile of EBUS-TBNA for this indication is very high, with the overall incidence of complications ranging from 0.1 to 0.5%.

Apart from adult patients with suspected TBLA, there are some fundamental limitations of the EBUS-TBNA [14] approach in the pediatric population due to anatomical reasons (i.e., the diameter of the trachea). In this situation, the EUS-b-FNA approach [27, 29] can be effectively used when an echo-bronchoscope is placed transesophageally (Fig. 7). The diagnostic effectiveness of both EBUS-TBNA and EUS-b-FNA in the pediatric population ranges from 50 to 89%, while complications range from 0.5 to 8.9%, with the majority being mild.

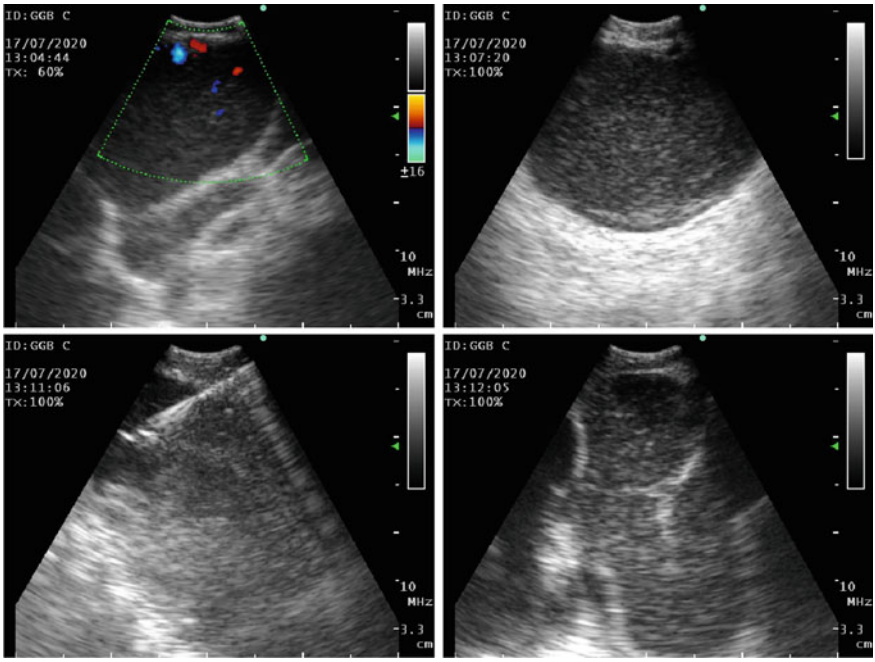

Fig. 6 Ultrasound view of EBUS-TBNA (Echobronchoscope Olympus BF UC180F, US center Olympus EU ME1): **a**, Endoscopic sonogram of huge subcarinal lymphatic node (note color flow Doppler is showing several vessels in the upper part of the screen); **b**, a round-shaped solitary node in the right hilum of the same patient, hypoechoic features; **c**, the moment of EBUS-TBNA, the needle tip is well-visualized inside the tissue of lymphatic node; **d**, view of target node immediately after biopsy. According to histopathology and microbiology, TBLA was confirmed

Another ultrasound technique, radial endobronchial ultrasound (rEBUS), allows navigation of peripheral lesions in pulmonary parenchyma (Fig. 8). After the localization of the lesion, ultrasound mini-probe can be removed, and biopsies are performed regularly. The inability to guarantee that biopsy will be performed in the same place where the lesion was detected by rEBUS is a disadvantage of this technique. Another option is to use an extended working channel, "guide sheath," which allows to remove mini-probe and fix the place for the further biopsy.

Recent data [6, 30] showed 77–85% sensitivity of navigated bronchoscopy using rEBUS and a guide sheath technique for detection of PTB, mostly using brush and TBLB as biopsy modalities.

3.7 Endoscopic Lung Volume Reduction in Cavitary Pulmonary Tuberculosis

Apart from the diagnostic role, modern bronchoscopy can be a salvation method for patients with cavitary forms of pulmonary TB. Performing endoscopic lung volume

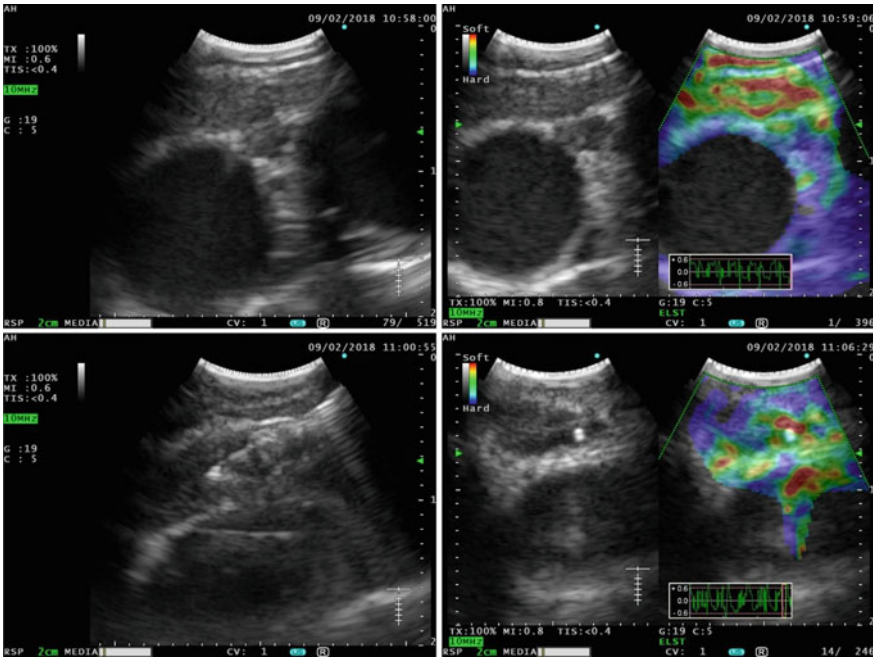

Fig. 7 Ultrasound view of EUS-b-FNA in a four-year-old child suspected of TBLA (Echobronchoscope Olympus BF UC180F, US center Olympus EU ME2): **a**, Endoscopic sonogram of the lymphatic node in position 4L with tiny hypoechoic lesions (necrosis); **b**, elastography-guided selection of area for biopsy—note the red areas, suggesting necrotic melting inside the node; **c**, the moment of EUS-b-FNA, the needle tip is well-visualized inside the tissue of lymphatic node; **d**, view of another target node before the biopsy–well-recognized areas of necrotic melting round the hyperechoic zones (calcification). According to histopathology and microbiology, TBLA was confirmed

reduction (ELVR) for TB indication (Fig. 9) has been widely accepted in Russian Federation for more than a decade [31, 32], and to some extent, in Italy [33], India, and China. Technically, during ELVR, a dedicated one-way endobronchial valve is placed with full obstruction of target lobar or segmental bronchus to gradually achieve local atelectasis and consequent closure of the cavity.

According to the recent data [31, 32], the efficacy of ELVR in cavitary MDR-TB reaches 95–96% for sputum conversion and 67–71% for cavity closure, with minimal complications and a high compliance rate. Interestingly, that procedure efficacy does not depend on mycobacteria resistance profile, and ELVR is effective in drug-sensitive, MDR-, and XDR-TB. The mean duration of valve placement is 12–15 months; afterward, the endobronchial valve can be removed via bronchoscopy under local or general anesthesia.

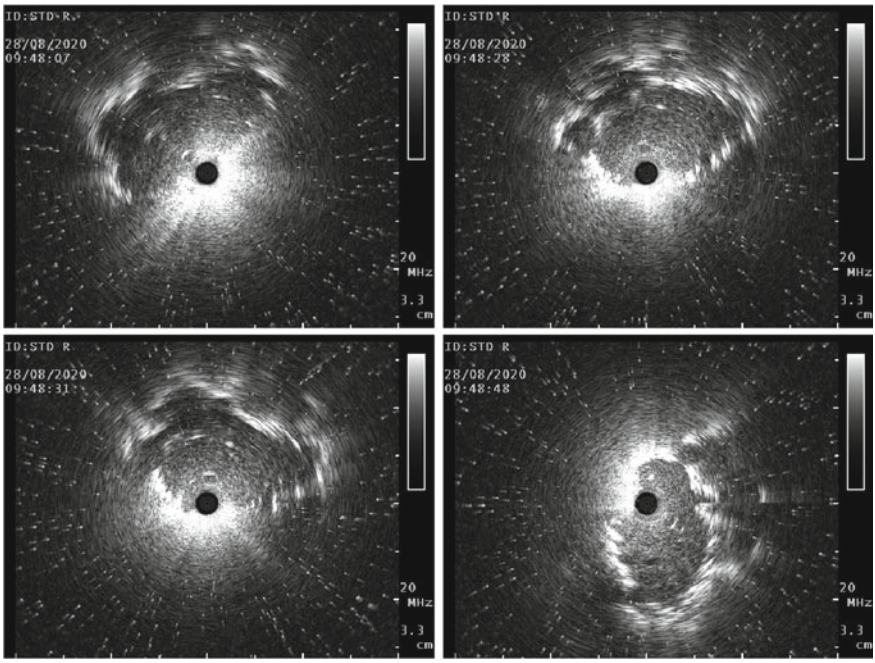

Fig. 8 Sonograms of radial EBUS of a patient with a solitary pulmonary nodule in RML (US center Olympus EU ME1): **a**–**c**, endoscopic sonograms of the peripheral pulmonary lesion, uneven shaped, with multiple air artifacts inside (terminal bronchioles). According to histopathology and microbiology, tuberculoma was confirmed

4 Bronchoscopy in Endobronchial Tuberculosis: From Diagnosis to Treatment

Endobronchial TB (EBTB) directly targets the bronchial tree, involving the tracheal and/or bronchial wall. This form of the disease is characterized by the extremely aggressive transmission of mycobacteria during cough or speech of a patient because of direct contact of infection locus with central airways. Data regarding the incidence of EBTB in PTB patients are controversial, and probably real incidence of this form is underestimated, but according to historical data, EBTB hits 5–45% of adult patients with PTB. In the pediatric population, EBTB is also an important clinical problem [27, 34], and its incidence reaches 4.7–8%. Early diagnostics of EBTB is extremely important to stop the disease transmission in the community and avoid possible complications, even life-treating, i.e., tracheobronchial stenoses and tracheomalacia. Diagnostic and therapeutic bronchoscopy plays a crucial role in establishing the diagnosis and performing local therapy.

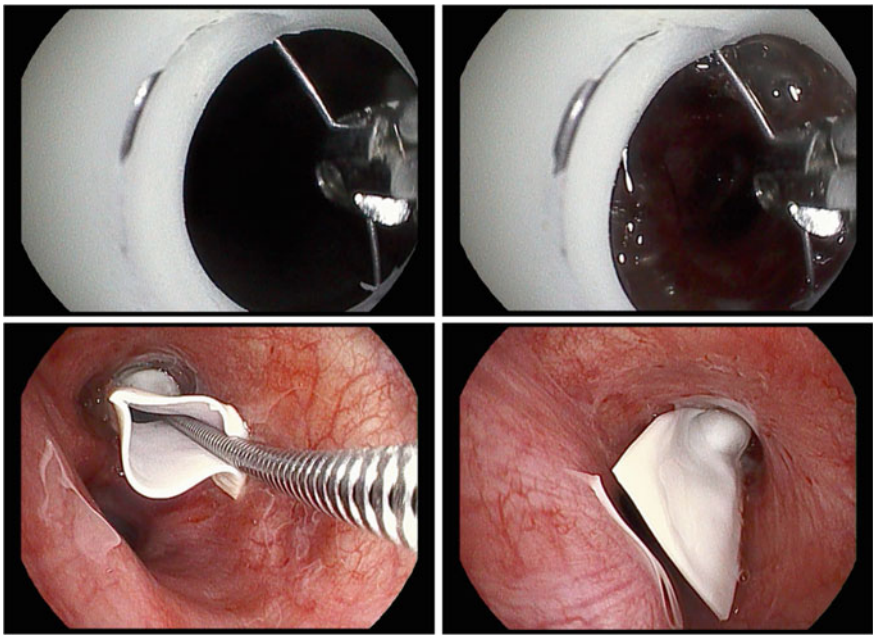

Fig. 9 Endoscopic view of ELVR (Videobronchoscope Pentax EB15 J10, endobronchial valve by Medlung): **a**, a valve is fixed like a 'cap' over the tip of the bronchoscope, and holds by forceps' cups; **b**, bronchoscope and valve *en bloc* inserted through the rigid barrel, going toward target bronchus; **c**, a valve is inserted in the orifice of left upper lobe bronchus, and gradually forceps are removing from it; **d**, immediately after removal of the forceps, an endobronchial valve is placed in the target bronchus (note that the 'tale' of the valve is not in contact with bronchial mucosa)

4.1 Bronchoscopic Findings in Endobronchial Tuberculosis and Possible Biopsies

Analyzing chest CT of patients with suspected EBTB, some signs of possible tracheobronchial injuries can be found, e.g., narrowing of the bronchial lumen, atelectasis, irregularity of inner margin of main bronchi and trachea, and peribronchial thickening. Unfortunately, these indirect signs of possible endobronchial lesions are not sensitive enough to confirm EBTB, so bronchoscopy in this situation should be performed.

According to the endoscopic picture, EBTB can be divided from five to seven forms, strongly associated with stages of bronchial wall injury [35]. These forms can be depicted as follows [36]:

- Non-specific bronchitis: minor changes and moderately hyperemia of bronchial surface;
- Edematous-hyperemic: remarkably bloated bronchial mucosa with definitive hyperemia;

- Actively caseating: bronchial mucosa with signs of edema and hyperemia, coated by different volumes of cheesy white plaques, often accompanied by tracheobronchial stenosis of different severity;
- Granular: extreme inflammatory changes of bronchial mucosa with multiple millet-like changes;
- Tumorous: visually detected tumor-like exophytic lesion with a cheesy surface, regularly leading to obstruction of target bronchus;
- Ulcerative: multiple ulcer-like lesions on the bronchial surface; and
- Fibrostenotic: different variants of cicatrical stenoses of bronchial tree

In some countries (East Europe and Russian Federation, for example), a shorter version of this classification is used, including hyperemic, ulcerative, tumorous, fibrostenotic, and one new specific form: the bronchonodular fistula, which can resemble an actively caseating form.

Interestingly, all presented forms (Fig. 10) reflect the natural course of EBTB from minimal lesions with microscopically-detectable mycobacterial invasion through the active process with caseation or granular/tumorous lesions to finalization of process in post-inflammatory fibrotic sequelae of the disease.

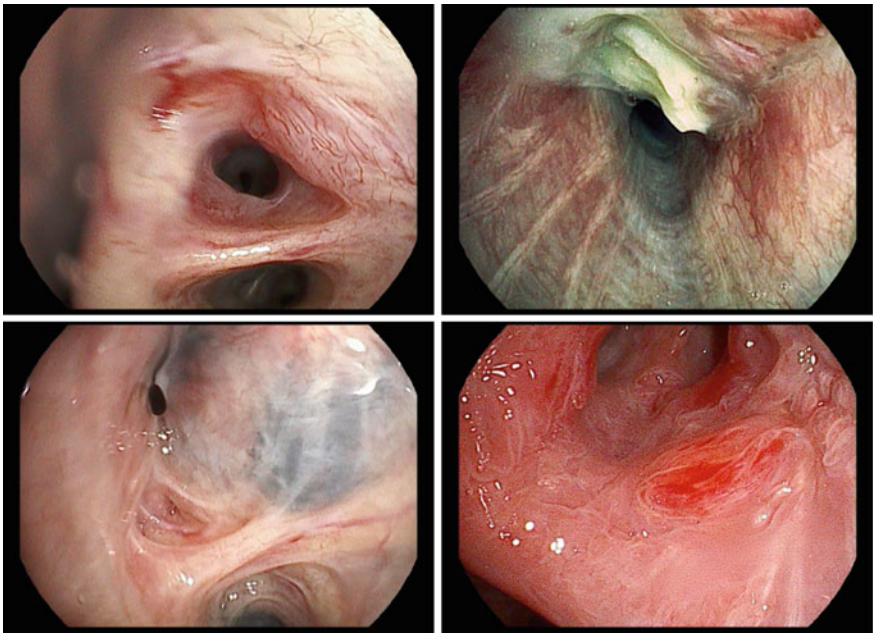

Fig. 10 Endoscopic view of different EBTB types (Videobronchoscope Pentax EB15 J10): **a**, tumorous type (note exophytic tissue on the right part of the picture); **b**, actively caseating type (necrotic tissue narrows the lumen of the left main bronchus); **c**, fibrostenotic type (severe post-inflammatory stenosis of the right main bronchus, note areas of anthracosis); **d** ulcerative type (note ulcerated, crater-like bronchial mucosa on the right side of the picture)

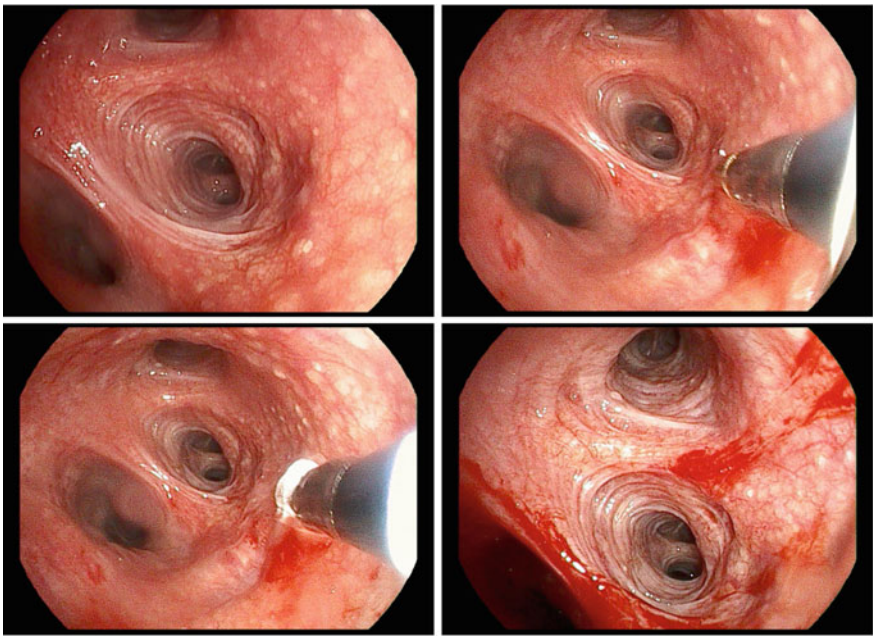

Fig. 11 Endoscopic view of EBCB in granular-type EBTB (Videobronchoscope Pentax EB15 J10, cryo-system ErbeCryo II): **a**, granular type of EBTB (note millet-like granular tissue on bronchial mucosa); **b**, 1,9-mm cryoprobe is inserted through the instrumental channel of the scope; **c**, the moment of endobronchial cryobiopsy of the lesions in the distal part of the left main bronchus (note ice tip formation around the probe); **d**, biopsy site after the removal of the specimen (moderate amount of blood after the intervention is observed)

Among all the biopsies previously mentioned, mostly EBB/EBCB is used to confirm EBTB and exclude possible malignancy or other granulomatosis (i.e., aspergillosis), followed by brushing and/or cTBNA in order of decline. The sensitivity of EBB is 75–100% [37, 38], whereas endobronchial cryobiopsy tends to be more effective in this situation (Fig. 11) due to larger samples obtained (Fig. 12). Brushing and cTBNA tend to be less effective in diagnosing EBTB [37, 38], with the sensitivity of 50% and 19%, respectively.

4.2 Endobronchial Treatment of Active Endobronchial Tuberculosis and Its Consequences

According to Chung et al. [35], who precisely observed the natural evolution of different EBTB variants, the cumulative incidence of fibrostenotic sequelae in this cohort is 53%, with almost two-thirds of caseating and hyperemic forms coming to stenosis. All depicted changes occurred within three months of systemic treatment. These facts highlight the importance of new endoscopic treatment options implementation. These options dedicated to active EBTB include:

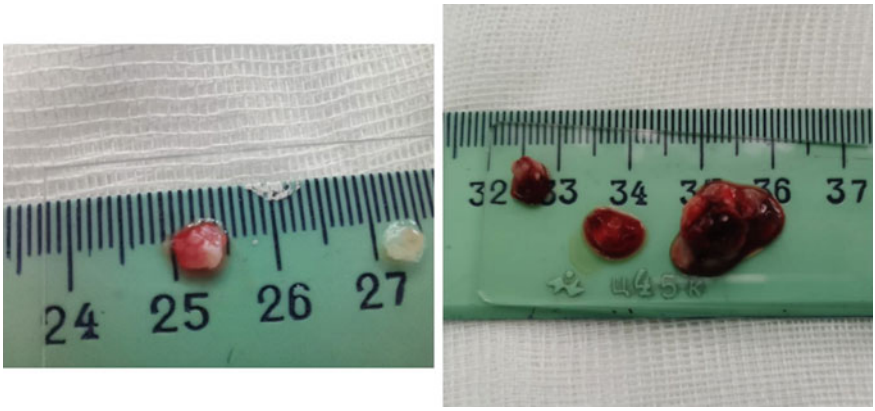

Fig. 12 Macroscopic view of tissue, retrieved by cryoprobes: **a**, specimens after TBCB with 1,9-mm cryoprobe; **b**, specimens retrieved after EBCB with 2,4-mm cryoprobe (note much larger size of specimens on the right picture due to larger cryoprobe)

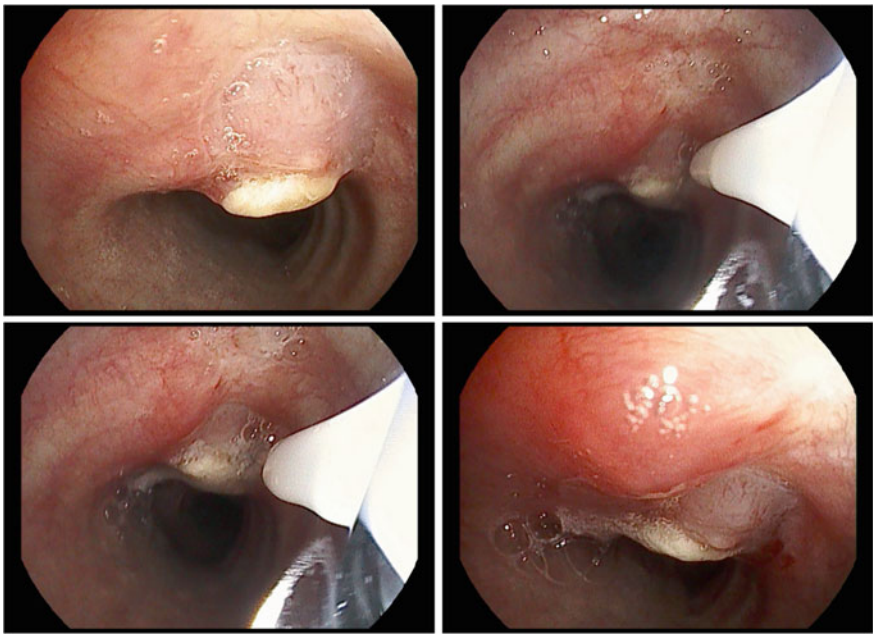

Fig. 13 Endoscopic view of cTBNI in a necrotic type of EBTB (Videobronchoscope Pentax EB15 J10): **a**, necrotic cap with granulations on tracheal mucosa, confirmed as EBTB; **b** and **c**, 22G cTBNI with an injection of capreomycin directly under the 'cap' of the tracheal lesion; **d**, site of injection immediately after the needle removal (note some swelling of granular tissue after the injection)

- local chemotherapy injections (Fig. 13);
- steroid injections; and
- photodynamic treatment of some localized forms [39].

Local injections of anti-TB drugs (mostly amikacin and capreomycin) usually are performed using a dedicated injection needle of 22–25G, with submucosal injection of active substance in the affected area, and done by series (from three to five bronchoscopies one week apart), gaining effective sputum conversion in two–three weeks, and accelerating healing of bronchial lesion in four to five weeks. Steroid injections can be added on late procedures to minimize the probability of fibrostenotic lesions. Photodynamic therapy, widely used in lung cancer patients, looks promising in EBTB healing because its effects do not need vascular supply to transfer to mycobacteria and do not depend on the level of mycobacterial resistance [39, 40]. In the case of the local granular type of EBTB, a single course of bronchoscopic photodynamic therapy led to full histologically and culture-proven healing of bronchial mucosa in three weeks without notable complications (Fig. 14).

In the case of bronchial obstruction due to active EBTB, many types of bronchoscopic recanalization can be used. Balloon dilatation [41] shows the immediate

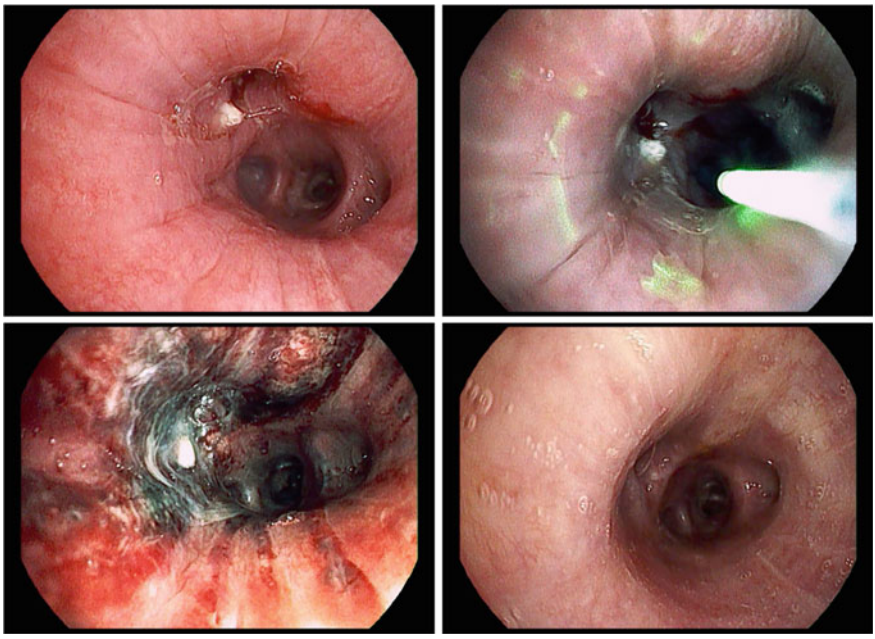

Fig. 14 Evolution of endoscopic view during PDT for EBTB (Video bronchoscope Pentax EB15 J10): **a**, necrotic cap with granulations on the bronchial mucosa, confirmed as EBTB; b, PDT irradiation of the affected zone; **c**, photoreaction with edema and necrotic changes one week after PDT; **d**, full restoration and healing of EBTB at three weeks after PDT procedure

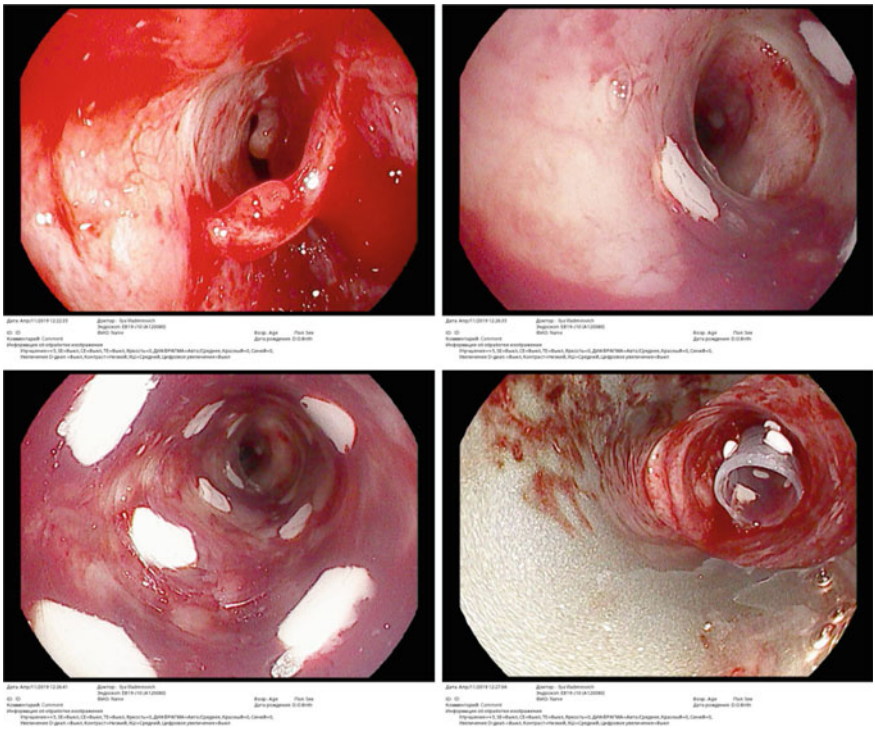

Fig. 15 Tracheobronchial stenting of severe fibrostenotic EBTB of right main bronchus in a patient after left pneumonectomy (Video bronchoscope Pentax EB15 J10, rigid barrel Karl Storz, custom-made silicone stent 'Medsil'): **a**, Initial presentation of a single-lung patient with extreme deformation and stenosis of right main bronchus; **b** and **c**, patency of RMB restored after custom-made J-shaped silicone tracheobronchial stent placement–view through-the-stent; **d**, proximal part of the silicone stent in the lower part of the trachea

but temporary effect; cryorecanalization can lead to a safe and rapid restoration of bronchial patency; also, different types of lasers and electrocautery were reported [42].

Endoscopic treatment of fibrostenotic sequelae of EBTB presents a challenging issue for clinicians, thoracic surgeons, and bronchoscopist. Until now, no definite and effective cure exists for this situation. Different combinations of dilation techniques have been used, e.g., rigid bronchoscope bougie, balloon dilatation, and tracheobronchial stenting (including custom silicone stents; Fig. 15). Also, quite often, "hot" (laser, cutter, argon-plasma coagulation) and "cold" (cryotherapy, cryo-spray) methods are used to restore bronchial patency (Fig. 16). Most often, complications after usage of "hot techniques" are restenosis and new scar formations, and for dilation techniques–again, restenosis, stent migrations and sputum congestion due to stent placement. Whereas possible, the general opinion is to avoid stenting in these situations to escape from acceleration of tracheomalacia and complications associated with stenting.

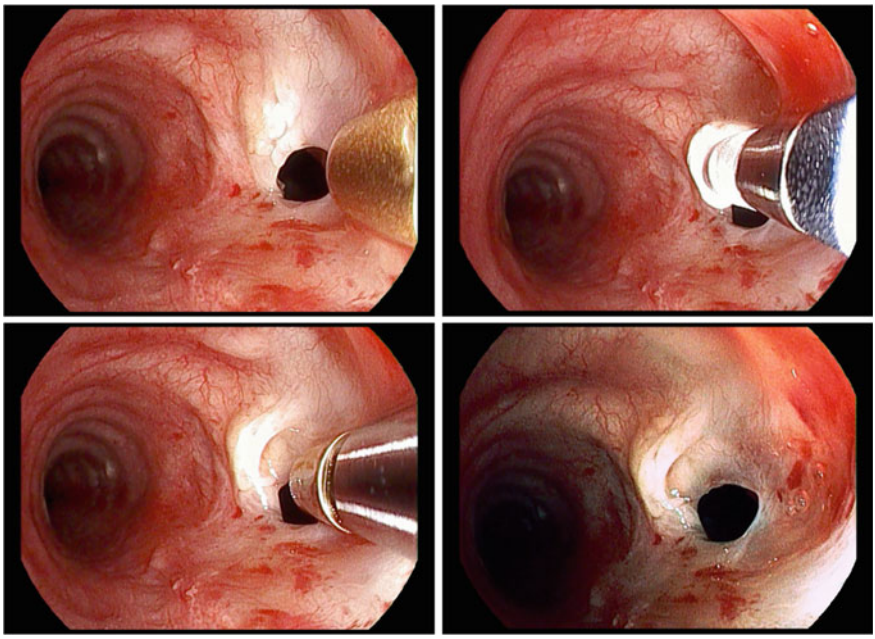

Fig. 16 Evolution of endoscopic view during endobronchial cryotherapy of severe stenosis of the right main bronchus after EBTB (Videobronchoscope Pentax EB15 J10, cryo-system ErbCryo II): **a**, Initial view of RMB stenosis; **b**, cryotherapy of the affected zone using 1,9-mm cryoprobe, exposition 60s, three cycles; **c**, cryoreaction several seconds after the first cycle of cryotherapy (note prominent softening of the treated area); **d**, an increase of lumen patency of RMB after the first cycle of cryotherapy

5 Conclusion

For more than a century of its history, bronchoscopy and interventional pulmonology have come a long way. In addition to outstanding advances in the minimally invasive diagnosis of TB, nowadays, we can see an incredible increase in the possibilities of endoscopic treatment of such a formidable infectious disease.

Core Messages

- Bronchoscopy is extremely useful both in the diagnostics and local treatment of EBTB.
- Bronchoscopic investigation can be accompanied by numerous biopsy types to confirm or rule out TB.
- Endoscopic treatment for EBTB and PTB includes local chemotherapy injections, ELVR procedures, and endoluminal PDT.

References

1. Meyer KC, Raghu G, Baughman RP, Brown KK, Costabel U, du Bois RM, Drent M, Haslam PL, Kim DS, Nagai S, Rottoli P, Saltini C, Selman M, Strange C, Wood B (2012) An official American thoracic society clinical practice guideline: the clinical utility of bronchoalveolar lavage cellular analysis in interstitial lung disease. Am J Respir Crit Care Med 185(9):1004–1014
2. Luo WX, Huang Y, Li QB, Han J (2014) Values of a combination of multiple less invasive or non-invasive examinations in the diagnosis of pediatric sputum-negative pulmonary tuberculosis. Zhongguo Dang Dai Er Ke Za Zhi 16(8):791–794
3. Kalawat U, Sharma KK, Reddy PN, Kumar AG (2010) Study of bronchoalveolar lavage in clinically and radiologically suspected cases of pulmonary tuberculosis. Lung India 27 (3):122–124. https://doi.org/10.4103/0970-2113.68307
4. Iyer VN, Joshi AY, Boyce TG, Brutinel MW, Scalcini MC, Wilson JW, McCoy K, Aksamit TR (2011) Bronchoscopy in suspected pulmonary TB with negative induced-sputum smear and MTD (®) Gen-probe testing. Respir Med 105(7):1084–1090. https://doi.org/10.1016/j.rmed.2011.03.003
5. Min JW, Yoon HI, Park KU, Song JH, Lee CT, Lee JH (2010) Real-time polymerase chain reaction in bronchial aspirate for rapid detection of sputum smear-negative tuberculosis. Int J Tuberc Lung Dis 14(7):852–858
6. Shabalina IY, Sivokozov IV, Evgushenko GV, Berezovsky YS, Andreevskaya SN, Karpina NL (2019) The use of rEBUS—radial endobronchial ultrasonography in the diagnosis of peripheral pulmonary lesions (PPLs) in single TB Centre. Ural Med J 11 (179):206–2015. https://doi.org/10.25694/URMJ.2019.11.30
7. Mehta J, Krish G, Berro E, Harvill L (1990) Fiberoptic bronchoscopy in the diagnosis of pulmonary tuberculosis. South Med J 83(7):753–755
8. Gupta N, Singh GC, Rana MK (2015) Histopathological yield in different types of bronchoscopic biopsies in proven cases of pulmonary tuberculosis. Indian J Pathol Microbiol 58(4):439–442. https://doi.org/10.4103/0377-4929.168881
9. Lai RS, Lee SS, Ting YM, Wang HC, Lin CC, Lu JY (1996) Diagnostic value of transbronchial lung biopsy under fluoroscopic guidance in solitary pulmonary nodule in an endemic area of tuberculosis. Respir Med 90(3):139–143. https://doi.org/10.1016/s0954-6111(96)90155-9
10. Mok Y, Tan TY, Tay TR et al (2016) Do we need transbronchial lung biopsy if we have bronchoalveolar lavage Xpert(1) MTB/RIF? Int J Tuberc Lung Dis 20:619–624
11. Maldonado F, Danoff SK, Wells AU, Colby TV, Ryu JH, Liberman M, Wahidi MM, Frazer L, Hetzel J, Rickman OB, Herth FJF, Poletti V, Yarmus LB (2020) Transbronchial cryobiopsy for the diagnosis of interstitial lung diseases: CHEST guideline and expert panel report. Chest 157(4):1030–1042. https://doi.org/10.1016/j.chest.2019.10.048
12. Sánchez-Cabral O, Martínez-Mendoza D, Fernandez-Bussy S, López-González B, Perea-Talamantes C, Rivera-Rosales RM, Luna-Rivero C, Martínez-Orozco JA, Flores-Suárez LF, Santillán-Doherty P, Reyes-Terán G (2017) Utility of transbronchial lung cryobiopsy in non-interstitial diseases. Respiration 94:285–292
13. Sánchez-Cabral O, Santillán-Díaz C, Flores-Bello ÁP, Herrera-Ortega MI, Sandoval-Gutiérrez JL, Santillán-Doherty P, Martínez-Mendoza D (2020) GeneXpert® MTB/RIF assay with transbronchial lung cryobiopsy for *Mycobacterium tuberculosis* diagnosis. Ann Transl Med 8(6):351. https://doi.org/10.21037/atm.2020.02.100
14. Cetinkaya E, Yildiz P, Altin S, Yilmaz V (2004) Diagnostic value of transbronchial needle aspiration by Wang 22-gauge cytology needle in intrathoracic lymphadenopathy. Chest 125 (2):527–531. https://doi.org/10.1378/chest.125.2.527
15. Cetinkaya E, Yildiz P, Kadakal F, Tekin A, Soysal F, Elibol S, Yilmaz V (2002) Transbronchial needle aspiration in the diagnosis of intrathoracic lymphadenopathy. Respiration 69(4):335–338. https://doi.org/10.1159/000063275

16. Gasparini S, Ferretti M, Secchi EB, Baldelli S, Zuccatosta L, Gusella P (1995) Integration of transbronchial and percutaneous approach in the diagnosis of peripheral pulmonary nodules or masses. Experience with 1,027 consecutive cases. Chest 108(1):131–137. https://doi.org/10.1378/chest.108.1.131
17. Wang KP, Haponik EF, Britt EJ, Khouri N, Erozan Y (1984) Transbronchial needle aspiration of peripheral pulmonary nodules. Chest 86(6):819–823. https://doi.org/10.1378/chest.86.6.819
18. Yasufuku K, Nakajima T, Motoori K, Sekine Y, Shibuya K, Hiroshima K, Fujisawa T (2006) Comparison of endobronchial ultrasound, positron emission tomography, and CT for lymph node staging of lung cancer. Chest 130(3):710–718. https://doi.org/10.1378/chest.130.3.710
19. von Bartheld MB, Dekkers OM, Szlubowski A, Eberhardt R, Herth FJ, in 't Veen JC, de Jong YP, van der Heijden EH, Tournoy KG, Claussen M, van den Blink B, Shah PL, Zoumot Z, Clementsen P, Porsbjerg C, Mauad T, Bernardi FD, van Zwet EW, Rabe KF, Annema JT (2013) Endosonography vs conventional bronchoscopy for the diagnosis of sarcoidosis: the GRANULOMA randomized clinical trial. JAMA 309(23):2457–2464. https://doi.org/10.1001/jama.2013.5823
20. Wahidi MM, Herth F, Yasufuku K, Shepherd RW, Yarmus L, Chawla M, Lamb C, Casey KR, Patel S, Silvestri GA, Feller-Kopman DJ (2016) Technical aspects of endobronchial ultrasound-guided transbronchial needle aspiration: CHEST guideline and expert panel report. Chest 149(3):816–835. https://doi.org/10.1378/chest.15-1216
21. Dedushkin D, Sivokozov I, Zaytsev A (2020) Efficacy of endoscopic fine-needle aspiration under ultrasound control in mediastinal adenopathy. Chest 157(6):A186
22. Lim CE, Steinfort DP, Irving LB (2020) Diagnostic performance of 19-gauge endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) in suspected lymphoma: a prospective cohort study. Clin Respir J. Published online April 19, 2020
23. Dhooria S, Aggarwal AN, Gupta D, Behera D, Agarwal R (2015) Utility and safety of endoscopic ultrasound with bronchoscope-guided fine-needle aspiration in mediastinal lymph node sampling: systematic review and meta-analysis. Respir Care 60(7):1040–1050. https://doi.org/10.4187/respcare.03779
24. Li W, Zhang T, Chen Y, Liu C, Peng W (2015) Diagnostic value of convex probe endobronchial ultrasound-guided transbronchial needle aspiration in mediastinal tuberculous lymphadenitis: a systematic review and meta-analysis. Med Sci Monit 21:2064–2072
25. Ye W, Zhang R, Xu X, Liu Y, Ying K (2015) Diagnostic efficacy and safety of endobronchial ultrasound-guided transbronchial needle aspiration in intrathoracic tuberculosis: a meta-analysis. J Ultrasound Med 34(9):1645–1650. https://doi.org/10.7863/ultra.15.14.06017
26. Abedini A, Razavi F, Farahani M, Hashemi M, Emami H, Mohammadi F, Kiani A (2020) The utility of elastography during EBUS-TBNA in a population with a high prevalence of anthracosis. Clin Respir J 14(5):488–494. https://doi.org/10.1111/crj.13159
27. Sivokozov IV, Shabalina IY, Evgushchenko GV, Gubkina M, Petrakova IY (2018) First experience of endoscopic ultrasound-guided fine-needle aspiration for mediastinal lymphadenopathy in a four-year-old child. CTRI Bulletin 3:71–76. https://doi.org/10.7868/S258766781803010X
28. Sivokozov IV, Silina TL, Korolev VN, Pravednikov PA, Lenskiĭ BS (2014) The first experience in using elastography in combination with endobronchial ultrasonography for mediastinal pathology: Preliminary assessment of feasibility and comparison of characteristics via different approaches. Vestn Rentgenol Radiol 4:13–19
29. Dhooria S, Madan K, Pattabhiraman V, Sehgal IS, Mehta R, Vishwanath G, Srinivasan A, Sivaramakrishnan M, Mohan A, Mathew JL, Kabra SK, Guleria R, Behera D, Agarwal RA (2016) A multicenter study on the utility and safety of EBUS-TBNA and EUS-B-FNA in children. Pediatr Pulmonol 51(10):1031–1039. https://doi.org/10.1002/ppul.23415
30. Chan A, Devanand A, Low SY, Koh MS (2015) Radial endobronchial ultrasound in diagnosing peripheral lung lesions in a high tuberculosis setting. BMC Pulm Med 15:90. https://doi.org/10.1186/s12890-015-0089-9

31. Levin A, Sklyuev S, Felker I, Tceymach E, Krasnov D (2016) Endobronchial valve treatment of destructive multidrug-resistant tuberculosis. Int J Tuberc Lung Dis 20(11):1539–1545. https://doi.org/10.5588/ijtld.16.0033
32. Lovacheva OV, Sivokozov IV, Ergeshov AE, Vasil'eva IA, Bagdasarian TR (2008) Use of a valvular bronchoblocker in the treatment of patients with destructive pulmonary tuberculosis. Probl Tuberk Bolezn Legk 10:58–61
33. Corbetta L, Tofani A, Montinaro F, Michieletto L, Ceron L, Moroni C, Rogasi PG (2016) Lobar collapse therapy using endobronchial valves as a new complementary approach to treat cavities in multidrug-resistant tuberculosis and difficult-to-treat tuberculosis: a case series. Respiration 92(5):316–328. https://doi.org/10.1159/000450757
34. Wang MS, Wang JL, Liu XJ (2020) Epidemiological trends in the form of childhood tuberculosis in a referral tuberculosis hospital in shandong, China. Biomed Res Int. Published online August 8, 2020
35. Chung HS, Lee JH (2000) Bronchoscopic assessment of the evolution of endobronchial tuberculosis. Chest 117(2):385–392. https://doi.org/10.1378/chest.117.2.385
36. Mondoni M, Repossi A, Carlucci P, Centanni S, Sotgiu G (2017) Bronchoscopic techniques in the management of patients with tuberculosis. Int J Infect Dis 64:27–37. https://doi.org/10.1016/j.ijid.2017.08.008
37. Altin S, Çikrikçioğlu S, Morgül M, Koşar F, Özyurt H (1997) 50 Endobronchial tuberculosis cases based on bronchoscopic diagnosis. Respiration 64:162–164. https://doi.org/10.1159/000196662
38. Turovtseva Y, Sivokozov I, Shabalina I, Dedushkin D (2020) Bronchoscopic findings in patients with pulmonary TB: single center experience. Chest 157(6):A103
39. Sivokozov I, Dedushkin D, Chesalina Y, Turovtseva Y, Shabalina I, Chernikh N, Bagdasaryan T (2020) Photodynamic therapy for endobronchial and laryngeal tuberculosis: initial experience. Chest 157(6):A97
40. Chung JE, Oak CH, Sung N, Jheon S (2015) The potential application of photodynamic therapy in drug-resistant tuberculosis. J Photochem Photobiol B: Biol 150:60–65
41. Ding WM, Wang JP, Fu Y, Zhang JY, Fu WX, Wang WJ (2010) [The clinical value of balloon dilatation through flexible bronchoscope in the management of tracheobronchial stenosis in 149 cases of endobronchial tuberculosis] Zhonghua Jie He He Hu Xi Za Zhi. 33 (7):510–514
42. Amat B, Esselmann A, Reichle G, Rohde HJ, Westhoff M, Freitag L (2012) The electrosurgical knife in an optimized intermittent cutting mode for the endoscopic treatment of benign web-like tracheobronchial stenosis. Arch Bronchoneumol 48:14–21

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Diagnosis of Childhood Tuberculosis in Low- and Middle-Income Countries

10

Basant Joshi

There can be no keener revelation of a society's soul than the way in which it treats its children.

Nelson Mandela

Summary

Pediatric tuberculosis (TB) is one of the neglected groups globally. Non-specific symptoms, the paucibacillary nature of the disease, and non-specific chest X-ray findings make childhood TB diagnosis challenging. Additionally, poor knowledge about childhood TB among healthcare workers (HCWs) and community people, especially in the lower level of the low- and middle-income countries (LMICs), contribute to the neglect of childhood TB in resource-limited settings, as they lack the pediatric specialist facility and also the important diagnostic tools. Smear microscopy–the most common diagnostic tool available in the resource-limited setting–is not present in all health facilities, and if present, sustainability is the frequent issue seen. Due to the lower sensitivity of the smear microscopy, TB cases are being missed to be diagnosed, resulting in increased missing cases of TB in the community, which makes TB disease transmit from one person to another in resource-limited high-burden settings of LMICs. Children are more vulnerable to TB in these settings as they acquire TB from

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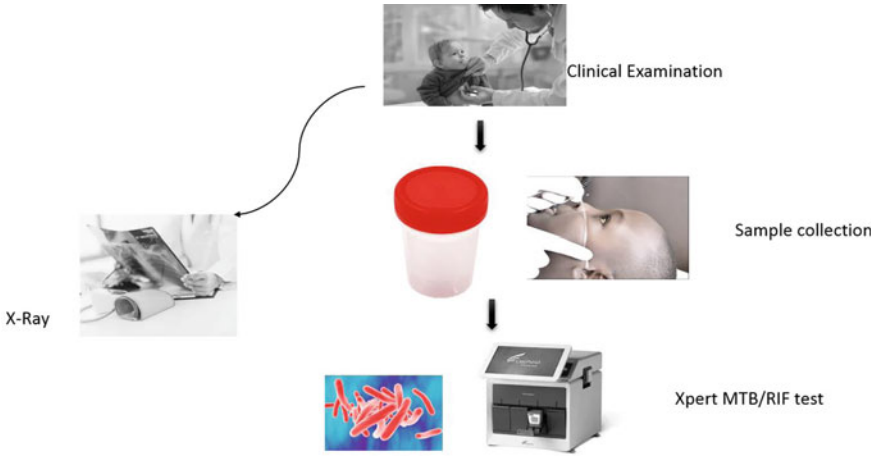
adult cases. As pediatric TB is difficult to diagnose even with advanced diagnostic tools, HCWs working in lower-level health centers should be trained to develop history-taking and clinical examination skills and to analyze laboratory and radiological findings for diagnosing pediatric TB. In addition, national tuberculosis programs (NTPs) should prioritize the scale-up of Xpert MTB/RIF and sputum extraction procedures for children suspected of TB in decentralized-level healthcare settings to improve childhood TB diagnosis and case notification in these areas.

Graphical Abstract



Diagnosis of pediatric tuberculosis

Keywords

Childhood TB · Diagnosis · LMICS · Tuberculosis

1 Introduction

Children are one of the vulnerable groups for Tuberculosis (TB) but are still neglected. Although children of any age can have TB, most commonly in TB-endemic settings, children of one to four years are more vulnerable to TB. The risk of developing TB is more common in children below two years of age whose immune system is immature compared to other age groups. Age-specific risk of

Table 1 Age-specific risk of developing tuberculosis (TB) following infection with *M. tuberculosis* in immunocompetent children. Adapted with permission from [7]

Child age at primary infection with TB (Year)	Risk of development to TB disease (%)		
	Pulmonary TB disease	Disseminated (Miliary) or Tuberculous meningitis	No TB disease
< 1	30–40	10–20	50
1–2	10–20	2–5	75–80
2–5	5	0.5	95
5–10	2	< 0.5	98
> 10	10–20	< 0.5	80–90

progression of TB following primary infection with an index case of infective TB is shown in Table 1. Pulmonary TB (PTB) is the most common type of TB in children. Extrapulmonary TB (EPTB) accounts for 30–40% and can occur in any body part. Children generally develop TB within one year of infection [1]; so, when TB is diagnosed in children, this indicates the recent TB transmission in the community. Immunocompetent children will be infected exclusively with TB after being exposed to a TB-infected case; however, in the case of infants, the illness will be more severe, with symptoms similar to pneumonia.

TB diagnosis in children is difficult compared to adults as there is a possibility of either under-diagnosis or overdiagnosis. So, healthcare workers (HCWs) should be careful while diagnosing children with TB. It is important to assess all the information gathered from careful history taking, clinical examination, and laboratory and radiological findings to confirm TB in children. PTB, the most common form of TB in children, is difficult to diagnose by using the techniques used to diagnose PTB in adults. Various factors contribute to the difficulty in diagnosing childhood TB:

- children present with non-specific symptoms; this will make it harder for HCWs to differentiate TB from other conditions such as malnutrition as well as HIV/AIDS;
- children tend to have a paucibacillary disease; this will make it difficult to detect TB bacilli, especially in smear microscopy;
- quality of sputum is also another concern, younger children cannot produce sputum of good quality, and this will result in misdiagnosis of TB by using laboratory procedures, such as sputum smear microscopy and even in Xpert MTB/RIF assay;
- X-ray is not present at primary healthcare centers, it will result in increased costs for parents to go to the referred X-ray centers. Moreover, the cost of chest X-rays is not covered by the national tuberculosis programs (NTPs); and
- there is also difficulty in interpreting chest X-rays in children due to non-specific findings in the radiography.

Many advancements have been made to diagnose TB in adults and children, which are being utilized more often in developed countries. More sensitive diagnostic technologies are being employed in resource-equipped countries to diagnose TB. However, low- and middle-income countries (LMICs) are still struggling for advanced diagnostic tools to be used throughout the country for TB diagnosis. In this context, pediatric TB is still neglected in LMICs. Conventional diagnostic tools for adults are not as sensitive for diagnosing TB in children as for adults. So, vulnerable children in resource-limited settings are far from appropriate sample collection and testing tools. There are many challenges for correctly diagnosing childhood TB in resource-limited settings, from screening to diagnosis. These challenges include poor knowledge about childhood TB among HCWs and children's parents in these settings. There are many operational challenges for implementing advanced diagnostic tools in lower-level healthcare settings of LMICs. Due to poor socioeconomic status, many NTPs of LMICs cannot scale up the novel diagnostic tools for childhood TB diagnosis at a decentralized level. Due to limited human resources, they also face challenges in deploying trained HCWs in lower-level healthcare settings.

Although research activities are performed to enhance childhood TB diagnosis in LMICs, there is still a lack of a mechanism for putting evidence into practice. This chapter will discuss the diagnostic tools used for pediatric TB in resource-limited settings, the challenges faced by health facilities in LMICs, and the way forward for improving childhood TB diagnosis in such settings. We will mainly focus on drug-susceptible (DS) TB in children.

2 Diagnosis of Childhood Tuberculosis in Resource-Limited Settings

TB is considered one of the oldest diseases in the world. Despite many advancements in the diagnosis and treatment of TB, this disease is still a global public health problem. The prevalence of TB is more in LMICs and highly populated countries like India, China, and Indonesia. There is a large gap between estimated cases and reported cases of TB. This is mainly due to the presence of a diagnosis gap which is more pronounced in resource-limited high-burden settings. As childhood TB is difficult to diagnose compared to adult TB, there is a significant case detection gap. Especially in resource-limited settings of LMICs, childhood TB is generally under-diagnosed due to the absence of proper diagnostic tools and the lack of pediatric specialty in lower-level healthcare settings.

Diagnosis of TB starts with a proper screening procedure at the healthcare center's entry point (triage), followed by clinical evaluation and laboratory and radiological investigation at the clinic. For pediatric tuberculosis diagnosis, information from screening, clinical assessment, and laboratory and radiographic data are all crucial for decision making. To differentiate TB from other disease conditions in children, it is sometimes better to see the patient's response to a course of

antibiotics before confirmation of TB. Appropriate specimens should be collected from the suspected site and tested in microscopy, culture, or Xpert MTB/RIF. More sensitive laboratory tools are needed to diagnose PTB in children because of the paucibacillary nature of the disease in children. PTB is the most common form of TB in children as in adults. The best sample for diagnosing PTB is sputum. It is challenging to collect sputum from children in resource-limited settings because children cannot easily produce sputum. This makes it difficult to diagnose PTB in children in these settings because they either do not have novel diagnostic tools such as Xpert MTB/RIF (which are more sensitive to diagnose TB bacilli) in their facility or if present, it is under-utilized for diagnosing pediatric TB due to lack of proper sputum collection methods such as induced sputum, gastric aspirate, nasopharyngeal aspirate and/or lack of trained HCWs to perform these methods to collect sputum from children. Smear microscopy, culture, and Xpert MTB/RIF are the widely used tests in LMICs to diagnose TB both in adults and children. Among these tests, smear microscopy is the only test available in most lower-level health facilities. Still, it is not sufficient to diagnose TB in children, which will be discussed later in this chapter. Other specimens from extrapulmonary sites are only being extracted in tertiary healthcare levels of LMICs. Chest X-ray findings help HCWs decide for the diagnosis of TB in children, but it alone is not a sufficient diagnostic tool for childhood TB. The culture of TB bacteria, *Mycobacterium tuberculosis (M. tb)*, is another TB diagnostic test, but it takes a long time to get a result, and it may not be as sensitive in the case of children as it is for adults. Apart from these diagnostic tests, tuberculin skin test (TST) or Mantoux test and interferon-gamma release assay (IGRAs) tests, along with other diagnostic tests, will help better diagnose childhood TB.

In LMICs and resource-limited settings, there is a high burden of TB, which leaves children at high risk for developing TB. Moreover, poor diagnostic facilities increase the challenge for diagnosing childhood TB in these settings. Although tertiary healthcare services in LMICs have the facility for diagnosing childhood TB, primary healthcare services and the majority of secondary healthcare services in these settings have difficulties diagnosing TB in children resulting in misdiagnosis of pediatric TB in these settings. There are many challenges for diagnosing TB in children in resource-limited settings described in the following paragraphs.

3 Poor Screening of Presumptive Child Tuberculosis Cases at Triage

Screening at triage is important for ruling out and providing an early diagnosis of TB. History taking is the most important part of TB diagnosis in children. In triage of health facilities, the child should be screened for signs and symptoms of TB. History of close contact with known adults and children with infectious TB should be taken into account because children usually acquire TB bacteria from pulmonary especially bacteriologically confirmed adult or adolescent TB cases known as the

index cases. Symptom-based screening is an effective approach to finding TB cases in TB endemic settings [2]. In many primary healthcare centers of LMICs, no proper screening for childhood TB is being done at triage. Some health facilities in these resource-limited settings do not practice screening at triage for various reasons such as lack of human resources, place, etc. In contrast, others practice screening with untrained HCWS or volunteers. This causes a delay in the diagnosis of TB in children, and children with TB may be overlooked in outpatient departments (OPD) by HCWs due to the clinic's high patient flow. In some cases, untrained HCWs at triage might miss the presumptive child TB cases because of non-specific signs and symptoms present in children.

To increase childhood TB case notification and minimize the gap in childhood TB case notification, proper screening of children at triage by trained HCWs is important. HCWs at the triage should be well educated on common signs and symptoms of TB and childhood TB to screen presumptive child cases and send them to the clinic for further investigation. If screening for TB and childhood TB could be made mandatory in all healthcare facilities and HCWs are trained for proper screening of children for TB, this will help diagnose more children with TB and minimize the diagnosis gap. In order to do this, each health institution should create infrastructure for triage in lower-level health facilities, as well as frequent training for HCWs working at triage should be planned to improve technical abilities on screening for childhood TB. Frequent monitoring and supervision visits and clinical mentoring from experts and managerial levels will encourage HCWs to develop the necessary skills for properly screening children for TB at triage.

4 Difficulties for Sustainability of Active Case Finding Strategies

Early diagnosis of TB and initiation of anti-TB drugs will help control TB transmission and achieve the ambitious targets set by the World Health Organization (WHO) and NTPs. Active case finding (ACF) has been an important approach for identifying children with TB infection and disease [3, 4]. Household contact tracing of index TB cases will help find the new cases of TB and vulnerable groups (children less than five years, malnourished children, household members with HIV) to start TB preventive therapy, which will break the chain of transmission. In LMICs, especially in high-burden and resource-limited settings, ACF interventions are seen to be started at some point of time in support of external funding and help for increasing case notification. But due to lack of regular funding, these interventions for ACF are not continued in these areas. This will lead to many TB cases missed in the community who will die because they are undiagnosed for TB and transmit TB to other people, resulting in an increased number of TB cases in the community.

ACF activities such as household contact tracing, mobile screening clinics, screening of children in schools, etc., will help increase childhood TB case detection in high-burden and resource-limited settings. To minimize the existing diagnostic gap for TB in these resource-limited settings, ACF interventions should be adopted as a routine program by each NTP, and an equal emphasis should be given to ACF for pediatric TB as well as adult TB. This will help increase TB case diagnosis from the high-burden community and break the chain of transmission and mortality due to TB in these settings.

4.1 Lack of Diagnostic Tools

TB can be diagnosed bacteriologically or clinically. Diagnostic tools are most important for confirmation of TB in adults and children. Diagnostic tools commonly used include microbiological tests and molecular tests to confirm TB bacteria and radiological findings. Childhood TB diagnosis needs all or majority of these diagnostic tests results/findings before confirmation of TB. Here we discuss various tests and tools used to diagnose childhood TB in LMICs, operational challenges for their effective implementation, and measures that can be adopted to overcome the challenges faced by health facilities in resource-limited settings.

4.1.1 Mantoux Test

Mantoux test (MT) is also known as tuberculin skin test (TST). It is a delayed-type hypersensitivity reaction to tuberculin purified protein derivative (PPD). Positive MT indicates the infection with *M. tb* but cannot confirm active TB disease. HCWs should be aware of the possibility of false-positive TST responses, particularly in the context of past BCG vaccination and nontuberculous mycobacterial (NTM) infection. Furthermore, a nonreactive TST result does not exclude *M. tb* or TB disease infection. This test can be helpful to confirm pediatric TB if it is considered with other laboratory tests and the history of the child. This test is available at some of the primary healthcare centers of resource-limited settings, but it can not be used as a confirmatory test for diagnosing childhood TB. So, this easy-to-perform test can be used in the lower-level healthcare setting along with other laboratory tests for confirming pediatric TB.

Another test to detect TB infection in children is the interferon-gamma release assay (IGRAs). Although this test has higher specificities than TST, especially in low burden settings and among BCG vaccinated children, it has poor sensitivities for immunocompromised individuals and children with severe forms of TB. This test also can not differentiate between TB infection and disease and is not commonly used in resource-limited settings.

4.1.2 Chest X-ray

Another diagnostic tool, i.e., chest X-ray, is not available in most of these settings. If available, there is a challenge for the regular availability of technical staff and X-ray interpreters. A regular power supply is also a challenge to running X-ray

machines in resource-limited settings. Most HCWs at primary healthcare centers lack the skills for interpreting chest X-ray findings [5]. Due to the poor economic condition of the parents of the children and lack of transportation, they do not prefer to go to the X-ray facility, which is usually very far from the community they belong to. Sometimes the referred cases will be lost and never come to the healthcare facility. This will lead to the condition that the children with presumptive TB will not get a complete diagnosis, increasing the diagnostic gap in this group. This will also increase the chance of rising mortality of children due to TB disease, which is otherwise curable.

4.1.3 Smear Microscopy

In most of the primary healthcare centers of LMICs, the only diagnostic tool for TB is sputum smear microscopy, which can diagnose adult PTB but cannot detect all children with PTB, resulting in missed diagnosis of TB in children. Children, especially young ones, do not produce quality sputum. Primary healthcare facilities in these settings do not have the capability of extracting sputum (induced sputum, gastric aspirate, nasopharyngeal aspirate) from children because they lack the appropriate equipment and/or qualified HCWs to execute sputum extraction procedures in children. They also do not have equipment for collecting extrapulmonary samples from children. So, in health facilities in these settings, HCWs need to refer the child who cannot produce sputum to higher centers to collect sputum or other samples for EPTB. Although smear microscopy clinics in lower-level healthcare settings have helped diagnose adult PTB, they have made a relatively minor contribution to pediatric PTB diagnosis.

4.1.4 Xpert MTB/RIF Assay

Xpert MTB/RIF assay can be used in vulnerable people, including children, to increase TB case detection and determine bacteria's sensitivity (sensitive or resistant) towards Rifampicin, an important TB drug. Xpert Ultra, a more sensitive test among the Xpert tests group, has the highest sensitivity among rapid diagnostic tests used for diagnosing childhood TB [6]. If these Xpert Ultra machines can be established in resource-limited high-burden settings, they will contribute to diagnosing pediatric TB. Sample collection by induced sputum and nasopharyngeal aspirate in children will provide better quality samples for testing in Xpert. If a combination of these sample collection techniques can be used and the sample is tested in Xpert Ultra, most children with PTB can be detected [6]. In some primary healthcare centers of LMICs, Xpert MTB/RIF is provided to diagnose pulmonary bacteriologically confirmed TB using sputum samples. Still, a majority of them have a challenge as interruption of Xpert facility occurs due to various causes such as frequent power cut-off, lack of sophisticated room for Xpert machine, frequent turnover of trained laboratory staff, interruption in the supply chain of the necessary consumables such as cartridges, delay in technical support in case of a module failure, and calibration of the machine. Battery-operated Xpert MTB/RIF machines might be the alternative for those settings having frequent power cut-off. NTPs should prioritize establishing Xpert MTB/RIF centers in high-burden settings to

increase TB case detection in adults and children. This will help minimize the morbidity and mortality of TB disease in high-burden resource-limited settings. There might be different challenges for effective implementation of the Xpert MTB/RIF. Some challenges faced by one health facility might not exist in others. So, operational and implementation research should be conducted to identify the implementation bottlenecks in different settings. These operational and implementation research will help to identify various barriers and facilitators for the implementation of Xpert MTB/RIF in specific settings and pave the path for better implementation of Xpert MTB/RIF.

4.1.5 Culture

Culture is also known as the gold standard for the diagnosis of TB as it grows the live bacteria present in the sample. Culture is used to isolate organisms (*M. tb*) and determine phenotypic drug susceptibility testing (DST). For this, samples from suspected sites are collected and grown in culture media. Because of the paucibacillary nature of the illness in children, the sensitivity of culture to detect *M. tb* in children samples is lower than in adults. There is no provision for culture in lower-level healthcare settings, and the sample should be transported to the laboratory situated in higher-level healthcare centers, especially tertiary-level healthcare facilities. Because the course of TB in children is quick, the time to get culture findings is lengthy, and the sensitivity of culture results in children is poor, it is preferable to begin TB therapy before bacteriological confirmation.

To increase pediatric TB diagnosis in resource-limited settings, sputum extraction techniques, such as induced sputum, gastric aspirate, or nasopharyngeal aspirate, and diagnostic techniques with increased sensitivity such as Xpert MTB/RIF should be available at lower-level health facilities in these settings. In addition, training and refresher training to HCWs and regular clinical mentoring is important to implement these interventions easily and contribute to increased pediatric TB case detection.

4.2 Poor Knowledge About Childhood Tuberculosis Among Parents and HCWs

Since most children with presumptive TB belong to a low-income family, they cannot visit the higher healthcare facility even when referred to the higher centers. Similarly, the out-of-pocket cost for X-rays also discourages children of low-income families from complete diagnosis. Apart from these, most of the parents of the lower level settings of LMICs have poor knowledge about TB, especially childhood TB. Due to this, they do not take childhood TB seriously. Instead of taking their children to healthcare facilities, they prefer visiting traditional healers first, which delays the diagnosis of TB and increases the severity of the disease. Even HCWs of lower-level settings have poor knowledge about childhood TB. This poor knowledge about childhood TB among HCWs will not only directly affect the childhood TB diagnosis in these settings but will also prevent HCWs from

sensitizing the parents about childhood TB and the importance of early diagnosis of childhood TB. Due to this reason, childhood TB remains neglected by parents and HCWs in lower-level healthcare settings. This will lead to a large number of children in the community who are undiagnosed and untreated for TB. So, training on childhood TB for HCWs in such settings is important to initiate and increase case detection. Apart from this, various programs based on community to sensitize community people on TB and childhood TB are necessary on a regular basis [8].

5 Conclusion

This chapter described diagnostic tools for childhood TB in the lower-level healthcare setting of LMICs. The challenges faced by HCWs in resource-limited settings for diagnosing childhood TB were discussed, along with the way forward for the increased and improved diagnosis of childhood TB. The discussion highlighted how operational and implementation research is important in these settings to identify bottlenecks for introducing better diagnostic tools for childhood TB in resource-limited settings. So, "*healthier children for better community*" should be accepted by community people to protect children from infectious diseases like TB.

Core Messages

- Childhood TB is still neglected.
- Childhood TB diagnosis tends to be missed due to the paucibacillary nature of the disease and the lack of specific diagnostic tools.
- Lack of diagnostic tools and experience makes diagnosing pediatric TB difficult in low- and middle-income countries.
- There is a need to study the possibilities of decentralizing childhood TB diagnostic technologies in resource-limited settings.

References

1. Marais BJ, Gie RP, Schaaf HS, Hesseling AC, Obihara CC, Starke JJ, Enarson DA, Donald PR, Beyers N (2004) The natural history of childhood intra-thoracic tuberculosis: A critical review of literature from the pre-chemotherapy era. Int J Tuberc Lung Dis 8:392–402
2. Triasih R, Robertson CF, Duke T, Graham SM (2015) A prospective evaluation of the symptom-based screening approach to the management of children who are contacts of tuberculosis cases. Clin Infect Dis 60:12–18. https://doi.org/10.1093/cid/ciu748
3. Joshi B, Chinnakali P, Shrestha A, Das M, Kumar AMV, Pant R, Lama R, Sarraf RR, Dumre SP, Harries AD (2015) Impact of intensified case-finding strategies on childhood TB case registration in Nepal. Public Heal action 5:93–98. https://doi.org/10.5588/pha.15.0004

4. Marais BJ (2015) Strategies to improve tuberculosis case finding in children. Public Heal Action 5:90–91. https://doi.org/10.5588/pha.15.0028
5. Christiansen JM, Gerke O, Karstoft J, Andersen PE (2014) Poor interpretation of chest X-rays by junior doctors. Dan Med J 61:1–5
6. Zar HJ, Workman LJ, Prins M, Bateman LJ, Mbhele SP, Whitman CB, Denkinger CM, Nicol MP (2019) Tuberculosis diagnosis in children using Xpert Ultra on different respiratory specimens. Am J Respir Crit Care Med 200:1531–1538. https://doi.org/10.1164/rccm.201904-0772OC
7. Marais BJ, Gie RP, Schaaf HS, Beyers N, Donald PR, Starke JR (2006) Childhood pulmonary tuberculosis: old wisdom and new challenges. Am J Respir Crit Care Med 173:1078–1090. https://doi.org/10.1164/rccm.200511-1809SO
8. Reuter A, Seddon JA, Marais BJ, Furin J (2020) Preventing tuberculosis in children: a global health emergency. Paediatr Respir Rev. https://doi.org/10.1016/j.prrv.2020.02.004

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Pediatric Tuberculosis: Current Evidence for Laboratory Diagnosis

11

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Many vague concepts on the pathogenesis of tuberculosis are attributable to an inadequate understanding of the basic facts and principles of the action of the bacteria and the reactions of the host.

Arnold Rich [1]

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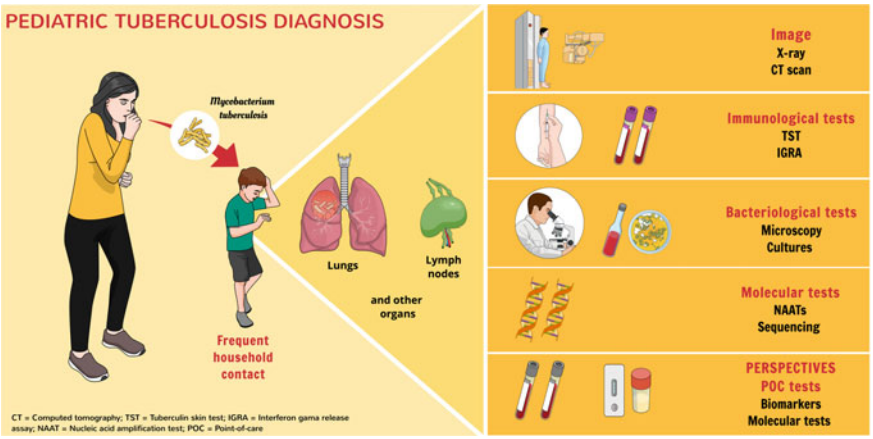
Summary

As children develop paucibacillary forms of tuberculosis (TB), bacteriological tests usually are negative. This chapter describes aspects of the laboratory tests available for clinical practice. It also discusses perspectives, such as biomarkers, which can be utilized in rapid tests, point of care (POC) tests, performed on samples other than sputum.

Graphical Abstract



Pediatric tuberculosis diagnosis

Keywords

Biomarker · Children · Diagnosis · GeneXpert® · Pediatric tuberculosis · Tuberculosis · Xpert Ultra®

1 Introduction

Children represent approximately 11% of worldwide tuberculosis (TB) cases. Despite Robert Koch's discovery of its principal etiologic agent, *Mycobacterium tuberculosis (M. tb)*, bacteriological confirmation remains a challenge in this age group, as children develop paucibacillary forms of the disease, primary TB, and young children are unable to expectorate [2]. Pulmonary TB (PTB) is the most prevalent form. Adolescents often present post-primary PTB similar to adults, they can expectorate, and bacteriological tests usually tend to be positive [3, 4].

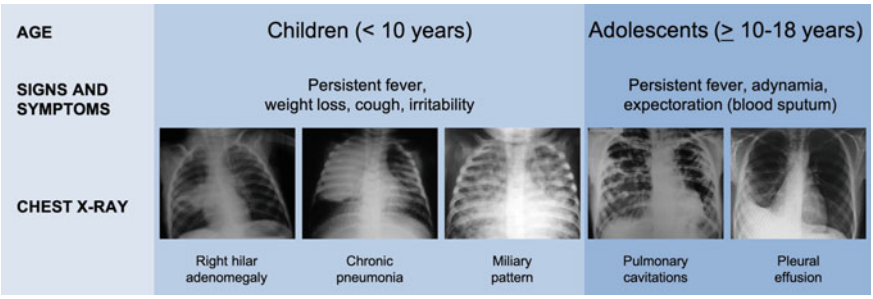


Fig. 1 Clinical and radiological characteristics of PTB in children and adolescents. Adapted with permission from [4]

The radiological changes predominant among children are hilar lymphadenopathy, nodular, micronodular (miliary) infiltrates, and chronic evolution of lesions characterizing expansive pneumonia. In adolescents (10–19 years of age), post-primary or adult-type TB is more frequent [4, 5]. These characteristics are displayed in Fig. 1. Also, the forms of extrapulmonary TB (EPTB) in human immunodeficiency virus (HIV) co-infected patients are paucibacillary [6]. Even today, especially in children, the diagnosis, mainly of PTB, is based on clinical, radiological, and epidemiological findings together with the positive result of the tuberculin skin test (TST) [6, 7].

cGraham et al. (2015) propose that the final classification of pediatric TB cases should be either confirmed TB (culture and/or polymerase chain reaction (PCR) positive for *M. tb*) or unconfirmed TB [8]. This categorization facilitates the comparison of results obtained in scientific studies. In recent years, various strategies, including the assessment regarding contacts of bacilliferous patients and diagnosis and treatment of latent TB infection (LTBI), have been used for "The End TB Strategy" of the World Health Organization (WHO) [9]. Individuals under five years old and with immunodeficiency are given priority in the treatment of LTBI. It is known that treating LTBI can diminish the risk of illness, with an efficiency of between 60 and 90% [10]. Primarily, LTBI is treated with one or two drugs; thus, both clinicians and laboratorians must differentiate it from active TB [6].

In 2016, the WHO projected 490,000 multidrug-resistant TB (MDR-TB) cases globally, which reinforces the need for the availability of laboratory tests to assess bacterial resistance [11].

The classic tests used to investigate suspected TB are TST, bacterioscopy for the acid-fast bacilli (AFB), and culture techniques for *M. tb* in clinical specimens [6]. Increased knowledge of the pathogenesis and immunology of the disease, with the development of molecular biology and biotechnology, has allowed the identification of molecules that can be used in diagnostic tests in clinical practice. Examples of new tools incorporated into clinical practice are detection in peripheral blood through interferon-gamma release assays (IGRA) and nucleic acid amplification tests (NAATs). Such tests provide fast results and accurately measure drug-resistant

(DR) [12]. Moreover, the development of rapid TB tests, point of care (POC) tests, is cardinal as they can provide a fast diagnosis, especially for children and co-infected patients [13]. New perspectives have emerged from the studies performed to evaluate the role of biomarkers in TB, including innovations such as "omics" technologies that identify molecules such as proteomics, metabolomics, and transcriptomics [14].

This chapter presents the laboratory tests currently available for diagnosing LTBI and active TB in children and adolescents and new perspectives in this era.

2 Non-specific Tests

The hematological abnormalities in pediatric TB are anemia, neutrophilia, or monocytosis. Although such hematological changes are quite prevalent in children with TB, in developing countries, they also occur frequently among children with other non-TB respiratory infections. Thus, the complete blood count has no predictive diagnostic value when investigating a child assumed to have TB [15].

Higher leukocytosis, neutrophilia, neutrophil/lymphocyte ratio, C-reactive protein (CRP), and red cell distribution width (RDW) were observed among adult patients with advanced PTB [16]. In this group, the effectiveness of CRP for the diagnosis and follow-up of TB cases has been demonstrated since its concentrations decrease throughout the anti-TB treatment (ATT) and will be normalized at the end of the therapy [17]. CRP is superior to erythrocyte sedimentation rate (ESR) [18]. In another study involving adults, after two months of treatment, patients with PTB and anemia were more likely to display bacteria in the sputum than TB cases without anemia, denoting a higher risk of a positive AFB with increasing severity of anemia [19].

3 Immunological Tests

3.1 Tuberculin Skin Test

TST, an indirect method to detect *M. tb* infection, is useful to detect LTBI and as a complementary test in suspected active TB cases [20–22]. Charles Mantoux developed TST in 1907. TST is performed by intradermal inoculation of five crude tuberculin units (TU) or a purified protein derivative of *M. tb* (PPD). The most commonly used is PPDRT23, which provides a reading after 48–72 h [20, 22]. For almost 100 years, TST was the only diagnostic tool available to detect *M. tb*, regardless of clinical presentation. Furthermore, TST has a high positive predictive value in regions where LTBI is prevalent and among those individuals with known risk factors [20, 23]. The TST is considered positive if the area of induration is greater than or equal to five millimeters [6]. Consideration should be given to the guidance of health authorities in each country concerning local norms and policies.

Usually, the interpretation of TST results for individuals vaccinated with Bacillus Calmette-Guérin (BCG) is similar to those not vaccinated [22]. BCG vaccine can cause false-positive results. However, evidence suggesting that the positive result is due to an LTBI can be affirmed by ascertaining known contacts with contagious TB, family history of TB, time since BCG vaccination (> five years), and TST reaction size ($\geq$ 15 mm) [20, 22]. Broadly, the specificity ranges from 49 to 65% among children vaccinated with BCG and between 95 to 100% for unvaccinated children [20]. Some disadvantages of TST include administration and interpretation of the process as a trained professional is required to apply and read the test. The patient is also required to return to read it. False-positive can result either due to the previous application of a BCG vaccination or because of a viable contact with non-tuberculous mycobacteria (NTM) [20].

Immunocompetent children with confirmed TB may present negative TST [22]. Other reasons for a false-negative TST result are young age, starvation, inadequate handling of PPD, viral infections (chickenpox, influenza, and measles), recent *M. tb* infection, immunosuppressive diseases, such as acquired immunodeficiency syndrome (AIDS), or because of other severe forms of TB [20, 22, 23]. Therefore, the lack of reaction to TST does not exclude LTBI or active TB [22].

3.2 Interferon-Gamma Release Assay

Since 2002, QuantiFERON-TB Gold In-Tube® (Cellestis International, Melbourne, Australia) and T-SPOT TB® (T-SPOT TB®; Oxford Immunotec, Abington, UK) are available for use in the United States of America. Both detect blood interferon-gamma (IFN-γ) synthesized by lymphocytes stimulated with *M. tb* antigens. These antigens are associated with *M. tb* rather than *M. bovis* (BCG vaccine) or several other NTMs. While the QuantiFERON-TB Gold In-Tube® analyzes the amount of IFN-γ produced by lymphocytes stimulated by 6-kDa early secretory antigenic target (ESAT6), 10-kDa culture filtrate protein (CFP-10), and Rv2654 (TB7.7), the T- Spot quantifies lymphocytes stimulated by ESAT-6 and CFP-10 [22, 23]. The possible results are positive, negative, or indeterminate, and T-SPOT TB® also has the possibility of yielding a borderline result [23]. In cases of indeterminate or negative results, the test must be repeated, or another method must be used [22, 23].

TST and IGRA sensitivity are similar for documenting *M. tb* infection in adults and children; however, there may be less sensitivity for IGRAs in countries with a high-TB burden [20, 22, 24]. The specificity of IGRA is greater than that of TST [20].

In children, who have signs and symptoms of TB, the IGRA has a sensitivity of 60–80%. Yet, among confirmed TB cases, it ranged between 80 and 85%. Specificity ranges from 89 to 100% in children vaccinated with BCG and from 90 to 95% among unvaccinated [20]. More recently, it has been observed that IGRA has consistent performance among children aged two years and over, and some data support its usage even for younger children [22].

While a positive IGRA result in children should be considered *M. tb* infection, a negative result does not rule it out. Conversely, indeterminate or invalid results

have several causes related to the patient, the test, or the performance itself. These results do not exclude infection by *M. tb* and may require the repetition of the test or the use of another method. Therefore, indeterminate/invalid IGRA results should not be solely employed to make clinical decisions [22].

3.3 Tuberculin Skin Test Versus Interferon-Gamma Release Assay

TST is the test of choice for children under 24 months of age. In those older than two years, either the TST or IGRAs can be used; the latter is even preferred if the child is vaccinated with BCG, thus avoiding false-positive TST results [22]. Both IGRA and TST depend on cellular immunity, so sensitivity may be lower in immunosuppressive conditions such as HIV/AIDS [20, 22, 23]. When comparing TST and IGRA, the second costs more and needs more specialized laboratories, so it is not regarded as a substitute for TST in endemic TB regions with limited resources, regardless of the HIV status [4, 9, 25].

4 Bacteriological Tests

4.1 Bacilloscopy (Direct Examination)

Bacilloscopy is a simple, safe, and fast method performed in laboratories by trained professionals. The procedure evaluates the AFB in material obtained from the airway and extrapulmonary lesions. For a positive result, at least 5000 bacilli per ml of respiratory secretion are required. However, this method does not allow a distinction between the *M. tb* and NTM [6, 25]. Therefore, it can be performed through conventional microscopy, with Ziehl–Neelsen staining, the most frequently used method, or through fluorescence microscopy, conventional or with light-emitting diode (LED). Fluorescence microscopy has slightly higher accuracy than conventional microscopy [6, 25]. Correspondingly, the reading and interpretation of bacilloscopy results are described in Table 1. The reading and interpretation of the bacilloscopy results of other materials are shown in Table 2.

4.2 Culture

Culture testing is a more complex, expensive, and time-consuming method; as a result, it can take up to 40 days in case of a solid medium, compared to bacilloscopy, but requires fewer bacilli in the material to show a positive result. It can be performed using a solid egg-based medium, Löwenstein-Jensen (LJ), Ogawa-Kudoh, or a liquid medium [25–27]. Particularly, this method tests the sensitivity of the infectious agent to antimicrobials.

Table 1 Reading and interpretation of sputum bacilloscopy AFB, acid-fast bacillus

Reading	Result
AFB not found in 100 fields observed	Negative
1–9 AFB in 100 fields observed	The quantity of bacilli found is reported
10–99 AFB in 100 fields observed	Positive +
1–10 AFB per field in 50 fields observed	Positive ++
On average more than 10 AFB per field in 20 fields observed	Positive +++

Adapted with permission from [6]

Table 2 Reading and interpretation of the bacilloscopy of several clinical specimens AFB, acid-fast bacillus

Finding	Result
AFB is not found in the examined material	Negative
AFB is found in any quantity of the examined material	Positive

Adapted with permission from [6]

The commonly used liquid media are Middlebrook 7H9 and Dubos Tween, as well as automated alternatives, such as microscopic observation drug susceptibility assay (MODS), BACTEC 460 TB, and the *Mycobacteria* growth indicator tube (MGIT) 960 system. Complementarily, secure laboratories with adequate equipment and trained professionals are needed. Also, there is a greater risk of contamination with such methods, but the waiting time for results when positive ranges from five to 12 days [25–27]. A study carried out in adults validated that the average waiting time for a result was 25.8 days for the LJ medium and 13.2 days for the concur that these data can be extrapolated to children MGIT [28, 29].

The performance of smear and culture varies according to age and the material analyzed. For bacterioscopy, positivity varies from 50 to 80% in sputum, 10–15% in gastric lavage (GL), and is lower in pleural fluid and cerebrospinal fluid (CSF) (less than 5–10%). Culture yield can be high in sputum (80%), between 20 and 77% in GL, less than 25–50% in pleural fluid, and less than 30–90% in CSF [26].

The culture test result confirms the diagnosis of mycobacteria. Specific identification is necessary to characterize whether it is a case of TB (*M. tb* complex) or NTM [6, 25]. The identification of sensitivity to anti-TB drugs can be conducted by the sensitivity test (ST). It can employ phenotypic (biochemical) or genotypic (molecular) methods. The most common phenotypic methods for ST are [6, 25, 30]:

i. a proportion method (the conventional method of Canetti, Rist, and Grosset) that utilizes a solid medium and provides results in up to 42 days of incubation; and
ii. an automated method that uses a liquid medium, with resistant results available in about five to 13 days and identifies sensitivity in 13 days.

The drugs commonly tested are streptomycin (S), isoniazid (H/Inh), rifampicin (R/Rif), ethambutol (E/Emb), and pyrazinamide (Z/Pza).

An expensive method for detecting *M. tb* growth and assessing resistance (R/Rif and H/Inh) is the MODS [31]. A study with 96 children, using the MODS and MGIT methods, showed sensitivity and specificity of 39.7% and 42.3%, respectively, for diagnosing TB. Besides, the average time to acquire the results was eight and 13 days, respectively [32]. The culture test, although of paramount importance, both for confirming the case and for detecting DR, does not help in the initial decision to treat a TB case due to the slowness of the result [33].

5 Nucleic Acid Amplification Tests

The Xpert® M. TB/RIF assay (Cepheid; Sunnyvale, CA, USA) consists of a molecular method that amplifies nucleic acids through real-time PCR using the Gene Xpert® platform. It integrates three processes (sample preparation, amplification, and detection) concerning *M. tb* deoxyribonucleic acid (DNA) and utilizes a technique that does not require manipulation of mycobacterial DNA after amplification [34]. It is possible to identify the *M. tb* DNA and R/Rif resistance (RR) within two hours. After amplification, the region responsible for the resistance gene, rpoβ, is examined to detect mutations [33]. Although this technique does not test I/Inh, most cases regarding the detection of RR by Xpert® are also resistant to this drug, named MDR-TB [35]. This is a fast, automated, and operator-independent test whose role is to add the reagent to the sample and then homogenize it for 15 min to transfer to the Xpert® cartridge [34]. Also, the machine requires relatively simple maintenance and has customized cartridges. Each machine has independent modules for the cartridges and can process various materials (organic liquids and aspirated peripheral lymph nodes) [36]. It should not be used for treatment control since it can identify live or dead bacilli [33]. With the incorporation of Xpert®, the identification of *M. tb* DNA and RR has increased [2].

In 2017, the Xpert M. TB-RIF Ultra® (Xpert Ultra) emerged with the expectation of presenting greater sensitivity, particularly in the paucibacillary population. The platform was maintained, and two modifications were made to the method cartridge, a larger recipient for nucleic acid amplification, enabling a larger sample and two more *M. tb* targets [37]. Additionally, other technical changes were made. Therefore, to enhance the detection of resistance to RMP, melt curve analysis is used instead of the real-time PCR, thus avoiding false-positive results among those with a small bacillary population [38].

The limit of detection by Xpert® is 4.5 copies of genomes or 131 colony-forming units (CFU) of *M. tb* per ml of sputum. These values are higher in comparison to the culture for *Mycobacteria* (10–100 CFU/ml) but substantially lower than bacilloscopy (10.000 CFU/ml) [34]. As a result, there is a good alignment between the positivity of Xpert® and the culture test, which was already evident among Brazilian children and adolescents with presumed PTB [39]. Moreover, the changes

in Ultra®, in contrast with Xpert®, provided a reduction in the detection limit to approximately 16 CFU per ml [40].

Detjen et al. (2015) performed a meta-analysis to evaluate the use of Xpert® in pediatric TB cases. When compared to culture, the sensitivity and specificity were 62% and 98% in sputum and induced sputum (IS) and 66% and 98% in GL, respectively [41]. Specificity is similar to that observed in adult clinical samples (98%) [42]. However, most studies involve the sensitivity of the method using culture as a gold standard from those with a clinical diagnosis and a negative culture (using clinical diagnosis as gold standard), whereas the ideal would be to evaluate all as a single group since there is no gold standard for diagnosis of TB in the pediatric age group. The sensitivity of Xpert® in children may vary according to the specimen evaluated, and collecting serial samples can increase performance. Ioos et al. (2018) evaluated the detection of *M. tb* by Xpert® and through cultures in alternative specimens (GL, IS, nasopharyngeal aspirate [NPA]) in children with symptoms suggestive of PTB. The detection of Xpert® was 2–17% in IS, 5% to 51% in GL, and 3% in NPA [43]. The use of Xpert® in bronchoalveolar lavage (BAL) among children with negative bacilloscopy in GL or sputum/IS samples and in those with suspected MDR-TB proved effective. Advice regarding this type of specimen collection would be to restrict only to patients who evolve without clinical or radiological improvement, seeking confirmation of PTB or proof of MDR-TB [44, 45].

Notably, in the forms of EPTB, Xpert® is also recommended by the WHO, even though few studies validate its positive effects for children and adolescents [46–48]. The sensitivity of the method in lymph nodes, compared to culture, for both children and adults, is 96%, with the specificity of 93% [49]. In an Ethiopian study evaluating patients with suggestive TB lymphadenitis, Xpert® detected *M. tb* in more than half of the samples with caseum [50]. The document referring to TB diagnosis published in 2020 by the WHO suggests Xpert® and Ultra® as an alternative for bacilloscopy evaluating cases suggestive of PTB in children and adults. These tests are recommended to be performed on samples other than sputum, such as NPA, GL, and stools [12]. It also emphasizes using these tests in cases of EPTB [12]. Professionals involved with pediatric TB should be aware of the different aspects of PTB among children and adolescents, the intricacy involved in collecting different specimens, especially from outpatients, and the lower accuracy of DNA amplification tests in children when compared to adults, so that TB in this group, especially among younger children, tends to be underdiagnosed.

In a study with children who presented signs suggestive of TB meningoencephalitis, the positivity of Xpert® was 21.4% [47]. Lopez et al. [48], when simultaneously analyzing urine and respiratory samples using Xpert®, were unsuccessful in getting a positive urine result, a reflection of PTB among children. WHO recommendations regarding specimen analysis by Ultra® are the same as those of Xpert®, including extrapulmonary samples. The semi-quantitative analysis of both methods is similar, with results presented as high, medium, low, and very low for *M. tb* DNA detection. A new category was included, "traces," presented only in Ultra® (corresponding to a level below the very low rating given by

Xpert®). This category, "traces," corresponds to a very small bacillary population, configuring a true-positive result in children, HIV-infected, and EPTB patients [38].

Investigating PTB Among hospitalized children in South Africa, samples from IS collected on the same day were submitted to Xpert® and Ultra® along with a culture test for comparison. For those with bacteriological confirmation (culture, Xpert or Ultra), the detection was 63.2% (Xpert®), 73.7% (Ultra®), and 82.9% (culture). However, using culture as a reference, the sensitivity of both molecular methods was similar (64.4% for Xpert® and 65.8% for Ultra®) [51]. To evaluate the higher performance of Ultra®, the combination of NPA and IS achieved 80% sensitivity, using culture as a reference [51].

WHO (2020) recommends other nucleic acid amplification methods, such as loop-mediated isothermal amplification (TB-LAMP), line probe assay (LPAs), and second-line LPAs, especially for adults. Second-line LPAs are highlighted to be used in cases with MDR-TB/RR to test for resistance to other drugs, such as fluoroquinolones (FQs) [12]. Table 3 illustrates the characteristics of the molecular tests available.

6 Serial Samples, Different Specimens, and Faster Methods

The evaluation of respiratory specimens on consecutive days can intensify the performance of Xpert®. This type of collection among pediatric patients with presumed PTB enabled the additional detection of 7.9% positivity through

Table 3 Characteristics of some molecular tests used for the diagnosis of TB, nucleic acid amplification tests (NAATs); Xpert M. TB/RIF assay, molecular test for *M. tb* and resistance to rifampin (R/Rif); isoniazid (I/Inh), line probe assay (LPA), fluoroquinolones (FQs) and second-line injectable drugs (SLID)

Method	Time (h)	Sensitivity	Specificity
Xpert MTB /RIF assay	< 2	85% (pooled) 96% (RIF)	99% (MTB detection) 98% (RIF resistance)
Xpert MTB/RIF Ultra	< 2	90% (pooled) 94% (RIFresistance)	96% (MTB detection) 98% (RIF resistance)
LPA (1 line) [INH and RIF]	5	98% (RIF resistance) 84% (INH resistance)	99% (RIF resistance) > 99% (INH resistance)
LPA (2 lines) [Fluo; SLID]	5	86% (FLQ resistance) 87% (SLID resistance)	99% (FLQ resistance) 99% (SLID resistance)
Loopamp™ MTBC assay	< 2	78% (pooled)	98% (MTB detection)
TrueNAT MTB Plus	< 2	89% (pooled)	98% (MTB detection)
TrueNAT MTB – RIF Dx		93% (RIF resistance)	95% (RIF resistance)

Adapted with permission from [52]

molecular methods [53]. The evaluation of paired samples on the same day, using an NPA sample and an IS sample using culture as a confirmed definition of TB, exemplified the sensitivity of Xpert® with the collection of a paired sample of 80.5%, similar to the percentage obtained with the Ultra® [54, 55]. However, collection on different days may be more difficult for outpatients, and in these cases, the analysis of two samples collected in one visit can be considered [35, 43].

The Xpert® and Ultra® can be performed on the stools and be helpful in paucibacillary patients who are unable to generate respiratory specimens. Simultaneous collection of IS and fecal sample, in children in South Africa, denoted sensitivity of 37.9% for Xpert® and 58.2% for Ultra®, when compared to a bacteriological confirmation according to culture, Xpert® or Ultra®, in the IS. These values increased to 77.8 and 88.9% for Xpert® and Ultra®, respectively, when the reference was bacteriologically confirmed only by a culture test [56].

Genomic sequencing can confirm the susceptibility of at least first-line drugs. The genotypic approach associated with the phenotype is necessary for the optimal diagnosis of DR [35]. Omni and Edge are portable modules (POC test) for a single Xpert cartridge, powered by batteries that facilitate the investigation of community TB cases [35].

7 Biomarkers

Biomarkers are substances or components that can serve as markers and express normal or pathogenic biological events. They can be used in laboratory tests, either for diagnostic purposes or to control the therapeutic response. They can be unique or represent a set of molecules characterizing the biosignature, and they originate from the pathogen or host [57, 58].

In the context of TB diagnosis, some aspects related to biomarkers deserve to be highlighted, such as using POC tests and analyzing different specimens of respiratory secretions like blood, urine, and exhaled air, among others. This characteristic of biomarkers is integral for children with TB and TB-HIV-co-infected patients in whom bacilloscopy has low sensitivity and sputum collection is proving to be difficult [13]. A good biomarker could allow rapid screening of patients, detect individuals more likely to develop TB from LTBI, and differentiate between healthy individuals and those with active TB [59, 60]. According to the WHO, a test to be used on a large scale for TB must have at least 90% sensitivity and 70% specificity [7].

7.1 Serology

M. tb can stimulate the humoral immune system resulting in the synthesis of different antibodies [61, 62]. In pediatric TB, serological tests arouse scientific interest since the tests can be performed in specimens other than respiratory secretions and other sites of infection. They also have the advantage of potentially low cost and the ability to provide quick results [63, 64].

In children, some aspects should be highlighted when assessing the dosage of immunoglobulins (Igs) in the diagnosis of TB. The immune system is still developing in individuals under two years of age. In newborns, IgM is the main immunoglobulin synthesized in response to antigenic stimuli; IgG levels result mainly from the mother-fetus transplacental passage and breast milk supplies yield IgA [65]. At two, six, and ten years, a child reaches the maximum levels of IgM, IgG, and IgA, respectively [66].

Achkar and Ziegenbalg [64] selected 23 articles on the use of antibodies in pediatric TB. They found differences in antibody levels in terms of sensitivity (14–85%) and specificity (86–100%). These authors highlighted the difficulty in comparing the results between studies due to different age groups, in house methods or use of commercial kits, distinct antigens (Ag5, "old tuberculin," A60 (ANDA-TB) 16KDA, 30 KDA, 38 KDA, PPD, ESAT-6, TBLG, LOS, DAT, PGBTb1, Ag 85 complex, and CFP-10), as well as the antibody isotypes evaluated and the type of final TB diagnosis (confirmed or not) in the studies.

Nonyane et al. [7] evaluated the IgG response to 119 antigens (i.e., multiplexed bead-based assay) in the serum of children aged from 12 to 152 months with confirmed PTB, unconfirmed PTB, and improbable PTB. When comparing patients with confirmed PTB, improbable PTB, and negative TST, for all ages, they found the best area under the curve (AUC) of the receiver operating characteristic (ROC) equal to 0.74 for the RV 3875_FIND antigen; with sensitivity and specificity of 70 and 71%. When comparing PTB versus improbable TB with positive TST, the antigen with the best result was DID64IDRI, with AUC of 0.59, the sensitivity of 60%, and specificity of 64% [7].

Schmidt et al. [67] evaluated IgG anti Mce1A (mammalian cell protein) as a biomarker to differentiate LTBI and active TB, finding sensitivity of 74% and specificity of 64%, respectively, when comparing pediatric patients with PTB with a positive bacteriological method (AFB, GeneXpert *M. tb*/RIF® or culture) and patients with LTBI.

The commercial serological methods are not recommended, by WHO, to be used in clinical practice due to their low accuracy [7, 68].

7.2 Lipoarabinomannan

The measurement of lipoarabinomannan (LAM), present in the cell wall of *M. tb*, in the urine (POC test), is an auxiliary tool in co-infected (TB-HIV) adults with advanced disease [69]. Nicol et al. [70], examining children co-infected or not, found low sensitivity and specificity, thus, discouraging its use in this group. However, in 2020, WHO recommended the use of LAM dosage through lateral flow in the following situations:

- children, adolescents, and adults living with HIV;
- hospitalized patients with suspected PTB or EPTB or with severe AIDS with a total CD4 less than 200 cells/mm^3; and

- children, adolescents, and adults living with HIV and treated on an outpatient basis, depicting signs and symptoms of PTB and EPTB or being seriously ill, regardless of clinical manifestations of TB, and with an unknown CD4, or with CD4 cells less than 200/mm^3, as well those with no symptoms but with CD4 cells of approximately 100–200 cells/mm^3 [12]

Despite these indications, its use in childhood is still limited.

7.3 Cytokines

Cells (T lymphocytes, macrophages, and non-specific T cells) activated by *M. tb* antigens secrete cytokines that can be used as biomarkers to further distinguish between LTBI, active TB, and other lung diseases. Some cytokines have already been detected in elevated levels in TB patients [71].

In a study with children carried out in India, the association of the dosage of interferon-gamma inducible protein 10 (IP-10) and IGRA exhibited promising results [72]. Sudbury et al. [73] published a study performed in Peru aiming to evaluate the effectiveness of some cytokines as a tool for diagnosing active TB and LTBI in pediatric participants, demonstrated higher levels of IFN-ɣ, interleukin (IL) 1ra, IL-2, IL-13, IP-10, macrophage inflammatory protein 1 beta (MIP-1β), and tumor necrosis factor α (TNF-α) when compared to control groups (healthy people and patients with other diseases). The best performance was obtained with IL-2, IL-13, and IP-10, with positive and negative predictive values above 90% for IL-2. Levels of IL-1ra and TNF-α differentiated LTBI and active TB. Togun et al. [74] studied Gambian children with a condition suggestive of intrathoracic TB, and identified a biosignature (IL-1Ra, IL-7, and IP-10) capable of differentiating TB from other respiratory diseases; the sensitivity was 72.2% (95% CI: 60.4–82.1%), the specificity was 75.0% (95% CI: 64.9–83.4%), and the AUC was 0.74 (95% CI: 67–81%). Lastly, different detection methods and evaluated cytokines in pediatric TB studies make it difficult to compare their results [71].

8 Omics Technologies

Biotechnology has enabled the development of "omics." The suffix "omics" has a Latin origin and is a branch of biology that seeks to characterize a set of molecules that translate into the structure, function, and dynamics of an organism [14]. These experiments measure the activities of genes that result in the identification of transcriptomics, proteomics, and metabolomics [75]. Since the 1990s, two-dimensional electrophoresis and mass spectrometry have allowed the identification of several microbial proteins. Specifically, proteomics mentions the set of proteins expressed by a given cell in different situations [76]. Likewise, metabolomics studies the metabolite repertoire of an organism [77]. Molecules of

ribonucleic acid (RNA) read from part of the DNA are the transcripts, which together form transcriptomics. Typically, it is formed by messenger RNA [78]. The studies of proteomics, metabolomics, and transcriptomics have been evaluated as potential biomarkers for use in diagnostic tests for TB.

Anderson et al. [79] in a prospective study in South Africa, Malawi, and Kenya, with children with suspected TB, infected or not with HIV, detected 51 transcripts that allowed differentiation between TB and other diseases. In patients with TB confirmed by culture, the sensitivity was 82.9% and the specificity was 83.6%.

Penn-Nicholson et al. [80] studied two proteomic signatures (i.e., TRM5 and 3PR) in the plasma of TB contacts in South Africa and the Gambia. Both were able to predict TB, although they did not meet the criteria designated by the WHO for use in large-scale testing, suggesting that further studies are required.

9 Conclusion

Despite technological advances, the difficulty in laboratory confirmation of TB persists in children. TST and IGRAs are used in cases of LTBI and active TB. However, traditional bacteriological tests (bacterioscopy and culture) perform poorly in children. Furthermore, Xpert *M. tb* RIF® and Ultra® have already been incorporated into clinical practice, but in children, they have not achieved the expected results as obtained in adults. New perspectives are being evaluated, such as the use of different clinical specimens and the discovery of potential biomarkers that achieve adequate accuracy for developing POC tests [81].

Pediatric tuberculosis requires much attention: we do not always find the bacillus, but it is there.

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Core Messages

- In children, PTB and EPTB are, commonly, paucibacillary.
- The bacteriological or molecular tests are less sensitive in children compared to adults.
- Although TST and IGRAs are useful for detecting *M. tb* infection, they do not differentiate LTBI from active TB.
- The new perspectives are different NAATs and biomarkers that can be used in POC tests.

References

1. Rich A (1946) Patogenia de la tuberculosis. Buenos Aires, Alfa
2. World Health Organization (2019) Global tuberculosis report 2019. Available https://www.who.int/tb/publications/global_report/en/. Acessed 22 Jun 2020
3. Sant'Anna CC, Schmidt CM, March MFBP, Pereira SM, Barreto ML (2013) Tuberculosis among adolescents in two Brazilian State capitals. Cad Saúde Pública 29(1):111–116
4. Carvalho ACC, Cardoso CAA, Martire TM, Migliori GB, Sant'Anna CC (2018) Aspectos epidemiológicos, manifestações clínicas e prevenção da tuberculose pediátrica sob a perspectiva da Estratégia End TB. J Bras Pneumol 44(2):134–144
5. Gie R (2003) Diagnostic atlas of intrathoracic tuberculosis in children. International Union Against Tuberculosis and Lung Disease, Paris
6. Ministério da Saúde (2019) Manual de Recomendações para o Controle da Tuberculose no Brasil. Available http://bvsms.saude.gov.br/bvs/publicacoes/manual_recomendacoes_controle_tuberculose_brasil_2_ed.pdf. Acessed 22 Jun 2020
7. Nonyane BAS, Nicol MP, Andreas NJ, Rimmele S, Schneiderhan-Marra N, Workman LJ, Perkins MD, Joos T, Broger T, Ellner JJ, Alland D, Kampmann B, Dorman SE, Zar HJ (2018) Serologic responses in childhood pulmonary tuberculosis. Pediatr Infect Dis J 37(1):1–9
8. Graham SM, Cuevas LE, Jean-Philippe P, Browning R, Casenghi RM, Detjen AK, Gnanashanmugam D, Hesseling AC, Kampmann B, Mandalakas A, Marais BJ, Schito M, Spiegel HML, Starke JR, Worrell C, Zar HJ (2015) Clinical case definitions for classification of intrathoracic tuberculosis in children: an update. Clin Infect Dis 61(Suppl 3):S179–S187
9. World Health Organization (2014) The End TB strategy global strategy and targets for tuberculosis prevention, care and control after 2015. Available https://www.who.int/tb/strategy/End_TB_Strategy.pdf?ua=1. Acessed 22 Jul 2020
10. Lobue P, Menzies D (2010) Treatment of latent tuberculosis infection: an update. Respirology 15(4):603–622
11. World Health Organization (2017) Multidrug-resistant tuberculosis (MDR-TB) 2017 update. Available https://www.who.int/tb/challenges/mdr/MDR-RR_TB_factsheet_2017.pdf?ua=1. Acessed 22 Jul 2020
12. World Health Organization (2020) WHO consolidated guidelines on tuberculosis module 3: diagnosis rapid diagnostics for tuberculosis detection. Available https://www.who.int/publications/i/item/who-consolidated-guidelines-on-tuberculosis-module-3-diagnosis—rapid-diagnostics-for-tuberculosis-detection. Acessed 22 Jul 2020
13. Yerlikaya S, Broger T, MacLean E, Yerlikaya S, Broger T, MacLean E, Pai M, Denkinger CM (2017) A tuberculosis biomarker database: the key to novel TB diagnostics. Int J Infect Dis 56:253–257
14. Haas CT, Roe JK, Pollara G, Mehta M, Noursadeghi M (2016) Diagnostic 'omics' for active tuberculosis. BMC Med 14:37
15. Wessels G, Schaaf HS, Beyers N, Gie RP, Nel E, Donald PR (1999) Haematological abnormalities in children with tuberculosis. J Trop Pediatr 45(5):307–310
16. Abakay O, Abakay A, Sen HS, Tanrikulu AC (2015) The relationship between inflammatory marker levels and pulmonary tuberculosis severity. Inflammation 38(2):691–696
17. Peresi E, Silva SMR, Calvi SA, Marcondes-Machado J (2008) Cytokines and acute phase serum proteins as markers of inflammatory regression during the treatment of pulmonary tuberculosis. J Bras de Pneumol 34(11):942–949
18. Martins C, Gama ACC, Valcarenghi D, Batschauer APB (2014) Markers of acute-phase response in the treatment of pulmonary tuberculosis. J Bras Patol Med Lab 50(6):428–433
19. Nagu TJ, Spiegelman D, Hertzmark E, Aboud S, Makani J, Matee MI, Fawzi W, Mugusi F (2014) Anemia at the initiation of tuberculosis therapy is associated with delayed sputum conversion among pulmonary tuberculosis patients in Dar-es-Salaam, Tanzania. PLoSOne 18;9(3):e91229

20. Starke JR, Committee on Infectious Diseases (2014) Interferon-γ release assays for diagnosisoftuberculosisinfectionanddisease in children. Pediatrics 134(6):e1763–e1773
21. Gonzalez NE, Ferrero F (2015). Diagnóstico da Tuberculose na Infância. In: Tuberculose em crianças e jovens, 1st ed. Editora Atheneu, São Paulo, pp 17–24
22. Committee on Infectious Diseases, American Academy of Pediatrics (2018) Tuberculosis: 2018–2021 report of the committee on infectious diseases. 31th ed. Elk Grove Village IL pp 829–834
23. Starke JR (2012) Interferon-gama release assays for the diagnosis of tuberculosis Infection in children. J Pediatrics 161(4):581–582
24. Machingaidze S, Wiysonge CS, Gonzalez-Angulo Y Hatherill M, Moyo S, Hanekom W, Mahomed H (2011) The utility of interferon-gamma release assay for diagnosis of latent tuberculosis infection and disease in children: a systematic review and meta-analysis. Pediatr Infect Dis J 30:694–700
25. World Health Organization (2015) Implementing tuberculosis diagnostic. Policy framework. Available https://apps.who.int/iris/bitstream/handle/10665/162712/9789241508612_eng.pdf;jsessionid=C5ACAB489566CAFC4731CEF90864F9A4?sequence=1. Acessed 01 Aug 2020
26. Schaaf HS, Reuter H (2009) Practical approaches to ordering diagnostic tests. In: Schaaf HS, Zumla A (eds) Tuberculosis: a comprehensive clinical reference. Saunders, Elsevier, pp 216–226
27. Martire TM (2009) Diagnóstico laboratorial da tuberculose na infância: métodos convencionais e métodos rápidos. Pulmão RJ Supl 1:20-S27
28. Cruciani M, Scarparo C, Malena M, Bosco O, Serpelloni G, Mengoli C (2004) Meta-analysis of BACTEC MGIT 960 and BACTEC 460 TB, with or without solid media, for detection of mycobacteria. J Clin Microbiol 42:2321–2325
29. Nicol MP, Zar HJ (2011) New specimens and laboratory diagnostics for childhood pulmonary TB: progress and prospects. Paediatr Respir Rev 12:16–21
30. Canetti G, Rist N, Grosset J (1963) Mesure de sensibilité du bacille tuberculeux aux drogues antibacillaire par la methode de proportions: methodologie, criterie de rèsitance, resultats et interpretations. Rev tub pneumol 27:217–272
31. Moore DA, Mendoza D, Gilman RH, Evans CAW, Delgado MGH, Guerra J, Caviedes L, Vargas D, Ticona E, Ortiz J, Soto G, Serpa J, Tuberculosis Working Group in Peru (2004) Microscopic observation drug susceptibility assay, a rapid, reliable diagnostic test for multidrug-resistant tuberculosis suitable for use in resource-poor settings. J Clin Microbiol 42 (10):4432–4437
32. Ha DT, Lan NT, Wolbers M, Duong TN, Quang ND, Thi Van Thinh T, Thi Hong Ngoc L, Thi Ngoc Anh N, Van Quyet T, Thi Bich Tuyen N, Thi Ha V, Day J, Thi Thanh Hang H, Kiet VS, Thi Nho N, Hoa DV, Dung NH, Huu Lan N, Farrar J, Caws M (2009) Microscopic observation drug susceptibility assay (MODS) for early diagnosis of tuberculosis in children. PLoS ONE 4:e8341
33. Ministério da Saúde (2011) Manual de Recomendações para o Controle da Tuberculose no Brasil. Available http://bvsms.saude.gov.br/bvs/publicacoes/manual_recomendacoes_controle_tuberculose_brasil_2_ed.pdf. Acessed 22 Jun 2020
34. Nicol M, Whitelaw A, Stevens W (2013) Using Xpert M. TB/RIF. Curr Respir Med Rev 9 (3):187–192
35. Lange C, Dheda K, Chesov D, Mandalakas AM, Udwadia Z, Horsburgh CR Jr (2019) Management of drug-resistant tuberculosis. The Lancet 394(10202):953–966
36. Ministério da Saúde (2016) Teste rápido molecular para tuberculose.Nova tecnologia para o diagnóstico da tuberculose. http://www.saude.gov.br/images/pdf/2016/maio/18/folder-TRM-TB-grafica-reduzido.pdf. Acessed 30 Aug 2020
37. Chakravorty S, Simmons AM, Rowneki M, Parmar H, Yuan Cao Y, Ryan J, Banada PP, Srinidhi Deshpande S, Shubhada Shenai S, Alexander Gall A, Glass , Krieswirth B, Schumacher SG, Nabeta P, Tukvadze N, Rodrigues C, Skrahina A, Tagliani E, Cirillo DM, Davidow A, Denkinger CM, Persing D, Kwiatkowski R, Jones M, Allanda D (2017) The new

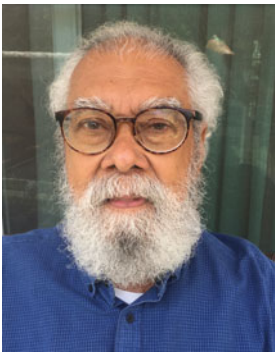
xpert M. TB/RIF ultra: improving detection of *Mycobacterium tuberculosis* and resistance to rifampin in an assay suitable for point-of-caretesting. mBio 29;8(4):e00812–e00817
38. World Health Organization (2017) Meeting report of a technical expert consultation: non-inferiority analysis of Xpert M. TB/RIF ultra compared toXpert M. TB/RIF. 11 p. Available https://www.who.int/tb/publications/2017/XpertUltra/en/. Acessed 01 Jul 2020
39. Sieiro TA, Aurilio RB, Soares ECC, Chiang SS, Sant`Anna CC (2018) The role of the Xpert M. TB/RIF assay among adolescents suspected of pulmonary tuberculosis in Rio de Janeiro, Brazil. Rev Soc Bras Med Trop 51(2):234–236
40. Atherton RR, Cresswell FV, Ellis J, Kitaka SB, Boulware DR (2019) Xpert M. TB/RIF Ultra for tuberculosis testing in children: a mini-review and commentary. Front Pediatr 7:34
41. Detjen AK, DiNardo AR, Leyden J, Steingart KR, Menzies D, Schiller I, Dendukuri N, Mandalakas AM (2015) Xpert M. TB/RIF assay for the diagnosis of pulmonary tuberculosis in children: a systematic review and meta-analysis. The Lancet Resp Med 3(6):451–61
42. Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N (2014) Xpert® M. TB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults (Review). Cochrane Database Of Systematic Reviews. Available https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4470349/pdf/CD009593.pdf. Acessed 10 May 2020
43. Ioos V, Cordel H, Bonnet M (2018) Alternative sputum collection methods for diagnosis of childhood intrathoracic tuberculosis: a systematic literature review. Arch Dis Child 104 (7):629–635
44. Walters E, Goussard P, Bosch C, Hesseling AC, Gie RP (2013) GeneXpert M. TB/RIF on bronchoalveolar lavage samples in children with suspected complicated intrathoracic tuberculosis: a pilot study. Pediatr Pulmonol 55:1133–1137
45. Saini I, Mukherjee A, Gautam H, Singla M (2018) Diagnostic yield of Xpert M. TB/RIF in Bronchoalveolar lavage in children with probable pulmonary tuberculosis. Indian Pediatr 55 (12):1062–1065
46. Bholla M, Kapalata N, Masika E, Chande H, Jugheli L, Sasamalo M, Glass TR, Peter Beck H-P, Reither K (2016) Evaluation of Xpert® M. TB/RIF and Ustar Easy NAT™ TB IAD for diagnosis of tuberculous lymphadenitis of children in Tanzania: a prospective descriptive study. BMC Infect Dis 16(1):1–9. Springer Science and Business Media LLC
47. Aruna J, Ratageri VH, Illalu S, Fattepur SR, Wari PK (2019) The utility of CSF xpert M. TB/RIF in diagnosis of tubercular meningitis in children. Indian J Pediatr 86(12):1089–1093
48. Lopez AL, Aldaba JG, Morales-Dizon M, Sarol JN, Daag JV, Ama MC, Sylim P, Salonga A, Nielsen-Saines K (2019) Urine Xpert M. TB/RIF for the diagnosis of childhood tuberculosis. Int J Infect Dis 79:44–46
49. Maynard-Smith L, Larke N, Peters JA, Lawn SD (2014) Diagnostic accuracy of the Xpert M. TB/RIF assay for extrapulmonary and pulmonary tuberculosis when testing non-respiratory samples: a systematic review. BMC Infect Dis 14(1):1–15
50. Fantahun M, Kebede A, Yenew B, Gemechu T, Mamuye Y, Tadesse M, Brhane B, Jibriel A, Solomon D, Yaregal Z (2019) Diagnostic accuracy of Xpert M. TB/RIF assay and non-molecular methods for the diagnosis of tuberculosis lymphadenitis. Shankar EM, PLoS ONE 14(9):e0222402
51. Nicol MP, Worman L, Prins M Bateman L, Ghebrekristos Y, Mbhele S, Denkinger CM, Zar HJ (2018) Accuracy of xpert M. tb/Rif ultra for the diagnosis of pulmonary tuberculosis in children. Pediatr Infect Dis J. 37(10):e261–e263
52. MacLean E, Kohli M, Weber SF, Suresh A, Schumacher SG, Denkinger CM, Pai M (2020) Advances in molecular diagnosis of TB. J Clin Microbiol. Accepted Manuscript posted on line
53. Singh S, Singh A, Prajapati S, Kabra SK, Lodha R, Mukherjee A, Singh V, Hesseling AC, Grewal HM (2015) Xpert M. TB/RIF assay can be used on archived gastric aspirate and induced sputum samples for sensitive diagnosis of paediatric tuberculosis. BMC Microbiol 15 (1):191

54. Zar HJ, Workman L, Isaacs W, Munro J, Black F, Eley B, Allen V, Boehme CC, Zemanay W, Nicol MP (2012) Rapid molecular diagnosis of pulmonary tuberculosis in children using nasopharyngeal specimens. Clin Infect Dis 55(8):1088–1095
55. Zar HJ, Workman LJ, Prins M, Bateman LJ, Mbhele SP, Whitman CB, Denkinger CM, Nicol MP (2019) Tuberculosis diagnosis in children using xpert ultra on different respiratory specimens. Am J Respir Crit Care Med 200(12):1531–1538
56. Kabir S, Rahman SMM, Ahmed S, Islam MS, Banu RS, Shewade HD, Thekkur P, Anwar S, Banu NA, Nasrin R, Uddin MKM, Choudhury S, Ahmed S, Paul KK, Khatun R, Chisti MJ, Banu S (2020) Xpert ultra assay on stool to diagnose pulmonary tuberculosis in children. Clin Infect Dis. May 18; ciaa583
57. Doherty TM, Wallis RS, Zumla A Biomarkers of disease activity, cure, and relapse in tuberculosis (2009). Clin Chest Med 30(4):783–96
58. Biomarkers Definitions Working Group (2001) Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 69(3):89–95
59. Wallis RS, Wang C, Doherty TM, Onyebujoh P, Vahedi M, Laang H, Olesen O, Parida S (2010) Zumla A (2010) Biomarkers for tuberculosis disease activity, cure, and relapse. Lancet Infect Dis 10(2):68–69. https://doi.org/10.1016/S1473-3099(10)70003-7
60. Loots DT (2016) TB or not TB? Improving the understanding and diagnosis of tuberculosis through metabolomics. Biomark Med 10(10):1025–1028
61. Goodridge A, Cueva C, Lahiff M, Muzanye G, Johnson JL, Nahid P, Riley LW (2012) Anti-phospholipid antibody levels as biomarker for monitoring tuberculosis treatment response. Tuberculosis 92:243–247
62. Takenami I, de Oliveira CC, Lima FR, Soares J, Machado A Jr, Riley LW, Arruda S (2016) Immunoglobulin G response to mammalian cell entry 1A (Mce1A) protein as biomarker of active tuberculosis. Tuberculosis 100:82–88
63. Clavijo E, Díaz R, Anguita A, García A, Pinedo A, Smits HL (2003) Comparison of a dipstick assay for detection of Brucella-specific immunoglobulin M antibodies with other tests for serodiagnosis of human brucellosis. Clin Diagn Lab Immunol 10:612–615
64. Achkar JM, Ziegenbalg A (2012) Antibody responses to mycobacterial antigens in children with tuberculosis: challenges and potential diagnostic value. Clin Vaccine Immunol 19 (12):1898–1906
65. Pan-Hammarström Q, Zhao Y, Hammarström L (2007) Class switch recombination: a comparison between mouse and human. Adv Immunol 93:1–61
66. Schroeder HW Jr, Mortari F, Shiokawa S, Kirkham PM, Elgavish RA (1995) Bertrand FE 3rd (1995) Developmental regulation of the human antibody repertoire. Ann NY Acad Sci 764:242–260
67. Schmidt CM, Lovero KL, Carvalho FR, Dos Santos DCM, Barros ACMW, Quintanilha AP, Barbosa AP, Pone MVS, Pone SM, Araujo JM, de Paula Martins C, Macedo SGD, Miceli AL, Vieira ML, Sias SMA, Queiroz A, Coca Velarde LG, Kritski AL, Silva AA, Sant'Anna CC, Riley LW, Araújo Cardoso CA (2020) Serum anti-Mce1A immunoglobulin detection as a tool for differential diagnosis of tuberculosis and latent tuberculosis infection in children and adolescents. Tuberculosis (Edinb) 120:101893
68. World Health Organization (2011) Tuberculosis serodiagnostic tests policy statement Available https://apps.who.int/iris/bitstream/handle/10665/44652/9789241502054_eng.pdf?sequence=1&isAllowed=y. Acessed 31 Aug 2020
69. Peter JG, Theron G, van Zyl-Smit R, Haripersad A, Mottay L, Kraus S, Binder A, Meldau R, Hardy A, Dheda K (2012) Diagnostic accuracy of a urine lipoarabinomannan strip-test for TB detection in HIV-infected hospitalised patients. Eur Respir J 40:1211–1220
70. Nicol MP, Allen V, Workman L, Isaacs W, Munro J, Pienaar S, Black F, Adonis L, Zemanay W, Ghebrekristos Y, Zar HJ (2014) Urine lipoarabinomannan testing for diagnosis of pulmonary tuberculosis in children: a prospective study. Lancet Glob Health May 2(5): e278–84

71. Wei M, Wu ZY, Lin JH, Li Y, Qian ZX, Xie YQ, Su H, Zhou W (2015) Regulation network of serum cytokines induced by tuberculosis-specific antigens reveals biomarkers for tuberculosis diagnosis. Genet Mol Res 14(4):17182–17192
72. Jenum S, Dhanasekaran S, Ritz C, Macaden R, Doherty TM, Grewal HM, Trials Study Group TB (2016) Added value of IP-10 as a read-out of mycobacterium tuberculosis: specific immunity in young children. Pediatr Infect Dis J 35(12):1336–1338
73. Sudbury EL, Otero L, Tebruegge M, Messina NL, Seas C, Montes M, Rìos J, Germano S, Gardiner K, Clifford V, Gotuzzo E, Curtis N (2019) *Mycobacterium tuberculosis*-specific cytokine biomarkers for the diagnosis of childhood TB in a TB-endemic setting. J Clin Tuberc Other Mycobact Dis 16:100102
74. Togun T, Hoggart CJ, Agbla SC, Gomez MP, Egere U, Sillah AK, Saidy B, Mendy F, Pai M, Kampmann B (2020) A three-marker protein biosignature distinguishes tuberculosis from other respiratory diseases in Gambian children. EBioMedicine 58:102909
75. Reimand J, Isserlin R, Voisin V, Kucera M, Tannus-Lopes C, Rostamianfar A, Wadi L, Meyer M, Wong J, Xu C, Merico D, Bader GD (2019) Pathway enrichment analysis and visualization of omics data using g:profiler. GSEA, Cytoscape and Enrichment Map Nat Protoc. 14(2):482–517
76. Flores-Villalva S, Rogríguez-Hernández E, Rubio-Venegas Y, Cantó-Alarcón JG, Milián-Suazo F (2015) What can proteomics tell us about tuberculosis? J Microbiol Biotechnol. 25(8):1181–1194
77. Oliver SG (2002) Functional genomics: lessons from yeast. Philos Trans R Soc Lond B Biol Sci 357(1417):17–23
78. National Human Genome Research Institute (NHGRI) (2017) Transcriptome Fact Sheet https://www.genome.gov/13014330/transcriptome-fact-sheet/. Acessed 30 Mar 2017
79. Anderson ST, Kaforou M, Brent AJ, Wright VJ, Banwell CM, Chagaluka G, Crampin AC, Dockrell HM, French N, Hamilton MS, Hibberd ML, Kern F, Langford PR, Ling L, Mlotha R, Ottenhoff THM, Pienaar S, Pillay V, Scott JAG, Twahir H, Wilkinson RJ, Coin LJ, Heyderman RS, Levin M, Eley B (2014) Diagnosis of childhood tuberculosis and host RNA expression in Africa. N Engl J Med 370(18):1712–1723
80. Penn-Nicholson A, Hraha T, Thompson EG, Sterling D, Mbandi SK, Wall KM, Fisher M, Suliman S, Shankar S, Hanekom WA, Janjic N, Hatherill M, Kaufmann SHE, Sutherland J, Walzl G, De Groote MA, Ochsner U, Zak DE, Scriba TJ; ACS and GC6–74 cohort study groups (2019) Discovery and validation of a prognostic proteomic signature for tuberculosis progression: a prospective cohort study. PLoS Med 16(4):e1002781
81. WHO consolidated guidelines on tuberculosis. Module 5: management of tuberculosis in children and adolescents. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO

Christiane Mello Schmidt has worked for 27 years as a general pediatrician and pediatric pulmonologist at Hospital Universitario Antônio Pedro, Universidade Federal Fluminense, Niterói, Brazil, where she is also a Professor of Pediatrics at the Maternal and Child Department of the Faculty of Medicine. Her interests are in childhood tuberculosis, among other diseases. During the Master's, she and colleagues studied the characteristics of tuberculosis (TB) in adolescents from two Brazilian capitals. The results helped to change the strategy of the Brazilian Ministry of Health regarding the approach to TB in this age group. In the Ph.D., the group studied the use of biomarkers (IgG Mce 1A) to diagnose pulmonary TB in pediatrics. Recently, she participated in a research group on pediatric TB. In addition to research projects,

they also develop activities in continuing education in TB. She currently works in an outpatient care clinic for pediatric TB at the University.



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A Multidisciplinary Approach Towards Finding and Treating All Tuberculosis Patients

12

Negussie Beyene, Georgies Mgode, Robert Burny, Cynthia D. Fast, Christophe Cox, and Lena Fiebig

A theoretical discovery has but the merit of its existence: it awakens hope, and that is all. But let it be cultivated, let it grow, and you will see what it will become.

Louis Pasteur

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Summary

Tuberculosis (TB) is an old disease but not a disease of the past. Despite a global plan to End TB, it is still among the leading causes of death. The main challenges are the gap in case detection and challenges along the cascade of care. According to the World Health Organization, in 2018, thirty percent of the people that were ill with TB were missed. Resource-limited settings are particularly affected. This chapter describes an unconventional, multidisciplinary scientific approach to tackle TB in high-burden settings. In 2001, the non-profit organization APOPO initiated the idea of training African giant pouched rats to diagnose TB. Informed by behavioral science and analytical chemistry of volatile organic compounds, this concept was put into practice with local health partners to impact health and inspire positive societal change. This spirit guided the development of an integrated TB detection model to increase TB detection rates by utilizing innovative TB detection rats alongside novel service elements, including new sample referral structures to speed up diagnostic services and critical efforts to reliably link newly diagnosed patients to care. This approach has resulted in more than 20,000 additionally diagnosed TB cases across three African countries. TB detection rats are one of the most sophisticated medical uses of animal scent detection. Yet, this is not the end of ambitions: the diagnostic tool and the service approach are continuously refined, and new sample materials and disease targets remain to be tackled.

Graphical Abstract

Tuberculosis (TB) detection rats technology: **a** presumptive TB cases provide sputum samples for microscopic examination at their local clinic; **b** once the microscopic examination is completed, APOPO's sample collectors pick up both TB-positive and TB-negative samples; **c** at the APOPO facility, samples are heat-inactivated to ensure that any TB-positive sputum is no longer contagious; **d** all the samples from the clinics are presented to the rats. If a rat shows positive indication (holding the nose over the sample for 3 s) and if this sample was TB-positive at the partner clinic, the rat receives a reward that keeps them accurate and eager to find TB in the samples; **e** if the rat indicates a sample marked negative by the clinics, this sample is flagged suspect; **f** the suspect samples are then confirmed at APOPO's lab using WHO-endorsed diagnostic methods; **g** confirmed positive results are reported to the clinic within 24 h. Adapted with permission from APOPO vzw. Copyright © APOPO

Keywords

Cricetomys ansorgei · Diagnostic · Same-day TB testing · Sample referral network · TB detection rats · Tuberculosis

1 Introduction

Tuberculosis (TB) is both treatable and preventable. Nonetheless, it remains one of the top causes of mortality. The United Nations (UN) and its member states are committed to "End TB." The "Find.Treat.All.#EndTB" initiative[1] was jointly developed by the World Health Organization (WHO), the Stop TB Partnership, and the Global Fund to Fight AIDS, Tuberculosis, and Malaria. However, finding and treating all patients remains a global challenge. Bold strategies and initiatives to improve the diagnosis and treatment follow-up are required to fulfill the ambitious disease elimination plan. Next to conventional national and international disease efforts, unusual paths were taken to challenge TB: training rats to diagnose TB by APOPO,[2] a Belgian NGO that develops and utilizes scent detection technology for humanitarian purposes.[3] Scent detection is a broad, interdisciplinary area of science and application. Odors play a very important role in the life of many species (olfaction is, evolutionary speaking, our oldest sense). Using animals such as dogs for scent detection, i.e., as biosensors, is rather common; however, deploying African giant pouched rats (*Cricetomys ansorgei*) for humanitarian tasks is unique. Work that proved these trained rats can detect explosives raised the question in 2001 if they could also find TB. Scientific studies followed, and the hypothesis was supported: rats can detect TB using their sense of smell. APOPO's projects do not end with proofs-of-concept but include the deployment of the detection rat technology as part of wider community response and services. Thus since 2007, a new area of research and application has emerged, with the development of an integrated TB detection model that incorporates rats as a test for research purposes, urban sample referral models, and community action to improve services alongside the cascade of TB care.

This chapter describes the multidisciplinary science behind the TB detection rat technology, the holistic approach envisioned, and the future outlook of this novel technology. The first section deals with the biology, behavior, and training procedures of *Cricetomys ansorgei* (animal behavior and psychology), the second section explains the evaluation cage designs developed (industrial engineering), the third section deals with the description of the TB-specific odors the rats are sniffing (organic analytical chemistry), the fourth section describes the urban sample referral network, the fifth section describes the diagnostic procedures and integrated diagnostic services (biomedicine and public health), and section six presents the social impact of the rats when combined with WHO-endorsed tests in three different countries (public health and epidemiology). Finally, a conclusion is drawn, and an outlook to the future is given.

[1] https://www.who.int/tb/joint-initiative/en/.
[2] Anti-Persoonsmijnen Ontmijnende Product Ontwikkeling, which stands for Anti-Personnel Landmines Removal Product Development.
[3] www.apopo.org.

2 Animal Behaviour and Training

APOPO works with giant African pouched rats (*Cricetomys ansorgei;* Fig. 1) belonging to the *Nesomyidae* family within the *Muroidea* superfamily. The omnivorous and nocturnal rats are native to sub-Saharan Africa. Like their hamster cousins, the pouched rats get their names from large cheek pouches in which they commonly store food or transport various items [1]. Their large size inspires the "giant" aspect of their name, averaging 60–90 cm in overall length (including their tail, which accounts for nearly half of this length) and weighing 1–1.5 kg (males slightly more) as adults. In captivity, the rats commonly live nine years or more.

APOPO maintains a breeding colony of *Cricetomys* alongside its training and research facilities at the Sokoine University of Agriculture in Morogoro, Tanzania. Genetic diversity of the colony is maintained by the occasional introduction of wild-caught rats that are re-released after litters are weaned at ten weeks post-natal age. After weaning, each animal is outfitted with a radio frequency identification (RFID) chip and given a unique name to highlight individual importance.

All APOPO rats are housed with littermates in ventilated home cages containing a locally sourced clay pot to mimic their natural underground burrow, clean wood shaving substrate, an untreated wood gnawing/climbing stick, free access to drinking water, and either an elevated platform or large running wheel. The rats are fed a variety of fresh fruits, vegetables, grains, and protein sources, including nuts, legumes, and sun-dried sardines supplemented by commercial rodent chow. During

Fig. 1 An adult African giant pouched rat (Cricetomys ansorgei). Adapted with permission from APOPO vzw. Copyright© APOPO/Marten Boersema

training sessions, most of their food is provided with extra rations supplied in the home cage during weekends and holidays. The rats are routinely examined by a veterinarian, who inspects rat health, welfare, and housing conditions and provides healthcare as needed.

Training begins when the eyes of young pups open at four weeks of age. This early training socializes the rats to humans through gentle handling three times a day and hand feeding them palatable foods, including bananas or peanuts. During these sessions, the trainer also habituates the young rats to the various sights, sounds, and smells they may encounter as a future scent detection rat by gradually exposing them to various objects and environments. Not only does this rich range of experiences minimize stress to the rat during more advanced training stages and help ensure they will not experience anxiety while later working in the scent detection context, but it also approximates what is often termed "environmental enrichment." Such enrichment has been shown to positively impact learning and memory abilities in other rat species [2–4]. This early training continues for approximately two weeks after the rat is weaned (roughly 12 weeks of age) or until it does not show any signs of fear, stress, or aggression when introduced to a novel context or person. Enrichment and socialization are then provided through three weekly exercise sessions of 15 min each in which the rat is allowed to freely explore a large, ventilated enclosure equipped with securely fastened climbing branches or ramps, a large running wheel, and a variety of objects presented in rotation, such as sisal climbing ropes, large diameter pipes, or cardboard boxes.

After the rat has been properly socialized, scent detection training proceeds through a series of stages (summarized below), beginning when it is approximately 13 weeks old. Training activities are typically conducted five days per week and are driven by the psychology principles of shaping, positive reinforcement, and discrimination learning.

2.1 Reinforcer Selection

Successful scent detection training requires carefully timed reinforcement of desired behaviors. While the target behavior may vary across stages of training as the rules of shaping are applied, the reinforcement must always represent a desirable outcome for the rat (a so-called "reward") to maintain proper learning motivation and encourage a recurrence of the target behavior. Early work at APOPO established a mixture of bananas, avocados, and crushed rodent chow pellets as optimal reinforcement because it not only meets nutritional demands but is also highly palatable for even mildly food-restricted rats (when 23 h has elapsed since prior access to food but with no deviations observed in free-feeding bodyweight). Furthermore, when delivered through a 20-cc syringe, this soft mixture is immediately consumed by the rat rather than stored in its cheek pouches, thereby avoiding motivation attenuation shown with other "storable" food items.

2.2 Clicker Training

Applying the classical principles of learning discovered by Pavlov [5], a previously neutral click sound acquires relevance to the rat by repeatedly pairing this sound with the biologically relevant food reinforcement (unconditional stimulus (US) in Pavlovian terms). Clicker training is accomplished by placing the rat inside a rectangular enclosure and triggering the click when the rat approaches the trainer positioned outside, who then immediately provides food reinforcement. Such click → food pairing occurs 15–20 times (until the rat appears sated) during each of two daily sessions. These pairings effectively form an association between the clicker sound and food which can be observed in the behavior of the rat. That is, prior to pairing, the clicker fails to evoke any response from the rat; however, after the rat has learned that the click reliably predicts food delivery, the click sound-alone provokes the rat to approach the trainer in anticipation of the food. Not only does this newly established value of the click serve as a signal for food to the rat, but it also establishes the sound of the click as a conditioned reinforcer [6] capable of supporting further learning. This association establishes a critical foundation for subsequent training because it provides the trainer with a means of communicating with the rat. Like most animals, rats engage in various natural behaviors within a short period. Therefore, the timing of reinforcement is essential to teach the rat which specific behavior triggered the reinforcement effectively. Such precise timing is difficult to achieve with a food-filled syringe that must be physically moved to the rat's vicinity; however, using a small handheld device, the trainer can quickly depress a button generating the click sound to instantly reinforce the rat's behavior at precisely the moment it occurs. This allows the trainer to shape the rat's natural behavior by reinforcing successive approximations towards the unnatural target response.

2.3 Indication Training

Explicit shaping begins by teaching the rat to approach and sniff a two-cm diameter hole in the floor of the training enclosure. A sputum sample confirmed by microscopy to contain *Mycobacterium tuberculosis* (*M. tb*, TB-positive sample) is placed beneath each of three holes spaced equidistant along the floor. Each hole is covered by a metal plate. After placing the rat within the enclosure, the trainer slides open one metal plate to reveal the hole and the sample it contains. The rat's natural exploratory behavior to approach this novel hole is reinforced by immediately sounding the click. As the rat moves away from the hole to retrieve its food reward, the hole over the container is slid closed. The hole is then re-opened, and the click is again sounded when the rat re-approaches it. During subsequent trials with the open hole, the trainer withholds clicking until the rat inserts its nose within the hole (nose pokes). Gradually, across trials, the trainer requires the rat to nose poke for progressively longer durations. By placing the rat in the same end of the apparatus at the start of each training session and opening each sample hole in succession,

starting with the hole nearest the rat, the rat also learns to navigate the apparatus to evaluate scent samples in sequence during this training stage. This process is repeated across days until the rat reliably nose pokes for at least three seconds in each of nine consecutive holes containing TB-positive samples (each of the three holes is visited three times).

2.4 Discrimination Training

Using the same three-hole rectilinear training apparatus, discrimination training establishes the unique odor profile emitted by TB-positive samples as a discriminative stimulus to signal when the rat's nose poke behavior will be reinforced. This is accomplished by introducing sputum samples deemed not to contain *M. tb* (TB-negative samples) and never sounding the click or delivering food reinforcement when the rat nose pokes in the hole containing these samples, regardless of how long the nose poke response occurs. Importantly, which hole contains TB-positive or TB-negative samples is randomized across sessions; thus, the only cue the rat can rely on to predict reward is the smell of the sample placed beneath the hole. Half of the holes contain TB-positive sputum samples during initial training sessions, while the rest are TB-negative samples. To prevent the cunning rat from relying on cues extraneous to the presence of TB and to ensure that the rat accurately identifies *M. tb* across its various presentations within samples (including bacterial load, how the sample was stored or otherwise handled, and unique patient characteristics including age, sex, diet, or comorbid infections), thousands of TB-positive and TB-negative samples from partner clinics are used throughout this and the following training stages. The total number of sputum samples included in the session gradually increases across days while the ratio of TB-positive to TB-negative samples decreases. To advance to the final training stage, the rat undergoes a blind test in which the trainer is not even aware which samples are TB-positive or TB-negative. This ensures the rat's nose poke behavior is unequivocally driven by the odors emitted by the samples without influence from any subtle behavioral cues of the trainer, such as reaching for the next hole before the rat has finished nose poking the current hole containing a TB-negative sample or leaving the hole open longer when a TB-positive sample is placed within it. This test includes 30 unique sputum samples (each of the three holes within the apparatus are visited ten times), from which only eight are TB-positive. To pass the test and advance to the final training stage, the rat must either 1) accurately indicate (nose poke of $\geq$ three seconds) all eight TB-positive samples while committing no more than one false indication of a TB-negative sample or 2) miss (nose poke < three seconds) no more than one of the eight TB-positive samples while committing no false indications of TB-negative samples.

2.5 Final Training

To capitalize on the rats' incredible speed in the scent-based evaluation of sputum samples and maximize throughput, rats are transitioned to a larger version of the rectilinear training apparatus containing ten holes instead of three. Daily training sessions follow the same procedures established during discrimination training; however, the total number of samples evaluated within each session gradually increases across days from 30 (each hole visited three times) to 100. To prepare the rat for research involving patient samples from local clinics, the prevalence of TB-positive samples is gradually reduced to 10% or less, while blind samples are introduced. Blind samples offer the benefit of avoiding inadvertent cueing from the trainer that may influence the rat's indication behavior and undermine the scent detection training while also teaching the rat that a reward does not always follow correct indications. This latter point is critical in positioning the rat to reliably identify new cases of TB that were previously undetected at the clinic. Without specific training, the behavioral phenomenon of extinction rapidly occurs when reinforcement is withheld under circumstances when it would otherwise be expected. Extinction learning causes the rat to stop performing the indication response. From the rat's perspective, it expects to be rewarded if its nose pokes a hole that smells like *M. tb*. However, if the clinic misses the presence of *M. tb* within the sputum sample, the sample will appear TB-negative for rat training purposes, and the trainer will not reinforce the rat's nose poke with the click sound and food. This can be surprising to the rat and reduces its motivation to indicate subsequent TB-positive samples (likewise reducing the likelihood of finding new cases). However, by only reinforcing a subset of known TB-positive samples during training, the rat's indication behavior can be protected from extinction by essentially teaching it that non-reinforcement occasionally occurs, but if the rat continues on the task of sniffing successive samples, it will eventually encounter another TB-positive sample for which it can earn the click and food.

This training continues for at least three months of daily sessions until the rat passes another blind test. During this final blind test, the rat is challenged to achieve 100% sensitivity and 95% specificity with 100 sputum samples of confirmed TB content (including nine or fewer TB-positive samples) or otherwise miss no more than one TB-positive sample while achieving 97% specificity. Allowing two separate criteria for passing this blind test allows highly sensitive and specific rats to join APOPO's operational research programs. Deploying the rats in a team strategy (where a pre-defined number of rats must all indicate any given sputum sample for it to be considered suspected of containing *M. tb* and subjected to laboratory confirmation) reduces the risk of missing a new TB-positive case by offsetting one rat's high specificity with the strong sensitivity of other rats. Likewise, relying on the indication behavior of only one or a few highly sensitive rats compromises efficiency through an increased risk of false indications, which a highly specific rat counters. Thus, including both sensitive and specific rats in operations ensures cost-efficient detection of new TB cases.

As with final training, operational TB detection research utilizes the ten-hole cage and includes all sputum samples collected by partnering clinics, including those which were already confirmed TB-positive and those diagnosed TB-negative. TB-positive samples permit within-session calibration of rat performance while providing an opportunity for the rat to earn food reinforcement, effectively maintaining its TB detection training and motivation for the task. TB-negative samples indicated (nose poke of $\geq$ three seconds) by the team of rats are then flagged for confirmatory analysis within APOPO's laboratory using WHO-endorsed TB tests.

3 Rat Evaluation Setups

Presently, all rat TB detection research is performed in manually operated training cages, including the rectilinear and semi-automated line cages. In these setups, trainers manually open and close the sample holes (by sliding the metal plate covering them) to provide access for the rat to sequentially sniff sputum samples placed beneath them.

3.1 Rectilinear Manual Cage

This is a custom-engineered apparatus consisting of a rectangular enclosure (210 cm long $\times$ 52 cm wide $\times$ 41 cm tall) elevated by four 85-cm steel legs and covered by a hinged wire-mesh door. All sides of the enclosure are constructed from clear Perspex panels allowing an unobstructed view of the rat. The stainless-steel floor contains ten holes (measuring two cm in diameter) spaced ten cm apart (as measured from the center of one hole to the center of the adjacent hole). Aluminum cassettes measuring 192 $\times$ 8 $\times$ 45 cm, which contain ten holes aligned with the ten holes along the enclosure floor, are loaded with plastic sputum sample containers. Cassettes are placed in a hinged bracket below the cage floor, which, when locked into the upward position, situate the samples beneath the holes of the cage floor. Each hole is covered by an aluminum plate that can be easily slid by the trainer to expose or re-cover it (and the sputum sample it contains).

When all samples have been received from partner clinics and autoclaved for the day (Section "Social Impact of Tuberculosis Detection Rat Technology"), the data coordinator enters coded sample, patient, and partner clinic information into a secure database. The database then randomly assigns samples to specific cassette positions using criteria defined by the data coordinator, including the overall prevalence of known TB-positive samples. If possible, a range of bacterial loads (from scanty to higher grades, 3+) is represented across the TB-positive samples included in each rat evaluation session, and at least one blind TB-positive sample is planned. A sample log detailing hole position and bacterial load of (non-blind) TB-positive samples is then generated for reference during the rat evaluation session.

Typically, three people must operate the manual cage during the rats' evaluation sessions. One person (designated as the "trainer") is responsible for opening and closing the sample holes in succession and carefully observing the rat's behavior as it navigates the cage and sniffs the sample holes. The trainer is also responsible for classifying the rat's nose poke responses as indications depending on the estimated time the rat pauses with its nose within a sample hole. If the rat nose pokes for three or more seconds, the trainer announces the hole number (labeled one through ten). A second person serves as a note-taker and relies on the announcements from the trainer to record samples indicated by the rat. The note-taker is equipped with the sample log on a clipboard and a clicker. If the trainer calls a rat indication on a sample known as TB-positive, the note-taker immediately activates the clicker and records the rat indication on the sample log. If the sample in question is marked TB-negative on the log (either found TB-negative at the partner clinic or a TB-positive sample from the clinic that was blinded prior to the session), the note-taker marks the sample as "suspect" on the log but does not activate the click. The third person involved in the session is the handler responsible for delivering food reinforcement after the note-taker has clicked by inserting the syringe tip through a hole in one wall of the line cage. The handler also assists the trainer with swapping sample cassettes throughout the session (typically ten cassettes housing ten sputum samples each) and transporting the rat to and from the line cage apparatus and its home cage. This video (https://www.youtube.com/watch?v=kuYpIUZIwAE) shows how the rectilinear manual cage works.

3.2 Semi-Automated Line Cage (ALC)

This apparatus resembles the rectilinear manual cage in design, overall dimensions, and materials but is outfitted with a mechanism (ENV-203-94, MedAssociates, Georgia, VT) to automatically deliver flavored pellets (5TCY OmniTreat, TestDiet) to one side of the enclosure when the rat has correctly indicated a known TB-positive sample (sample position tracked by MS Visual Basic). A through-beam photoelectric sensor (ENV-254, MedAssociates) inside each sample hole records beam break duration (when the rat nose pokes) to the nearest millisecond to determine when a sample has been indicated (sniff duration exceeds a threshold defined prior to the start of the session). Each sample indicated is recorded regardless of known TB status (TB-positive or TB-negative). In this way, the ALC minimizes the potential for error in data collection, ensures consistent timing of rat indication responses and delivery of reinforcement, and reduces the personnel required to just two: a trainer and handler. Figure 2 shows the schematic design of the ALC, and this video (https://www.youtube.com/watch?v=VrzpEfCUnjc) illustrates how it works.

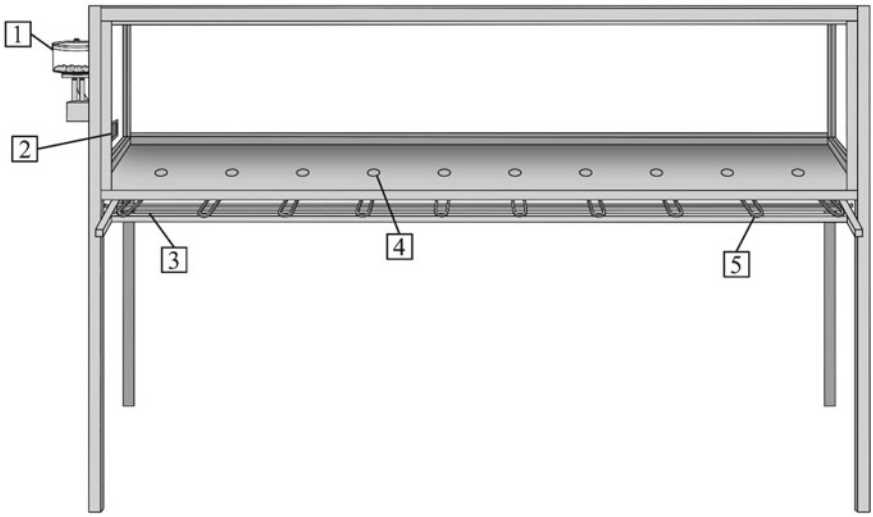

Fig. 2 Design scheme of an automated line cage: 1, pellet dispenser; 2, pellet delivery port; 3, through-beam photoelectric sensor; 4, sniffing hole; 5, sliding metal plate

3.3 Fully-Automated Line Cage Odor Nose Poke Holes Apparatus

The number of people needed to operate a rat evaluation session is further reduced to just one with this newest version of APOPO's evaluation cage. Like the ALC, the Fully-Automated Line Cage Odor Nose-poke holes (FALCON) apparatus utilizes controlling software, photoelectric beams and sensors, and automated pellet delivery mechanisms. Additionally, solenoids attached to the metal sample hole plates beneath the cage's floor are programmed to automatically open and close the holes in succession, dependent on the rat's nose poke behavior. When the session initiates, Hole 1 opens, and an LED positioned outside the glass wall near the hole is illuminated. When the rat nose pokes in Hole 1, Hole 2 immediately opens, and a second LED positioned near it is illuminated. Notably, Hole 1 remains open until the rat inserts its nose into Hole 2, at which time Hole 3 is then opened, and a third LED near this hole is lit. Holes 4–10 operate similarly, with Hole 10 closing after a 0.5-s delay after the photobeam is restored (the rat removes its nose). The trainer then changes the sample cassettes and selects the appropriate bar on the software graphical user interface (GUI) to again open Hole 1 containing a new sputum sample. Interested readers can view this *video* (https://www.apopo.org/en/latest/2019/10/Unlocking-the-full-potential-of-the-HeroRATs-with-FALCON) for a better understanding.

In summary, we have gone through the developments of a three-person manual rat evaluation cage to a one-person fully automated cage, which is a huge step in our research and to availing the technology for those interested in state-of-the-art detection rat technology. Most high-TB burdened countries are in Sub-Saharan

Africa, where electric supply is intermittent. The manual cage has a practical advantage over the semi-automated and fully-automated ones because it does not require electricity. Moreover, the manual cage can be produced locally with a limited investment cost. Therefore, its simplicity, low cost, no electricity requirement, and of course, more job creation by involving three people makes it the preferred setup used in our operational sites (Section "Social Impact of Tuberculosis Detection Rat Technology").

3.4 Tuberculosis-Specific Odor

APOPO has been conducting a series of organic analytical chemistry researches to determine what the rats rely on to discriminate TB-positive samples from the negatives. The first study aimed to find out whether the TB detection rats could correctly identify clinical samples with *M. tb* and ignore others with non-tuberculous mycobacteria (NTM) and with other microorganisms that are commonly found in the respiratory tract (*Nocardia, Rhodococcus, Streptomyces, Moraxella, Candida, and Streptococcus pneumonia*). The *Nocardia* and *Rhodococcus* species can look like the *Mycobacteria* species (acid-fast) under microscopic examination. We found that the rats discriminate clinical samples with *M. tb* from those with NTM and other microorganisms [7]. In another study, we compared the performance of fully trained TB detection rats (trained with TB-positive sputum samples) on cultures of standard *M. tb*, NTM, and other microorganisms (*Nocardia*, *Rhodococcus*, *Streptomyces*, *Bacillus*, *Candida*, and *Saccharomyces*). We found that trained rats could distinguish *M. tb* cultures from that of NTM and other microorganisms [8]. Furthermore, analysis of volatile organic compounds (VOCs) from *M. tb*, NTM, and other respiratory tract microorganisms revealed that a group of VOCs from *M. tb* is distinct from NTM and other microorganisms. There were 13 *M. tb*-specific compounds (most of them were aromatic compounds), and 13 others were also found in NTM and other respiratory tract microorganisms. Among the 13 M. *tb*-specific compounds, the following six were relatively higher in concentration and selected to test the rats' response on spiked samples: methyl nicotinate, methyl 4-anisate, 2-phenylanisol, 4-methyl anisole, ethyl 4-anisate, and benzothiazole. Sputum samples were spiked with individual compounds as well as different combinations of the six compounds, and the spiked samples were presented to trained rats. The best detection was observed when samples were spiked with a mixture of all six compounds suggesting that the rats relied on a bouquet of VOCs rather than a few specific compounds [9–11]. The result also indicates the complexity of investigating a few *M. tb*-specific VOC biomarkers towards developing olfaction-based diagnostic tools such as e-noses. Rats, as a living biosensor, are going for a bouquet of VOCs rather than the smell of a few specific compounds that simplifies their task [12]. Further research is ongoing to compare the chemical signatures of known TB-positive sputum samples with the standard culture isolates.

The research and development aspects of the TB detection technology have been described in the preceding sections. And yet, APOPO is also conducting ongoing

field research using TB detection rats as a diagnostic tool and providing diagnostic services in agreement and partnership with national TB programs and local TB clinics and health care facilities in mostly urban settings of three high-TB burden African countries. Our main approach is supporting partnering clinics in enhancing their TB case detection by re-evaluating the patients' samples at our labs and promptly returning results to the clinics. Thus, the subsequent sections describe the practical aspect undergoing operational research.

3.5 Urban Model for a Sample Referral Network

The TB detection rats are designed for high speed and sample throughput. This can be achieved best when testing is centralized. Under the premise that patients should not face extra efforts (e.g., time to travel, costs), it was clear that samples, not patients, had to be referred for re-evaluation. To this effect, sample collectors are trained on the basics of TB, e.g., mode of transmission, infection control, prevention measures (laboratory safety, road safety, triple packaging of infectious materials), and data collection. Triple packaging refers to the three layers of protection as follows:

i. samples are placed in tightly-locked lunch-box type plastic boxes;
ii. the plastic boxes are placed in a tightly-locked large cool box; and
iii. the cool box is put in an aluminum box that is fitted in the rear of the motorcycle.

Sample collectors are equipped with personal protection equipment (lab coat, N-95 mask, gloves, tissue paper, 70% alcohol) as well as accident protection gears.

The sample referral network was first conceived and tested in Dar es Salaam, Tanzania, in 2013. Sputum samples from 12 TB clinics in Dar es Salaam were stored at a central location at the laboratory of one of the participating hospitals collected daily by a trained laboratory assistant riding a motorcycle. APOPO then transported these samples weekly to its research facility in Morogoro, 200 km from Dar es Salaam. However, the long delay in the result turnaround time (TAT) forced us to change course and started daily transport of samples to the research facility using public transport but with strict adherence to international guidelines for transporting biological materials (triple packaging). Instead of the motorcycle-fitted aluminum box, a lockable and portable aluminum box was used. Although the latest development reduced the TAT to a few days, it was still unsatisfactory. Time matters with respect to receiving the additional results for patients who were newly diagnosed by the project (who were initially tested negative by the TB clinics) for eventual treatment initiation. This led us to strive for a result TAT of 24 h. That means results from APOPO are released on the same day of collection to the respective clinics so that the clinicians can use the result for clinical decision-making. In 2016, APOPO opened a new TB diagnostic laboratory facility in Dar es Salaam, i.e., closer to where the largest number of patients live, which

Fig. 3 APOPO's urban sample referral network (model). Adapted with permission from APOPO vzw. Copyright © APOPO

helped build the same-day testing and reporting scheme. After the success in Dar es Salaam, the same scheme has been replicated in a similar operational research program established in 2013 in Maputo, Mozambique, and in 2018 in Addis Ababa, in Ethiopia. This has partly improved the treatment initiation and thereby reduced the loss to follow-up. The infographic in Fig. 3 shows the urban sample referral model [13].

3.6 How the Tuberculosis Detection Rats Technology Is Deployed in the Field

Trained rats are currently not a stand-alone diagnostic test and complement rather than replace the standard diagnostic services at local TB clinics. Because of this, we collect samples once the primary (first-line) testing is completed at the clinics, and thus the testing done by the trained rats is termed "second-line testing." The second-line testing model incorporates not only rat testing but also confirmation of rats' positive results by WHO-endorsed methods. Those patients that are negative by the clinics' test but positive by the second-line testing are considered as "additional cases."

Patients with signs and symptoms of TB (e.g., cough) visit their local TB clinic or center. There, not only a medical exam and diagnostics are offered (mostly sputum smear microscopy), but also patients are registered and asked to provide contact details such as telephone numbers (their own, a family member's, friend's,

or co-worker's) and addresses. Patient contact details are also made available to trained community health workers (CHW) for the purpose of tracking the patients. Once the sputum samples are examined by microscopy at the clinic, the clinics' lab personnel keep the remaining samples for the APOPO project. The project's sample collectors pick up the samples with the following corresponding data: lab serial number of the patient, gender, age, and microscopy result at the clinics.

Once the sample is picked up, it is transported to a central detection rats' facility where all the data (clinic's name, patient number, gender, age, and smear results) are fed to a dedicated Laboratory Information Management System (TB-LIMS). This database generates a random list of samples from different clinics to be checked by the rats. The rat evaluation session is conducted, and those samples that are negative at the clinic's lab but positive by the rats are further confirmed by a more sensitive method. If the samples indicated as positive by the rats are confirmed, then the corresponding patient number is notified to the TB coordinator of the clinic for eventual patient tracking and treatment initiation using the contact details collected during the initial consultation. Collaboration with CHWs from patient organizations ensures that more newly diagnosed TB patients are supported and tracked, if needed, to make sure they start the free standard TB care at their clinics. The following infographics (Graphical Abstract) and animation *video* (https://youtu.be/Z_vc5BtPPQ0) illustrate the whole process.

4 Social Impact of Tuberculosis Detection Rat Technology

The first proof of principle for the TB detection rats technology was completed in 2007 and published in 2009 [14]. Since 2007, APOPO has been steadily improving this technology while using it as an add-on diagnostic tool to contribute to the efforts of finding missed TB cases. Over the years, it has expanded its reach to a huge number of TB clinics and towns in Tanzania, as well as opened TB detection programs in Maputo, Mozambique (2013 onwards) and Addis Ababa, Ethiopia (2018 onwards).

4.1 Tanzanian Enhanced Case Finding Programs

This program started with four participating TB clinics in 2007 and gradually increased to 21 in 2013 and four regions (Coastal, Dar es Salaam, Dodoma, and Morogoro) and a total of 75 TB clinics in 2020. Until the end of June 2020, more than 516,220 samples collected from 289,406 patients were screened, from which 13,467 patients were found on top of the 24,101 found by the participating TB clinics. To intensify the social impact, APOPO initiated a collaboration with a local NGO called Mapambano ya Kifua Kikuu na Ukimwi Tanzania (MKUTA), which was established by former TB patients. They have been involved in the additional case tracking and linking to care, and they enhanced the treatment initiation rate

from a baseline of 10% (prior to the involvement of MKUTA) to an average annual of 81% in 2017.

In Dar es Salaam, a special opportunity emerged to add a digital element to the integrated TB detection model and pilot the digital treatment adherence technology e-Compliance (from OpASHA, India) in the Temeke District. This allowed going beyond linkage to care and follow-up the patients until their treatment completion. Overall, 3250 TB patients were reached by the digital technology e-Compliance. The enrolled TB patients benefited (after an initial clinic-based treatment phase) from daily drug doses, digital monitoring, and support delivered to the patients' homes by MKUTA community health workers, which led to a steady increase of the treatment adherence (in % of daily doses taken) from 65 to 92% and above. This pilot described elsewhere [15] illustrates that the innovative TB detection model is modular, complementary, and interoperable both with standard care in the respective countries (without duplicating efforts) yet also with newly emerging approaches alongside the cascade of care. The general ambition and theme stay to make services more integrated and patient-centered.

4.2 Mozambican Enhanced Case Finding Programs

This is APOPO's first program outside of Tanzania and was initiated in 2013 in collaboration with Eduardo Mondlane University, the National Institute of Health, the national TB program, and the Maputo city health directorate. In Mozambique, APOPO started its case detection operation in 2013 with eight centers in Maputo city and covered 100% of the TB centers in the city. The coverage of clinics increased over time, and as of June 2020, there were 23 participating TB centers from Maputo city that supplied more than 109,629 sputum samples collected from around 60,880 patients. APOPO's trained rats found 4067 TB cases that were negative by the conventional diagnostic method conducted at their respective health facilities.

The Mozambique program emulated the Tanzanian one not only in finding the missed TB cases with a faster result delivery but also in the effort of tracking newly diagnosed patients for rapid treatment initiation. To this effect, APOPO signed a partnership agreement with a community-based local organization called Kenguelekezé in 2017. This partnership, in conjunction with the introduction of the rapid diagnostic TAT in 2015, increased the patient tracking and treatment initiation rate from a baseline of 54% in 2013 to 78% in mid-2018 [13].

4.3 Ethiopian Enhanced Case Finding Programs

The Ethiopian APOPO facility started in 2018 with the aim of finding the missed cases among presumptive TB cases (enhanced case finding) who are diagnosed as negative by their health facility laboratory. There are currently 58 TB diagnostic centers participating in this program, and up to the end of June 2020, the trained rats

screened more than 54,374 samples collected from around 27,794 patients. The TB centers found 1038 smear-positive cases (microscopy), but APOPO-Ethiopia found additional 707 patients that were missed by the health facility diagnostic center. Apart from being just a two-and-a-half-year-old program, there is no community-based local organization involved in patient tracking and treatment initiation (unlike the Tanzanian and Mozambique programs), and therefore the treatment initiation rate is currently around 50–60%. Consensus has been reached with the national program and the Addis Ababa City Administration Health Bureau that the confirmatory method employed is nationally and internationally accepted with better sensitivity than direct smear microscopy, and thus clinicians can safely utilize the confirmatory diagnostic results coming from this project. Moreover, the 24 h TAT, which is already implemented in Tanzania and Mozambique, is not yet practiced in Ethiopia, which is another factor impeding the timely treatment initiation.

5 Conclusion

This chapter described an avenue starting from an original idea, training rats to diagnose TB, and its scientific underpinning. In line with Pasteur's wise words, we let the idea grow into an application within the TB control ecosystem. APOPO's enhanced case-finding programs started with one TB lab in Morogoro, Tanzania, has successfully been replicated in a new APOPO lab facility in Maputo, Mozambique, in Dar es Salaam, Tanzania, and in Addis Ababa, Ethiopia.

This operational TB research in the three countries has resulted in the evaluation of more than 581,649 sputum samples from around 378,512 presumptive TB patients and resulted in the identification of 18,872 additional TB cases averting 188,720–283,080 potential infections (as of June 2020). The data from Tanzania shows that young children and PLHIV are benefiting most from the enhanced case-finding programs [16]. In parallel to researching TB detection rats under field conditions, service delivery models have been created and implemented to maximize benefits for patients. These include sample collection networks, same-day testing, shorter turnaround time (24-h), and patient tracking for prompt treatment initiation through partnering patient organizations and CHWs [13, 17].

APOPO has a strong scientific record and active lines of research in the investigation of odor patterns (volatile organic compounds) specific to *M. tb*, rat odor discrimination learning and performance, as well as the possibility of using other biological materials such as saliva, urine, sweat, and sebum (instead of sputum) that are less invasive and more child-friendly, and thus can potentially benefit pediatric patients with signs and symptoms of TB. The research also involves master and Ph.D. students contributing to the local capacity-building efforts. APOPO, as a humanitarian non-profit organization, will continue improving its methodology and make its steady contribution to finding and treating more TB patients in the three countries until the TB challenge is overcome.

Core Messages

- Utilizing an unconventional, multidisciplinary, and integrated science approach can help find missed TB cases.
- Diagnostic innovations integrated with the combination of tools and approaches can generate social and health impacts.
- Expertise in chemistry, behavioral sciences, engineering, and public health led to scent detection animal technology.
- Advances in scent detection animal technology may lead to synthetic scent detection devices such as electronic noses.

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Compliance with Ethical Standards

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Studies involving humans' ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Approval was granted by the Tanzanian Medical Research Coordination Committee (NIMR/HQ/R.8c/Vol.I/1386); the Institutional Review Board of Armauer

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Studies involving animals' ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Furthermore, APOPO obtained Animal Welfare Assurance from the Department of Health and Human Service, National Institute of Health, USA.

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References

1. Ajayi SS (1977) Field observations on the African giant rat *Cricetomys gambianus* Waterhouse in southern Nigeria. Afr J Ecol 15:191–198
2. Hutchinson E, Avery A, VandeWoude S (2005) Environmental enrichment for laboratory rodents. ILAR J 48:148–161
3. Nithianantharajah J, Hannan AJ (2005) Enriched environments, experience-dependent plasticity and disorders of the nervous system. Nat Rev Neurosci 7:697–709
4. Van Praag H, Kempermann G, Gage FH (2000) Neural consequences of environmental enrichment. Nat Rev Neurosci 1:191–198
5. Pavlov IP (1927) Conditioned reflexes. Oxford University Press, London
6. Pryor K (2002) Don't shoot the dog! The new art of teaching and training. Ringpress Books, Ltd., Gloucestershire, UK
7. Mgode GF, Weetjens BJ, Nawrath T, Cox C, Jubitana M, Machang'u RS, Cohen-Bacrie S, Bedotto M, Drancourt M, Schulz S, Kaufmann SH (2011) Diagnosis of tuberculosis by trained African giant pouched rats and confounding impact of pathogens and microflora of the respiratory tract. J Clin Microbiol 50:274–280
8. Mgode GF, Weetjens BJ, Cox C, Jubitana M, Machang'u RS, Lazar D, Weiner J, Van Geertruyden JP, Kaufmann SH (2012) Ability of Cricetomys rats to detect Mycobacterium tuberculosis and discriminate it from other microorganisms. Tuberculosis 92:182–186
9. Mgode GF (2012) Determination of Mycobacterium tuberculosis odour compounds detected by Cricetomys gambianus rats for diagnosis of pulmonary tuberculosis in low-income settings. Ph.D. Dissertation, Technical University of Berlin, Germany
10. Mgode GF, Weetjens BJ, Nawrath T, Lazar D, Cox C, Jubitana M, Mahoney A, Kuipers D, Machang'u RS, Weiner J, Schulz S, Kaufmann SHE (2012) *Mycobacterium tuberculosis* volatiles for diagnosis of tuberculosis by *Cricetomys* rats. Tuberculosis 92:535–542
11. Nawrath T, Mgode GF, Weetjens B, Kaufmann SHE, Schulz S (2012) The volatiles of pathogenic and nonpathogenic mycobacteria and related bacteria. Beilstein J Org Chem 8:290–299
12. Fiebig L, Beyene N, Burny B, Fast C, Cox C, Mgode G (2020) From pests to tests: training rats to diagnose tuberculosis. Eur Respir J 55:1902243. https://doi.org/10.1183/13993003.02243-2019
13. Burny R, Manhiça I, Magaia P, de Abreu A et al (2020) Rapid TB diagnostic service and community action to FIND.TREAT.ALL#EndTB—experiences from Maputo, Mozambique, 2013–2018. Public Health Action 10:4–6
14. Weetjens BJ, Mgode GF, Machang'u RS, Kazwala R, Mfinanga G, Lwilla F et al (2009) African pouched rats for the detection of pulmonary tuberculosis in sputum samples. Int J Tuberc Lung Dis 13:737–743
15. Mgode et al. Springer Book Chapter, Chapter in preparation

16. Mgode GF, Cox CL, Mwimanzi S, Mulder C (2018) Pediatric tuberculosis detection using trained African giant pouched rats. Pediatr Res 84:99–103
17. Mgode G, Cox C, Bwana D, Mtui L, Magesa D, Kahwa A, Mfinanga G, Mulder C (2017) Enhancing tuberculosis detection by trained rats and tracking of missed patients through community-based strategy in TB high-burden countries. BMJ Global Health 2 (Suppl 2): A34-5

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Chemotherapy for Drug-Susceptible Tuberculosis

13

Vinayak Singh, Nicole Cardoso, and Stanislav Huszár

I was in jail when they took a specimen of my sputum and sent it to hospital. I was diagnosed with TB … Fortunately we sent the specimen before there were holes in the lung.

Nelson Mandela

Summary

The decade of 1940s brought one of the major achievements when streptomycin was discovered to treat tuberculosis (TB)–the first effective chemotherapy for the treatment of TB. However, even today, millions are still affected by this dreadful disease. In this chapter, basic principles in TB chemotherapy and information on all approved drugs used to treat drug-susceptible TB are reviewed. A brief overview of the drugs to treat drug-resistant TB is also discussed. The

Nicole Cardoso, Stanislav Huszár—equally contributed to the work.

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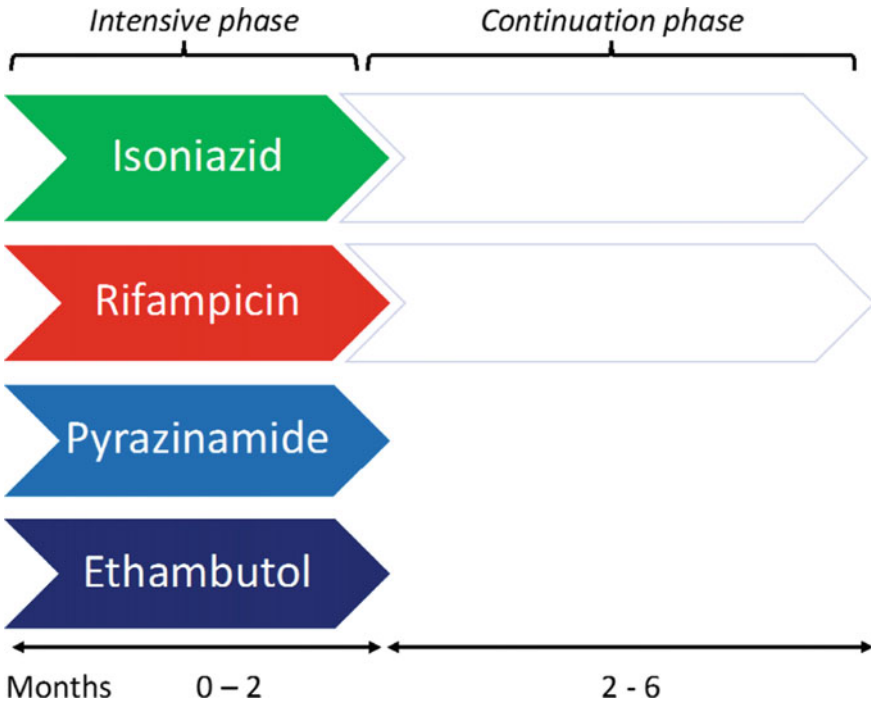
information includes discovery, mechanism of action, efficacy, and safety in humans. Treatment regimens are discussed in accordance with the current WHO guidelines.

Graphical Abstract



Drug-susceptible tuberculosis treatment. It shows that the treatment of drug-susceptible tuberculosis requires a combination of different drugs and standardly takes place over six months. During the two-month intensive phase, isoniazid, rifampicin, pyrazinamide, and ethambutol are administered. The continuation phase lasts four months and includes the administration of isoniazid and rifampicin

Keywords

Chemotherapy · DOTS · First-line anti-tuberculars · Tuberculosis

1 Introduction

Before discovering streptomycin in 1946, tuberculosis (TB) was considered incurable [1]. The first clinical trial performed by the British Medical Research Council (BMRC) demonstrated the ability of streptomycin to significantly reduce mortality; however, resistance emerged soon after that [2]. In a second BMRC clinical trial, it was shown that the use of para-aminosalicylic acid in combination with streptomycin could prevent or delay the onset of resistance. Para-aminosalicylic acid is bacteriostatic but shown to be useful as monotherapy; however, resistance development and associated side effects limited the use [3]. The end of the 1940s witnessed the evolution of TB chemotherapy to combined therapy with streptomycin-para-aminosalicylic acid, typically given for 12–24 months. Following the discovery of the wonder drug isoniazid, with its anti-TB bactericidal activity and low cytotoxicity in 1952 [4], it became well accepted that the combination of isoniazid with streptomycin and para-aminosalicylic acid was more effective than either drug on its own, and led to the first standardized combination therapy regimen for the treatment of TB. Except for a rash or hepatitis, isoniazid was generally well tolerated in patients.

In the 1950s, there were other drugs discovered, such as cycloserine [5], viomycin [6], kanamycin [7], ethambutol, ethionamide [8], and very importantly, pyrazinamide [9] (Fig. 1). However, due to the widespread use and effectiveness of isoniazid-para-aminosalicylic acid-streptomycin, the alternate drugs were not prioritized for the standardized regimen and were used only in patients in which the standard therapy was ineffective. At that time, TB care was limited to sanatoria or hospitals. A 12-month randomized controlled trial of isoniazid-para-aminosalicylic acid was launched at the Tuberculosis Chemotherapy Centre of Madras (India), which showed that an equivalent level of care and treatment efficacy could be obtained in the home as in a sanatorium or hospital [10]. Thereafter, TB care was shifted to the home. However, it raised the issue of how to ensure patients adhere to therapy during a year of home treatment [11]. Many years later, this led to the implementation of Directly Observed Therapy (DOT), a strategy by the World Health Organization (WHO) which involves trained healthcare personnel watching the patient swallow their medications and maintaining the record at a mutually agreeable location. Other significant TB drugs introduced during this era were thioacetazone [12], capreomycin [13], and clofazimine [14]. However, there were several associated side effects to these drugs. Ethambutol subsequently replaced para-aminosalicylic acid in the standard drug regimen as it was better tolerated than para-aminosalicylic acid. This also significantly shortened the treatment duration to 18 months [15].

In the 1970s, the discovery of rifampicin revolutionized TB chemotherapy. It was rifampicin that decreased the treatment duration from 18 to nine months after the groundbreaking clinical trials performed in East Africa and Hong Kong. The data strongly suggested that rifampicin/isoniazid/ethambutol/streptomycin or rifampicin/isoniazid/streptomycin drug regimens were effective in controlling the

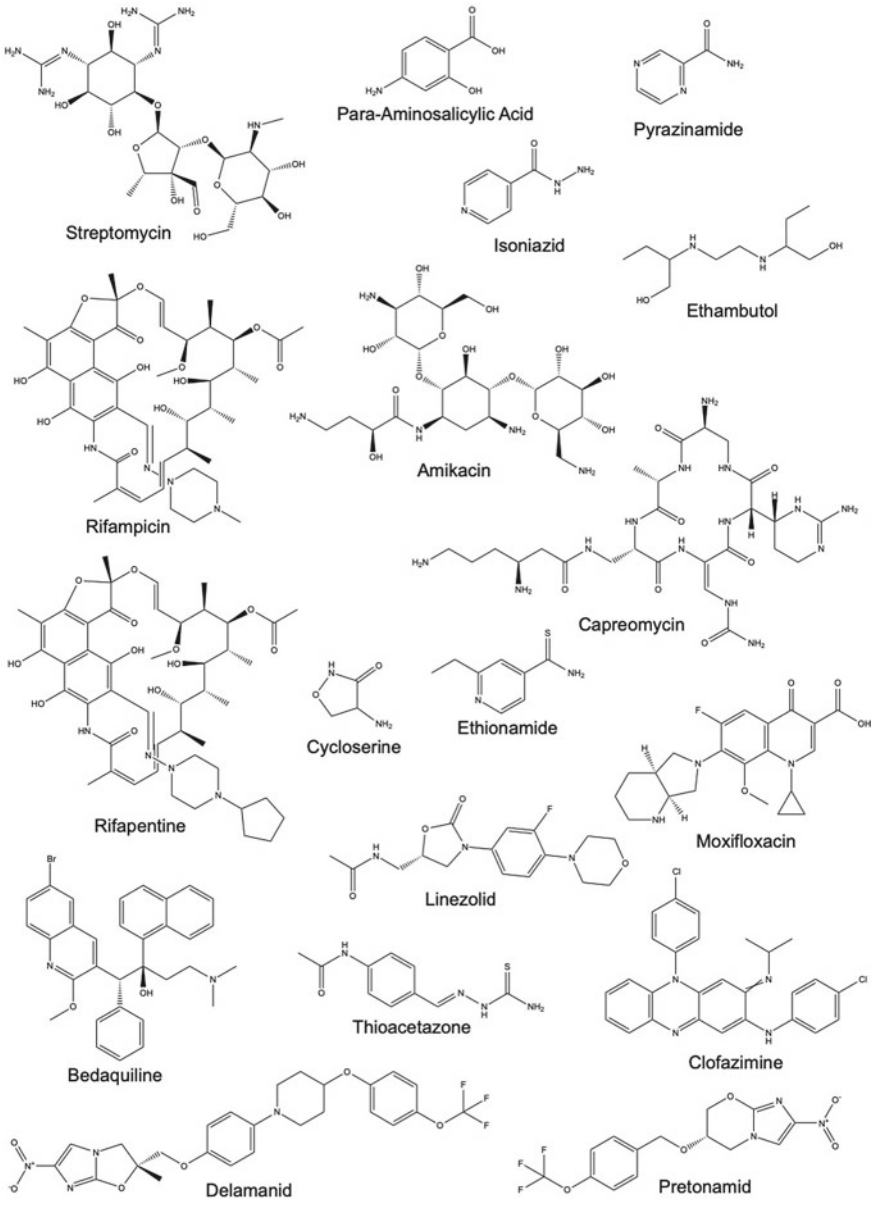


Fig. 1 Structures of the clinically used TB drugs

relapse rate [16–18]. The clinical trials performed during the 1970s and the 1980s suggested that pyrazinamide was instrumental in reducing the TB treatment duration from nine to six months [19–21]. Later on, streptomycin was replaced by ethambutol due to the intravenous route of streptomycin administration. These

studies were the basis of the modern, highly effective "short-course" chemotherapy for drug-susceptible (DS) TB, and the world celebrated the end of the "White Plague." The first two months of treatment, known as the "intensive phase," uses pyrazinamide, isoniazid, ethambutol, and rifampicin, in combination and the subsequent "continuation phase" of four months includes additional treatment with isoniazid and rifampicin (Fig. 2).

Based on the WHO estimation, TB is declining both in terms of treatment and the emergence of new cases. However, there were still 1.5 million deaths in 2018 [22]. The major problems with the current chemotherapy are:

- the six-month treatment regimen, it is too long, risking poor adherence to treatment which may lead to the emergence of drug resistance;
- increasing prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *Mycobacterium tuberculosis (M. tb)*; and
- drug-drug interaction with antiretrovirals and anti-diabetics

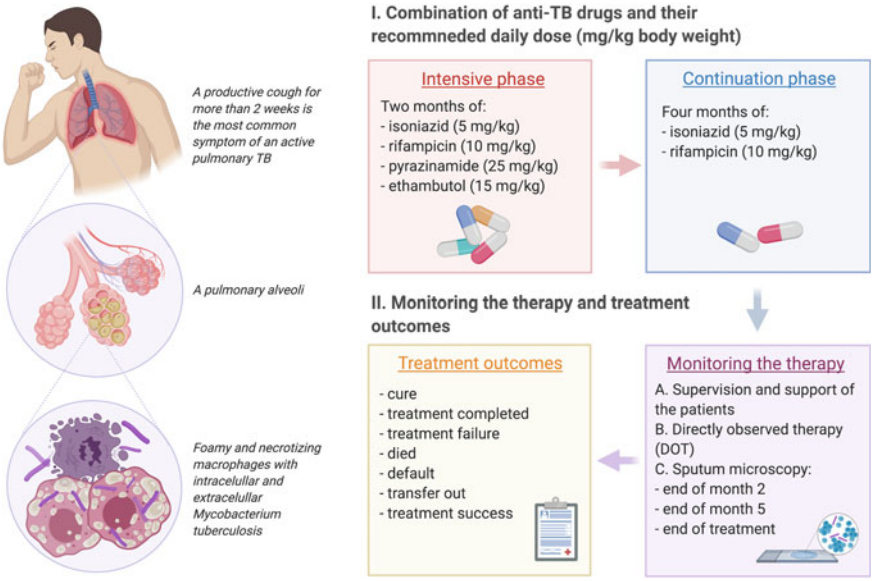


Fig. 2 The standard treatment regimen for new TB patients with drug-susceptible TB. The standard treatment regimen for new TB patients with drug-susceptible TB includes 2-month intensive phase drug therapy with four first-line anti-TB drugs (rifampicin, isoniazid, pyrazinamide, and ethambutol), followed by a 4-month continuation phase with two drugs (rifampicin and isoniazid). The patient's response to the treatment is continuously monitored to evaluate the disease progression by sputum microscopy to manage the possible adverse reactions of the drugs and prevent undesired discontinuation of the therapy by the patient. Several treatment outcomes are classified for TB treatment. If a TB patient was smear or culture positive at the beginning of the treatment but is not smear or culture positive in the last month of the procedure, the patient is declared as cured. The figure was created using BioRender

2 Anti-Tuberculosis Drugs

2.1 The First-Line Anti-Tuberculosis Drugs

The first-line anti-TB drugs isoniazid, rifampicin, pyrazinamide, and ethambutol, are the most effective clinically approved anti-TB agents (Fig. 1). Their combination constitutes the standard treatment regimen for newly diagnosed TB patients with known or suspected drug-susceptible TB. All first-line anti-TB drugs are given orally.

2.1.1 Isoniazid

Isoniazid is the hydrazide of isonicotinic acid that has been used for seventy years in TB therapy since its identification as a potent anti-TB agent in 1952 [4]. Isoniazid is specifically active only on mycobacteria and blocks mycobacterial cell wall formation by inhibiting mycolic acid biosynthesis. Isoniazid is a pro-drug that is activated by the *M. tb* catalase-peroxidase, KatG. This activation leads to the interaction of the isonicotinyl radical with nicotinamide adenine dinucleotide (NAD^+) to form isoniazid-NAD^+ adducts that in turn inhibit *M. tb* enoyl-ACP reductase InhA, a crucial component of the mycobacterial fatty acid synthase type II (FAS-II) system [23–26]. The FAS-II system is essential for building the precursors of mycolic acids; thus, inhibition of this process leads to the total loss of mycolic acids. Isoniazid has an irreplaceable role in TB treatment; however, the worldwide expansion of *M. tb* isoniazid-resistant strains makes it unusable for MDR/XDR-TB. Thus, novel and direct InhA inhibitors (without the necessity of pro-drug activation) may represent a powerful tool in the TB treatment regimen for drug-resistant (DR) strains of *M. tb*.

2.1.2 Rifampicin

One of the current anti-TB drug therapy pillars, rifampicin, was introduced into clinical practice for TB treatment in 1968 [27]. Rifampicin is a semisynthetic antibiotic derived from natural products–rifamycins (formerly rifomycins), isolated from *Streptomyces mediterranei* [28, 29]. Rifampicin is a broad-spectrum antimicrobial agent which inhibits bacterial RNA synthesis by blocking the DNA-dependent RNA polymerase [30, 31]. The structural study has shown that rifampicin hampers the elongation of RNA when the transcript is one to three nucleotides long [32]. Resistance to rifampicin in many bacterial species, including *M. tb*, developed via mutations in the *rpoB* gene, which encodes the RNA polymerase β-subunit [33]. Moreover, the combination of *M. tb* cell wall inhibitors with rifampicin increases the accumulation of rifampicin in *M. tb* due to the increased cell wall permeability [34], validating the synergy of the current drugs in the multidrug treatment regimen.

2.1.3 Pyrazinamide

Pyrazinamide, the structural analog of nicotinamide, is a pro-drug that is hydrolyzed into its active form, pyrazinoic acid, by pyrazinamidase encoded by the *pncA* gene in *M. tb* [35]. Pyrazinamide is active only at slightly acidic pH and specifically inhibits *M. tb*, which can be explained by the lack of pyrazinoic acid efflux mechanisms in *M. tb* compared to other mycobacteria [36]. The primary target of activated pyrazinamide is fatty acid synthase type I (FAS-I), as it was shown that pyrazinamide inhibits the biosynthesis of fatty acids in mycobacteria [37]. However, the mechanism of action of pyrazinamide is more complex. Pyrazinamide binds to the ribosomal protein RpsA, thus inhibiting mycobacterial trans-translation [38], and also disrupts the membrane potential [39]. Interestingly, it was subsequently shown that pyrazinamide activation might not require the *M. tb* pyrazinamidase PncA, thus providing a possible reason for the reduced activity observed in vitro compared to in vivo activity [40]. Pyrazinamide is active in replicating, non-replicating, and intracellular *M. tb*. The synergy of pyrazinamide with isoniazid allowed for reduction of the standard treatment of DS-TB from nine to six months and lowered the relapse rate when pyrazinamide was included in the treatment regimen [41, 42].

2.1.4 Ethambutol

The uniqueness of the mycobacterial cell wall composition and its clinical significance is underlined by another cell wall inhibitor–Ethambutol, which acts exclusively on mycobacteria. Ethambutol was discovered in 1961 [43], and thanks to its good water solubility, selectivity, and high potency, it has been used for TB treatment for more than 50 years. Several subsequent studies have shown that ethambutol affects the biosynthesis of the major cell wall polysaccharide–arabinogalactan (AG), which leads to the lack of attachment sites for mycolic acids, impaired cell wall integrity, and a bacteriostatic effect [44–47]. Accumulation of the arabinose precursor (decaprenyl-P-arabinose, DPA) in ethambutol-treated mycobacterial cells as well as ethambutol-mediated mutations in *M. tb* genes *embA*, *embB,* and *embC*, and the recent structural study have shown that ethambutol inhibits the enzymatic activity of arabinofuranosyl transferases in mycobacteria [48–51]. Since arabinosyl sugar residues are also a structural part of the mycobacterial lipoarabinomannan (LAM), the synthesis of LAM is also affected by ethambutol [44, 46].

2.2 The Second-Line Injectable Anti-Tuberculosis Drugs

2.2.1 Streptomycin

First isolated in 1943, Streptomycin is an aminoglycoside antibiotic produced by *Streptomyces griseus* (formerly *Actinomyces griseus*) with broad-spectrum activity against gram-positive and gram-negative bacteria [52]. Streptomycin binds to the 16S rRNA of the 30S small subunit of the bacterial ribosome, which causes the inhibition of protein synthesis. Streptomycin is administrated intramuscularly and is

recommended by the WHO in a retreatment regimen for TB patients with relapse of the disease, in TB patients who continue treatment following defaulting, or for the treatment of DR-TB [53].

2.2.2 Amikacin, Kanamycin, and Capreomycin

Amikacin, kanamycin, and capreomycin are the second-line injectable anti-TB drugs used to treat DR-TB. All three drugs interfere with the bacterial ribosome and inhibit bacterial protein synthesis. Amikacin and kanamycin are aminoglycosides that bind to the 16S rRNA of the 30S small ribosomal subunit, while capreomycin is a cyclic polypeptide antibiotic that blocks the ribosomal protein interaction within the 50S ribosomal subunit [54, 55].

2.2.3 Fluoroquinolones

Fluoroquinolones are a broad-spectrum, synthetic group of antimicrobials that inhibit the essential activity of bacterial type II topoisomerases, i.e., DNA gyrase and topoisomerase IV [56]. Unlike most bacteria, *M. tb* only contains DNA gyrase encoded by *gyrA* and *gyrB*, which possesses characteristics of both type II topoisomerases [57]. The fluoroquinolones levofloxacin, moxifloxacin, gatifloxacin, and ofloxacin are second-line anti-TB drugs that are regularly included in regimens for the treatment of MDR-TB; however, levofloxacin and moxifloxacin have been used in place of either isoniazid or ethambutol when those first-line drugs cannot be tolerated by patients [58]. This practice is not a standard recommendation as it has been shown that the replacement of ethambutol with moxifloxacin or gatifloxacin, or of isoniazid with moxifloxacin, is less effective than the standard treatment [58].

2.2.4 Ethionamide/Prothionamide

The thioamides are pro-drugs that inhibit InhA by forming adducts with NAD^+ following activation, similar to isoniazid [59]. The activation of ethionamide or prothionamide is facilitated by flavin adenine dinucleotide (FAD)-containing Baeyer–Villiger monooxygenase, encoded by *ethA* [59, 60]. Resistance to both drugs can arise either through mutations in *ethA* or *inhA*, with the latter leading to cross-resistance with isoniazid. Ethionamide/prothionamide is usually included in the treatment regimen for MDR-TB due to the low cost and forms part of the combination used to treat pediatric TB meningitis [58]. However, severe gastrointestinal side-effects are observed, making the thioamides poorly tolerated among patients. Although limited, some evidence points out that prothionamide causes fewer adverse gastric effects, suggesting that it should be the preferred choice when designing an MDR-TB regimen [61].

2.2.5 Cycloserine/Terizidone

D-cycloserine is a cyclic analog of the peptidoglycan pentapeptide component, D-alanine, and is naturally produced by *Streptomyces garyphalus* and *S. lavendulae* [62]. The structural analog, terizidone, is a combination of two cycloserine molecules [63]. D-cycloserine targets two enzymes involved in the biosynthesis of peptidoglycan: alanine racemase (Alr) and D-alanine:D-alanine ligase (Ddl) [62,

64]. D-cycloserine and terizidone are approved only for the treatment of MDR-TB due to adverse neurological side-effects as a result of interaction with the *N*-methyl-D-aspartic acid (NMDA) receptor in the brain [62, 63]. The emergence of resistance to D-cycloserine is extremely low and mainly attributed to Alr overproduction [65]. This favorable characteristic and the possibility that phosphorylated forms of D-cycloserine would not interact with NMDA and thus be less toxic [62] make D-cycloserine a promising candidate for structure-activity relationship (SAR) studies to develop new anti-TB drugs for susceptible and resistant strains.

2.2.6 Para-Aminosalicylic Acid/Para-Aminosalicylic Acid Sodium

The acid salt form, para-aminosalicylic acid sodium (Na), was the first aminosalicylic acid compound used to treat TB, with para-aminosalicylic acid being introduced later due to better gastric tolerance [66]. However, due to the side effects of persistent gastrointestinal disruption and possible hypothyroidism, as well as the high cost, the use of para-aminosalicylic acid is restricted to the treatment of DR-TB [67]. Although available for clinical use since the 1940s, a possible mechanism of action (MoA) of para-aminosalicylic acid was only recently identified [68]. It has been shown that para-aminosalicylic acid is a pro-drug that, when metabolized, disrupts the essential folate biosynthesis pathway by competing with dihydrofolate as a substrate for dihydrofolate reductase (DHFR), leading to the inhibition of DHFR and poisoning of the pathway [68]. The high potency of para-aminosalicylic acid is also attributed to the inhibition of the second target in the folate pathway, the flavin-dependent thymidylate synthase. In addition, the decreased pool of S-adenosylmethionine (SAM) leads to the impaired synthesis of the methoxy-mycolic acids, implying the multi-targeting effect of para-aminosalicylic acid [69].

2.2.7 Bedaquiline

Bedaquiline was the first drug with a novel MoA to be approved for TB treatment in over 50 years, but only under extreme circumstances. Initially, bedaquiline could only be used as “compassionate care” for the treatment of patients in which all other available drugs had failed [70]. More recently, South Africa became one of the first countries to include bedaquiline for the treatment of MDR-TB [71]. However, there are still apprehensions regarding the use of bedaquiline, including the increasing prevalence of resistance [72–74] and safety concerns due to QT prolongation in patients being treated with bedaquiline [75]. Due to good efficacy and a novel MoA, bedaquiline is currently in clinical trials to assess a new regimen made up of bedaquiline, pretomanid, moxifloxacin, and pyrazinamide, for the treatment of DS-TB [22]. Bedaquiline is a diarylquinoline that targets the respiratory chain by inhibiting the activity of ATP synthase [76] and has sparked interest in the development of compounds targeting oxidative phosphorylation in mycobacteria.

2.2.8 Delamanid

Delamanid, similar to bedaquiline, received expedited approval from the US Food and Drug Administration (FDA) and can be included in an extended treatment program for MDR-TB [22]. Delamanid is a dihydro-nitroimidazooxazole derivative

and a pro-drug that targets mycolic acid synthesis in *M. tb*, specifically inhibiting the biosynthesis of keto- and methoxy-mycolic acids [77].

2.2.9 Linezolid

The oxazolidinone, linezolid, is a synthetic antibacterial licensed to treat skin and soft tissue infections, bacteremia, and pneumonia caused by DR-gram-positive organisms [78, 79]. Recently, linezolid has been used to treat MDR-TB and is currently being evaluated in different clinical trials to optimize dosing and combination therapies [22]. Linezolid has moderate early bactericidal activity and acts by inhibiting translation initiation by binding to rRNA on both the 30S and 50S ribosomal subunits [79].

2.2.10 Clofazimine

Clofazimine is a rhimophenazine with a broad-spectrum antibacterial activity used to treat leprosy [22, 75]. Clofazimine was originally developed to treat DS-TB; however, due to the adverse effect of skin discoloration, associated depression in patients, and poor treatment outcomes in trials, the use of clofazimine for DS-TB was abandoned [75]. Due to the increasing emergence of drug resistance, clofazimine was reconsidered and is currently available for the treatment of XDR-TB [70]. In addition, clofazimine is being tested in phase II clinical trials to develop new and/or shortened regimens for the treatment of MDR-TB [22]. Clofazimine is thought to exert its antibacterial effect by more than one mechanism, including the production of reactive oxygen species (ROS) during its reduction by NADH dehydrogenase and associated spontaneous oxidation by oxygen and by inhibition of K^+ transporters due to membrane disruption [75, 80].

2.2.11 Augmentin

Augmentin is a combination of the β-lactam, amoxicillin, and the β-lactamase inhibitor clavulanate and is a broad-spectrum antibiotic commonly used for the treatment of a variety of bacterial infections. The drug targets the bacterial cell wall by disrupting the biosynthesis of peptidoglycan via inhibition of the penicillin-binding protein, DD-transpeptidase. Augmentin has been shown to lack early bactericidal activity in patients with TB and is restricted for use in the case of XDR-TB when the preferred options are not viable [67, 81].

2.2.12 Thioacetazone

In combination with isoniazid, Thioacetazone was widely used for the treatment of TB in the past but currently is only used for the treatment of XDR-TB when better-tolerated options cannot be used [67]. Thioacetazone is not recommended for HIV-positive patients due to more severe side effects and possibly death. Thioacetazone is a pro-drug and, like ethionamide, is activated by EthA and inhibits the cyclopropanation of mycolic acids in the cell wall [82].

3 Treatment in Adults

Shortly after the first anti-TB drug, streptomycin, began to be used in clinical practice, patients developed drug resistance during the treatment process. Resistance has also occurred after the use of other new anti-TB drugs. The reason for this phenomenon was the administration of only one medication at a time in the treatment regimen. It is now widely accepted that TB should never be treated with only one drug [83]. TB treatment requires the administration of anti-TB drug combinations to prevent the development of drug resistance or relapse of the disease. Instead of monotherapy, multidrug treatment has become a standard. However, not every combination of anti-TB agents has favorable results, as some of them might have an antagonistic effect when combined or may interfere with the antiretroviral drugs for HIV patients. According to the current WHO guidelines for TB treatment, all new TB patients (not treated previously for TB) with drug-susceptible pulmonary TB should receive the standard drug combination therapy consisting of isoniazid/rifampicin/pyrazinamide/ethambutol for two months (intensive phase) followed by a combination of isoniazid/rifampicin for four months (continuation phase) (Fig. 2) [84]. The internationally recommended daily doses (in mg/kg of body weight) of anti-TB drugs are following: isoniazid (5 mg/kg), rifampicin (10 mg/kg), pyrazinamide (25 mg/kg), ethambutol (15 mg/kg), and streptomycin (15 mg/kg). If anti-TB drugs are taken three times per week instead of daily usage, the recommended doses are following: isoniazid (10 mg/kg), rifampicin (10 mg/kg), pyrazinamide (35 mg/kg), ethambutol (30 mg/kg), and streptomycin (15 mg/kg) [67]. The daily administration of anti-TB drugs is suggested because the administration of drugs thrice-weekly throughout the TB therapy can raise the chance of developing DR strains of *M. tb* [85].

However, the importance of multidrug therapy carries one of the most common problems in TB treatment itself, which is the administration of a considerable number of medicines at once, often nine to 16 pills a day, for a TB patient. This can lead to the accidental or intentional omission of some tablets or, in general, problems with their ingestion, which ultimately results in a lower effective dose of the drugs and opens a space for developing *M. tb* resistant strains. Therefore, the WHO recommends administering fixed-dose combination tablets instead of separate drug formulations for the treatment of patients with DS-TB [84]. Fixed-dose medicines are the combinations of two anti-TB drugs (isoniazid + rifampicin or isoniazid + ethambutol), three anti-TB drugs (isoniazid + rifampicin + pyrazinamide or isoniazid + rifampicin + ethambutol), or four anti-TB drugs (isoniazid + rifampicin + pyrazinamide + ethambutol) in a single tablet. Administration of the fixed-dose formulation allows for reduction of the number of pills that need to be taken by the patient to only three or four ones daily during the entire period of the TB therapy, while the recommended dose per kg body weight of each drug is well balanced (Table 1) [86].

Table 1 Currently recommended fixed-dose combinations (FDCs) of anti-tuberculosis (TB) drugs for the treatment of drug-susceptible TB in HIV negative patients [105]

	Administration	Body weight	FDCs (mg)	Tablets	Period
Children					
	Daily	≤ 7 kg	Rifampicin/Isoniazid/Pyrazinamide (60/30/150)[a]	1	2 months
			Rifampicin/Isoniazid (60/30)[a]	1	4 months
		8–9 kg	Rifampicin/Isoniazid/Pyrazinamide (60/30/150)[a]	1.5	2 months
			Rifampicin/Isoniazid (60/30)[a]	1.5	4 months
		10–14 kg	Rifampicin/Isoniazid/Pyrazinamide (60/30/150)[a]	2	2 months
			Rifampicin/Isoniazid (60/30)[a]	2	4 months
		15–19 kg	Rifampicin/Isoniazid/Pyrazinamide (60/30/150)[a]	3	2 months
			Rifampicin/Isoniazid (60/30)[a]	3	4 months
		20–29 kg	Rifampicin/Isoniazid/Pyrazinamide/Ethambutol (150/75/400/275)	2	2 months
			Rifampicin/Isoniazid (150/75)	2	4 months
	Thrice-weekly	≤ 7 kg	Rifampicin/Isoniazid/Pyrazinamide (60/30/150)*	1	2 months
			Rifampicin/Isoniazid (60/60 mg)[a]	1	4 months
		8–9 kg	Rifampicin/Isoniazid/Pyrazinamide (60/30/150)*	1.5	2 months
			Rifampicin/Isoniazid (60/60)[a]	1.5	4 months
		10–14 kg	Rifampicin/Isoniazid/Pyrazinamide (60/30/150)[a]	2	2 months
			Rifampicin/Isoniazid (60/60)[a]	2	4 months
		15–19 kg	Rifampicin/Isoniazid/Pyrazinamide (60/30/150)[a]	3	2 months
			Rifampicin/Isoniazid (60/60)[a]	3	4 months
Adults					
	Daily[b]	30–37 kg	Rifampicin/Isoniazid/Pyrazinamide/Ethambutol (150/75/400/275)	2	2 months
			Rifampicin/Isoniazid (150/75)	2	4 months
		38–54 kg	Rifampicin/Isoniazid/Pyrazinamide/Ethambutol (150/75/400/275)	3	2 months
			Rifampicin/Isoniazid (150/75)	3	4 months

(continued)

Table 1 (continued)

	Administration	Body weight	FDCs (mg)	Tablets	Period
		55–70 kg	Rifampicin/Isoniazid/Pyrazinamide/Ethambutol (150/75/400/275)	4	2 months
			Rifampicin/Isoniazid (150/75)	4	4 months
		≥ 71 kg	Rifampicin/Isoniazid/Pyrazinamide/Ethambutol (150/75/400/275)	5	2 months
			Rifampicin/Isoniazid (150/75)	5	4 months
	Thrice-weekly	30–37 kg	Rifampicin/Isoniazid/Pyrazinamide (150/150/500)	2	2 months
			Rifampicin/Isoniazid (150/150)	2	4 months
		38–54 kg	Rifampicin/Isoniazid/Pyrazinamide (150/150/500)	3	2 months
			Rifampicin/Isoniazid (150/150)	3	4 months
		55–70 kg	Rifampicin/Isoniazid/Pyrazinamide (150/150/500)	4	2 months
			Rifampicin/Isoniazid (150/150)	4	4 months
		≥ 71 kg	Rifampicin/Isoniazid/Pyrazinamide (150/150/500)	5	2 months
			Rifampicin/Isoniazid (150/150)	5	4 months

[a]dispersible tablet for pediatric TB
[b]if ethambutol is used in a single-drug form, FDCs contains Rifampicin/Isoniazid/Pyrazinamide (150/75/400 mg)

The most significant side effects of the first-line anti-TB drugs include skin rash with or without itching (streptomycin, isoniazid, rifampicin, pyrazinamide); deafness (streptomycin); vertigo and nystagmus (streptomycin); hepatitis (isoniazid, pyrazinamide, rifampicin); confusion caused by acute liver failure (most anti-TB drugs); visual impairment (Ethambutol); shock, purpura and acute renal failure (rifampicin) or decreased urine output (streptomycin). If patients with DS-TB develop serious side effects, the drug administration is stopped, and the clinician decides on the next course of the treatment. All patients with severe side effects should be treated in the hospital [67].

Apart from the effective anti-TB drugs, their drug combination, and a sufficient treatment period, the successful treatment of active pulmonary DS-TB requires the proper management of the entire treatment process. The response to TB therapy should be monitored for every TB patient. The continuous monitoring of TB treatment enables primary caregivers to identify and manage the possible adverse reactions of the drugs and evaluate the disease progression and patient's response to the medicines. To prevent administration errors, omission of some of the drugs, or interruption of the treatment by patients, DOT is advised by the WHO. Indeed, the implementation of DOT allowed achieving significant progress in global TB control [67]. If possible, the community-based or home-based DOT is recommended by the WHO; however, DOT should be managed by a healthcare worker or a trained community member rather than by a family member [84]. All medications given to the patient, adverse reactions, bacteriological tests, and all changes during the treatment procedure should be documented [87].

The remission or progression of the disease is monitored mainly by sputum microscopy. Patients should be examined for sputum smear microscopy before starting drug therapy and during the treatment, specifically at the end of the second month of treatment, the end of the fifth month, and the end of the sixth month. Smear microscopy confirms or refutes the presence of *M. tb* and provides additional information on the bacillary load during the monitoring of the treatment response, thus helping to direct further treatment. The patient's body weight is also a useful indicator of the treatment progress and should be monitored every month. When the patient's body weight has changed, the doses of the individual drugs are adjusted accordingly.

WHO has classified up to seven different treatment outcomes for TB therapy (Fig. 1) [67]. One of the recurrent events during TB treatment is the discontinuation of prescription medication by the patient mainly due to the side effects that become intolerable. When treatment discontinuation lasts for two or more months, the WHO refers to this treatment outcome as a *default*. If the patient is taking the prescribed medication but is smear or culture positive in the fifth or sixth month of the treatment, this outcome is referred to as *treatment failure*. This also includes the detection of any resistance to the prescribed anti-TB drugs at any time during the patient's treatment. Drug susceptibility testing (DST) is necessary to confirm and specify the acquired drug resistance in this scenario. On the other hand, *treatment success* is attributed to the patient who has completed the whole treatment regimen and is not smear or culture positive in the last month of the regimen [67].

Patients who have not completed treatment (*default*) or patients with relapse of the disease may undergo a *retreatment regimen*, in which streptomycin is added to the drug combination. The retreatment regimen consists of a two-month isoniazid/rifampicin/pyrazinamide/ethambutol/streptomycin combination, followed by a one-month isoniazid/rifampicin/pyrazinamide/ethambutol combination and a continued five-month isoniazid/rifampicin/ethambutol combination. If the patient is diagnosed with a DR form of TB during the retreatment regimen, patients switch to the MDR-TB treatment regimen [67].

4 Treatment in Children

Children are a vulnerable group of TB patients. Approximately 1.1 million new TB infections (11% of total new TB cases) and 14% of TB deaths in HIV-negative and 13% in HIV-positive TB cases are attributed to children (aged < 15 years) [22]. However, children can be protected from TB by vaccination with the Bacillus Calmette–Guérin (BCG) vaccine.

If children are diagnosed with active DS-TB, the treatment consists of administering the standard first-line anti-TB drugs isoniazid, rifampicin, pyrazinamide, and ethambutol. The treatment of TB in children is thus not fundamentally different from the treatment in adults, although there are a few exceptions. In the treatment of pediatric TB, it is necessary to modify the drug doses due to the different pharmacokinetics in the young organism. Specifically, higher doses are administered in children to reach the serum drug concentrations equal to adults. Therefore, the WHO recommends the daily doses (in mg/kg body weight) of the first-line anti-TB drugs for treatment of TB in children as follows: isoniazid (10 mg/kg), rifampicin (15 mg/kg), pyrazinamide (35 mg/kg), and ethambutol (20 mg/kg) [88, 89]. Importantly, it has been established that higher doses of first-line anti-TB drugs for pediatric TB do not increase the risk of anti-TB drug-induced hepatotoxicity [90, 91]. The anti-TB drug combination and the length of the therapy (intensive and continuation phase) for children may differ depending on their living settings (HIV and MDR/XDR-TB prevalence in the country) (Box 1) [89].

Box 1: Treatment of Tuberculosis (TB) in Children [89]
Treatment regimen for TB in children depends on the patient's HIV status, local drug resistance status and type, or severity of the disease:

Isoniazid/Rifampicin/Pyrazinamide (2 months), Isoniazid/Rifampicin (4 months).

- for children in low HIV prevalence and low isoniazid resistance settings with smear-negative pulmonary TB, intrathoracic lymph node TB, or tuberculous peripheral lymphadenitis

Isoniazid/Rifampicin/Pyrazinamide/Ethambutol (2 months), Isoniazid/Rifampicin (4 months).

- for children in low HIV prevalence and low isoniazid resistance settings with extensive pulmonary disease, smear-positive pulmonary TB, or severe forms of extrapulmonary TB (other than tuberculous meningitis/osteoarticular TB).
- for children in high HIV prevalence or high isoniazid resistance settings or both, with smear-positive pulmonary TB, smear-negative pulmonary TB with or without extensive parenchymal disease, or with all forms of extrapulmonary TB except tuberculous meningitis and osteoarticular TB.

Isoniazid/Rifampicin/Pyrazinamide/Ethambutol (2 months), Isoniazid/Rifampicin (10 months).

- for children with tuberculous meningitis and osteoarticular TB, regardless of the local HIV or isoniazid resistance settings.

DS-TB in children is thus treated with the same first-line anti-TB drugs as TB in adults, except for streptomycin. Streptomycin should not be used to treat pediatric TB due to the higher risk of toxicity in children and problems associated with injection-based drug regimens for children [88]. The daily administration of anti-TB drugs is recommended; however, children without HIV infection living in settings with well-established DOT may receive drugs thrice weekly during the continuation phase [88]. Generally, the adverse effects of TB treatment are less common in children than adult TB patients. Hepatotoxicity is the most severe adverse effect in children treated with first-line anti-TB drugs isoniazid, rifampicin, and pyrazinamide. Ethambutol administration in children may cause the impairment of vision due to optic neuritis. When detected early, the ethambutol side effects are reversible after stopping treatment. The ocular toxicity in children caused by ethambutol is very rare and often dose-dependent [92]. Isoniazid may also cause neurological problems associated with pyridoxine deficiency, especially among malnourished children; however, pyridoxine supplementation can compensate this [89]. The available fixed-dose combinations of anti-TB drugs and their recommended doses for children are summarized in Table 1. The dispersible tablet should be the preferable choice in pediatric TB treatment.

5 Treatment in HIV-Positive Patients

Co-infection with HIV accounted for ~ 5% of TB cases globally in 2018, with ~ 71% of co-infections found in Africa alone [22]. The efficient treatment of TB in HIV-positive individuals is crucial in eradicating TB. Several factors affect

the treatment of TB in HIV-positive individuals, with the potential for drug interactions with antiretroviral therapies (ART) and the possibility of developing immune reconstitution inflammatory syndrome (IRIS) being two of the most important. The WHO guidelines state that all new TB patients with a known HIV-positive status are to be on ART, regardless of their CD4 count [67]. The drug combination, dosage, and duration for HIV-positive individuals are the same as for HIV-negative individuals, as described above [67]. However, intermittent dosing via a thrice-weekly administration during the intensive phase is not an option for HIV-positive patients, as it has been shown to increase the incidence of relapse, treatment failure, and the risk of acquiring rifampicin resistance [84].

In order to minimize the risk of developing TB-IRIS, it is recommended that TB treatment be started before ART in patients that are not already receiving treatment for HIV [67]. Furthermore, ART initiation should be within eight weeks of the start of TB treatment, but in patients with a CD4 count below 50 cells/mm^3, this should be shortened to within two weeks [84]. Regardless of CD4 count, ART initiation in patients with TB meningitis is not recommended within eight weeks of the start of TB treatment [58, 93]. The ART regimen recommended by the WHO for TB patients should include first-line HIV drugs made up of two nucleoside reverse transcriptase inhibitors and one non-nucleoside reverse transcriptase inhibitor (NNRTI) [67]. Additional considerations need to be made for new TB patients that are on ART, such as modification of the regimen to reduce drug interactions or increased side effects due to similar toxicities and evaluation of whether the new TB disease is a result of activation of latent TB infection, and thus represents ART treatment failure [67]. In order to prevent infection with opportunistic bacterial pathogens, the WHO recommends that all HIV-positive TB patients begin a prophylactic course of cotrimoxazole [67, 93].

TB-IRIS is an acute inflammatory response to *M. tb* antigens. It occurs in patients receiving TB treatment upon initiation of ART (paradoxical) or in HIV-positive patients with undiagnosed TB who start ART (unmasked) [94]. The treatment of TB in patients who develop IRIS is not modified or stopped in order to reduce the possibility of DR. However, due to the difficulty in distinguishing between clinical symptoms caused by TB-IRIS and by MDR-TB, DR must be ruled out as soon as possible in patients experiencing worsening symptoms while on treatment for both TB and HIV [93, 94]. At present, only corticosteroids such as prednisone are promising for managing TB-IRIS symptoms [95].

Rifamycins display clinically significant drug-drug interactions with several classes of drugs due to the induction of metabolic pathways that metabolize drugs, e.g., the cytochrome P450 system. Rifamycins cause a decrease in serum concentrations of NNRTI's, integrase strand transfer inhibitors (INSTI's), and protease inhibitors used for the treatment of HIV [58, 67]. Dependent on the HIV treatment cocktail, the type of rifamycin used should be altered. Rifampicin is not recommended for use with protease inhibitors, and a 150 mg daily dose of rifabutin is recommended instead [58]. While rifampicin can be used with non-nucleoside reverse transcriptase inhibitors, it does decrease their exposure and thus requires higher doses of the NNRTI's. An exception is the NNRTI efavirenz, which requires

higher doses of rifabutin and is therefore preferably used in conjunction with rifampicin. Similarly, the dose of INSTI's should be increased when administered with rifampicin, or rifabutin can be used [58].

6 Treatment of Latent Infections

Latent TB infection (LTBI) is a persistent immune response to stimulation with *M. tb* antigens in the absence of clinical TB symptoms [96]. Estimates indicate that ~ 1.75 billion people harbor LTBI globally, with a 5–10% probability of developing active disease in their lifetime [22]. In order to meet the goal set by the United Nations of obtaining an 80% reduction in TB incidence by 2035, the treatment of LTBI has become of utmost importance. Currently, the only diagnostic tests available for LTBI are the tuberculin skin test (TST) and interferon-gamma release assay (IGRA), but neither of these is routinely performed for active case finding, and both have disadvantages, e.g., false-positive TST results due to previous BCG vaccination and the high cost of IGRAs. Therefore, the WHO has identified high-risk groups that should be prioritized for the treatment of LTBI [96]. These groups are adults and adolescents living with HIV, infants and children living with HIV, household contacts of pulmonary TB, contacts of MDR-TB patients, and individuals initiating anti-TNF treatment, receiving dialysis, preparing for organ/hematological transplant or who have silicosis [96].

The most widely used treatment for LTBI has been isoniazid preventative therapy (IPT), which consists of the daily administration of 5 mg/kg of INH for six or nine months with the recommendation to supplement with 25 mg Vitamin B6 (pyridoxine) in patients more susceptible to developing peripheral neuropathy [97]. The WHO has recently updated the recommended guidelines to include additional regimens for LTBI treatment. In addition to IPT and based on the outcomes from several clinical trials, the available options for the treatment of LTBI in adults include the following:

- daily rifampicin at 10 mg/kg/day for four months;
- daily rifampicin and isoniazid for three months;
- weekly isoniazid and rifapentine for three months; and
- rifapentine (600 mg) and isoniazid (300 mg) daily for one month.

The regimens listed above are also recommended for children over two years old but with slight adjustments to doses [96]. Except for combinations with rifapentine, the treatment plans listed above are also applicable for pregnant women, but it is encouraged that liver function tests be performed where feasible to monitor possible toxicity [96]. Special consideration is provided for LTBI treatment in HIV-positive individuals located in high-TB transmission environments, e.g., mines or prisons. In these cases, 36 months of daily isoniazid monotherapy is conditionally recommended, but not in those with a negative TST [96]. When choosing which regimen to prescribe, clinicians need to evaluate several factors, including patient age, drug

interactions, existing comorbidities, availability of the drugs, and patient preference. Prevailing evidence points to improved adherence with shorter regimens and, if possible, should be the preferred option [96, 97]. Interestingly, it has been observed that socio-economic factors significantly contribute to LTBI treatment outcomes, e.g., immigrant status, distance to the health care facility, alcohol and drug abuse, social stigma, unemployment, and history of incarceration [97]. These observations highlight the importance of taking a holistic approach to the treatment of TB that considers the clinical practice and the social situations of individuals.

7 Factors Associated with Treatment Outcome

The main objectives of TB treatment include to:

i. rapidly reduce the bacterial load in order to lessen disease severity, prevent death and stifle transmission;
ii. obtain complete bacterial sterilization, including persister populations, in order to prevent relapse; and
iii. prevent the development of antibiotic resistance during treatment [58, 98]

Unfortunately, due to the complexity of the disease as well as the requirement of efficient collaboration during treatment, it is likely that one of these objectives will not be met and thus result in treatment failure. Although the treatment success rate has increased from 81% in 2016 to 85% in 2017, based on the estimated number of TB cases, $\sim$ 1.5 million people failed treatment in 2018 alone [22]. Including the socio-economic factors mentioned above for LTBI treatment, other patient-associated factors which contribute to treatment outcome include age, immunological competence, nutritional status, tobacco smoking, the presence of comorbidities, such as diabetes, and genetic factors, e.g., genetic features associated with drug absorption and metabolism [58, 98]. Advanced age has been associated with unsuccessful treatment outcomes in different populations [99, 100].

Pathological features associated with relapse include extensive disease due to an increased number and/or size of cavities [58]. Similarly, an increased baseline colony count and slow culture conversion are also associated with relapse. Pathogen-specific characteristics that contribute to therapy outcome include drug tolerance, susceptibility to the drugs in the regimen, and the strain genotype, with the Beijing strain being associated with unfavorable outcomes [101]. Patient adherence largely dictates the outcome of a treatment regimen, and the healthcare system should be designed to promote this. To this end, the implementation of DOT has improved patient adherence. However, health facility-based DOT poses challenges for patients, e.g., having access to transport, loss of income due to missed work, and physical hardship. Therefore, it is recommended that a flexible combination of health facility- and community-based DOT be available to help ensure patient adherence [98]. As mentioned above, the large number of pills taken at a time and adverse drug reactions also contribute to patient adherence and outcome.

It is now accepted that adherence should not be placed solely on the patient. Interventions that are predicted to improve adherence include educating communities about the disease and available treatment programs, minimizing costs and unpleasant environments at clinics, educating patients and involving them in the decision-making process regarding treatment design and side-effects, providing support in the form of food or travel vouchers or monetary compensation in the form of grants during times of lost income and addressing employment policies to reduce job losses [98].

8 Conclusion

Although the treatment of DS-TB is readily available, the time taken to treat TB is frustrating for both the physicians and patients. This is fueled by the emergence of resistance from single-drug resistance to multidrug resistance, which is further extended to extensive drug resistance and, in some cases, total drug resistance where no available drug can kill the resistant pathogen. Currently, the key to TB therapy is the dissemination of knowledge about therapy and the associated risk of resistance to the patients and healthcare workers who might be involved in the treatment of TB. Although the WHO has DST for all TB patients to guide treatment decisions, improve treatment outcomes, and indirectly assist with drug resistance surveillance, resources availability is limited [102, 103]. Concerted efforts in improving the diagnosis of TB are vital to achieving the targets of the End TB Strategy. Additionally, significant efforts are underway to discover novel chemical classes and new drug targets to address the treatment shortening of DS-TB and DR-TB [104].

We are determined that we will overcome Tuberculosis.

Vinayak Singh, Nicole Cardoso, Stanislav Huszár

Core Messages

- DS-TB is curable.
- Standard TB treatment requires combination therapy for a total period of six months.
- TB treatment in children and adults is largely similar but differs in drug dosage.
- Steps are urgently needed to address the DR-TB problem regarding its treatment regimen, side effects, and duration.
- A patient-specific regimen is encouraged.

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References

1. Marshall G, Blacklock J, Cameron C, Capon N, Cruickshank R, Gaddum J et al (1948) Streptomycin treatment of pulmonary tuberculosis: a medical research council investigation. Br Med J 2(4582):769–782
2. Fox W, Sutherland I, Daniels M (1954) A five-year assessment of patients in a controlled trial of streptomycin in pulmonary tuberculosis. Quart J Med 23(91):347–366
3. Dooneief AS, Buchberg A, Steinbach MM (1950) Para-aminosalicylic acid (PAS) in chronic pulmonary tuberculosis. N Engl J Med 242(22):859–862
4. Marshall G, Crofton J, Cruickshank R, Daniels M, Geddes J, Heaf F et al (1952) The treatment of pulmonary tuberculosis with isoniazid. BMJ 2:735–746
5. Hudgins PC, Patnode RA, Cummings MM (1955) The effect of cycloserine on growing and resting tubercle bacilli. Am Rev Tuberc Pulmonary Dis 72(5):685–686
6. Bartz QR, Ehrlich J, Mold JD, Penner MA, Smith RM (1951) Viomycin, a new tuberculostatic antibiotic. Am Rev Tuberc 63(1):4–6
7. Patnode R, Hudgins P (1958) Effect of kanamycin on Mycobacterium tuberculosis in vitro. Am Rev Tuberc Pulmonary Diseases 78(1):138–139
8. Hutton P, Tonkin IM (1960) Ethionamide ('1314') with streptomycin in acute tuberculosis of recent origin in Uganda Africans: a pilot study. Tubercle 41(4):253–256
9. Kaida K, Sugiyama K (1959) Clinical experience with PZA-INH therapy: report on study of resected specimens following the above therapy in particular. CHEST 36(4):378–388
10. Centre TC (1959) A concurrent comparison of home and sanatorium treatment of pulmonary tuberculosis in South India. Bull World Health Organ 21(1):51
11. Fox W (1958) The problem of self-administration of drugs; with particular reference to pulmonary tuberculosis. Tubercle 39(5):269–274
12. Bienenstock J, Shaldon S (1963) Thiacetazone in tuberculosis. Lancet (London, England) 2 (7312):817
13. Cuthbert J, Bruce L (1964) Treatment of pulmonary tuberculosis by capreomycin and PAS: a small preliminary trial. Tubercle 45(3):205–210
14. Development GAfTD (2008) Clofazimine. Tuberculosis (Edinb) 88:96–99
15. Doster B, Murray FJ, Newman R, Woolpert SF (1973) Ethambutol in the initial treatment of pulmonary tuberculosis: US Public Health Service tuberculosis therapy trials. Am Rev Respir Dis 107(2):177–190
16. African E, Councils BMR (1974) Controlled clinical trial of four short-course (6-month) regimens of chemotherapy for treatment of pulmonary tuberculosis: third report. The Lancet 304(7875):237–240
17. Fisher L (1971) Rifampin–new and potent drug for TB treatment. Bull-Nat Tuberc Respir Dis Assoc 57(8):11–12
18. Grosset J (1978) The sterilizing value of rifampicin and pyrazinamide in experimental short-course chemotherapy. Bull Int Union Tuberc 53(1):5–12
19. Long-term follow-up of a clinical trial of six-month and four-month regimens of chemotherapy in the treatment of pulmonary tuberculosis. Singapore tuberculosis service/British medical research council (1986). Am Rev Respir Dis 133:779–783
20. Council STSBMR (1979) Clinical trial of six-month and four-month regimens of chemotherapy in the treatment of pulmonary tuberculosis. Am Rev Respir Dis 119 (4):579–585

21. East, Study CABMRCFC (1986) Controlled clinical trial of 4 short-course regimens of chemotherapy (three 6-month and one 8-month) for pulmonary tuberculosis. Tubercle 67 (1):5–15
22. WHO (2019) Global Tuberculosis Report 2019
23. Banerjee A, Dubnau E, Quemard A, Balasubramanian V, Um KS, Wilson T et al (1994) inhA, a gene encoding a target for isoniazid and ethionamide in Mycobacterium tuberculosis. Science 263(5144):227–230
24. Dessen A, Quemard A, Blanchard JS, Jacobs WR, Sacchettini JC (1995) Crystal structure and function of the isoniazid target of Mycobacterium tuberculosis. Science 267 (5204):1638–1641
25. Marrakchi H, Lanéelle G, Ak Q (2000) InhA, a target of the antituberculous drug isoniazid, is involved in a mycobacterial fatty acid elongation system FAS-II. Microbiology 146 (2):289–296
26. Zhang Y, Heym B, Allen B, Young D, Cole S (1992) The catalase—peroxidase gene and isoniazid resistance of Mycobacterium tuberculosis. Nature 358(6387):591–593
27. Sensi P (1983) History of the development of rifampin. Rev Infect Dis 5(Supplement_3): S402-S406
28. Margalith P, Beretta G (1960) Rifomycin. XI. taxonomic study on streptomyces mediterranei nov. sp. Mycopathologia Mycologia Applicata 13(4):321–330
29. Sensi P, Greco A, Ballotta R (1959) Rifomycin. I. Isolation and properties of rifomycin B and rifomycin complex. Antibiot Annu 7:262
30. Hartmann G (1967) The specific inhibition of DNA-directed RNA synthesis by rifamycin. Biochem Biophys Acta 145:843–844
31. White R, Lancini G, Silvestri L (1971) Mechanism of action of rifampin on Mycobacterium smegmatis. J Bacteriol 108(2):737–741
32. Campbell EA, Korzheva N, Mustaev A, Murakami K, Nair S, Goldfarb A et al (2001) Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. Cell 104 (6):901–912
33. Telenti A, Imboden P, Marchesi F, Matter L, Schopfer K, Bodmer T et al (1993) Detection of rifampicin-resistance mutations in Mycobacterium tuberculosis. The Lancet 341 (8846):647–651
34. McNeil MB, Chettiar S, Awasthi D, Parish T (2019) Cell wall inhibitors increase the accumulation of rifampicin in Mycobacterium tuberculosis. Access Microbiology 1(1): e000006
35. Scorpio A, Zhang Y (1996) Mutations in pncA, a gene encoding pyrazinamidase/ nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. Nat Med 2(6):662–667
36. Zhang Y, Scorpio A, Nikaido H, Sun Z (1999) Role of acid pH and deficient efflux of pyrazinoic acid in unique susceptibility of Mycobacterium tuberculosis to pyrazinamide. J Bacteriol 181(7):2044–2049
37. Zimhony O, Cox JS, Welch JT, Vilchèze C, Jacobs WR (2000) Pyrazinamide inhibits the eukaryotic-like fatty acid synthetase I (FASI) of Mycobacterium tuberculosis. Nat Med 6 (9):1043–1047
38. Shi W, Zhang X, Jiang X, Yuan H, Lee JS, Barry CE et al (2011) Pyrazinamide inhibits trans-translation in Mycobacterium tuberculosis. Science 333(6049):1630–1632
39. Zhang Y, Wade MM, Scorpio A, Zhang H, Sun Z (2003) Mode of action of pyrazinamide: disruption of Mycobacterium tuberculosis membrane transport and energetics by pyrazinoic acid. J Antimicrob Chemother 52(5):790–795
40. Via LE, Savic R, Weiner DM, Zimmerman MD, Prideaux B, Irwin SM et al (2015) Host-mediated bioactivation of pyrazinamide: implications for efficacy, resistance, and therapeutic alternatives. ACS Infect Dis 1(5):203–214

41. Council STSBMR (1985) Clinical trial of three 6-month regimens of chemotherapy given intermittently in the continuation phase in the treatment of pulmonary tuberculosis. Am Rev Respir Dis 132(2):374–378
42. Gopal P, Sarathy JP, Yee M, Ragunathan P, Shin J, Bhushan S et al (2020) Pyrazinamide triggers degradation of its target aspartate decarboxylase. Nat Commun 11(1):1661. https://doi.org/10.1038/s41467-020-15516-1
43. Thomas J, Baughn C, Wilkinson R, Shepherd R (1961) A new synthetic compound with antituberculous activity in mice: Ethambutol (dextro-2, 2′-(ethylenediimino)-di-1-butanol). Am Rev Respir Dis 83(6):891–893
44. Deng L, Mikusová K, Robuck KG, Scherman M, Brennan PJ, McNeil MR (1995) Recognition of multiple effects of ethambutol on metabolism of mycobacterial cell envelope. Antimicrob Agents Chemother 39(3):694–701
45. Kilburn JO, Takayama K, Armstrong EL, Greenberg J (1981) Effects of Ethambutol on phospholipid metabolism in Mycobacterium smegmatis. Antimicrob Agents Chemother 19 (2):346–348
46. Mikusova K, Slayden RA, Besra GS, Brennan PJ (1995) Biogenesis of the mycobacterial cell wall and the site of action of ethambutol. Antimicrob Agents Chemother 39(11):2484–2489
47. Takayama K, Kilburn JO (1989) Inhibition of synthesis of arabinogalactan by Ethambutol in Mycobacterium smegmatis. Antimicrob Agents Chemother 33(9):1493–1499
48. Belanger AE, Besra GS, Ford ME, Mikusová K, Belisle JT, Brennan PJ et al (1996) The embAB genes of Mycobacterium avium encode an arabinosyl transferase involved in cell wall arabinan biosynthesis that is the target for the antimycobacterial drug ethambutol. Proc Natl Acad Sci 93(21):11919–11924
49. Goude R, Amin A, Chatterjee D, Parish T (2009) The arabinosyltransferase EmbC is inhibited by Ethambutol in Mycobacterium tuberculosis. Antimicrob Agents Chemother 53 (10):4138–4146
50. Wolucka BA, McNeil MR, de Hoffmann E, Chojnacki T, Brennan PJ (1994) Recognition of the lipid intermediate for arabinogalactan/arabinomannan biosynthesis and its relation to the mode of action of ethambutol on mycobacteria. J Biol Chem 269(37):23328–23335
51. Zhang L, Zhao Y, Gao Y, Wu L, Gao R, Zhang Q et al (2020) Structures of cell wall arabinosyltransferases with the anti-tuberculosis drug ethambutol. Science 368(6496):1211–1219
52. Schatz A, Bugle E, Waksman SA (1944) Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative Bacteria. Proc Soc Exp Biol Med 55 (1):66–69
53. Organization WH (2017) Guidelines for treatment of drug-susceptible tuberculosis and patient care
54. Lin Y, Li Y, Zhu N, Han Y, Jiang W, Wang Y et al (2014) The Antituberculosis antibiotic capreomycin inhibits protein synthesis by disrupting interaction between ribosomal proteins L12 and L10. Antimicrob Agents Chemother 58(4):2038–2044. https://doi.org/10.1128/aac.02394-13
55. Suzuki Y, Katsukawa C, Tamaru A, Abe C, Makino M, Mizuguchi Y et al (1998) Detection of Kanamycin-Resistant *Mycobacterium tuberculosis* by identifying mutations in the 16S rRNA Gene. J Clin Microbiol 36(5):1220–1225. https://doi.org/10.1128/jcm.36.5.1220-1225.1998
56. Correia S, Poeta P, Hebraud M, Capelo JL, Igrejas G (2017) Mechanisms of quinolone action and resistance: where do we stand? J Med Microbiol 66(5):551–559. https://doi.org/10.1099/jmm.0.000475
57. Aldred KJ, Blower TR, Kerns RJ, Berger JM, Osheroff N (2016) Fluoroquinolone interactions with Mycobacterium tuberculosis gyrase: Enhancing drug activity against wild-type and resistant gyrase. Proc Natl Acad Sci U S A 113(7):E839-846. https://doi.org/10.1073/pnas.1525055113

58. Nahid P, Dorman SE, Alipanah N, Barry PM, Brozek JL, Cattamanchi A et al (2016) Official American thoracic society/centers for disease control and prevention/infectious diseases society of America clinical practice guidelines: treatment of drug-susceptible tuberculosis. Clin Infect Dis 63(7):e147–e195. https://doi.org/10.1093/cid/ciw376
59. Wang F, Langley R, Gulten G, Dover LG, Besra GS, Jacobs WR Jr et al (2007) Mechanism of thioamide drug action against tuberculosis and leprosy. J Exp Med 204(1):73–78. https://doi.org/10.1084/jem.20062100
60. DeBarber AE, Mdluli K, Bosman M, Bekker L-G, Barry CE (2000) Ethionamide activation and sensitivity in multidrug-resistant *Mycobacterium tuberculosis*. Proc Natl Acad Sci 97 (17):9677–9682. https://doi.org/10.1073/pnas.97.17.9677
61. Scardigli A, Caminero JA, Sotgiu G, Centis R, D'Ambrosio L, Migliori GB (2016) Efficacy and tolerability of ethionamide versus prothionamide: a systematic review. Eur Respir J 48 (3):946–952. https://doi.org/10.1183/13993003.00438-2016
62. Batson S, de Chiara C, Majce V, Lloyd AJ, Gobec S, Rea D et al (2017) Inhibition of D-Ala: D-Ala ligase through a phosphorylated form of the antibiotic D-cycloserine. Nat Commun 8 (1):1939. https://doi.org/10.1038/s41467-017-02118-7
63. Hwang TJ, Wares DF, Jafarov A, Jakubowiak W, Nunn P, Keshavjee S (2013) Safety of cycloserine and terizidone for the treatment of drug-resistant tuberculosis: a meta-analysis. Int J Tuberc Lung Dis 17(10):1257–1266. https://doi.org/10.5588/ijtld.12.0863
64. Prosser GA, de Carvalho LP (2013) Kinetic mechanism and inhibition of Mycobacterium tuberculosis D-alanine:D-alanine ligase by the antibiotic D-cycloserine. FEBS J 280 (4):1150–1166. https://doi.org/10.1111/febs.12108
65. Evangelopoulos D, Prosser GA, Rodgers A, Dagg BM, Khatri B, Ho MM et al (2019) Comparative fitness analysis of D-cycloserine resistant mutants reveals both fitness-neutral and high-fitness cost genotypes. Nat Commun 10(1):4177. https://doi.org/10.1038/s41467-019-12074-z
66. Caminero JA, Sotgiu G, Zumla A, Migliori GB (2010) Best drug treatment for multidrug-resistant and extensively drug-resistant tuberculosis. Lancet Infect Dis 10 (9):621–629. https://doi.org/10.1016/S1473-3099(10)70139-0
67. WHO (2010) Treatment of tuberculosis: guidelines 4th edition
68. Zheng J, Rubin EJ, Bifani P, Mathys V, Lim V, Au M et al (2013) para-Aminosalicylic acid is a pro-drug targeting dihydrofolate reductase in Mycobacterium tuberculosis. J Biol Chem 288(32):23447–23456. https://doi.org/10.1074/jbc.M113.475798
69. Hajian B, Scocchera E, Shoen C, Krucinska J, Viswanathan K, G-Dayanandan N, et al (2019) Drugging the folate pathway in Mycobacterium tuberculosis: the role of multi-targeting agents. Cell Chem Biol 26(6):781-791.e786. https://doi.org/10.1016/j.chembiol.2019.02.013
70. WHO (2014) Companion handbook to the WHO guidlines for the programmatic management of drug-resistant tuberculosis
71. Bouton TC, de Vos M, Ragan EJ, White LF, Van Zyl L, Theron D et al (2019) Switching to bedaquiline for treatment of rifampicin-resistant tuberculosis in South Africa: a retrospective cohort analysis. PLoS ONE 14(10):e0223308. https://doi.org/10.1371/journal.pone.0223308
72. Ghajavand H, Kargarpour Kamakoli M, Khanipour S, Pourazar Dizaji S, Masoumi M, Rahimi Jamnani F et al (2019) High prevalence of bedaquiline resistance in treatment-naive tuberculosis patients and verapamil effectiveness. Antimicrob Agents Chemother 63(3). https://doi.org/10.1128/AAC.02530-18
73. Villellas C, Coeck N, Meehan CJ, Lounis N, de Jong B, Rigouts L et al (2017) Unexpected high prevalence of resistance-associated Rv0678 variants in MDR-TB patients without documented prior use of clofazimine or bedaquiline. J Antimicrob Chemother 72(3):684–690. https://doi.org/10.1093/jac/dkw502

74. Zimenkov DV, Nosova EY, Kulagina EV, Antonova OV, Arslanbaeva LR, Isakova AI et al (2017) Examination of bedaquiline- and linezolid-resistant Mycobacterium tuberculosis isolates from the Moscow region. J Antimicrob Chemother 72(7):1901–1906. https://doi.org/10.1093/jac/dkx094
75. Cholo MC, Mothiba MT, Fourie B, Anderson R (2017) Mechanisms of action and therapeutic efficacies of the lipophilic antimycobacterial agents clofazimine and bedaquiline. J Antimicrob Chemother 72(2):338–353. https://doi.org/10.1093/jac/dkw426
76. Andries K, Verhasselt P, Guillemont J, Gohlmann HW, Neefs JM, Winkler H et al (2005) A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. Science 307(5707):223–227. https://doi.org/10.1126/science.1106753
77. Liu Y, Matsumoto M, Ishida H, Ohguro K, Yoshitake M, Gupta R et al (2018) Delamanid: From discovery to its use for pulmonary multidrug-resistant tuberculosis (MDR-TB). Tuberculosis (Edinb) 111:20–30. https://doi.org/10.1016/j.tube.2018.04.008
78. Dietze R, Hadad DJ, McGee B, Molino LP, Maciel EL, Peloquin CA et al (2008) Early and extended early bactericidal activity of linezolid in pulmonary tuberculosis. Am J Respir Crit Care Med 178(11):1180–1185. https://doi.org/10.1164/rccm.200806-892OC
79. Hashemian SMR, Farhadi T, Ganjparvar M (2018) Linezolid: a review of its properties, function, and use in critical care. Drug Des Devel Ther 12:1759–1767. https://doi.org/10.2147/DDDT.S164515
80. Yano T, Kassovska-Bratinova S, Teh JS, Winkler J, Sullivan K, Isaacs A et al (2011) Reduction of clofazimine by mycobacterial type 2 NADH:quinone oxidoreductase: a pathway for the generation of bactericidal levels of reactive oxygen species. J Biol Chem 286(12):10276–10287. https://doi.org/10.1074/jbc.M110.200501
81. Donald PR, Sirgel FA, Venter A, Parkin DP, Van de Wal BW, Barendse A et al (2001) Early bactericidal activity of amoxicillin in combination with clavulanic acid in patients with sputum smear-positive pulmonary tuberculosis. Scand J Infect Dis 33(6):466–469. https://doi.org/10.1080/00365540152029954
82. Alahari A, Trivelli X, Guerardel Y, Dover LG, Besra GS, Sacchettini JC et al (2007) Thiacetazone, an antitubercular drug that inhibits cyclopropanation of cell wall mycolic acids in mycobacteria. PLoS ONE 2(12):e1343. https://doi.org/10.1371/journal.pone.0001343
83. Toman K (2004) Toman's Tuberculosis: case detection, treatment, and monitoring: questions and answers. World Health Organ
84. WHO (2017) Treatment of Tuberculosis Guidelines for treatment of drug-susceptible tuberculosis and patient care 2017 update
85. Menzies D, Benedetti A, Paydar A, Martin I, Royce S, Pai M et al (2009) Effect of duration and intermittency of rifampin on tuberculosis treatment outcomes: a systematic review and meta-analysis. PLoS Med 6(9):e1000146
86. Blomberg B, Spinaci S, Fourie B, Laing R (2001) The rationale for recommending fixed-dose combination tablets for treatment of tuberculosis. Bull World Health Organ 79:61–68
87. Hopewell PC, Pai M, Maher D, Uplekar M, Raviglione MC (2006) International standards for tuberculosis care. Lancet Infect Dis 6(11):710–725
88. WHO (2012) Rapid advice: treatment of tuberculosis in children 2010. WHO, Geneva
89. WHO (2014) Guidance for national tuberculosis programmes on the management of tuberculosis in children. World Health Organ
90. Donald PR (2011) Antituberculosis drug-induced hepatotoxicity in children. Pediatr Rep 3 (2)
91. Organization WH (2006) Ethambutol efficacy and toxicity: literature review and recommendations for daily and intermittent dosage in children. World Health Organ
92. Graham S, Daley H, Banerjee A, Salaniponi F, Harries A (1998) Ethambutol in tuberculosis: time to reconsider? Arch Dis Child 79(3):274–278
93. WHO (2007) Tuberculosis care with TB-HIV co-management: Integrated management of adolescent and adult illness

94. Meintjes G, Sonderup MW (2011) A practical approach to the diagnosis and management of paradoxical tuberculosis immune reconstitution inflammatory syndrome. 29(10)
95. Walker NF, Stek C, Wasserman S, Wilkinson RJ, Meintjes G (2018) The tuberculosis-associated immune reconstitution inflammatory syndrome: recent advances in clinical and pathogenesis research. Curr Opin HIV AIDS 13(6):512–521. https://doi.org/10.1097/COH.0000000000000502
96. WHO (2020) WHO consolidated guidlelines on tuberculosis. Module 1: prevention-tuberculosis preventive treatment
97. WHO (2015) Guidelines on the management of latent tuberculosis infection
98. I TC (2014) International standards for tuberculosis care. In: I TC (ed)
99. Tola A, Minshore KM, Ayele Y, Mekuria AN (2019) Tuberculosis treatment outcomes and associated factors among TB Patients Attending Public Hospitals in Harar Town, Eastern Ethiopia: a five-year retrospective study. Tuberc Res Treat 2019:1503219. https://doi.org/10.1155/2019/1503219
100. Wen Y, Zhang Z, Li X, Xia D, Ma J, Dong Y et al (2018) Treatment outcomes and factors affecting unsuccessful outcome among new pulmonary smear positive and negative tuberculosis patients in Anqing, China: a retrospective study. BMC Infect Dis 18(1):104. https://doi.org/10.1186/s12879-018-3019-7
101. Liu Q, Wang D, Martinez L, Lu P, Zhu L, Lu W et al (2020) Mycobacterium tuberculosis Beijing genotype strains and unfavourable treatment outcomes: a systematic review and meta-analysis. Clin Microbiol Infect 26(2):180–188. https://doi.org/10.1016/j.cmi.2019.07.016
102. Schon T, Miotto P, Koser CU, Viveiros M, Bottger E, Cambau E (2017) Mycobacterium tuberculosis drug-resistance testing: challenges, recent developments and perspectives. Clin Microbiol Infect 23(3):154–160. https://doi.org/10.1016/j.cmi.2016.10.022
103. WHO (2014) The end TB strategy: global strategy and targets for tuberculosis prevention, care and control after 2015. World Health Organization, Geneva
104. Huszár S, Chibale K, Singh V (2020) The quest for the holy grail: new antitubercular chemical entities, targets and strategies. Drug Discovery Today 25(4):772–780
105. WHO (2003) Stop TB Initiative & Stop TB Partnership. Global Drug Facility. Frequently asked questions about the 4-drug fixed-dose combination tablet recommended by the World Health Organization for treating tuberculosis

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Nicole Cardoso is a molecular- and micro-biologist with several years of experience in mycobacterial research. She obtained her Ph.D. from the University of the Witwatersrand in 2018 at the Centre of Excellence for Biomedical TB Research (CBTBR). Her research focused on the electron transport chain to identify novel drug targets and specifically investigate the role of the cytochrome bd oxidase in adaptation to stress, growth, and survival of mycobacteria. She remained at the CBTBR for a Postdoctoral Fellowship and continued her research on respiration and nitrogen metabolism in mycobacteria. Currently, Nicole is a part of the Drug Discovery and Development Centre (H3D) TB Biology team as a senior research scientist. She is involved in the different TB drug discovery screening projects and determining the mechanism of action of promising compounds.

Stanislav Huszár is a biochemist with more than ten years of research expertise focused on *Mycobacterium tuberculosis*. He obtained a Ph.D. (2016) in Biochemistry from the Faculty of Natural Sciences, Comenius University in Bratislava, Slovakia. After finishing the Ph.D., he continued his postdoctoral research in the same laboratory on the various projects centered on the mycobacterial cell wall assembly and identification of the mechanism of action of novel antimycobacterial agents. In 2019 he joined the H3D TB Biology unit as a postdoctoral fellow (2019–2020). He currently works as an Assistant Professor/lecturer at the Department of Biochemistry, Faculty of Natural Sciences, Comenius University in Bratislava, where he is interested in the biochemistry of mycobacteria.

14 The Pharmacokinetic and Pharmacodynamic Properties of Antitubercular Medications

Ashlan J. Kunz Coyne, Anthony M. Casapao, and Eric F. Egelund

The thoughtless person playing with penicillin treatment is morally responsible for the death of the man who succumbs to infection with the penicillin-resistant organism.

Sir Alexander Fleming

Summary

Pharmacokinetics and pharmacodynamics operate in a partnership that can be used to maximize a drug's potential for increasing treatment efficacy, reducing side effects, and minimizing drug interactions; maximizing these parameters will help decide on an optimal dose, ideal administration timing, and an appropriate frequency of the drug. We reviewed individual medications used in active tuberculosis (TB), the pharmacokinetic parameters of relevance, and the association of pharmacokinetics with pharmacodynamics with treatment efficacy. First-line agents, including rifamycins, isoniazid, pyrazinamide, and ethambutol, were evaluated on how to optimize their use. First-line agents highlight the effectiveness of eradicating active TB by providing an effective dose from various pharmacokinetic studies. Second-line antibiotics include moxifloxacin, amikacin,

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cycloserine, bedaquiline, and delamanid. The unique pharmacokinetic properties of each agent were explored. An overview of the standard doses is featured in this review, along with the pharmacokinetic parameter associated with efficacy in addition to therapeutic drug monitoring considerations. One of the factors that clinicians can manipulate in managing TB is the pharmacokinetic parameters via dosing changes, food administration, management of drug interactions, and adherence through directly observed therapy.

Graphical Abstract

Common keywords related to pharmacokinetic and pharmacodynamic properties of anti-tuberculosis (TB) medications

Keywords

Pharmacodynamics · Pharmacokinetics · Tuberculosis

1 Introduction

The interplay between the patient, drug, and infectious organism is complex. Physiochemical properties dictate a drug's pharmacokinetics (PK) and pharmacodynamics (PD). Knowledge of PK, defined as the study of the absorption, distribution, metabolism, and elimination of medications, combined with PD, defined as the study of the effects a drug has on the body, is essential to optimizing drug treatment, to increase efficacy, reduce both short- and long-term side effects, and minimize drug interactions. Specifically, we attempt to maximize parameters to

decide on an optimal dose, the ideal timing of that dose, and how often the dose should be given. Numerous factors can affect a drug's PK parameters: the presence or absence of food, gastric function (e.g., pH), liver metabolism (e.g., cytochrome enzymes, transporters), and drug-drug interactions, among others. In an ideal world, we focus on individualized approaches that incorporate patient factors, drug properties, and microbe characteristics to maximize patient care and improve outcomes. Herein, we discuss the individual medications used in active tuberculosis (TB) treatment, PK parameters of relevance, and the associated PK parameters in conjunction with PD with treatment efficacy.

2 First-Line Medications

2.1 Rifampin

Rifamycins used to treat TB include rifampin, rifapentine, and rifabutin. In addition to isoniazid, the introduction of rifampin in 1967 (FDA approved in 1971) to the treatment of TB dramatically changed the time necessary to treat TB [1]. The rifamycins' bactericidal activity is associated with concentration. Specifically, they are considered concentration-dependent killers of *Mycobacterium tuberculosis* (*M. tb*) [2]. Rifampin, the most bactericidal agent used for TB, is key to preventing disease relapse following treatment [3].

Its PK parameter of efficacy is either area under the curve (AUC) or the peak concentration (Cmax) (Table 1). Early studies examined rifampin point to Cmax/minimum inhibitory concentration (MIC) as the PK/PD parameter for bactericidal activity. However, studies conducted more recently show AUC/MIC may be the PK/PD parameter we should optimize.

The rifamycins differ in regards to absorption properties. Rifampin's absorption is decreased by approximately 36% in the presence of food; thus, if possible, rifampin should be taken on an empty stomach [4]. Additionally, different formulations have variable absorption, as will several disease states. Patients with the following comorbid conditions may experience lower than expected absorption: diabetes, cystic fibrosis, and human immunodeficiency virus (HIV) [5–7]. Some studies suggest that the more advanced the disease, the greater the degree of malabsorption with HIV-TB-co-infected persons [8].

The standard initial dose for rifampin is 600 mg/day (10 mg/kg). The choice of a 600 mg dose had little data supporting it at the time. Investigating the rationale behind the selection of this dosing strategy, Van Ingen et al. detail three points of the rationale for the choice:

i. a 600 mg dose averages concentrations, as measured in the serum, substantially above the MIC;
ii. concern existed that adverse events are dose-related, and the lowest effective dose could mitigate rifampin's adverse events; and
iii. rifampin's expense required judicial use in treatment [9].

Table 1 Pharmacokinetic parameters and concentrations associated with efficacy

Drug	Standard dose (mg)	PK parameter associated w/ efficacy	Peak concentration w/ standard dose (mcg/mL) for TDM considerations	TDM post-dose sample times (h)
Rifampin	8–12 mg/kg	Cmax, AUC	8–24	2 and 6
Rifapentine	600 mg twice daily	Cmax, AUC	8–30	5 and 6
Rifabutin	5 mg/kg once daily 15 mg/kg intermittent	AUC	0.45–0.90	3 and 7
Isoniazid	4–6 mg/kg (300 mg) daily 15 mg/kg (900 mg) biweekly	Cmax	3–6 9–15	2 and 6
Pyrazinamide	25 mg/kg daily 50 mg/kg biweekly If CrCl is less than 30 mL/min/1.73 m^2, consider 25- to 35-mg/kg/dose thrice weekly	AUC	20–50 60–90	24[a]
Ethambutol	15–25 mg/kg daily 50 mg/kg twice weekly	Cmax	2–6 4–12	2 and 6[b]
Moxifloxacin	400 mg daily	AUC	3–5 AUC 55 (36–79)	2 and 6
Levofloxacin	1000 mg daily	AUC	8–13 AUC 129 (103–358)	2 and 6
Amikacin	15–20 mg/kg daily	Cmax	35–45	1.8 mcg/mL
Streptomycin	15–20 mg/kg daily	Cmax	35–45	2 and 6
Cycloserine	15–20 mg/kg (divided dosing)	%T > MIC	20–35	2[c]
Bedaquiline	400 mg daily for two weeks, then 200 mg thrice weekly	AUC	2.5–8.5	2 and 6
Delamanid	100 mg twice daily	AUC	1–4	2 and 6

Prepared with data from [89, 105–108]
CrCL Creatinine clearance; *TDM* Therapeutic drug monitoring
[a] Serum uric acid concentration is used as an adherence detector
[b] 3- and 7-h samples may be preferred if the patient is concomitantly taking rifabutin and/or clarithromycin
[c] 6-h sample if not taken on an empty stomach. Consider collecting samples after 3–4 days of administration to allow for a steady state to be reached

Several recent studies indicated the 600 mg once daily dose may be too low on the dose–effect curve. A reduction in early bactericidal activity (EBA) was seen when doses were reduced from 600 to 300 mg [10]. This was supported by clinical data from a randomized control trial which showed 450 mg was less effective than 600 mg [11]. Data suggest that increasing doses beyond 600 mg will increase bactericidal activity. Additionally, higher doses of rifampin are currently used in

treating other disease states such as brucellosis and leprosy [12]. Based on the accumulating evidence from these in vitro, EBA, and animal studies, the HIRIF trial was conducted to examine doses beyond 600 mg in TB patients [13]. One-hundred eighty TB patients in Peru were randomized to receive 10 mg/kg (600 mg), 15 mg/kg (900 mg), or 20 mg/kg (1200 mg) of rifampin in addition to isoniazid, pyrazinamide, and ethambutol. Pharmacokinetic findings from the trial showed an increase in concentrations above the MIC. Importantly, adverse events were similar between the three groups. Therefore, increasing drug concentrations with higher doses (900 and 1200 mg) maximizes the PK/PD parameter of efficacy without associated increase in adverse effects and guides initial rifampin dosing.

2.2 Rifapentine

Rifapentine is the cyclo-pentyl ring-substituted cogener of rifampin; therefore, the drug has similar PK properties to its predecessor. These properties include a similar side effects profile but an advantage in PD with a lower MIC than rifampin [3]. However, the cyclo-pentyl group creates a difference in some PK properties, such as half-life. Rifapentine's half-life is approximately 15 h, leading researchers to examine intermittent dosing with active TB. However, intermittent therapy may increase rifamycin resistance, particularly in persons living with HIV [14].

Rifapentine is highly protein-bound (98–99%) and, as with rifampin, efficacy may require higher doses [15]. Similar to the HIRIF study, a randomized, dose-ranging study by Dorman et al. examined 10, 15, and 20 mg/kg rifapentine daily [16]. The study showed that antimicrobial activity was associated with AUC. Importantly, the study showed no increased adverse events due to dose increase. It is important to note that food, which increases the absorption of rifapentine, was co-administered with the dose. Additionally, rifapentine concentrations experience high inter-individual variability; as weight does not affect clearance, dosing by a specific milligram dose (rather than mg/kg) would help decrease AUC variability. The optimum AUC appears to be achieved between 900 and 1200 mg for active TB.

It is unknown if doses beyond 1200 mg will result in increased efficacy. Early bactericidal studies by Sirgel et al. indicated a minimal increase in antibacterial activity beyond 1200 mg, and toxicity might prevent higher doses [17, 18]. Currently, rifapentine seems to have found its niche as an option to treat latent TB when combined with high-dose isoniazid.

2.3 Rifabutin

Rifabutin's chemical structure differs from that of rifampin and rifapentine. Rifabutin's side effect profile differs from the other two rifamycins in one substantial aspect: rifabutin concentrations are limited due to dose-related side effects (e.g., anterior uveitis and neutropenia), which are more prominent beyond 1 mcg/mL.

Unlike rifampin and rifapentine, food does not impact rifabutin's absorption to any great extent [3].

Rifabutin is used in TB treatment when drug interactions preclude the use of rifampin (e.g., HIV antiretrovirals, anti-seizure medications). Rifabutin induces enzymes approximately sixty percent less than that of rifampin and rifapentine. However, rifabutin is a CYP3A substrate, and well-noted bidirectional interactions are possible. The potential drug interactions with CYP inducers or inhibitors, as well as the concentration-related side effects, lends rifabutin to therapeutic drug monitoring if logistically and economically feasible [19].

Rifabutin's efficacy is believed to be similar to that of rifampin and rifapentine despite what some would consider an unfavorable Cmax/MIC ratio, which is lower than that of rifampin and rifapentine. It is theorized that rifabutin's intracellular penetration may be superior to that of its rifamycin counterpart with an up to nine-fold concentration within cells [3].

2.4 Isoniazid

For over 60 years, isoniazid has been a primary drug used to treat both active and latent TB. In the first six months of active TB treatment, isoniazid is used in combination with rifampin, pyrazinamide, and ethambutol for two months, then with rifampin for the following four months [20]. For latent TB, isoniazid may be used as monotherapy for six to nine months or combined with rifampin for three months [21]. Isoniazid's bactericidal activity is driven by its concentration-dependent rapid killing. Its efficacy and affordability assist attainability and prevent companion drug resistance (DR) [22].

Isoniazid is usually administered orally with intramuscular (IM) and intravenous (IV) formulations also available. The typical dose is 300 mg daily with an alternative 900 mg dose administered one, two, or three times per week [23]. When taken with food, isoniazid's bioavailability decreases. Thus, it should be taken on an empty stomach [24]. Within 2 h of administration, isoniazid exhibits a Cmax serum concentration of 3 mcg/mL, sufficient for bactericidal activity against sensitive *M. tb* strains. Furthermore, increasing the Cmax to 5 mcg/mL may facilitate killing of isolates that withstand Cmax concentrations of 3 mcg/mL [25]. In situ permeability studies in rats demonstrated that isoniazid has low gastric but high small intestine permeability. However, isoniazid bioavailability did not differ significantly in TB patients following stomach or intestinal surgical resections [26].

Isoniazid has the propensity to cause severe hepatotoxicity, which has been linked to gene alterations affecting N-acetyl transferase 2 (NAT2) metabolism [24, 27]. Greater than 40 NAT2 variants have been identified, resulting in high inter-individual isoniazid concentrations [22]. Genotyping NAT2 may make it possible to individualize isoniazid dosing by lowering the dose in slow acetylators who are more prone to liver enzyme elevations, thus achieving optimal efficacy without increasing the risk of hepatotoxicity [28]. Despite evidence for genotype-guided dosing, it is not commonly performed in current practice.

2.5 Pyrazinamide

Pyrazinamide, combined with isoniazid, rifampin, and ethambutol for the first two months, is another essential first-line TB treatment. Its sterilizing effect is most efficacious during this time against *M. tb*, allowing a duration of treatment of six instead of nine months [29]. Unlike other TB treatments, pyrazinamide has bactericidal activity in acidic environments, which may improve its susceptibility in immunocompromised hosts [30]. Of all anti-TB therapies, pyrazinamide has likely the most predictable absorption [29]. It demonstrates high bioavailability with serum Cmax achieved within 1–2 h after administration and intracellular accumulation within 3 h, irrespective of cellular metabolism [29, 31]. Only minor changes in bioavailability and Cmax are demonstrated when pyrazinamide is taken with meals [32]. Approximately 10% of pyrazinamide is protein-bound, and it is not known whether pyrazinamide crosses the placental barrier, but distribution into breast milk has been documented [33, 34]. The elimination half-life of pyrazinamide, which is primarily excreted in the urine, is 9–10 h in healthy adults; however, metabolite accumulation and a prolonged elimination to 26 h occur in patients with severe renal dysfunction [35].

Weight-based pyrazinamide dosing is recommended for drug-susceptible TB (25 mg/kg) and multidrug-resistant (MDR) TB (35 mg/kg) to achieve a goal AUC of $\geq$ 363 mg h/L, which has demonstrated improved patient outcomes. However, traditional pyrazinamide dosing often results in suboptimal PK target attainment; thus, higher doses may be warranted [32]. In a study by Pasipanodya et al., pyrazinamide AUC values < 363 g h/ml resulted in poor patient outcomes, including treatment failure, disease relapse, and death [36]. Alsultan et al. demonstrated in a one-compartment model that weight and sex substantially impact pyrazinamide PK. Specifically, when comparing fixed- versus weight-adjusted dosing in a 90 kg and 40 kg patient, the model estimated that a fixed dose of pyrazinamide would decrease the AUC two-fold in the 90 kg patient and that weight-adjusted doses of 50 mg/kg and 60–70 mg/kg were needed to reach AUC goals in 80% and 90% patients weighing 40–90 kg, respectively. Based on these data, the universal use of a fixed dose of pyrazinamide may not be appropriate [29]. However, higher dosing with a weight-adjusted approach may increase levels of protonated pyrazinoic acid and the subsequent risk of pyrazinamide parent drug- or metabolite-induced hepatotoxicity [37]. Thus, the risk of hepatotoxicity should be balanced against using higher doses to improve outcomes.

2.6 Ethambutol

Ethambutol is a first-line TB treatment that protects rifampin from developing DR during the first two months of therapy in case isoniazid demonstrates resistance. Ethambutol may be stopped at two months if the other first-line agents demonstrate susceptibility. However, if it is a rifampin- or isoniazid-resistant strain, ethambutol therapy may be prolonged [38].

Ethambutol's optimal PD parameter has yet to be determined; however, dose–response efficacy has been observed [39]. Microbial kill has been linked to both AUC/MIC and Cmax/MIC, while resistance has been associated with time-dependent activity (%T/MIC) [2].

Ethambutol AUC is decreased in obese patients, reducing the risk of toxicity while increasing the risk of therapeutic failure. This was demonstrated by Hall et al. in a prospective study that evaluated the impact of weight on ethambutol PK after one oral dose of 1600 mg in 18 adults with normal renal function and body mass index ranging from less than 25 to greater than 40 kg/m^2. Using a two-compartment model, they identified that ethambutol AUC was lower for obese versus lean adults. These data concluded that weight-based ethambutol dosing regimens might be warranted for obese and extremely obese patients to improve treatment outcomes [40].

Ethambutol activity against *M. tb* requires concentrations of 1 mcg/mL and 5 mcg/mL to achieve a static and cidal effect, respectively [41]. Following oral administration of a 25 mg/kg dose of ethambutol, approximately 80% is absorbed with Cmax serum concentrations of 4–5 mcg/mL at approximately 2–4 h post-dose. Although serum concentrations are not significantly affected by food ingestion, they are reduced when ethambutol is taken with aluminum hydroxide. Ethambutol demonstrates low protein binding and rapid gastrointestinal absorption, resulting in high concentrations at various sites (e.g., kidneys, lungs, erythrocytes, and saliva) [42]. Ethambutol undergoes partial hepatic metabolism, resulting in 50–80% of unchanged drug and 8–15% of its metabolites being excreted in the urine, while 25% of the unabsorbed drug is excreted in feces at 24 h [43]. Its elimination has two phases: a fast decline in the initial 12 h and a slow decrease after that [44]. The usual plasma half-life is 3.5 h, which can extend to 15 h with renal or hepatic dysfunction.

Optic neuritis is a serious adverse effect of ethambutol. The concern of ocular toxicity increases substantially in patients with severe renal dysfunction due to an approximately two-fold increase in drug half-life [45].

3 Second-Line Medications

3.1 Fluoroquinolones

Fluoroquinolones have been tested in various regimens to determine if they may be used as first-line treatment. However, most guidelines and clinicians agree that they should be considered second-line therapy [46, 47].

Fluoroquinolones have high oral bioavailability and low protein binding; additionally, the plasma half-lives range from 5 to 7 h [48]. These PK properties allow for once-daily dosing. Its PK efficacy parameter is AUC [49, 50]. Studies show that the fluoroquinolones' early bactericidal activity was less than that of isoniazid but greater than rifampin [51, 52]. Based on these studies, various trials were initiated

to determine if using a fluoroquinolone in place of a first-line agent (e.g., ethambutol or isoniazid) shortens the duration of therapy [46, 47, 53]. Thus far, study results have not shown a clear advantage in switching to fluoroquinolones in regards to shortening treatment [46, 47, 54, 55].

Moxifloxacin is active against *M. tb* isolates, including MDR-*M. tb* isolates [49]. The oral bioavailability is approximately 92% with little first-pass effect [48]. Moxifloxacin has a half-life of 11–15 h, and oral absorption may be delayed when given with food [56]. Similar to most fluoroquinolones, moxifloxacin significantly penetrates body tissues and bronchial secretions, a result of its substantial volume of distribution [57–59]. There are no significant drug interactions through the cytochrome P450 pathway; however, absorption may be decreased when concomitantly administered orally with multivalent cations. Specifically, aluminum or magnesium ions decrease the absorption by 40%, and iron ions may decrease as much as 60% [60]. Moxifloxacin is metabolized and excreted primarily by the hepatobiliary system by sulfate (38%) and glucuronide (14%) conjugation [61]. The remainder is excreted in feces (25%) and urine (20%) unchanged.

Clinical efficacy with moxifloxacin has been correlated with the AUC/MIC ratio [62]. In particular, moxifloxacin has been the focus of studies testing its use as an alternative agent to be used in a standard first-line regimen. Current literature failed to meet non-inferiority on shorter duration as well as replacing the first-line regimens. Yet, if a patient cannot tolerate one of the first-line regimens, and if found susceptible, moxifloxacin may be an option. Currently, the CDC and WHO recommend the use of moxifloxacin for MDR-TB. Levofloxacin and ofloxacin are also alternative treatment options. Most in vitro studies show the MICs for moxifloxacin and levofloxacin are less than 0.5 mcg/mL and ofloxacin around 1 mcg/mL [63]. Ciprofloxacin's antitubercular activity is not adequate for efficacy [64].

Additionally, concerns exist regarding fluoroquinolones' resistance due to their widespread use for multiple disease states. Poor adherence and indiscriminate prescribing have contributed to this resistance. In regards to the treatment of TB, previous use can limit their usefulness.

Overall, the newer fluoroquinolones generally have a good safety profile, and their antimycobacterial drug interactions are limited to rifampin, which results in decreased moxifloxacin plasma concentration (approximately 31%) [65, 66]. Higher than the standard 400 mgmoxifloxacin dose may be required when taken concomitantly with rifampin, and therapeutic drug monitoring may be considered, if feasible. In regards to moxifloxacin dosing, data suggest that the current 400 mg dose may be suboptimal in eradicating *M. tb* in the absence of drug interactions and that 600–800 mg should be used [50, 67, 68].

3.2 Aminoglycosides

The aminoglycosides, once mainstays in the treatment of MDR-TB, are no longer utilized as often due to the availability of drugs with superior safety profiles and ease of administration (oral vs. IV). Kanamycin and capreomycin, in particular, are

not often used, and the current ATS/CDC/ERS/IDSA guidelines recommend against their use due to an association with poorer outcomes in MDR-TB patients [69]. Streptomycin and amikacin currently are still recommended for MDR-TB. Streptomycin, the first isolated aminoglycoside, has been used extensively, but due to its frequent use, it is estimated that more than 50% of MDR-*M. tb* isolates may be resistant in certain regions [70].

Amikacin is active against *M. tb* [71] and has shown high susceptibility rates in MDR-*M. tb* strains [72, 73]. Amikacin has the lowest MIC of the aminoglycosides by the absolute concentration methods [74]. The likelihood of cross-resistance between aminoglycosides is not fully consistent. The incidence of amikacin resistance seems to be lower with MDR-*M. tb* isolates, especially those that are streptomycin-resistant [75].

Amikacin demonstrates poor gastrointestinal absorption, whereas, with IM injection, the bioavailability is approximately 95%. Amikacin is, therefore, commonly administered as an IV agent. Amikacin has a half-life of 1.9–2.6 h with adults in normal renal function, whereas it will be prolonged to 44 h in patients with decreased renal function [76–78]. Its volume of distribution is approximately 30% of the total body weight, indicating that amikacin is primarily distributed in the extracellular fluids [76]. Amikacin has been shown to penetrate bronchial secretions in patients with pneumonia and has shown that once-daily administration at higher concentrations is more effective than divided doses [79].

The Cmax is attained approximately one hour after the start of IV administration. Amikacin's PK parameter most associated with killing and efficacy is Cmax; thus, adjusting the aminoglycoside dose to optimize the Cmax/MIC ratio is necessary to increase effectiveness. Therapeutic drug monitoring of peak concentrations may aid in increasing efficacy and potentially reduce the risk of adverse events (e.g., nephrotoxicity and ototoxicity) [80, 81]. Amikacin has been shown to concentrate in the renal cortex and accumulate with repeated doses [82]. Both nephrotoxicity and ototoxicity are associated with the duration of aminoglycoside use [83]. In a retrospective analysis of eighty patients, van Altena et al. found that cumulative drug concentrations correlated with the extent of hearing loss [80]. Modongo et al. showed its association with AUC and ototoxicity [84]. In their analysis, an amikacin AUC greater than 87,332 mg h/L resulted in a significant increase in ototoxicity. Post-antibiotic effect duration has been seen as far as 17.4 h in an in vitro setting [85]. The post-antibiotic effect may increase to 95–97 h in combination with other antimycobacterial agents.

Amikacin is excreted entirely from the kidneys and has tubular reabsorption [77]. Within 24 h of administration, approximately 94% of amikacin is excreted unchanged in the urine [76, 77].

3.3 Cycloserine

Cycloserine, structurally related to D-alanine, shows activity against several gram-positive bacteria in addition to *M. tb*. Data on cycloserine PK is sparse

compared to other anti-TB medications. Neuropsychiatric side effects prevent cycloserine's widespread use, and it remains a second-line agent. The standard dose of cycloserine is 250–500 mg, taken one to two times daily. The MIC for cycloserine is approximately 10 mcg/mL in Lowenstein-Jensen media [42]. Regarding efficacy, studies indicate that time > MIC is the PK/PD parameter that should be maximized [42].

Studies indicate that cycloserine's absorption is influenced by food and antacids. Cycloserine is water-soluble and is highly bioavailable. A healthy volunteer study by Zhu et al. demonstrated decreased cycloserine absorption when taken concomitantly with a high-fat meal, which resulted in a decreased Cmax and prolonged Tmax. In the same study, antacids and orange juice had little effect on PK parameters. A population PK model by Alghamdi et al. collated cycloserine concentrations from 247 individuals from three different countries [33]. The authors noted that dividing the dose did not improve PK/PD breakpoints, except when patients received a 750 mg dose divided into 500 and 250 mg. As stated, the central nervous system (CNS) toxicity limits the use of cycloserine. Some common CNS side effects include headache, lethargy, dizziness, dysarthria, and depression. Assuming CNS toxicity is associated with the Cmax, then dividing the dose can reduce the risk for CNS toxicity while maintaining a time > MIC associated with efficacy [33].

3.4 Bedaquiline

Bedaquiline, formerly TMC207, received approval to treat pulmonary MDR-*M. tb* in 2012 [86]. It has a different mechanism of action compared to other antimycobacterial agents, making it an attractive treatment option for MDR-TB. Bedaquiline has high in vitro activity against susceptible and resistant strains of *M. tb* [87]. Following oral administration, bedaquiline is well absorbed with a median Tmax at 5 h, which is dose-independent. Food increases absorption and is recommended to be given with the administration of Bedaquiline [88].

Bedaquiline displays linear kinetics with both single-dose and multiple-dose administration; thus, Cmax and AUC both increase in proportion with single and multiple doses. Per animal studies, it is believed that the primary PK parameter associated with efficacy is AUC. Bedaquiline dosing is 400 mg every day for two weeks, then 200 mg three days per week. Intermittent dosing is utilized to prevent the accumulation of bedaquiline while maintaining the average plasma concentration of 0.6 mcg/mL (based on murine models). The terminal half-life of bedaquiline is approximately 164 days, which is substantially longer than the 24 h considered to be its "effective half-life." Heeswijk et al. describe the effective half-life as: "the half-life associated with drug accumulation in plasma" [89].

Aside from its long elimination half-life, two other PK features of bedaquiline stand out: protein binding and its metabolism. Similar to rifapentine, bedaquiline is highly protein-bound, approximately 99.9% [90]. Bedaquiline's active metabolite, M2 (N-monodesmethyl metabolite), is also highly protein-bound, approximately

99.7% [89]. In regards to metabolism, bedaquiline is primarily metabolized by CYP3A4 into M2. As a substrate of CYP3A4, the potential for drug-drug interactions is apparent. Notably, bedaquiline is not known to be an inducer or inhibitor of phase I or II enzymes [91].

3.5 Delamanid

Delamanid, an inhibitor of mycolic acid biosynthesis, was approved by the European Medicines Agency (EMA) in 2014 for MDR-TB. Delamanid shows activity against actively replicating *M. tb* as well as dormant and intracellular bacilli [92]. Delamanid has greater than 99% plasma protein binding and a substantial volume of distribution [93]. Food increases absorption versus a fasted state. Peak delamanid concentrations occur between 4 and 8 h. In regards to distribution, delamanid can achieve high tissue levels, including bone tissue and the CNS; thus, delamanid passes the blood–brain barrier [94].

One of the more unique PK properties of delamanid is how the drug is metabolized. Plasma albumin is the primary metabolic pathway, which is seen with a few drug products [93, 95]. Additionally, delamanid neither induces nor inhibits CYP enzymes or efflux/influx pumps; thus, a few drug interactions is expected [96]. The plasma half-life ranges from 30 to 38 h, which lends itself to once-daily dosing [97]. Dosing is nonlinear; however, in a dose-ranging study by Diacon et al., delamanid's AUC increased disproportionately from 100 to 400 mg; the authors believed it was due to dose-limited absorption [98]. Importantly, delamanid was found to be well-tolerated with few significant signs of toxicity. Common side effects are nausea, vomiting, headache, and QT prolongation [99]. Though studies show that QT prolongation has not led to any clinical cardiac events, even when given in conjunction with other QT-prolonging medications (e.g., fluoroquinolones), patients should be monitored closely [99].

The MICs range between 0.006 and 0.03 mcg/mL, depending on the assay used [100, 101]. Resistance is low, approximately 1.3%, based on a study of 744 MDR or extensively drug-resistant (XDR) isolates with ten of those isolates having MICs at or greater than 0.5 mcg/mL [102]. Critical concentrations of 0.2 and 0.125 mcg/mL have been proposed previously [103, 104].

4 Conclusion

Alexander Fleming's prescient comments regarding the penicillin drug resistance ring are as true now as he spoke them 90 years ago. TB is a perfectly curable disease if treated appropriately; yet, it is currently the number one infectious killer globally. Many factors have contributed to its dissemination throughout the globe, including logistical and socioeconomic factors. One of the factors that clinicians can manipulate to a certain extent is the pharmacokinetic parameters via dosing

changes, food administration, management of drug interactions, and optimizing adherence via directly observed therapy. Until a viable vaccine is available or newer, more potent agents are developed, we are left with maximizing today's medications to the best of our ability.

Core Messages

- Optimized PK/PD parameters increases anti-TB treatment efficacy while minimizing side effects and drug interactions.
- TB therapy is two months of isoniazid, rifamycin, pyrazinamide, and ethambutol, then four months of isoniazid and rifampin.
- Alternative therapy options for TB include fluoroquinolones, aminoglycosides, cycloserine, bedaquiline, and delamanid.
- PK parameters associated with efficacy for all agents are Cmax and AUC, except cycloserine, for which it is T > MIC.

References

1. Rothstein DM (2016) Rifamycins, alone and in combination. Cold Spring Harb Perspect Med 6:1–20. https://doi.org/10.1101/cshperspect.a027011
2. Gumbo T, Louie A, Deziel MR et al (2007) Concentration-dependent Mycobacterium tuberculosis killing and prevention of resistance by rifampin. Antimicrob Agents Chemother 51:3781–3788. https://doi.org/10.1128/AAC.01533-06
3. Burman WJ, Gallicano K, Peloquin C (2001) Comparative pharmacokinetics and pharmacodynamics of the rifamycin antibacterials. Clin Pharmcokinetics 40:327–341
4. Peloquin CA, Namdar R, Singleton M, Nix D (1999) Pharmacokinetics of rifampin under fasting conditions, with food, and with antacids. Chest 115:12–18
5. Peloquin CA, Nitta A, Burman WJ et al (1996) Low antituberculosis drug concentrations in patients with AIDS. Ann Pharmacother 30:919–925
6. Kimmerling M, Phillips P, Patterson P et al (1998) Low serum antimycobacterial drug levels in non-HIV-infected tuberculosis patients. Chest 113:1178–1183
7. Alfarisi O, Mave V, Gaikwad S et al (2018) Effect of diabetes mellitus on the pharmacokinetics and pharmacodynamics of tuberculosis treatment. Antimicrob Agents Chemother 62:1–14. https://doi.org/10.1128/AAC.01383-18
8. Gurumurthy P, Ramachandran G, Hemanth Kumar AK et al (2004) Decreased bioavailability of rifampin and other antituberculosis drugs in patients with advanced human immunodeficiency virus disease. Antimicrob Agents Chemother 48:4473–4475. https://doi.org/10.1128/AAC.48.11.4473-4475.2004
9. Van Ingen J, Aarnoutse RE, Donald PR, et al (2011) Why do we use 600 mg of rifampicin in tuberculosis treatment? Clin Infect Dis 52. https://doi.org/10.1093/cid/cir184
10. Mitnick C (2009) Tuberculosis pharmacotherapy: strategies to optimize patient care. Expert Opin Pharmacother 10:381–401
11. Dawson R, Narunsky K, Carman D, et al (2019) intensive phase treatment of pulmonary tuberculosis. 19:780–786. https://doi.org/10.5588/ijtld.14.0868.Two-stage

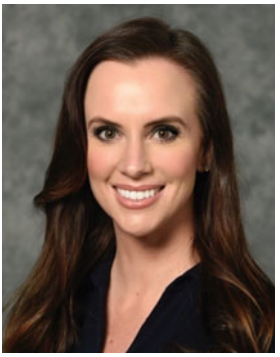
12. Drancourt M, Stein A, Argenson JN et al (1993) Oral rifampin plus ofloxacin for treatment of Staphylococcus-infected orthopedic implants. Antimicrob Agents Chemother 37:1214–1218. https://doi.org/10.1128/AAC.37.6.1214
13. Peloquin CA, Velásquez GE, Lecca L et al (2017) Pharmacokinetic evidence from the HIRIF Trial to support increased doses of rifampin for tuberculosis. Antimicrob Agents Chemother 61:1–6. https://doi.org/10.1128/AAC.00038-17
14. Benator D, Bhattacharya M, Bozeman L et al (2002) Rifapentine and isoniazid once a week versus rifampicin and isoniazid twice a week for treatment of drug-susceptible pulmonary tuberculosis in HIV-negative patients: a randomised clinical trial. Lancet 360:528–534. https://doi.org/10.1016/S0140-6736(02)09742-8
15. Egelund EF, Weiner M, Singh RP et al (2014) Protein binding of rifapentine and its 25-desacetyl metabolite in patients with pulmonary tuberculosis. Antimicrob Agents Chemother 58:4904–4910. https://doi.org/10.1128/AAC.01730-13
16. Dorman SE, Savic RM, Goldberg S et al (2015) Daily rifapentine for treatment of pulmonary tuberculosis: a randomized, dose-ranging trial. Am J Respir Crit Care Med 191:333–343. https://doi.org/10.1164/rccm.201410-1843OC
17. Sirgel FA, Fourie PB, Donald PR et al (2005) The early bactericidal activities of rifampin and rifapentine in pulmonary tuberculosis. Am J Respir Crit Care Med 172:128–135. https://doi.org/10.1164/rccm.200411-1557OC
18. Savic RM, Weiner M, MacKenzie WR et al (2017) Defining the optimal dose of rifapentine for pulmonary tuberculosis: exposure–response relations from two phase II clinical trials. Clin Pharmacol Ther 102:321–331. https://doi.org/10.1002/cpt.634
19. Mouton JW (2016) General concepts of pharmacodynamics for anti-infective agents
20. Denholm J (2010) The use of anti-tuberculosis therapy for latent TB infection. Infect Drug Resist 63. https://doi.org/10.2147/idr.s8994
21. Norton BL, Holland DP (2012) Current management options for latent tuberculosis: a review. Infect Drug Resist 5:163–173. https://doi.org/10.2147/IDR.S29180
22. Seng K, Hee K, Soon G et al (2015) Population pharmacokinetic analysis of isoniazid, acetylisoniazid, and isonicotinic acid in healthy volunteers. Antimicrob Agents Chemother 59:6791–6799. https://doi.org/10.1128/AAC.01244-15
23. National Institute of Diabetes and Digestive and Kidney Diseases (2012) LiverTox: clinical and research information on drug-induced liver injury
24. Wang P, Pradhan K, Zhong X-b, Ma X (2016) Isoniazid metabolism and hepatotoxicity. Acta Pharm Sin B 6:384–392. https://doi.org/10.1016/j.apsb.2016.07.014
25. Curry International Tuberculosis Center, California Department of Health Services, Office of AIDS (2016) Drug-resistant tuberculosis: a survival guide for clinicians
26. Klein DJ, Boukouvala S, McDonagh EM et al (2016) PharmGKB summary. Pharmacogenet Genomics 26:436–444. https://doi.org/10.1097/fpc.0000000000000232
27. Epic Pharma LLC (2018) Isoniazid Tablets, USP
28. Cordes H, Thiel C, Aschmann HE et al (2016) A physiologically based pharmacokinetic model of isoniazid and its application in individualizing tuberculosis chemotherapy. Antimicrob Agents Chemother 60:6134–6145. https://doi.org/10.1128/AAC.00508-16
29. Alsultan A, Savic R, Dooley KE et al (2017) Population pharmacokinetics of pyrazinamide in patients with tuberculosis. Antimicrob Agents Chemother 61:1–11. https://doi.org/10.1128/AAC.02625-16
30. Lamont EA, Baughn AD (2019) Impact of the host environment on the antitubercular action of pyrazinamide. EBioMedicine 49:374–380. https://doi.org/10.1016/j.ebiom.2019.10.014
31. Zimic M, Loli S, Gilman RH et al (2012) A new approach for pyrazinamide susceptibility testing in mycobacterium tuberculosis. Microb Drug Resist 18:372–375. https://doi.org/10.1089/mdr.2011.0207
32. Chirehwa MT, McIlleron H, Rustomjee R et al (2017) Pharmacokinetics of pyrazinamide and optimal dosing regimens for drug-sensitive and -resistant tuberculosis. Antimicrob Agents Chemother 61:8–13. https://doi.org/10.1128/AAC.00490-17

33. Alghamdi WA, Alsultan A, Al-Shaer MH et al (2019) Cycloserine population pharmacokinetics and pharmacodynamics in patients with tuberculosis. Antimicrob Agents Chemother 63:1–11. https://doi.org/10.1128/AAC.00055-19
34. Bothamley G (2001) Drug treatment for tuberculosis during pregnancy. Drug Saf 24:553–565. https://doi.org/10.2165/00002018-200124070-00006
35. Malone RS, Fish DN, Spiegel DM et al (1999) The effect of hemodialysis on isoniazid, rifampin, pyrazinamide, and ethambutol. Am J Respir Crit Care Med 159:1580–1584. https://doi.org/10.1164/ajrccm.159.5.9810034
36. Pasipanodya JG, McIlleron H, Burger A et al (2013) Serum drug concentrations predictive of pulmonary tuberculosis outcomes. J Infect Dis 208:1464–1473. https://doi.org/10.1093/infdis/jit352
37. Ramappa V, Aithal GP (2013) Hepatotoxicity related to anti-tuberculosis drugs: mechanisms and management. J Clin Exp Hepatol 3:37–49. https://doi.org/10.1016/j.jceh.2012.12.001
38. Dorman S, Gupta A (2017) Treatment of pulmonary tuberculosis. Handb Tuberc 3536:35–90. https://doi.org/10.1007/978-3-319-26273-4_3
39. Denti P, Jeremiah K, Chigutsa E et al (2015) Pharmacokinetics of isoniazid, pyrazinamide, and ethambutol in newly diagnosed pulmonary TB patients in Tanzania. PLoS ONE 10:1–19. https://doi.org/10.1371/journal.pone.0141002
40. Hall RG, Swancutt MA, Meek C et al (2012) Ethambutol pharmacokinetic variability is linked to body mass in overweight, obese, and extremely obese people. Antimicrob Agents Chemother 56:1502–1507. https://doi.org/10.1128/AAC.05623-11
41. Dias-Freedman I, Chen C, Dietzold J, et al (2017) Ethambutol partitioning in tuberculous pulmonary lesions explains its clinical efficacy. Antimicrob Agents Chemother 61:1–12
42. Meal H, Juice O, Peloquin CA, Pharm D (2001) Pharmacokinetics of cycloserine under fasting conditions and with high-fat meal, orange juice, and antacids. Pharmacotherapy: J Hum Pharmacol Drug Therapy
43. Baietto L, Corcione S, Pacini G et al (2014) A 30-years review on pharmacokinetics of antibiotics: is the right time for pharmacogenetics? Curr Drug Metab 15:581–598
44. Egelund EF, Alsultan A, Peloquin CA (2015) Optimizing the clinical pharmacology of tuberculosis medications. Clin Pharmacol Ther 98:387–393. https://doi.org/10.1002/cpt.180
45. Leibold JE (1966) The ocular toxicity of ethambutol and its relation to dose. Ann N Y Acad Sci 135:904–909. https://doi.org/10.1111/j.1749-6632.1966.tb45532.x
46. Burman WJ, Goldberg S, Johnson JL et al (2006) Moxifloxacin versus ethambutol in the first 2 months of treatment for pulmonary tuberculosis. Am J Respir Crit Care Med 174:331–338. https://doi.org/10.1164/rccm.200603-360OC
47. Dorman SE, Johnson JL, Goldberg S et al (2009) Substitution of moxifloxacin for isoniazid during intensive phase treatment of pulmonary tuberculosis. Am J Respir Crit Care Med 180:273–280. https://doi.org/10.1164/rccm.200901-0078OC
48. Ballow C, Lettieri J, Agarwal V et al (1999) Absolute bioavailability of moxifloxacin. Clin Ther 21:513–522. https://doi.org/10.1016/S0149-2918(00)88306-X
49. Shandil RK, Jayaram R, Kaur P et al (2007) Moxifloxacin, ofloxacin, sparfloxacin, and ciprofloxacin against *Mycobacterium tuberculosis*: evaluation of in vitro and pharmacodynamic indices that best predict in vivo efficacy. Antimicrob Agents Chemother 51:576–582. https://doi.org/10.1128/AAC.00414-06
50. Gumbo T, Louie A, Deziel MR et al (2004) Selection of a moxifloxacin dose that suppresses drug resistance in *Mycobacterium tuberculosis*, by use of an in vitro pharmacodynamic infection model and mathematical modeling. J Infect Dis 190:1642–1651. https://doi.org/10.1086/424849
51. Gillespie SH, Billington O (1999) Activity of moxifloxacin against mycobacteria. J Antimicrob Chemother 44:393–395. https://doi.org/10.1093/jac/44.3.393
52. Sirgel FA, Donald PR, Odhiambo J et al (2000) A multicentre study of the early bactericidal activity of anti-tuberculosis drugs. J Antimicrob Chemother 45:859–870. https://doi.org/10.1093/jac/45.6.859

53. Rustomjee R, Lienhardt C, Kanyok T et al (2008) A phase II study of the sterilising activities of ofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis. Int J Tuberc Lung Dis 12:128–138
54. Gillespie SH, Crook AM, McHugh TD et al (2014) Four-month Moxifloxacin-based regimens for drug-sensitive tuberculosis. N Engl J Med 371:1577–1587. https://doi.org/10.1056/NEJMoa1407426
55. Merle CS, Fielding K, Sow OB et al (2014) A four-month gatifloxacin-containing regimen for treating tuberculosis. N Engl J Med 371:1588–1598. https://doi.org/10.1056/NEJMoa1315817
56. Sullivan JT, Woodruff M, Lettieri J et al (1999) Pharmacokinetics of a once-daily oral dose of moxifloxacin (Bay 12-8039), a new enantiomerically pure 8-methoxy quinolone. Antimicrob Agents Chemother 43:2793–2797. https://doi.org/10.1128/aac.43.11.2793
57. Chatzika K, Manika K, Kontou P et al (2014) Moxifloxacin pharmacokinetics and pleural fluid penetration in patients with pleural effusion. Antimicrob Agents Chemother 58:1315–1319. https://doi.org/10.1128/AAC.02291-13
58. Müller M, Staß H, Brunner M et al (1999) Penetration of moxifloxacin into peripheral compartments in humans. Antimicrob Agents Chemother 43:2345–2349. https://doi.org/10.1128/aac.43.10.2345
59. Wirtz M, Kleeff J, Swoboda S et al (2004) Moxifloxacin penetration into human gastrointestinal tissues. J Antimicrob Chemother 53:875–877. https://doi.org/10.1093/jac/dkh173
60. Stass H, Kubitza D (1999) Interaction profile of moxifloxacin. Drugs 58:235–236. https://doi.org/10.2165/00003495-199958002-00073
61. Stass H, Kubitza D (1999) Pharmacokinetics and elimination of moxifloxacin after oral and intravenous administration in man. J Antimicrob Chemother 43:83–90. https://doi.org/10.1093/jac/43.suppl_2.83
62. Drusano GL, Preston SL, Owens RC Jr, Ambrose PG (2001) Fluoroquinolone pharmacodynamics. Clin Infect Dis 33:2091–2092. https://doi.org/10.1086/323748
63. Ruiz P, Causse M, Vaquero M, Casal M (2019) In vitro activity of tedizolid against *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 17:229–231. https://doi.org/10.1128/AAC.01939-1
64. Gumbo T, Louie A, Deziel MR, Drusano GL (2005) Pharmacodynamic evidence that ciprofloxacin failure against tuberculosis is not due to poor microbial kill but to rapid emergence of resistance. Antimicrob Agents Chemother 49:3178–3181. https://doi.org/10.1128/AAC.49.8.3178-3181.2005
65. Naidoo A, Naidoo K, Mcilleron H et al (2017) A review of moxifloxacin for the treatment of drug-susceptible tuberculosis. J Clin Pharmacol 57:1369–1386. https://doi.org/10.1002/jcph.968.A
66. Nijland HMJ, Ruslami R, Juwono Suroto A et al (2007) Rifampicin reduces plasma concentrations of moxifloxacin in patients with tuberculosis. Clin Infect Dis 45:1001–1007. https://doi.org/10.1086/521894
67. Zvada SP, Denti P, Sirgel FA et al (2014) Moxifloxacin population pharmacokinetics and model-based comparison of efficacy between moxifloxacin and ofloxacin in African patients. Antimicrob Agents Chemother 58:503–510. https://doi.org/10.1128/AAC.01478-13
68. Pranger AD, Van Altena R, Aarnoutse RE et al (2011) Evaluation of moxifloxacin for the treatment of tuberculosis: 3 years of experience. Eur Respir J 38:888–894. https://doi.org/10.1183/09031936.00176610
69. Nahid P, Mase SR, Migliori GB et al (2019) Treatment of drug-resistant tuberculosis an official ATS/CDC/ERS/IDSA clinical practice guideline
70. Krause KM, Serio AW, Kane TR, Connolly LE (2016) Aminoglycosides: an overview. Cold Spring Harb Perspect Med 6:1–18. https://doi.org/10.1101/cshperspect.a027029
71. Gangadharam PRJ, Candler ER (1977) Tubercle 58 (1977) 35–38

72. Balabanova Y, Ruddy M, Hubb J et al (2005) Multidrug-resistant tuberculosis in Russia: clinical characteristics, analysis of second-line drug resistance and development of standardized therapy. Eur J Clin Microbiol Infect Dis 24:136–139. https://doi.org/10.1007/s10096-004-1268-4
73. Abubakar I, Moore J, Drobniewski F et al (2009) Extensively drug-resistant tuberculosis in the UK: 1995 to 2007. Thorax 64:512–515. https://doi.org/10.1136/thx.2008.108712
74. Dijkstra JA, Van der Laan T, Akkerman OW et al (2018) In vitro susceptibility of *Mycobacterium tuberculosis* to Amikacin, kanamycin, and capreomycin. Antimicrob Agents Chemother 62:1–6. https://doi.org/10.1128/AAC.01724-17
75. Banerjee R, Allen J, Westenhouse J et al (2008) Extensively drug-resistant tuberculosis in California, 1993–2006. Clin Infect Dis 47:450–457. https://doi.org/10.1086/590009
76. Cabana BE, Taggart JG (1973) Comparative pharmacokinetics of BB-K8 and kanamycin in dogs and humans. Antimicrob Agents Chemother 3:478–483. https://doi.org/10.1128/AAC.3.4.478
77. Kirby WMM, Clarke JT, Libke RD, Regamey C (1976) Clinical pharmacology of Amikacin and kanamycin. J Infect Dis 134:312–315. https://doi.org/10.1093/infdis/135.supplement_2.s312
78. Lode H, Grunert K, Koeppe P, Langmaack H (1976) Pharmacokinetic and clinical studies with Amikacin, a new aminoglycoside antibiotic. J Infect Dis 134:316–322. https://doi.org/10.1093/infdis/135.supplement_2.s316
79. Santre C, Georges H, Jacquier JM et al (1995) Amikacin levels in bronchial secretions of 10 pneumonia patients with respiratory support treated once daily versus twice daily. Antimicrob Agents Chemother 39:264–267. https://doi.org/10.1128/aac.39.1.264
80. van Altena R, Dijkstra JA, van der Meer ME et al (2017) Reduced chance of hearing loss associated with therapeutic drug monitoring of amino. Antimicrob Agents Chemother 61:1–10. https://doi.org/10.1128/AAC.01400-16
81. Dijkstra JA, Van Altena R, Akkerman OW et al (2015) Limited sampling strategies for therapeutic drug monitoring of Amikacin and kanamycin in patients with multidrug-resistant tuberculosis. Int J Antimicrob Agents 46:332–337. https://doi.org/10.1016/j.ijantimicag.2015.06.008
82. Edwards CQ, Smith CR, Baughman KL et al (1976) Concentrations of gentamicin and Amikacin in human kidneys. Antimicrob Agents Chemother 9:925–927. https://doi.org/10.1128/AAC.9.6.925
83. De Jager R, Van Altena R (2002) Hearing loss and nephrotoxicity in long-term aminoglycoside treatment in patients with tuberculosis. Int J Tuberc Lung Dis 6:622–627
84. Modongo C, Pasipanodya JG, Zetola NM et al (2015) Amikacin concentrations predictive of ototoxicity in multidrug-resistant tuberculosis patients. Antimicrob Agents Chemother 59:6337–6343. https://doi.org/10.1128/AAC.01050-15
85. Chan CY, Au-Yeang C, Yew WW et al (2001) Post-antibiotic effects of antituberculosis agents alone and in combination. Antimicrob Agents Chemother 45:3631–3634. https://doi.org/10.1128/AAC.45.12.3631-3634.2001
86. Lakshmanan M, Xavier AS (2013) Bedaquiline—the first ATP synthase inhibitor against multi drug resistant tuberculosis. J Young Pharm 5:112–115. https://doi.org/10.1016/j.jyp.2013.12.002
87. Hoagland D, Liu J, Lee RB, Lee RE (2017) New agents for the treatment of drug-resistant *Mycobacterium tuberculosis*. Adv Drug Deliv Rev 55–72. https://doi.org/10.1016/j.addr.2016.04.026.New
88. McLeay SC, Vis P, Van Heeswijk RPG, Green B (2014) Population pharmacokinetics of bedaquiline (TMC207), a novel antituberculosis drug. Antimicrob Agents Chemother 58:5315–5324. https://doi.org/10.1128/AAC.01418-13
89. van Heeswijk RPG, Dannemann B, Hoetelmans RMW (2014) Bedaquiline: a review of human pharmacokinetics and drug-drug interactions. J Antimicrob Chemother 69:2310–2318. https://doi.org/10.1093/jac/dku171

90. Svensson RJ, Simonsson USH (2016) Application of the multistate tuberculosis pharmacometric model in patients with rifampicin-treated pulmonary tuberculosis. CPT Pharmacometrics Syst Pharmacol 5:264–273. https://doi.org/10.1002/psp4.12079
91. Kempker RR, Alghamdi WA, Al-Shaer MH et al (2019) A pharmacology perspective on simultaneous tuberculosis and hepatitis C treatment. Antimicrob Agents Chemother 63:1–14
92. Liu Y, Matsumoto M, Ishida H et al (2018) Delamanid: from discovery to its use for pulmonary multidrug-resistant tuberculosis (MDR-TB). Tuberculosis 111:20–30. https://doi.org/10.1016/j.tube.2018.04.008
93. Sasahara K, Shimokawa Y, Hirao Y et al (2015) Pharmacokinetics and metabolism of delamanid, a novel anti-tuberculosis drug, in animals and humans: importance of albumin metabolism in vivo. Drug Metab Dispos 43:1267–1276. https://doi.org/10.1124/dmd.115.064527
94. Shibata M, Shimokawa Y, Sasahara K et al (2017) Absorption, distribution and excretion of the anti-tuberculosis drug delamanid in rats: extensive tissue distribution suggests potential therapeutic value for extrapulmonary tuberculosis. Biopharm Drug Dispos 38:301–312. https://doi.org/10.1002/bdd.2064
95. Shimokawa Y, Sasahara K, Koyama N et al (2015) Metabolic mechanism of delamanid, a new anti-tuberculosis drug, in human plasma. Drug Metab Dispos 43:1277–1283. https://doi.org/10.1124/dmd.115.064550
96. Shimokawa Y, Sasahara K, Yoda N et al (2014) Delamanid does not inhibit or induce cytochrome P450 enzymes in vitro. Biol Pharm Bull 37:1727–1735. https://doi.org/10.1248/bpb.b14-00311
97. Rustomjee R, Zumla A (2015) Delamanid expanded access novel treatment of drug resistant tuberculosis. Infect Drug Resist 8:359–366. https://doi.org/10.2147/IDR.S62119
98. Diacon AH, Dawson R, Hanekom M et al (2011) Early bactericidal activity of delamanid (OPC-67683) in smear-positive pulmonary tuberculosis patients. Int J Tuberc Lung Dis 15:949–954. https://doi.org/10.5588/ijtld.10.0616
99. Gler M, Skripconoka V, Sanchez-Garavito E (2012) Delamanid for multidrug-resistant pulmonary tuberculosis. N Engl J Med 366:2151–2160
100. Matsumoto M, Hashizume H, Tomishige T et al (2006) OPC-67683, a nitro-dihydro-imidazooxazole derivative with promising action against tuberculosis in vitro and in mice. PLoS Med 3:2131–2144. https://doi.org/10.1371/journal.pmed.0030466
101. Pang Y, Zheng H, Tan Y, Song Y, Zhao Y (2017) In vitro activity of bedaquiline against nontuberculous mycobacteria in China. Antimicrobial Agents Chemother 61(5):e02627-16
102. Liu Q, Ma A, Wei L et al (2018) China's tuberculosis epidemic stems from historical expansion of four strains of *Mycobacterium tuberculosis*. Nat Ecol Evol 2:1982–1992. https://doi.org/10.1038/s41559-018-0680-6
103. Stinson K, Kurepina N, Venter A et al (2016) MIC of delamanid (OPC-67683) against *Mycobacterium tuberculosis* clinical isolates and a proposed critical concentration. Antimicrob Agents Chemother 60:3316–3322. https://doi.org/10.1128/AAC.03014-15
104. Hoffmann H, Borroni E, Schena E et al (2016) Delamanid susceptibility testing of Mycobacterium tuberculosis using the resazurin microtitre assay and the BACTEC™ MGIT™ 960 system-authors' response. J Antimicrob Chemother 71:3625. https://doi.org/10.1093/jac/dkw365
105. Alsultan A, Peloquin CA (2014) Therapeutic drug monitoring in the treatment of tuberculosis: an update. Drugs 74:839–854. https://doi.org/10.1007/s40265-014-0222-8
106. Maller R, Ahrne H, Holmen C et al (1993) Once- versus twice-daily amikacin regimen: efficacy and safety in systemic gram-negative infections. J Antimicrob Chemother 31:939–948. https://doi.org/10.1093/jac/31.6.939
107. Peloquin CA, Hadad DJ, Molino LPD et al (2008) Population pharmacokinetics of levofloxacin, gatifloxacin, and moxifloxacin in adults with pulmonary tuberculosis. Antimicrob Agents Chemother 52:852–857. https://doi.org/10.1128/AAC.01036-07
108. Fields S (2013) Safety and efficacy of delamanid in the treatment of multidrug-resistant tuberculosis (MDR-TB). Clin Med Insights 5:137–149



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Immune Approaches in Tuberculosis Treatment

15

Dmytro Butov, Valeriy Myasoedov, Anton Tkachenko, and Tetiana Butova

If you change the way you look at things, the things you look at change.

Max Planck

Summary

Tuberculosis (TB) is a dangerous infectious disease caused by *Mycobacterium tuberculosis* (*M. tb*). Although approximately 90% of individuals are infected with *M. tb*, the disease develops only in 5–10% of the infected people. Other persons infected with *M. tb* remain healthy during their entire life, i.e., it can be assumed that the symbiotic host–pathogen interactions emerge to prevent not only TB but also other diseases. We believe that the host immune system is important in developing TB-associated inflammation and another response of this system to *M. tb* significantly contributes to TB development. Modulation of immunity via immunotherapeutic agents may balance host–pathogen interactions. Conventional chemotherapeutic anti-TB drugs aim at eliminating *M. tb*, whereas the imbalance in the immunopathological process should be modified by immunotherapy. Adjunctive immunotherapy may enhance the treatment

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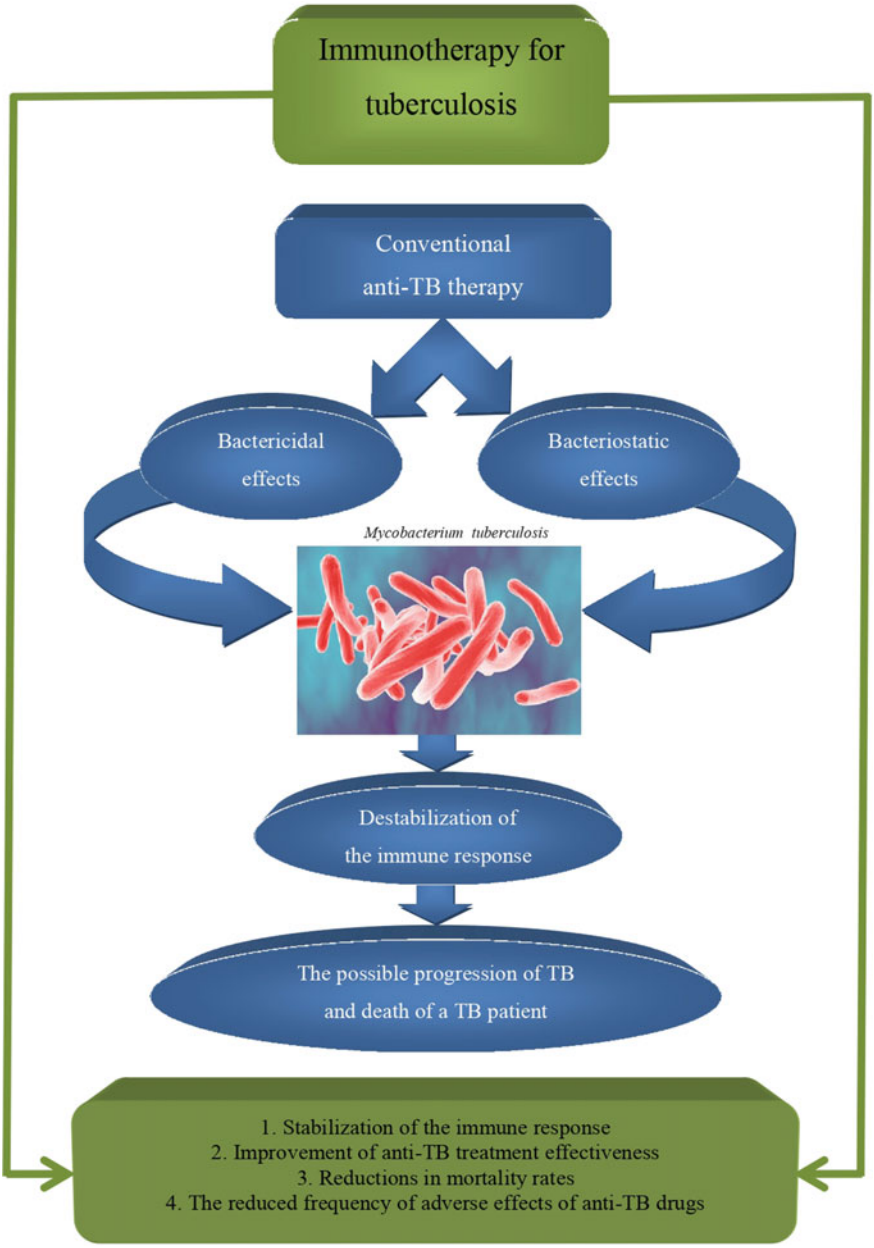
effectiveness, reduce the duration of chemotherapy, and improve the host immunity preventing TB relapses. This chapter deals with the most common types of immunotherapeutic strategies that have been used in clinical trials.

Graphical Abstract



Immunotherapy for tuberculosis (TB)

Keywords

Corticosteroids · Cytokines · Dexamethasone · DNA vaccine · Extensively drug-resistant · Immunomodulators · Immunity · Immunotherapy · Interferons · Interleukins · Multidrug-resistant · *Mycobacterium tuberculosis* · Prednisolone · TB · Tuberculosis · Vaccine · Vitamin D

1 Introduction

Tuberculosis (TB) is a global problem of mankind, which is one of the most common causes of death worldwide [1]. The drug-resistant (DR) forms of *Mycobacterium tuberculosis* (*M. tb*) are one of the main problems for the End TB Strategy of the World Health Organization (WHO) to eliminate TB, which aims to reduce the incidence of TB by 2035 [2]. The main common and most dangerous forms of resistant TB are multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB). MDR-TB is due to *M. tb* strains resistant to the most effective drugs for the treatment of TB, namely rifampicin and isoniazid [3, 4]. XDR-TB is caused by *M. tb* strains which are resistant not only to isoniazid and rifampicin but also to a drug from the fluoroquinolone group and one additional group A drug as defined by WHO [5]. XDR-TB and MDR-TB are characterized by lower treatment effectiveness, the need for increased treatment duration, and more prevalence of side effects to anti-TB drugs, as compared with susceptible pulmonary TB (PTB) [6, 7].

Chemotherapy remains an optimal therapeutic approach for TB patients [8]. However, drugs for anti-TB chemotherapy can lose their efficiency over time due to the development of DR mutants. For example, the recently introduced drugs delamanid and bedaquiline are becoming less effective since cases with delamanid and bedaquiline resistance have already been reported [9, 10]. It is important to note that it took ten years for these drugs to be approved and enter the market, and during that period, no other novel anti-TB drugs have become available for patients [11]. This situation has probably emerged since pharmaceutical companies are not particularly interested in developing anti-TB drugs due to high drug development costs, time-requiring drug testing, low profits, rapid resistance development, and other reasons. Furthermore, the treatment effectiveness should also depend on the combined therapeutic action, for example, on the immunity [12, 13]. Some authors emphasize that the role of immune therapy in TB treatment is rather limited compared with chemotherapy [8]. However, there is evidence that the human immune response is crucial for determining the infection outcome, i.e., complete clearance of the pathogen, reconvalescence, or relapse [4, 14]. Furthermore, the susceptibility of HIV-infected persons to PTB infection highlights the role of immunity in TB development [15]. Immune cells involved in the antimycobacterial

immune response are affected by HIV, which explains the high incidence of TB among HIV-infected individuals (50%) and the fact that TB is a leading cause of death in patients with HIV infection [1]. It is worth mentioning immunological parameters have prognostic values for treatment effectiveness in TB-HIV co-infection [16].

One-third of the population is infected with *M. tb*, and this parameter reaches 90% in countries with high TB prevalence (Ukraine, Russia, India, China, etc.). However, the disease develops only in 10% of *M. tb*-infected individuals [17, 18]. Therefore, healthy *M. tb*-infected people exist in an immunological symbiosis with *M. tb* without the development of TB.

The host–pathogen interactions result in a complex immune response whose intensity and efficiency are dependent on multiple factors. The survival and reproduction of mycobacteria in the body and the release of pathogen antigens from cells are balanced by evolutionary immune-escaping mechanisms developed by *M. tb* and host immunological mechanisms that implicate cells of monocyte/macrophage lineage, lymphocytes, and granuloma formation [19]. The immune response can significantly affect the amount of replicating microorganisms and, hence, the disease outcome [20]. The knowledge of the nature of interactions between the host and pathogen underlies the development of anti-TB immunotherapeutic strategies.

It is important to note that *M. tb* primarily infects airway epithelial cells, dendritic cells, alveolar macrophages, and monocytes [21]. The effective elimination of *M. tb* by the cells mentioned above is crucial for infection abortion. Thus, *M. tb*-host immune cell interactions are of paramount importance for determining whether the disease develops or early clearance occurs. The survival of *M. tb* within macrophages is mediated by the phagosome-lysosome fusion (PLF) [22, 23]. The phagosomal vacuole should fuse with lysosomes during phagocytosis to provide *M. tb* destruction under the action of low pH values and lysosomal hydrolytic enzymes. In addition to *M. tb* destruction, this process results in the formation of mycobacterial antigens, which are presented to T cells via major histocompatibility complex (MHC) type II molecules [24]. PLF is prevented by *M. tb* mediating the latent infection. During its evolution, *M. tb* has developed several strategies to avoid PLF and enable its persistence inside immune cells. These strategies allow the pathogen to manipulate macrophages instead of killing them. Given that *M. tb* is an intracellular microorganism, it is hidden and cannot be recognized by other immune cells, which fail to destroy it. Therefore, *M. tb* has the time to reproduce inside the cell until the next step of immunological reaction occurs.

In addition to PLF, the pathogen has developed other mechanisms responsible for its survival and reproduction in macrophages. In particular, *M. tb* can down-regulate antigen-presenting molecules, prevent cell apoptosis, induce macrophage necrosis, block oxidative damage, and inhibit autophagy [22, 23, 25].

Another important factor in TB pathogenesis is the phagosome disruption that promotes the release of *M. tb* into the cytosol. The cytosolic translocation of bacilli facilitates the development of infection via several mechanisms, including NLRP3 inflammasome activation, type I IFN secretion, nucleotide-binding oligomerization

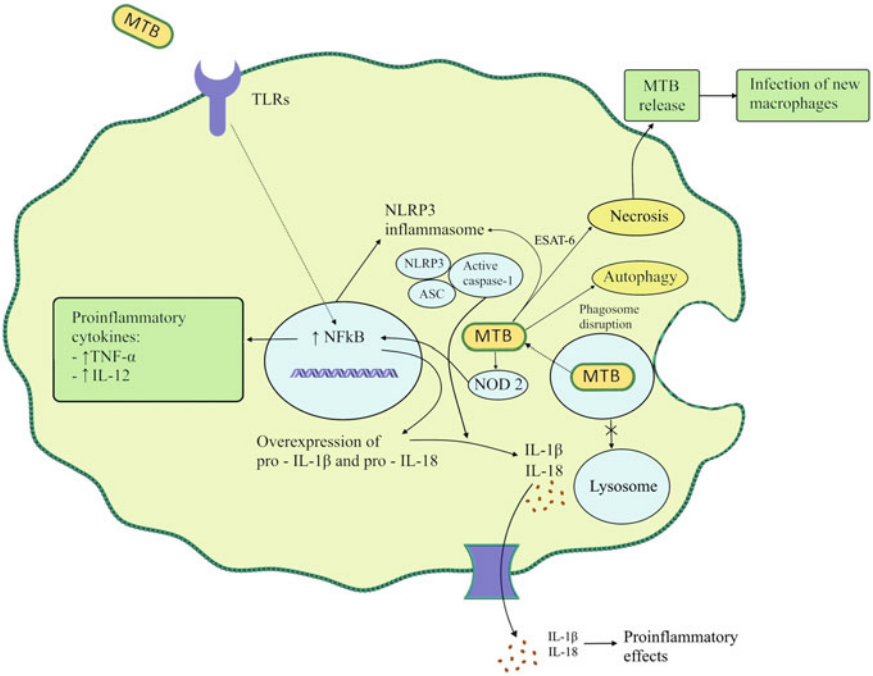


Fig. 1 *Mycobacterium tuberculosis* (*M. tb*) infects a macrophage. Initially, it resides inside a phagosome. *M. tb* prevents phagosomal-lysosomal fusion and its destruction by lysosomal enzymes. Instead, *M. tb* promotes the phagosomal rupture with the subsequent translocation of the pathogen in the cytosol. In the cytosol, it binds to NLRP3, activating the formation of the inflammasome, which is accompanied by the activation of caspase-1 and caspase-1-mediated cleavage of IL-1β and IL-18 precursors. The binding of *M. tb* to NOD2 activates the synthesis of pro-inflammatory cytokines, which are also upregulated when extracellular *M. tb* interacts with TLRs. In addition, intracytosolic *M. tb* activates necrosis to provide the spread and infection of new immune cells. The pathogen blocks autophagy, which is protective for the host

domain-containing protein 2 (NOD2) signaling, and autophagy suppression [26]. NLRP3 inflammasome is activated via the ESAT-6 secretion system-1 (ESX-1), which is believed to be a major virulence determinant in *M. tb*. ESX-1-mediated NLRP3 inflammasome assembly results in the proteolytic activation of caspase-1, which cleaves pro-IL-1β and pro-IL-18 to produce active pro-inflammatory cytokines released from the infected macrophages (Fig. 1). It is worth mentioning that ESX-1 stimulates NLRP3 inflammasome activation by promoting potassium ion efflux [26, 27]. Moreover, ESX-1 mediates the necrosis of macrophages (Fig. 1). This highly pro-inflammatory mode of cell death is necessary for the release of *M. tb* from the macrophages to infect new cells [28].

The phagosome disruption also promotes type I IFN release from the infected cells. The leakage of mycobacterial components into the cytosol, in particular extracellular DNA, activates the STING-TBK1-IRP3 signaling axis, which drives

the expression of type I IFNs [29]. When the pathogen is translocated to the cytosol and, thus, enters the intracellular environment, its structural fragments become exposed to pattern recognition receptors (PRRs). PRPs are components of the innate immune system, whose ligands are pathogen-associated molecular patterns (PAMPs), including mycobacterial nucleic acids and fragments of the cell wall [25, 30, 31]. Toll-like receptors (TLRs) and NOD2 protein are among the most widely studied PRRs involved in the antimycobacterial immune response. Several studies have demonstrated the association between TLRs and NOD2 polymorphisms and susceptibility to TB [32, 33]. TLRs, especially TLR2 and TLR4, are responsible for triggering the adaptive immunity to *M. tb* [34]. TLRs are expressed primarily on the surface of immune cells and are responsible for recognizing extracellular mycobacterial antigens. In particular, TLR2 and TLR4 signaling induced by mycobacterial PAMPs, such as cell wall glycolipids, promotes activation of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), its translocation to the nucleus and subsequent upregulation of pro-inflammatory cytokines, primarily tumor necrosis factor-α (TNF-α) [35]. In addition, TLR signaling promotes the intracellular synthesis of calcitriol, an active form of vitamin D, in macrophages. Calcitriol shows mycobactericidal effects and promotes anti-*M. tb* immune response [36]. This allows considering vitamin D supplementation for a combined pulmonary TB treatment.

In contrast to TLRs, NOD2 is an intracellular protein receptor. It is expressed in monocyte/macrophage lineage cells and is activated upon exposure to bacterial components. NOD2 downstream effects include the induction of NF-κB and the release of cytokines [37]. After the phagosome disruption and *M. tb* translocation to cytosol, NOD2 recognizes and binds to the components of mycobacterial cell wall peptidoglycans such as muramyl dipeptide. This leads to activation of NF-κB transcriptional factors and NF-κB-mediated upregulation of cytokines, including TNF-α, IL-1β, and IL-12 [25, 38]. TNF-α and IL-12 derived from macrophages induce IFN-γ secretion by T lymphocytes and NK cells. This cytokine plays a pivotal role in antimycobacterial immunity suggesting its implication in TB immunotherapy. IFN-γ promotes phagosome maturation in the infected macrophages, improves antigen-presentation, and activates host autophagy [23, 25]. Autophagy is a defense mechanism developed in eukaryotic cells against *M. tb*. This process in *M. tb*-infected immune cells is activated in response to the translocation of *M. tb* into the cytosol via its ESX-1 system [39]. In addition, mycobacterial DNA interacts with the STING protein mentioned above, which results in ubiquitination of bacterial components followed by ubiquitin recognition by autophagy adaptor molecules and autophagy activation [40].

As outlined above, it is obvious that the cell-mediated immunity significantly contributes to the *M. tb*-induced immune response. It is mediated primarily by CD4 and CD8 T cells [19]. CD4 T lymphocytes can produce Th1 cytokines, including TNF-α, IFN-γ, and IL-2 [41]. IL-2 is of crucial importance for the differentiation of T lymphocytes. It is a pleiotropic cytokine responsible for promoting NK cell cytotoxic properties and the formation of memory T cells [42, 43]. A growing body of evidence suggests that the continuous antigen stimulation in TB results in T cell

exhaustion associated with the loss of T cell function [42]. This is accompanied by the reduced cytotoxicity of T lymphocytes, inadequate secretion of Th1 cytokines (primarily IL-2, but also TNF-α and IFN-γ), and insufficient proliferation of T cells. Thus, IL-2 and IFN-γ are promising adjunctive immunotherapeutic agents for TB treatment.

In contrast to cell-mediated immunity, the impact of antibody-mediated immunity is low in TB. This is explained by the fact that the beneficial effects of antibodies are limited due to the intracellular localization of mycobacteria [44]. Humoral immunity does not determine the disease outcome in TB patients.

However, immunosuppression does not seem to be optimal for PTB development, evidenced by the major immunological tests used in TB (tuberculin and IFN-γ release assay). These tests are based on immunological hypersensitization. It should be noted that these tests allow detecting the positive or pronounced immunological reaction to the pathogen, and, in this case, severe TB is usually not observed. *M. tb* provokes a constant response to bacillary antigens, and its intensity does not depend on the number of viable mycobacteria in patients [45]. Thus, immunotherapy should prevent host tissue damage by promoting an adequate and balanced immune response instead of reinforcing the inflammatory reaction. In particular, TNF-α, whose role in anti-*M. tb* response can hardly be overestimated, acts as a double-edged sword, and promotes tissue damage [46]. This cytokine regulates tissue remodeling and the expression of tissue-degrading enzymes. In addition, significantly elevated concentrations of TNF-α can promote macrophage necrosis, which is highly pro-inflammatory due to the release of damage-associated molecular patterns (DAMPs) in the extracellular space due to the loss of membrane integrity. This contributes to pulmonary parenchyma damage and the formation of cavities in the lungs [47]. TNF-α inhibitors have been reported to prevent such unfavorable effects.

Modern chemotherapy has bacteriostatic and bactericidal effects. It alters the immunological status of TB patients. Thus, the task of restoring the immune system to the level it used to be prior to the disease cannot be achieved by only chemotherapy without administering adjunctive immunotherapeutic agents [48].

Since the TB course and outcome are determined by the patient's immune response, biological properties of the pathogen (genotype, susceptibility, and resistance to drugs), and its interactions with the host immune system, it is of huge importance to take into account all the factors outlined above when developing the methodology of personalized prevention and immunomodulatory treatment in PTB as a component of combined chemotherapy-based anti-TB treatment [18]. Thus, we believe that the altered immune response in TB is an indication of immunotherapeutic interventions that can improve the effectiveness of anti-TB treatment [12, 49].

2 Cytokines and Their Inhibitors as Adjunctive Immunotherapeutic in TB

There is strong evidence that TB is characterized by the prevalence of cell-mediated immunity with the implication of cytokine-secreting T cells [20]. Th1 cells are among the crucial players in the *M. tb*-induced immune response [50]. It is believed that the hallmark of TB is the inability to eliminate or restrain the pathogen due to the inadequate host cell-mediated immune response [51]. Thus, one of the strategies for cytokine-based adjunct immunotherapy in TB is to modulate the immunity by promoting Th1 cytokine action (IFN-γ, IL-2, and IL-12) [52]. On the other hand, excessive secretion of pro-inflammatory cytokines may promote damage to the pulmonary tissue, such as the formation of cavities and fibrosis development, in TB patients [47]. Thus, prevention of cytokine-mediated tissue damage seems to be the second approach in cytokine-based adjunctive TB immunotherapy [52]. Indeed, the effectiveness of TNF-α blockers in immunotherapy against TB has been studied since recently. The TNF-α-driven immune response is of paramount importance in TB patients. TNF-α has been reported to enhance phagocytosis in macrophages, as well as promote intracellular destruction of *M. tb* in a reactive oxygen species (ROS)- and reactive nitrogen species (RNS)-dependent manner and induce apoptosis of *M. tb*-infected macrophages to restrict the release of *M. tb* and infection of new macrophages [53, 54]. The protective role of TNF-α in TB patients is supported by the observations of higher risks for TB development against the background of anti-TNF-α-based treatment of rheumatic diseases and inflammatory bowel disease (IBD) [55]. However, TNF-α overgeneration can be detrimental because it can cause pulmonary tissue damage in TB patients.

Promising results of cytokine-based TB immunotherapy were initially demonstrated in rodent models. Aerosolized IFN-γ has been shown to have bacteriostatic effects against *M. tb,* reducing its growth in *M. tb*-infected mice [56]. In addition, an animal experiment has confirmed that IL-12 can reduce mortality of *M. tb*-infected mice, decrease the amount and size of granulomas in the lungs of animals, as well as stimulate the synthesis of antimycobacterial IFN-γ [57]. Data collected using murine models show that recombinant IL-2 has antimycobacterial effects [58, 59]. Administration of TNF-α inhibitors against the background of chemotherapy was reported to enhance *M. tb* clearance in a murine model of TB [60]. Encouraging results of animal studies are supplemented by the fact that IL-2- and IFN-γ-deficient mice are more susceptible to TB [61, 62].

However, literature data analysis has revealed that human recombinant IL-2, IFN-γ, IL-12, and TNF-α inhibitors show controversial results. The number of randomized trials that have analyzed the effectiveness and safety of human recombinant cytokines and their inhibitors in adjunctive immunotherapy in TB is limited. It is important to note that most studies use the following criteria for assessing the cytokine-based anti-TB treatment: proportion of sputum smear and culture conversion, radiographic improvements, changes in clinical manifestations, and weight gain. The difference in assessing approaches may cause biases when

evaluating the results of clinical trials. Moreover, various doses of recombinant cytokines used in the clinical studies, their origin, route of administration, duration of treatment, and features of conventional anti-TB therapy regimens impede the comparison of the results.

Data obtained from clinical studies suggest that recombinant IL-2 can increase the rate of sputum culture and smear conversion among TB patients [41, 63, 64]. However, a randomized trial performed by Johnson et al. has revealed that IL-2-based immunotherapy results in neither changes in clinical symptoms nor bacillary clearance improvements in individuals with drug-susceptible TB [65]. In addition, recombinant IL-2 administration has no effects on X-ray parameters of TB patients in most cases [41].

Clinical studies have proven that immunotherapy with recombinant human IFN-γ benefits TB patients [66–68]. IFN-γ has been shown to promote sputum conversion, alleviate the clinical course of the disease, and provide chest X-ray improvements [67–70]. Furthermore, adjunctive immunotherapy with IFN-γ was well tolerated by patients and was not associated with serious adverse effects [68].

Wallis et al. reported that TNF-α inhibitors were safe, promoted sputum conversion, and increased CD4 cell count in HIV-infected patients with TB [71]. In addition, anti-TNF-α antibodies could alter the granuloma integrity facilitating the action of anti-*M. tb* antibiotics and, hence, improving the bacillary clearance [72]. Despite the reported favorable effects of anti-TNF-α adjunctive immunotherapy, TNF-α blockers suppress the host antimycobacterial immune response, which impedes the determination of the harm-benefit balance of their administration as adjunctive immunotherapeutic agents in TB patients. This hypothesis is supported by a higher TB incidence against the background of TNF-α blocker-based treatment strategies in patients with IBD and rheumatic diseases.

It is worth mentioning that the route of administration significantly affects the efficiency of cytokines in immunotherapy against TB. Aerosol delivery seems to be the most efficient [56, 67]. Analysis of available data indicates that cytokine-based adjunct immunotherapy is promising in the combined treatment of TB patients. However, it has certain limitations. First, large clinical trials with a greater sample size should be performed to assess the effectiveness of cytokines and their antagonists, as well as determine the optimal doses and treatment duration, evaluate both short-term and long-term adverse effects, and propose the best optimal combinations with conventional anti-TB chemotherapeutic drugs. In addition, therapeutic modulation of the antimycobacterial host immune response is a complicated task due to the complex networks of interactions between cytokines and immunocompetent cells in TB immunopathogenesis. Since cytokines may act as double-edged swords, the outcome of therapeutic interventions with them or their blockers in TB patients can hardly be predicted.

3 Immunotherapeutic Vaccines Against Tuberculosis

Immunotherapeutic vaccines against TB imply the administration of mycobacterial components to boost the specific anti-*M. tb* immune response in patients with manifestations of TB to improve the treatment outcome. Several approaches have been used as immunotherapeutic vaccines against TB. The history of immunotherapeutic vaccines dates back to the early twentieth century when Friedrich Franz Friedmann developed a vaccine based on *Mycobacterium chelonae* isolated from the pulmonary tissue of turtles. This vaccine proved to be effective for TB treatment in both children and adults [73]. Other mycobacterial species such as *M. indicus pranii (M. w)*, *M. vaccae*, and *M. bovis* have been reported to be used to develop immunotherapeutic vaccines. In particular, an *M. indicus pranii*-based vaccine action aims at inducing pro-inflammatory cytokines (IFN-γ, IL-2, IL-12, and TNF-α) and downregulating anti-inflammatory IL-10 and pro-fibrotic transforming growth factor-beta (TGF-β), regulating the balance between pro-inflammatory and anti-inflammatory cytokines to promote the immune response in TB patients [74]. An *M. vaccae*-based vaccine has shown its clinical and radiographic effectiveness in some clinical trials [75–77]. In addition, the standard Bacillus Calmette–Guérin (BCG; *M. bovis*), which is used for vaccination of non-infected individuals, has been tested as an adjunctive therapy to improve the effectiveness of chemotherapy and to prevent MDR-TB development [78].

M. tb bacteria are also promising candidates for developing new therapeutic vaccines. In particular, RUTI is a therapeutic vaccine prepared from non-viable, detoxified *M. tb* components encapsulated in liposomes. There is evidence that this vaccine can reduce the bacterial load in animal models. Furthermore, it is safe and immunogenic for healthy adults and patients with latent TB. However, its effectiveness in TB patients is still under investigation [79].

Another relatively novel approach that can be successfully used for TB adjunctive therapy is gene-based vaccination. DNA vaccines against TB contain the genes of selected mycobacterial antigens inserted into a bacteria-derived plasmid vector. Over 60 mycobacterial genes have been considered candidates to be included in immunotherapeutic DNA vaccines against TB. However, plasmid DNA vaccines encoding heat shock protein 65 (HSP65), ESAT-6, antigen 85A (Ag85A), and antigen 85B (Ag85B) are the most common and widely studied, at least using experimental animal models [80]. In addition, combined chimeric immunotherapeutic DNA vaccines expressing two mycobacterial components have been demonstrated to be promising as adjuncts to conventional chemotherapy [81]. DNA vaccines expressing *M. tb* antigens can induce the cellular Th1-mediated and CD8 T-cell-mediated antimycobacterial immune response [80]. In particular, a DNA vaccine that encodes HSP65 of *M. leprae* has been proven to activate lymphocytes and show immunoregulatory properties in healthy individuals and TB patients, making it a valuable adjunctive immunotherapeutic agent [82]. Moreover, it has been shown in vitro that a DNA vaccine expressing *M. tb*-derived Ag85A can stimulate both humoral and cellular Th1-mediated immune responses [83]. DNA

vaccines encoding mycobacterial ESAT-6, Ag85A, and Hsp65 have positive effects in animal models [84–86]. Besides, controversial findings have been observed in studies of chimeric Ag85A/ESAT-6 DNA vaccines. There is evidence that they are beneficial and can improve immune response in TB [87], whereas the findings of the other study indicate that this chimeric vaccine increases the mortality of TB-infected mice [81]. According to the latter study's authors, ESAT-6 cannot even be used for adjunctive immunotherapeutic vaccines [81].

All the immunotherapeutic vaccines mentioned above are intended to be administered as adjuncts to the standard anti-TB chemotherapy. Despite the first promising results of their administration to laboratory animals, further research is required to assess their effectiveness and safety for healthy individuals and TB patients in detail. However, some immunotherapeutic vaccines are already suitable for clinical trials that allow evaluating their effects on patients with TB and implementing them in clinical practice.

4 Corticosteroids as Adjunctive Immunotherapeutic Agents for Tuberculosis Treatment

Tissue damage with the formation of caseous masses is typical for caseous pneumonia, military TB, and tuberculous meningitis (TBM). They are formed not only as a result of the pathogen's activities but also due to the self-destructive immune response caused by the altered host–pathogen interactions [88]. Such inflammation-induced tissue damage may provoke organ dysfunction. Thus, the anti-inflammatory properties of corticosteroids (CSs) can be used to alleviate the negative consequences of TB-associated inflammation [89]. In addition, CSs can be used to reduce inflammation due to their immunosuppressant effects. CSs are known to exert anti-inflammatory and immunosuppressant actions via several mechanisms. Their intracellular effects are mediated through binding to the cytosolic glucocorticoid receptor (GR). As a result, the glucocorticoid (GC)-GR complex is translocated to the nucleus and affects the expression of anti-inflammatory proteins at the transcriptional level. It has been reported that annexin 1, IL-10—an anti-inflammatory cytokine—, and IkB-alpha—a regulatory protein that inhibits the pro-inflammatory transcriptional factor (NF-κB)—are upregulated in response to the GC-GR complex DNA binding [90]. In addition, GCs can directly inhibit the DNA-binding capacity of NF-κB, preventing the expression of pro-inflammatory NF-κB target genes and, hence, reducing inflammation [91]. Furthermore, it has been recently reported that CSs can prevent *M. tb*-induced necrosis of host macrophages by activating mitogen-activated protein kinase phosphatase 1 (MKP-1) that dephosphorylates p38 MAPK [92]. Necrosis inhibition prevents the release of *M. tb* into the extracellular space and, thus, infection of new macrophages. Moreover, given the lytic nature of this cell death mode, necrosis prevention reduces the release of DAMPs that activate *M. tb*-induced inflammation.

There is evidence that CSs can increase the survival of TB patients [93, 94]. However, there is a controversy. TB develops in the context of pathological immunity. Thus, the question that arises is how CSs-mediated immunosuppression can improve the outcome of the disease. We believe that TB is associated with the abnormal host-*M. tb* interactions and tissue damage is provoked by the host immune system. TB-associated lung injury includes the development of cavitation and fibrosis. The key role in the emergence of cavitation is played by matrix metalloproteinases (MMPs), especially MMP-1 and MMP-9. They are proteolytic enzymes responsible for the degradation of extracellular matrix components and tissue remodeling. Overexpression of MMP-1 and MMP-9 in TB patients is observed in response to the action of pro-inflammatory cytokines TNF-α and IL-1β via an NF-κB-dependent way, as well as hypoxia that acts through a hypoxia-inducible factor 1α (HIF-1α)-mediated manner. In addition to their role in cavitation formation, TNF-α and IL-1β have fibrogenic properties and, along with TGF-β, significantly contribute to fibrosis [47]. Pulmonary tissue damage in TB is also mediated by TB-specific CD4 cells that generate IL-1β and IFN-γ and neutrophils [47, 95]. Thus, CSs seem to be able to reduce lung injury in TB. However, it has been reported that the administration of CSs in PTB is doubtful and more studies are required to assess their effectiveness despite some reports on the positive effects of CSs in patients with TB [96]. In particular, CSs have been shown to cause radiographic improvements in PTB [97].

There is accumulating evidence that CSs reduce TBM mortality [93, 94] without affecting the survival rate in TB pericarditis [98]. However, some data indicate that this group of anti-inflammatory drugs is effective in TB pericarditis [89]. It has been demonstrated that CSs are of limited effectiveness in TB exudative pleurisy at the intensive phase of treatment with the insignificant reduction of the risk for developing pleural thickening or adhesions [99]. However, another study shows that prednisolone decreases the risk of having severe radiographic adverse events or functional consequences in patients with TB pleurisy [100].

CSs are recommended as adjuvant immunotherapeutics for children who have TB of mediastinal lymph nodes complicated by stenosis and atelectasis against the background of endobronchial obstruction [89].

Given the prevalence of TB among HIV-infected patients, it is important to mention that the treatment with CSs in such patients has certain peculiarities. The evaluation of steroid treatment effectiveness in HIV infection and TBM is under debate [101]. There is some evidence that CSs increased the risk for developing Kaposi's sarcoma in HIV-infected individuals with TB pleurisy. Thus, GCs are recommended not to be added to the combined treatment of such patients [102]. Furthermore, short-term steroid therapy of TB and HIV co-infection results in immune activation, an increase in CD4 cell count, preservation of the immune function in these patients, as well as a short-term enhancement of viral load [103].

Collectively, these data indicate that the effectiveness of CSs in TB is controversial, which raises the question of the feasibility of their administration. However, dexamethasone remains the only approved adjunctive immunotherapeutic agent with the greatest clinical experience of its administration [92]. In addition, our

clinical experience suggests that CSs reduce mortality, improve radiographic parameters, and accelerate the treatment in patients with severe and disseminated PTB (caseous pneumonia) and TBM, despite the high percentage of disability caused by TB due to the disseminated process. Furthermore, it is difficult to assess the effectiveness of CS-based immunotherapy due to heterogeneous designs of studies. In particular, clinical trials aimed at evaluating the effects of CSs in the combined TB therapy have been reported to have insufficient sample sizes, various endpoints (weight gain, radiographic improvement, sputum smear, culture conversion, adverse effects, etc.), and different basic TB treatment regimens [90].

5 Vitamin D Adjunctive Treatment in Tuberculosis

Antimycobacterial and immunomodulatory properties of vitamin D (ergocalciferol-D2 and cholecalciferol-D3) have been reported for decades [104]. There is strong evidence that altered vitamin D status is associated with a higher susceptibility to TB [105–107]. The immunomodulatory effects of vitamin D are attributed to its active hydroxylated form calcitriol (1,25-dihydroxycholecalciferol or 1,25-(OH)2D3)). Vitamin D of either exogenous (dietary) or endogenous (produced in the skin under the influence of sunlight) origin is metabolized to 1,25-(OH)2D3 by two consecutive hydroxylation reactions catalyzed by hepatic vitamin D 25-hydroxylase and renal 1α-hydroxylase. In addition to its well-recognized implication in calcium and phosphate metabolism regulation, 1,25-(OH)2D3 affects immunity. Cells of macrophage/monocyte origin, including macrophages and dendritic cells, express 1α-hydroxylase and, thus, are capable of synthesizing calcitriol [105]. Its intracellular effects in immunocompetent cells are mediated by binding to vitamin D receptors (VDR). The calcitriol-VDR complex is a transcriptional factor that upregulates the corresponding target genes such as cathelicidin (LL-37) and β-defensins [106, 108, 109]. Besides the direct antimycobacterial activity of LL-37, this cationic antimicrobial host defense protein can induce the expression of cytokines modulating the immune response [110]. Moreover, LL-37 has been reported to block the PLF, critical for *M. tb* elimination within macrophages [109].

It is important to mention that TLR signaling and IFN-γ promote upregulation of both 1α-hydroxylase and VDR [36, 105], while 1,25-(OH)2D3, in its turn, blocks NF-κB activation and, therefore, NF-κB-induced overexpression of TNF-α, IFN-γ, and IL-12 by T lymphocytes and macrophages [105, 111]. Thus, 1,25(OH)2D3-VDR-mediated downregulation of the pro-inflammatory cytokines reduces inflammation and prevents host tissue damage facilitating the resolution. In addition, vitamin D may reduce inflammation by downregulation of TLR-2, TLR-4 and, therefore, TLR-mediated production of pro-inflammatory cytokines [112]. Compelling evidence indicates that 1,25(OH)2D3 promotes autophagy in *M. tb*-infected macrophages, including in an LL-37-dependent manner [105, 113, 114].

Vitamin D has shown synergistic effects with a conditionally essential amino acid L-arginine [105]. This amino acid is a precursor of biologically active nitric oxide (NO), which plays an important role in the antimycobacterial defense of macrophages. NO generated by inducible NO-synthase (iNOS) from L-arginine in macrophages is converted to peroxynitrite radical (ONOO) interacting with superoxide ion (O_2^-). Peroxynitrite is a highly pro-oxidant RNS, which along with ROS produced by NADPH-oxidase, is formed in the phagosome of *M. tb*-infected phagocytic cells to promote oxidative burst and oxidative damage to mycobacteria [115]. However, the efficiency of oxidative burst is limited in *M. tb*-infected macrophages since several protective mechanisms have evolved in *M. tb* that can help the pathogen to avoid RNS- and ROS-mediated destruction [116].

iNOS in immune cells is upregulated under a plethora of pro-inflammatory stimuli in TB [117]. In addition, there is accumulating evidence that 1,25(OH)2D3 can promote iNOS induction in *M. tb*-infected macrophages contributing to NO-mediated mycobacteriostatic and mycobactericidal effects [118]. Moreover, it has been shown that TB is accompanied by reduced NO bioavailability [119, 120]. Thus, complex vitamin D and L-arginine supplementations may be beneficial for TB patients. Nevertheless, clinical studies have demonstrated that neither L-arginine nor vitamin D administration benefits in TB [120].

However, the results of several trials summarized in meta-analyses indicate that vitamin D supplementation increased the percentage of sputum smear and culture conversion [121, 122]. In addition, vitamin D intake by TB patients results in gaining weight, reducing inflammation, and elevating IFN-γ concentrations [106]. It should be noted that these data are inconsistent with other studies, which have demonstrated neither radiographic improvements nor IFN-γ overproduction [120, 123, 124]. Some trials show that vitamin D adjunctive immunotherapy has been associated with no serious adverse events in TB [122, 125–127].

Despite controversial clinical data on vitamin D efficacy, we believe that vitamin D has positive immunomodulatory and clinical effects in TB patients. In addition, it is cost-effective. Thus, cholecalciferol supplementation is of limited importance for TB treatment, but it can be added to the combined anti-TB therapy as an immunotherapeutic agent in patients with compromised vitamin D status. TB may also promote vitamin D insufficiency, suggesting its supplementation requirements in TB [36]. Literature indicates that further studies are needed to elucidate the role of cholecalciferol in TB immunopathogenesis and its effectiveness in anti-TB adjunctive immunotherapy.

6 Other Immunotherapeutic Agents

In addition to those strategies of TB immunotherapy discussed above, the global pharmaceutical industry offers other approaches and groups of drugs that may improve the treatment outcome in TB patients. The findings of several clinical studies suggest that the V5 immunitor is well tolerated by TB patients and efficient

as an immunotherapeutic agent when used along with the standard chemotherapy [13, 128]. Butov et al. demonstrated that the V5 immunitor reduced the duration of treatment to one month [13]. This therapeutic vaccine was initially intended for patients with chronic hepatitis B and C since it is obtained from blood samples of patients with those infections after blood inactivation with chemicals and high temperature [129]. There is no data concerning the precise immunomodulatory effects of the V5 immunitor. However, it is tempting to speculate that some donors might have latent TB infection with circulating *M. tb* antigens, which could induce the immune response [130].

Another immunotherapeutic approach implies the use of mesenchymal stem cells (MSCs). These cells are mainly found in the bone marrow [131]. However, their localization is not restricted to the bone marrow, and MSCs are present in different body organs, including the pulmonary tissue [132]. It should be mentioned that MSCs have reparative features and can contribute to repairing tissue injuries [133]. A growing body of evidence suggests that immunological effects of MSCs are mediated by growth factors and prostaglandin E2 [134]. Administration of MSCs to patients with MDR-TB and XDR-TB during four weeks after the use of anti-TB has shown their safety [131].

Monoclonal antibodies to various microorganisms can enhance the immune response via several mechanisms, including toxin neutralization, opsonization, complement activation, cytokine release, antibody-dependent cytotoxicity promotion, and antigen presentation provision [135]. An immunotherapeutic approach in which monoclonal antibodies against *M. tb*-specific antigens are used has demonstrated controversial results [136, 137]. Such controversy may be associated with different types of monoclonal antibodies selected for the studies. It is worth noting that monoclonal antibodies isolated from BCG-vaccinated individuals have enhanced *M. tb* intracellular killing in macrophages and improved *M. tb*-specific cell-mediated immune response [135].

A combination of *quercetin* and *polyvinylpyrrolidone* that acts as a capillary stabilizing agent with antioxidant and immunomodulatory properties [49] results in the following morphological changes in a murine model of TB: caseous necrosis in granulomas was separated from unaffected areas, and infiltration with Langhans cells and lymphocytes was observed. In addition, a combination of *quercetin* and *polyvinylpyrrolidone* with anti-TB drugs has promoted fibrillization of epithelioid cellular tubercles and tissue remodeling with separation of TB granulomas from the intact tissue via the connective tissue [88, 138]. A clinical study demonstrates that the combination of quercetin and *polyvinylpyrrolidone* against the background of the conventional anti-TB therapy promotes clinical and radiographic improvements, including earlier closure of cavities and resolution of infiltrative and focal TB-associated changes in the lungs, as well as faster bacillary clearance in patients with the newly diagnosed drug-susceptible disease [139].

In addition, several plant-based herbal medications have been tested. One of them is Dzherelo or Immunoxel. Immunoxel is an alcohol-water phytoconcentrate of medicinal plants. It has demonstrated positive effects combined with the standard chemotherapy in clinical trials, promoting faster sputum conversion in TB patients.

Furthermore, Immunoxel is characterized by anti-inflammatory and hepatoprotective properties [12, 140].

A plethora of immunotherapeutic drugs against TB registered in Ukraine and other post-Soviet states can be used in combination with chemotherapy. This diversity of immunomodulatory agents is widely available for the therapy of TB patients. Most studies that focus on the effectiveness of immunomodulators in post-Soviet countries are published in Russian or Ukrainian and are available as clinical bulletins or manufacturer's instructions [141, 142]. Such diversity of immunomodulating agents in the post-Soviet states can be due to a high prevalence of DR-TB in this region, low effectiveness of treatment, and inadequate supply of resources required for the treatment of TB patients [7]. Compared to the post-Soviet world, administration of immunomodulators is less common in the Western countries due to a higher level of medical care, a lower amount of TB patients, especially those with MDR-TB, and greater treatment efficiency [48]. Demand creates its own supply. It explains why most Western researchers and clinicians are skeptical towards immunotherapy. They do not have experience administering this group of drugs, so they focus on chemotherapy [8]. Our clinical experience of TB immunotherapy depicted in the publications mentioned above indicates that adjunct immunotherapy along with standard chemotherapy can be beneficial for TB patients.

It is interesting to note that Mycobacterium-based vaccines such as the BCG vaccine and anti-TB vaccine (attenuated viable *M. bovis*) can improve the body's defense from a wide range of other infections and can be used even to treat bladder cancer [143]. This is explained by the symbiotic host-*M. tb* co-existence in which the host protects the pathogen from unfavorable environmental factors, while *M. tb* maintains the indirect immunological defense from causative agents of other diseases.

7 Conclusion

TB, especially MDR-TB and XDR-TB, as well as its co-infection with HIV, are a major public health burden. The future of novel chemotherapy regimens and the effectiveness of new anti-TB drugs seem to be rather obscure primarily due to the fulminant development of resistance to anti-TB agents. Skeptical attitude towards immunotherapy in the medical community is not currently uncommon, and chemotherapy is believed to have no alternative in TB treatment.

However, TB is a typical chronic infectious disease characterized by persistent inflammation with autoimmune components. This contributes to the alteration of immune response and enhancement of *M. tb*-specific inflammation, leading to irreversible consequences [48, 144]. Anti-TB therapy cannot improve the immunity and can even worsen the *M. tb*-associated immune response, which can result in the death of TB patients with wide dissemination of the process, such as caseous pneumonia and miliary TB. Analysis of papers and our personal clinical experience

indicate that immunotherapy against TB may alleviate clinical manifestations, fasten closure of cavities and the disappearance of focal and infiltrative changes, as well as reduce the time to sputum culture and smear conversion and the frequency of chemotherapy-associated adverse effects. These findings suggest that adjunct immunotherapy is a promising approach in TB treatment. Immunomodulating agents can stabilize the immune response, shortening the duration of chemotherapy despite not having antimycobacterial properties. However, clinicians should be careful when prescribing immunomodulating agents since it is difficult to predict their action, and they can be detrimental to TB patients altering the immune response. We believe that more well-designed clinical trials with a large number of patients enrolled may help to elucidate the effectiveness of adjunctive immunotherapeutic agents, their optimal doses, and possible beneficial combinations with chemotherapeutic drugs. In addition, this type of treatment seems to be suitable for personalized medicine when immune profiling can determine the feasibility and a way of adjunctive therapy in a particular case. In addition, there is accumulating evidence that adjunctive immunotherapy against TB is mainly well-tolerated and safe. Collectively, these data show that adjunct TB immunotherapy is promising for TB patients, including MDR-TB and XDR-TB. It can shorten the time of TB treatment, improve immunity, and prevent relapses. Further studies that focus on the search for optimal immunotherapeutic anti-TB agents are required.

Core Messages

- The outcome of TB depends on a complex host–pathogen interaction and is strongly linked with the host immune status.
- Adjunctive immunotherapy can modulate the host immune system and improve the treatment outcome.
- Immunotherapies can increase the effectiveness of treatment and reduce anti-TB therapy-related side effects.
- Intensified efforts are required to study the effectiveness of existing immunotherapeutic agents and to develop new ones.
- The standard anti-TB treatment strategies should be re-evaluated to provide a personalized approach to TB patients.

References

1. Global Tuberculosis Report 2019: WHO Report 2021 (2021) World Health Organization. https://www.who.int/publications/i/item/9789240037021
2. The End TB Strategy (2015) Global strategy and targets for tuberculosis prevention, care and control after 2015. World Health Organisation. https://www.who.int/tb/post2015_TBstrategy.pdf

3. Dudnyk A, Butov D, Crudu V, Lange C, Chesov D (2017) MDR-TB in Eastern Europe in the era of the TB elimination action framework. Int J Tuberc Lung Dis 21:2–3
4. Butov D, Myasoedov V, Gumeniuk M, Gumeniuk G, Choporova O, Tkachenko A, Akymenko O, Borysova O, Goptsii O, Ye V, Butova T (2020) Treatment effectiveness and outcome in patients with a relapse and newly diagnosed multidrug-resistant pulmonary tuberculosis. Med Glas (Zenica) 17(2):356–362. https://doi.org/10.17392/1179-20
5. WHO announces updated definitions of extensively drug-resistant tuberculosis (2021) World Health Organisation. https://www.who.int/news/item/27-01-2021-who-announces-updated-definitions-of-extensively-drug-resistant-tuberculosis
6. World Health Organisation consolidated guidelines on drug-resistant tuberculosis treatment (2019) World Health Organisation. https://apps.who.int/iris/bitstream/handle/10665/311389/9789241550529-eng.pdf?ua=1
7. Butov D, Lange C, Heyckendorf J, Kalmykova I, Butova T, Borovok N, Novokhatskaya M, Chesov D (2020) Multidrug-resistant tuberculosis in the Kharkiv Region, Ukraine. Int J Tuberc Lung Dis 24(5):485–491
8. Yew WW, Lange C, Leung CC (2011) Treatment of tuberculosis: update 2010. Eur Respir J 37(2):441–462
9. Nguyen TVA, Anthony RM, Bañuls AL, Nguyen TVA, Vu DH, Alffenaar JC (2018) Bedaquiline resistance: its emergence, mechanism, and prevention. Clin Infect Dis 66 (10):1625–1630
10. Fujiwara M, Kawasaki M, Hariguchi N, Liu Y, Matsumoto M (2018) Mechanisms of resistance to delamanid, a drug for *Mycobacterium tuberculosis* [published correction appears in Tuberculosis (Edinb). 2018 Mar 31]. Tuberculosis (Edinb) 108:186–194
11. Olaru ID, von Groote-Bidlingmaier F, Heyckendorf J, Yew WW, Lange C, Chang KC (2015) Novel drugs against tuberculosis: a clinician's perspective. Eur Respir J 45(4):1119–1131
12. Zaitzeva SI, Matveeva SL, Gerasimova TG, Pashkov YN, Butov DA, Pylypchuk VS, Frolov VM, Kutsyna GA (2019) Treatment of cavitary and infiltrating pulmonary tuberculosis with and without the immunomodulator Dzherelo. Clin Microbiol Infect 15 (12):1154–1162
13. Butov DA, Efremenko YV, Prihoda ND, Yurchenko LI, Sokolenko NI, Arjanova OV, Stepanenko AL, Butova TS, Zaitzeva SS, Jirathitikal V, Bourinbaiar AS, Kutsyna GA (2012) Adjunct immune therapy of first-diagnosed TB, relapsed TB, treatment-failed TB, multidrug-resistant TB and TB/HIV. Immunotherapy 4(7):687–695
14. Butov D, Gumenuik M, Gumeniuk G, Tkachenko A, Kikinchuk V, Stepaniuk R, Peshenko A, Butova T (2019) Effectiveness of anti-tuberculosis chemotherapy in patients with tuberculosis relapse compared with newly diagnosed patients. Int J Mycobacteriol 8 (4):341–346
15. Adepoju P (2020) Tuberculosis and HIV responses threatened by COVID-19. Lancet HIV 7 (5):e319–e320
16. Bock P, Jennings K, Vermaak R, Cox H, Meintjes G, Fatti G, Kruger J, De Azevedo V, Maschilla L, Louis F, Gunst C, Grobbelaar N, Dunbar R, Limbada M, Floyd S, Grimwood A, Ayles H, Hayes R, Fidler S, Beyers N (2018) Incidence of tuberculosis among HIV-positive individuals initiating antiretroviral treatment at higher CD4 counts in the HPTN 071 (PopART) trial in South Africa. J Acquir Immune Defic Syndr 77(1):93–101
17. Abel L, El-Baghdadi J, Bousfiha AA, Casanova JL, Schurr E (2014) Human genetics of tuberculosis: a long and winding road. Philos Trans R Soc Lond B Biol Sci 369 (1645):20130428
18. Butov DO, Kuzhko MM, Makeeva NI, Butova TS, Stepanenko HL, Dudnyk AB (2016) Association of interleukins genes polymorphisms with multi-drug resistant tuberculosis in Ukrainian population. Pneumonol Alergol Pol 84(3):168–173

19. de Martino M, Lodi L, Galli L, Chiappini E (2019) Immune response to *Mycobacterium tuberculosis*: a narrative review. Front Pediatr 7:350. https://doi.org/10.3389/fped.2019.00350 [Published online 2019 Aug 27]
20. Abate G, Hoft DF (2016) Immunotherapy for tuberculosis: future prospects. Immunotargets Ther 5:37–45. https://doi.org/10.2147/ITT.S81892
21. Li Y, Wang Y, Liu X (2012) The role of airway epithelial cells in response to mycobacteria infection. Clin Dev Immunol 2012:791392. https://doi.org/10.1155/2012/791392
22. Carranza C, Chavez-Galan L (2019) Several routes to the same destination: inhibition of phagosome-lysosome fusion by *Mycobacterium tuberculosis*. Am J Med Sci 357(3):184–194. https://doi.org/10.1016/j.amjms.2018.12.003
23. Jamwal SV, Mehrotra P, Singh A, Siddiqui Z, Basu A, Rao KV (2016) Mycobacterial escape from macrophage phagosomes to the cytoplasm represents an alternate adaptation mechanism. Sci Rep 6:23089. https://doi.org/10.1038/srep23089
24. Upadhyay S, Mittal E, Philips JA (2018) Tuberculosis and the art of macrophage manipulation. Pathog Dis 76(4):fty037. https://doi.org/10.1093/femspd/fty037
25. Stamm CE, Collins AC, Shiloh MU (2015) Sensing of *Mycobacterium tuberculosis* and consequences to both host and bacillus. Immunol Rev 264(1):204–219. https://doi.org/10.1111/imr.12263
26. Wong KW (2017) The role of ESX-1 in *Mycobacterium tuberculosis* pathogenesis. Microbiol Spectr 5(3): https://doi.org/10.1128/microbiolspec.TBTB2-0001-2015
27. Wawrocki S, Druszczynska M (2017) Inflammasomes in *Mycobacterium tuberculosis*-driven immunity. Can J Infect Dis Med Microbiol 2017:2309478. https://doi.org/10.1155/2017/2309478
28. Pesu M (2016) New insights into the host cell necrosis in tuberculosis. Virulence 7(1):1–2. https://doi.org/10.1080/21505594.2015.1122167
29. Watson RO, Bell SL, MacDuff DA, Kimmey JM, Diner EJ, Olivas J, Vance RE, Stallings CL, Virgin HW, Cox JS (2015) The cytosolic sensor cGAS detects *Mycobacterium tuberculosis* DNA to induce type I interferons and activate autophagy. Cell Host Microbe 17(6):811–819. https://doi.org/10.1016/j.chom.2015.05.004
30. Hossain MM, Norazmi MN (2013) Pattern recognition receptors and cytokines in *Mycobacterium tuberculosis* infection—the double-edged sword? Biomed Res Int 2013:179174. https://doi.org/10.1155/2013/179174
31. Kleinnijenhuis J, Oosting M, Joosten LA, Netea MG, Van Crevel R (2011) Innate immune recognition of *Mycobacterium tuberculosis*. Clin Dev Immunol 2011:405310. https://doi.org/10.1155/2011/405310
32. Zhou Y, Zhang M (2020) Associations between genetic polymorphisms of TLRs and susceptibility to tuberculosis: a meta-analysis. Innate Immun 26(2):75–83. https://doi.org/10.1177/1753425919862354
33. Wang C, Chen ZL, Pan ZF, Wei LL, Xu DD, Jiang TT, Zhang X, Ping ZP, Li ZJ, Li JC (2013) NOD2 polymorphisms and pulmonary tuberculosis susceptibility: a systematic review and meta-analysis. Int J Biol Sci 10(1):103–108. https://doi.org/10.7150/ijbs.7585
34. Faridgohar M, Nikoueinejad H (2017) New findings of Toll-like receptors involved in *Mycobacterium tuberculosis* infection. Pathog Glob Health 111(5):256–264. https://doi.org/10.1080/20477724.2017.1351080
35. Biyikli OO, Baysak A, Ece G, Oz AT, Ozhan MH, Berdeli A (2016) Role of toll-like receptors in tuberculosis infection. Jundishapur J Microbiol 9(10):e20224. https://doi.org/10.5812/jjm.20224
36. Maceda EB, Gonçalves CCM, Andrews JR, Ko AI, Yeckel CW, Croda J (2018) Serum vitamin D levels and risk of prevalent tuberculosis, incident tuberculosis and tuberculin skin test conversion among prisoners. Sci Rep 8(1):997. https://doi.org/10.1038/s41598-018-19589-3
37. Negroni A, Pierdomenico M, Cucchiara S, Stronati L (2018) NOD2 and inflammation: current insights. J Inflamm Res 11:49–60. https://doi.org/10.2147/JIR.S137606

38. Brooks MN, Rajaram MV, Azad AK, Amer AO, Valdivia-Arenas MA, Park JH, Núñez G, Schlesinger LS (2011) NOD2 controls the nature of the inflammatory response and subsequent fate of *Mycobacterium tuberculosis* and *M. bovis* BCG in human macrophages. Cell Microbiol 13(3):402–418. https://doi.org/10.1111/j.1462-5822.2010.01544.x
39. Paik S, Kim JK, Chung C, Jo EK (2019) Autophagy: a new strategy for host-directed therapy of tuberculosis. Virulence 10(1):448–459. https://doi.org/10.1080/21505594.2018.1536598
40. Bento CF, Empadinhas N, Mendes V (2015) Autophagy in the fight against tuberculosis. DNA Cell Biol 34(4):228–242. https://doi.org/10.1089/dna.2014.2745
41. Zhang R, Xi X, Wang C, Pan Y, Ge C, Zhang L, Zhang S, Liu H (2018) Therapeutic effects of recombinant human interleukin 2 as adjunctive immunotherapy against tuberculosis: a systematic review and meta-analysis. PLoS ONE 13(7):e0201025. https://doi.org/10.1371/journal.pone.0201025
42. Liu X, Li F, Niu H, Ma L, Chen J, Zhang Y, Peng L, Gan C, Ma X, Zhu B (2019) IL-2 restores T-cell dysfunction induced by persistent *Mycobacterium tuberculosis* antigen stimulation. Front Immunol 10:2350. https://doi.org/10.3389/fimmu.2019.02350
43. Waters RS, Perry JSA, Han S, Bielekova B, Gedeon T (2018) The effects of interleukin-2 on immune response regulation. Math Med Biol 35(1):79–119. https://doi.org/10.1093/imammb/dqw021
44. Jacobs A, Wilkinson RJ (2015) Humoral immunity in tuberculosis [published correction appears in Eur J Immunol. 2015 Apr;45(4):1274]. Eur J Immunol 45(3):647–649. https://doi.org/10.1002/eji.201570034
45. Dannenberg AM Jr, Collins FM (2001) Progressive pulmonary tuberculosis is not due to increasing numbers of viable bacilli in rabbits, mice and guinea pigs, but is due to a continuous host response to mycobacterial products. Tuberculosis (Edinb) 81(3):229–242
46. Mootoo A, Stylianou E, Arias MA, Reljic R (2009) TNF-alpha in tuberculosis: a cytokine with a split personality. Inflamm Allergy Drug Targets 8(1):53–62. https://doi.org/10.2174/187152809787582543
47. Ravimohan S, Kornfeld H, Weissman D, Bisson GP (2018) Tuberculosis and lung damage: from epidemiology to pathophysiology. Eur Respir Rev 27(147):170077. https://doi.org/10.1183/16000617.0077-2017
48. Bourinbaiar AS, Mezentseva MV, Butov DA, Nyasulu PS, Efremenko YV, Jirathitikal V, Mishchenko VV, Kutsyna GA (2012) Immune approaches in tuberculosis therapy: a brief overview. Expert Rev Anti Infect Ther 10(3):381–389
49. Butov D, Zaitseva S, Butova T, Stepanenko G, Pogorelova O, Zhelezniakova N (2016) Efficacy and safety of quercetin and polyvinylpyrrolidone in treatment of patients with newly diagnosed destructive pulmonary tuberculosis in comparison with standard antimycobacterial therapy. Int J Mycobacteriol 5(4):446–453
50. Zeng G, Zhang G, Chen X (2018) Th1 cytokines, true functional signatures for protective immunity against TB? Cell Mol Immunol 15(3):206–215. https://doi.org/10.1038/cmi.2017.113
51. Amaral EP, Lasunskaia EB, D'Império-Lima MR (2016) Innate immunity in tuberculosis: how the sensing of mycobacteria and tissue damage modulates macrophage death. Microbes Infect 18(1):11–20. https://doi.org/10.1016/j.micinf.2015.09.005
52. Young C, Walzl G, Du Plessis N (2020) Therapeutic host-directed strategies to improve outcome in tuberculosis. Mucosal Immunol 13(2):190–204. https://doi.org/10.1038/s41385-019-0226-5
53. Zhang Z, Fan W, Yang G, Xu Z, Wang J, Cheng Q, Yu M (2017) Risk of tuberculosis in patients treated with TNF-α antagonists: a systematic review and meta-analysis of randomised controlled trials. BMJ Open 7(3):e012567. https://doi.org/10.1136/bmjopen-2016-012567 [Published online 2017 Mar 22]

54. Olsen A, Chen Y, Ji Q, Zhu G, De Silva AD, Vilchèze C, Weisbrod T, Li W, Xu J, Larsen M, Zhang J, Porcelli SA, Jacobs WR Jr, Chan J (2016) Targeting *Mycobacterium tuberculosis* tumor necrosis factor alpha-downregulating genes for the development of antituberculous vaccines. mBio 7(3):e01023-15. https://doi.org/10.1128/mBio.01023-15.
55. Esmail H, Wilkinson RJ (2017) Minimizing tuberculosis risk in patients receiving anti-TNF therapy. Ann Am Thorac Soc 14(5):621–623. https://doi.org/10.1513/AnnalsATS.201701-055ED
56. Denis M, Ghadirian E (1993) Immunotherapy of airborne tuberculosis in mice via the lung-specific delivery of cytokines. Can J Infect Dis 4(1):38–42. https://doi.org/10.1155/1993/954372
57. Nolt D, Flynn JL (2004) Interleukin-12 therapy reduces the number of immune cells and pathology in lungs of mice infected with *Mycobacterium tuberculosis*. Infect Immun 72 (5):2976–2988. https://doi.org/10.1128/iai.72.5.2976-2988.2004
58. Bermudez LE, Stevens P, Kolonoski P, Wu M, Young LS (1989) Treatment of disseminated *Mycobacterium avium* complex infection in mice with recombinant interleukin-2 and tumor necrosis factor. J Immunol 143:2996–3002
59. Bermudez LE, Young LS (1988) Tumor necrosis factor, alone or in combination with IL-2, but not IFN-gamma, is associated with macrophage killing of *Mycobacterium avium* complex. J Immunol 9:3006–3013
60. Skerry C, Harper J, Klunk M, Bishai WR, Jain SK (2012) Adjunctive TNF inhibition with standard treatment enhances bacterial clearance in a murine model of necrotic TB granulomas. PLoS ONE 7(6):e39680. https://doi.org/10.1371/journal.pone.0039680
61. Nikitina IY, Panteleev AV, Sosunova EV, Karpina NL, Bagdasarian TR, Burmistrova IA, Andreevskaya SN, Chernousova LN, Vasilyeva IA, Lyadova IV (2016) Antigen-specific IFN-γ responses correlate with the activity of *M. tuberculosis* infection but are not associated with the severity of tuberculosis disease. J Immunol Res 2016:7249369. https://doi.org/10.1155/2016/7249369
62. Sakai S, Kauffman KD, Sallin MA, Sharpe AH, Young HA, Ganusov VV, Barber DL (2016) CD4 T cell-derived IFN-γ plays a minimal role in control of pulmonary *Mycobacterium tuberculosis* infection and must be actively repressed by PD-1 to prevent lethal disease. PLoS Pathog 12(5):e1005667. https://doi.org/10.1371/journal.ppat.1005667
63. Tan Q, Min R, Dai GQ, Wang YL, Nan L, Yang Z, Xia J, Pan SY, Mao H, Xie WP, Wang H (2017) Clinical and immunological effects of rhIL-2 therapy in Eastern Chinese patients with multidrug-resistant tuberculosis. Sci Rep 7(1):17854. https://doi.org/10.1038/s41598-017-18200-5
64. Johnson BJ, Bekker LG, Rickman R, Brown S, Lesser M, Ress S, Willcox P, Steyn L, Kaplan G (1997) rhuIL-2 adjunctive therapy in multidrug resistant tuberculosis: a comparison of two treatment regimens and placebo. Tuber Lung Dis 78(3–4):195–203. https://doi.org/10.1016/s0962-8479(97)90026-5
65. Johnson JL, Ssekasanvu E, Okwera A, Mayanja H, Hirsch CS, Nakibali JG, Jankus DD, Eisenach KD, Boom WH, Ellner JJ, Mugerwa RD, Uganda-Case Western Reserve University Research Collaboration (2003) Randomized trial of adjunctive interleukin-2 in adults with pulmonary tuberculosis. Am J Respir Crit Care Med 168(2):185–191. https://doi.org/10.1164/rccm.200211-1359OC
66. Khan TA, Mazhar H, Saleha S, Tipu HN, Muhammad N, Abbas MN (2016) Interferon-gamma improves macrophages function against *M. tuberculosis* in multidrug-resistant tuberculosis patients. Chemother Res Pract 2016:7295390. https://doi.org/10.1155/2016/7295390
67. Gao XF, Yang ZW, Li J (2011) Adjunctive therapy with interferon-gamma for the treatment of pulmonary tuberculosis: a systematic review. Int J Infect Dis 15(9):e594–e600. https://doi.org/10.1016/j.ijid.2011.05.002

68. Suárez-Méndez R, García-García I, Fernández-Olivera N, Valdés-Quintana M, Milanés-Virelles MT, Carbonell D, Machado-Molina D, Valenzuela-Silva CM, López-Saura PA (2004) Adjuvant interferon gamma in patients with drug-resistant pulmonary tuberculosis: a pilot study. BMC Infect Dis 4:44. https://doi.org/10.1186/1471-2334-4-44
69. Zhang YQ, He CW, Li HQ, Zhao HL, Li BJ (2009) Effects of aerosolized interferon-γ in previously treated patients with smear-positive pulmonary tuberculosis. Med Recapitulate 15:306–307
70. Li D, Du DB, Qiu SQ, Meng QH, Luo SZ, Yuan RJ (2008) The early effectiveness and cellular immune function of interferon-γ combined with *Mycobacterium vaccae* for previously untreated pulmonary tuberculosis. J Chinese Anti-tuberc Assoc 30:460–461
71. Wallis RS, Kyambadde P, Johnson JL, Horter L, Kittle R, Pohle M, Ducar C, Millard M, Mayanja-Kizza H, Whalen C, Okwera A (2004) A study of the safety, immunology, virology, and microbiology of adjunctive etanercept in HIV-1-associated tuberculosis. AIDS 18(2):257–264. https://doi.org/10.1097/00002030-200401230-00015
72. Bourigault ML, Vacher R, Rose S, Olleros ML, Janssens JP, Quesniaux VF, Garcia I (2013) Tumor necrosis factor neutralization combined with chemotherapy enhances *Mycobacterium tuberculosis* clearance and reduces lung pathology. Am J Clin Exp Immunol 2(1):124–134
73. Vilaplana C, Cardona PJ (2010) Tuberculin immunotherapy: its history and lessons to be learned. Microbes Infect 12(2):99–105
74. Chahar M, Rawat KD, Reddy PVJ, Gupta UD, Natrajan M, Chauhan DS, Katoch K, Prasad GBKS, Katoch VM (2018) Potential of adjunctive Mycobacterium w (MIP) immunotherapy in reducing the duration of standard chemotherapy against tuberculosis. Indian J Tuberc 65(4):335–344
75. Butov DA, Efremenko YV, Prihoda ND, Zaitzeva SI, Yurchenko LV, Sokolenko NI, Butova TS, Stepanenko AL, Kutsyna GA, Jirathitikal V, Bourinbaiar AS (2013) Randomized, placebo-controlled Phase II trial of heat-killed *Mycobacterium vaccae* (Immodulon batch) formulated as an oral pill (V7). Immunotherapy 5(10):1047–1054
76. Yang XY, Chen QF, Li YP, Wu SM (2011) *Mycobacterium vaccae* as adjuvant therapy to anti-tuberculosis chemotherapy in never-treated tuberculosis patients: a meta-analysis. PLoS ONE 6(9):e23826
77. Bourinbaiar AS, Batbold U, Efremenko Y, Sanjagdorj M, Butov D, Damdinpurev N, Grinishina E, Mijiddorj O, Kovolev M, Baasanjav K, Butova T, Prihoda N, Batbold O, Yurchenko L, Tseveendorj A, Arzhanova O, Chunt E, Stepanenko H, Sokolenko N, Makeeva N, Tarakanovskaya M, Borisova V, Reid A, Kalashnikov V, Nyasulu P, Prabowo SA, Jirathitikal V, Bain AI, Stanford C, Stanford J (2019) Phase III, placebo-controlled, randomized, double-blind trial of tableted, therapeutic TB vaccine (V7) containing heat-killed *M. vaccae* administered daily for one month. J Clin Tuberc Other Mycobact Dis 18:100141
78. Lei JP, Xiong GL, Hu QF, Li Y, Zong PL, Tu SH, Tu RY (2008) Immunotherapeutic efficacy of BCG vaccine in pulmonary tuberculosis and its preventive effect on multidrug-resistant tuberculosis. Zhonghua Yu Fang Yi Xue Za Zhi 42(2):86–89
79. Prabowo SA, Painter H, Zelmer A, Smith SG, Seifert K, Amat M, Cardona PJ, Fletcher HA (2019) RUTI vaccination enhances inhibition of mycobacterial growth *ex vivo* and induces a shift of monocyte phenotype in mice. Front Immunol 10:894
80. Bruffaerts N, Huygen K, Romano M (2014) DNA vaccines against tuberculosis. Expert Opin Biol Ther 14(12):1801–1813. https://doi.org/10.1517/14712598.2014.951630
81. Liang Y, Bai X, Zhang J, Song J, Yang Y, Yu Q, Li N, Wu X (2016) Ag85A/ESAT-6 chimeric DNA vaccine induces an adverse response in tuberculosis-infected mice. Mol Med Rep 14(2):1146–1152. https://doi.org/10.3892/mmr.2016.5364
82. Wowk PF, Franco LH, Fonseca DMD, Paula MO, Vianna ÉDSO, Wendling AP, Augusto VM, Elói-Santos SM, Teixeira-Carvalho A, Silva FDC, Vinhas SA, Martins-Filho OA, Palaci M, Silva CL, Bonato VLD (2017) Mycobacterial Hsp65 antigen

upregulates the cellular immune response of healthy individuals compared with tuberculosis patients. Hum Vaccin Immunother 13(5):1040–1050
83. Xu Z, Hu T, Liu Z, Shen X, Liu J, Yin Y, Sun L, Chen X, Jiao X (2016) Expression and immunogenicity of Ag85A protein of *Mycobacterium tuberculosis*. Wei Sheng wu xue bao = Acta Microbiol Sinica 56(5):804–813
84. Mir SA, Verma I, Sharma S (2014) Immunotherapeutic potential of recombinant ESAT-6 protein in mouse model of experimental tuberculosis. Immunol Lett 158(1–2):88–94
85. Okada M, Kita Y, Nakajima T, Kanamaru N, Hashimoto S, Nagasawa T, Kaneda Y, Yoshida S, Nishida Y, Nakatani H, Takao K, Kishigami C, Nishimatsu S, Sekine Y, Inoue Y, Matsumoto M, McMurray DN, De la Cruz EC, Tan EV, Abalos RM, Burgos JA, Saunderson P, Sakatani M (2011) Novel therapeutic vaccine: granulysin and new DNA vaccine against tuberculosis. Hum Vaccin 7:60–67. https://doi.org/10.4161/hv.7.0.14563
86. Tanghe A, D'Souza S, Rosseels V, Denis O, Ottenhoff TH, Dalemans W, Wheeler C, Huygen K (2001) Improved immunogenicity and protective efficacy of a tuberculosis DNA vaccine encoding Ag85 by protein boosting. Infect Immun 69(5):3041–3047
87. Derrick SC, Yang AL, Morris SL (2004) A polyvalent DNA vaccine expressing an ESAT6-Ag85B fusion protein protects mice against a primary infection with *Mycobacterium tuberculosis* and boosts BCG-induced protective immunity. Vaccine 23(6):780–788
88. Butov DO, Zaitseva SI, Pitenko MM, Stepanenko GL, Butova TS (2015) Morphological changes in experimental tuberculosis resulting from treatment with quercetin and polyvinylpyrrolidone. Int J Mycobacteriol 4(4):296–301
89. Senderovitz T, Viskum K (1994) Corticosteroids and tuberculosis. Respir Med 88(8):561–565
90. Schutz C, Davis AG, Sossen B, Lai RP, Ntsekhe M, Harley YX, Wilkinson RJ (2018) Corticosteroids as an adjunct to tuberculosis therapy. Expert Rev Respir Med 12(10):881–891. https://doi.org/10.1080/17476348.2018.1515628
91. Liu T, Zhang L, Joo D, Sun SC (2017) NF-κB signaling in inflammation. Signal Transduct Target Ther 2:17023. https://doi.org/10.1038/sigtrans.2017.23
92. Gräb J, Suárez I, van Gumpel E, Winter S, Schreiber F, Esser A, Hölscher C, Fritsch M, Herb M, Schramm M, Wachsmuth L, Pallasch C, Pasparakis M, Kashkar H, Rybniker J (2019) Corticosteroids inhibit *Mycobacterium tuberculosis*-induced necrotic host cell death by abrogating mitochondrial membrane permeability transition. Nat Commun 10(1):688. https://doi.org/10.1038/s41467-019-08405-9
93. Butov D, Feshchenko Y, Kuzhko M, Gumenuik M, Yurko K, Grygorova A, Tkachenko A, Nekrasova N, Tlustova T, Kikinchuk V, Peshenko A, Butova T (2020) Effectiveness of intravenous isoniazid and ethambutol administration in patients with tuberculosis meningoencephalitis and HIV infection. Tuberc Respir Dis (Seoul) 83(1):96–103
94. Prasad K, Singh MB, Ryan H (2016) Corticosteroids for managing tuberculous meningitis. Cochrane Database Syst Rev 4(4):CD002244
95. Muefong CN, Sutherland JS (2020) Neutrophils in tuberculosis-associated inflammation and lung pathology. Front Immunol 11:962. https://doi.org/10.3389/fimmu.2020.00962
96. Critchley JA, Orton LC, Pearson F (2014) Adjunctive steroid therapy for managing pulmonary tuberculosis. Cochrane Database Syst Rev 2014(11):CD011370
97. Johnson JR, Turk TL, Macdonald FM (1967) Corticosteroids in pulmonary tuberculosis. Am Rev Respir Dis 96:6–73
98. George IA, Thomas B, Sadhu JS (2018) Systematic review and meta-analysis of adjunctive corticosteroids in the treatment of tuberculous pericarditis. Int J Tuberc Lung Dis 22(5):551–556
99. Ryan H, Yoo J, Darsini P (2017) Corticosteroids for tuberculous pleurisy. Cochrane Database Syst Rev 3(3):CD001876
100. Sun F, Li L, Liao X, Yan X, Han R, Lei W, Cao H, Feng M, Cao G (2018) Adjunctive use of prednisolone in the treatment of free-flowing tuberculous pleural effusion: a retrospective cohort study. Respir Med 139:86–90

101. Donovan J, Phu NH, Mai NTH, Dung LT, Imran D, Burhan E, Ngoc LHB, Bang ND, Giang DC, Ha DTM, Day J, Thao LTP, Thuong NT, Vien NN, Geskus RB, Wolbers M, Hamers RL, van Crevel R, Nursaya M, Maharani K, Hien TT, Baird K, Lan NH, Kestelyn E, Chau NVV, Thwaites GE (2018) Adjunctive dexamethasone for the treatment of HIV-infected adults with tuberculous meningitis (ACT HIV): study protocol for a randomised controlled trial. Wellcome Open Res 3:31
102. Elliott AM, Luzze H, Quigley MA, Nakiyingi JS, Kyaligonza S, Namujju PB, Ducar C, Ellner JJ, Whitworth JA, Mugerwa R, Johnson JL, Okwera A (2004) A randomized, double-blind, placebo-controlled trial of the use of prednisolone as an adjunct to treatment in HIV-1-associated pleural tuberculosis. J Infect Dis 190(5):869–878
103. Mayanja-Kizza H, Jones-Lopez E, Okwera A, Wallis RS, Ellner JJ, Mugerwa RD, Whalen CC, Uganda-Case Western Research Collaboration (2005) Immunoadjuvant prednisolone therapy for HIV-associated tuberculosis: a phase 2 clinical trial in Uganda. J Infect Dis 191(6):856–865
104. Ralph AP, Kelly PM, Anstey NM (2008) L-Arginine and vitamin D: novel adjunctive immunotherapies in tuberculosis. Trends Microbiol 16(7):336–344. https://doi.org/10.1016/j.tim.2008.04.003
105. Brighenti S, Bergman P, Martineau AR (2018) Vitamin D and tuberculosis: where next? J Intern Med. https://doi.org/10.1111/joim.12777.doi:10.1111/joim.12777
106. Kearns MD, Tangpricha V (2014) The role of vitamin D in tuberculosis. J Clin Transl Endocrinol 1(4):167–169. https://doi.org/10.1016/j.jcte.2014.08.002
107. Talat N, Perry S, Parsonnet J, Dawood G, Hussain R (2010) Vitamin D deficiency and tuberculosis progression. Emerg Infect Dis 16(5):853–855. https://doi.org/10.3201/eid1605.091693
108. Chung C, Silwal P, Kim I, Modlin RL, Jo EK (2020) Vitamin D-cathelicidin axis: at the crossroads between protective immunity and pathological inflammation during infection. Immune Netw 20(2):e12. https://doi.org/10.4110/in.2020.20.e12
109. Junaid K, Rehman A (2019) Impact of vitamin D on infectious disease-tuberculosis—a review. Clin Nutr Exp 25:1–10. https://doi.org/10.1016/j.yclnex.2019.02.003
110. Torres-Juarez F, Cardenas-Vargas A, Montoya-Rosales A, González-Curiel I, Garcia-Hernandez MH, Enciso-Moreno JA, Hancock RE, Rivas-Santiago B (2015) LL-37 immunomodulatory activity during *Mycobacterium tuberculosis* infection in macrophages. Infect Immun 83(12):4495–4503. https://doi.org/10.1128/IAI.00936-15
111. Chen Y, Zhang J, Ge X, Du J, Deb DK, Li YC (2013) Vitamin D receptor inhibits nuclear factor κB activation by interacting with IκB kinase β protein. J Biol Chem 288(27):19450–19458. https://doi.org/10.1074/jbc.M113.467670
112. Adamczak DM (2017) The role of toll-like receptors and vitamin D in cardiovascular diseases—a review. Int J Mol Sci 18(11):2252. https://doi.org/10.3390/ijms18112252
113. Panwar A, Garg RK, Malhotra HS, Jain A, Singh AK, Prakash S, Kumar N, Garg R, Mahdi AA, Verma R, Sharma PK (2016) 25-Hydroxy vitamin D, vitamin D receptor and toll-like receptor 2 polymorphisms in spinal tuberculosis: a case-control study. Medicine (Baltimore) 95(17):e3418. https://doi.org/10.1097/MD.0000000000003418
114. Campbell GR, Spector SA (2012) Autophagy induction by vitamin D inhibits both *Mycobacterium tuberculosis* and human immunodeficiency virus type 1. Autophagy 8(10):1523–1525. https://doi.org/10.4161/auto.21154
115. Sarkar K, Sil PC (2019) Infectious lung diseases and endogenous oxidative stress. Oxidative Stress in Lung Dis 125–148. https://doi.org/10.1007/978-981-13-8413-4_7
116. Shastri MD, Shukla SD, Chong WC, Dua K, Peterson GM, Patel RP, Hansbro PM, Eri R, O'Toole RF (2018) Role of oxidative stress in the pathology and management of human tuberculosis. Oxid Med Cell Longev 2018:7695364. https://doi.org/10.1155/2018/7695364
117. Wang CH, Lin HC, Liu CY, Huang KH, Huang TT, Yu CT, Kuo HP (2001) Upregulation of inducible nitric oxide synthase and cytokine secretion in peripheral blood monocytes from pulmonary tuberculosis patients. Int J Tuberc Lung Dis 5(3):283–291

118. Rockett KA, Brookes R, Udalova I, Vidal V, Hill AV, Kwiatkowski D (1998) 1,25-Dihydroxyvitamin D3 induces nitric oxide synthase and suppresses growth of *Mycobacterium tuberculosis* in a human macrophage-like cell line. Infect Immun 66 (11):5314–5321
119. Butov DO, Kuzhko MM, Kalmykova IM, Kuznetsova IM, Butova TS, Grinishina OO, Maksimenko OA (2014) Changes in nitric oxide synthase and nitrite and nitrate serum levels in patients with or without MDR-TB undergoing the intensive phase of anti-tuberculosis therapy. Int J Mycobacteriol 3(2):139–143
120. Ralph AP, Waramori G, Pontororing GJ, Kenangalem E, Wiguna A, Tjitra E, Sandjaja LDB, Yeo TW, Chatfield MD, Soemanto RK, Bastian I, Lumb R, Maguire GP, Eisman J, Price RN, Morris PS, Kelly PM, Anstey NM (2013) L-Arginine and vitamin D adjunctive therapies in pulmonary tuberculosis: a randomised, double-blind, placebo-controlled trial. PLoS ONE 8(8):e70032. https://doi.org/10.1371/journal.pone.0070032
121. Jolliffe DA, Ganmaa D, Wejse C, Raqib R, Haq MA, Salahuddin N, Daley PK, Ralph AP, Ziegler TR, Martineau AR (2019) Adjunctive vitamin D in tuberculosis treatment: meta-analysis of individual participant data. Eur Respir J 53(3):1802003. https://doi.org/10.1183/13993003.02003-2018
122. Wu HX, Xiong XF, Zhu M, Wei J, Zhuo KQ, Cheng DY (2018) Effects of vitamin D supplementation on the outcomes of patients with pulmonary tuberculosis: a systematic review and meta-analysis. BMC Pulm Med 18(1):108. https://doi.org/10.1186/s12890-018-0677-6 [Published online 2018 Jun 28]
123. Martineau AR, Wilkinson RJ, Wilkinson KA, Newton SM, Kampmann B, Hall BM, Packe GE, Davidson RN, Eldridge SM, Maunsell ZJ, Rainbow SJ, Berry JL, Griffiths CJ (2007) A single dose of vitamin D enhances immunity to mycobacteria. Am J Respir Crit Care Med 176(2):208–213. https://doi.org/10.1164/rccm.200701-007OC
124. Nursyam EW, Amin Z, Rumende CM (2006) The effect of vitamin D as supplementary treatment in patients with moderately advanced pulmonary tuberculous lesion. Acta Med Indones 38(1):3–5
125. Zhang J, Chen C, Yang J (2019) Effectiveness of vitamin D supplementation on the outcome of pulmonary tuberculosis treatment in adults: a meta-analysis of randomized controlled trials. Chin Med J (Engl) 132(24):2950–2959. https://doi.org/10.1097/CM9.0000000000000554
126. Wang J, Feng M, Ying S, Zhou J, Li X (2018) Efficacy and safety of vitamin D supplementation for pulmonary tuberculosis: a systematic review and meta-analysis. Iran J Public Health 47(4):466–472
127. Ganmaa D, Munkhzul B, Fawzi W, Spiegelman D, Willett WC, Bayasgalan P, Baasansuren E, Buyankhishig B, Oyun-Erdene S, Jolliffe DA, Xenakis T, Bromage S, Bloom BR, Martineau AR (2017) High-dose vitamin D3 during tuberculosis treatment in Mongolia. A randomized controlled trial. Am J Respir Crit Care Med 196(5):628–637. https://doi.org/10.1164/rccm.201705-0936OC
128. Feng M, Ding Q, Zhong C, Li J, Wang Q, Yuan Z, Dong Y (2016) Adjunctive therapy with V-5 Immunitor (V5) for the treatment of tuberculosis patients: a meta-analysis. Pharmazie 71 (9):499–503. https://doi.org/10.1691/ph.2016.6051
129. Batdelger D, Dandii D, Jirathitikal V, Bourinbaiar AS (2008) Open-label trial of therapeutic immunization with oral V-5 Immunitor (V5) vaccine in patients with chronic hepatitis C. Vaccine 26(22):2733–2737
130. Butov DA, Pashkov YN, Stepanenko AL, Choporova AI, Butova TS, Batdelger D, Jirathitikal V, Bourinbaiar AS, Zaitzeva SI (2011) Phase IIb randomized trial of adjunct immunotherapy in patients with first-diagnosed tuberculosis, relapsed and multi-drug-resistant (MDR) TB. J Immune Based Ther Vaccines 9:3

131. Skrahin A, Ahmed RK, Ferrara G, Rane L, Poiret T, Isaikina Y, Skrahina A, Zumla A, Maeurer MJ (2014) Autologous mesenchymal stromal cell infusion as adjunct treatment in patients with multidrug and extensively drug-resistant tuberculosis: an open-label phase 1 safety trial. Lancet Respir Med 2(2):108–122
132. Hogan BL, Yingling JM (1998) Epithelial/mesenchymal interactions and branching morphogenesis of the lung. Curr Opin Genet Dev 8(4):481–486
133. Sinclair K, Yerkovich ST, Chambers DC (2013) Mesenchymal stem cells and the lung. Respirology 18(3):397–411
134. Joshi L, Chelluri LK, Gaddam S (2015) Mesenchymal stromal cell therapy in MDR/XDR tuberculosis: a concise review. Arch Immunol Ther Exp (Warsz) 63(6):427–433
135. de Vallière S, Abate G, Blazevic A, Heuertz RM, Hoft DF (2005) Enhancement of innate and cell-mediated immunity by antimycobacterial antibodies. Infect Immun 73(10):6711–6720
136. Roy E, Stavropoulos E, Brennan J, Coade S, Grigorieva E, Walker B, Dagg B, Tascon RE, Lowrie DB, Colston MJ, Jolles S (2005) Therapeutic efficacy of high-dose intravenous immunoglobulin in *Mycobacterium tuberculosis* infection in mice. Infect Immun 73(9):6101–6109
137. Lopez Y, Yero D, Falero-Diaz G, Olivares N, Sarmiento ME, Sifontes S, Solis RL, Barrios JA, Aguilar D, Hernández-Pando R, Acosta A (2009) Induction of a protective response with an IgA monoclonal antibody against *Mycobacterium tuberculosis* 16kDa protein in a model of progressive pulmonary infection. Int J Med Microbiol 299(6):447–452
138. Butova T, Zaitseva S, Butov D, Stepanenko G (2016) Morphological changes in experimental tuberculosis resulting from treatment with quercetin and polyvinylpyrrolidone. Int J Mycobacteriol 5(Suppl 1):S103–S104
139. Butov D, Zaitseva S, Butova T (2016) Efficacy and safety of quercetin and polyvinylpyrrolidone in treatment of patients with newly diagnosed destructive pulmonary tuberculosis in comparison with standard antimycobacterial therapy. Int J Mycobacteriol. 5(Suppl 1):S110–S111
140. Batbold U, Butov DO, Kutsyna GA, Damdinpurev N, Grinishina EA, Mijiddorj O, Kovolev ME, Baasanjav K, Butova TS, Sandagdorj M, Batbold O, Tseveendorj A, Chunt E, Zaitzeva SI, Stepanenko HL, Makeeva NI, Mospan IV, Pylypchuk VS, Rowe JL, Nyasulu P, Jirathitikal V, Bain AI, Tarakanovskaya MG, Bourinbaiar AS (2017) Double-blind, placebo-controlled, 1:1 randomized Phase III clinical trial of Immunoxel honey lozenges as an adjunct immunotherapy in 269 patients with pulmonary tuberculosis. Immunotherapy 9(1):13–24
141. Mezentseva MV, Stakhanov VA, Zakharova MV, Zotova IF, Shapoval IM, Tregubova MI, Russu L (2011) Prospects of immunotherapy in the complex treatment of the infiltrative pulmonary tuberculosis. Biopreparats (Biopharmaceuticals) 2:20–25
142. Svistunova AS, Pinegin BV, Selitskaia RP (2002) Primenenie immunomoduliatora likopida v kompleksnom lechenii tuberkuleza legkikh [The use of immunomodulator likopid in the combined treatment pulmonary tuberculosis]. Probl Tuberk 3:21–25
143. Curtis N, Sparrow A, Ghebreyesus TA, Netea MG (2020) Considering BCG vaccination to reduce the impact of COVID-19. Lancet 395(10236):1545–1546
144. Grange JM, Brunet LR, Rieder HL (2011) Immune protection against tuberculosis—when is immunotherapy preferable to vaccination? Tuberculosis (Edinb) 91(2):179–185

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Inhalation Therapy in Pulmonary Tuberculosis

16

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In the mortality bills, pneumonia is an easy second, to tuberculosis; indeed, in many cities the death-rate is now higher and it has become, to use the phrase of Bunyan, 'the Captain of the men of death.

William Osier

Summary

Tuberculosis (TB) is a historical disease that can trace its impact on humanity back in time thousands of years. A bacterial infection by *Mycobacterium tuberculosis* (*M. tb*) that primarily strikes the pulmonary system appears in patients as latent, active, drug-resistant, and multidrug-resistant forms. While

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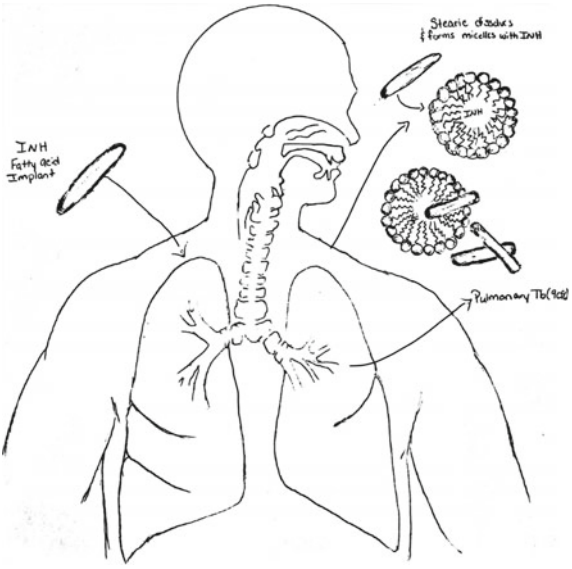
regiments recommended by medical institutions focus primarily on tablets, inhalation offers an approach that should deliver lower doses resulting in lower side effects. This chapter will review different approaches to inhalation therapy over the past one hundred years and their impact on the different forms of *M. tb* infection. It will conclude by suggesting a method to apply specific dosages of a front-line anti-TB drug to treat latent, active, and resistant forms of *M. tb* using an electronic vaporization technique.

Graphical Abstract



Fatty acid (stearic acid) nanoparticles encasing isoniazid are pictured being inhaled by a patient *(Made by Jenu Thomas Richardson)*

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Keywords

Mycobacterium tuberculosis · Bacterial infection · Delivery · Inhalation therapy · Inhalers · Pulmonary tuberculosis · Resistance · Treatment · Vaporization

1 Introduction

The inhalation of medicinal compounds has been tested and documented in the peer-reviewed scientific literature as a delivery method to rid a patient of *Mycobacterium tuberculosis* (*M. tb*) infection for over a century [1–77]. *M. tb infections* have ravaged humanity for at least ten thousand years [76]. TB is spread through the air by someone expelling water droplets infected with *M. tb* through a cough or sneeze. Water droplets containing *M. tb* can float in the air for several hours, making it possible for someone else nearby to inhale the bacteria. The most common form of TB, pulmonary TB (PTB), occurs when the bacteria attack the lungs. Extrapulmonary TB (EPTB) occurs when the bacteria infect other parts of the body, including the brain [11]. Contradictory to PTB, EPTB is rarely transmitted to others. The combination of isoniazid (INH), rifampin (RIF), and other first- and second-line anti-TB medications, are widely used to treat *M. tb* infections and can take six to nine months to complete the regimen. Combination therapy reduces the risk of the bacteria becoming drug-resistant (DR) [4]. DR-TB is a type of infection in which the bacteria become resistant to the primary drugs used to treat TB. This condition can arise from improper use of medications and regimens and not completing the full course of treatment. Totally DR-TB and extensively drug-resistant (XDR) TB are the most severe forms of *M. tb* infections. Bacille Calmette-Guérin (BCG) is a vaccine used for TB but has limited efficacy. BCG is recommended for children and adults at an increased risk of infection or exposure to TB disease.

Infection by *M. tb* does not necessarily result in immediate disease expression. When a subject is infected, granulomas are formed around the bacterium [84] to isolate and prevent its growth. The human body is efficient in containing outbreaks of TB after infection by sealing the disease in lesions. Although the TB is contained, the body cannot eradicate it, so latent TB infection (LTBI), or inactive TB, is expressed once the immune system is compromised. This can be observed in the syndemic relationship between TB and HIV; HIV drastically weakens the immune system, causing a TB outbreak to proliferate. Tuberculin-based skin tests are used to test for TB in travelers. There are two types of TB tests that are economical and routinely utilized by medical professionals: the skin test and the blood test. A positive test indicates that the patient has been infected with the *M. tb* but does not determine whether or not it is latent or active TB. Further diagnostic tests are needed to determine whether TB is latent or active in the patient [10]. Lifestyle, as

well as environmental factors, play a significant role in TB recovery. People with active TB self-quarantine to avoid infecting others. Patients may be recommended directly observed therapy (DOT), which requires you to take your medicine in front of your doctor several times a week. DOT helps prevent DR; however, it tends to add staggering costs to the patient.

2 History

Before the first mass production of penicillin in the 1940s and the introduction of antibiotics used to treat *M. tb* infections in the 1950s through the 1970s, there was no established treatment for the disease. The following three papers give an insight into some of the inhalation treatments tested. In a paper published in 1898, a medical professional had up to 2000 TB patients under his care. The infectious disease caused by *M. tb* was typically advanced in the patients [1]. The inhalation treatment was focused on the application of a vapor composed of:

- eucalyptol ($C_{10}H_{18}O$), which is a cyclic ether and a monoterpenoid, also called 1,8-cineol;
- oil of cloves (oil extracted from the clove plant, *Syzygium aromaticum* found in Southeast Asia, and is composed of β-caryophyllene, α-humulene, eugenyl acetate, and eugenol; and other ingredients

A quote from the author summarizes the results:

> "symptoms have improved in all cases, though some have improved significantly more than others".

These were a most unfavorable class of patients to treat, being in the advanced stage of the tubercular process with hemorrhages, emaciation, night sweats, anemia, and, in fact, scarcely able to breathe at all. In the absence of a specific symptom for TB, we believe that with proper apparatus and skillful and continued administration, much is to be hoped for in this class of patients by inhaling the antiseptic vapor. The historical literature does not explain why this promising approach was abandoned.

Benzol was the name for benzene and could be inhaled as a vapor. To put the study in perspective, penicillin was the first mass-produced antibiotic, which happened in the 1940s. The concept of an atom was not fully understood, emphasizing the Nobel prize in physics awarded to James Chadwick to discover the neutron in 1935. Benzene is now recognized by IARC as a human carcinogen that causes leukemia and non-Hodgkin lymphoma. The study did report changes in changes in the function of kidneys, liver, and hearts muscles in the mice, and the paper concluded [2]:

> that with further study of this rather specific poison, we may get a little nearer the solution of some of the unsolved problems of infection and immunity.

Studies of this nature, before the modern era of antibiotics, emphasize the desperation in seeking treatments using toxic compounds that were very poorly understood.

In a 1935 paperback entitled "Inhalation Therapy Technique" by W. Collison [3], there is a chapter dedicated to inhalation therapy in PTB. First, the author outlined some oils and liquids cannot be used for inhalation therapy because the large particle sizes generated allow minimal penetration of the substance deep into the lungs. Likewise, there are medical sprayers used in that time period for treating the nasal cavity or throat maladies that do not provide the parameters needed for the mist to penetrate deep into the lungs. An Apneu Inhaling Apparatus was used to deliver the vapor to a mask that covers the patients' faces and mouths. There is a discussion on the parameters that impact the size of the particles, as small as "1/5000th of an inch." The apparatus is used to deliver compounds such as adrenalin, camphor, menthol, and creosote. The substances are delivered with a purpose; adrenalin is used to reduce congestion by dilating the bronchial tubes; camphor is stated to stimulate blood circulation and breathing; thymol and menthol are antiseptic; creosote is a disinfectant; pine and cypress soothed the inflamed mucous membrane, etc. A typical prescription is described as three minutes of adrenalin therapy, a three-minute rest, a three-minute camphor therapy, a three-minute rest, followed by a three-minute adrenalin dosage. While not described as a cure, it did help patients feel better or "obtain relief."

3 Modern Work

Currently, there are three types of inhalers used for lung infection treatments:

i. Small-volume nebulizer (SVN), it generates a liquid into an aerosol, and the droplets are propelled by a compressed gas, typically air or pure oxygen;
ii. Dry powder nebulizer delivers the medication as a powder or small solid particles; and
iii. The pressurized metered-dose inhaler (MDI), it is the most common type.

Although not placed in the same category of inhalers, anesthetics are gases that can be inhaled but are not used directly to treat a medical condition. There are some unsuccessful ventures mentioned in the literature in which gases such as carbon dioxide were used unsuccessfully.

For a medication to function properly, it should have the ability to reduce or eliminate the bacterial load contained within a macrophage without eliminating a large percentage of the macrophages residing within the lungs. Often heavy doses of antibiotics have to be given to patients to overwhelm physiological barriers such as macrophages and granuloma to eliminate the *M. tb*. This results in significant side effects for the patient.

We have identified the following pharmacokinetic and pharmacodynamics aspects related to the administration of inhaled agents with medicinal activity for a viral infection that need to be considered if a large fraction of the droplets will reach the desired region of the lungs and be effective in reducing the bacterial load in the lungs and entering the bloodstream to eliminate its presence throughout the body (Box 1).

Box 1 A sequential outline of the biological and chemical processes that could impact the drug delivery efficiency when inhalation is utilized

Some electronic vaporization processes can potentially cause a fraction of the medication or molecule to react or degrade;
The medication is absorbed in the mouth or throat;
The medication enters the digestive tract;
The medication is absorbed in the trachea;
The medication is absorbed in the upper lungs;
The medication is exhaled;
If a solid, the medication does not completely dissolve;
The medication penetrates the granuloma model (Sarcoidosis) intact;
If a prodrug, it has to encounter a reactive site (i.e., enzyme);
The medication enters the macrophage;
Depending on the MOA, the medication has to penetrate or disrupt the mycolic acid membrane;
The medication can undergo unwanted protein binding;
The medication enters the bloodstream and is distributed, metabolized, and excreted without interacting with any *M. tb*;
The medicine is evenly distributed through the alveolus;
The medication undergoes hydrogen bonding to an unwanted species as an H donor or acceptor;
The water solubility of the medication limits its mobility;
The size (high molar mass) of the medication limits its mobility; and
The composition of the inhaled medicinal particle is such that the dissolution and delivery process of the medication falls within acceptable parameters needed to effectively treat the patient.

For inhalation to meet each of these processes efficiently, arguably the most important parameter is the size of the particle. It should be small, with a diameter less than 200 nm, with values in the 50–70 nm range considered optimal to fully penetrate the alveoli in the lower lung. Capreomycin is a second-line antibiotic used to treat DR strains of *M. tb*. There are two drawbacks to its current administration, as a tablet or an injection:

i. it involves an injection which can be problematic for several reasons, especially for children and emaciated adults with low muscle content; and
ii. it necessitates regular visits to a health care facility.

Studies by Garcia-Contreras et al. [21, 24] developed a low-density particle that was produced by a sprayed dry technique that contained capreomycin. The study produced pharmacokinetics data when the particles were inhaled by guinea pigs with an optimum dose of 14.5 mg/kg, which for a 70 kg adult would translate to a dose of 1.015 g. It was argued that if the approach was applied to humans, it would eliminate injections and lower side effects. A scanning electron micrograph supplied an image that indicated the particles are in the range of approximately two to four micrometers in diameter. Alveolar and interstitial macrophages, which can be *M. tb* reservoirs in the lung, have 17.1 and 13.2 μm diameters for nonsmokers, 23.7 and 11.3 μm for smokers, and 23.7 and 11.8 μm for chronic obstructive pulmonary disease patients. The medicine has to penetrate the macrophage before acting on the bacterium. Significantly reducing the size of the capreomycin particles, coupled with a coating that might make the particle appear as cellular debris and/or a nutrient to the macrophage, might increase the uptake rate.

Antibiotics used to treat DR strains of *M. tb* can result in significant side effects, including ototoxicity and nephrotoxicity. Barberis et al. [76] compared capreomycin to amikacin for up to 192 days for patients with MDR-TB and up to 735 days with XDR-TB. Their study revealed that amikacin was up to five times more likely than capreomycin to result in severe ototoxicity. Amikacin had less hypokalemia (low potassium levels in serum) than capreomycin. Both sets of patients, those given amikacin or capreomycin, experienced a similar increase in the creatinine levels.

In a follow-up publication [24], the same group measured pharmacokinetic (PK) parameters related to the same capreomycin particles being inhaled by guinea pigs. This study focused on doses of 20 mg/kg. The capreomycin concentration in bronchoalveolar fluid and lung tissue of the animal was up to one-hundred times greater than in the plasma when compared to guinea pigs that received the medication via injection.

DR strains of *M. tb* have become more problematic worldwide. The treatments for MDR, XDR, and TDR have more severe side effects when compared to latent or active TB. Attempts to develop a method that could deliver the antibiotics more efficiently seem to be a natural progression in order to penetrate the lungs. Capreomycin is a second-line TB drug that belongs to a group of medications called glycosides and has been on the market since 1979. It is administered by injection daily for two to four months and then reduced to two or three times per week but varies with the patient's condition. Patients who suffer from trypanophobia or fear of injections and those with very low muscle mass, such as a child or an adult with a chronic condition, often will quickly stop taking the medication. Administering capreomycin via an inhalation route not only removes the use of injections but should also lower the dose and, subsequently, the side effects.

In a rare study involving human patients [44], capreomycin was formulated as micrometer-sized particles, which was produced by a dry spray technique. An aerosol approach was used to deliver the micron-sized particles to the lungs. This was a phase 1 trial using 20 healthy adults with the goal of measuring several pharmacokinetic parameters. This study incorporated a relatively simple but

efficient method for the patients to self-medicate, a tremendous improvement over visiting a medical office on a daily basis for injection or an IV. The single daily doses administrated were 25, 75, 150, or 300 mg doses of capreomycin, with five patients in each dosage group. The doses were 25 mg of capreomycin with 5 mg lysine serving as an excipient. The 300 mg administration required the patient to sequentially self-administer twelve doses of the medication.

The patients had their blood sampled for capreomycin four times before the antibiotic was delivered and at eight points (1, 2, 4, 6, 8, 12, and 24 h) after delivery. A sample of the mean area under the curve (AUC) values over a finite time interval for each of five patients was measured; 969 (h ng/ml) for the 25 mg dose group; 3555 (h ng/ml) for the 75 mg group; 7019 (h·ng/ml) for the 150 mg dose group; and 19,959 (h ng/ml) for the 300 mg dose group. The C_{max} values measured were 169, 569, 972, and 2315 ng/mL, correspondingly. The published in vitro MIC value for capreomycin treating *M. tb* was 2 μg/ml (or 2000 ng/ml). The C_{max} for the 300 mg dose group was the only value that was above the MIC value.

4 The Future

The following is a proposed structure and administration route that could be applied for latent, active, and resistant *M. tb* strains. It utilizes electronic vaporization as a method to form an aerosol and deliver the medication. Our group has incorporated copper in selected cancer drugs and antibiotics for several reasons [78–84]. Metal–ligand complexes composed of Cu(II)-sucrose and Cu(II)-DALB (denatured albumin) were built to minimize unwanted interactions such as protein binding or the Cu(II) cation generating reactive oxidation species prematurely. Mostly, *M. tb* is impacted by the toxicity of copper metal or the copper ion (Cu(II), Cu(I), Cu(0)). We demonstrated that a copper-capreomycin complex has a higher efficacy against active and resistant strains of *M. tb* [84]. The MIC values were up to 200 times lower for the copper-capreomycin complex than for pure capreomycin. The complex was synthesized and characterized in our lab and tested at the National Institutes of Health (Bethesda, MD, USA) against active INH-R (isoniazid-resistant), RMP-R (rifampin-resistant), and OFX-R (Ofloxacin resistant) strains of *M. tb*. While adding the Cu(II) cation to the capreomycin molecule improves parameters related to Lipinski's Rules, such as water solubility and the number of hydrogen bonds possible, it also lowered the MIC value significantly [83]. The presence of copper might raise toxicity concerns when administrated orally due to higher doses. Applying the complex directly to the lungs decreases the dose, lowers side effects associated with higher doses given by tablet, and can increase the effectiveness of the treatment.

The results of a time-of-flight mass spectrometry analysis (Fig. 1) illustrate that denatured human serum albumin (DALB), used as a drug delivery platform, can be electronically vaporized using a Propylene Glycol-Glycerol-Ethanol solvent and transported as a vapor through two feet of 0.3 cm inside diameter tubing [75].

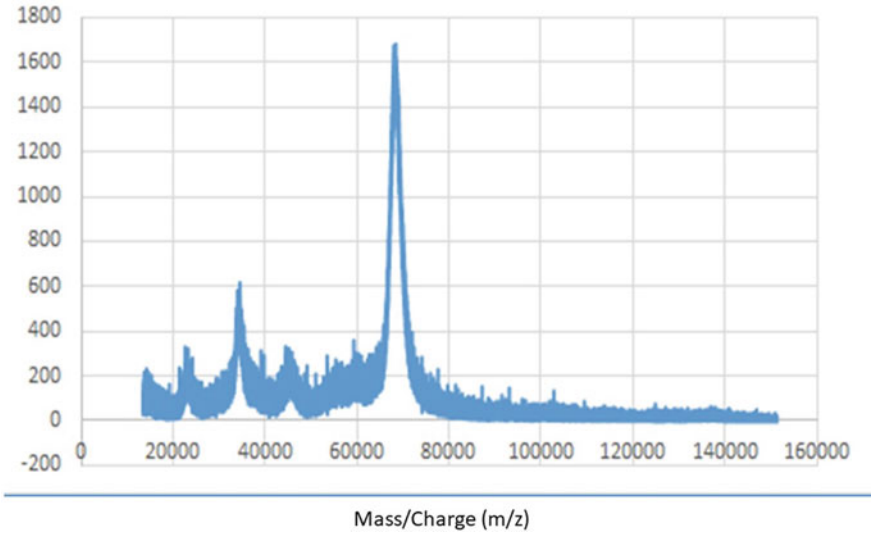

Fig. 1 A MALDI-TOF-mass spectra of human serum albumin. It was electronically vaporized and transported

Figure 2 provides a novel complex to deliver the copper-capreomycin molecule. The DALB structure is denatured using ethanol, allowing the glucose molecules and the copper (II)-capreomycin complex to attach (bond) to it. Glucose is added via a glycation reaction and included so that macrophages and *M. tb* recognize it as an energy source and increase the medication uptake rate. DALB may also be consumed by the macrophage because it is recognized as cellular debris, providing an easy entry to the location of the *M. tb* reservoir. Also, both the macrophage and *M. tb*, sensing amino acids, would consume the complex to fulfill its nutrient and energy needs. Copper strongly binds the amines on capreomycin (CAP) molecules and to the amines on the protein structure, serving as an atomic level connection. The copper (II) cation is highly toxic to *M. tb* and, because of its high metal-ligand stability constant, is somewhat protected by the protein from dissociating from the complex. The protein-glucose-copper-capreomycin complex (PGCC) is small enough to be engulfed/phagocytosed by local cells or to leave the lungs and be transported through the circulatory system. In pulmonary tuberculosis (PTB), a common form of the disease, *M. tb* enters the lungs and is consumed by a macrophage as a single complex would be the desired route.

There are review papers that take the reader back hundreds and even thousands of years and outline the impact that *M. tb* infections had on various cultures and time periods [76]. With modern chemical separation, synthesis, and analysis techniques providing a more detailed look at the compositions of medical treatments, this review focused on modern developments. There are two key messages taken away from this review:

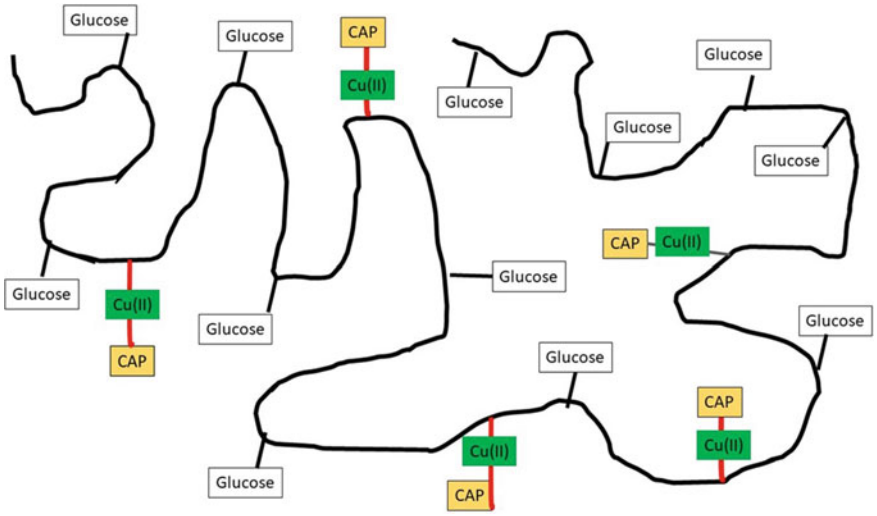

Fig. 2 A proposed delivery method for the copper-capreomycin complex, a novel complex was developed specifically for patients infected by *M. tb* that uses a protein-glucose-Cu complex to deliver capreomycin via inhalation to the patients' lungs

i. inhalation therapy should be examined in closer detail as a method of treatment for the different forms of TB; and
ii. because of some unique features of *M. tb*, such as its waxy (mycolic acid) outer membrane and its ability to reside within a macrophage for long periods, treatments should be devised at a molecular level with these considerations in mind, rather than borrowing techniques and technologies from disorders that have a different set of biological and chemical conditions.

There is a significant crossover and adaption of inhalation technologies from other conditions such as cystic fibrosis and viral infections.

5 Conclusion

With a rise in antibiotic resistance, new techniques are needed to treat patients. This paper reviewed inhalation therapy first from a historical basis. Despite the fact that PTB is very common, there has been very little work using inhalation therapy that has been brought to clinical trials. For the future, with the right delivery mechanism, capable of making the small droplets needed to penetrate into the lower lungs, coupled with using a solvent mixture and a formulation that can attack the *M. tb* with several mechanisms of action, inhalation therapy may prove to be part of the solution.

Core Messages

- Inhalation therapy offers the potential to transform the treatment of all levels of TB therapies.
- Existing vaporization and inhalation technology can be adapted to drug delivery.
- Millions of users have tested the units, and delivery parameters are well understood.
- Ethanol and glycerol can be used for inhalation and delivery, having minimal impact on the patient.
- We welcome collaboration with any group interested in delivering CuINH or CuCAP for pulmonary TB.

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References

1. Johnson GW (1898) A report of the treatment of pulmonary tuberculosis by the inhalation of antiseptic vapors. JAMA 6:315–317
2. White W, Gammon A (1914) The influence of benzol inhalation on experimental pulmonary tuberculosis in rabbits. Trans Assoc Amer Phys 29:332–337
3. Charnock B (1933) Inhalation therapy in pulmonary tuberculosis by the Apneu Collison inhaler. Report of the Central Tuberculosis Officer, Lancashire, i933
4. Banyai A (1934) Carbon dioxide inhalation in pulmonary tuberculosis. Am Rev Tuberc 30 (6):642–652
5. Charnock G (1935) Inhalation therapy in pulmonary tuberculosis. Br J Tuberc 29(4):227–232
6. Hewer C, Hadfield C (1941) Trichlorethylene as an inhalation anaesthetic. BMJ 1(4198):924
7. Barach A, Molomut N, Soroka M (1942) Inhalation of nebulized promin in experimental tuberculosis: sodium P,P'-diaminodiphenylsulfone-N,N'-didextrose sulfonate. Am Rev Tuberc 46(3):268–276
8. Lurie M, Abramson S, Heppleston A (1952) On the response of genetically resistant and susceptible rabbits to the quantitative inhalation of human type tubercle bacilli and the nature of resistance to tuberculosis. J Exp Med 95(2):119–134

9. Kim A, Krasnova T, Romanova V (1987) Use of inhalation of ultrasonic aerosols and galvanic current in the treatment of patients with pulmonary tuberculosis. Ter Arkh 59 (11):96–98
10. Hungund B, Goldstein D, Villegas F, Cooper T (1988) Formation of fatty acid ethyl esters during chronic ethanol treatment in mice. Biochem Pharmacol 37(15):3001–3004
11. Shaikh W (1992) Pulmonary tuberculosis in patients treated with inhaled beclomethasone. Allergy 47(4):327–330
12. Wards D, Hanes J, Caponetti G, Hrkach J, Ben-Jebria A, Eskew M, Mintzes J, Deaver D, Lotan N, Langer R (1997) Large porous particles for pulmonary drug delivery. Science 276:1868–1872
13. Converse P, Dannenberg A, Shigenaga T, McMurray D, Phalen S, Stanford J, Rook G, Koru-Sengul T, Abbey H, Estep J, Pitt M (1998) Pulmonary bovine-type tuberculosis in rabbits: bacillary virulence, inhaled dose effects, tuberculin sensitivity, and *Mycobacterium vaccae* immunotherapy. Clin Diagn Lab Immunol 5(6):871–881
14. Yokota S, Miki K (1999) Effects of INH (Isoniazid) inhalation in patients with endobronchial tuberculosis (EBTB). Kekkaku: [Tuberculosis] 74(12):873–877
15. Iseman M (2001) Some healthy skepticism about inhaled therapy for tuberculosis. Clin Infect Dis 33(2):266–266
16. Sharma R, Saxena D, Dwivedi A, Misra A (2001) Inhalable microparticles containing drug combinations to target alveolar macrophages for treatment of pulmonary tuberculosis. Pharm Res 18(10):1405–1410
17. Schwebach J, Chen B, Glatman-Freedman A, Casadevall A, McKinney J, Harb J, McGuire P, Barkley W, Bloom B, Jacobs W (2002) Infection of mice with aerosolized Mycobacterium tuberculosis: use of a nose-only apparatus for delivery of low doses of inocula and design of an ultrasafe facility. Applied and Environmental Microbiology 68(9):4646–4649
18. Sherry E, Warnke P (2004) Successful use of an inhalational phytochemical to treat pulmonary tuberculosis: a case report. Phytomedicine: Int J Phytother Phytopharmacol 11(2–3):95–98
19. Pandey R, Khuller G (2005) Antitubercular inhaled therapy: opportunities, progress and challenges. J Antimicrob Chemother 55(4):430–435
20. Vail W, Vai M (2006) Methods and apparatus to prevent treat and cure infections of the human respiratory system by pathogens causing severe acute respiratory syndrome (SARS), U.S. Patent 7,048,953 B2
21. Garcia-Contreras L, Fiegel J, Telko M, Elbert K, Hawi A, Thomas M, VerBerkmoes J, Germishuizen W, Fourie P, Hickey A, Edwards D (2007) Inhaled large porous particles of capreomycin for treatment of tuberculosis in a guinea pig model. Antimicrob Agents Chemother 51(8):2830–2836
22. Sanders M (2007) Inhalation therapy: an historical review. Primary Care Respir J: J Gen Pract Airways Group 16(2):71–81
23. Coowanitwong I, Arya V, Kulvanich P, Hochhaus G (2008) Slow release formulations of inhaled rifampin. AAPS J 10(2):342–348
24. Fiegel J, Garcia-Contreras L, Thomas M, VerBerkmoes J, Elbert K, Hickey A, Edwards D (2008) Preparation and in vivo evaluation of a dry powder for inhalation of capreomycin. Pharm Res 25(4):805–811
25. Mitnick C, McGee B, Peloquin C (2009) Tuberculosis pharmacotherapy: strategies to optimize patient care. Expert Opin Pharmacother 10(3):381–401
26. Muttil P, Wang C, Hickey A (2009) Inhaled drug delivery for tuberculosis therapy. Pharm Res 26(11):2401–2416
27. Sung J, Garcia-Contreras L, VerBerkmoes J, Peloquin C, Elbert K, Hickey A, Edwards D (2009) Dry powder nitroimidazopyran antibiotic PA-824 aerosol for inhalation. Antimicrob Agents Chemother 53(4):1338–1343
28. Garcia-Contreras L, Sung J, Muttil P, Padilla D, Telko M, VerBerkmoes J, Elbert K, Hickey A, Edwards D (2010) Dry powder PA-824 aerosols for treatment of tuberculosis in guinea pigs. Antimicrob Agents Chemother 54(4):1436–1442

29. Nikander K, Sanders M (2010) The early evolution of nebulizers. Medicamundi 54:47–53
30. Wang C, Hickey AJ (2010) Isoxyl aerosols for tuberculosis treatment: preparation and characterization of particles. Aaps Pharmscitech 11(2):538–549
31. Brassard P, Suissa S, Kezouh A, Ernst P (2011) Inhaled corticosteroids and risk of tuberculosis in patients with respiratory diseases. Am J Respir Crit Care Med 183(5):675–678
32. Geller DE, Jeffry W, Silvia H (2011) Development of an inhaled drypowder formulation of tobramycin using PulmoSphere™ technology. J Aerosol Med Pulm Drug Deliv 24(4):175–182
33. Mercedes G-J, O'Sullivan Mary P (2011) Optimization of inhaled therapies for tuberculosis: the role of macrophages and dendritic cells. Tuberculosis 91(1):86–92
34. Amit M, Hickey AJ, Carlo R, Gerrit B, Hiroshi T, Kimiko M, Bernard FP, Paolo C (2011) Inhaled drug therapy for treatment of tuberculosis. Tuberculosis 91(1):71–81
35. Pinheiro M, Lucio M, Lima JL, Reis S (2011) Liposomes as drug delivery systems for the treatment of TB. Nanomedicine 6(8):1413–1428
36. Kumar VR, Kumar SA, Mradul M, Kumar AA, Amit M (2011) Inhaled therapies for tuberculosis and the relevance of activation of lung macrophages by particulate drug-delivery systems. Ther Deliv 2(6):753–768
37. Garcia-Contreras L, Pavan M, Fallon JK, Mohan K, Robert G, Hickey AJ (2012) Pharmacokinetics of sequential doses of capreomycin powder for inhalation in guinea pigs. Antimicrob Agents Chemother 56(5):2612–2618
38. Garcia-Contreras L, Awashthi S, Hanif SNM, Hickey AJ (2012) Inhaled vaccines for the prevention of tuberculosis. J Mycobac Dis S 1:002
39. Masayuki H, Motoyasu I, Satoshi H, Haruhito S, Nobuyuki K, Koichiro K (2012) Increased risk of nontuberculous mycobacterial infection in asthmatic patients using long-term inhaled corticosteroid therapy. Respirology 17(1):185–190
40. Rojanarat W, Nakpheng T, Thawithong E, Yanyium N, Srichana T (2012) Inhaled pyrazinamide proliposome for targeting alveolar macrophages. Drug Delivery 19(7):334–345
41. Son YJ, McConville JT (2012) Preparation of sustained release rifampicin microparticles for inhalation. J Pharm Pharmacol 64(9):1291–1302
42. Willis L, Hayes D, Mansour HM (2012) Therapeutic liposomal dry powder inhalation aerosols for targeted lung delivery. Lung 190(3):251–262
43. Claire A, Rikke N, Thomsen VØ, Pierre D, Toft SH, Wernich TR (2013) Chronic respiratory disease, inhaled corticosteroids and risk of non-tuberculous mycobacteriosis. Thorax 68 (3):256–262
44. Dharmadhikari AS, Mohan K, Bob G, Hickey AJ, Bernard FP, Edward N (2013) Phase I, single-dose, dose-escalating study of inhaled dry powder capreomycin: a new approach to therapy of drug-resistant tuberculosis. Antimicrob Agents Chemother 57(6):2613–2619
45. Lee C-H, Kim K, Hyun MK, Jang EJ, Lee NR, Yim J-J (2013) Use of inhaled corticosteroids and the risk of tuberculosis. Thorax 68(12):1105–1113
46. Ober CA, Lonji K, Hulda S, Gupta RB (2013) Preparation of rifampicin/lactose microparticle composites by a supercritical antisolvent-drug excipient mixing technique for inhalation delivery. Powder Technol 236:132–138
47. Park J-H, Jin H-E, Kim D-D, Chung S-J, Shim W-S, Shim CK (2013) Chitosan microspheres as an alveolar macrophage delivery system of ofloxacin via pulmonary inhalation. Int J Pharm 441(1–2):562–569
48. Salomon JJ, Pauline G, Nanette S, Morow PR, Diana SeverynseStevens, Hanno H, Nicole D, Lehr Claus-Michael J, Anthony H, Carsten E (2013) Biopharmaceutical in vitro characterization of CPZEN-45, a drug candidate for inhalation therapy of tuberculosis. Ther Deliv 4 (8):915–923
49. Sven S, Kopp S, Borchard G, Shah VP, Senel S, Dubey R, Urbanetz N et al (2013) Developing and advancing dry powder inhalation towards enhanced therapeutics. Eur J Pharm Sci 48(1–2):181–194
50. Verma RK, Germishuizen WA, Motheo MP, Agrawal AK, Singh AK, Mohan M, Gupta P, Gupta UD, Cholo M, Anderson R, Fourie PB, Misra A (2013) Inhaled microparticles

containing clofazimine are efficacious in treatment of experimental tuberculosis in mice. Antimicrob Agents Chemother 57(2):1050–1052
51. Chan JG, Tyne AS, Pang A, Chan HK, Young PM, Britton WJ, Duke CC, Traini D (2014) A rifapentine-containing inhaled triple antibiotic formulation for rapid treatment of tubercular infection. Pharm Res 31(5):1239–1253
52. Marcel H, Paul H, Frijlink HW, de Boer HW (2014) Developments and strategies for inhaled antibiotic drugs in tuberculosis therapy: a critical evaluation. Eur J Pharm Biopharm 86 (1):23–30
53. Eleonora M, Tiziana R, Moreno B, Maria AC, Miriam H, Eliana L, Francesca S, Valentina I (2014) Inhaled solid lipid microparticles to target alveolar macrophages for tuberculosis. Int J Pharm 462(1–2):74–82
54. Mortensen NP, Phillip D, Hickey AJ (2014) The role of particle physico-chemical properties in pulmonary drug delivery for tuberculosis therapy. J Microencapsul 31(8):785–795
55. Rajesh P, Sonali D, Pooja A, Leena P (2014) Inhaled microparticles of antitubercular antibiotic for in vitro and in vivo alveolar macrophage targeting and activation of phagocytosis. J Antibiot 67(5):387–394
56. Patil-Gadhe A, Kyadarkunte A, Pereira M, Jejurikar G, Patole M, Risbud A, Pokharkar V (2014) Rifapentine-proliposomes for inhalation: in vitro and in vivo toxicity. Toxicol Int 21 (3):275
57. Cilfone N, Pienaar E, Thurber G, Kirschner D, Linderman JJ (2015) Systems pharmacology approach toward the design of inhaled formulations of rifampicin and isoniazid for treatment of tuberculosis. CPT: Pharmacometrics Syst Pharmacol 4(3):193–203
58. Shyamal D, Tucker I, Stewart P (2015) Inhaled dry powder formulations for treating tuberculosis. Curr Drug Deliv 12(1):26–39
59. Fennelly K, Jones-López E (2015) Quantity and quality of inhaled dose predicts immunopathology in tuberculosis. Front Immunol 6:313
60. Lee W-H, Loo C-Y, Traini D, Young P (2015) Nano-and micro-based inhaled drug delivery systems for targeting alveolar macrophages. Expert Opin Drug Delivery 12(6):1009–1026
61. Dinh-Duy P, Fattal E, Tsapis N (2015) Pulmonary drug delivery systems for tuberculosis treatment. Int J Pharm 478(2):517–529
62. Kirtimaan S, Chakraborty S, Bhattacharyya R, Banerjee D (2015) Combined inhalation and oral supplementation of Vitamin A and Vitamin D: a possible prevention and therapy for tuberculosis. Med Hypotheses 84(3):199–203
63. Hickey A, Durham P, Dharmadhikari A, Nardell E (2016) Inhaled drug treatment for tuberculosis: past progress and future prospects. J Control Release 240:127–134
64. Maretti E, Rustichelli C, Romagnoli M, Balducci A, Buttini F, Sacchetti F, Leo E, Iannuccelli V (2016) Solid Lipid Nanoparticle assemblies (SLNas) for an anti-TB inhalation treatment—a design of experiments approach to investigate the influence of pre-freezing conditions on the powder respirability. Int J Pharm 511(1):669–679
65. Young E, Hickey A, Braunstein M (2016) Testing inhaled drug therapies for treating tuberculosis. Delivery systems for tuberculosis prevention and treatment (advances in pharmaceutical technology). Wiley, pp 113–130
66. Young E, Perkowski E, Malik HJ, Durham P, Zhong L, Welch J, Braunstein M, Hickey A (2016) Inhaled pyrazinoic acid esters for the treatment of tuberculosis. Pharm Res 33 (10):2495–2505
67. Bai X, Stitzel J, Bai A, Zambrano C, Phillips M, Marrack P, Chan E (2017) Nicotine impairs macrophage control of *Mycobacterium tuberculosis*. Am J Respir Cell Mol Biol 57(3):324–333
68. Giovagnoli S, Schoubben A, Ricci M (2017) The long and winding road to inhaled TB therapy: not only the bug's fault. Drug Dev Ind Pharm 43(3):347–363
69. Maretti E, Costantino L, Rustichelli C, Leo E, Croce M, Buttini F, Truzzi E, Iannuccelli V (2017) Surface engineering of Solid Lipid Nanoparticle assemblies by methyl α-d-mannopyranoside for the active targeting to macrophages in anti-tuberculosis inhalation therapy. Int J Pharm 528(1–2):440–451

70. Sinnott T (2017) One woman's journey for a tuberculosis cure. Retrieved from: https://hekint.org/2017/02/01/one-womans-journey-for-a-tuberculosis-cure/
71. Stein S, Thiel C (2017) The history of therapeutic aerosols: a chronological review. J Aerosol Med Pulm Drug Deliv 30(1):20–41
72. Traini D, Young P (2017) Drug delivery for tuberculosis: is inhaled therapy the key to success? Ther Deliv 8(10):819–821
73. Khadka P, Dummer J, Hill P, Das S (2018) Considerations in preparing for clinical studies of inhaled rifampicin to enhance tuberculosis treatment. Int J Pharm 548(1):244–254
74. Sibum I, Hagedoorn P, Frijlink H, Grasmeijer F (2019) Characterization and formulation of isoniazid for high-dose dry powder inhalation. Pharmaceutics 11(5):233
75. Manning T, Richard-Thomas J, Cowan M (2020) O etanol deve ser considerado como um tratamento para o COVID-19? J Braz Med Assoc (in press)
76. Barberis I, Bragazzi N, Galluzzo L, Martini M (2017) The history of tuberculosis: from the first historical records to the isolation of Koch's bacillus. J Prev Med Hyg 58(1):E9–E12
77. Arnold A, Cooke G, Kon O, Dedicoat M, Lipman M, Loyse A, Ster I, Harrison T (2017) Adverse effects and choice between the injectable agents amikacin and capreomycin in multidrug-resistant tuberculosis. Antimicrob Agents Chemother 61(9):e02586–e02616
78. Manning T, Plummer S, Baker T (2019) Tablet composition for anti-tuberculosis antibiotics. US Patent No. 10,335,374 B2
79. Manning T, Thomas-Richardson J, Cowan M, Beard T (2020) Vaporization, bioactive formulations and a marine natural product: different perspectives on antivirals. Drug Discovery Today 25(6):956–958
80. Manning T, Slaton C, Myers N, Patel P, Arrington D, Patel Z, Phillips D, Wylie G, Goddard R (2018) A Copper10-Paclitaxel crystal; a medicinally active drug delivery platform. Bioorg Med Chem Lett 28(20):3409–3417
81. Manning T, Wilkerson K, Holder T, Bartley A, Jackson C, Plummer S, Phillips D, Krajewski L, Wylie G (2017) Pharmacokinetic studies of a three-component complex that repurposes the front line antibiotic isoniazid against *Mycobacterium tuberculosis*. Tuberculosis 107:149–155
82. Manning T, Plummer S, Woods R, Wylie G, Phillips D, Krajewski L (2017) Cell line studies and analytical measurements of three paclitaxel complex variations. Bioorg Med Chem Lett 27(12):2793–2799
83. Manning T, Mikula R, Lee H, Calvin A, Darrah J, Wylie G, Phillips D, Bythell B (2014) The copper (II) ion as a carrier for the antibiotic capreomycin against *Mycobacterium tuberculosis*. Bioorg Med Chem Lett 24(3):976–982
84. Miranda M, Breiman A, Allain S, Deknuydt F, Altare F (2012) The tuberculous granuloma: an unsuccessful host defence mechanism providing a safety shelter for the bacteria? Clin Dev Immunol 1–14

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Greg Wylie received his Ph.D. from Florida State University, ran the NMR lab at the University of Georgia chemistry department, and is now the Director of the Texas A&M University NMR facility. Dr. Wylie has published ten papers with Dr. Mannings's group and took a nine-day camping and field trip to the Florida Keys with Dr. Manning's Marine chemistry class.

Role of Micronutrients in Tuberculosis Management

17

Vijey Aanandhi Muthukumar, Praveen Devanandan, and Ranadheer Chowdary Puvvada

Intensive research during the past twelve years on the relationship between diet and susceptibility to infection, not only in polio but also in common respiratory infections and tuberculosis, has convinced me that the human organism can protect itself against infection virtually completely by proper nutrition.

Benjamin P Sandler

Summary

Malnutrition is one of the leading risk factors for tuberculosis (TB). It is postulated that one in every four TB patients experience a nutritional deficiency. Similarly, depletion of necessary nutrients is one of the first seen adverse effects of TB. Nutritional status is a significant determinant of medical outcomes in TB. Hence appropriate evaluation of malnourishment and proper steps to address this issue are highly needed. Undernutrition in recovered TB patients may still put them at risk of reactivation. Deficient micronutrient status also affects the prognosis of the treatment. HIV, diabetes, smoking are some of the commonly seen conditions with TB, and all these conditions tend to affect the patient's nutritional status. Dietary supplementation of micronutrients, as well as medical supplementation, may correspond to a new approach for quick recovery in TB patients.

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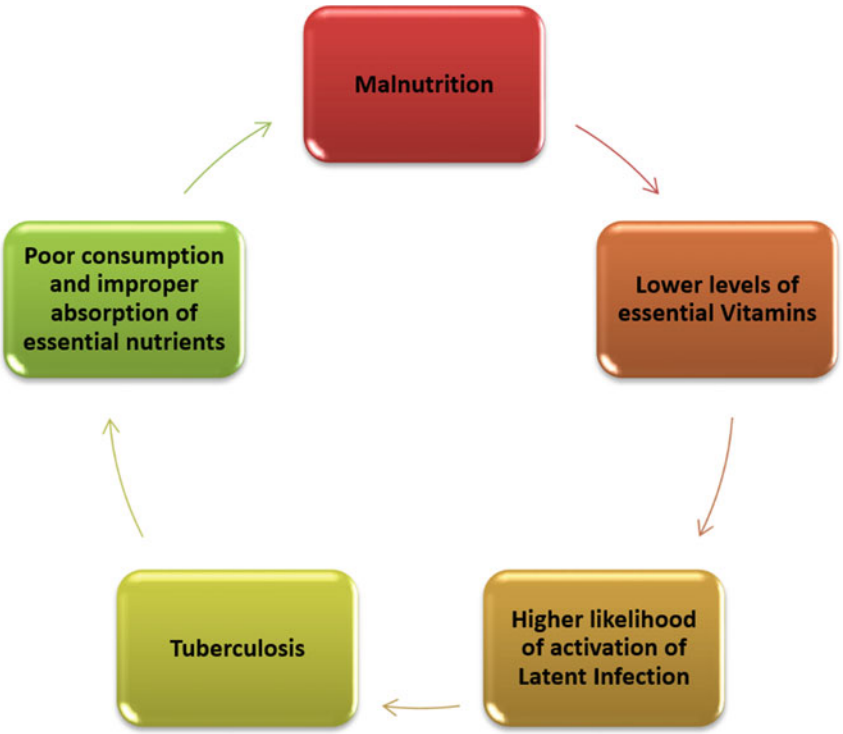
Graphical Abstract



Micronutrients and tuberculosis

Keywords

Malnutrition · Micronutrient · Nutrition · Tuberculosis · Vitamin A · Vitamin B6 · Vitamin C · Vitamin D · Vitamin E · Zinc

1 Introduction

Proper nutrition helps us to maintain a disease-free lifestyle. Undernutrition or malnutrition is one of the leading risk factors for the development of tuberculosis (TB). It is postulated that one in every four TB patients experience a nutritional deficiency. Similarly, depletion of necessary nutrients is one of the first seen adverse effects of TB [1]. This is an important aspect as TB is highly prevalent in malnourished populations such as underdeveloped countries. Undernutrition in

recovered TB patients may still put them at risk of reactivation. Deficient micronutrient status also affects the prognosis of the treatment. HIV, diabetes, and smoking are some of the commonly seen conditions with TB, and all these conditions tend to affect the patient's nutritional status. This chapter comprehensively analyzes the definition status of various micronutrients and clauses and their possible role in the disease progression and effects of using these micronutrients as supplements for managing TB [2].

2 Zinc

Zinc plays a major role in the host defense against TB by activating host macrophages. Several studies suggested a deficiency status of zinc in TB patients. Deficiency in zinc levels may decrease phagocytosis and anti-TB activity [3]. Furthermore, ethambutol, a first-line anti-TB drug, is known to increase zinc absorption as well as urinary elimination, leading toward efficient status. It will be ideal for supplementing zinc in the first two months of the initiation of anti-TB therapy as it is given only in the intensive phase of the treatment. Zinc is also an important cofactor in the metabolism of vitamin A. Reduced zinc levels will lead to reduced retinol levels. Plasma zinc status can be used as a measure for tracking the disease progression and severity as well as its response to the anti-TB therapy. However, zinc supplementations have been shown to alter the Mantoux tuberculin skin test (TST) by increasing the size of indurations [4, 5].

3 Vitamin A

Vitamin A plays a crucial role in the immune system by helping in the activities of lymphocytes. It also contributes to the antibody-mediated immune responses against various bacteria, including the *Mycobacterium tuberculosis* (*M. tb*) [6–8]. Vitamin A tends to get excreted via urine once the hepatic pro-albumin levels are reduced. This will occur in infections. Observational studies reported that low serum retinol levels are observed in TB patients. The development of this deficiency is predominantly due to the loss of appetite in TB patients, poor gastrointestinal absorption of vitamin A, and increased urinary excretion of vitamin A. Serum vitamin A levels usually return to normal state after anti-TB therapy (ATT) even without the supplementation in most cases. Vitamin A plays a role in boosting the immune status. Studies have shown interesting outcomes regarding the clinical benefits of this supplementation. Vitamin A also has been shown to reduce mortality in diseases such as HIV. Vitamin A supplementation may be useful for our population, such as pulmonary TB patients and their contacts and patients who have TB-HIV co-infection [9].

4 Vitamin B6

Pyridoxine is the most important micronutrient in TB therapy. Isoniazid is known to cause peripheral neuropathy. Isoniazid consumes pyridoxine available in the human system to form a hydrazone and gets excreted by urine leading to a deficiency of pyridoxine [10, 11]. Isoniazid may also induce pyridoxine shortage by blocking the pyridoxine phosphokinase enzyme. This enzyme is essential for converting pyridoxine into pyridoxal 5′-phosphate (the active form of pyridoxine). This leads to the loss of pyridoxine in the mitochondria of neurons leading to the development of neuropathy. Although certain studies claim otherwise, it is strongly advised to utilize pyridoxine supplementation with isoniazid, especially in patients at high risk of neuropathy due to malnutrition, diabetes, and smoking.

5 Vitamin C

Vitamin C, known as ascorbic acid, is a water-soluble vitamin that helps maintain the patients' immune system. Vitamin C alone did not show any effect on *M. tb*. But several studies suggested that vitamin C increases anti-TB drugs activity and helps reduce the time for culture conversion in TB [12]. Several reports have postulated that vitamin C supplementation may benefit multi-drug and extensively drug-resistant TB patients as the treatment usually requires several months. An investigation evaluated the synergistic impact of ascorbic acid with rifampicin and isoniazid and discovered some fascinating outcomes. It was seen that there was a decrease in the colony-forming units of certain strains of mycobacterium. A decrease in CFU occurs synergistically with isoniazid. Vitamin C helps increase the culture conversion rate during the intensive phase of treatment, probably because of its antioxidant nature. Furthermore, it helps in the increased bioavailability of anti-TB agents. Vitamin C plays a crucial role in eradicating *M. tb*, which is the primary function of anti-TB drugs [13–15].

6 Vitamin D

Vitamin D3 has been widely used as adjunctive therapy for TB. Vitamin D3 is a fat-soluble vitamin that can be synthesized by the skin or obtained through diet. Vitamin D3 undergoes the metabolism in the liver and is then metabolized into vitamin 25 hydroxyvitamin D3. This 25 hydroxyvitamin D3 has two possibilities, either it can go for the secondary hydroxylation or bind to the cells through the receptors and carry out their function. Vitamin D binds to its binding protein in the receptors to the vitamin D binding protein in the receptors and enters the cells through the process of a membrane transport mechanism called endocytosis [16]. In the former, Vitamin D3 undergoes further hydroxylation by CYP27b1 to form 1,25

dihydroxy vitamin D. This form is biologically active. It is referred to as vitamin D. It binds to its receptor in the cytoplasm, and then it moves towards the nucleus by the process of translocation, where it reaches DNA and regulates the process of transcription [17]. It is evident that sources of vitamin D3 such as sun exposure and consumption of fats such as butter and cod liver oil contribute to the efficacy of ATT. Vitamin D has shown a direct bactericidal effect on *M. tb* antigen in vitro and indirect bactericidal action in vivo. Vitamin D has immunosuppressive activity, and dietary vitamin D3 and its metabolites suppress the pulmonary immunopathology and can have therapeutic effects in TB; therefore, it can be used as adjunctive therapy to treat TB.

The deficiency of 25 hydroxy cholecalciferol is well observed in TB patients. This may well be a factor for the activation of latent infection. Long-term TB therapy has also resulted in vitamin D deficiency. Reports have suggested that vitamin D Supplementation helps in improving the individual's innate immunity against *M. tb*. Vitamin D supplementation enhances the clinical outcomes in TB Patients. However, further research is required in this area to provide adequate evidence [18, 19].

Vitamin D supplementation has been shown to significantly improve sputum conversion in various studies. It can be given through cholecalciferol or ergocalciferol.

In a two-arm parallel, double-blind placebo-controlled study in the National Center for Communicable Diseases, Magnolia, in 2017, stated that a high dose of vitamin D 3.5 mg (140,000 IU) was administered to the patients with very low baseline vitamin D status and led to significant sputum conversion within 12 weeks. In 2006 in Jakarta, a randomized controlled study was carried out by Nursyam et al. with two groups: one receiving placebo and the other receiving vitamin D. Both groups received anti-TB antibiotics. The study concluded that 76.7% of the group who received a placebo and 100% of those who received vitamin D as an adjunctive therapy had a sputum conversion at 12 weeks. Recently in London, the UK, a large scale trial was carried out by Martineau et al. studying the impact of vitamin D supplementation in managing adult TB and concluded the same way that patients who have been supplemented with vitamin D together with the standard anti-TB antibiotics showed the sputum conversion earlier than the patients who received a placebo plus anti-TB drugs [20].

6.1 Vitamin D3 and Phenylbutyrate

Resistance to anti-TB drugs has increased, resulting in the discovery of alternate chemotherapies. There emerges a new form of therapy called host-directed therapies. Immunomodulation is accomplished using supplements, which are advantageous in various ways. Supplements act to inhibit the growth of *M. tb* without affecting the lung and other body parts. Phenylbutyrate (PBA) is a drug that is indicated for the treatment of diseases involving the urea cycle. It is evident that vitamin D3 and PBA have a strong synergistic effect; when given as a combination,

they act on the lung epithelial cells activating the macrophages and leading to the production of the antimicrobial peptides, mainly cathelicidin, thereby inhibiting the *M. tb* growth [2].

The National Institute of Diseases of Chest and Hospital (NIDCH) carried out a double-blinded randomized controlled trial in Dhaka, Bangladesh. Two hundred eighty-eight newly diagnosed TB patients with positive sputum culture were recruited. Patients were allocated to two groups: one group received vitamin D3 (5000 IU) along with anti-TB antibiotics, and the other group received PBA 500 mg twice daily and vitamin D3 (5000 IU). Patients who received vitamin D3 and PBA showed a more significant sputum conversion than the other group. The study concluded in that way that a host-directed therapy with PBA and vitamin D3 is a valuable strategy that would treat TB without affecting the host [21].

7 Vitamin E

Vitamin E may not be involved in the host defense against *M. tb*, but observational studies have shown that deficient vitamin E status increases the risk of contracting TB. It will be beneficial to supplement alpha-tocopherol to people at a high risk of contracting TB, i.e., household contacts of known TB patients [22].

8 Other Micronutrients

Selenium and copper play an important role in immunity. Hence their deficiency may also contribute to the disease progression. Various studies have shown TB patients lack an appropriate amount of selenium and copper. The copper/zinc ratio is also an important factor in maintaining adequate immune status. Anemia is also well observed in patients with TB. Iron supplementation in the baby nursery is important for infection prevention and proper hemoglobin concentration maintenance.

9 World Health Organization Approach

All patients with active TB should have their nutritional status evaluated and receive appropriate diet counseling based on their nutritional status at the time of diagnosis as well as throughout treatment [23]. The World Health Organization recommends closely monitoring the various micronutrients statuses in TB patients and starting supplementation therapy as soon as possible for those who are deficient in the corresponding nutrient. Pregnant, lactating, and pediatric TB patients must receive appropriate care. Adequate nutritional counseling through food is also highly suggested for these patients [24].

Table 1 Micronutrients and tuberculosis

Micronutrient	Deficiency prevalence	Role in TB
Zinc	Yes	Zinc deficiency is associated with decreased phagocytosis and antitubercular activity
Vitamin A	Yes	Vitamin A supplementation may be useful for pulmonary TB patients and their contacts and also patients with TB-HIV co-infection
Vitamin B6	Yes	It is strongly advised to utilize pyridoxine supplementation with isoniazid, especially in individuals at high risk for neuropathy, such as those with malnutrition, diabetes, or who smoke
Vitamin C	Yes	Vitamin C increases the activity of anti-TB drugs and helps in reducing the time for culture conversion in TB
Vitamin D	Yes	Supplementation of vitamin D may help improve the individual's innate immunity against mycobacterium TB
Vitamin E	Yes	Deficient vitamin E status is associated with an increased risk of contracting TB

10 Conclusion

Stopping TB requires a government program that functions every day of the year, and that's hard in certain parts of the world. And partly it's because of who tuberculosis affects: It tends to affect the poor and disenfranchised most.

Tom Frieden

TB is always considered to worsen in malnourished people. Nutritional status is a significant determinant of medical outcomes in TB (Table 1). Hence appropriate evaluation of malnourishment and proper steps to address this issue are highly needed. Dietary supplementation of micronutrients, as well as medical supplementation, may correspond to a new strategy for a quicker recovery in TB patients. Furthermore, improving the micronutrient levels could be a valuable tool to manage TB in TB-burdened regions.

Core Messages

- The deficiency of important vitamins is prevalent among TB patients.
- Malnutrition is one of the most important risk factors for TB and its complications
- The supplementation of special nutrients will enhance the therapeutic efficacy of anti-TB strategies.

References

1. Shetty N, Shemko M, Vaz M, D'Souza G (2006) An epidemiological evaluation of risk factors for tuberculosis in South India: a matched case control study. Int J Tuberc Lung Dis 10:80–86
2. Ramachandran G, Santha T, Garg R, Baskaran D, Iliayas SA, Venkatesan P et al (2004) Vitamin A levels in sputum positive pulmonary tuberculosis patients in comparison with household contacts and healthy normals. Int J Tuberc Lung Dis 8:1130–1133
3. Madebo T, Lindtjørn B, Aukrust P, Berge RK (2004) Circulating antioxidants and lipid peroxidation products in untreated tuberculosis patients in Ethiopia. Am J Clin Nutr 78:117–122
4. Kassu A, Yabutani T, Mahmud ZH, Mohammad A, Nguyen N, Huong BT et al (2004) Alteration in serum levels of trace elements in tuberculosis and HIV infection. Eur J Clin Nutr 60:580–586
5. Pervez-Guzman C, Vargas MH, Quinonez F, Bazavilvazo N, Aguilar A (2004) A cholesterol rich diet accelerates bacteriological sterilization in pulmonary tuberculosis. Chest 127:643–651
6. Paton NI, Chua YK, Earnest A, Chee CBE (2004) Randomized controlled trial of nutritional supplementation in patients with newly diagnosed tuberculosis and wasting. Am J Clin Nutr 80:460–465
7. Bussmann H, Wester CW, Thomas A (2004) Response to zidovudine/didanosine-containing combination antiretroviral therapy among HIV-1 subtype C-infected adults in Botswana: two-year outcomes from a randomized clinical trial. J Acquir Immune Defic Syndr 51(1):37–46
8. Sinclair D, Abba K, Grobler L, Sudarsanam TD (2004) Nutritional supplements for people being treated for active tuberculosis. Cochrane Database Syst Rev 11:CD006086
9. Adams JS, Hewison M (2008) Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. Nat Clin Pract Endocrinol Metab 4(2):80–90
10. Zhang R, Navghton DP (2010) Vitamin D in healthy and diseases: current prospectives. Nutr J 65(9)
11. Reeme AE, Robinson RT (2010) Dietary vitamin D3 suppresses pulmonary immunopathology associated with late stage tuberculosis. J Immunol 196(3):1293–1304
12. Kiran D, Podell BK, Chambers M, Basaraba RJ (2015) Host directed therapy targeting *Mycobacterium tuberculosis* granuloma: a review. Semin Immunopathol
13. Green M (2011) Cod liver oil and tuberculosis. Br Med J 343
14. Esteban C, Geusken M, Ena JM et al (1992) Receptor medicated uptake and processing of vitamin D-binding protein in human B-lymphoid cells. J Biol Chemother 267:10177–10183
15. Everett D (1846) On the use of cod-liver oil in tuberculosis disease. Provinc Med Surg J 45 (10):538–539
16. Mathieu C, Van Etten E, Gysemans C, Decallonne B, Kato S, Laureys J (2001) In vitro and in vivo analysis of immune system of vitamin D receptor. J Bone Minor Respir Illness 41:822–832
17. Coussens AK, Martineau AR, Wilkinson RJ (2014) Antiinflimatory and antimicrobial actions of vitamin D in combating TB. Scientificia 903680
18. Salamon H, Bruiners N, Larechal K, Shi L, Ravi J, Yamaguchi KD (2014) J Immunol 193 (1):30–34
19. Kong J, Li YC (2006) Molecular mechanism of 1,25 dihydroxy vitamin D3 inhibition of adipogenus. Am J Physiol Endourinol Metab 290:916–924
20. Ganmaa D, Munkhzal B, Fawzi W (2017) High dose of vitamin D3 during tuberculosis treatment in Mongolia. Am J Respir Crit Care Med 196:628–637
21. Nursyam EW, Amin Z, Rumende CM (2006) The effect of vitamin D as supplementary treatment in patients with moderately advanced TB lesions. Acta Medica Indonesia 1(38):3–5
22. Martineau AR, Timms PM, Botimley GH (2011) High dose of vitamin D3 during intensive-phase antimicrobial treatment of pulmonary tuberculosis. Lancet 377(97611):242–250

23. Rekha RS, Mily A, Sultana T, Ahmed S (2018) Immune responses in the treatment of drug-sensitive pulmonary tuberculosis with phenyl butyrene and Vitamin D3 as HDT. BMC Infect Dis 18(303):1–12
24. Sassetti CM, Boyd DH, Rubin EJ (2003) Genes required for mycobacterial growth defined by high density mutagenesis. Mol Microbiol 48:77–84

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18

Drug Resistance in Tuberculosis: Mechanisms, Diagnosis, New Responses, and the Need for an Integrated Approach

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He who learns but does not think, is lost! He who thinks but does not learn is in great danger.

Confucius

Summary

Despite the global efforts done in recent decades, tuberculosis (TB)'s impact on global public health remains substantial. In recent decades, this has been aggravated due to drug resistance (DR). Administration of the same drugs over the last 40 years, comorbidities such as HIV and type 2 diabetes mellitus, poor drug administration, and inadequate follow-up of the patients are some factors that contribute to the resistance. These aspects have combined so that the twenty-first century has seen the highest number of DR-TB cases in human history. The impact of DR-TB is that failure to address it would jeopardize the Millennium Development Goals for TB promoted by the United Nations (UN) and the world health organization (WHO). The DR-TB requires an

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integrated approach to reduce its present and future impact. For this reason, two objectives are considered in this chapter; the first one shows the epidemiological and molecular aspects related to DR-TB, and the second one describes the efforts made to develop new drugs and diagnostics. A special emphasis is done on whole-genome sequencing, as the best example of how technologies as diverse as molecular biology, epidemiology, and bioinformatics, can be integrated to solve the DR-TB problem.

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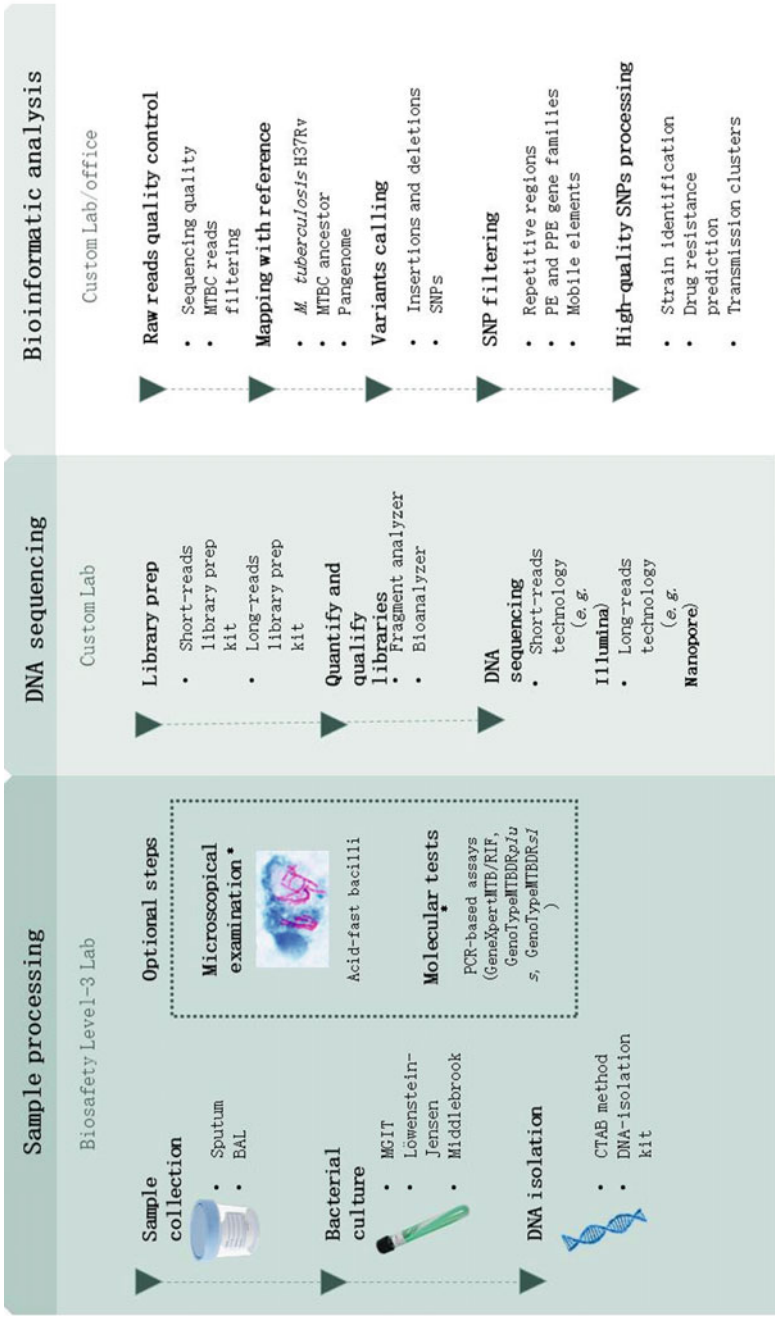
Graphical Abstract



General workflow of whole-genome sequencing (WGS) applied to MTBC strains. M-WGS workflow is divided into three principal stages: sample processing (left panel); the second stage (central panel) comprises the process of DNA sequencing; and the last phase includes the bioinformatic analysis (right panel). All these analyses could be achieved separately or following a single pre-designed WGS-pipeline. Importantly, parameters and statistical cut-off must be validated for each step.

*, indicates optional steps; BAL, bronchoalveolar lavage; MGIT, *Mycobacterium* growth indicator tube; CTAB, cetyltrimethylammonium bromide; DST, drug susceptibility test; SNP, single nucleotide polymorphism; MTBC, *Mycobacterium tuberculosis* complex.

Keywords

Diagnostic · Drug resistance · Tuberculosis · Whole-genome sequence

1 Introduction

Tuberculosis (TB) remains a principal source of stress for public health providers. If current conditions continue, it is expected that by 2030, more than 120 million people will be infected, and more than 15 million will die from the disease. There will also be an increase in the occurrence of epidemic outbreaks caused by TB that is resistant to all known drugs. One of the key factors with the greatest impact on the current standing of the disease is drug resistance (DR). The increase has been of such a great magnitude that the world health organization (WHO) has strongly recommended redoubling efforts to develop new drugs and procedures for accurate and early diagnosis.

This chapter provides updates on the situation and epidemiological factors related to DR in TB. It is followed by an overview of molecular mechanisms and techniques traditionally used to diagnose DR-TB. A second section shows how the integration of all knowledge related to DR is being used to design new drugs and diagnostic procedures, with special emphasis on whole-genome sequencing.

The information presented here will help to understand the complexity of the DR process in TB, how the newly generated knowledge, correctly integrated, helps create new solutions against TB, and decrease the impact as a health problem.

2 Epidemiological Situation of Tuberculosis and the Increasing Problem of Drug-Resistance

TB is an infectious disease with great epidemiological importance worldwide. Until the appearance of COVID-19 pandemic disease, this was the main cause of death by a single infectious disease. Annually close to ten million cases and 1.4 million deaths are reported. About 90% of individuals affected are adults, although it also

affects children. It affects both sexes, even though it has more presence in males than females. Worldwide, close to 60% of the patients are male adults, 30% female, and 10% children. The main comorbidity observed is HIV, present in 8.6% of the population infected with TB, with increasing numbers of type 2 diabetes mellitus comorbidity [1].

In terms of the distribution of TB, 86% of cases show up in three regions:

i. South-East Asia with 44%, and it includes two of the most affected countries, China and India;
ii. the second region with more cases is Africa with 24%; and
iii. the last one is the West-Pacific, with 18% of the worldwide TB cases.
iv. the remaining numbers are distributed in the Eastern Mediterranean (8%) and Americas and Europe (3%) [1].

TB is mainly caused by members of the *Mycobacterium tuberculosis* complex (MTBC), a group of acid-fast bacilli bacteria that consists of two human-adapted species (*Mycobacterium tuberculosis* (*M. tb*) and *M. africanum*) and nine animal-adapted species (*M. bovis, M. caprae, M. microti, M. mungi, M. orygis, M. pinnipedii, M. suricattae*, the '*dassie' bacillus*, and the '*chimpanzee' bacillus*) [2, 3]. It is acquired by inhaling mycobacteria expelled into the environment by an infected person when coughing, speaking, or sneezing. Mostly TB species affect mainly the lungs given place to the pulmonary TB (PTB), although also can be developed anywhere in the body, which is called extrapulmonary TB (EPTB) [1]. PTB is the most contagious form of the disease, and it is estimated that a sick person infects from three to ten people/year [4]. Furthermore, according to WHO, one in four people in the world is infected with *M. tb* in a latent mode and will develop active TB during some stage of their life; mechanisms related to this activation are not well known; however, a healthy immune system seems to be a key element [1].

This disease is diagnosed by detecting the causative agent directly (microscopy and culture) or indirectly (nucleic acid amplification or protein identification, etcetera) in fluids or tissues [5]. TB can be fatal if it is not treated promptly and efficiently. WHO recommends the use of four drugs administered for a period of four to six months: isoniazid, rifampicin, pyrazinamide, and ethambutol. When these drugs fail, it gives way to DR-TB, and the patient requires the use of the second group of drugs consisting of ethionamide, cycloserine, capreomycin, kanamycin, amikacin, ofloxacin, levofloxacin, moxifloxacin, and para-aminosalicylic acid, among others (Table 1) [6].

Currently, 85% of TB cases are successfully cured. However, several factors can promote the development of DR. Depending on the degree; it falls into five classes:

- mono-resistant (mono-TB), it is resistant to only one of the first-line drugs;
- poly-resistant (poly-TB), it is resistant to two or more drugs except for isoniazid and rifampicin;
- multidrug-resistant (MDR-TB), it is simultaneously resistant to isoniazid and rifampicin;

Table 1 Drugs used against tuberculosis infection

Drug	Symbol	Gen involved	Canonical mutations	Encoding product
First-line drugs				
Isoniazid	H	*katG*	315	Encodes catalase-peroxidase enzyme, inhibit synthesis mycolic acid
		inhA	−15, −8	Encodes enoyl ACP reductase, block production fatty acids
Rifampicin	R	*rpoB, rpoA, rpoC*	526, 531	Encodes the β-subunit of RNA polymerase
Ethambutol	E	*embB, embA, embC*	306	Encodes arabinosyltransferase involved in mycobacterial cell wall biosynthesis
Pyrazinamide	Z	*pncA*	−11, 96, 120	Encodes pyrazinaminidase produces pyracinoic acid, decrease pH
Second-line drugs				
Levofloxacin	Lfx	*gyrA*	80, 95	Encodes the DNA-gyrase A subunit
Moxifloxacin	Mxf	*gyrB*	512	Encodes the DNA-gyrase B subunit
Streptomycin	S	*rpsL*	43	Encodes RNAr 12S, related with inhibition of protein synthesis
		gidB	527	N/D
		rrS	906	Encodes RNAr 16S, related with inhibition of protein synthesis
Amikacin	Am	rrS	514, 517, 1401	
Para araminosalicilic acid (PAS)	Pas	*thyA*	75	N/D
		folC	43	
		ribD	11	
Cycloserin	Cs	*alr*	10	Encodes for an enzyme related to riboflavin biosynthesis
		ddlA		Encodes D-alanine ligase incorporates A to synthesis peptidoglycans
Ethionamide	Eto	*ethA*	397	Encodes a mono-oxygenase enzyme, which processes the pro-drug
		mabA	609	Encodes a 3-ketoacyl reductase, related synthesis mycolic acids
		inhA	21	Encodes an enoyl-ACP reductase, related synthesis mycolic acid
Rifabutin	Rfb	*rpoB*	531	Encodes the β-subunit of RNA polymerase
Linezolid	Lz	*rrl*	2061, 2576	Encodes ribosomal RNA 23S
		rplC	460	Encodes ribosomal protein 50S L3
Proteonamid	Ptn	*ddn*	–	Encodes deazaflavin-dependent nitroreductase
Bedaquiline	Bdq	*atpE*	63	Encodes for an ATP synthase and modify the ATP synthesis
Delamanid	Dlm	*fgd1*	–	Encodes F420-dependent glucose-6-phosphate dehydrogenase Probably encodes the biosynthetic protein F420
		fbiC, fbiA, fbiB		

- pre-extensive resistance (P-XDR-TB), it is a multidrug-resistant strain with additional resistance to any fluoroquinolone; and
- extensively drug-resistant (XDR-TB), it is a strain with additional resistance to at least one additional drug such as; Clofazimine, Cycloserin, Amikacin, Ethionamide, Rifabutin, among others [7].

According to WHO estimations, in 2018, there were more than ten million new TB cases, with 4% developing DR-TB. This number increases to 18% in previously treated cases. Besides, from 500,000 cases with confirmed resistance to rifampicin, 187,000 were confirmed cases of MDR-TB, with treatment effectiveness of 50% [1].

In addition to the fact that, virtually, the same antibiotics have been administered in a treatment scheme for the past 40 years, several risk factors have been identified as key contributors for the development of DR-TB, including socio-economic conditions, previous contact, or cohabitate with someone affected by a DR-TB, the presence of comorbidities such as HIV, and recently, type 2 diabetes mellitus [8]. Finally, the efficiency of health programs to provide drugs and surveillance to the TB-affected patients to adequately comply with their treatment is one of the most important factors that need to be considered. This last point has shown greater concern due to the dynamics imposed by the recent appearance of COVID-19 (SARS-CoV-2), which is expected to negatively influence the epidemiology of DR-TB, significantly increasing the number of this type of cases in the coming years [9].

TB remains the most important infectious disease affecting humankind. For that reason, in May 2014, WHO created the End of TB Strategy, and one year later, the Global Plan to Stop TB [10]. The aim is to end the current paradigm of TB and change the way the fight against the disease has developed by ensuring the medical care of 29 million people and preventing 45 million from becoming infected. This global plan is framed in the United Nations (UN)' Sustainable Development Goals (SDGs). This plan establishes the goal to end TB as an epidemic disease for 2030 and to reduce the deaths by 90% and the number of new cases per 100,000 inhabitants per year by 80%. To achieve these goals, it has been recognized that it is essential to increase research and technological development in TB, prioritizing the generation of new vaccines [11, 12], shorter and more effective treatment schemes for latent TB, novel mechanisms of drug administration [13], as well as the development of rapid diagnostic tests, and most specific ones for diagnostic of drug resistance [1].

3 Molecular Mechanisms Associated with Tuberculosis Drug Resistance

M. tb is a gram-positive bacillus, with three to five μm in length, without mobility and slow growth. It is characterized by a species-specific cell wall composed of abundant peptidoglycans, glycolipids, mycolic acids, carbohydrates, proteins, and

lipids [14], responsible for various infectious and physiological aspects of the bacterium [15].

The genome of *M. tb* H37Rv is ~ 4.4 million bp with 66% of G + C content, encoding around 4000 genes with a particularly high amount of proteins related to lipid metabolic pathways [16]. In-depth comparative and evolutionary analysis of thousands of genomic sequences has regarded *M. tb* as monomorphic bacteria, i.e., members of a low-sequence variability group of pathogens [17]. MTBC strains are considered genetically identical because they share 99% nucleotide identity and have a maximum genetic distance of 2500 single nucleotide polymorphisms (SNP) [18]. In contrast, related species out of the MTBC, such as *M. canettii*, share on average 98% nucleotide identity and differ by tens of thousands of SNPs [19].

The main cause of acquired DR is due to chromosomal mutations, which can appear as a consequence of stress in bacteria, either due to a high level of reactive oxygen species (ROSs), the host environment, or due to the inappropriate use of anti-TB drugs. These mutations are caused by SNPs, which consist of substituting a nucleotide at specific positions in the DNA [20]. SNPs are, therefore, the main source of variation in the *M. tb* genome, followed by insertions and deletions (INDELS) [21].

MTBC strains cannot undergo horizontal gene transfer, suggesting that the DR phenotypes rely on acquiring and maintaining beneficial mutations in core genes or promoter regions [22].

Nevertheless, both SNPs and INDELS can affect the mycobacterial genome, generating clone diversity that, together with natural selection, will determine which polymorphisms persist in the population. More than 60 genes have been related to the development of antibiotic resistance in TB. Table 1 describes the most associated genes with resistance against drugs, the mechanisms involved, the mutations considered most important, and molecular diagnostic tools. These mutations related to resistance usually are transmitted from one generation of *bacilli* to another. Consequently, one isolate can develop resistance to multiple drugs by accumulating individual mutations in various genes, each responsible for resistance to a specific antibiotic, explaining the occurrence of MDR-TB and XDR-TB. This information evidences the great complexity of mechanisms participating in DR-TB; besides, the last years have shown the occurrence of novel mechanisms that can contribute to the development of DR-TB.

4 Novel Mechanisms Related to Drug-Resistant Tuberculosis: Efflux Bumps and DNA Repair Systems

Recently, two new mechanisms have been described as having a role in developing DR-TB; the first involves the efflux pump systems and the second is the DNA repair system.

The first mechanism is confirmed by a set of proteins, "efflux pumps," that gives place to an "efflux system," which is responsible for transporting a wide variety of substrates from the interior to the exterior of the cell [23, 24]. Some of these pumps can be induced by specific substrates, including antibiotics, so a susceptible bacterium can produce an excess of this pump and become resistant [23]. This overexpression can be induced by acquiring one or several polymorphisms in the respective promoter or gene, increasing the efficiency for the drug exportation, decreasing the respective concentration, and inducing resistance to this drug [24].

In general terms, these pumps have been classified, into five categories, according to the type of energy source they use to perform the expulsion and the specificity they have for the substrate:

- *resistance-nodulation-division (RND);*
- *major facilitator superfamily (MFS);*
- *multidrug and toxic compound extrusion (MATE);*
- *small multidrug resistance (SMR); and*
- *ATP-binding cassette (ABC)* [25, 26].

Specifically, three families have been described in mycobacteria: ABC, MFS, and RND [27]. ABC transporter proteins are coupled with ATP hydrolysis to transport substrates. They are the most active transporters in mycobacteria. The 2.5% of the *M. tb* genome encodes for ABC efflux pumps, highlighting the importance of this family of proteins in the biology of mycobacteria. The implications for the decrease of drug sensitivity have been described for a while [28], and its direct relationship in the development of MDR-TB has also been demonstrated [29].

The families of pumps MFS and RND are considered secondary active carriers because they are driven by a protonic-motor force. MFS is a large and diverse family of carriers; most of the characterized pumps in mycobacteria belong to this family. An example is the Tap protein (Rv1258c), which in mycobacteria confers resistance to tetracycline and rifampicin [28], and the P55 pump (Rv1410c), which confers resistance to aminoglycosides, tetracycline, and rifampicin [30]. The RND family can promote resistance to an important range of antibiotics in TB. Within this family, 14 *mmpL* genes have been described, which code for various efflux pumps that transport lipids and other molecules. Some of them have been reported as responsible for "efflux" drugs and promoting resistance and virulence in TB. It has been shown that exposure of TB to isoniazid causes susceptible isolates to become highly resistant due to *mmpL7* gene overexpression (Rv2942) [31].

The second mechanism related to DR-TB has been identified as the DNA damage repair system, which also plays a fundamental role in the protection and genomic diversification of *M. tb* [32, 33]. In natural infective conditions, the defense mechanism of the host ROSs and reactive nitrogen intermediates is mostly produced by macrophages. Consequently, the infecting *M. tb* faces constant DNA damage [34], which requires multiple repair mechanisms to ensure its survival and promote its spread in the population [35, 36].

The characterization of the genes and mechanisms that participate in the DNA damage repair system in TB has been an important research issue in recent years [33, 37–62]. Table 2 shows some examples of how the damage repair system can generate hyper mutagenic phenotypes that promote or induce the generation of DR in TB.

Table 2 Genes involved in the DNA damage repair system and their association with drug resistance in tuberculosis

Gene	Function	Description
neiL, nei2 and nth	Exonucleases, Nei1 is specific for oxidized pyrimidines and uracil [37]. Nth removes damaged nucleotides [16]. The function of Nei2 is unknown [37]	The joint absence decreases survival and increases the mutation rate. Not observed in single gene deletion [37]
mutY and fpG	A combination of *mutY* and *fpG* is crucial in preventing mutations from C(G) to A(T) [38]	Elimination of both increases the mutation rate fourfold [38]
mutT1	Participates in the hydrolysis of 8-oxo-dGTP [39] and di-adenosine polyphosphates in cleaning the nucleotide pool [40]	A mutated gene increases the mutation rate [41]
uvrB	Participates in the NER pathway, recognizing damaged DNA through a UvrA2-UvrB ternary complex [42], as well as in the HR pathway in conjunction with UvrD1 [43]	Mutated gene linked to increased resistance [44]
dnaE1	Replicative polymerase, its elimination is lethal for the bacteria [45]	The absence of exonuclease of DnaE1 increases the mutation rate from 2300 to 3700 times [45]
dnaE2	DNA polymerase from trans lesions [46]	Its absence sensitizes and eliminates damage-induced mutagenesis [47]. Together with proteins ImuA and ImuB generate damage-induced mutagenesis [63]
dinB2	Low fidelity DNA polymerase [48, 49, 64]	Has a preference for ribonucleotides, capable of incorporating oxo-rGTP and 8-oxo-dGMP bases against 8-oxodG [48, 49, 64]
polD1 and polD2	Low fidelity polymerases [50]	They have a preference for the incorporation of ribonucleotides [50]
ogT	O6-alkylguanine DNA alkyltransferase which reverses the O^6-alkylguanine [51]	Mutated gene increases sensitivity to isoniazid [52]
adA/alkA, ogT and unG	*Alka* synthesizes a 3-methyladenine DNA glycosylase II with broad recognition of methylated bases. Ada control adaptive response to damage [53]. Ung removes uracil from DNA and participates in virulence [54–56]	Mutations in these genes have been linked to resistant strains of the Haarlem lineage [57, 58]
mutT4, mutT2 and ogT	MutT2 hydrolyzes dCTP, 5-methylCTP and 8-oxoGTP. Both participate in the cleaning of the nucleotide pool [16]	Mutations linked to resistant strains of the Beijing lineage [33, 59]

(continued)

Table 2 (continued)

Gene	Function	Description
recA	It catalyzes the exchange of threads in the HR pathway, thus initiating the recombination process [60]	Its elimination increases the sensitivity of *M. bovis* BCG to metronidazole [61]
nucS	Putative mismatch-specific endonuclease, necessary to prevent mutation and anti-recombination in vivo [62]	Its inactivation increases the generation of mutations up to 31 times and the development of SNPs up to 41 times more. Their alterations give a greater adaptation to drugs. Its expression is regulated adaptively [62]

M. tb has shown the presence of homologs systems to the traditional DNA repair systems that have been found in other bacteria. It has been identified as a base excision repair (BER) or nucleotide excision repair (NER) pathway. In this system, when the DNA double chain is broken, three major mechanisms participate:

i. homologous recombination pathways (HR);
ii. non-homologous end junction (NHEJ); and
iii. strand alignment (SSA).

The last two are considered the only pathways available during the pre-replicative stages of the cell [36]. On the other hand, although *M. tb* lacks the canonical mismatch repair genes (Miss Match Repair), recently has been found an alternate pathway mediated by *nucS*, a putative endonuclease specific for mismatches, necessary to avoid mutation and anti-recombination in vivo, with adaptive regulation that influences the generation of mutations [62].

Although some repair pathways are error-prone such as NHEJ and SSA [36], this can be increased especially when mutated genes or polymerases with low levels of fidelity such as *dnaE2* [63] and *dnB2* [64] participate. In the different *M. tb* lineages, the high polymorphic presence in the genes that make up the damage repair system has also been a focus of interest in recent years [36], and it was observed that in the Beijing lineage, this system could help explain its high propensity to rapidity develop DR [33].

5 The Drug-Resistant Tuberculosis Diagnosis

According to WHO, only 55% of diagnosed TB cases worldwide are bacteriologically confirmed. Furthermore, 51% of the new cases had a rifampicin sensitivity test; on the other hand, 40% of the confirmed MDR strains lack a second-line drug sensitivity test, so it is unknown if any could be further classified as XDR. Besides, the healing ratio for MDR is close to 50%, decreasing to 15–20% when is an XDR-TB; these are the major concerns that the TB programs deal against these

aggravated forms of TB. These figures clearly show that the global decrease of TB requires innovation for the development of new, sensitive, specific, reproducible, and non-expensive diagnostic tests that allow the identification of DR-TB, with an emphasis on MDR-TB and XDR-TB cases, and with this information to implement adequate treatments that improve the probability of cure and limit the transmission of such highly contagious strains [1].

With nearly 30–40 years of being used, the current diagnostics of DR-TB are largely outdated. Reference methods are mainly culture-based protocols; while these techniques are effective, they are time-consuming and require costly infrastructure, biosafety level-3 laboratory, and highly trained personnel.

These assays are recognized as obsolete, with limited resolution in the DR-TB diagnostics, and no longer suitable in the context of the Millennium Development Goals. Besides, they also present serious limitations if a new dynamic of attention or early identification of DR-TB cases wants to be incorporated.

6 The Response: Development of New Drugs and Diagnostic Assays for Drug-Resistant Tuberculosis

6.1 New Drugs Against Tuberculosis

Nowadays, the medical community is facing important challenges in the treatment of DR-TB. The need for new drugs that could be useful to treat aggravated forms of resistance in TB is increasingly urgent. To achieve this, it is important to understand the biology of *M. tb* and discover and validate new TB targets and their specific inhibitors. Also important is to formulate novel drug regimens, reduce standard therapy time, and improve the cost-effectiveness of therapies. Currently, research is focused on three major biological aspects of *M. tb*:

i. DNA replication and protein synthesis;
ii. cell wall biosynthesis; and
iii. energy metabolism.

The DNA replication and protein synthesis in *M. tb* has been well understood and include an important range of drugs used in the context of several drugs categories, working at different levels of inhibiting replication, transcription, and translation of DNA (rifampicin, streptomycin, amikacin, kanamycin, and capreomycin). The DNA gyrase is a topoisomerase II, encoded by *gyrA* and *gyrB genes*. It is recognized as an important element in mycobacteria DNA replication and is the target of fluoroquinolones and the next generation of these drugs. Additional series of drugs have been in development, considering the replication and protein synthesis. One of these is SPR719/SPR720: SPR719, an aminobenzimidazole that inhibits the action of DNA *gyrB*. It is currently in phase I of clinical study [65]. Several experimental compounds bind RNA polymerase in preliminary analysis phases, such as Na-aroyl-N-aryl-phenylalanine amides [66]. Sutezolid and

delpazolid are two new drugs derived from oxazolidinone; they are more potent than linezolid against sensible and MDR-TB [67, 68].

The cell wall of *M. tb* is a complex three-dimensional structure essential for pathogenesis and survival. This is confirmed by several types of lipids and molecules such as; mycolic acids (a layer of branched arabinogalactan polysaccharide) and a coat of peptidoglycans or mycolyl-arabinogalactan-peptidoglycan (mAGP).

Ethambutol, isoniazid, and, recently, delamanid are drugs used to inhibit the biosynthesis of the cell wall. Besides, a new series of drugs addressed to new targets, specifically enzymes, whose function is to produce the metabolic precursors or elements that make up the cell membrane, are on phase II-III clinical trial: Ramoplanin [69], Enduracidin, [70], SQ109: SQ109 [71], BM212/BM635 [72], and Pretomanid (PA824) [73], are just some of these new candidates.

According to the energy metabolism, *M. tb* generates adenosine triphosphate (ATP) via mainly metabolic pathways, such as substrate-level phosphorylation and oxidative phosphorylation. An important number of current TB candidate drugs are targeted against these metabolic pathways. Bedaquiline is the representative drug in this category; this is a diarylquinoline that blocks ATP synthesis [74] and has been recently approved by WHO to be used against MDR-TB. Nowadays, second-generation diarylquinolines, TBAJ-587 and TBAJ-876, are in preclinical development [75]. Another candidate, Q203, is an imidazopyridine-based drug that interferes with bacterial ATP production and is in a phase IIa clinical trial [76].

The drugs previously mentioned are just a few examples of the new generation of drugs in evaluation; today, the number of these new drugs and candidates increases constantly. The Stop TB Partnership's Working Group on New TB Drugs initiative aims to

> help coordinate guide, and accelerate the speed of worldwide development of lifesaving new cures to improve TB therapy.

This significantly impacts the support for the development, evaluation, and analysis of new drugs and trials. In the present day, more than 30 drugs are evaluated in several combinations of treatments and administration conditions in such a way that more than the same number of clinical trials are in development [77].

Considering all the mentioned above, it is essential to recognize the relevance of integrating different biological, molecular, medical, and clinical knowledge to solve the need for new antibiotics against TB. In the coming years, it is expected that there will be different drugs to contribute to this new fight against TB to accomplish the SDGs proposed by WHO.

7 New Approaches for the Molecular Diagnostic of Drug-Resistant Tuberculosis

In recent years, technological advances such as the polymerase chain reaction (PCR) have allowed the development of more sensitive tests for diagnosing DR-TB. These assays are mainly based on DNA amplification and further analysis (nucleic acid amplification tests, NAATs) and focused on analyzing those mutations/genes related to the DR phenomenon in TB (Table 3). The main advantages of these procedures are the highest levels of specificity and safety; they are fast and allow the identification of specific polymorphisms related to DR. Unfortunately, these assays require a large investment in infrastructure, equipment, supplies, and highly trained staff, which prevents many low-income countries from conducting them, precisely those most affected by the aggravated variants of TB [5, 78]. Despite these, many molecular tests are widely used as culture-free alternatives for detecting *M. tb* and indexing their most common DR genotypes. Table 3 summarizes the most important examples of these new procedures.

Table 3 Techniques and assays involved in the molecular diagnostic of drug-resistance in tuberculosis

Assay	Technique	Gene/drug	Sensitivity (%)	Specificity (%)	References
INNO-LiPA Rif. TB	Line probe assay	*rpoB*/rifampicin	96.9	100	[80]
Genotype MTBDR	Line probe assay	*rpoB*/rifampicin, *katG*/isoniazid	91–100, 67–100	100, 100	[81, 82]
Genotype MTBDRplus VER 1.0 and VER 2.0	Line probe assay	*rpoB*/rifampicin *katG* + *inhA*/ isoniazid	95–98 84–95	99 99	[83, 84]
Genotype MTBDRs/VER 1.0	Line probe assay	*girA*/fluoroquinolone, *embB*/ethambutol	85, 75, 55	99, 99, 78	[79, 85, 86]
Genotype MTBDRs/VER 2.0	Line probe assay	*girA* + *girB*/ fluoroquinolone, *eiS*/ kanamycin	93–100, 83–91, 89–96	98, 91–100, 92–98	[87, 88]
Nipro Genoscholar TB-NTM + MDR	Line probe assay	*rpoB*/rifampicin *katG* + *inhA*/ isoniazid	98 61	97 98.5	[89, 90]
Nipro Genoscholar INH TB	Line probe assay	*katG* + *mabA*/ isoniazid	90	100	[91]
AID TB resistance	Line probe assay	*rpoB*/rifampicin, *katG* + *Inha*/ isoniazid, *rpsL* + *rrS*/ streptomycin, *girA*/fluoroquinolone, *embB*/ethambutol	100, 98, 98, 100, 91, 72	–	[92]

(continued)

Table 3 (continued)

Assay	Technique	Gene/drug	Sensitivity (%)	Specificity (%)	References
Xpert MTB/RIF	Real time PCR Molecular beacon	*rpoB*/rifampicin	78–89	98	[106–108]
Abbott RealTime MTB RIF/INH resistance	Real time PCR	*rpoB*/rifampicin, *katG* + *inhA*/ isoniazid	87–96, 78–87	100, 94–100	[96, 97]
Flurotype MTBDR	Real time PCR	*rpoB*/rifampicin, *katG* + *InhA*/ isoniazid	97–98, 91–98	95–100, 97–100	[98, 99]
VerePLEX Biosystem	Molecular chip-based STMicroelectronics	*rpoB*/rifampicin, *katG* + *InhA*/ isoniazid	97, 73	100, 100	[100]
MID-DRS assay	PCR multiplex and sequencing	*rpoB*/rifampicin, *katG* + *InhA*/isoniazid, *pncA*/ pyrazinamide	97, 60, 75	98, 100, 98	[101]
Gene drive system	PCR-highlighter probes	*rpoB*/rifampicin	72	–	[102]
Melting curve analysis		*rpoB*/rifampicin *katG* + *inhA*/ isoniazid	90.3 90.2	90.4 93.9	[103]
Multiplex allele-specific polymerase chain reaction		*rpoB*/rifampicin *katG* + *inhA*/ isoniazid	98 82	100 100	[104]
Multi-PCR-single-strand conformational polymorphism analysis		*rpoB*/rifampicin *katG* + *inhA*/ isoniazid	84 85	92 100	[105]
Real time PCR, sloppy molecular beacon probes		*rpoB*/rifampicin	98	99	[93]
Amplification refractory mutation system PCR		*rpoB*/rifampicin	86–94	87.2–100	[94, 95]
Loop-mediated isothermal amplification		Only TB diagnosis	81–93	92–97	[110]
Whole genome sequencing		rpoB, A, C/rifampicin	98–100	98–100	[112]
		katG, *inhA*...[a]/ isoniazid	97–100	93–100	
		embB...[b]/ethambutol	71–100	15–95	
		pncA...[c]/ pyrazinamide	43–100	67–100	
		rpsL, *rrS*...[d]/ streptomycin	57–100	40–100	
		Second-line drugs[e]	–		

[a] *oxyR-ahpC, fpbC, Rv1592C, Rv1772, Rv2242, fabD, fabG1, kasA, accD, oxyR, ndh, fadE24, nat, kasA, mabA, accD6, accD*

[b] *embA, embC, embR, iniA, iniB, iniC, Rv3124, manB, PPE49, rmlD, manB*

[c] *rpsA, panD*

[d] *glidB*

[e] *rrs, eis, gidB, tlyA, gyrA, gyrB, ethA, ethR, folC, ribB, dfrA, whiB7, Rrl, rplC, Rv0678, Rv0678*

Among the most recommended tests by WHO are the Line probe assay tests (LIPA), which diagnose the presence of the *M. tb* and can help determine the resistance profile, mainly to rifampicin and isoniazid. Mutations are detected by binding amplicons (generated by PCR) to probe, targeting the most common mutations fixed on membrane strips. The final result of the hybridization is expressed in colored bands on the test strip [79–92].

There are various tests based on real-time PCR (RT-PCR), some of them using Sloppy Molecular Beacon; they are capable of detecting low concentrations of bacteria and a greater number of mutations [93], as well as based on endpoint PCR, such as the amplification refractory mutation system (ARMS), which uses nested primers and two or more internal primers that detect wild and mutant variants. The generated PCR product is analyzed by capillary electrophoresis, where the retention time in the column of the amplified DNA indicates the presence or absence of mutations and even heteroresistance [94, 95]. An additional set of probes using methods derivated from RT-PCR are included in Table 3 [93, 96–105].

The GeneXpert MTB/RIF assay was launched in 2010. This diagnostic procedure performs an heminested RT-PCR analysis for the simultaneous diagnosis of TB and rifampicin resistance directly from the clinical specimens. Specifically, the assay detects mutations associated with rifampicin resistance by RT-PCR amplification of the 81-bp fragment of the *rpoB* gene. This test has been approved by WHO and is currently one of the most widely used diagnostic procedures [106–108].

Recently, loop-mediated isothermal amplification (LAMP) has come out as a diagnostic test for TB that does not include DR markers. It uses a DNA polymerase with high chain displacement activity and four primers (two internal and two external) that recognize six different regions of the target DNA. Due to its cost, technical ease, infrastructure requirements, and speed (approximately one hour to the diagnostic), it is highly recommended by the WHO as an alternative to microscopy tests in developing countries, distant hospitals, or care centers [109, 110]. It is expected that modifications to this assay can identify mutations related to drug resistance in the incoming years.

Finally, it should be noted that the molecular diagnostic protocols for DR-TB are diverse and with variations in the specificity and sensitivity according to the molecular assay and the target used for it, mainly due to the high specificity for the nucleotide change or specific mutation/polymorphism. Additionally, another set of variables has been implied in the efficiency of most of the molecular tests; the most important are: the characteristic of the sputum or culture used, the age of the individual (children or elderly), the co-occurrence of other morbidities such as HIV/AIDS and type 2 diabetes mellitus, and the contamination by human factor. Despite these, the new generation of assays has a better future than the traditional procedures.

8 Whole-Genome Sequencing of *Mycobacterium tuberculosis*: Towards a Fast and Affordable Diagnostic of Drug-Resistant Tuberculosis

One of the major drawbacks of the molecular and high-throughput diagnostic methods for DR-TB is the lack of information on the strain, such as lineage identification and the high false-negative rate, as they are inherently limited to detecting a pre-designed subset of mutations. This also represents a huge problem as emerging antibiotic-resistant strains remain out of the scope. For instance, there is no rapid molecular test or a targeted amplicon design to detect strains resistant to many of the second-line drugs, including the drugs such as bedaquiline and delamanid–the latest Food and drug administration-approved antibiotics for XDR-TB treatment [111, 112].

For the last two decades, advances in sequencing technologies have monitored the entire suite of genes within chromosomes. The whole-genome sequencing (WGS) in TB has positioned itself as a versatile molecular tool that overcomes most molecular assays' limitations for diagnosing TB and pharmacological resistance of the infecting strain [112]. Their results reveal the existence of SNPs and INDELS throughout the genome, where the most frequent mutations related to DR are identified, as well as those of less frequency. It also makes it possible to explore other important variants such as lineage [113], mutation rate [114], the existence of compensatory mutations [20], allelic variation and fixation of variants [115], diversification of strains in the host [116], and analysis of the transmission of the disease in the population [117], among others advantages.

A general WGS workflow could be divided into three stages (Graphical Abstract):

i. sample processing, the first stage consists of collecting sputum samples, isolating the bacteria using selective media, and purifying the genomic DNA. Additional microscopy or molecular tests could be applied during this stage to verify the presence of *M. tb*. Importantly, all these first-stage steps must be performed in a laboratory with BSL-3 facilities;
ii. sequencing, during the second stage, DNA library preparation and sequencing are carried out. The Illumina platform (paired-end format) has proven effective in sequencing strains from the *M. tb* complex due to its high-quality sequencing and relatively low cost; and
iii. bioinformatic analysis, this stage includes quality control of reads, mapping reads against a reference genome, and identifying variants, e.g., SNPs, INDELS, repetitive regions, and mobile elements.

In the last decades, an increasing number of newly developed bioinformatic pipelines have facilitated and optimized the *M. tb*-WGS process of analysis. Overall, most pipelines are effective; however, it is important to identify key differences to ensure high-quality and reproducible analysis. Usually, the workflow of WGS-pipelines includes:

i. reads validation, that is, the removal of reads other than MTBC sequences avoiding data contamination;
ii. selection of the reference genome for comparison (*M. tb* H37Rv, ancestor-MTBC, pangenome);
iii. selection of the resistance-associated SNP catalogs;
iv. selection of the lineage-associated SNP catalog [118–121];
v. alignment, it is mapping and SNPs-calling processes adjusting their specific parameters, i.e., coverage, depth, and etcetera;
vi. high-quality SNPs filtration, it includes SNP support, determination of statistical cut-off and allele frequency, and excluding specific genomic regions, resistance genes, PE and PPE gene families, repetitive regions, mobile elements;
vii. selection of the maximum SNPs distance defining a transmission cluster; and
viii. batch processing for multiple samples.

Recently, Schleusener and collaborators compared five MTBC-WGS pipelines, CASTB, KvarQ, Mykrobe TB Predictor, PhyResSE, and TB Profiler, in terms of accuracy for lineage typing and antibiotic-resistance predictions [122]. They found that most of the pipelines provide similar results; however, PhyResSE stood out due to their high-resolution predictions. In another comparative report, Jajou and collaborators reported similar results for transmission cluster identification of five *M. tb*-WGS pipelines [123]. While those results indicate a certain level of reproducibility, the scientific community is currently seeking to standardize WGS-pipelines for a uniform characterization of MTBC strains to minimize noise and prediction errors that current pipelines present.

The Relational Sequencing Tuberculosis Knowledge base (ReSeqTB) is the most comprehensive international database of curated genomic information from *M. tb* strains [124]. ReSeqTB database contains antibiotic resistance genotyping information complemented with phenotyping data, metadata, and clinical outcomes. The Comprehensive Resistance Prediction for Tuberculosis: An International Consortium (CRyPTIC) is another worldwide initiative created to achieve MDR-TB proficient predictions [125]. CRyPTIC is a repository of high-confidence genetic variants associated with drug resistance and information about minimum inhibitory concentrations for most anti-TB drugs. On the other hand, in recent years, the integration of immunodynamics, genomics, epidemiology, and evolutionary biology, also called phylodynamics, has emerged to tackle key TB issues such as the development of massive detection protocols coupled to strain genotyping, transmission characterization, and outbreak mapping [126]. Overall, *M. tb*-WGS protocols have proven great potential for high-resolution strain typing prediction, accurate detection of transmission clusters, sequence-based analysis of virulence and pathogenic factors, efficient vaccine design, evolution analysis, and most importantly, drug-susceptibility [127]. The more complete genomes of MTBC are published, the better the mysteries of this organism can be understood.

All these attributes in the WGS place this procedure as the most important tool for diagnosing and characterizing TB and DR-TB for the coming years. However, there are two major obstacles that WGS have to be addressed;

i. one of them has to do with the costs related to the equipment and materials needed for the analysis. Fortunately, this assay is becoming more popular, so it is expected that the costs will decrease even more in the next years. Also, this system is not regulated by any pharmaceutical, as is the case with most diagnostic kits; this guarantees the maintenance of the low costs levels and total availability for the development of this assay. This will have remarkable repercussions in low- and middle-income countries, those more affected by TB and specifically DR-TB; and
ii. the second obstacle is the need for a previous culture of the clinical isolate to have enough DNA, in sufficient quantity and quality, to perform the sequencing and subsequent analysis to obtain reliable results. In this regard, recent advances allow the WGS and further analysis directly from clinical samples, reducing diagnostic times to only a few days [128]; the impact of this procedure will be determined in the coming years.

Considering all the mentioned above, the introduction of the WGS in the dynamic of TB will surely transform the diagnosis of DR-TB and the clinical, epidemiological scenarios associated, creating an eminently preventive orientation of these complex cases and development of individualized treatments, decreasing the impact and negative effects of the DR-TB.

9 Conclusion

There has been a duel between humans and mycobacteria to survive from each other for a long time. The development of antibiotics has changed the balance for several decades. However, the increase in TB isolates with resistance has modified the balance, again placing TB as an infectious disease of difficult management. Treatment effectiveness in aggravated cases, such as XDR-TB, is 15–20%, with no treatable cases.

In recent years it has been possible to characterize the multiple and complex mechanisms used by mycobacteria to adapt to the adverse conditions represented by the presence of an antibiotic. As described in this chapter, DR-TB is mainly associated with mutations in specific genes that usually codify for the enzyme and other mechanisms such as DNA reparation and membrane efflux pumps. This information contributes to the development of new anti-TB drugs, many of which are in different levels of clinical trials, so it is expected, in the coming years, to have a new repertoire of antibiotics used under new treatment schemes, and thus place the balance in our favor.

It is demonstrated that the current diagnostic systems are inadequate to address and solve the problem of DR-TB. It is essential to have an early diagnosis of the drug sensitivity profile. It allows performing interventions that would limit the development and progression of a resistant infection and avoid the dispersion of such strain, thus cutting the transmission chain. A new generation of resistance diagnostic procedures has been developed in response, based on DNA analysis and identification of mutations and other mechanisms involved in this process.

Undoubtedly, WGS will have the greatest impact since, in addition to being fast and inexpensive, identify the mutations present in all genes associated with resistance.

In conclusion, studies that integrate the information generated from such diverse areas of knowledge as bioinformatics, molecular biology, and epidemiology will allow developing tools that support the control and potentially eradicate TB as a global public health problem.

Tuberculosis clearly highlights our selfishness as humanity and evidence, like no other disease, our economic and social differences. Its eradication will undoubtedly be a great demonstration of our ability to evolve into a just and egalitarian society.

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Core Messages

- The number of TB-DR cases is increasing each year.
- A new generation of TB diagnostic molecular tools is emerging.
- WGS is a new TB diagnostic method with great potential to become the future standard.
- More than 30 clinical trials using new anti-TB drugs are in development.

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References

1. WHO (2020) WHO | Global tuberculosis report 2019. World Health Organization, Geneva
2. Malone KM, Gordon SV (2017) *Mycobacterium tuberculosis* complex members adapted to wild and domestic animals. Advances in experimental medicine and biology. Springer, New York, pp 135–154
3. Chiner-Oms Á, Sánchez-Busó L, Corander J, Gagneux S, Harris SR, Young D, González-Candelas F, Comas I (2019) Genomic determinants of speciation and spread of the *Mycobacterium tuberculosis* complex. Sci Adv 5. https://doi.org/10.1126/sciadv.aaw3307
4. Van Leth F, Van Der Werf MJ, Borgdorff MW (2008) Prevalence of tuberculous infection and incidence of tuberculosis; a re-assessment of the Styblo rule. Bull World Health Organ. https://doi.org/10.2471/BLT.06.037804
5. Machado D, Couto I, Viveiros M (2019) Advances in the molecular diagnosis of tuberculosis: from probes to genomes. Infect Genet Evol. https://doi.org/10.1016/j.meegid.2018.11.021
6. World Health Organization (2019) WHO consolidated guidelines on drug-resistant tuberculosis treatment. WHO Consol Guidel Drug-Resistant Tuberc Treat
7. WHO (2020) WHO | Drug-resistant tuberculosis. World Health Organization
8. WHO (2019) WHO | TB comorbidities and risk factors. World Health Organization
9. Glaziou P (2020) Predicted impact of the COVID-19 pandemic on global tuberculosis deaths in 2020. medRxiv 2020.04.28.20079582. https://doi.org/10.1101/2020.04.28.20079582

10. Stop TB Partnership W (2016) The Global Plan to Stop TB 2016–2020 2020:1–4
11. Zenteno-Cuevas R (2014) Update on the development of TB vaccines. Curr Pharm Biotechnol 14:940–946. https://doi.org/10.2174/1389201014666131226124940
12. Sarmiento ME, Alvarez N, Chin KL, Bigi F, Tirado Y, García MA, Anis FZ, Norazmi MN, Acosta A (2019) Tuberculosis vaccine candidates based on mycobacterial cell envelope components. Tuberculosis 115:26–41. https://doi.org/10.1016/J.TUBE.2019.01.003
13. Eduardo Pérez-Martínez D, Zenteno-Cuevas R (2020) Nanotechnology as a potential tool against drug- and multidrug-resistant tuberculosis. In: Nanotechnology based approaches for tuberculosis treatment. Elsevier, pp 37–52
14. Boshoff HIM, Barry CE (2005) Tuberculosis—metabolism and respiration in the absence of growth. Nat Rev Microbiol 3:70–80. https://doi.org/10.1038/nrmicro1065
15. Howard NC, Khader SA (2020) Immunometabolism during *Mycobacterium tuberculosis* infection. Trends Microbiol
16. Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon S V, Eiglmeier K, Gas S, Barry CE, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Krogh A, McLean J, Moule S, Murphy L, Oliver K, Osborne J, Quail MA, Rajandream MA, Rogers J, Rutter S, Seeger K, Skelton J, Squares R, Squares S, Sulston JE, Taylor K, Whitehead S, Barrell BG (1998) Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature 393:537–544. https://doi.org/10.1038/31159
17. Achtman M (2008) Evolution, population structure, and phylogeography of genetically monomorphic bacterial pathogens. Annu Rev Microbiol 62:53–70
18. Comas I, Coscolla M, Luo T, Borrell S, Holt KE, Kato-Maeda M, Parkhill J, Malla B, Berg S, Thwaites G, Yeboah-Manu D, Bothamley G, Mei J, Wei L, Bentley S, Harris SR, Niemann S, Diel R, Aseffa A, Gao Q, Young D, Gagneux S (2013) Out-of-Africa migration and Neolithic coexpansion of *Mycobacterium tuberculosis* with modern humans. Nat Genet 45:1176–1182. https://doi.org/10.1038/ng.2744
19. Supply P, Marceau M, Mangenot S, Roche D, Rouanet C, Khanna V, Majlessi L, Criscuolo A, Tap J, Pawlik A, Fiette L, Orgeur M, Fabre M, Parmentier C, Frigui W, Simeone R, Boritsch EC, Debrie AS, Willery E, Walker D, Quail MA, Ma L, Bouchier C, Salvignol G, Sayes F, Cascioferro A, Seemann T, Barbe V, Locht C, Gutierrez MC, Leclerc C, Bentley SD, Stinear TP, Brisse S, Medigue C, Parkhill J, Cruveiller S, Brosch R (2013) Genomic analysis of smooth tubercle bacilli provides insights into ancestry and pathoadaptation of *Mycobacterium tuberculosis*. Nat Genet 45:172–179. https://doi.org/10.1038/ng.2517
20. Dookie N, Rambaran S, Padayatchi N, Mahomed S, Naidoo K (2018) Evolution of drug resistance in *Mycobacterium tuberculosis*: a review on the molecular determinants of resistance and implications for personalized care. J Antimicrob Chemother. https://doi.org/10.1093/jac/dkx506
21. Godfroid M, Dagan T, Merker M, Kohl TA, Diel R, Maurer FP, Niemann S, Kupczok A (2020) Insertion and deletion evolution reflects antibiotics selection pressure in a *Mycobacterium tuberculosis* outbreak. bioRxiv. https://doi.org/10.1101/2020.01.28.922765
22. Nebenzahl-Guimaraes H, Jacobson KR, Farhat MR, Murray MB (2014) Systematic review of allelic exchange experiments aimed at identifying mutations that confer drug resistance in *Mycobacterium tuberculosis*. J Antimicrob Chemother 69:331–342. https://doi.org/10.1093/jac/dkt358
23. Rodrigues L, Parish T, Balganesh M, Ainsa JA (2017) Antituberculosis drugs: reducing efflux = increasing activity. Drug Discov Today 22:592–599. https://doi.org/10.1016/j.drudis.2017.01.002
24. Piddock LJV (2006) Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. Clin Infect Dis 19:382–402. https://doi.org/10.1128/CMR.19.2.382

25. Blanco P, Hernando-Amado S, Reales-Calderon J, Corona F, Lira F, Alcalde-Rico M, Bernardini A, Sanchez M, Martinez J (2016) Bacterial multidrug efflux pumps: much more than antibiotic resistance determinants. Microorganisms 4:14. https://doi.org/10.3390/microorganisms4010014
26. Li XZ, Plésiat P, Nikaido H (2015) The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. Clin Microbiol Rev 28:337–418. https://doi.org/10.1128/CMR.00117-14
27. Lentz F, Reiling N, Martins A, Molnár J, Hilgeroth A (2018) Discovery of novel enhancers of isoniazid toxicity in *Mycobacterium tuberculosis*. Molecules 23:1–9. https://doi.org/10.3390/molecules23040825
28. Braibant M, Gilot P, Content J (2000) The ATP binding cassette (ABC) transport systems of *Mycobacterium tuberculosis*. FEMS Microbiol Rev 24:449–467. https://doi.org/10.1111/j.1574-6976.2000.tb00550.x
29. Wang K, Pei H, Huang B, Zhu X, Zhang J, Zhou B, Zhu L, Zhang Y, Zhou FF (2013) The expression of ABC efflux pump, Rv1217c-Rv1218c, and its association with multidrug resistance of *Mycobacterium tuberculosis* in China. Curr Microbiol 66:222–226. https://doi.org/10.1007/s00284-012-0215-3
30. de la Paz Santangelo M, Romano MI, Silva PEA, Bigi F, Martin C, Cataldi A (2001) Characterization of P55, a multidrug efflux pump in *Mycobacterium bovis* and *Mycobacterium tuberculosis*. 45:800–804. https://doi.org/10.1128/AAC.45.3.800
31. Rodrigues L, Baptista P, Veigas B, Amaral L, Viveiros M (2012) Contribution of efflux to the emergence of isoniazid and multidrug resistance in *Mycobacterium tuberculosis*. 7. https://doi.org/10.1371/journal.pone.0034538
32. Chopra I, O'Neill AJ, Miller K (2003) The role of mutators in the emergence of antibiotic-resistant bacteria. Drug Resist Updat 6:137–145
33. Ebrahimi-Rad M, Bifani P, Martin C, Kremer K, Samper S, Rauzier J, Kreiswirth B, Blazquez J, Jouan M, van Soolingen D, Gicquel B (2003) Mutations in putative mutator genes of *Mycobacterium tuberculosis* strains of the W-Beijing family. Emerg Infect Dis 9:838–845. https://doi.org/10.3201/eid0907.020589
34. Adams LB, Dinauer MC, Morgenstern DE, Krahenbuhl JL (1997) Comparison of the roles of reactive oxygen and nitrogen intermediates in the host response to *Mycobacterium tuberculosis* using transgenic mice. Tuber Lung Dis. https://doi.org/10.1016/S0962-8479(97)90004-6
35. Gorna AE, Bowater RP, Dziadek J (2010) DNA repair systems and the pathogenesis of *Mycobacterium tuberculosis*: varying activities at different stages of infection. Clin Sci
36. Singh A (2017) Guardians of the mycobacterial genome: A review on DNA repair systems in *Mycobacterium tuberculosis*. Microbiol (United Kingdom)
37. Moolla N, Goosens VJ, Kana BD, Gordhan BG (2014) The contribution of Nth and nei DNA glycosylases to mutagenesis in *Mycobacterium smegmatis*. DNA Repair (Amst). https://doi.org/10.1016/j.dnarep.2013.11.003
38. Hassim F, Papadopoulos AO, Kana BD, Gordhan BG (2015) A combinatorial role for MutY and Fpg DNA glycosylases in mutation avoidance in *Mycobacterium smegmatis*. Mutat Res Fundam Mol Mech Mutagen. https://doi.org/10.1016/j.mrfmmm.2015.06.002
39. Arif SM, Patil AG, Varshney U, Vijayan M (2017) Biochemical and structural studies of *Mycobacterium smegmatis* MutT1, a sanitization enzyme with unusual modes of association. Acta Crystallogr Sect D Struct Biol. https://doi.org/10.1107/S2059798317002534
40. Arif SM, Varshney U, Vijayan M (2017) Hydrolysis of diadenosine polyphosphates. Exploration of an additional role of *Mycobacterium smegmatis* MutT1. J Struct Biol. https://doi.org/10.1016/j.jsb.2017.07.002
41. Dos Vultos T, Blázquez J, Rauzier J, Matic I, Gicquel B (2006) Identification of nudix hydrolase family members with an antimutator role in *Mycobacterium tuberculosis* and *Mycobacterium smegmatis*. J Bacteriol. https://doi.org/10.1128/JB.188.8.3159-3161.2006

42. Verhoeven EEA, Wyman C, Moolenaar GF, Goosen N (2002) The presence of two UvrB subunits in the UvrAB complex ensures damage detection in both DNA strands. EMBO J. https://doi.org/10.1093/emboj/cdf396
43. Güthlein C, Wanner RM, Sander P, Davis EO, Bosshard M, Jiricny J, Böttger EC, Springer B (2009) Characterization of the mycobacterial NER system reveals novel functions of the uvrDl helicase. J Bacteriol. https://doi.org/10.1128/JB.00216-08
44. Eldholm V, Norheim G, von der Lippe B, Kinander W, Dahle UR, Caugant DA, Mannsåker T, Mengshoel AT, Dyrhol-Riise AM, Balloux F (2014) Evolution of extensively drug-resistant *Mycobacterium tuberculosis* from a susceptible ancestor in a single patient. Genome Biol 15:490. https://doi.org/10.1186/s13059-014-0490-3
45. Rock JM, Lang UF, Chase MR, Ford CB, Gerrick ER, Gawande R, Coscolla M, Gagneux S, Fortune SM, Lamers MH (2015) DNA replication fidelity in *Mycobacterium tuberculosis* is mediated by an ancestral prokaryotic proofreader. Nat Genet 47:677–681. https://doi.org/10.1038/ng.3269
46. Fuchs RP, Fujii S (2013) Translesion DNA synthesis and mutagenesis in prokaryotes. Cold Spring Harb Perspect Biol. https://doi.org/10.1101/cshperspect.a012682
47. Mizrahi V, Warner D, Ndwandwe D, Abrahams G, Venclovas C (2012) A novel inducible mutagenesis system in *Mycobacterium tuberculosis*. FASEB J 26
48. Sharma A, Nair D (2012) MsDpo4—a DinB homolog from *Mycobacterium smegmatis*—is an error-prone DNA polymerase that can promote G:T and T:G mismatches. J Nucleic Acids 1–8
49. Ordonez H, Uson ML, Shuman S (2014) Characterization of three mycobacterial DinB (DNA polymerase IV) paralogs highlights DinB2 as naturally adept at ribonucleotide incorporation. Nucleic Acids Res. https://doi.org/10.1093/nar/gku752
50. Pitcher RS, Brissett NC, Picher AJ, Andrade P, Juarez R, Thompson D, Fox GC, Blanco L, Doherty AJ (2007) Structure and function of a mycobacterial NHEJ DNA repair polymerase. J Mol Biol. https://doi.org/10.1016/j.jmb.2006.10.046
51. Miggiano R, Casazza V, Garavaglia S, Ciaramell M, Perugino G, Rizzi M, Rossia F (2013) Biochemical and structural studies of the Mycobacterium tuberculosis O6-methylguanine methyltransferase and mutated variants. J Bacteriol. https://doi.org/10.1128/JB.02298-12
52. Wiid I, Grundlingh R, Bourn W, Bradley G, Harington A, Hoal-van Helden E, van Helden P (2002) O6-alkylguanine-DNA alkyltransferase DNA repair in mycobacteria: pathogenic and non-pathogenic species differ. Tuberculosis 82:45–53
53. O'Brien P, Ellenberger T (2004) The *Escherichia coli* 3-methyladenine DNA glycosylase AlkA has a remarkably versatile active site. J Biol Chem 279:26876–26884
54. Sassetti CM, Rubin EJ (2003) Genetic requirements for mycobacterial survival during infection. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.2134250100
55. Purnapatre K, Varshney U (1998) Uracil DNA glycosylase from *Mycobacterium smegmatis* and its distinct biochemical properties. Eur J Biochem. https://doi.org/10.1046/j.1432-1327.1998.2560580.x
56. Venkatesh J, Kumar P, Krishna PSM, Manjunath R, Varshney U (2003) Importance of Uracil DNA glycosylase in *Pseudomonas aeruginosa* and *Mycobacterium smegmatis*, G +C-rich bacteria, in mutation prevention, tolerance to acidified nitrite, and endurance in mouse macrophages. J Biol Chem. https://doi.org/10.1074/jbc.M302121200
57. Nouvel LX, Kassa-Kelembho E, Dos Vultos T, Zandanga G, Rauzier J, Lafoz C, Martin C, Blazquez J, Talarmin A, Gicquel B (2006) Multidrug-resistant *Mycobacterium tuberculosis*, Bangui, Central African Republic. Emerg Infect Dis. https://doi.org/10.3201/eid1209.060361
58. Olano J, Lopez B, Reyes A, Lemos MP, Correa N, Del Portillo P, Barrera L, Robledo J, Ritacco V, Zambrano MM, López B, Reyes A, del Pilar LM, Correa N, Del Portillo P, Barrera L, Robledo J, Ritacco V, Mercedes Zambrano M (2007) Mutations in DNA repair genes are associated with the Haarlem lineage of *Mycobacterium tuberculosis* independently of their antibiotic resistance. Tuberculosis (Edinb) 87:502–508. https://doi.org/10.1016/j.tube.2007.05.011

59. Lari N, Rindi L, Bonanni D, Tortoli E, Garzelli C (2006) Mutations in mutT genes of *Mycobacterium tuberculosis* isolates of Beijing genotype. J Med Microbiol. https://doi.org/10.1099/jmm.0.46261-0
60. Cox MM (1999) Recombinational DNA repair in bacteria and the RecA protein. Prog Nucleic Acid Res Mol Biol
61. Sander P, Papavinasasundaram KG, Dick T, Stavropoulos E, Ellrott K, Springer B, Colston MJ, Böttger EC (2001) *Mycobacterium bovis* BCG recA deletion mutant shows increased susceptibility to DNA-damaging agents but wild-type survival in a mouse infection model. Infect Immun. https://doi.org/10.1128/IAI.69.6.3562-3568.2001
62. Castañeda-García A, Martín-Blecua I, Cebrián-Sastre E, Chiner-Oms A, Torres-Puente M, Comas I, Blázquez J (2020) Specificity and mutagenesis bias of the mycobacterial alternative mismatch repair analyzed by mutation accumulation studies. Sci Adv. https://doi.org/10.1126/sciadv.aay4453
63. Boshoff HIM, Reed MB, Barry CE, Mizrahi V (2003) DnaE2 polymerase contributes to in vivo survival and the emergence of drug resistance in *Mycobacterium tuberculosis*. Cell 113:183–193. https://doi.org/10.1016/S0092-8674(03)00270-8
64. Ordonez H, Shuman S (2014) *Mycobacterium smegmatis* DinB2 misincorporates deoxyribonucleotides and ribonucleotides during templated synthesis and lesion bypass. Nucleic Acids Res. https://doi.org/10.1093/nar/gku1027
65. Brown-Elliott BA, Rubio A, Wallace RJ (2018) In vitro susceptibility testing of a novel benzimidazole, SPR719, against nontuberculous mycobacteria. Antimicrob Agents Chemother 62. https://doi.org/10.1128/AAC.01503-18
66. Maffioli SI, Zhang Y, Degen D, Carzaniga T, Del Gatto G, Serina S, Monciardini P, Mazzetti C, Guglierame P, Candiani G, Chiriac AI, Facchetti G, Kaltofen P, Sahl HG, Dehò G, Donadio S, Ebright RH (2017) Antibacterial nucleoside-analog inhibitor of bacterial RNA polymerase. Cell 169:1240-1248.e23. https://doi.org/10.1016/j.cell.2017.05.042
67. Wallis RS, Jakubiec W, Kumar V, Bedarida G, Silvia A, Paige D, Zhu T, Mitton-Fry M, Ladutko L, Campbell S, Miller PF (2011) Biomarker-assisted dose selection for safety and efficacy in early development of PNU-100480 for tuberculosis. Antimicrob Agents Chemother 55:567–574. https://doi.org/10.1128/AAC.01179-10
68. Choi Y, Lee SW, Kim A, Jang K, Nam H, Cho YL, Yu KS, Jang IJ, Chung JY (2018) Safety, tolerability and pharmacokinetics of 21 day multiple oral administration of a new oxazolidinone antibiotic, LCB01-0371, in healthy male subjects. J Antimicrob Chemother 73:183–190. https://doi.org/10.1093/jac/dkx367
69. Helm JS, Chen L, Walker S (2002) Rethinking Ramoplanin: the role of substrate binding in inhibition of peptidoglycan biosynthesis. J Am Chem Soc 124:13970–13971. https://doi.org/10.1021/ja021097n
70. Wu MC, Styles MQ, Law BJC, Struck AW, Nunns L, Micklefield J (2015) Engineered biosynthesis of enduracidin lipoglycopeptide antibiotics using the ramoplanin mannosyltransferase Ram29. Microbiol (United Kingdom) 161:1338–1347. https://doi.org/10.1099/mic.0.000095
71. Sacksteder KA, Protopopova M, Barry CE, Andries K, Nacy CA (2012) Discovery and development of SQ109: a new antitubercular drug with a novel mechanism of action. Future Microbiol 7:823–837
72. La Rosa V, Poce G, Canseco JO, Buroni S, Pasca MR, Biava M, Raju RM, Porretta GC, Alfonso S, Battilocchio C, Javid B, Sorrentino F, Ioerger TR, Sacchettini JC, Manetti F, Botta M, De Logu A, Rubin EJ, De Rossi E (2012) MmpL3 is the cellular target of the antitubercular pyrrole derivative BM212. Antimicrob Agents Chemother 56:324–331. https://doi.org/10.1128/AAC.05270-11
73. Palmer BD, Thompson AM, Sutherland HS, Blaser A, Kmentova I, Franzblau SG, Wan B, Wang Y, Ma Z, Denny WA (2010) Synthesis and structure-activity studies of biphenyl analogues of the tuberculosis drug (6S)-2-nitro-6-{[4-(trifluoromethoxy)benzyl]oxy}-

6,7-dihydro- 5H-imidazo[2,1-b][1,3]oxazine (PA-824). J Med Chem 53:282–294. https://doi.org/10.1021/jm901207n
74. Andries K, Verhasselt P, Guillemont J, Göhlmann HWH, Neefs JM, Winkler H, Van Gestel J, Timmerman P, Zhu M, Lee E, Williams P, De Chaffoy D, Huitric E, Hoffner S, Cambau E, Truffot-Pernot C, Lounis N, Jarlier V (2005) A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. Science (80-) 307:223–227 . https://doi.org/10.1126/science.1106753
75. Sutherland HS, Tong AST, Choi PJ, Blaser A, Conole D, Franzblau SG, Lotlikar MU, Cooper CB, Upton AM, Denny WA, Palmer BD (2019) 3,5-Dialkoxypyridine analogues of bedaquiline are potent antituberculosis agents with minimal inhibition of the hERG channel. Bioorg Med Chem 27:1292–1307. https://doi.org/10.1016/j.bmc.2019.02.026
76. Berube BJ, Parish T (2018) Combinations of respiratory chain inhibitors have enhanced bactericidal activity against mycobacterium tuberculosis. Antimicrob Agents Chemother 62. https://doi.org/10.1128/AAC.01677-17
77. Working Group on New TB Drugs (2016) Pipeline | Working Group for New TB Drugs. https://www.newtbdrugs.org/. Accessed 24 Aug 2020
78. Pai M, Nicol MP, Boehme CC (2016) Tuberculosis diagnostics: state of the art and future directions. Microbiol Spectr. https://doi.org/10.1128/microbiolspec.tbtb2-0019-2016
79. WHO (2013) The use of molecular line probe assay for the detection of resistance to second-line anti-tuberculosis drugs. Who Expert Gr Meet Rep 1–52
80. Viveiros M, Leandro C, Rodrigues L, Almeida J, Bettencourt R, Couto I, Carrilho L, Diogo J, Fonseca A, Lito L, Lopes J, Pacheco T, Pessanha M, Quirim J, Sancho L, Salfinger M, Amaral L (2005) Direct application of the INNO-LiPA Rif.TB line-probe assay for rapid identification of *Mycobacterium tuberculosis* complex strains and detection of rifampin resistance in 360 smear-positive respiratory specimens from an area of high incidence of multidrug. J Clin Microbiol. https://doi.org/10.1128/JCM.43.9.4880-4884.2005
81. Hillemann D, Weizenegger M, Kubica T, Richter E, Niemann S (2005) Use of the genotype MTBDR assay for rapid detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* complex isolates. J Clin Microbiol. https://doi.org/10.1128/JCM.43.8.3699-3703.2005
82. Miotto P, Piana F, Penati V, Canducci F, Migliori GB, Cirillo DM (2006) Use of genotype MTBDR assay for molecular detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* clinical strains isolated in Italy. J Clin Microbiol. https://doi.org/10.1128/JCM.00083-06
83. Asencios L, Galarza M, Quispe N, Vásquez L, Leo E, Valencia E, Ramírez J, Acurio M, Salazar R, Mendoza-Ticona A, Cáceres O (2012) Prueba molecular Genotype® MTBDRplus, una alternativa para la detección rápida de tuberculosis multidrogorresistente. Rev Peru Med Exp Salud Publica. https://doi.org/10.1590/s1726-46342012000100014
84. Ling DI, Zwerling AA, Pai M (2008) GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta-analysis. Eur Respir J. https://doi.org/10.1183/09031936.00061808
85. Theron G, Peter J, Richardson M, Barnard M, Donegan S, Warren R, Steingart KR, Dheda K (2014) The diagnostic accuracy of the GenoType® MTBDRsl assay for the detection of resistance to second-line anti-tuberculosis drugs. Cochrane Database Syst Rev.
86. Cheng S, Cui Z, Li Y, Hu Z (2014) Diagnostic accuracy of a molecular drug susceptibility testing method for the anti-tuberculosis drug, ethambutol: a systematic review and meta-analysis. J Clin
87. Tagliani E, Cabibbe AM, Miotto P, Borroni E, Toro JC, Mansjö M, Hoffner S, Hillemann D, Zalutskaya A, Skrahina A, Cirillo DM (2015) Diagnostic performance of the new version (v2.0) of GenoType MTBDRsl assay for detection of resistance to fluoroquinolones and second-line injectable drugs: a multicenter study. J Clin Microbiol. https://doi.org/10.1128/JCM.01257-15

88. Gardee Y, Dreyer AW, Koornhof HJ, Omar SV, Da Silva P, Bhyat Z, Ismail NA (2017) Evaluation of the GenoType MTBDRsl Version 2.0 assay for second-line drug resistance detection of *Mycobacterium tuberculosis* Isolates in South Africa. J Clin Microbiol. https://doi.org/10.1128/JCM.01865-16
89. Mitarai S, Kato S, Ogata H, Aono A, Chikamatsu K, Mizuno K, Toyota E, Sejimo A, Suzuki K, Yoshida S, Saito T, Moriya A, Fujita A, Sato S, Matsumoto T, Ano H, Suetake T, Kondo Y, Kirikae T, Moria T (2012) Comprehensive multicenter evaluation of a new line probe assay kit for identification of Mycobacterium species and detection of drug-resistant *Mycobacterium tuberculosis*. J Clin Microbiol. https://doi.org/10.1128/JCM.05638-11
90. Nathavitharana RR, Hillemann D, Schumacher SG, Schlueter B, Ismail N, Omar SV, Sikhondze W, Havumaki J, Valli E, Boehme C, Denkinger CM (2016) Multicenter noninferiority evaluation of hain GenoType MTBDRplus Version 2 and Nipro NTM +MDRTB line probe assays for detection of rifampin and isoniazid resistance. J Clin Microbiol. https://doi.org/10.1128/JCM.00251-16
91. Technologies G meeting stakeholders roundtable on D diagnostic (2015) Diagnosis for choosing the appropriate remedy, Genoscholar. Nipro Corp
92. Molina-Moya B, Lacoma A, Prat C, Diaz J, Dudnyk A, Haba L, Maldonado J, Samper S, Ruiz-Manzano J, Ausina V, Dominguez J (2015) AID TB resistance line probe assay for rapid detection of resistant *Mycobacterium tuberculosis* in clinical samples. J Infect. https://doi.org/10.1016/j.jinf.2014.09.010
93. Chakravorty S, Kothari H, Aladegbami B, Cho EJ, Lee JS, Roh SS, Kim H, Kwak H, Lee EG, Hwang SH, Banada PP, Safi H, Via LE, Cho SN, Barry CE, Alland D (2012) Rapid, high-throughput detection of rifampin resistance and heteroresistance in *Mycobacterium tuberculosis* by use of sloppy molecular beacon melting temperature coding. J Clin Microbiol. https://doi.org/10.1128/JCM.00143-12
94. Fan XY, Hu ZY, Xu FH, Yan ZQ, Guo SQ, Li ZM (2003) Rapid detection of rpoB gene mutations in rifampin-resistant *Mycobacterium tuberculosis* isolates in Shanghai by using the amplification refractory mutation system. J Clin Microbiol. https://doi.org/10.1128/JCM.41.3.993-997.2003
95. Shi X, Zhang C, Shi M, Yang M, Zhang Y, Wang J, Shen H, Zhao G, Ma X (2013) Development of a single multiplex amplification refractory mutation system PCR for the detection of rifampin-resistant *Mycobacterium tuberculosis*. Gene 530:95–99 . https://doi.org/10.1016/j.gene.2013.07.060
96. Kostera J, Leckie G, Abravaya K, Wang H (2018) Performance of the Abbott RealTime MTB RIF/INH resistance assay when used to test *Mycobacterium tuberculosis* specimens from Bangladesh. Infect Drug Resist 11:695–699
97. Kostera J, Leckie G, Tang N, Lampinen J, Szostak M, Abravaya K, Wang H (2016) Analytical and clinical performance characteristics of the Abbott RealTime MTB RIF/INH Resistance, an assay for the detection of rifampicin and isoniazid resistant *Mycobacterium tuberculosis* in pulmonary specimens. Tuberculosis. https://doi.org/10.1016/j.tube.2016.09.006
98. Hillemann D, Haasis C, Andres S, Behn T, Kranzer K (2018) Validation of the FluoroType MTBDR assay for detection of rifampin and isoniazid resistance in mycobacterium tuberculosis complex isolates. J Clin Microbiol. https://doi.org/10.1128/JCM.00072-18
99. De Vos M, Derendinger B, Dolby T, Simpson J, Van Helden PD, Rice JE, Wangh LJ, Theron G, Warren RM (2018) Diagnostic accuracy and utility of FluoroType MTBDR, a new molecular assay for multidrug-resistant tuberculosis. J Clin Microbiol. https://doi.org/10.1128/JCM.00531-18
100. Cabibbe AM, Miotto P, Moure R, Alcaide F, Feuerriegel S, Pozzi G, Nikolayevskyy V, Drobniewski F, Niemann S, Reither K, Cirillo DM, Di Pietro P, San Biagio F, Alessi E, Barbuzzi TG, Tafaj S, Bachiyska E, Kontsevaya I, Balabanova Y, Lazzeri E, Sserunkuma J, Aloi F, Nsubuga M, Sasamalo M (2015) Lab-on-chip-based platform for fast molecular diagnosis of multidrug-resistant tuberculosis. J Clin Microbiol. https://doi.org/10.1128/JCM.01824-15

101. Pérez-Osorio AC, Boyle DS, Ingham ZK, Ostash A, Gautom RK, Colombel C, Houze Y, Leader BT (2012) Rapid identification of mycobacteria and drug-resistant *Mycobacterium tuberculosis* by use of a single multiplex PCR and DNA sequencing. J Clin Microbiol. https://doi.org/10.1128/JCM.05570-11
102. Castan P, De Pablo A, Fernández-Romero N, Rubio JM, Cobb BD, Mingorance J, Toro C (2014) Point-of-care system for detection of *Mycobacterium tuberculosis* and rifampin resistance in sputum samples. J Clin Microbiol. https://doi.org/10.1128/JCM.02209-13
103. Galarza M, Guio H, Reques J, Piscoya O, Rodriguez M (2018) Diagnóstico molecular de tuberculosis multidrogorresistente en muestras de esputo mediante el análisis de curvas de melting. Rev Peru Med Exp Salud Publica. https://doi.org/10.17843/rpmesp.2018.353.3402
104. Chia BS, Lanzas F, Rifat D, Herrera A, Kim EY, Sailer C, Torres-Chavolla E, Narayanaswamy P, Einarsson V, Bravo J, Pascale JM, Ioerger TR, Sacchettini JC, Karakousis PC (2012) Use of multiplex allele-specific polymerase chain reaction (MAS-PCR) to detect multidrug-resistant tuberculosis in Panama. PLoS ONE. https://doi.org/10.1371/journal.pone.0040456
105. Cheng X, Zhang J, Yang L, Xu X, Liu J, Yu W, Su M, Hao X (2007) A new Multi-PCR-SSCP assay for simultaneous detection of isoniazid and rifampin resistance in *Mycobacterium tuberculosis*. J Microbiol Methods. https://doi.org/10.1016/j.mimet.2007.05.002
106. Lawn SD, Nicol MP (2011) Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. Future Microbiol
107. Detjen AK, DiNardo AR, Leyden J, Steingart KR, Menzies D, Schiller I, Dendukuri N, Mandalakas AM (2015) Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children: a systematic review and meta-analysis. Lancet Respir Med. https://doi.org/10.1016/S2213-2600(15)00095-8
108. Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N (2014) Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev
109. World Health Organization (2016) The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis: policy guidance. WHO Libr Cat Data 1–40
110. Nagai K, Horita N, Yamamoto M, Tsukahara T, Nagakura H, Tashiro K, Shibata Y, Watanabe H, Nakashima K, Ushio R, Ikeda M, Narita A, Kanai A, Sato T, Kaneko T (2016) Diagnostic test accuracy of loop-mediated isothermal amplification assay for *Mycobacterium tuberculosis*: systematic review and meta-analysis. Sci Rep. https://doi.org/10.1038/srep39090
111. Nieto-Ramirez LM, Vargas KQ, Diaz G (2020) Whole genome sequencing for the analysis of drug resistant strains of mycobacterium tuberculosis: a systematic review for bedaquiline and delamanid. Antibiotics 9
112. Papaventsis D, Casali N, Kontsevaya I, Drobniewski F, Cirillo DM, Nikolayevskyy V (2017) Whole genome sequencing of *Mycobacterium tuberculosis* for detection of drug resistance: a systematic review. Clin Microbiol Infect 23:61–68. https://doi.org/10.1016/j.cmi.2016.09.008
113. Ford CB, Shah RR, Maeda MK, Gagneux S, Murray B, Cohen T, Johnston JC, Gardy J, Lipsitch M, Fortune S (2014) *Mycobacterium tuberculosis* mutation rate estimates from different lineages predict substantial differences in the emergence of drug resistant tuberculosis. Nat Genet 45:784–790. https://doi.org/10.1038/ng.2656.Mycobacterium
114. Ford CB, Lin P, Chase M, Shah RR, Iartchouk O, Galagan J, Mohaideen N, Ioerger T, Sacchettini J, Lipsitch M, Flynn J, Fortune S (2015) Use of whole genome sequencing to estimate the mutation rate of *Mycobacterium tuberculosis* during latent infection. Nat Genet 40:1291–1296. https://doi.org/10.1097/CCM.0b013e31823da96d.Hydrogen

115. Sun G, Luo T, Yang C, Dong X, Li J, Zhu Y, Zheng H, Tian W, Wang S, Barry CE, Mei J, Gao Q (2012) Dynamic population changes in *Mycobacterium tuberculosis* during acquisition and fixation of drug resistance in patients. J Infect Dis 206:1724–1733. https://doi.org/10.1093/infdis/jis601
116. Liu Q, Liu Q, Wei J, Li Y, Li Y, Wang M, Su J, Lu Y, López MG, Qian X, Zhu Z, Wang H, Gan M, Jiang Q, Jiang Q, Fu YX, Takiff HE, Takiff HE, Comas I, Comas I, Li F, Lu X, Lu X, Fortune SM, Fortune SM, Fortune SM, Gao Q, Gao Q (2020) *Mycobacterium tuberculosis* clinical isolates carry mutational signatures of host immune environments. Sci Adv. https://doi.org/10.1126/sciadv.aba4901
117. Zakham F, Laurent S, Esteves Carreira AL, Corbaz A, Bertelli C, Masserey E, Nicod L, Greub G, Jaton K, Mazza-Stalder J, Opota O (2019) Whole-genome sequencing for rapid, reliable and routine investigation of Mycobacterium tuberculosis transmission in local communities. New Microbes New Infect 31. https://doi.org/10.1016/j.nmni.2019.100582
118. Homolka S, Projahn M, Feuerriegel S, Ubben T, Diel R, Nübel U, Niemann S (2012) High resolution discrimination of clinical *Mycobacterium tuberculosis* complex strains based on single nucleotide polymorphisms. PLoS One 7. https://doi.org/10.1371/journal.pone.0039855
119. Coll F, McNerney R, Guerra-Assunção JA, Glynn JR, Perdigão J, Viveiros M, Portugal I, Pain A, Martin N, Clark TG, Guerra-Assuncao JA, Glynn JR, Perdigao J, Viveiros M, Portugal I, Pain A, Martin N, Clark TG (2014) A robust SNP barcode for typing *Mycobacterium tuberculosis* complex strains. Nat Commun 5:4–8. https://doi.org/10.1038/ncomms5812
120. Comas I, Homolka S, Niemann S, Gagneux S (2009) Genotyping of genetically monomorphic bacteria: DNA sequencing in *Mycobacterium tuberculosis* highlights the limitations of current methodologies. PLoS ONE 4:e7815. https://doi.org/10.1371/journal.pone.0007815
121. Stucki D, Malla B, Hostettler S, Huna T, Feldmann J, Yeboah-Manu D, Borrell S, Fenner L, Comas I, Coscolla M, Gagneux S (2012) Two new rapid SNP-typing methods for classifying *Mycobacterium tuberculosis* complex into the main phylogenetic lineages. PLoS ONE 7:e41253. https://doi.org/10.1371/journal.pone.0041253
122. Schleusener V, Koser CU, Beckert P, Niemann S, Feuerriegel S (2017) Mycobacterium tuberculosis resistance prediction and lineage classification from genome sequencing: comparison of automated analysis tools. Sci Rep 7:46327. https://doi.org/10.1038/srep46327
123. Jajou R, Kohl TA, Walker T, Norman A, Cirillo DM, Tagliani E, Niemann S, De Neeling A, Lillebaek T, Anthony RM, Van Soolingen D (2019) Towards standardisation: comparison of five whole genome sequencing (WGS) analysis pipelines for detection of epidemiologically linked tuberculosis cases. Eurosurveillance 24. https://doi.org/10.2807/1560-7917.ES.2019.24.50.1900130
124. Starks AM, Aviles E, Cirillo DM, Denkinger CM, Dolinger DL, Emerson C, Gallarda J, Hanna D, Kim PS, Liwski R, Miotto P, Schito M, Zignol M, Avilés E, Cirillo DM, Denkinger CM, Dolinger DL, Emerson C, Gallarda J, Hanna D, Kim PS, Liwski R, Miotto P, Schito M, Zignol M (2015) Collaborative effort for a centralized worldwide tuberculosis relational sequencing data platform. Clin Infect Dis 61(Suppl 3):S141–S146. https://doi.org/10.1093/cid/civ610
125. CRyPTIC-consortium T (2018) Prediction of susceptibility to first-line tuberculosis drugs by DNA sequencing. N Engl J Med NEJMoa1800474. https://doi.org/10.1056/NEJMoa1800474
126. Baele G, Suchard MA, Rambaut A, Lemey P (2017) Emerging concepts of data integration in pathogen phylodynamics. In: Systematic biology. Oxford University Press, pp e47–e65
127. Meehan CJ, Goig GA, Kohl TA, Verboven L, Dippenaar A, Ezewudo M, Farhat MR, Guthrie JL, Laukens K, Miotto P, Ofori-Anyinam B, Dreyer V, Supply P, Suresh A, Utpatel C, van Soolingen D, Zhou Y, Ashton PM, Brites D, Cabibbe AM, de Jong BC, de Vos M, Menardo F, Gagneux S, Gao Q, Heupink TH, Liu Q, Loiseau C, Rigouts L,

Rodwell TC, Tagliani E, Walker TM, Warren RM, Zhao Y, Zignol M, Schito M, Gardy J, Cirillo DM, Niemann S, Comas I, Van Rie A (2019) Whole genome sequencing of *Mycobacterium tuberculosis*: current standards and open issues. Nat Rev Microbiol 17:533–545

128. Goig GA, Cancino-Muñoz I, Torres-Puente M, Villamayor LM, Navarro D, Borrás R, Comas I (2020) Whole-genome sequencing of *Mycobacterium tuberculosis* directly from clinical samples for high-resolution genomic epidemiology and drug resistance surveillance: an observational study. Lancet Microbe 1:e175–e183. https://doi.org/10.1016/S2666-5247(20)30060-4

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Resistance in Tuberculosis: Molecular Mechanisms and Modulation

19

Himanshu Verma, Shalki Choudhary, and Om Silakari

Science has no idea how much it owns the imagination.

Ralph Emerson

Summary

Since the time of years following World War II, tuberculosis (TB) has become a treatable disease due to the discovery of antibiotics such as streptomycin. But now, TB has poised to make a dramatic and deadly comeback again owing to the emergence of some drug-resistant (DR) strains, including multidrug-resistant (MDR), extensively drug-resistant (XDR), and totally drug-resistant (TDR) strains. Health professionals are alarmed that these TB strains are so virulent that they are called "virtually untreatable" even with the most potent anti-TB drugs available. Inappropriate TB practice conducted, mainly in developing countries, is considered the main reason behind DR. These malpractices include inadequate treatment of TB patients due to incorrect drug combinations, insufficient dose irregularities, or poor adherence. Consequently, both public and non-public sectors contribute to DR-TB. The current chapter sheds light on various DR mechanisms that hinder effective treatment with existing anti-TB drugs. Multiple strategies to overcome the problem of DR-TB have also been discussed. The chapter provides recent updates on newly developed drugs, clinical trials in the pipeline, and

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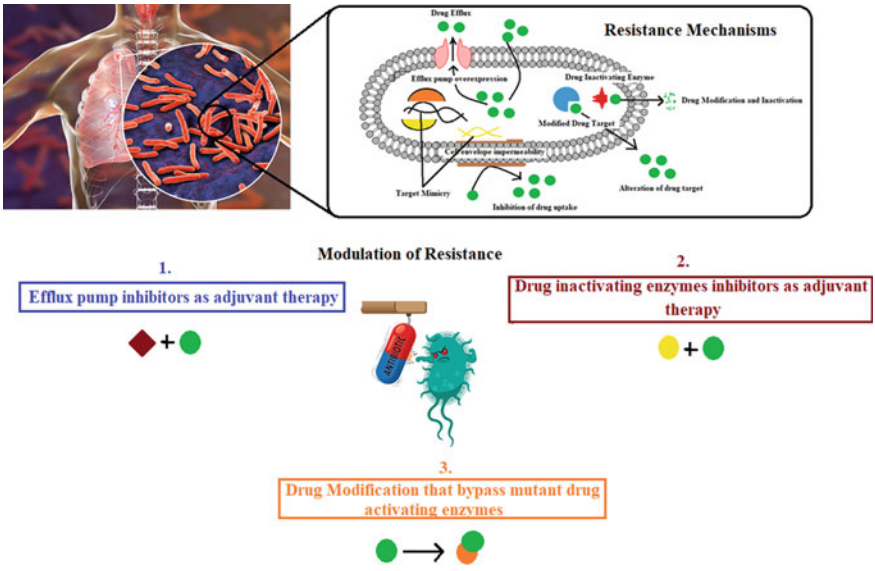
international recommendations to manage resistance. This information is essential for developing a more effective therapy that is potent against DR-TB and can shorten the antibiotic course required for both drug-susceptible TB and DR-TB.

Graphical Abstract



Mechanisms of resistance to anti-tubercular drugs and their possible modulation

Keywords

Chemotherapy · Clinical trials · Resistance · Tuberculosis

1 Introduction

Tuberculosis (TB) is caused by *M. tb*, and resistance to anti-TB drugs has severely threatened its control and management. Seventy years ago, streptomycin was discovered by American biochemists Waksman and coworkers, who suggested this drug as a chemotherapeutic approach for TB treatment as an alternative to diet, physical exercise, and fresh air [1]. Soon after the beginning of the chemotherapy era with streptomycin, it was observed that not all cases of TB were curative with the emergence of streptomycin-resistant strains of *M. tb* [2]. Later, first-line drug therapy, including isoniazid, pyrazinamide, ethambutol, or rifampin, was developed, which were very active against TB. The success rate of this short-course

chemotherapeutics became approximately 100% as the drug regimen complied with both the physician and the patient [3–7]. However, the past 20 years have spotted the worldwide appearance of multidrug-resistant (MDR) [8, 9], succeeded by extensively drug-resistant (XDR) [10], and totally drug-resistant (TDR) strains of TB [11–13]. The emergence of these resistant strains has raised the chance to return to an era in which drugs remain ineffective [14]. The MDR strain of *M. tb* is resistant to two of the most routinely used front-line anti-TB drugs, i.e., rifampicin and isoniazid. According to World Health Organization (WHO) XDR-TB Task Force meeting organized in October 2006, XDR strain of *M. tb* is not only resistant to first-line anti-TB drugs such as rifampicin and isoniazid but also to fluoroquinolones and at a certain extent to one of three injectable second-line anti-TB drugs including amikacin, kanamycin, and capreomycin [15]. TDR strain of *M. tb* is resistant to all existing first and second-line anti-TB drugs (rifampicin, streptomycin, pyrazinamide, isoniazid, ethambutol, para-aminosalicylic acid, ethionamide, cycloserine, amikacin, capreomycin, ofloxacin, ciprofloxacin, kanamycin) [13].

As per the WHO global TB report 2019, about 10,000,000 people were infected with TB in 2018, with 1,500,000 deaths and 484,000 drug resistant-tuberculosis (DR-TB) cases. The three countries, i.e., the Russian Federation (9%), China (14%), and India (27%), contribute to the major share of the global burden. Globally, 18% of previously treated cases and 3.4% of new TB cases had rifampicin-resistant TB (RR-TB) or MDR-TB, observed among previously treated patients with the highest proportion, i.e., >50%, in countries of the former Soviet Union. Throughout 15 years, 128 countries have published representative data obtained with continual surveillance or surveys about the proportion of MDR-TB cases. Integrating the data obtained from these surveys, about 6.2% of the average proportion of MDR-TB cases were observed with XDR-TB [16]. In light of the above, new therapeutic interventions are promptly needed to address the existing epidemic of resistant TB.

Anti-TB drug treatment is long, expensive, and toxic, and the percentage of undesirable outcomes is very high. Drug-susceptible (DS)-TB can be treated with supervised therapy for $\geq$ six months in more than 95% of cases. While, the majority of poor nations have difficulties purchasing second and third-line medications for MDR-TB treatment, which needs a $\geq$24-month course of supervised therapy with five to seven less costly, effective, and toxic treatments. XDR-TB and TDR-TB are generally untreatable in developing countries [17]. Factors responsible for creating a massive pool of individuals defenseless to DR-TB are increasing population, poverty, malnutrition, and the contemporary outbreak of acquired immunodeficiency syndrome (AIDS) [18]. At the molecular level, the resistance to first-line and second-line anti-TB drug therapy is caused due to a chromosomal mutation, drug inactivation, induction of efflux pump, target mimicry, or target modification [19].

Research efforts need to be stimulated to develop entirely new antibiotics that are least affected by the ongoing resistance mechanisms. Alternatively, adjuvant therapies such as efflux pump inhibitors [20], drug inactivating enzyme inhibitors [21], etc., with the existing therapies can also be suggested to overcome the problem of

drug-resistant (DR)-TB. Additionally, other non-traditional approaches, including repurposing old drugs or targeting resistance mechanisms, need to be further examined. For the success of these approaches, these DR mechanisms in *M. tb* need to be comprehensively studied and well understood. The current chapter provides mechanistic insight into the resistance mechanism at the molecular level, various counter-strategies to resolve the resistance problem, and an update on the drugs that are being developed or under clinical trials for treating DR-TB.

2 Molecular Mechanisms of Resistance in Tuberculosis

Bacteria have adopted several common strategies to acquire resistance to antibiotics or antibacterial agents. These strategies include target overexpression, modification, and inactivation via drug-inactivating enzymes and also barrier mechanisms (reduced uptake or increased efflux). These molecular mechanisms account for resistance in *M. tb* towards distinct crucial groups in antimycobacterial drugs, as summarized in Fig. 1.

2.1 Permeability Associated Resistance

The cell wall of *M. tb* is highly thick with multiple layers; it consists of layers of peptidoglycan that is covalently linked via an L-Rha-D-GlcNAC-P as a linker unit to linear galactofuran. In succession, it is attached to highly branched arabinofuran, which is further linked to mycolic acids [22]. Peptidoglycan is covalently linked to arabinogalactan, which due to its hydrophilic nature, acts as an obstacle to the

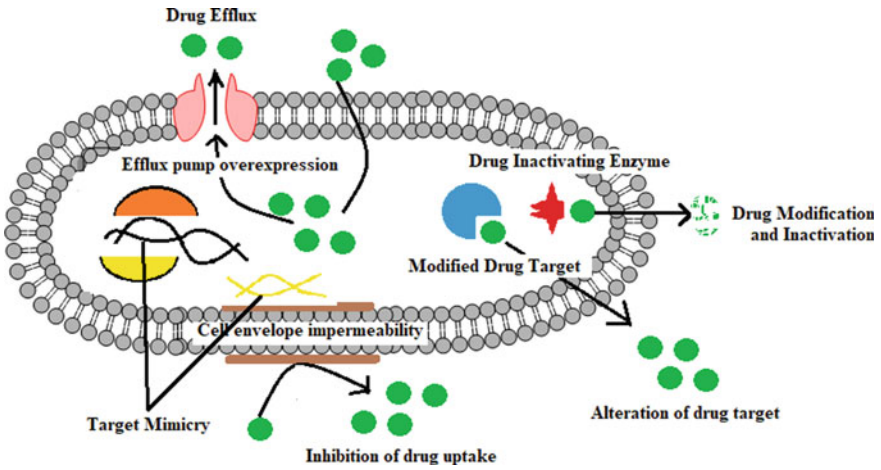


Fig. 1 Molecular mechanisms are responsible for resistant TB

penetration of hydrophobic drugs [23]. As arabinogalactan is further attached to the hydrophobic compound mycolic acid, it acts as an obstacle for the penetration of hydrophilic drugs [24]. Thus, the cell wall of *M. tb* acts as a barrier to the permeability of both the hydrophilic and hydrophobic drugs. This way, peptidoglycan-arabinogalactan-mycolic acid complex acts as a barrier and allows *M. tb* to survive inside host macrophages (phagocytic cells). For instance, the *OmpATb* gene encodes a pore-forming protein that affects both *M. tb* growth at reduced pH (known as acid resistance) and its permeability to several water-soluble substances. This is very challenging to develop an efficient antibiotic to cross the cell wall of *M. tb* [25, 26]. *M. tb* encodes porin-like proteins that expedite the antibiotics import via an outer layer of the mycobacterial cell wall, thus influencing DR.

2.2 Acquired Resistance by a Genetic Mutation

In 1952, a renowned microbiologist, Renee Dubos, anticipated that bacteria might develop antibiotic resistance owing to natural selection and random mutations [27]. *M. tb* favored Dubos' pre-trial judgment, as *M. tb* underwent a major evolution leading to the development of various *M. tb*-resistant strains. Random genetic mutation is one of the major causes of DR [19]. Chromosomal mutations show up at a frequency of 10^{-6} to 10^{-8} mycobacterial replications, with a probability of 10^{-18} to 10^{-20} of developing DR [28]. Anti-TB drugs that undergo resistance through this mechanism have been briefly explained below.

M. tb is resistant to most first-line drugs (pyrazinamide, isoniazid, rifampicin, and ethambutol) and second-line drugs (kanamycin, amikacin, and capreomycin) due to mutation, either in the drug target itself or activator of pro-drug. Reported mutations that are accountable for resistance to some anti-TB drugs are mentioned in Table 1. For instance, isoniazid, a first-line drug, enters the mycobacterial cell by passive diffusion. Being a pro-drug, it undergoes activation to highly reactive species via the catalase-peroxidase enzyme (KatG) encoded by the *KatG* gene. KatG couples the active species (isonicotinic acyl radical) with nicotinamide adenine dinucleotide (NADH) to form the isoniazid-NADH complex and subsequently inhibits its target InhA (enoyl-acyl carrier protein reductase); consequently, the synthesis of cell wall mycolic acid is inhibited [29]. A single-point mutation is the most frequent cause of resistance in isoniazid-resistant strains, accounting for 50–95% of isoniazid-resistant clinical isolates [30]. Table 1 illustrates G315C single point mutation resulted in amino-acid substitution (Ser → Thr) in target protein (KatG). One of the clinical studies reported that Ser315Thr mutation in *KatG* was highly prevalent among isoniazid-resistant *M. tb* clinical isolates in northwestern Russia from 1996 to 2001 [31]. Another mechanism of resistance to isoniazid reported is InhA overexpression owing to a mutation in the promoter region of *fabG1inhA*. Additionally, in some Isoniazid-resistant strains, resistance to isoniazid occurred due to mutation in the *furAKatG* promoter region, which is a well-known target required for Isoniazid activation [32]. Another example of a drug that undergoes resistance through this mechanism is rifampicin, one of the principal first-line drugs discovered in 1957 by

Table 1 Mutations responsible for resistance to some of the anti-tubercular drugs

S. No.	Gene	Alteration in gene after mutation or overexpressed gene region	Encoded protein	Affected drug	Mechanism of resistance	References
1	KatG	Codon 315, AGC → ACC	Catalase-peroxidase	Isoniazid	Ser → Thr substitution	[38]
2	InhA	C15T mutation	Enoyl ACP reductase	Isoniazid and Ethionamide	S94A, I194T substitution	[39]
3	Ndh	Codon 18, GTG → GCG	NADH dehydrogenase 2	Isoniazid	Val → Ala substitution	[40]
4	RpoB	Codon 381, GCG → GTG	Beta-subunit of RNA polymerase	Rifampicin	Ala → Val substitution in the N-terminal region	[41]
5	PncA	C → G point mutation in nucleotide 169	Pyrazinamidase	Pyrazinamide	H57D substitution	[42]
6	EmbB	A → G transition at nucleotide position 916	Arabinosyl transferase B	Ethambutol	Met306Val	[43]
7	EmbR	CGT → TGG ATT → ACC	embCAB transcription regulator	Ethambutol	R230W I369T	[44]
8	EmbC	ACC → ATT ATT → ACC	Arabinosyl transferase C	Ethambutol	T270I I297T	[44]
9	RpsL	A128G	S12 ribosomal protein	Streptomycin	K43R	[45]
10	Rrs	T23C	16S rRNA	Amikacin/Kanamycin	A29V	[46]
11	GidB	C233A	16S rRNA methyltransferase	Streptomycin	P75T	[45]
12	WhiB7	Mutation in the 5′Untranslated region (UTR) region of the WhiB7 gene	MDR transcription regulator	Streptomycin and Kanamycin	Increased expression of Eis and Tap (efflux pump)	[47]
13	MshA	A332G	Glycosyltransferase (involved in mycothiol biosynthesis)	Ethionamide	N111S	[48]
14	GyrA	GAG → CAG	DNA gyrase subunit A	Fluoroquinolones	E21Q	[49]
15	ethA	G127A	Flavin monooxygenase	Ethionamide	G43S	[50]
16	gyrB	Codon: 495GAC → AAC	DNA gyrase subunit B	Fluoroquinolones (Ofloxacin)	D437N	[51]
17	thyA	ACC → GCC	Thymidylate synthase A	P-aminosalicylic acid	T202V	[52]
18	FolC	GAG → GCG	Dihydrofolate synthase	P-aminosalicylic acid	E153A	[53]

Piero Sensi [33]. Rifampicin acts as an antimycobacterial drug by binding to the β-subunit of RNA polymerase that subsequently interferes with the transcription and RNA elongation. More than 90% of *M. tb* strains resistant to rifampicin possessed genetic alterations within the rifampicin-determining region of the *rpoB* gene (81 bp fragment), which encodes β-subunit of RNA polymerase [34]. One of the computational studies provided in silico evidence to explain the mechanism of rifampicin resistance among *rpoB* mutant strains that explained the resistance mechanism mutation at codon 450 (S450L) and 445 (H445Y) due to mutation in the well-defined central region of the *rpoB* gene with 81-base pair [35]. Pyrazinamide, another well-known first-line drug, possesses antimycobacterial properties by exerting action on non-specific targets to disrupt the membrane energetic translation process, cytoplasmic acidification, or coenzyme A synthesis. The emergence of resistance to pyrazinamide in *M. tb* is due to a mutation in *rpsA*, *panD*, and *pncA* gene encoding proteins that are targeted by pyrazinamide. One such example is a double mutation, i.e., H21R and I29V, within the non-active site of the *panD* gene [36]. Pro-drug pyrazinamide is a chemical analog of nicotinamide that requires an enzyme pyrazinamidase to convert pyrazinamide into its active form pyrazinoic acid which subsequently disrupts bacterial membrane energy. It was confirmed in 1996 that *pncA* gene mutation is also associated with pyrazinamide resistance against *M. tb*. Studies revealed a ten-fold decrease in enzymatic activity with mutations in recombinant *pncA*. The important residues associated with the normal functioning of *pncA* are active site amino acid residues, including Asp8, Ile133, Ala134, and Cys138. Therefore, the mutation in these residues affects the enzymatic activity of pncA [37].

Novel target decaprenylphosphoryl-β-D-ribose 2′epimerase (DprE1) has been reported to be a potential target to treat MDR-TB and XDR-TB. Benzothiazinone, especially BTZ043 and pBTZ169, inhibits DprE1 with nanomolar potency. These covalent inhibitors of DprE1 initiate the reduction of the nitro group to an active intermediate 'nitroso' induced by the transfer of hydride from flavin adenine dinucleotide ($FADH_2$), as a consequence of which a semimercaptal adduct is formed with the thiol group in key residue, i.e., Cys387 of DprE1 [11, 54]. Makarov et al. reported that the *M. tb* NTB1 strain (which includes Cys387Ser mutation in *DprE1*) is resistant to BTZ043; this mutation resulted in >10,000-fold increase in resistance to BTZ043 [55].

Novel *ubiA* mutations encoding 5-phospho-α-D-ribose-1-diphosphate: decaprenyl phosphate 5-phosphoribosyltransferase, one of the enzymes involved in decaprenylphosphoryl-Darabinose (DPA) pathway for cell wall synthesis, has been reported to undergo overexpression that subsequently results in elevated DPA levels available for EmbB and competes with the drug ethambutol. This competitive binding promotes the resistance to ethambutol [56].

2.3 Drug Efflux

Proteins bound to membranes as efflux pumps play a major role in the antibiotic-unrelated metabolism or physiology, for example, transport of toxins, wastes, nutrients, or signaling molecules through the bacterial cell wall. Their functions in antibiotic resistance might be subsidiary due to non-specific transportation [38]. These proteins expel antibiotics from the cell and prevent the drug from reaching the site of action, allowing the *M. tb* to survive. Efflux pumps can be classified into five families based on their energy source, structure, and type of substrates. ABC ATP-binding cassette (ABC) superfamily and major facilitator superfamily (MFS) are larger and former families among the five families. While other three belong to smaller families, i.e., the multidrug and toxic compound extrusion (MATE) family, the resistance-nodulation cell division family (RND), and the small multidrug resistance (SMR) family [39].

The ABC efflux pump family is a group of transmembrane proteins comprised of a cytosolic ATP-binding site with a channel structure. ATP hydrolysis enables drug transportation in the presence of magnesium. Two ABC family efflux pumps, including Rv1456c-Rv1457c-Rv1458c and Rv2686c-Rv2687c-Rv2688c transport systems, have been recently identified in *M. tb* H37Rv. Overexpression of these two pumps was detected in the presence of first-line anti-TB drugs, which led to H37Rv resistance to at least one of ethambutol, isoniazid, streptomycin, and rifampicin [40]. Danilchanka et al. identified the molecular mechanisms of *M. tb* resistance to β-lactam antibiotics via Rv0194 efflux pump in *M. tb* strain [41]. In 2017, Zhang et al. discovered four efflux proteins (Rv3756c, Rv0191, Rv1667c, and Rv3008) responsible for binding pyrazinoic acid using *M. tb* proteome microarray. They concluded that overexpression of these four genes encoding efflux pump in *M. tb* cause low-level resistance to pyrazinamide and pyrazinoic acid but not to other drugs [42]. Duan et al. recently reported a novel ABC efflux pump, Rv1473, involved in Mycobacterium intrinsic resistance to macrolides via efflux mechanism [43]. DrrAB is another example of an ABC efflux pump accountable for resistance to a wide range of clinically relevant antibiotics, including erythromycin, tetracycline, norfloxacin, ethambutol, chloramphenicol, and streptomycin. This suggested a possible role of DrrAB that it might play in antibiotic resistance of *M. tb* [44].

The MFS efflux pump is another largest class of secondary transporters that facilitate the transfer of target substrate across the cellular membrane of *M. tb* in response to the osmotic ion gradient. Some of the efflux pumps that belong to the MFS family in *M. tb* are Tap, P55, JefA, EfpA, Rv2994, Rv2477, etc. [45]. A gene expression study of efflux pump by Gupta et al. reported that under drug stressed conditions, an open reading frame Rv2459 encoding JefA efflux pump are over-expressed, leading to an increase in MIC of the two former drugs, ethambutol and isoniazid, thereby marking the basis of resistance [46]. In the consecutive year, the same research group performed a microarray analysis of efflux pump genes in MDR-TB and reported increased activities of Rv2994 and Rv2477 associated with resistance to fluoroquinolones and streptomycin for the first time [47]. Silva et al. identified P55, a putative multidrug efflux pump from *M. tb* responsible for the

efflux of tetracycline and aminoglycosides, including streptomycin and gentamicin [48]. Garcia et al. reported that deletion of the *Rv1410c* gene encoding P55 efflux pump increased the risk of resistance in the case of TB strain susceptible to rifampicin and clofazimine [49]. Transcription of the Tap-encoding gene (*rv2158c*) is responsible for encoding Tap transporter efflux of several drugs, including aminoglycosides, spectinomycin, tetracycline, and para-aminosalicylic acid [50]. The gene expression for this transporter is controlled by the WhiB7 (antibiotic-responsive MDR regulator) [51]. A recent study reported that efflux pump's like Tap expression is induced in mycobacterial cells that reside within host granulomas. This expression contributes to the drug tolerance of *M. tb* during latent TB infection (LTBI). Doran et al. [52] reported a putative efflux protein, EfpA, responsible for resistance to ethambutol and isoniazid encoded by the *EfpA* gene in the H37Rv strain of *M. tb* [52, 53]. Ramon-Garcia et al. used *Mycobacterium bovis* BCG as a model organism to assess the contribution of intrinsic antibiotic resistance via Stp efflux protein encoded by the *Rv2333c* from *M. tb* to spectinomycin and tetracycline [57]. Some of the other efflux pumps that belong to the MFS family and are associated with DR are Rv0783, Rv3008, Rv3728, and Rv1634 [58].

Multidrug and toxic compound extrusion (MATE) is one more family of efflux pump proteins that efflux out drugs employing proton motive force (H^+ or Na^+) [59]. Among anti-TB drugs, ethambutol is the substrate of MATE1 and MATE2K [60].

RND efflux pumps are proton-dependent pumps that constitute inner and outer membrane protein and a membrane fusion protein. The mycobacterial small membrane protein (MmpS) and mycobacterial membrane proteins (MmpL) are two major efflux pumps of the RND family in *M. tb*. *Mycobacterium smegmatis* (*M. sm*) strain with MmpL7 overexpression is resistant to ethionamide and isoniazid [61]. Where MmpL5–MmpS5 has diverse antibiotic substrates, its overexpression is a cause of resistance to bedaquiline and clofazimine [62].

Moreover, SMR belongs to a family of small multidrug resistance proteins (100–200 amino acids) that form four transmembrane helical structures with amino acids which bind directly to the hydrophobic regions of macrolide antibiotics and aminoglycoside [63]. The first identified efflux pump from an SMR family was Mmr (Rv3065) [64]. It has been reported that strains exposed to high levels of isoniazid significantly overexpress the Mmr efflux pump [65].

2.4 Target Mimicry

Fluoroquinolones have gained importance to treat TB; however, expression of MfpA, a member of the pentapeptide repeat family, results in resistance to this class of antibiotics, especially sparfloxacin and ciprofloxacin [66]. This protein binds to DNA gyrase by DNA mimicry and further inhibits its activity. It is reported that it mimics the B-form of DNA due to its similarity based on size, shape, and electrostatic properties with DNA. The mechanism by which *M. tb* develops resistance to fluoroquinolones via DNA mimicry has been briefly described below.

DNA gyrase (Topoisomerase II) comprises two subunits, A and B, responsible for replication and repair. Among these two, the DNA gyrA subunit has a site for DNA breakage and reunion, whereas the gyrB site is responsible for hydrolyzing ATP for an energy generation required for enzyme activity [67]. The MfpA dimer is highly specific for DNA gyrase; it extends across the entire gyrA and provides an effective inhibition of gyrase activity. MfpA competes with B-form DNA to reach the gyrase surface. Since fluoroquinolones bind to only the DNA gyrase-DNA complex, the binding of MfpA to DNA gyrase averts the complex formation. It comes out with an explanation of the molecular cause of resistance to fluoroquinolones [66].

2.5 Drug Degradation and Modification

Another resistance mechanism by which pathogenic bacteria, including *M. tb,* lower the quality of treatment with antibiotics is to degrade them via drug inactivating enzymes directly. *M. tb* with drug inactivating enzymes expression has developed the ability to modify or degrade different classes of antibiotics, including aminoglycosides, β-lactams, and macrolides [19, 38]. Among these, β-lactam antibiotics are one of the best examples that have undergone resistance via enzymatic degradation due to β-lactamases (BlaC) [68].

β-lactam antibiotics have been the classical and most general classes of antibacterial agents which can be used in treating numerous bacterial infections. These antibiotics disrupt the cell wall biogenesis to result in cell death by binding to penicillin-binding proteins (PBPs) and subsequently inhibiting its activity by hindering the assembling of the peptidoglycan network. However, these antibiotics are ineffective against *M. tb* owing to the expression of an intrinsic enzyme called BlaC [69]. These BlaC hydrolyze the β-lactam ring present in these antibiotics by following three major steps:

i. activation of important amino acid Ser70, which further initiates the nucleophilic attack at carbonyl center of the β-lactam ring;
ii. formation of Michaelis complex followed by activation of conserved water by Glu166 and Lys73; and
iii. hydrolysis of covalently acylated enzyme intermediate [70, 71].

These molecular events lead to the opening of the β-lactam ring and drug inactivation. Another example of such a resistance mechanism includes the acetylation of isoniazid by arylamine N-acetyltransferase (NAT) [72]. Studies have reported that with the overexpression of *nat* gene in *M. sm* enhanced resistance to isoniazid is observed [73], while knockout of the same gene resulted in escalating sensitivity to isoniazid [74]. Inactivation of Isoniazid via NAT involves the following steps:

i. abstraction of a proton by Asp127 from –NH of imidazole ring present in His110;

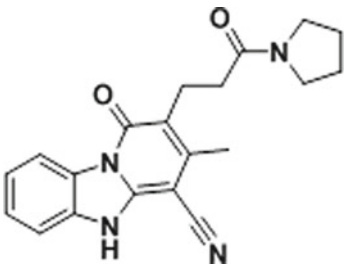


Fig. 2 Structure of compound I

ii. deprotonation of Cys70 by His110;
iii. formation of tetrahedral transition state between acetyl CoA and activated Cys70;
iv. acetylation of Cys70 and release of CoA; and
v. finally, the Acetyl group transfers at NH of the isoniazid [75].

Kanamycin exerts its anti-TB action on resistant strains by generally interfering with the bacterial protein synthesis. However, structural modification of kanamycin due to the enhanced intracellular survival enzyme (Eis) finally results in its resistance. This enzyme inactivates kanamycin by catalyzing the reposition of the acetyl group from Acetyl-CoA to the free amine group of aminoglycosides in five steps:

i. positioning of an amino group of aminoglycoside by His119 backbone to perform a nucleophilic attack on CoA thioester;
ii. stabilization of tetrahedral transition state through the hydrogen bonds with Phe84 and Val85 by the polarization of the thioester carbonyl;
iii. interaction between the C-terminal carboxylate of Phe402 and the amino group of aminoglycoside through a bridging water molecule; and
iv. protonation of CoA thiolate hydroxyl group of conserved residues, i.e., Tyr126 [76].

Decaprenylphosphoryl-β-D-ribose 2-oxidase (DprE1) is a potential target for the treatment of DR-TB [77]. One of the classes of DprE1 inhibitors, i.e., fused pyrido-benzimidazole, especially compound 1 with cyano substitution, is reported to inhibit *M. tb* growth with an IC_{90} value of 0.39 μM [78]. However, a study conducted by Warrier and coworkers in 2016 reported that S-adenosyl-L-methionine-dependent methyltransferase (Rv0560c) causes N-methylation of this pyrido-benzimidazole compound and abolishes its mycobactericidal activity. Some in vitro studies revealed a two-fold reduction in the activity of compound 1 with the overexpression of Rv0560c ([79]; Fig. 2).

3 Strategies to Modulate Resistance in Tuberculosis

3.1 Structural Modification in Existing Drugs to Address Mutation-Associated Resistance

Analog designing is often defined as modifying a drug molecule or any other bioactive component to form a new molecule that reflects the chemical and biological similarities with the parent compound [80]. This approach has been utilized to overcome the resistance issue and is briefly discussed below.

Being a pro-drug, isoniazid needs to get bio-transformation-based activation by the well-known *M. tb* target KatG. Nearly 70% of isoniazid resistance strains acquire resistance due to mutation in *katG*, thus resulting in the inability of KatG to activate this pro-drug. To overcome this problem, various research teams have made some efforts to create compounds that can achieve moderate clinical efficacy similar to isoniazid and prevent much of the current resistance by exceeding the requirement for getting activated (bypass the activation) via KatG and directly inhibiting InhA. Some of the reported isoniazid analogs which may bypass KatG activation and target InhA directly have been mentioned below.

Vosátka et al. [81] synthesized a series of N-alkyl-2-iso-nicotinoyl hydrazine-1-carboxamides from isoniazid which were later cyclized to N-alkyl-5-(pyridin-4-yl)-1,3,4-oxadiazole-2-amines. Among all oxadiazole, N-Dodecyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amine (compound II; Fig. 3) was the most efficacious one with an ability to inhibit the growth of both susceptible as well as DR-*M. tb* strains with MIC values in the range of 4–8 μM. Structural activity relationship (SAR) revealed that the introduction of shorter alkyls results in the least active or inactive compounds while incorporating a long aliphatic chain enhanced the activity. It targets InhA and further inhibits the synthesis of mycolic acids to influence the growth of both DS-TB and DR-TB, including MDR-TB and XDR-TB strains, at the same

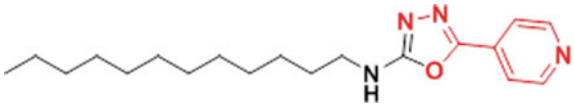


Fig. 3 Structure of compound II

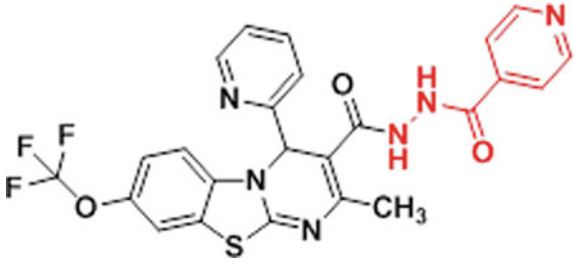


Fig. 4 Structure of compound III

concentration as that of isoniazid devoid of any cross-resistance to already existing antimycobacterial drug [81].

Bhoi et al. [82] synthesized a series of novel N′-isonicotinoyl-2-methyl-4-(pyridin2-yl)-4H-benzo[4,5]thiazolo[3,2-a]pyrimidine-3-carbohydrazide analogs, which were evaluated against *M. tb* strain. In vitro results concluded that compound III (Fig. 4) was the most potent antimycobacterial agent with a MIC value of 6.25 mg/mL. Molecular docking and dynamics studies revealed that this compound displayed about three hydrophobic interactions and four hydrogen bonding interactions within the active pocket of the InhA enzyme. SAR study revealed that the analog possessing trifluoromethoxy (–OCF_3) group at the eighth position is the most potent antimycobacterial agent. While the presence of heterocyclic nuclei such as isoniazid, pyridine, and pyrimido[2,1-b]benzothiazole as pharmacophores significantly improved the lipophilic character of the molecule. The analog with electron-donating and electron-withdrawing group substitutions at the sixth, seventh, and eighth position of pyrimido [2,1-b]benzothiazole fused ring system facilitated the permeability through the microorganism's biological membrane, thus successfully inhibiting the bacterial growth [82].

De et al. [83] reported some isoniazid-cinnamic acid hybrids, among which the most active compound IV (MIC: 0.3 Mm; Fig. 5) manifested potency twice the parent drug isoniazid (MIC 0.6 mM) against *M. tb* H37Rv with low toxicity (IC_{50} 168 mM) towards THP1 cell and the selectivity index (SI) of 560. This isoniazid-cinnamic acid hybrid was further assessed for the inhibitory activity against two isoniazid-resistant strains, MYC5165 (*M. tb* conferring mutation in *InhA*) and 1400 (*M. tb* conferring mutation in *katG*). The SAR data revealed that for the R group, the short-chain and electron-donation groups more favored the inhibitory activity than the longer one (Me > Et > i-pentenyl) and electron-withdrawing groups (Me > CF3 > Et > CF3CH2) [83].

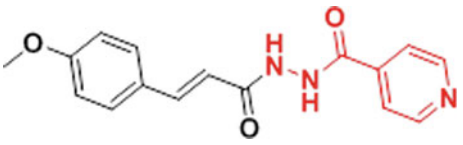


Fig. 5 Structure of compound IV

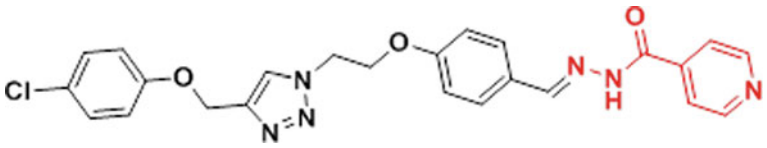
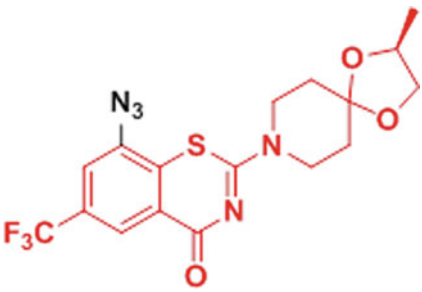


Fig. 6 Structure of compound V

Fig. 7 Structure of compound VI



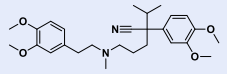
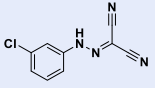
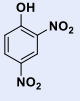
Kumar et al. [84] developed a novel 1,2,3-triazole based analog of isoniazid and assessed the in vitro activity of these compounds against *M. tb*. Results revealed these five compounds to be equally potent to that of reference compound isoniazid. Molecular docking and dynamics simulations results indicated that compound V retained crucial interactions with key amino acids in both wild-type *InhA* and mutant *InhA* harboring D148G mutation. Compound V (Fig. 6) also exhibited significant stability in the binding pocket of wild-type *InhA* and mutant *InhA*, which may be considered a positive indication for its potential to be an effective therapy against isoniazid-resistant strains of *M. tb*. While in vivo studies further revealed its safety when evaluated in terms of toxicity profile [84].

The promising new class of anti-TB compounds like BTZ043 and PBTZ169 constitutes a nitroaromatic system as an electron-deficient core, which causes the inactivation of *DprE1* by forming a covalent semimercaptal adduct. However, Cys387Ser mutation in *DprE1* resulted in resistance to these drugs. Some efforts have been made to overcome this problem by modifying BTZ043. Tiwari et al. [85] reported 1,3-benzothiazinone azide (compound VI; Fig. 7) as an effective reversible and non-covalent inhibitor of DprE1 that was found effective against both wild-type and mutant (harboring Cys387Ser) *DprE1*. The MIC value of the *M. tb* H37Rv or NTB1 (harboring Cys387Ser mutation in *DprE1*) strains to BTZ-N_3 compound was found to be 0.5 μg/ml [85], with equivalent potency in both wild-type and resistant strains of *M. tb*.

3.2 Targeting Efflux Pumps Responsible for Drug Efflux

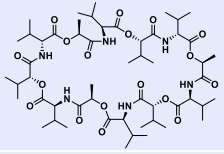
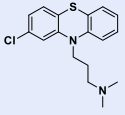
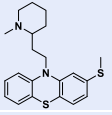
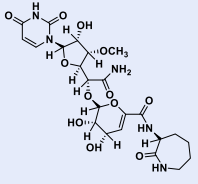
Over the past few years, there has been a growing interest in searching and developing efflux pump inhibitors. Efflux pump inhibitors are a potential adjuvant therapy with existing anti-TB drugs against DR-TB. They have the high potential to raise the intracellular concentration of anti-TB drugs. This raised intracellular concentration in blood may improve or restore standard antimycobacterial agents' activity in resistant strains and reduce the treatment duration. However, some concern is associated with the efflux pump inhibitors, i.e., toxicity. Since these agents target both prokaryote and eukaryote transporters, the clinical development of multiple efflux inhibitors must be carried out by considering toxic complications.

Table 2 Some of the drugs repurposed as efflux pump inhibitors

S. No.	Name of the drug	Chemical structure	Drug target	Anti-tuberculosis effects
1	Verapamil		**ABC (ATP binding cassette) family** PstB, Rv2686c-2687c-2688c, DrrAB **MFS (multifacilitator superfamily)** Rv1634, lfrA, Rv1258c, Rv2846c, Rv1877 **RND (resistance nodulation division)** Rv1145, Rv1146, Rv0678 **SMR (small mutiresistance) family** Rv3065 (mmr)	(1) MIC value of anti-TB drugs is reduced (2) Anti-TB drug dose is reduced (3) Synergy with anti-TB drugs exists (4) Duration of anti-TB drugs retention in *M. tb* is elongated (5) Treatment duration is reduced (6) Growth and tolerance of *M. tb* are inhibited
2	Protonophore (CCCP)		**ABC (ATP binding cassette) family** Rv2936-Rv2937 (DrrAB), Rv2686c-2687c-2688c, Rv0933(PstB) **MFS (multifacilitator superfamily)** Rv2459 (jefA), Rv1410c (P55), lfrA, Rv1258c, Rv1634, Rv1877, Rv1410c, Rv2846c **RND (resistance nodulation division)** Rv1146, Rv0676c-Rv0677c (MmpS5-MmpL5), Rv1145 **SMR (small mutiresistance) family** Rv3065 (mmr)	(1) MIC value is reduced (2) Resistance to anti-TB drugs is reduced (3) Synergy with anti-TB drugs exists
3	Protonophore (DNP)		**ABC (ATP binding cassette) family** Rv2936-Rv2937(DrrAB), Rv0933 (PstB), Rv2686c-2687c-2688c **MFS (multifacilitator superfamily)** lfrA, Rv1634, Rv1258c	(1) MIC value of anti-TB drugs is reduced

(continued)

Table 2 (continued)

S. No.	Name of the drug	Chemical structure	Drug target	Anti-tuberculosis effects
4	Protonophore (Valinomycin)		**MFS (multifacilitator superfamily)** Rv1410c (P55)	(1) Sensitivity to anti-TB drugs is enhanced (2) Duration of anti-TB drugs retention in *M. tb* is elongated
5	Phenothiazine (Chlorpromazine)		**RND (resistance nodulation division)** Rv1145, Rv1146 **MFS (multifacilitator superfamily)** Rv1877, Rv2846c **SMR (small mutiresistance) family** Rv3065(mmr)	(1) It can have effects on both drug-sensitive and drug-resistant TB strains (2) Synergy with anti-TB drugs exists (3) *M. tb* sensitivity to anti-TB drugs is enhanced
6	Phenothiazine (Thioridazine)		**RND (resistance nodulation division)** Rv3160c-Rv3161c	(1) It can have effects on both drug-sensitive and drug-resistant TB strains
7	Capuramycin and analogs		Phosphor-N-acetylmuramyl-pentapeptide-translocase (translocase)	(1) It can have effects on both drug-sensitive and drug-resistant TB strains (2) Synergy with anti-TB drugs exists (3) It has been shown to reduce bacterial load in mouse lungs

(continued)

Table 2 (continued)

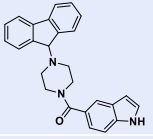
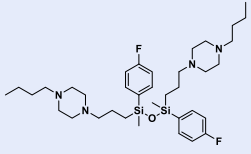
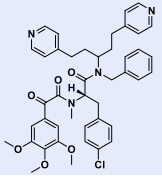
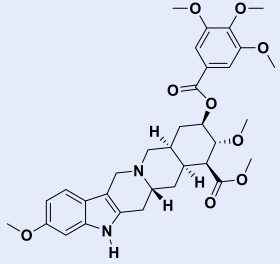
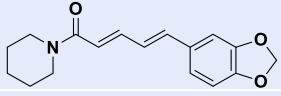
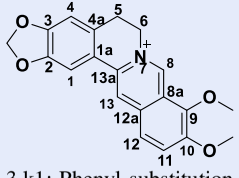
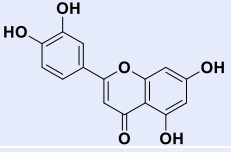
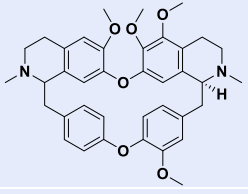
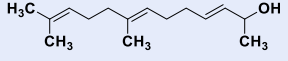
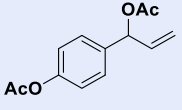
S. No.	Name of the drug	Chemical structure	Drug target	Anti-tuberculosis effects
8	GEQ compound Phe-Are-β-napthylamide (MC-207110, MC-02595, MC-04124, BU-005)		**RND (resistance nodulation division)** Rv2942 (MmpL7), CmlA (cmlR1), FloR (cmlR2)	(1) MIC value of anti-TB drugs is reduced (2) *M. tb* growth is hampered (3) It causes reverse *M. tb* resistance to anti-TB drugs
9	SILA 421		Mdr-1	(1) Synergy with anti-TB drugs exists (2) Macrophage ability to kill *M. tb* is enhanced (3) It causes reverse *M. tb* resistance to anti-TB drugs
10	Timcodar		**ABC (ATP binding cassette) family** DrrAB, PstB, Rv2686c-2687c-2688c **MFS (multifacilitator superfamily)** IfrA, Rv1634, Rv1258c, Pgp, MDR-associated protein (MRP)	(1) *M. tb* growth is hampered (2) Synergy with anti-TB drugs exists (3) It has been shown to reduce bacterial load in the lungs

Table 3 Natural product as efflux pump inhibitors

S. No.	Phytoconstituent efflux-pump inhibitors	Chemical structure	Drug target	How these drugs has got potential for the treat of tuberculosis
1	Reserpine		**ABC (ATP binding cassette) family** (1) DrrABC encoded byRv2936-Rv2937-Rv2938 (2) PstA-encoded by Rv0933 **RND: (resistance nodulation division)** Mmpl7 encoded by Rv0678, Rv1145, Rv1196, Rv2942 **MFS: (multifacilitator family)** Rv1410c(P55) **SMR: (small mutiresistance family)** Rv3065(mmr)	(1) Reduced MIC value of anti-tubercular drugs (2) The MIC resistance to anti-tubercular drugs inMtb for long duration, as there is elevation in its concentration intracellularly
2	Piperine		**MFS: (multifacilitator family)** Rv1258c	(1) Reduction in MIC value ofAntitubercular drugs (2) Cellualr immunity unregulated
3	Berberine	 3.k1:-Phenyl substitution at 8 position 3.k2:-4-chlorobenzyl 3.k3:-2,4-dichlorobenzyl 3.k4:-4-fluorobenzyl 3.k5:-3′,3′-dimethyl allyl	NorA, RamR	Reverse the drug resistance

(continued)

Table 3 (continued)

S. No.	Phytoconstituent efflux-pump inhibitors	Chemical structure	Drug target	How these drugs has got potential for the treat of tuberculosis
4	Quercetin		**SMR: (small mutiresistance family)** Rv3065(mmr), Isocitrate Lyase	(1) *M. tb* growth is hampered
5	Tetrandrine		**MFS: (multifacilitator family)** Rv2459(jefA),Rv3728 **SMR: (small mutiresistance family)** Rv3065(mmr)	(1) MIC value of anti-tubercular drug's is reduced (2) Synergy withanti-tubercular drug's
6	Farnesol		Not determined	(1) Synergy with anti-tubercular drug's (2) Retention ofAntitubercular drug's intracellularly, thus is accumulated
7	Phenylpropanoids		**RND: (resistance nodulation division)** Rv1145,Rv1146 MFS:Rv1877,Rv2846c **SMR: (small mutiresistance family)** Rv3065(mmr)	(1) Reduced MIC value of anti-tubercular drug's (2) Accumulation of anti-tubercular drug's intracellularly

For therapeutic purposes, an efflux inhibitor must display insubstantial toxicity to the host cells at its therapeutic dose. A way to overcome this problem would be to use inhibitors that are selective towards efflux pumps absent in human cells. A drug discovery strategy, i.e., drug repurposing, could help overcome this limitation. Some of the drugs repurposed as efflux pump inhibitors have been mentioned in Table 2, and some natural products-based efflux pump inhibitors are summarized in Table 3.

3.3 Bypassing Drug Inactivating Enzymes

3.3.1 N-acetyltransferase

Isoniazid acetylation via NAT illustrates a major cause of its biotransformation-based inactivation in human beings. As mentioned above, acetylation considerably minimizes the drug's therapeutic activity due to underdosing, diminished bioavailability, and the development of acquired isoniazid resistance. It was assumed that structural modification of isoniazid with different functional groups might prevent acetylation while maintaining significant anti-TB action, ameliorate clinical outcomes, and help overcome isoniazid resistance. Hearn and coworkers reported modified isoniazid as lipophilic Schiff base N^2-cyclohexylidenyl isonicotinic acid hydrazide (compound VII; Fig. 8) such that the hydrazine moiety provides hindrance to acetylation by NAT. The Isoniazid Schiff base demonstrated good in vitro activity against *M. tb* strains H37Rv and Erdman with MICs of 0.03 mg/L. The Schiff base was found to be slightly more active in comparison to isoniazid [86].

Shingnapurkar et al. [87] reported NAT-protected Schiff bases as analogs of pyruvate–isoniazid, as well as their copper complexes as anti-TB agents that might also block enzymatic acetylation via NAT. These metal complexes with pyruvate–isoniazid enhanced lipophilicity and improved the uptake of isoniazid and analogous hydrazides to access both susceptible and resistant mycobacteria. Compound VIII (Fig. 9) was the most active complex that improved the therapeutic activity of isoniazid [87].

O-ester derivatives of plumbagin have been reported to possess good inhibitory activity compared to plumbagin against both *M. sm* and *M. tb* H37Rv, respectively. Considering the importance of O-esters of plumbagin, Dandawate et al. [88] reported potent conjugate of isoniazid and plumbagin, which prevented enzymatic acetylation of isoniazid by NAT. Under low-iron conditions, the anti-TB activity of plumbagin–isoniazid conjugate (compound IX; Fig. 10) and its β-cyclodextrin inclusion complex yielded MIC values of 0.5 μg/ml, which is better than isoniazid (MIC 32 μg/ml), suggesting their significance in countering isoniazid resistance [88].

A series of 5-substituted-(1,1-dioxo-2,3-dihydro-1H-$1\lambda^6$-benzo[e][1,2]thiazin-4-ylidene)-thiazolidine-2,4-dione derivatives represents as competitive inhibitors of recombinant bacterial arylamine NATs. SAR results indicated that R-group substituted with large hydrophobic (planar aromatic and long-chain aliphatic) on the sultam-TZD adduct manifested good inhibition due to non-covalent bonding

π-stacking and hydrophobic–hydrophobic interactions with key residues within the binding pocket of NAT. Kinetic analysis of the inhibitors by varying the concentrations of isoniazid substrate and inhibitors revealed that the most potent compound (compound X; Fig. 11) displayed 14.41 μM as inhibition constant (K_i) value [89], while displayed half-maximal inhibitory concentration (IC_{50}) value with 20 ± 1 μM against NAT [89].

3.3.2 Acetyltransferase Eis

The upregulation of *M. tb* acetyltransferase Eis is majorly responsible for resistance to the aminoglycoside kanamycin. This enzyme inactivates kanamycin by acetylating the amino groups. Willby et al. [90] identified potent isothiazole S,S-dioxide heterocyclic core containing inhibitors that overlapped within the aminoglycoside binding site of this enzyme to prevent the acetylation of kanamycin. Isothiazole S, S-dioxide-based derivative with a p-dimethylphenyl group as R_1 substitution and a diamine separated by three carbons at the R_2 position was a highly potent Eis inhibitor with IC_{50} value 0.054 ± 0.002 μM (Fig. 12). These inhibitors interacted with key amino acid residues of Eis via hydrophobic, steric complementarity, and H-bonding interactions. These inhibitors also conferred potencies in the range of mid-nanomolar [90].

To combat kanamycin resistance owing to Eis, analogs of pyrrolo[1,5-a]pyrazine have been developed. These inhibitors contain acetophenone moiety enriched with π electrons and the fully aromatic pyrrolo[1,5-a]pyrazine that is important for binding with crucial amino acid residues with aromatic property within the active binding pocket of Eis via π−π stacking interactions. SAR study revealed that acetophenone moiety substituted with bigger halogen groups like Cl and Br at the meta position was generally more suitable. While the substitution of the acetophenone at the para position with a smaller F group was observed to display the best Eis inhibitory potential (Fig. 13). Among all possible pyrrolo[1,5-a] pyrazine-based analogs, compound with m-Cl substitution resulted in activity

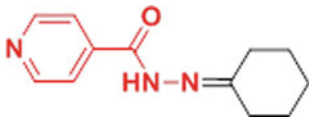


Fig. 8 Structure of compound VII

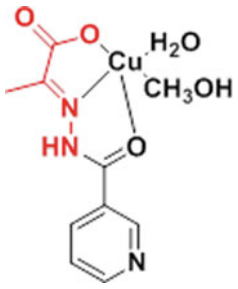


Fig. 9 Structure of compound VIII

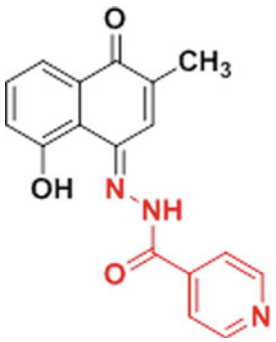


Fig. 10 Structure of compound IX

around three-fold less than the parent compound while conferring thirteen times better activity than compound with m-Br substitution because Br substituent was not well supported in the binding pocket of Eis owing to steric hindrance. R_2-substituted acetophenone fits mainly within the hydrophobic environment of Leu63, Trp36, and Arg37. Hence, the substitution of R_2 with a polar hydroxyl group was unfavorable for binding within the hydrophobic environment of Eis and consequently reduced the Eis inhibitory activity. While the aromaticity of the pyrrolo [1,5-a]pyrazine core is important for inhibitory activity due to the stacking of the pyrazine ring between the side chain of Glu401 of Eis and its C-terminal carboxyl group. The aromatic pyrazine ring demonstrated $\pi-\pi$ interactions with the indole ring of Trp36, which is the main reason for improved inhibitory activity of pyrrolo [1,5-a]pyrazine-based analogs. This class of inhibitors in combination therapy with kanamycin may combat kanamycin resistance, especially in MDR and XDR strains of *M. tb*; thus, they may act as adjuvant molecules [91].

Sulfonamide-based derivatives (one of them is displayed in Fig. 14) have been reported to restore kanamycin susceptibility in the kanamycin-resistant strain of *M. tb* by efficiently inhibiting the Eis. Sulfonamide derivative with N-methyl and 2-naphthyl substitution manifested a substantial increase in Eis inhibitory activity (IC_{50} 0.00024 ± 0.00010 μM) to attenuate kanamycin resistance in *M. tb* K204 (MIC $\leq$ 1.25 μg/mL). SAR study revealed that removal of N-methyl group is unfavorable as it is responsible for optimal Van der Waals interaction with key residue, i.e., Trp36. While electron-donating groups, including naphthalene ring or

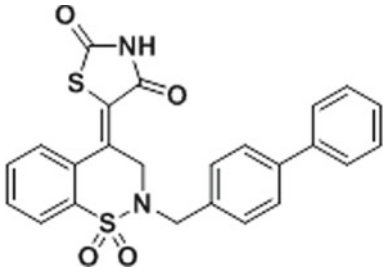


Fig. 11 Structure of compound X

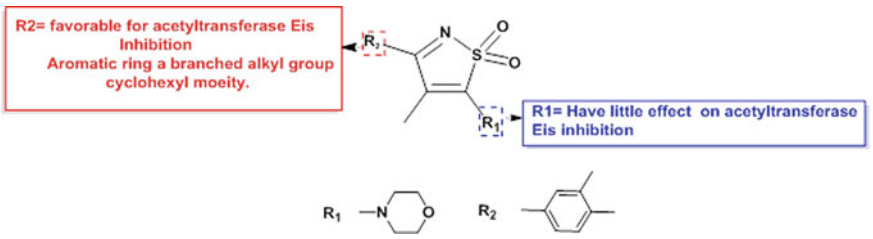


Fig. 12 SAR for isothiazoleS, S-dioxide based Eis inhibitors

a methoxy group, enhanced the π−π interaction between aniline ring and key amino acid residues Phe84 and Trp36 within the aminoglycosides (AG)-binding site of Eis. These derivatives can be used as promising kanamycin adjuvants (Fig. 4) [92].

Recently, Eis inhibitors comprising 1,2,4-Triazino[5,6b]indole-3-thioether have been reported, which can be given as an adjuvant therapy to treat kanamycin-resistant TB. These 1,2,4-triazino[5,6]indole-3-thioether-based Eis inhibitors constitute a tricyclic core with a long flexible linker, which allows easy access to the active site of Eis. SAR study revealed that the length of the linker up to a 2-carbon linker attached to a larger six-membered heterocycle such as piperidinyl ring occupied the binding pocket more efficiently, which resulted in a two-fold improvement in Eis inhibitory activity. While methyl group as R_1 substituent was the most favorable among all tested N_5-modifications (Fig. 15). The flexible C3-linker with a piperidinyl group at the end outstretches toward a large opening within the AG-binding site to gain hydrophobic contact with an aliphatic stem of Glu401. The compound with the modifications mentioned above conferred $IC_{50K\text{-}}$ anamycin of 0.03 ± 0.005 μM [93].

Garzan et al. [94] identified two new scaffolds as potent Eis inhibitors, i.e., 4H-fluoro[3,2-b]pyrrole-5-carboxylate and the 3-(1,3-dioxolano)-2-indolinone through high-throughput screening (HTS). The MIC value of kanamycin improved to 5 μg/mL when 3-(1,3-dioxolano)-2-indolinone based BlaC inhibitor with m-fluorophenyl substitution (compound XII; Fig. 16) was given as adjuvant therapy. The inhibitory activity of m-chloro phenyl substituted 3-(1,3-dioxolano)-2-indolinone derivative was less than p-fluoro substituted phenyl group derivative,

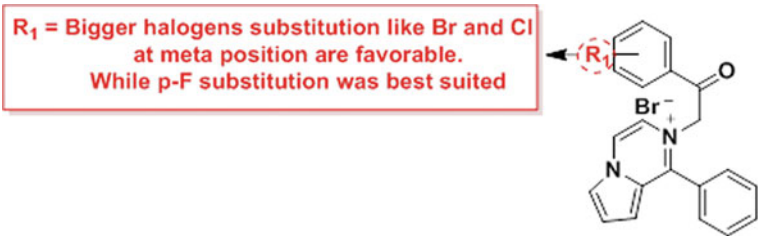


Fig. 13 SAR for pyrrolo[1,5-a]pyrazine based Eis inhibitors

and it did not sensitize MtbK204 to kanamycin ($MIC_{Kanamycin}$ 5–10 g/mL). On the other hand, 4H-furo[3,2-b]pyrrole-5-carboxylate derivative with phenyl substitution (compound XI; Fig. 17) showed slight inhibition of Eis *M. tb* in the HTS campaign by displaying satisfactory Eis-*M. tb* inhibitory activity with an IC_{50} value in the range of 3 ± 1 μM. This derivative combined with kanamycin manifested MIC values of 5−10 μg/mL against *M. tb* K204 [94].

3.3.3 β-Lactamases

β-lactam was initially developed for treating infections associated with gram-positive bacteria by inhibiting transpeptidases involved in peptidoglycan cross-linking. Previously, penicillins, including ampicillin, amoxicillin, and cephalosporins, have been reported to be active in vitro against *M. tb*. However, membrane-associated BlaC results in their deactivation by breaking the β-lactam ring and resulting in ring-opening. The single chromosomally encoded β-lactamase, i.e., BlaC, is a promising target to develop novel inhibitors. Some of the BlaC inhibitors developed to overcome the resistance associated with the β-lactam class of anti-TB drugs have been briefly discussed below.

Kurz and coworkers reported a cefoperazone analog as boronic acid transition state inhibitors that bind to the binding pocket of BlaC to form an adduct of boronate, which sterically and electronically resemble the highly-energetic intermediate with tetrahedral geometry formed as a resultant of the β-lactam hydrolysis reaction. The most potent compound (compound XIII; Fig. 18) was found to be equally potent to that of clavulanate (K_i 0.65 ± 0.05 μM), inhibiting BlaC in a slow and time-dependent manner [95].

Hazra et al. [96] reported 6-methylidene β-lactam capable of irreversibly inhibiting BlaC, i.e., 70 times better than clavulanate. This penem manifested synergism with meropenem and ampicillin against a growing culture of *M. tb* by inhibiting BlaC, transpeptidase, and peptidoglycan cross-linking. β-lactamase acts on β-lactam by promoting ring-opening and generating a C6 thiolate intermediate by acting upon the later formed thiazoline ring of the covalently attached inhibitor, which resembles opened oxazole ring system in clavulanate. The thiolate subsequently attacks the C6-C7 ester bond that is conjugated unsaturated bond to form 1,4-thiazepine species. With the formation of cyclized thiazepine at the BlaC active

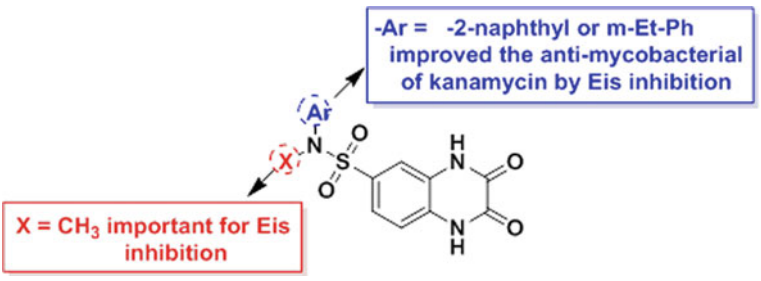


Fig. 14 SAR for sulfonamide based Eis inhibitors

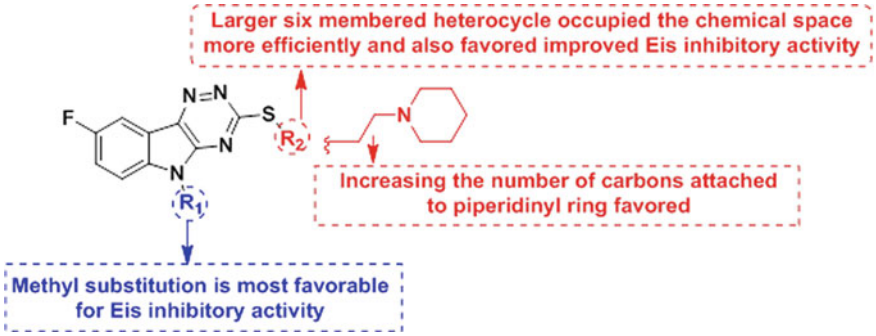
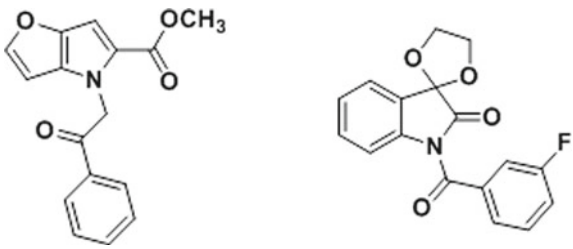


Fig. 15 SAR for 1,2,4-Triazino[5,6]indole-3-thioether based Eis inhibitors

Fig. 16 Structure of compound XII



site, no more subsequent hydrolysis of β-lactam drugs takes place. The later formed 1,4-thiazepine species interacted covalently with BlaC (Fig. 19). Penem-2 inhibited not only BlaC but also the L-D- and D-D-transpeptidases and subsequent peptidoglycan cross-linking, leading to a weak cell wall arrangement in *M. tb*. These designed penems had K_i values of 0.8 and 0.2 μM, respectively, lower than those observed with clavulanate [96].

Former Carbapenem, such as meropenem, is given in combination with clavulanate (β-lactamase inhibitor). Carbapenems have never been efficient enough to escape hydrolysis by β-lactamase BlaC. Iannazzo et al. [97] attempted to modify and generate a new Carbapenem that is superior to meropenem in the context of both reduced hydrolysis by BlaC and efficiency in terms of in vitro inactivation of L-D-transpeptidases Ldt_{Mt1}. They reported modification in two side chains substitutions on either side of the five-membered rings present in the Carbapenem core and β-lactam. The SAR studies revealed that the triazole ring present in Carbapenem (2a–f) chains mimicked the amide that is present in meropenem (1a) and Ertapenem (1b). The triazole linker was favorably stable within the active site of $Ldt_{Mt1,}$ as indicated by mass spectroscopy. Thus, triazole is well supported for significant Ldt_{Mt1} inhibition. Moreover, it was observed that the carboxylic group significantly enhanced the solubility of these classes of drugs ([97]; Fig. 20).

Xu et al. [98] identified NXL104 as a novel β-lactamase inhibitor comprising a nonlactam structural scaffold. As per the reported mechanism, NXL104 inhibits

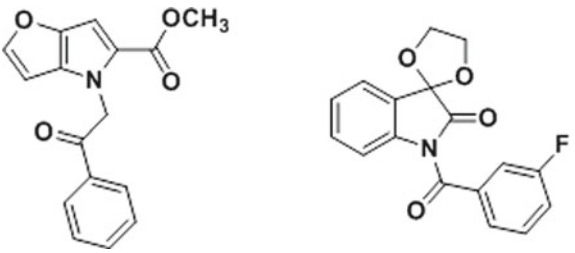


Fig. 17 Structure of compound XI

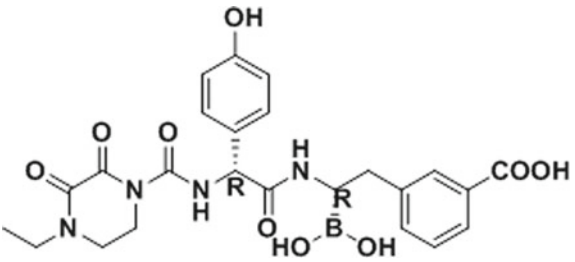


Fig. 18 Structure of compound XIII

BlaC by initiating deprotonation of key residue Ser70 by Lys73, following which the nucleophile hydroxyl group present in Ser70 facilitates electrophilic attack at carbonyl carbon present in NXL104 to form an inactive carbamyl BlaC intermediate (Fig. 21). Herein, decarbamylation of this formed intermediate is the rate-limiting step as it is much slower than carbamylation. The decarbamylation is mediated by Glu166 and a catalytic water molecule, as a consequence of which inactivation of BlaC occurs. The observed inhibition efficiency, i.e., k2/K of NXL104, was reported to be 100-fold lower in comparison to clavulanate (which is a classical β-lactamase inhibitor) as a resultant of the bulky rings present in NXL104. Reactivation assay results revealed that BlaC (2 μM) was completely inactivated when incubated with 5 μM NXL104 for 24 h. On the other hand, BlaC was inactivated with 5 μM clavulanate only for two hours at room temperature [98].

4 New Drugs Under Development or in Clinical Trials for the Treatment of Tuberculosis

The research of new anti-TB drug discovery and repurposing has been accelerated in the past five years with the approval of the Bedaquiline (a new anti-TB drug). Some of the recently developed classes of drugs that can be used to treat TB are diarylquinoline, nitroimidazole, oxazolidinone, 1,2-ethylenediamine, benzothiazinone, imidazopyridine, 1–4 azaindoles, oxaborole, carbostyril, and iminophenazine. The development of potent nitroimidazoles and the diarylquinoline class of drugs provides new hope as an oral pan-TB regimen to handle the DR-TB problem. The

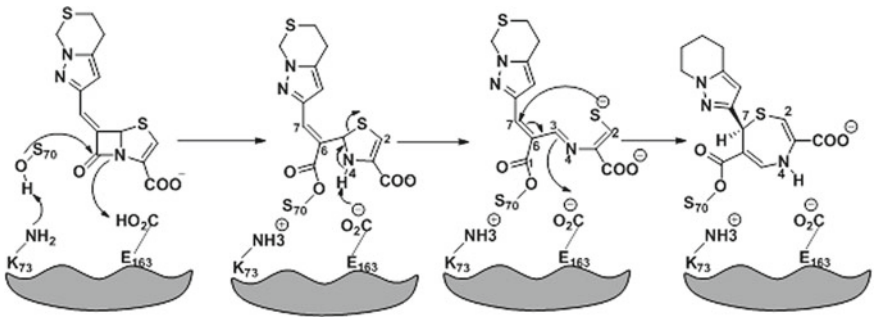


Fig. 19 Mechanism of BlaC inhibition by 6-methylidene β-lactam

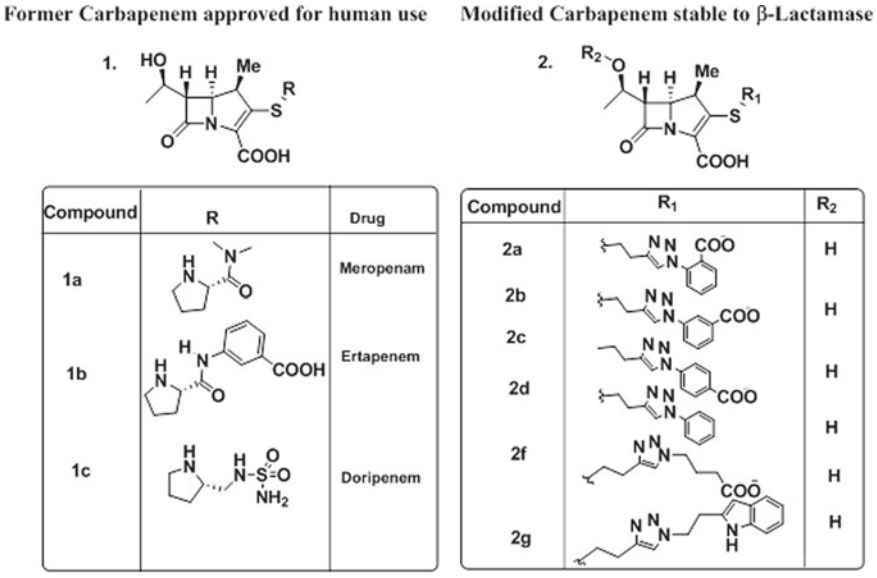
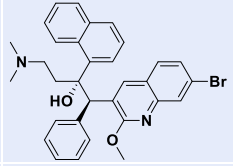
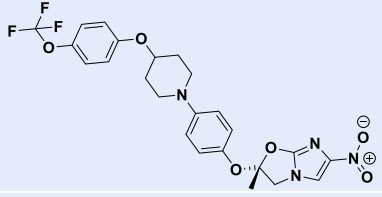
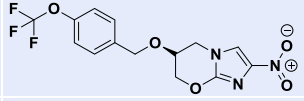
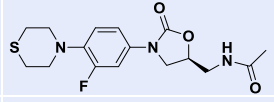
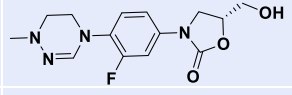
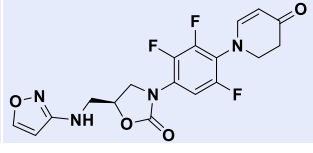


Fig. 20 Carbapenem before and after modification

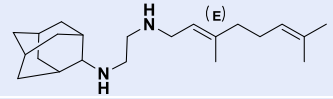
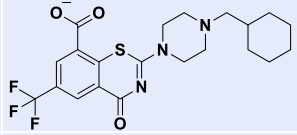
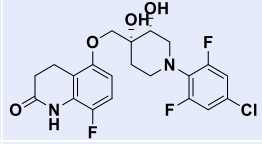
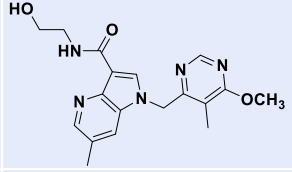
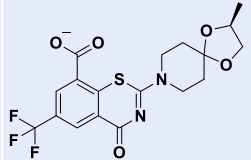
class of drugs, their mechanisms of action, trial phase, and their structure are mentioned in Table 4. PBTZ169 is now in the early stage of clinical trials, i.e., phase 2 for antimycobacterial activity. A new compound, Q203, was assessed in a phase 1 trial that finished in 2017, while TBA7371 was introduced in phase 1. SQ109 appears to be active in vitro but is not used with rifampicin, as such a combination substantially reduces levels of rifampicin. As per the provisional results of a double-blind placebo-controlled trial in Russia, about 80% of patients suffering from MDR-TB received SQ109 plus optimized background regimen and were tested as sputum negative within 24 weeks in comparison to 61% of patients who received placebo plus regimen. Despite these advances, there are some

Table 4 New drugs being developed or under clinical trial for the treatment of TB

S. No.	Drug	Structure	Target	Clinical Trial	Class
1	Bedaquiline		ATP synthase	Phase-3	Diarylquinoline
2	Delamanid		Cell wall synthesis and cell respiration	Phase-3	Nitroimidazole
3	Pretomanid		Cell wall synthesis and cell respiration	Phase-3	Nitroimidazole
4	Sutezolid		Protein synthesis (23 s ribosome)	Phase-2a	Oxazolidinone
5	Delpazolid		Protein synthesis (23 s ribosome)	Phase-2	Oxazolidinone
6	Contezolid		Protein synthesis (23 s ribosome)	Phase-1	Oxazolidinone

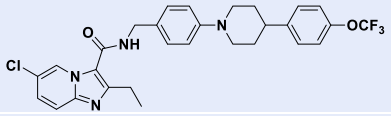
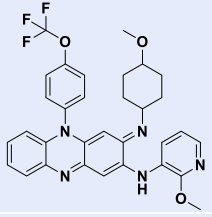
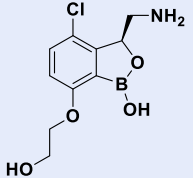
(continued)

Table 4 (continued)

S. No.	Drug	Structure	Target	Clinical Trial	Class
7	SQ109		Cell wall synthesis (MmpL3)	Phase-2	1,2-ethylene diamine
8	PBTZ169		DprE1 inhibitor Cell wall synthesis	Phase-2	Benzothiazinone
9	OPC-167832		DprE1 inhibitor Cell wall synthesis	Phase-1	Carbostyril
10	TBA7371		DprE1 inhibitor Cell wall synthesis	Phase-1	1–4 azaindoles
11	BTZ 043		DprE1 inhibitor Cell wall synthesis	Phase-1	Benzothiazinone

(continued)

Table 4 (continued)

S. No.	Drug	Structure	Target	Clinical Trial	Class
12	Q203			Phase-1	Imidazopyridine
13	TBI-166		Cytochrome QcrB and cell respiration	Phase-1	Riminophenazine
14	GSK070		Protein synthesis (leucyl-tRNA synthetase)	Phase-1	Oxaborole

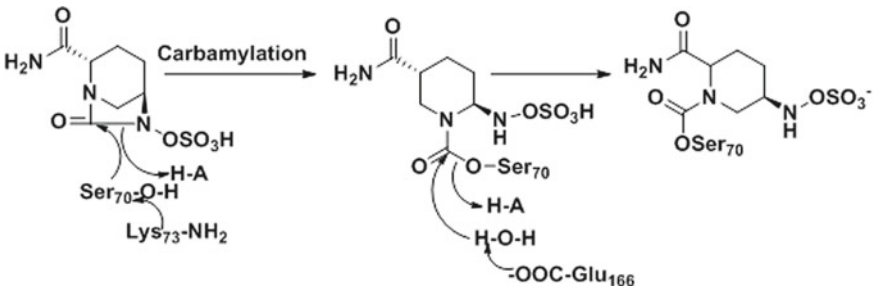


Fig. 21 Mechanism of BlaC inhibition by NXL104

setbacks: for instance, Sutezolid yielded favorable results in the phase 2 trial; however, phase 1 studies must be repeated due to licensing issues. TBA–354 and AZD5847 are two further examples of such setbacks, with no substantial potency in phase 1 investigations that revealed neurotoxicity.

5 Current Therapy Available for Drug-Resistant Tuberculosis

The taxonomy of anti-TB drugs and combinations is rapidly evolving due to new clinical trials data and meta-analyses [99, 100]. As per WHO, anti-TB drugs used as second-line therapy have been categorized as panel 1 (Table 5) for treating MDR-TB and rifampicin-resistant TB [101]. The treatment for rifampicin-resistant

Table 5 Different categories of second-line anti-TB drugs recommended by WHO for the treatment of rifampicin-resistant and MDR-TB

Group A Fluoro quinolones	Group B Second-line injectable drugs	Group C Other core second-line drugs	Group D Add-on drugs		
			D1	D2	D3
Gatifloxacin Moxifloxacin Levofloxacin	Kanamycin Capreomycin Streptomycin Amikacin	Ethionamide or prothionamide Linezolid Cycloserine or Terizidone Clofazimine	High-dose isoniazid Ethambutol Pyrazinamide	Delamanid Bedaquiline	Para-aminosalicylic acid Meropenem (requires clavulanate) Imipenem plus cilastatin (requires clavulanate) Thioacetazone Amoxicillin plus clavulanate

and MDR-TB includes two core drugs, i.e., injectable aminoglycosides and fluoroquinolones, along with other core drugs, i.e., prothionamide or ethionamide, terizidone or cycloserine, clofazimine, and linezolid. Under resistance or intolerance conditions, if further drugs are required, then non-core drugs like bedaquiline or delamanid can be added. However, these two non-core drugs cannot be given in combination. On the other hand, non-core drugs, such as carbapenems and para-aminosalicylic acid (PABA) with clavulanate combination are reserved for patients with XDR-TB having few therapeutic options [102, 103]. Standard drug regimen preserved for patients with pre-XDR-TB or XDR-TB has been categorized as panel 2 and mentioned in Table 6 [99].

TDR strain of *M. tb* is resistant to anti-TB drugs from both the first and second-line generations. Under such conditions, WHO proposed to explore novel drugs on high priority in the extreme cases resembling TDR-TB. A clinical study suggested that XDR-TB patients can be treated with thioridazine (TDZ) as these patients do not respond to any antibiotic therapy and manifest a poor prognosis. TDZ potentially cured ten out of 12 XDR-TB patients, while the remaining two patients were dropped out of the program [104, 105].

6 Conclusion

DR-TB treatment demands steady advancement in developing new anti-TB agents and repurposing of already existing drugs against resistant strains of *M. tb*. The resistance problem can efficiently be managed, and the complete eradication of resistant strains of TB may become an actuality if there is a persistent effort at both national and international levels. This can only be achieved by promoting proper TB management programs, availing sufficient funding for research, and fruitful collaborations between academia and industry. The development of new drugs or drug repurposing should ensure that the treatments given to the patient are affordable, effective, safe, and devoid of the resistance problem. Also, the developed drugs must shorten the treatment duration. To corroborate the effective development of this type of therapy, various resistance mechanisms were discussed at the molecular level, and an update was provided on different strategies to overcome the resistance problem with anti-TB drugs. The current chapter also furnished a brief account of drugs currently undergoing clinical trials. Based on the information provided in this chapter, it can be inferred that molecular methods can be used in identifying resistance to different anti-TB drugs. Nevertheless, literature reports also suggest that diversity in the mutation may challenge the molecular diagnosis of resistance. Therefore, integrated genotypic and phenotypic monitoring is needed to understand the root cause of resistance in anti-TB drugs. Understanding different aspects of resistance mechanisms may help the researchers cater to better treatment options for DR-TB.

Table 6 Standardized regimen for patients with pre-XDR-TB or XDR-TB

S. No.	Regimen composition	Drug	Target	Activity	Important recommendation
1	Two core drugs	Linezolid	23S ribosomal RNA [104]	Bactericidal and Sterilising	Linezolid or bedaquiline could be given in place of Delamanid if: One of these drugs has been previously used and suffers with problem of resistance and severe toxicity
		Bedaquiline	ATP synthase [105]		Bedaquiline can potentially prolong QT
2a	One companion (one of the following)	Clofazimine (first choice)	Guanine bases of bacterial DNA [106]	Sterilising	This is recommended among MDR-TB patients who were never been treated with this drug before and neither respond to conventional 21–24 months MDR regimen. Potentially QT prolonging drug
		Cycloserine	Inhibit Alanin ligase and alanine racemose [107]	No bactericidal and sterilizing	To be prescribed to patients who were not treatment with this drug before, and when clofazimine was administered in a previous failing regimen (shorter 9–12 months RR/MDR-TB regimen)
2b	*One companion drug* (one of the following)	One carbapenem + amoxicillin /clavulanate	Synthesis of the bacterial cell wall	Bactericidal	*3 options*
			β-lactamase inhibitor		Meropenem
					Imipenam/cilastatin
					Ertapenam
		Delamanid	Cell wall synthesis and cell respiration [105]	Bactericidal and sterilizing	ECG monitoring needed
		PAS (Para-aminosalicyclic scid)	Hamper folate metabolism via competitive binding with dihydrofolate reductase [108]	Bacteriostatic	Carbapenams and delamanid are not used in low income countries
					Line Probe Assay to second line drugs showing possible susceptibility to Amikacin or moxifloxacin

(continued)

Table 6 (continued)

S. No.	Regimen composition	Drug	Target	Activity	Important recommendation
3	Three supporting drugs	One FQ (moxifloxacin)	Topoisomerase IV, DNA gyrase and [109]	Bactericidal and Sterilizing	Based on the previous treatment history of a patient
					For an instance: pre-XDR or XDR-TB patients who were not previously treated with a FQ
					QTc interval close monitoring can be achieved with bedaquiline and clofazimine
	One FQ One second line injectables		Inhibit protein synthesis by affecting the 30S ribosomal subunit [110]		If levofloxacin is used previously
					If Moxifloxacin is used peviously
		One FQ (high dose moxifloxacin)		Bactericidal and Sterilizing	Recommended in case pre-XDR or XDR-TB patient have not been previously treated for TB with a Second line injectables
		One FQ (high dose levofloxacin)	Enoyl-acyl carrier protein reductase [111]		If kanamycin or capreomycin have been used previously to treat TB
	High-dose isoniazid	One second line injectable (amikacin)		Bactericidal	Drug is discontinued from the regimen in case of resistance development to all the second line injectables
					Dose: 15–20 $mg \cdot kg^{-1}$ Isonazid is discontinued when: high level of resistance is confirmed in vitro
		High-dose isoniazid		Bactericidal	When the Line probe assay demonstrates double mutation in katG and inhA

Core Messages

- TB patients require better-scheduled antibiotics as well as supervision.
- Affordable, effective, and safe anti-TB medications may enhance treatment quality.
- Using current anti-TB medications as adjuvants may save substantial costs in drug research and development.
- To address latent TB infection, efforts may involve public health systems and advanced diagnostic tools.

References

1. Kresge N, Simoni RD, Hill RL (2004) Selman Waksman: the father of antibiotics. J Biol Chem 279(48):e7
2. Waksman SA, Lechevalier HA (1949) Neomycin, a new antibiotic active against streptomycin-resistant bacteria, including tuberculosis organisms. Science 109(2830):305–307
3. Society AT (1992) Control of tuberculosis in the United States. Am Rev Respir Dis 146:1623–1633
4. Grosset JH (1989) Present status of chemotherapy for tuberculosis. Rev Infect Dis 11(Suppl 2):S347–S352
5. Hopewell P (1994) The cure: organization and administration of therapy for tuberculosis. In: Tuberculosis: back to the future. Wiley, Chichester, pp 99–120
6. Iseman MD, Cohn DL, Sbarbaro JA (1993) Directly observed treatment of tuberculosis—we can't afford not to try it. Mass Med Soc
7. Iseman MD (1994) Evolution of drug-resistant tuberculosis: a tale of two species. Proc Natl Acad Sci 91(7):2428–2429
8. CfD C (1991) Nosocomial transmission of multidrug-resistant tuberculosis among HIV-infected persons–Florida and New York, 1988–1991. MMWR Morb Mortal Wkly Rep 40(34):585
9. Frieden TR, Sterling T, Pablos-Mendez A, Kilburn JO, Cauthen GM, Dooley SW (1993) The emergence of drug-resistant tuberculosis in New York City. N Engl J Med 328(8):521–526
10. Shah NS, Wright A, Bai G-H, Barrera L, Boulahbal F, Martín-Casabona N, Drobniewski F, Gilpin C, Havelková M, Lepe R: Worldwide emergence of extensively drug-resistant tuberculosis. Emerg Infect Dis 13(3):380
11. Verma H, Choudhary S, Singh PK, Kashyap A, Silakari O (2019) Decoding the signature of molecular mechanism involved in mutation associated resistance to 1,3-benzothiazin-4-ones (Btzs) based DprE1 inhibitors using BTZ043 as a reference drug. Mol Simul 45(18):1515–1523
12. Velayati AA, Farnia P, Masjedi MR (2013) The totally drug resistant tuberculosis (TDR-TB). Int J Clin Exp Med 6(4):307
13. Velayati AA, Masjedi MR, Farnia P, Tabarsi P, Ghanavi J, ZiaZarifi AH, Hoffner SE (2009) Emergence of new forms of totally drug-resistant tuberculosis bacilli: super extensively drug-resistant tuberculosis or totally drug-resistant strains in Iran. Chest 136(2):420–425

14. Gandhi NR, Nunn P, Dheda K, Schaaf HS, Zignol M, Van Soolingen D, Jensen P, Bayona J (2010) Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. Lancet 375(9728):1830–1843
15. Jain A, Mondal R (2008) Extensively drug-resistant tuberculosis: current challenges and threats. FEMS Immunol Med Microbiol 53(2):145–150
16. Annabel B, Anna D, Hannah M (2019) Global tuberculosis report 2019. World Health Organization, Geneva
17. Ahmad S, Mokaddas E (2014) Current status and future trends in the diagnosis and treatment of drug-susceptible and multidrug-resistant tuberculosis. J Infect Public Health 7(2):75–91
18. Hargreaves JR, Boccia D, Evans CA, Adato M, Petticrew M, Porter JD (2011) The social determinants of tuberculosis: from evidence to action. Am J Public Health 101(4):654–662
19. Smith T, Wolff KA, Nguyen L (2012) Molecular biology of drug resistance in *Mycobacterium tuberculosis*. In: Pathogenesis of *Mycobacterium tuberculosis* and its interaction with the host organism. Springer, pp 53–80
20. Sharma S, Kumar M, Sharma S, Nargotra A, Koul S, Khan IA (2010) Piperine as an inhibitor of Rv1258c, a putative multidrug efflux pump of *Mycobacterium tuberculosis*. J Antimicrob Chemother 65(8):1694–1701
21. Hugonnet J-E, Blanchard JS (2007) Irreversible inhibition of the *Mycobacterium tuberculosis* β-lactamase by clavulanate. Biochemistry 46(43):11998–12004
22. Meena S, Shivangi ML (2018) Interaction of *Mycobacterium tuberculosis* H37Rv with microfold cell leads to a New Era of infection in host. Ann Clin Lab Res 6(3):246
23. Nikaido H, Brennan P (1995) The envelope of mycobacteria. Annu Rev Biochem 64:29–63
24. Liu J, Rosenberg EY, Nikaido H (1995) Fluidity of the lipid domain of cell wall from *Mycobacterium chelonae*. Proc Natl Acad Sci 92(24):11254–11258
25. Vandal OH, Nathan CF, Ehrt S (2009) Acid resistance in *Mycobacterium tuberculosis*. J Bacteriol 191(15):4714–4721
26. Raynaud C, Papavinasasundaram K, Speight RA, Springer B, Sander P, Böttger EC, Colston MJ, Draper P (2002) The functions of OmpATb, a pore-forming protein of *Mycobacterium tuberculosis*. Mol Microbiol 46(1):191–201
27. Sequoia Ecosystem and Recreation Preserve Act of 1999 (1999) 106th Congress edn
28. Dye C, Williams BG (2010) The population dynamics and control of tuberculosis. Science 328(5980):856–861
29. Timmins GS, Deretic V (2006) Mechanisms of action of isoniazid. Mol Microbiol 62 (5):1220–1227
30. Aslan G, Tezcan S, Serin MS, Emekdas G (2008) Genotypic analysis of isoniazid and rifampin resistance in drug-resistant clinical *Mycobacterium tuberculosis* complex isolates in southern Turkey. Jpn J Infect Dis 61(4):255–260
31. Mokrousov I, Narvskaya O, Otten T, Limeschenko E, Steklova L, Vyshnevskiy B (2002) High prevalence of KatG Ser315Thr substitution among isoniazid-resistant *Mycobacterium tuberculosis* clinical isolates from northwestern Russia, 1996 to 2001. Antimicrob Agents Chemother 46(5):1417–1424
32. Zhang Y, Yew W (2015) Mechanisms of drug resistance in *Mycobacterium tuberculosis*: update 2015. Int J Tuberc Lung Dis 19(11):1276–1289
33. Lancini G (2014) In memory of Piero Sensi (1920–2013). J Antibiot 67(9):609–611
34. Herrera L, Jiménez S, Valverde A, García-Aranda MA, Sáez-Nieto JA (2003) Molecular analysis of rifampicin-resistant *Mycobacterium tuberculosis* isolated in Spain (1996–2001). Description of new mutations in the rpoB gene and review of the literature. Int J Antimicrobial Agents 21(5):403–408
35. Kumar S, Jena L (2014) Understanding rifampicin resistance in tuberculosis through a computational approach. Genomics Inform 12(4):276
36. Pandey B, Grover S, Tyagi C, Goyal S, Jamal S, Singh A, Kaur J, Grover A (2016) Molecular principles behind pyrazinamide resistance due to mutations in panD gene in *Mycobacterium tuberculosis*. Gene 581(1):31–42

37. Njire M, Tan Y, Mugweru J, Wang C, Guo J, Yew W, Tan S, Zhang T (2016) Pyrazinamide resistance in *Mycobacterium tuberculosis*: review and update. Adv Med Sci 61(1):63–71
38. Nguyen L (2016) Antibiotic resistance mechanisms in *M. tuberculosis*: an update. Arch Toxicol 90(7):1585–1604
39. Kanji A, Hasan R, Hasan Z (2019) Efflux pump as alternate mechanism for drug resistance in *Mycobacterium tuberculosis*. Indian J Tuberc 66(1):20–25
40. Hao P, Shi-Liang Z, Ju L, Ya-Xin D, Biao H, Xu W, Min-Tao H, Shou-Gang K, Ke W (2011) The role of ABC efflux pump, Rv1456c-Rv1457c-Rv1458c, from *Mycobacterium tuberculosis* clinical isolates in China. Folia Microbiol 56(6):549–553
41. Danilchanka O, Mailaender C, Niederweis M (2008) Identification of a novel multidrug efflux pump of *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 52(7):2503–2511
42. Zhang Y, Zhang J, Cui P, Zhang Y, Zhang W (2017) Identification of novel efflux proteins Rv0191, Rv3756c, Rv3008, and Rv1667c involved in pyrazinamide resistance in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 61(8)
43. Duan W, Li X, Ge Y, Yu Z, Li P, Li J, Qin L, Xie J (2019) *Mycobacterium tuberculosis* Rv1473 is a novel macrolides ABC efflux pump regulated by WhiB7. Future Microbiol 14 (1):47–59
44. Choudhuri BS, Bhakta S, Barik R, Basu J, Kundu M, Chakrabarti P (2002) Overexpression and functional characterization of an ABC (ATP-binding cassette) transporter encoded by the genes drrA and drrB of *Mycobacterium tuberculosis*. Biochem J 367(1):279–285
45. Li P, Gu Y, Li J, Xie L, Li X, Xie J (2017) *Mycobacterium tuberculosis* major facilitator superfamily transporters. J Membr Biol 250(6):573–585
46. Gupta AK, Reddy VP, Lavania M, Chauhan D, Venkatesan K, Sharma V, Tyagi A, Katoch V (2010) jefA (Rv2459), a drug efflux gene in *Mycobacterium tuberculosis* confers resistance to isoniazid & ethambutol. Indian J Med Res 132(2):176–188
47. Gupta AK, Katoch VM, Chauhan DS, Sharma R, Singh M, Venkatesan K, Sharma VD (2010) Microarray analysis of efflux pump genes in multidrug-resistant *Mycobacterium tuberculosis* during stress induced by common anti-tuberculous drugs. Microb Drug Resist 16(1):21–28
48. Silva PE, Bigi F, de la Paz Santangelo M, Romano MI, Martín C, Cataldi A, Aínsa JA (2001) Characterization of P55, a multidrug efflux pump in *Mycobacterium bovis* and *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 45(3):800–804
49. Ramón-García S, Martín C, Thompson CJ, Aínsa JA (2009) Role of the *Mycobacterium tuberculosis* P55 efflux pump in intrinsic drug resistance, oxidative stress responses, and growth. Antimicrob Agents Chemother 53(9):3675–3682
50. Cloete R, Kapp E, Joubert J, Christoffels A, Malan SF (2018) Molecular modelling and simulation studies of the *Mycobacterium tuberculosis* multidrug efflux pump protein Rv1258c. PLoS ONE 13(11):e0207605
51. Katoch VM (2019) Molecular basis of drug resistance in Mycobacteria. In: Pathogenicity and drug resistance of human pathogens. Springer, pp 3–31
52. Doran JL, Pang Y, Mdluli KE, Moran AJ, Victor TC, Stokes RW, Mahenthiralingam E, Kreiswirth BN, Butt JL, Baron GS (1997) *Mycobacterium tuberculosis* efpA encodes an efflux protein of the QacA transporter family. Clin Diagn Lab Immunol 4(1):23–32
53. Li X-Z, Zhang L, Nikaido H (2004) Efflux pump-mediated intrinsic drug resistance in *Mycobacterium smegmatis*. Antimicrob Agents Chemother 48(7):2415–2423
54. Batt SM, Jabeen T, Bhowruth V, Quill L, Lund PA, Eggeling L, Alderwick LJ, Fütterer K, Besra GS (2012) Structural basis of inhibition of *Mycobacterium tuberculosis* DprE1 by benzothiazinone inhibitors. Proc Natl Acad Sci 109(28):11354–11359
55. Malliaras K, Zhang Y, Seinfeld J, Galang G, Tseliou E, Cheng K, Sun B, Aminzadeh M, Marbán E (2013) Cardiomyocyte proliferation and progenitor cell recruitment underlie therapeutic regeneration after myocardial infarction in the adult mouse heart. EMBO Mol Med 5(2):191–209

56. He L, Wang X, Cui P, Jin J, Chen J, Zhang W, Zhang Y (2015) ubiA (Rv3806c) encoding DPPR synthase involved in cell wall synthesis is associated with ethambutol resistance in *Mycobacterium tuberculosis*. Tuberculosis 95(2):149–154
57. Ramón-García S, Martín C, De Rossi E, Aínsa JA (2007) Contribution of the Rv2333c efflux pump (the Stp protein) from *Mycobacterium tuberculosis* to intrinsic antibiotic resistance in *Mycobacterium bovis* BCG. J Antimicrob Chemother 59(3):544–547
58. Rodrigues L, Cravo P, Viveiros M (2020) Efflux pump inhibitors as a promising adjunct therapy against drug resistant tuberculosis: a new strategy to revisit mycobacterial targets and repurpose old drugs. Exp Rev Anti-Infect Ther, pp 1–17
59. Singh R, Dwivedi SP, Gaharwar US, Meena R, Rajamani P, Prasad T (2020) Recent updates on drug resistance in *Mycobacterium tuberculosis*. J Appl Microbiol 128(6):1547–1567
60. Te Brake LH, van den Heuvel JJ, Buaben AO, van Crevel R, Bilos A, Russel FG, Aarnoutse RE, Koenderink JB (2016) Moxifloxacin is a potent in vitro inhibitor of OCT-and MATE-mediated transport of metformin and ethambutol. Antimicrob Agents Chemother 60 (12):7105–7114
61. Pasca MR, Guglierame P, De Rossi E, Zara F, Riccardi G (2005) mmpL7 gene of *Mycobacterium tuberculosis* is responsible for isoniazid efflux in *Mycobacterium smegmatis*. Antimicrob Agents Chemother 49(11):4775–4777
62. Briffotaux J, Huang W, Wang X, Gicquel B (2017) MmpS5/MmpL5 as an efflux pump in Mycobacterium species. Tuberculosis 107:13–19
63. Poulsen BE, Deber CM (2012) Drug efflux by a small multidrug resistance protein is inhibited by a transmembrane peptide. Antimicrob Agents Chemother 56(7):3911–3916
64. De Rossi E, Branzoni M, Cantoni R, Milano A, Riccardi G, Ciferri O (1998) mmr, a *Mycobacterium tuberculosis* gene conferring resistance to small cationic dyes and inhibitors. J Bacteriol 180(22):6068–6071
65. Rodrigues L, Villellas C, Bailo R, Viveiros M, Aínsa JA (2013) Role of the Mmr efflux pump in drug resistance in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 57 (2):751–757
66. Hegde SS, Vetting MW, Roderick SL, Mitchenall LA, Maxwell A, Takiff HE, Blanchard JS (2005) A fluoroquinolone resistance protein from *Mycobacterium tuberculosis* that mimics DNA. Science 308(5727):1480–1483
67. Hameed PS, Raichurkar A, Madhavapeddi P, Menasinakai S, Sharma S, Kaur P, Nandishaiah R, Panduga V, Reddy J, Sambandamurthy VK (2014) Benzimidazoles: novel mycobacterial gyrase inhibitors from scaffold morphing. ACS Med Chem Lett 5(7):820–825
68. Flores AR, Parsons LM, Pavelka MS Jr (2005) Genetic analysis of the β-lactamases of *Mycobacterium tuberculosis* and *Mycobacterium smegmatis* and susceptibility to β-lactam antibiotics. Microbiology 151(2):521–532
69. Page MG (2012) Beta-lactam antibiotics. In: Antibiotic discovery and development. Springer, pp 79–117
70. Kashyap A, Singh PK, Silakari O (2018) Mechanistic investigation of resistance via drug-inactivating enzymes in *Mycobacterium tuberculosis*. Drug Metab Rev 50(4):448–465
71. Tremblay LW, Xu H, Blanchard JS: Structures of the Michaelis complex (1.2 Å) and the covalent acyl intermediate (2.0 Å) of cefamandole bound in the active sites of the *Mycobacterium tuberculosis* β-lactamase K73A and E166A mutants. Biochemistry 49 (45):9685–9687
72. Upton A, Mushtaq A, Victor T, Sampson S, Sandy J, Smith DM, Van Helden P, Sim E (2001) Arylamine N-acetyltransferase of *Mycobacterium tuberculosis* is a polymorphic enzyme and a site of isoniazid metabolism. Mol Microbiol 42(2):309–317
73. Payton M, Auty R, Delgoda R, Everett M, Sim E (1999) Cloning and characterization of arylamine N-acetyltransferase genes from *Mycobacterium smegmatis* and *Mycobacterium tuberculosis*: increased expression results in isoniazid resistance. J Bacteriol 181(4):1343–1347

74. Payton M, Gifford C, Schartau P, Hagemeier C, Mushtaq A, Lucas S, Pinter K, Sim E (2001) Evidence towards the role of arylamine N-acetyltransferase in *Mycobacterium smegmatis* and development of a specific antiserum against the homologous enzyme of *Mycobacterium tuberculosis*. Microbiology 147(12):3295–3302
75. Sikora AL, Frankel BA, Blanchard JS (2008) Kinetic and chemical mechanism of arylamine N-acetyltransferase from *Mycobacterium tuberculosis*. Biochemistry 47(40):10781–10789
76. Chen W, Biswas T, Porter VR, Tsodikov OV, Garneau-Tsodikova S (2011) Unusual regioversatility of acetyltransferase Eis, a cause of drug resistance in XDR-TB. Proc Natl Acad Sci 108(24):9804–9808
77. Gao C, Peng C, Shi Y, You X, Ran K, Xiong L, Ye T-H, Zhang L, Wang N, Zhu Y (2016) Benzothiazinethione is a potent preclinical candidate for the treatment of drug-resistant tuberculosis. Sci Rep 6(1):1–9
78. Warrier T, Martinez-Hoyos M, Marin-Amieva M, Colmenarejo G, Porras-De Francisco E, Alvarez-Pedraglio AI, Fraile-Gabaldon MT, Torres-Gomez PA, Lopez-Quezada L, Gold B (2015) Identification of novel antimycobacterial compounds by screening a pharmaceutical small-molecule library against nonreplicating *Mycobacterium tuberculosis*. ACS Infect Dis 1 (12):580–585
79. Warrier T, Kapilashrami K, Argyrou A, Ioerger TR, Little D, Murphy KC, Nandakumar M, Park S, Gold B, Mi J (2016) N-methylation of a bactericidal compound as a resistance mechanism in *Mycobacterium tuberculosis*. Proc Natl Acad Sci 113(31):E4523–E4530
80. Wermuth CG (2003) Analog design. In: Burger's medicinal chemistry and drug discovery, pp 167–180
81. Vosátka R, Krátký M, Švarcová M, Janoušek J, Stolaříková J, Madacki J, Huszár S, Mikušová K, Korduláková J, Trejtnar F (2018) New lipophilic isoniazid derivatives and their 1,3,4-oxadiazole analogues: synthesis, antimycobacterial activity and investigation of their mechanism of action. Eur J Med Chem 151:824–835
82. Bhoi MN, Borad MA, Jethava DJ, Acharya PT, Pithawala EA, Patel CN, Pandya HA, Patel HD (2019) Synthesis, biological evaluation and computational study of novel isoniazid containing 4H-Pyrimido [2,1-b] benzothiazoles derivatives. Eur J Med Chem 177:12–31
83. De P, Koumba Yoya G, Constant P, Bedos-Belval F, Duran H, Saffon N, Daffé M, Baltas M (2011) Design, synthesis, and biological evaluation of new cinnamic derivatives as antituberculosis agents. J Med Chem 54(5):1449–1461
84. Kumar D, Khare G, Kidwai S, Tyagi AK, Singh R, Rawat DS (2014) Synthesis of novel 1,2,3-triazole derivatives of isoniazid and their in vitro and in vivo antimycobacterial activity evaluation. Eur J Med Chem 81:301–313
85. Tiwari R, Miller PA, Chiarelli LR, Mori G, Šarkan M, Centárová I, Cho S, Mikušová K, Franzblau SG, Oliver AG (2016) Design, syntheses, and anti-TB activity of 1,3-benzothiazinone azide and click chemistry products inspired by BTZ043. ACS Med Chem Lett 7(3):266–270
86. Hearn MJ, Cynamon MH (2004) Design and synthesis of antituberculars: preparation and evaluation against *Mycobacterium tuberculosis* of an isoniazid Schiff base. J Antimicrob Chemother 53(2):185–191
87. Shingnapurkar D, Dandawate P, Anson CE, Powell AK, Afrasiabi Z, Sinn E, Pandit S, Swamy KV, Franzblau S, Padhye S (2012) Synthesis and characterization of pyruvate–isoniazid analogs and their copper complexes as potential ICL inhibitors. Bioorg Med Chem Lett 22(9):3172–3176
88. Dandawate P, Vemuri K, Swamy KV, Khan EM, Sritharan M, Padhye S (2014) Synthesis, characterization, molecular docking and anti-tubercular activity of plumbagin-isoniazid analog and its β-cyclodextrin conjugate. Bioorg Med Chem Lett 24(21):5070–5075
89. Brooke EW, Davies SG, Mulvaney AW, Okada M, Pompeo F, Sim E, Vickers RJ, Westwood IM (2003) Synthesis and in vitro evaluation of novel small molecule inhibitors of bacterial arylamine N-acetyltransferases (NATs). Bioorg Med Chem Lett 13(15):2527–2530

90. Willby MJ, Green KD, Gajadeera CS, Hou C, Tsodikov OV, Posey JE, Garneau-Tsodikova S (2016) Potent inhibitors of acetyltransferase Eis overcome kanamycin resistance in *Mycobacterium tuberculosis*. ACS Chem Biol 11(6):1639–1646
91. Garzan A, Willby MJ, Ngo HX, Gajadeera CS, Green KD, Holbrook SY, Hou C, Posey JE, Tsodikov OV, Garneau-Tsodikova S (2017) Combating enhanced intracellular survival (Eis)-mediated kanamycin resistance of *Mycobacterium tuberculosis* by novel pyrrolo [1,5-a] pyrazine-based Eis inhibitors. ACS Infect Dis 3(4):302–309
92. Garzan A, Willby MJ, Green KD, Gajadeera CS, Hou C, Tsodikov OV, Posey JE, Garneau-Tsodikova S (2016) Sulfonamide-based inhibitors of aminoglycoside acetyltransferase Eis abolish resistance to kanamycin in *Mycobacterium tuberculosis*. J Med Chem 59 (23):10619–10628
93. Ngo HX, Green KD, Gajadeera CS, Willby MJ, Holbrook SY, Hou C, Garzan A, Mayhoub AS, Posey JE, Tsodikov OV (2018) Potent 1,2,4-triazino [5,6b] indole-3-thioether inhibitors of the kanamycin resistance enzyme Eis from *Mycobacterium tuberculosis*. ACS Infect Dis 4(6):1030–1040
94. Garzan A, Willby MJ, Green KD, Tsodikov OV, Posey JE, Garneau-Tsodikova S (2016) Discovery and optimization of two Eis inhibitor families as kanamycin adjuvants against drug-resistant *M. tuberculosis*. ACS Med Chem Lett 7(12):1219–1221
95. Kurz SG, Hazra S, Bethel CR, Romagnoli C, Caselli E, Prati F, Blanchard JS, Bonomo RA (2015) Inhibiting the β-lactamase of *Mycobacterium tuberculosis* (*M. tb*) with novel boronic acid transition-state inhibitors (BATSIs). ACS Infect Dis 1(6):234–242
96. Hazra S, Kurz SG, Wolff K, Nguyen L, Bonomo RA, Blanchard JS (2015) Kinetic and structural characterization of the interaction of 6-methylidene penem 2 with the β-lactamase from *Mycobacterium tuberculosis*. Biochemistry 54(36):5657–5664
97. Iannazzo L, Soroka D, Triboulet S, Fonvielle M, Compain F, Dubée V, Mainardi J-L, Hugonnet J-E, Braud E, Arthur M (2016) Routes of synthesis of carbapenems for optimizing both the inactivation of l,d-transpeptidase LdtMt1 of *Mycobacterium tuberculosis* and the stability toward hydrolysis by β-lactamase BlaC. J Med Chem 59(7):3427–3438
98. Xu H, Hazra S, Blanchard JS (2012) NXL104 irreversibly inhibits the β-lactamase from *Mycobacterium tuberculosis*. Biochemistry 51(22):4551–4557
99. Caminero JA, Piubello A, Scardigli A, Migliori GB (2017) Proposal for a standardised treatment regimen to manage pre-and extensively drug-resistant tuberculosis cases. Eur Respir Soc
100. Tiberi S, Scardigli A, Centis R, D'Ambrosio L, Munoz-Torrico M, Salazar-Lezama MA, Spanevello A, Visca D, Zumla A, Migliori GB (2017) Classifying new anti-tuberculosis drugs: rationale and future perspectives. Int J Infect Dis 56:181–184
101. Falzon D, Schünemann HJ, Harausz E, González-Angulo L, Lienhardt C, Jaramillo E, Weyer K (2017) World Health Organization treatment guidelines for drug-resistant tuberculosis, 2016 update. Eur Respir J 49(3)
102. World Health Organization (2016) WHO treatment guidelines for drug-resistant tuberculosis. World Health Organization
103. Caminero JA, Scardigli A (2015) Classification of antituberculosis drugs: a new proposal based on the most recent evidence. Eur Respir Soc
104. Abbate E, Vescovo M, Natiello M, Cufré M, García A, Ambroggi M, Poggi S, Símboli N, Ritacco V (2007) Tuberculosis extensamente resistente (XDR-TB) en Argentina: aspectos destacables epidemiológicos, bacteriológicos, terapéuticos y evolutivo
105. Amaral L, Boeree MJ, Gillespie SH, Udwadia ZF, Van Soolingen D (2010) Thioridazine cures extensively drug-resistant tuberculosis (XDR-TB) and the need for global trials is now! Int J Antimicrob Agents 35(6):524–526
106. Gopal M, Padayatchi N, Metcalfe J, O'Donnell M (2013) Systematic review of clofazimine for the treatment of drug-resistant tuberculosis. Int J Tuberc Lung Dis 17(8):1001–1007
107. Chhabra N, Aseri M, Dixit R, Gaur S (2012) Pharmacotherapy for multidrug resistant tuberculosis. J Pharmacol Pharmacother 3(2):98

108. Zhang T, Jiang G, Shu'an Wen FH, Wang F, Huang H, Pang Y (2019) Para-aminosalicylic acid increases the susceptibility to isoniazid in clinical isolates of Mycobacterium tuberculosis. Infect Drug Resist 12:825
109. Fan Y-L, Wu J-B, Cheng X-W, Zhang F-Z, Feng L-S (2018) Fluoroquinolone derivatives and their anti-tubercular activities. Europ J Med Chem 146:554–563
110. Kumar B, Sharma D, Sharma P, Katoch VM, Venkatesan K, Bisht D (2013) Proteomic analysis of Mycobacterium tuberculosis isolates resistant to kanamycin and amikacin. J Proteomics 94:68–77
111. Rozwarski DA, Grant GA, Barton DH, Jacobs WR, Sacchettini JC (1998) Modification of the NADH of the isoniazid target (InhA) from Mycobacterium tuberculosis. Science 279 (5347):98–102

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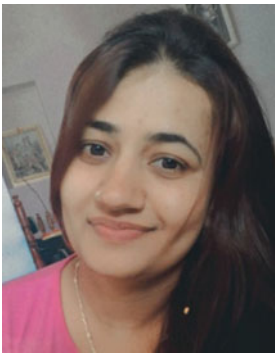

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Personalized Tuberculosis Care for Drug-Resistant Tuberculosis

20

Tjip S. van der Werf and Yvette A. de Reus

...I am launching a new Precision Medicine Initiative... to give us all access to the personalized information we need to keep ourselves and our families healthier.

Barack Obama

Summary

Drug-resistant tuberculosis (DR-TB) includes mono-resistant forms of TB and multidrug-resistant tuberculosis (MDR-TB), defined by loss of susceptibility to Rifampicin and Isoniazid. MDR-TB is subdivided along a gradient of further loss of susceptibility, with extensively drug-resistant tuberculosis (XDR-TB) characterized by resistance to any fluoroquinolones and Linezolid or Bedaquiline. Even XDR-TB is far from homogeneous, and neither are patient groups affected by these different forms of DR-TB, with co-infections and comorbidities, differences in genetic background, disease severity, nutritional status, gender, and body composition. Drug exposure relative to minimal inhibitory concentrations for each regimen drug, including core- and companion drugs, determines the outcome. Inter- and intra-individual drug exposure are highly variable; therapeutic drug monitoring (TDM) by measuring drug exposure in multiple blood samples following drug administration is helpful in fine-tuning treatment. Apart from TB drugs, patients may benefit from host-directed

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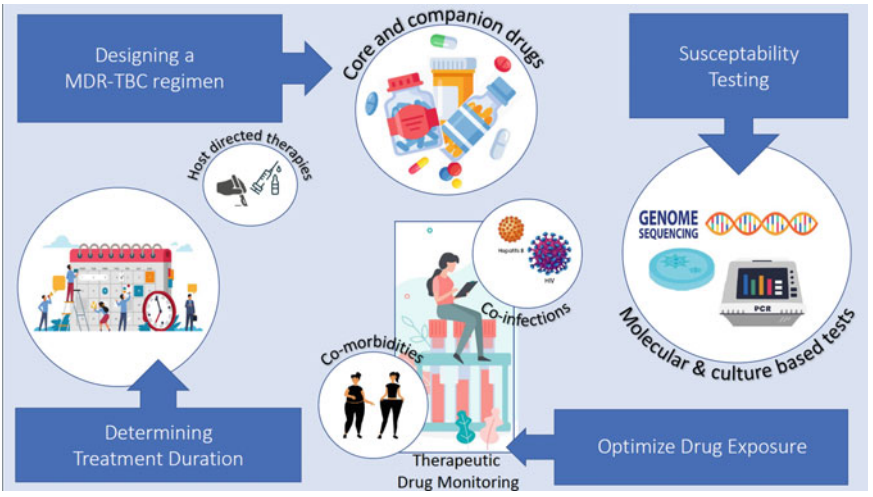
therapies, including therapeutic vaccinations and surgical interventions. TDM is still under development, but appropriate technologies have been developed to apply TDM even in low-resource settings.

Graphical Abstract



The basic components of diagnosing and treating multidrug-resistant tuberculosis (MDR-TB)

Keywords

Individualized treatment · Minimal inhibitory concentration · Multi-drug resistant · Pharmacodynamic modeling · Pharmacokinetic · Precision medicine · Therapeutic drug monitoring · Tuberculosis

1 Introduction

The standard treatment approach for drug-susceptible tuberculosis (DS-TB), as well as drug-resistant tuberculosis (DR-TB), has been extensively addressed. In this chapter, the focus is on the wide range of phenotypes that have one feature in common; drug resistance. Earlier clinical trials have clearly established the strength of standardized treatment, based on Rifampicin and Isoniazid for six months, with the addition of Pyrazinamide and Ethambutol for the first two months for DS-TB [1]. Loss of susceptibility of *Mycobacterium tuberculosis* (*M. tb*) against any of the companion drugs, and even Isoniazid alone, did not seem to alter the response to

treatment [2]. Only by adding Rifampicin, in combination with two months of Pyrazinamide, high cure rates were achieved with a treatment duration of six months [3]. It has long been thought that loss of susceptibility would be accompanied by fitness loss of the organism, which would, in turn, reduce transmission of these less susceptible strains. The focus of TB control was, therefore, on early detection of smear-positive (pulmonary) TB and improved treatment outcome by improving adherence to treatment, primarily by promoting witnessed drug ingestion [4]. Fitness loss has, however, largely been overestimated, and also compensatory fitness gain among DR-TB strains has been identified, which in turn explained how drug resistance could emerge and be transmitted [5, 6]. It has become clear that even Isoniazid mono-resistance is associated with less favourable outcome than TB caused by fully susceptible *M. tb* strains [5].

When Rifampicin resistance emerged unprecedentedly in the 1990s, with the first report from New York City [6, 7], awareness of a real global threat imposed by DR-TB emerged [8]. The problem with drug resistance is that for each pattern of susceptibility to anti-TB drugs, a unique phenotype is established: DR-TB is by definition heterogeneous in nature. Rifampicin-resistant TB (RR-TB) and TB resistant to both Isoniazid and Rifampicin, referred to as multidrug-resistant TB (MDR-TB), may seem homogeneous, but obviously, treatment outcome entirely depends on the completion of a regimen to which the causative microorganism is still susceptible. In general, the successful outcome has been reported at around 50% [9–11]. Clearly, with increasing numbers of drugs to which an isolate is no longer susceptible, the chances to establish an effective drug combination diminish [12]. This happens with XDR-TB, defined as TB caused by an *M. tb* resistant to not only Rifampicin and Isoniazid–making it MDR-TB–but also resistant to one of the fluoroquinolones, as well as–at least–one other group A drug (Bedaquiline or Linezolid). Group A drugs are ranked as the most potent second-line drugs in the treatment of DR-TB (Table 1).[1] XDR-TB carries an even worse prognosis, with the unsuccessful outcome resulting from toxicity of second-line drugs with inherent poor adherence [13] and a loss of core drugs with a high bactericidal and sterilizing capacity [12, 14, 15]. A successful treatment schedule consists of a combination of TB drugs during the intensive phase and a combination that follows during the continuation phase. During the intensive phase, bactericidal activity is primordial to bring the bacterial load down as quickly as possible. In the continuation phase, the selection of drugs is based on its potential to sterilize persister phenotype organisms to eradicate the residual bacterial load of *M. tb*, including difficult-to-reach sites like pulmonary cavities [16, 17] and meningeal [18] and cerebral lesions. Ideally, for each phenotype of MDR-TB, a randomized comparison would allow for evidence-based treatment, whereas individualized treatment is at best guided by expert opinion; see Fig. 1. The tremendous heterogeneity of drug susceptibility makes it extremely challenging, if not virtually impossible, to design randomized trials, to address all the different questions for each subset of patients with

[1] https://www.who.int/news/item/27-01-2021-who-announces-updated-definitions-of-extensively-drug-resistant-tuberculosis.

Table 1 Grouping of medicines recommended for use in longer MDR-TB regimens according to the WHO consolidated guidelines on drug-resistant tuberculosis treatment intended to guide the design of individualized, longer MDR-TB regimens

Group: step	Medicine	Abbreviation
Group A: include all three medicines	Levofloxacin or moxifloxacin	Lfx Mfx
	Bedaquiline	Bdq
	Linezolid	Lzd
Group B: add one or both medicines	Clofazimine	Cfz
	Cycloserine *OR* terizidone	Cs Trd
Group C: add to complete the regimen and when medicines from Groups A and B cannot be used	Ethambutol	E
	Delamanid	Dlm
	Pyrazinamide	Z
	Imipenem–cilastatin or meropenem	Ipm–Cln Mpm
	Amikacin or streptomycin	Am (S)
	Ethionamide or prothionamide	Eto Pto
	p-aminosalicylic acid	PAS

MDR-TB. With the high success rates of individualized treatment in some affluent parts of the world [11, 19–21] and a small margin of non-inferiority, such trials would require large sample sizes for each phenotype of drug resistance. Most drugs in current use are repurposed antimicrobials. There is a paucity of new anti-TB drugs; only two products–Delamanid and Bedaquiline–have reached the market in the last decades, and only a few drugs are expected to be registered in the next few years [22]. Only one treatment schedule with repurposed drugs has been tested in three different settings without a parallel control arm [23–25]. Only one randomized study was conducted with a standard care treatment arm as a comparator [26]. Only one study evaluating a treatment schedule including one repurposed and two novel agents was published, but this study lacked a parallel control group for comparison [27]. Therefore, during the last three decades, the evidence for MDR-TB treatment has been predominantly based on observational, retrospective data [11, 20]. The recommendations following these publications typically provide basic rules, without detailed treatment instructions to be followed [28, 29]. Here, we explain further why individualized treatment is inevitable and necessary for treating MDR-TB, in its different forms, in different patient populations [30–32]. Individualized treatment is widely accepted and appreciated, at least in settings where advanced technologies like molecular testing for mutations in *M. tb* isolates predicting susceptibility or resistance and dosing guidance based on measuring drug exposure are available.

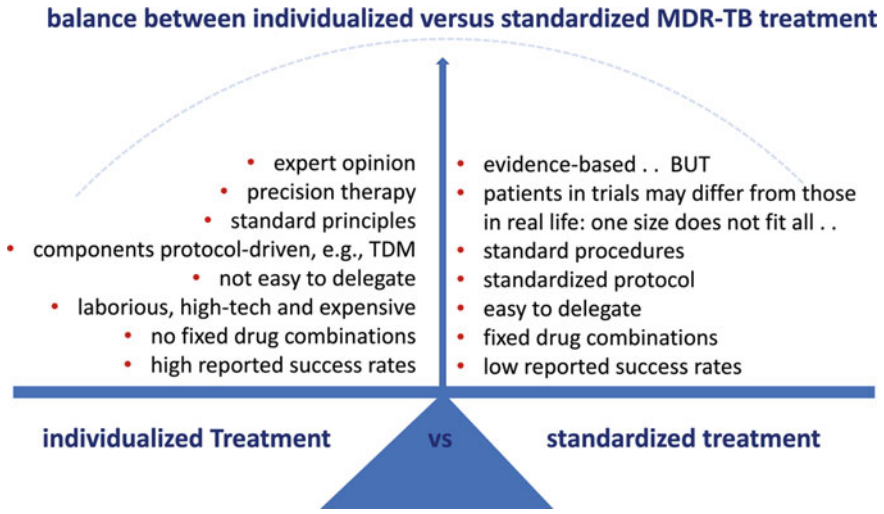


Fig. 1 A balanced discussion of standardized and individualized treatment for MDR-TB

In this chapter, we first discuss the general rules for the treatment of MDR-TB. Next, we focus on phenotypic and molecular tests to select drugs to be used as core drugs and additional drugs as companion agents, both during an intensive phase and during continuation treatment. Then, we discuss the evidence and the logic of therapeutic drug monitoring (TDM), i.e., the science of drug exposure measurement, followed by adjustments in drug dosing to optimize treatment, considering the target drug exposure–related to the minimal inhibitory drug concentration for a given drug, for a particular *M. tb* isolate [33]. Next, we discuss treatment of MDR-TB in patients with co-infections and comorbid conditions; we discuss host-directed therapies; the potential of immunotherapy, using therapeutic vaccinations; the role of surgery; and finally, we provide some general ideas to further advance the field, with a summary and conclusions.

2 Treating Multidrug-Resistant Tuberculosis: Basic Rules of Engagement and Designing a Regimen

The basic rules to design an appropriate regimen for MDR-TB are the following (Fig. 2):

- inclusion of highly potent drugs (core drugs) that
  - rapidly kill and reduce the bacterial burden; and
  - sterilize slowly replicating, persister organisms to prevent relapse;

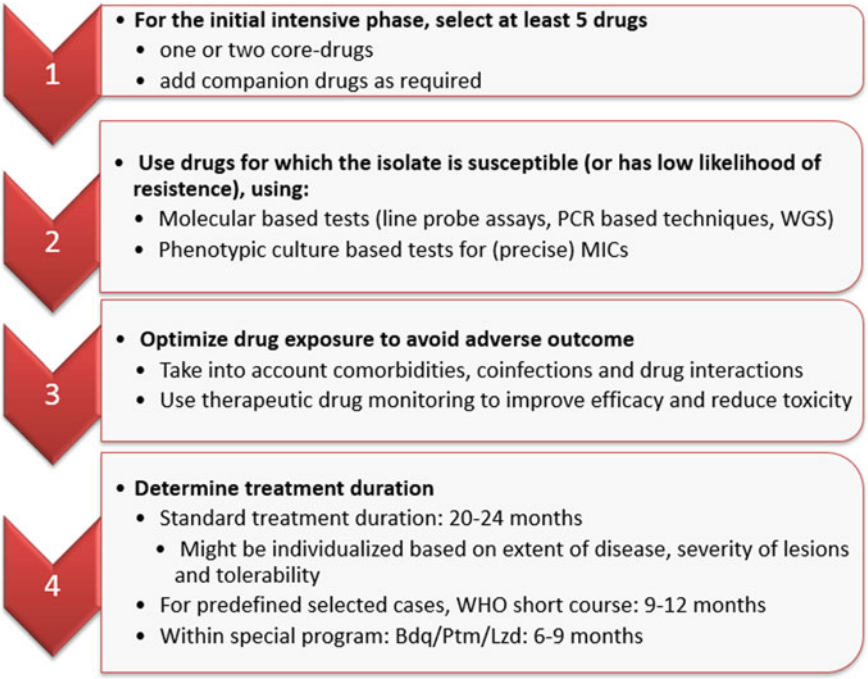


Fig. 2 Strategy on how to design a regimen for MDR-TB

- adding companion drugs to prevent sub-populations of resistant mutants from repopulating;
- drugs to be selected based on susceptibility testing;
- dosing should be adequate, and as drug exposure (i.e., pharmacokinetics) is highly variable, dosing should be guided by measuring drug exposure to improve effectiveness and to reduce the chance of toxicity; and
- treatment duration should be adequate to sterilize lesions, and no longer than necessary, to secure adherence and avoid unnecessary toxicity.

2.1 Individual Drugs

2.1.1 Core Drugs

Some of the TB drugs have high bactericidal activity, which is very important at the onset of treatment to quickly reduce the bacterial burden, and also sterilizing features that are extremely important to attack the sub-population of organisms with low metabolic activity and slow replication rate. This phenotype is referred to as the persister population [34]. Drugs with excellent sterilizing properties reduce the persister organisms and prevent relapse. Core TB drugs have excellent bactericidal

and sterilizing properties. Besides core drugs, several companion drugs are needed to suppress mutant organisms in the bacterial population. For these resistant mutants, it is important to prevent single drug treatment, as this inevitably results in treatment failure [35]. In MDR-TB, the critically important core drug Rifampicin is lost by definition; therefore, a treatment schedule for MDR-TB needs to have at least one, and preferably two, alternative core drugs (see Table 1, on how to build a regimen). Fluoroquinolones are critically important in managing MDR-TB [36]; loss of susceptibility results in a severely reduced chance of favorable outcome, at least with a combination of repurposed drugs [11, 37]. Although an entirely novel schedule with Bedaquiline, Pretomanid, and Linezolid provided promising results, resistance is luring even for XDR-TB [27]. For MDR-TB treatment, the second-line injectables (notably, the aminoglycosides Amikacin, Kanamycin, and the amino-peptide Capreomycin) have long been considered core agents [28]. However, it has been challenged by the analysis of a large observational database, where Kanamycin was associated with impaired outcomes [11]. Fourth-generation fluoroquinolones and the novel agent Bedaquiline may be considered core drugs [38], but also Linezolid is a highly effective bactericidal and sterilizing agent [39, 40].

Bedaquiline has indeed become a cornerstone of treatment for MDR-TB [41–45]. From different parts of the world, Bedaquiline resistance has, however, been reported [46, 47], sometimes in combination with the emergence of resistance to other TB drugs [48]. Bedaquiline is lipophilic, with a long secondary half-life; initial loading is necessary to establish adequate drug exposure in the early phase of treatment [37].

2.1.2 Companion Drugs

High-dose Isoniazid has potential value as a bactericidal agent to quickly reduce the bacterial load in the initial intensive phase of treatment. This may be especially helpful if MDR-TB strains are borderline susceptible for Isoniazid [49], e.g., in most *inh*A mutations, and also in many of the *kat*G mutations–except for mutations in codon 315 [41]. Clofazimine has been identified as an important companion drug with sterilizing capacity [42]. One study showed earlier sputum culture conversion than the control arm, while shorter treatment with clofazimine resulted in an equally successful outcome rate compared to standard treatment for MDR-TB [43]. Clofazimine is highly lipophilic; it binds to fat tissue and causes orange skin discoloration. In many areas in Asia, fair skin color is preferred, and the transient effect on skin color has a stigmatizing effect, with subsequently potentially impaired adherence. We have used this companion drug for a long time now and have not observed major problems in its use, even among patients from Asian descent.

2.2 Susceptibility Testing

The selection of agents in the (individualized) treatment regimen should preferentially be guided by the susceptibility of the organism and tolerability of the patient. Susceptibility testing using in vitro culture and susceptibility testing typically requires several weeks; ideally, not only susceptibility should be assessed,

defined as a minimal inhibitory concentration (MIC) below the breakpoint, but rather, a precise MIC, to enable optimal dosing, considering that the effect of any antimicrobial agent is predicted by drug exposure relative to the susceptibility of the organism [44]. Breakpoints are drug concentrations for which the vast majority of wild-type *M. tb* isolates is still susceptible. There are different breakpoints issued by different international organizations for Clinical Microbiology, the European community on antimicrobial susceptibility testing (EUCAST) being the most commonly followed by clinical microbiologists [45]. Breakpoints for anti-TB drugs issued by EUCAST have been criticized based on in vitro experiments using a so-called hollow fiber model for infections; these models imitate the fluctuating drug concentrations over time as they typically occur in patients following ingestion of anti-TB medication [50–52]. An inherent limitation with culture-based susceptibility testing results is the loss of detection of important drug-resistant subpopulations [53]. The bacterial population–especially in patients with advanced disease–is large; within this large bacterial load, naturally occurring mutants result in a subset of organisms resistant to at least one of the drugs in the treatment regimen [54]; this phenomenon has also been referred to as hetero-resistance [55].

2.3 Adequate Dosing

Some of the drugs in current use against *M. tb* are dosed in the low range of the therapeutic window. An important example is Rifampicin, where costs were an important concern when it was first introduced in the clinic in the early 1970s [2, 56], and its dosing was set at ten mg/kg body weight [57]. Later studies showed that Rifampicin was much more effective in eradicating and sterilizing lesions when administered at 30 mg/kg in a murine infection model [58], a dose that appeared to be well tolerated in humans with TB [59].

Low drug exposure results in the risk that by chance, 5% of the patient population exposed to standard dosing has inadequate drug exposure, allowing for naturally occurring mutant *M. tb* with a replication advantage under antimicrobial pressure that suppresses susceptible organisms, facilitating drug-resistant mutants to expand and repopulate lesions [60, 61]. Under persisting antimicrobial pressure, large numbers of drug-resistant organisms thrive and may, in turn, be transmitted in the population [62]. Adequate dosing to suppress borderline susceptible organisms is critically important to improve and optimize treatment outcomes [63]. Adequate dosing of multiple drugs is therefore primordial in designing a treatment schedule.

For most second-line drugs, drug exposure probably correlates better with the area under the concentration–time curve, $AUC_{0-24\ h}$, than the peak plasma concentration, or C_{max}. $AUC_{0-24\ h}$ is the computed drug exposure measured by drug blood concentrations following the first 24 h after drug ingestion; see Fig. 3. This pharmacokinetic (PK) parameter is divided by the susceptibility of the *M. tb* isolate–the MIC–reflecting the pharmacodynamic (PD) parameter, to arrive at the PK/PD equation: AUC/MIC, for each drug in any given treatment schedule. AUC/MIC targets have not been established for most second-line TB drugs; for

Linezolid, a target AUC/MIC >100 has been proposed [64]; for Moxifloxacin, a target of >53 based on the hollow fiber infection model was computed, and >100, based on animal studies.[2] Because of the potential of variability within the microbial population, the anticipated time delay before final MIC data become available, and the time required to fine-tune dosing based on PK measurements, the preferred design of the initial intensive treatment schedule comprises at least four to five drugs, including one or two core drugs [29, 35]. Drug resistance and susceptibility of *M. tb* are entirely genetically driven; mutations are relatively rare events, but the absolute number of mutants occurring after cell division is considerable in a large bacterial population. Mutants can be detected using molecular methods that, unlike phenotypic culture-based assays, can be made readily available. However, currently, still, amplification using culture is required to harvest sufficient quantities of bacterial DNA to run the tests. Currently, whole-genome analysis is gradually replacing older, PCR-based techniques [65]. Selecting drugs based on molecular assays could be done fairly rapidly, thereby avoiding exposure to unnecessarily toxic and ineffective agents and optimizing dosing based on PK measurement [66]. In summary, TDM has the potential to detect apparently low and apparently high drug exposures, which is a tremendous asset to improve effectiveness and reduce toxicity in patients with MDR-TB [33].

2.4 Treatment Duration

Treatment duration, both for the intensive initial phase as well for the continuation phase, might at some point also be individualized; this is perhaps the most difficult aspect of individualized treatment. The default duration of therapy for MDR-TB, 20–24 months, reflects the duration of therapy before the introduction of Rifampicin and Pyrazinamide, and by convention, the standard duration of MDR-TB treatment has been established at 20–24 months [29]. In practice, clinicians appear to make decisions on treatment duration based on the extent of disease and severity of lesions (e.g., large cavities, or severe forms of the extrapulmonary disease, e.g., meningeal, cerebral, or bone involvement) [16], as well as on tolerability and adverse effects of medication(s). Shorter duration–nine to eleven months and even six months–has been shown to yield successful outcomes in a number of studies. In a cohort study conducted in Bangladesh, a nine-month treatment schedule was tested, which included an initial phase of four to six months of Prothionamide, high-dose Isoniazid, Kanamycin, Moxifloxacin, Clofazimine, Pyrazinamide, and Ethambutol followed by five months of Moxifloxacin, Clofazimine, Pyrazinamide, and Ethambutol. Of the 206 study participants that received this treatment, 87.9% (95% confidence interval, 82.7–91.6) were cured without relapse [23].

The ‘Bangladesh’ regimen was later tested in several African countries, again with generally favorable outcomes [24, 25]. In a multi-center randomized clinical trial (STREAM), participants with MDR-TB in the experimental arm were treated

[2] Mathieu Bolhuis, unpublished data.

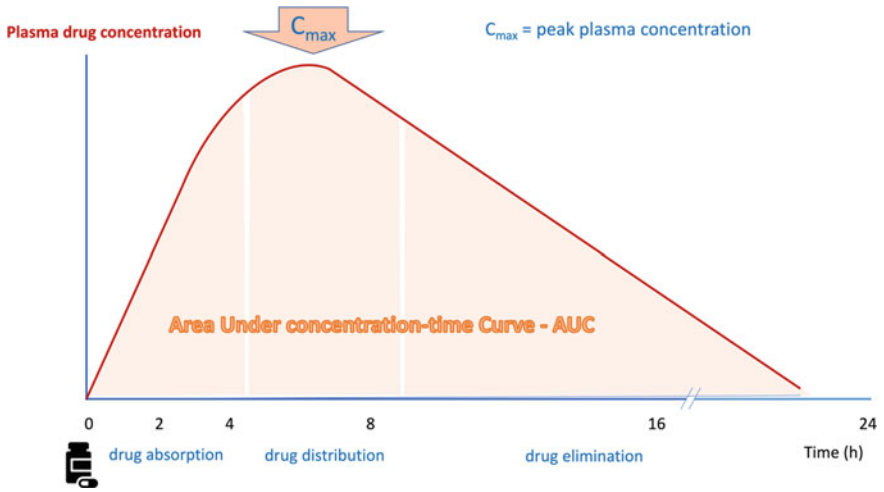


Fig. 3 Basics of pharmacokinetic (PK) analysis, based on drug concentrations measurement over time, following ingestion of a (TB) drug; the curve is the result of fitting, using specific PK software

for 40 weeks with high-dose Moxifloxacin, Clofazimine, Ethambutol, and Pyrazinamide supplemented by Kanamycin, Isoniazid, and Prothionamide in the first 16 weeks; at the pre-defined time point in week 132, 78.8% of study participants in the experimental arm had a favorable outcome, compared to 79.8% in the standard treatment arm; overall effectiveness, as well as adverse effects, were similar in both arms, but among participants receiving high-dose Moxifloxacin, dose adjustments were occasionally necessary if rate-corrected QT (QTc) intervals exceeded 500 ms [26]. Among the fluoroquinolones, exposure to Moxifloxacin relative to MIC is often too low; increased dosing of Moxifloxacin is more problematic than the increase in dosing for Levofloxacin [67], which for that reason might be the preferred fluoroquinolone. Gatifloxacin is probably even better [68], but unfortunately, this drug is currently not available on the market.

In their guidelines, the world health organization (WHO) has accepted a 'short-course' treatment for MDR-TB since 2017, provided that several different criteria were fulfilled, especially susceptibility to core drugs including fluoroquinolones and earlier also, the second-line injectables [69, 70]. Following the considerations mentioned above, a treatment duration between nine and twelve months appears justified, provided that:

- sufficient drug exposure (i.e., PK/PD), as well as drug penetration to all diseased sites, can be safely assumed;
- one or two core drugs with sufficient susceptibility are included in the treatment;
- adverse effects are acceptable; and
- adherence with therapy is optimal.

The major concern of all treatment schedules mentioned above is drug toxicity. Linezolid use is predominantly limited by its neurotoxicity and bone marrow suppression to a lesser extent. In our center, using PK/PD considerations with AUC/MIC targets and following patients with a process referred to as TDM, we have used Linezolid for prolonged periods without appreciable toxicity [71, 72], and we consider this agent as a core anti-TB drug [40].

The use of second-line injectable drugs is perhaps even more challenging. Daily painful intramuscular injections or risks associated with daily intravenous administration may be temporary inconveniences, but nephrotoxicity [73], ototoxicity, and vestibulotoxicity are perhaps even more worrying [74]. In a clinical collection of MDR-TB strains, MIC for Amikacin was one log-step lower than for Kanamycin [75], and therefore, among the injectables, Amikacin would be the preferred drug. In our center, we have meticulously followed patients with audiograms in the past and tailored dosing of aminoglycoside injectables using PK/PD directed dosing [76] with excellent clinical outcome, without any appreciable hearing loss [21, 77]. Still, under the new WHO guideline [70], injectables have only been used sporadically in our center. Figure 4a–c illustrate effective treatment using individualized treatment with TDM in extensive disease, with low toxicity despite prolonged use of Linezolid and Amikacin, and even without adjunctive (surgical) treatment.

If for some reason or other, no reasonable companion drug schedule can be constructed, other repurposed drugs might be considered. Ethionamide/Prothionamide is problematic because of (intestinal) adverse effects; Cycloserine may affect mood severely; and para-aminosalicylic acid is problematic by its large volume and intestinal adverse effects. Drugs to be considered in individualized schedules though currently not included in the list of WHO include Cotrimoxazole [78, 79] and carbapenems, e.g., Imipenem-Cilastatin, Meropenem [80], or Ertapenem [81, 82], preferably in combination with a beta-lactamase inhibitor.

Bedaquiline is definitively in the forefront now for all patients with MDR-TB, although even for this relatively novel agent, drug resistance is emerging [46]; the question of whether penetration into sanctuary sites like the cerebrospinal fluid is sufficient or not remains unresolved [83]. Delamanid is still considered an important companion drug, but its position may change over time [84]. Pretomanid has been included in the novel BPaL regimen [27, 85]; it has been added as a companion drug with low toxicity, fair bioavailability, and no major drug–drug interactions [86].

3 Pharmacokinetic/Pharmacodynamic Modeling: Therapeutic Drug Monitoring

As mentioned above, an important aspect of treatment individualization and precision treatment is monitoring drug exposure (PK) and adjusting dosing accordingly, using the principles of TDM [33]; see Fig. 3. The problem with the PK of drugs is complex but highly relevant [87]: there is a large inter-individual variation

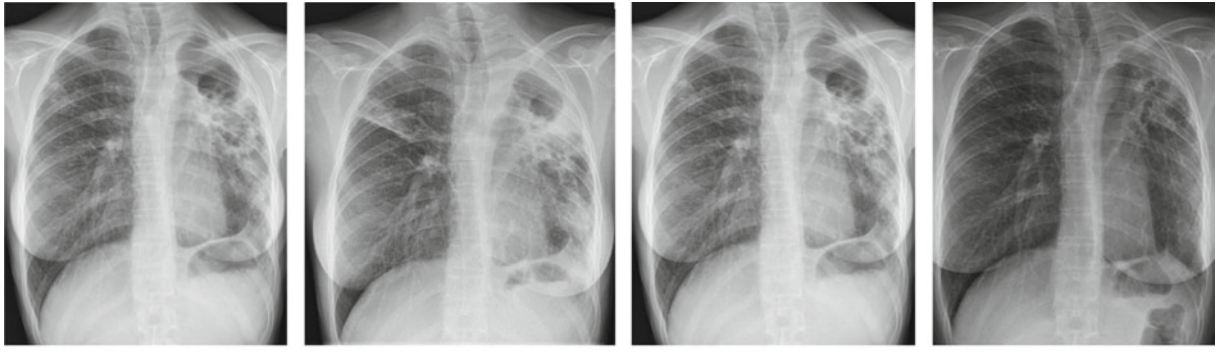


Fig. 4 A patient with severe multidrug-resistant cavitary pulmonary tuberculosis, successfully treated in 2015 with injectable Amikacin, combined with Linezolid, both dosed based on therapeutic drug monitoring; and Pyrazinamide, Prothionamide, Moxifloxacin, Clofazimine, and Cycloserine

in drug handling for many of the second-line TB drugs. For drugs like Linezolid and Moxifloxacin, which are eliminated by liver cytochrome enzymes, genetic polymorphisms vary across the human population; there are many drug–drug interactions. For drugs with renal elimination, the renal function determines the drug half-life. Nutritional status may vary, and drug absorption may be variable between individuals, but also within a patient who may gradually improve clinically gain weight with subsequent increase of the volume of distribution. Finally, concurrent food intake may have a considerable effect on drug absorption, both for the first-line drugs [88] and for various different second-line drugs. To sample venous blood over time and measure drug concentrations using high-tech equipment, like liquid chromatography combined with tandem mass spectrometry, followed by computation of $AUC_{0–24\ h}$ for each drug is challenging, even in affluent settings. MIC for each drug is initially assumed below the EUCAST breakpoint if, based on molecular testing, a wild-type gene is detected in the *M. tb* genome. If MIC is very low, drug dosing can subsequently be further reduced [44, 72]. In clinical practice, the largest asset is by detecting those individuals with toxic or subtherapeutic drug exposure, as they run the highest risk of adverse outcomes. These individuals may even be detected with appropriate TDM technology, using limited sampling combined with dried blood spots that can be sent by ordinary mail under ambient temperatures to a central-based lab facility, even in less affluent settings [89–91]. Patients' prioritization for TDM involves factors such as HIV co-infection, impaired renal clearance, hepatic dysfunction, diabetes mellitus, malnutrition, critical illness, TB meningitis, TB of the skeleton, drug–drug interactions, and poor response to TB treatment despite optimized adherence [33].

4 Host Factors: Coinfections and Comorbidities

Susceptibility to TB is driven by genetic susceptibility [92]. Likewise, pharmacogenetics is also important to explain inter-individual PK variability. To date, most studies have addressed first-line drugs, especially acetylator status variations for Isoniazid drug metabolism, driven by polymorphisms in genes coding for N-acetyltransferase [93, 94] and CYP polymorphisms driving PK and toxicity of Rifampicin [95, 96]. Few studies report on the impact of pharmacogenetics of second-line TB drugs [97]. PK variability is large, even in relatively homogeneous patient populations. However, many potential drug–drug interactions may influence exposure to second-line drugs in patients with chronic viral infections such as HIV and hepatitis B and C, in turn impacting on outcome of co-infected individuals [98]. Obesity is an emerging condition around the world, and it has become increasingly important among patients with TB and MDR-TB as well. For drugs with hydrophilic properties and low volumes of distribution, like aminoglycosides and Ethambutol, dosing should best be calculated on lean body mass [99, 100]. An important and emerging comorbid condition is diabetes mellitus [101, 102], which is notorious for changes in PK of first-line TB drugs [103]; few data on second-line

TB drugs have been published [104]. An association between diabetes and MDR-TB has also been reported [105]. In summary, we propose awareness of risks in drug exposure and therefore advise TDM and individualized therapy in patients with MDR-TB and comorbid conditions and co-infections.

5 Host-Directed Therapies

Statistics on the outcome of TB treatment have conventionally been dichotomized into favorable or successful and unfavorable or unsuccessful [106]. Although these outcome criteria have been extremely helpful to compare treatment schedules, these criteria largely obscure what happens after the successful completion of therapy. Fibrotic pulmonary sequelae may impair exercise capacity, and persistent cavitary lesions and bronchiectatic airways may become secondarily infected by *Pseudomonas* and *Aspergillus* spp. Patients may die prematurely from massive hemoptysis from aspergillomas and infected bronchiectasis long after they have been cured for their MDR-TB. Patients surviving TB meningitis may suffer from neurologic sequela, and patients with TB of the spinal column may experience spinal cord injury [107]. Indeed, many patients experience limitations in daily life, as they suffer sequela and impaired quality of life–and even reduced life expectancy after TB treatment completion [108].

Excessive and transient inflammation may be harmful and treated with anti-tumor necrosis factor-alpha agents, e.g., Infliximab, or anti-inflammatory agents such as corticosteroids [109]. In TB meningitis, the impact of concurrent treatment with Dexamethasone with optimized TB drug treatment though widely practiced has remained controversial [110]. In pulmonary TB, steroids have been discouraged, although severe paradoxical inflammation may respond favorably [111]. In MDR-TB, steroids are even riskier, especially in the early stages of treatment when paradoxical reactions are most common, and MICs for drugs chosen in the initial empirical treatment are less certain. Whether certain co-medications like macrolides might reduce fibrotic changes is currently under investigation in our center. Non-steroidal anti-inflammatory agents may have added benefits [112], but many clinicians hesitate to add these agents in standard care.

6 Therapeutic Vaccination

With the emergence of drug resistance, now even for the novel drug Bedaquiline [46], the daunting prospect of totally DR-TB is looming. With limited treatment options, the concept of enhancing host immune responses might be advantageous [113, 114]. Only a few vaccine products currently under investigation have reached phase III clinical development, and even fewer have been developed as therapeutic vaccine products [115]. Stimulating the immune system during active disease might

seem hazardous and even counter-intuitive because some of the host-directed therapies discussed above try to reduce exaggerated immune responses involved in paradoxical reactions, excessive and harmful inflammation in TB meningitis and central nervous system and spinal cord compression. The evidence from clinical studies with *M. vaccae* vaccination [116] and *Mycobacterium indicus pranii* [117] show no evident harm and some benefit in terms of earlier sputum culture conversion. One ongoing phase IIa trial is in progress, evaluating the immunogenicity and safety of RUTI, a novel anti-tuberculosis vaccine product expressing antigens of an inactivated *M. tb* strain cultured under stress conditions, thereby aiming at enhanced immune responses toward an antigenic repertoire associated with *M. tb* persisters [118, 119]. Clearly, targeting persister organisms–in essence, addressing the issue of sterilizing lesions–is critical to curing TB [34], and any attempt to address this is critically important to achieving a relapse-free cure for MDR-TB.

7 Surgery

For individual patients, individual teams may decide on the potential added value of surgical resection of tissues damaged beyond repair; such decisions are hardly evidence-based and typically depend on locally available skills and experience of surgical teams. Surgery is important in TB of the axial skeleton, and drainage of large pleural effusions and abscesses associated with TB of bone and lymph nodes is equally important. The role of resection surgery in pulmonary MDR-TB has predominantly been addressed in anecdotal case reports and series. A database of MDR-TB patients comprising over 6000 individual patient records was analyzed to detect any potential benefit of added surgery [120]. In this large database, partial but not total pneumonectomy was associated with improved outcomes. Observational data like these are potentially highly confounded by indication, and selection bias cannot be ruled out [121]. The position of surgery, therefore, remains adjunctive, as it may have added value in selected cases [122].

8 Conclusion

With the unprecedented emergence of drug resistance, individualized treatment for MDR-TB is inevitable and necessary. The down-side is obviously the limited scientific evidence; each individual group of resistance, and each identifiable group of patients with unique comorbid conditions, genetic background, nutritional status, the severity of disease, mix of pulmonary and extrapulmonary disease, and co-medications, would obscure any potential positive effect if groups of patients are too small and too heterogeneous to conduct a meaningful randomized trial with a relevant control regimen. Perhaps the only way to provide evidence for individual patients to entrust the proposed individualized treatment regimen would be to randomize individualized against standardized treatment for MDR-TB. The

evidence would otherwise need to come from the notion that in vitro and molecular testing of drug susceptibilities, combined with measured drug exposure, provides the best possible way for successful treatment outcomes. The evidence for the efficacy of individual drugs is only derived from large retrospective database analyses. Many questions remain unanswered, but the general principle that even the worst forms of MDR-TB are potentially curable should set the scene to aim high and attain high cure rates. New drugs are in the pipeline, like the Linezolid analog, Sutezolid [123], with lower MIC and potentially lower toxicity that might therefore be a suitable replacement for Linezolid [124]. Not only survival following treatment completion and cure, but also reduction of sequela and improved quality and quantity of life after completion of therapy are important. TDM is critically important, and it should be included in international guidelines for the management of MDR-TB. Providing adequate exposure to each of the drugs included in the regimen is critically important to improve the outcome of individual patients as well as to reduce the transmission of MDR-TB. During treatment, PK parameters may change over time, and in certain conditions like pregnancy, these changes may be dramatic, therefore requiring multiple interventions of TDM [125]. Molecular tests predicting susceptibility are gradually improving, but their weakness at this point in time is that they do not (yet) predict MIC reliably, and MIC for each drug in the regimen is critically important for those drugs that have a relatively narrow therapeutic window. The ambitious targets set by the UNION and the WHO to eradicate TB in the next two decades cannot be met without an increased effort to target MDR-TB; individualized treatment is essential to combat this daunting condition.

Core Messages

- MDR-TB is a daunting novel epidemic that frustrates efforts to defeat TB any time soon.
- MDR-TB management requires novel molecular diagnostic tools with fast lab turn-around times.
- MDR-TB treatment requires measures such as TDM to optimize efficacy and reduce toxicity.
- Host-directed therapies hold promise to further improve outcomes.

References

1. Fox W, Ellard GA, Mitchison DA (1999) Studies on the treatment of tuberculosis undertaken by the British Medical Research Council tuberculosis units, 1946–1986, with relevant subsequent publications. Int J Tuberc Lung Dis 3:S231–S279
2. Anonymous (1986) Controlled clinical trial of 4 short-course regimens of chemotherapy (three 6-month and one 8-month) for pulmonary tuberculosis: final report. East and Central African/British Medical Research Council fifth collaborative study. Tubercle 67:5–15

3. Anonymous (1972) Controlled clinical trial of short-course (6-month) regimens of chemotherapy for treatment of pulmonary tuberculosis. Lancet 1:1079–1085
4. Fox W (1983) Compliance of patients and physicians: experience and lessons from tuberculosis-II. BMJ 287:101–105
5. Fregonese F, Ahuja SD, Akkerman OW, Arakaki-Sanchez D, Ayakaka I, Baghaei P, Bang D, Bastos M, Benedetti A, Bonnet M, Cattamanchi A, Cegielski P, Chien J-Y, Cox H, Dedicoat M, Erkens C, Escalante P, Falzon D, Garcia-Prats AJ, Gegia M, Gillespie SH, Glynn JR, Goldberg S, Griffith D, Jacobson KR, Johnston JC, Jones-López EC, Khan A, Koh WJ, Kritski A, Lan ZY, Li JH, Li PZ, Maciel EL, Galliez RM, Merle CS, Munang M, Narendran G, Nguyen VN, Nunn A, Ohkado A, Park JS, Phillips PJ, Ponnuraja C, Reves R, Romanowski K, Seung K, Schaaf HS, Skrahina A, van Soolingen D, Tabarsi P, Trajman A, Trieu L, Barunekha VV, Viiklepp P, Wang J-Y, Yoshiyama T, Menzies D (2018) Comparison of different treatments for isoniazid-resistant tuberculosis: an individual patient data meta-analysis. Lancet Respir Med 6:265–275
6. Frieden TR, Sterling T, Pablos-Mendez A, Kilburn JO, Cauthen GM, Dooley SW (1993) The emergence of drug-resistant tuberculosis in New York City. N Engl J Med 328:521–526
7. Moss AR, Alland D, Telzak E, Hewlett D, Sharp V, Chiliade P, LaBombardi V, Kabus D, Hanna B, Palumbo L, Brudney K, Weltman A, Stoeckle K, Chirgwin K, Simberkoff M, Moghazeh S, Eisner W, Lutfey M, Kreiswirth B (1997) A city-wide outbreak of a multiple-drug-resistant strain of *Mycobacterium tuberculosis* in New York. Int J Tuberc Lung Dis 1:115–121
8. Pablos-Mendez A, Raviglione MC, Laszlo A, Binkin N, Rieder HL, Bustreo F, Cohn DL, Lambregts-van Weezenbeek CS, Kim SJ, Chaulet P, Nunn P (1998) Global surveillance for antituberculosis-drug resistance, 1994–1997. World Health Organization-International Union against tuberculosis and lung disease working group on anti-tuberculosis drug resistance surveillance. N Engl J Med 338:1641–1649
9. Johnston JC, Shahidi NC, Sadatsafavi M, Fitzgerald JM (2009) Treatment outcomes of multidrug-resistant tuberculosis: a systematic review and meta-analysis. PLoS ONE 4:e6914
10. World Health Organization (2017) Global tuberculosis report 2017. WHO, Geneva, p 2017
11. Collaborative Group for the Meta-Analysis of Individual Patient Data in MDR-TB treatment–2017, Ahmad N, Ahuja SD, Akkerman OW, Alffenaar J-WC, Anderson LF, Baghaei P, Bang D, Barry PM, Bastos ML, Behera D, Benedetti A, Bisson GP, Boeree MJ, Bonnet M, Brode SK, Brust JCM, Cai Y, Caumes E, Cegielski JP, Centis R, Chan P-C, Chan ED, Chang K-C, Charles M, Cirule A, Dalcolmo MP, D'Ambrosio L, de Vries G, Dheda K, Esmail A, Flood J, Fox GJ, Fréchet-Jachym M, Fregona G, Gayoso R, Gegia M, Gler MT, Gu S, Guglielmetti L, Holtz TH, Hughes J, Isaakidis P, Jarlsberg L, Kempker RR, Keshavjee S, Khan FA, Kipiani M, Koenig SP, Koh WJ, Kritski A, Kuksa L, Kvasnovsky CL, Kwak N, Lan Z, Lange C, Laniado-Laborín R, Lee M, Leimane V, Leung CC, Leung EC, Li PZ, Lowenthal P, Maciel EL, Marks SM, Mase S, Mbuagbaw L, Migliori GB, Milanov V, Miller AC, Mitnick CD, Modongo C, Mohr E, Monedero I, Nahid P, Ndjeka N, O'Donnell MR, Padayatchi N, Palmero D, Pape JW, Podewils LJ, Reynolds I, Riekstina V, Robert J, Rodriguez M, Seaworth B, Seung KJ, Schnippel K, Shim TS, Singla R, Smith SE, Sotgiu G, Sukhbaatar G, Tabarsi P, Tiberi S, Trajman A, Trieu L, Udwadia ZF, van der Werf TS, Veziris N, Viiklepp P, Vilbrun SC, Walsh K, Westenhouse J, Yew WW, Yim JJ, Zetola NM, Zignol M, Menzies D (2018) Treatment correlates of successful outcomes in pulmonary multidrug-resistant tuberculosis: an individual patient data meta-analysis. Lancet 392:821–834
12. Bastos ML, Hussain H, Weyer K, Garcia-Garcia L, Leimane V, Leung CC, Narita M, Penã JM, Ponce-de-Leon A, Seung KJ, Shean K, Sifuentes-Osornio J, Van der Walt M, van der Werf TS, Yew WW, Menzies D, Collaborative Group for Meta-analysis of Individual Patient Data in MDR-TB (2014) Treatment outcomes of patients with multidrug-resistant and extensively drug-resistant tuberculosis according to drug susceptibility testing to first- and second-line drugs: an individual patient data meta-analysis. Clin Infect Dis 59:1364–1374

13. Borisov S, Danila E, Maryandyshev A, Dalcolmo M, Miliauskas S, Kuksa L, Manga S, Skrahina A, Diktanas S, Codecasa LR, Aleksa A, Bruchfeld J, Koleva A, Piubello A, Udwadia ZF, Akkerman OW, Belilovski E, Bernal E, Boeree MJ, Cadiñanos Loidi J, Cai Q, Cebrian Gallardo JJ, Dara M, Davidavičienė E, Forsman LD, De Los RJ, Denholm J, Drakšienė J, Duarte R, Elamin SE, Escobar Salinas N, Ferrarese M, Filippov A, Garcia A, García-García JM, Gaudiesiute I, Gavazova B, Gayoso R, Gomez Rosso R, Gruslys V, Gualano G, Hoefsloot W, Jonsson J, Khimova E, Kunst H, Laniado-Laborín R, Li Y, Magis-Escurra C, Manfrin V, Marchese V, Martínez Robles E, Matteelli A, Mazza-Stalder J, Moschos C, Muñoz-Torrico M, Mustafa Hamdan H, Nakčerienė B, Nicod L, Nieto Marcos M, Palmero DJ, Palmieri F, Papavasileiou A, Payen MC, Pontarelli A, Quirós S, Rendon A, Saderi L, Šmite A, Solovic I, Souleymane MB, Tadolini M, van den Boom M, Vescovo M, Viggiani P, Yedilbayev A, Zablockis R, Zhurkin D, Zignol M, Visca D, Spanevello A, Caminero JA, Alffenaar JW, Tiberi S, Centis R, D'Ambrosio L, Pontali E, Sotgiu G, Migliori GB (2019) Surveillance of adverse events in the treatment of drug-resistant tuberculosis: first global report. Eur Respir J 54:1901522
14. Leimane V, Dravniece G, Riekstina V, Sture I, Kammerer S, Chen MP, Skenders G, Holtz TH (2010) Treatment outcome of multidrug/extensively drug-resistant tuberculosis in Latvia, 2000–2004. Eur Respir J 36:584–593
15. Liu CH, Li L, Chen Z, Wang Q, Hu YL, Zhu B, Woo PCY (2011) Characteristics and treatment outcomes of patients with MDR and XDR tuberculosis in a TB referral hospital in Beijing: a 13-year experience. PLoS ONE 6:e19399
16. Dheda K, Lenders L, Magombedze G, Srivastava S, Raj P, Arning E, Ashcraft P, Bottiglieri T, Wainwright H, Pennel T, Linegar A, Moodley L, Pooran A, Pasipanodya JG, Sirgel FA, van Helden PD, Wakeland E, Warren RM, Gumbo T (2018) Drug-penetration gradients associated with acquired drug resistance in patients with tuberculosis. Am J Respir Crit Care Med 198:1208–1219
17. Akkerman OW, van Altena R, Klinkenberg T, Brouwers AH, Bongaerts AHH, van der Werf TS, Alffenaar J-W (2013) Drug concentration in lung tissue in multidrug-resistant tuberculosis. Eur Respir J 42:1750–1752
18. Alffenaar JWC, Van Altena R, Bökkerink HJ, Luijckx GJ, van Soolingen D, Aarnoutse RE, van der Werf TS (2009) Pharmacokinetics of Moxifloxacin in cerebrospinal fluid and plasma in patients with tuberculous meningitis. Clin Infect Dis 49:1080–1082
19. Geerligs WA, van Altena R, De Lange WCM, van Soolingen D, van der Werf TS (2000) Multidrug-resistant tuberculosis: long-term treatment outcome in the Netherlands. Int J Tuberc Lung Dis 4:758–764
20. Ahuja SD, Ashkin D, Avendano M, Banerjee R, Bauer M, Bayona JN, Becerra MC, Benedetti A, Burgos M, Centis R, Chan ED, Chiang C-Y, Cox H, D'Ambrosio L, DeRiemer K, Dung NH, Enarson D, Falzon D, Flanagan K, Flood J, Garcia-Garcia ML, Gandhi N, Granich RM, Hollm-Delgado MG, Holtz TH, Iseman MD, Jarlsberg LG, Keshavjee S, Kim H-R, Koh WJ, Lancaster J, Lange C, de Lange WC, Leimane V, Leung CC, Li J, Menzies D, Migliori GB, Mishustin SP, Mitnick CD, Narita M, O'Riordan P, Pai M, Palmero D, Park SK, Pasvol G, Peña J, Pérez-Guzmán C, Quelapio MI, Ponce-de-Leon A, Riekstina V, Robert J, Royce S, Schaaf HS, Seung KJ, Shah L, Shim TS, Shin SS, Shiraishi Y, Sifuentes-Osornio J, Sotgiu G, Strand MJ, Tabarsi P, Tupasi TE, van Altena R, Van der Walt M, Van der Werf TS, Vargas MH, Viiklepp P, Westenhouse J, Yew WW, Yim JJ, Collaborative Group for Meta-Analysis of Individual Patient Data in MDR-TB (2012) Multidrug resistant pulmonary tuberculosis treatment regimens and patient outcomes: an individual patient data meta-analysis of 9,153 patients. PLoS Med 9:e1001300
21. van Altena R, de Vries G, Haar CH, de Lange WCM, Magis-Escurra C, van den Hof S, van Soolingen D, Boeree MJ, van der Werf TS (2015) Highly successful treatment outcome of multidrug-resistant tuberculosis in the Netherlands, 2000–2009. Int J Tuberc Lung Dis 19:406–412

22. Tiberi S, du Plessis N, Walzl G, Vjecha MJ, Rao M, Ntoumi F, Mfinanga S, Kapata N, Mwaba P, McHugh TD, Ippolito G, Migliori GB, Maeurer MJ, Zumla A (2018) Tuberculosis: progress and advances in development of new drugs, treatment regimens, and host-directed therapies. Lancet Infect Dis 18(7):e183–e198
23. Van Deun A, Maug AKJ, Salim MAH, Das PK, Sarker MR, Daru P, Rieder HL (2010) Short, highly effective, and inexpensive standardized treatment of multidrug-resistant tuberculosis. Am J Respir Crit Care Med 182:684–692
24. Piubello A, Harouna SH, Souleymane MB, Boukary I, Morou S, Daouda M, Hanki Y, Van Deun A (2014) High cure rate with standardised short-course multidrug-resistant tuberculosis treatment in Niger: no relapses. Int J Tuberc Lung Dis 18:1188–1194
25. Aung KJM, Van Deun A, Declercq E, Sarker MR, Das PK, Hossain MA, Rieder HL (2014) Successful "9-month Bangladesh regimen" for multidrug-resistant tuberculosis among over 500 consecutive patients. Int J Tuberc Lung Dis 18:1180–1187
26. Nunn AJ, Phillips PPJ, Meredith SK, Chiang C-Y, Conradie F, Dalai D, Van Deun A, Dat P-T, Lan N, Master I, Mebrahtu T, Meressa D, Moodliar R, Ngubane N, Sanders K, Squire SB, Torrea G, Tsogt B, Rusen ID, STREAM Study Collaborators (2019) A trial of a shorter regimen for Rifampin-resistant tuberculosis. N Engl J Med 380:1201–1213
27. Conradie F, Diacon AH, Ngubane N, Howell P, Everitt D, Crook AM, Mendel CM, Egizi E, Moreira J, Timm J, McHugh TD, Wills GH, Bateson A, Hunt R, van Niekerk C, Li M, Olugbosi M, Spigelman M, Nix-TB Trial Team (2020) Treatment of highly drug-resistant pulmonary tuberculosis. N Engl J Med 382:893–902
28. Falzon D, Jaramillo E, Schünemann HJ, Arentz M, Bauer M, Bayona J, Blanc L, Caminero JA, Daley CL, Duncombe C, Fitzpatrick C, Gebhard A, Getahun H, Henkens M, Holtz TH, Keravec J, Keshavjee S, Khan AJ, Kulier R, Leimane V, Lienhardt C, Lu C, Mariandyshev A, Migliori GB, Mirzayev F, Mitnick CD, Nunn P, Nwagboniwe G, Oxlade O, Palmero D, Pavlinac P, Quelapio MI, Raviglione MC, Rich ML, Royce S, Rüsch-Gerdes S, Salakaia A, Sarin R, Sculier D, Varaine F, Vitoria M, Walson JL, Wares F, Weyer K, White RA, Zignol M (2011) WHO guidelines for the programmatic management of drug-resistant tuberculosis: 2011 update. Eur Respir J 38:516–528
29. WHO (2016) WHO treatment guidelines for drug-resistant tuberculosis. WHO, Geneva, pp 1–61
30. Bolhuis MS, Akkerman OW, Sturkenboom MGG, de Lange WCM, van der Werf TS, Alffenaar J-WC (2016) Individualized treatment of multidrug-resistant tuberculosis using therapeutic drug monitoring. Int J Mycobact 5(Suppl 1):S44–S45
31. Alffenaar J-WC, Akkerman OW, Anthony RM, Tiberi S, Heysell S, Grobusch MP, Cobelens FG, van Soolingen D (2017) Individualizing management of extensively drug-resistant tuberculosis: diagnostics, treatment, and biomarkers. Exp Rev Anti Infect Ther 15:11–21
32. Cox H, Hughes J, Black J, Nicol MP (2018) Precision medicine for drug-resistant tuberculosis in high-burden countries: is individualised treatment desirable and feasible? Lancet Infect Dis 18(9):e282–e287
33. Zuur MA, Bolhuis MS, Anthony R, den Hertog A, van der Laan T, Wilffert B, de Lange W, van Soolingen D, Alffenaar J-WC (2016) Current status and opportunities for therapeutic drug monitoring in the treatment of tuberculosis. Exp Opin Drug Metab Toxicol 12:509–521
34. Zhang Y, Yew WW, Barer MR (2012) Targeting persisters for tuberculosis control. Antimicrob Agents Chemother 56:2223–2230
35. Caminero JA, Scardigli A, van der Werf TS, Tadolini M (2018) Treatment of drug-susceptible and drug-resistant tuberculosis. In: Migliori GB, Bothamley G, Duarte R, Rendon A (eds) Tuberculosis. ERS monograph. ERS, pp 152–187 (Chapter 10)
36. Pranger AD, van der Werf TS, Kosterink JGW, Alffenaar JWC (2019) The role of fluoroquinolones in the treatment of tuberculosis in 2019. Drugs 79:161–171

37. Drusano GL, Neely MN, Kim S, Yamada WM, Schmidt S, Duncanson B, Nole J, Mtchedlidze N, Peloquin CA, Louie A (2020) Building optimal 3-drug combination chemotherapy regimens. Antimicrob Agents Chemother 64(11)
38. van Deun A, Decroo T, Piubello A, de Jong BC, Lynen L, Rieder HL (2018) Principles for constructing a tuberculosis treatment regimen: the role and definition of core and companion drugs. Int J Tuberc Lung Dis 22:239–245
39. Zhang M, Sala C, Dhar N, Vocat A, Sambandamurthy VK, Sharma S, Marriner G, Balasubramanian V, Cole ST (2014) In vitro and in vivo activities of three oxazolidinones against nonreplicating *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 58:3217–3223
40. Drusano GL, Myrick J, Maynard M, Nole J, Duncanson B, Brown D, Schmidt S, Neely M, Scanga CA, Peloquin C, Louie A (2018) Linezolid kills acid-phase and nonreplicative-persister-phase *Mycobacterium tuberculosis* in a hollow-fiber infection model. Antimicrob Agents Chemother 62
41. Rivière E, Whitfield MG, Nelen J, Heupink TH, Van Rie A (2020) Identifying isoniazid resistance markers to guide inclusion of high-dose Isoniazid in tuberculosis treatment regimens. Clin Microbiol Infect 26:1332–1337
42. Xu J, Lu Y, Fu L, Zhu H, Wang B, Mdluli K, Upton AM, Jin H, Zheng M, Zhao W, Li P (2012) In vitro and in vivo activity of clofazimine against *Mycobacterium tuberculosis* persisters. Int J Tuberc Lung Dis 16:1119–1125
43. Du Y, Qiu C, Chen X, Wang J, Jing W, Pan H, Chen W, Liu Y, Li C, Xi X, Yin H, Zeng J, Zhang X, Xu T, Wang Q, Guo R, Wang J, Pang Y, Chu N (2020) Treatment outcome of a shorter regimen containing clofazimine for multidrug-resistant tuberculosis: a randomized control trial in China. Clin Infect Dis 71:1047–1054
44. Alffenaar JWC, Akkerman OW, Bolhuis MS, Boeree MJ, de Lange WCM, van der Werf TS (2015) Breakpoints and drug exposure are inevitably closely linked. Antimicrob Agents Chemother 59:1384–1384
45. Schön T, Werngren J, Machado D, Borroni E, Wijkander M, Lina G, Mouton J, Matuschek E, Kahlmeter G, Giske C, Santin M, Cirillo DM, Viveiros M, Cambau E (2020) Antimicrobial susceptibility testing of *Mycobacterium tuberculosis* complex isolates—the EUCAST broth microdilution reference method for MIC determination. Clin Microbiol Infect
46. Nguyen TVA, Anthony RM, Bañuls A-L, Vu DH, Alffenaar J-WC (2018) Bedaquiline resistance: its emergence, mechanism, and prevention. Clin Infect Dis 66:1625–1630
47. Mokrousov I, Akhmedova G, Molchanov V, Fundovnaya E, Kozlova E, Ostankova Y, Semenov A, Maslennikova N, Leontev D, Zhuravlev V, Turkin E, Vyazovaya A (2020) Frequent acquisition of bedaquiline resistance by epidemic XDR *Mycobacterium tuberculosis* strains in Russia during long-term treatment. Clin Microbiol Infect
48. Nimmo C, Millard J, van Dorp L, Brien K, Moodley S, Wolf A, Grant AD, Padayatchi N, Pym AS, Balloux F, O'Donnell M (2020) Population-level emergence of bedaquiline and clofazimine resistance-associated variants among patients with drug-resistant tuberculosis in southern Africa: a phenotypic and phylogenetic analysis. Lancet Microbe 1:e165–e174
49. Dooley KE, Miyahara S, Groote-Bidlingmaier von F, Sun X, Hafner R, Rosenkranz SL, Ignatius EH, Nuermberger EL, Moran L, Donahue K, Swindells S, Vanker N, Diacon AH, A5312 Study Team (2020) Early bactericidal activity of different isoniazid doses for drug resistant TB (INHindsight): a randomized open-label clinical trial. Am J Respir Crit Care Med
50. Cavaleri M, Manolis E (2015) Hollow fiber system model for tuberculosis: the European medicines agency experience. Clin Infect Dis 61(Suppl 1):S1–S4
51. Pasipanodya J, Srivastava S, Gumbo T (2012) New susceptibility breakpoints and the regional variability of MIC distribution in *Mycobacterium tuberculosis* isolates. Antimicrob Agents Chemother 56:5428–5428

52. Gumbo T, Angulo-Barturen I, Ferrer-Bazaga S (2015) Pharmacokinetic-pharmacodynamic and dose-response relationships of anti-tuberculosis drugs: recommendations and standards for industry and academia. J Infect Dis 211(Suppl 3):S96–S106
53. Nimmo C, Shaw LP, Doyle R, Williams R, Brien K, Burgess C, Breuer J, Balloux F, Pym AS (2019) Whole genome sequencing *Mycobacterium tuberculosis* directly from sputum identifies more genetic diversity than sequencing from culture. BMC Genomics 20:389–389
54. Grobbelaar M, Louw GE, Sampson SL, van Helden PD, Donald PR, Warren RM (2019) Evolution of rifampicin treatment for tuberculosis. Infect Genet Evol 74:103937
55. Nimmo C, Brien K, Millard J, Grant AD, Padayatchi N, Pym AS, O'Donnell M, Goldstein R, Breuer J, Balloux F (2020) Dynamics of within-host *Mycobacterium tuberculosis* diversity and heteroresistance during treatment. EBioMedicine 55:102747
56. Wicha SG, Clewe O, Svensson RJ, Gillespie SH, Hu Y, Coates ARM, Simonsson USH (2018) Forecasting clinical dose-response from preclinical studies in tuberculosis research: translational predictions with Rifampicin. Clin Pharmacol Ther 104:1208–1218
57. van Ingen J, Aarnoutse RE, Donald PR, Diacon AH, Dawson R, Plemper van Balen G, Gillespie SH, Boeree MJ (2011) Why do we use 600 mg of Rifampicin in tuberculosis treatment? Clin Infect Dis 52:e194–e199
58. Liu Y, Pertinez H, Ortega-Muro F, Alameda-Martin L, Harrison T, Davies G, Coates A, Hu Y (2018) Optimal doses of Rifampicin in the standard drug regimen to shorten tuberculosis treatment duration and reduce relapse by eradicating persistent bacteria. J Antimicrob Chemother 73:724–731
59. Boeree MJ, Diacon AH, Dawson R, Narunsky K, Bois du J, Venter A, Phillips PPJ, Gillespie SH, McHugh TD, Hoelscher M, Heinrich N, Rehal S, van Soolingen D, van Ingen J, Magis-Escurra C, Burger D, Plemper van Balen G, Aarnoutse RE, PanACEA Consortium (2015) A dose-ranging trial to optimize the dose of rifampin in the treatment of tuberculosis. Am J Respir Crit Care Med 191:1058–1065
60. Pasipanodya JG, Srivastava S, Gumbo T (2012) Meta-analysis of clinical studies supports the pharmacokinetic variability hypothesis for acquired drug resistance and failure of anti-tuberculosis therapy. Clin Infect Dis 55:169–177
61. Pasipanodya JG, McIlleron H, Burger A, Wash PA, Smith P, Gumbo T (2013) Serum drug concentrations predictive of pulmonary tuberculosis outcomes. J Infect Dis 208:1464–1473
62. Gumbo T (2013) Biological variability and the emergence of multidrug-resistant tuberculosis. Nat Genet 45:720–721
63. Zuur MA, Pasipanodya JG, van Soolingen D, van der Werf TS, Gumbo T, Alffenaar J-WC (2018) Intermediate susceptibility dose-dependent breakpoints for high dose rifampicin, isoniazid and pyrazinamide treatment in multidrug-resistant tuberculosis programmes. Clin Infect Dis 29:565
64. Bolhuis MS, Akkerman OW, Sturkenboom MGG, Ghimire S, Srivastava S, Gumbo T, Alffenaar J-WC (2018) Linezolid-based regimens for multidrug-resistant tuberculosis (TB): a systematic review to establish or revise the current recommended dose for TB treatment. Clin Infect Dis 67:S327–S335
65. CRyPTIC Consortium and the 100,000 Genomes Project, Allix-Béguec C, Arandjelovic I, Bi L, Beckert P, Bonnet M, Bradley P, Cabibbe AM, Cancino-Muñoz I, Caulfield MJ, Chaiprasert A, Cirillo DM, Clifton DA, Comas I, Crook DW, De Filippo MR, de Neeling H, Diel R, Drobniewski FA, Faksri K, Farhat MR, Fleming J, Fowler P, Fowler TA, Gao Q, Gardy J, Gascoyne-Binzi D, Gibertoni-Cruz A-L, Gil-Brusola A, Golubchik T, Gonzalo X, Grandjean L, He G, Guthrie JL, Hoosdally S, Hunt M, Iqbal Z, Ismail N, Johnston J, Khanzada FM, Khor CC, Kohl TA, Kong C, Lipworth S, Liu Q, Maphalala G, Martinez E, Mathys V, Merker M, Miotto P, Mistry N, Moore DA, Murray M, Niemann S, Omar SV, Ong RT-H, Peto TE, Posey JE, Prammananan T, Pym A, Rodrigues C, Rodrigues M, Rodwell T, Rossolini GM, Sánchez Padilla E, Schito M, Shen X, Shendure J, Sintchenko V, Sloutsky A, Smith EG, Snyder M, Soetaert K, Starks AM, Supply P, Suriyapol P, Tahseen S,

Tang P, Teo Y-Y, Thuong TNT, Thwaites G, Tortoli, van Soolingen D, Walker AS, Walker TM, Wilcox M, Wilson DJ, Wyllie D, Yang Y, Zhang H, Zhao Y, Zhu B (2018) Prediction of susceptibility to first-line tuberculosis drugs by DNA sequencing. N Engl J Med 379:1403–1415

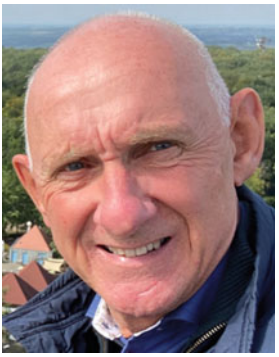
66. Gröschel MI, Walker TM, van der Werf TS, Lange C, Niemann S, Merker M (2018) Pathogen-based precision medicine for drug-resistant tuberculosis. PLoS Pathog 14: e1007297
67. Ghimire S, Van't Boveneind-Vrubleuskaya N, Akkerman OW, de Lange WCM, van Soolingen D, Kosterink JGW, van der Werf TS, Wilffert B, Touw DJ, Alffenaar J-WC (2016) Pharmacokinetic/pharmacodynamic-based optimization of levofloxacin administration in the treatment of MDR-TB. J Antimicrob Chemother 164
68. Van Deun A, Decroo T, Kuaban C, Noeske J, Piubello A, Aung KJM, Rieder HL (2019) Gatifloxacin is superior to levofloxacin and moxifloxacin in shorter treatment regimens for multidrug-resistant TB. Int J Tuberc Lung Dis 23:965–971
69. WHO (2020) WHO consolidated guidelines on tuberculosis. Module 4: treatment—drug-resistant tuberculosis treatment. World Health Organization, Geneva
70. Van Deun A, Decroo T, Tahseen S, Trébucq A, Schwoebel V, Ortuño-Gutiérrez N, de Jong BC, Rieder HL, Piubello A, Chiang C-Y (2020) World Health Organization 2018 treatment guidelines for rifampicin-resistant tuberculosis: uncertainty, potential risks and the way forward. Int J Antimicrob Agents 55:105822
71. Bolhuis MS, Tiberi S, Sotgiu G, De Lorenzo S, Kosterink JGW, van der Werf TS, Migliori GB, Alffenaar J-WC (2015) Linezolid tolerability in multidrug-resistant tuberculosis: a retrospective study. Eur Respir J 46:1205–1207
72. Bolhuis MS, van der Werf TS, Kerstjens HAM, de Lange WCM, Alffenaar J-WC, Akkerman OW (2019) Treatment of MDR-TB using therapeutic drug monitoring: first experiences with sub-300 mg linezolid dosages using in-house made capsules. Eur Respir J 1900580
73. Shean K, Streicher E, Pieterson E, Symons G, van Zyl SR, Theron G, Lehloenya R, Padanilam X, Wilcox P, Victor TC, van Helden P, Grobusch MP, Groubusch M, Warren R, Badri M, Dheda K (2013) Drug-associated adverse events and their relationship with outcomes in patients receiving treatment for extensively drug-resistant tuberculosis in South Africa. PLoS ONE 8:e63057
74. Arnold A, Cooke GS, Kon OM, Dedicoat M, Lipman M, Loyse A, Chis Ster I, Harrison TS (2017) Adverse effects and choice between the injectable agents Amikacin and Capreomycin in multidrug-resistant tuberculosis. Antimicrob Agents Chemother 61
75. Dijkstra JA, van der Laan T, Akkerman OW, Bolhuis MS, de Lange WCM, Kosterink JGW, van der Werf TS, Alffenaar JWC, van Soolingen D (2018) In vitro susceptibility of *Mycobacterium tuberculosis* to Amikacin, Kanamycin, and Capreomycin. Antimicrob Agents Chemother 62
76. Dijkstra JA, Van Altena R, Akkerman OW, de Lange WCM, Proost JH, van der Werf TS, Kosterink JGW, Alffenaar JWC (2015) Limited sampling strategies for therapeutic drug monitoring of amikacin and kanamycin in patients with multidrug-resistant tuberculosis. Int J Antimicrob Agents 46:332–337
77. van Altena R, Dijkstra JA, van der Meer ME, Borjas Howard JF, Kosterink JGW, van Soolingen D, van der Werf TS, Alffenaar JWC (2017) reduced chance of hearing loss associated with therapeutic drug monitoring of aminoglycosides in the treatment of multidrug-resistant tuberculosis. Antimicrob Agents Chemother 61
78. Alsaad N, Van Altena R, Pranger AD, van Soolingen D, de Lange WCM, van der Werf TS, Kosterink JGW, Alffenaar JWC (2012) Evaluation of cotrimoxazole in treatment of multidrug-resistant tuberculosis. Eur Respir J 42:504–512
79. Alsaad N, van der Laan T, Van Altena R, Wilting KR, van der Werf TS, Stienstra Y, van Soolingen D, Alffenaar JWC (2013) Trimethoprim/sulfamethoxazole susceptibility of *Mycobacterium tuberculosis*. Int J Antimicrob Agents 42:472–474

80. van Rijn SP, Zuur MA, Anthony R, Wilffert B, van Altena R, Akkerman OW, de Lange WCM, van der Werf TS, Kosterink JGW, Alffenaar J-WC (2019) Evaluation of carbapenems for treatment of multi- and extensively drug-resistant *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 63
81. van Rijn SP, van Altena R, Akkerman OW, van Soolingen D, van der Laan T, de Lange WCM, Kosterink JGW, van der Werf TS, Alffenaar J-WC (2016) Pharmacokinetics of ertapenem in patients with multidrug-resistant tuberculosis. Eur Respir J 47:1229–1234
82. Srivastava S, van Rijn SP, Wessels AMA, Alffenaar J-WC, Gumbo T (2016) Susceptibility testing of antibiotics that degrade faster than the doubling time of slow-growing mycobacteria: ertapenem sterilizing effect versus *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 60:3193–3195
83. Akkerman OW, Odish OFF, Bolhuis MS, de Lange WCM, Kremer HPH, Luijckx G-JR, van der Werf TS, Alffenaar J-W (2016) Pharmacokinetics of bedaquiline in cerebrospinal fluid and Serum in multidrug-resistant tuberculous meningitis. Clin Infect Dis 62:523–524
84. Skripconoka V, Danilovits M, Pehme L, Tomson T, Skenders G, Kummik T, Cirule A, Leimane V, Kurve A, Levina K, Geiter LJ, Manissero D, Wells CD (2013) Delamanid improves outcomes and reduces mortality in multidrug-resistant tuberculosis. Eur Respir J 41:1393–1400
85. Dawson R, Diacon AH, Everitt D, van Niekerk C, Donald PR, Burger DA, Schall R, Spigelman M, Conradie A, Eisenach K, Venter A, Ive P, Page-Shipp L, Variava E, Reither K, Ntinginya NE, Pym A, von Groote-Bidlingmaier F, Mendel CM (2015) Efficiency and safety of the combination of moxifloxacin, pretomanid (PA-824), and pyrazinamide during the first 8 weeks of anti-tuberculosis treatment: a phase 2b, open-label, partly randomised trial in patients with drug-susceptible or drug-resistant pulmonary tuberculosis. Lancet 385(9979):1738–1747
86. Salinger DH, Subramoney V, Everitt D, Nedelman JR (2019) Population pharmacokinetics of the antituberculosis agent pretomanid. Antimicrob Agents Chemother 63:5516
87. van der Burgt EPM, Sturkenboom MGG, Bolhuis MS, Akkerman OW, Kosterink JGW, de Lange WCM, Cobelens FGJ, van der Werf TS, Alffenaar J-WC (2016) End TB with precision treatment! Eur Respir J 47:680–682
88. Saktiawati AMI, Sturkenboom MGG, Stienstra Y, Subronto YW, Sumardi KJGW, van der Werf TS, Alffenaar J-WC (2016) Impact of food on the pharmacokinetics of first-line anti-TB drugs in treatment-naive TB patients: a randomized cross-over trial. J Antimicrob Chemother 71:703–710
89. Vu DH, Alffenaar JWC, Edelbroek PM, Brouwers JRBJ, Uges DRA (2011) Dried blood spots: a new tool for tuberculosis treatment optimization. Curr Pharm Des 17:2931–2939
90. Vu DH, Koster RA, Bolhuis MS, Greijdanus B, Altena RV, Nguyen DH, Brouwers JRBJ, Uges DRA, Alffenaar JWC (2014) Simultaneous determination of Rifampicin, clarithromycin and their metabolites in dried blood spots using LC-MS/MS. Talanta 121:9–17
91. Ghimire S, Bolhuis MS, Sturkenboom MGG, Akkerman OW, de Lange WCM, van der Werf TS, Alffenaar J-WC(2016) Incorporating therapeutic drug monitoring into the World Health Organization hierarchy of tuberculosis diagnostics. Eur Respir J 47:1867–1869
92. Abel L, Fellay J, Haas DW, Schurr E, Srikrishna G, Urbanowski M, Chaturvedi N, Srinivasan S, Johnson DH, Bishai WR (2018) Genetics of human susceptibility to active and latent tuberculosis: present knowledge and future perspectives. Lancet Infect Dis 18:e64–e75
93. Yuliwulandari R, Prayuni K, Susilowati RW, M Sofro AS, Tokunaga K, Shin J-G (2019) NAT2 slow acetylator is associated with anti-tuberculosis drug-induced liver injury severity in indonesian population. Pharmacogenomics 20:1303–1311
94. Mthiyane T, Millard J, Adamson J, Balakrishna Y, Connolly C, Owen A, Rustomjee R, Dheda K, McIlleron H, Pym AS (2020) N-acetyltransferase 2 genotypes among Zulu-speaking South Africans and isoniazid and n-acetyl-isoniazid pharmacokinetics during antituberculosis treatment. Antimicrob Agents Chemother 64

95. Sharma SK, Jha BK, Sharma A, Sreenivas V, Upadhyay V, Jaisinghani C, Singla R, Mishra HK, Soneja M (2014) Genetic polymorphisms of CYP2E1 and GSTM1 loci and susceptibility to anti-tuberculosis drug-induced hepatotoxicity. Int J Tuberc Lung Dis 18:588–593
96. Abulfathi AA, Decloedt EH, Svensson EM, Diacon AH, Donald P, Reuter H (2019) Clinical pharmacokinetics and pharmacodynamics of Rifampicin in human tuberculosis. Clin Pharmacokinet 58:1103–1129
97. Bolhuis MS, Akkerman OW, Sturkenboom MGG, de Lange WCM, van der Werf TS, Touw DJ, Alffenaar J-WC (2019) Different underlying mechanism might explain the absence of a significant difference in area under the concentration-time curve of linezolid for different ABCB1 genotypes. Ther Drug Monit 41:253–254
98. Bisson GP, Bastos M, Campbell JR, Bang D, Brust JC, Isaakadis P, Lange C, Menzies D, Migliori GB, Pape JW, Palmero D, Baghei P, Tabarsi P, Viiklepp P, Vilbrun S, Walsh J, Marks SM (2020) Mortality in adults with multidrug-resistant tuberculosis and HIV by antiretroviral therapy and tuberculosis drug use: an individual patient data meta-analysis. Lancet 396:402–411
99. Hasenbosch RE, Alffenaar JWC, Koopmans SA, Kosterink JGW, van der Werf TS, van Altena R (2008) Ethambutol-induced optical neuropathy: risk of overdosing in obese subjects. Int J Tuberc Lung Dis 12:967–971
100. Alffenaar J-W, van der Werf TS (2010) Dosing ethambutol in obese patients. Antimicrob Agents Chemother 54:404–405
101. Baker MA, Lin HH, Chang HY, Murray MB (2012) The risk of tuberculosis disease among persons with diabetes mellitus: a prospective cohort study. Clin Infect Dis 54:818–825
102. McAllister SM, Koesoemadinata RC, Santoso P, Soetedjo NNM, Kamil A, Permana H, Ruslami R, Critchley JA, van Crevel R, Hill PC, Alisjahbana B (2020) High tuberculosis incidence among people living with diabetes in Indonesia. Trans R Soc Trop Med Hyg 114:79–85
103. Ruslami R, Nijland HMJ, Adhiarta IGN, Kariadi SHKS, Alisjahbana B, Aarnoutse RE, van Crevel R (2010) Pharmacokinetics of anti-tuberculosis drugs in pulmonary tuberculosis patients with type 2 diabetes. Antimicrob Agents Chemother 54:1068–1074
104. Dekkers BGJ, Bolhuis MS, Beek Ter L, de Lange WCM, van der Werf TS, Alffenaar J-WC, Akkerman OW (2019) Reduced moxifloxacin exposure in patients with tuberculosis and diabetes. Eur Respir J 54
105. Ruesen C, Chaidir L, Ugarte-Gil C, van Ingen J, Critchley JA, Hill PC, Ruslami R, Santoso P, Huynen MA, Dockrell HM, Moore DAJ, Alisjahbana B, van Crevel R (2020) Diabetes is associated with genotypically drug-resistant tuberculosis. Eur Respir J 55
106. Laserson KF, Thorpe LE, Leimane V, Weyer K, Mitnick CD, Riekstina V, Zarovska E, Rich ML, Fraser HSF, Alarcón E, Cegielski JP, Grzemska M, Gupta R, Espinal M (2005) Speaking the same language: treatment outcome definitions for multidrug-resistant tuberculosis. Int J Tuberc Lung Dis 9:640–645
107. Wouda EMN, Stienstra Y, van der Werf TS, Kerstjens H, de Lange WCM, Coppes M, Kuijlen J, Tepper M, Akkerman OW (2017) Neurological and functional recovery in tuberculosis patients with spinal cord injury in The Netherlands. Neuro Rehabil 40:439–445
108. Romanowski K, Baumann B, Basham CA, Ahmad Khan F, Fox GJ, Johnston JC (2019) Long-term all-cause mortality in people treated for tuberculosis: a systematic review and meta-analysis. Lancet Infect Dis 19:1129–1137
109. Molton JS, Huggan PJ, Archuleta S (2015) Infliximab therapy in two cases of severe neurotuberculosis paradoxical reaction. Med J Aust 202:156–157
110. Prasad K, Singh MB, Ryan H (2016) Corticosteroids for managing tuberculous meningitis. Cochrane Database Syst Rev 4:CD002244
111. Done MM, Akkerman OW, Al-Kailany W, de Lange WCM, de Jonge G, Kleinnijenhuis J, Stienstra R, van der Werf TS (2020) Corticosteroid therapy for the management of paradoxical inflammatory reaction in patients with pulmonary tuberculosis. Infection 48:641–645

112. Kroesen VM, Gröschel MI, Martinson N, Zumla A, Maeurer M, van der Werf TS, Vilaplana C (2017) Non-steroidal anti-inflammatory drugs as host-directed therapy for tuberculosis: a systematic review. Front Immunol 8:772
113. Prabowo SA, Gröschel MI, Schmidt EDL, Skrahina A, Mihaescu T, Hastürk S, Mitrofanov R, Pimkina E, Visontai I, de Jong B, Stanford JL, Cardona P-J, Kaufmann SHE, van der Werf TS (2013) Targeting multidrug-resistant tuberculosis (MDR-TB) by therapeutic vaccines. Med Microbiol Immunol 202:95–104
114. Gröschel MI, Prabowo SA, Cardona P-J, Stanford JL, van der Werf TS (2014) Therapeutic vaccines for tuberculosis—a systematic review. Vaccine 32:3162–3168
115. Schrager LK, Vekemens J, Drager N, Lewinsohn DM, Olesen OF (2020) The status of tuberculosis vaccine development. Lancet Infect Dis 20:e28–e37
116. Huang C-Y, Hsieh W-Y (2017) Efficacy of *Mycobacterium vaccae* immunotherapy for patients with tuberculosis: a systematic review and meta-analysis. Hum Vaccin Immunother 13:1960–1971
117. Sharma SK, Katoch K, Sarin R, Balambal R, Kumar Jain N, Patel N, Murthy KJR, Singla N, Saha PK, Khanna A, Singh U, Kumar S, Sengupta A, Banavaliker JN, Chauhan DS, Sachan S, Wasim M, Tripathi S, Dutt N, Jain N, Joshi N, Penmesta SRR, Gaddam S, Gupta S, Khamar B, Dey B, Mitra DK, Arora SK, Bhaskar S, Rani R (2017) Efficacy and safety of *Mycobacterium indicus pranii* as an adjunct therapy in category II pulmonary tuberculosis in a randomized trial. Sci Rep 7:3354
118. Cardona P-J, Amat I, Gordillo S, Arcos V, Guirado E, Díaz J, Vilaplana C, Tapia G, Ausina V (2005) Immunotherapy with fragmented *Mycobacterium tuberculosis* cells increases the effectiveness of chemotherapy against a chronical infection in a murine model of tuberculosis. Vaccine 23(11):6
119. Vilaplana C, Montané E, Pinto S, Barriocanal AM, Domenech G, Cardona PJ, Costa J (2010) Double-blind, randomized, placebo-controlled phase I clinical trial of the therapeutical antituberculous vaccine RUTI®. Vaccine 28:11
120. Fox GJ, Mitnick CD, Benedetti A, Chan ED, Becerra M, Chiang C-Y, Keshavjee S, Koh WJ, Shiraishi Y, Viiklepp P, Yim J-J, Pasvol G, Robert J, Shim TS, Shin SS, Menzies D, Collaborative Group for Meta-analysis of Individual Patient Data in MDR-TB, Ahuja S, Ashkin D, Avendaño M, Banerjee R, Bauer M, Burgos M, Centis R, Cobelens F, Cox H, D'Ambrosio L, de Lange WCM, DeRiemer K, Enarson D, Falzon D, Flanagan K, Flood J, Gandhi N, Garcia-Garcia L, Granich RM, Hollm-Delgado MG, Holtz TH, Hopewell P, Iseman M, Jarlsberg LG, Kim HR, Lancaster J, Lange C, Leimane V, Leung CC, Li J, Menzies D, Migliori GB, Narita M, Nathanson E, Odendaal R, O'Riordan P, Pai M, Palmero D, Park SK, Pena J, Pérez-Guzmán C, Ponce-de-Leon A, Quelapio MID, Quy HT, Riekstina V, Royce S, Salim M, Schaaf HS, Seung KJ, Shah L, Shean K, Sifuentes-Osornio J, Sotgiu G, Strand MJ, Sung SW, Tabarsi P, Tupasi TE, Vargas MH, van Altena R, van der Walt M, van der Werf TS, Westenhouse J, Yew WW (2016) Surgery as an adjunctive treatment for multidrug-resistant tuberculosis: an individual patient data metaanalysis. Clin Infect Dises 62:887–895
121. Fox GJ, Benedetti A, Mitnick CD, Pai M, Menzies D, Collaborative group for meta-analysis of individual patient data in MDR-TB (2016) propensity score-based approaches to confounding by indication in individual patient data meta-analysis: non-standardized treatment for multidrug resistant tuberculosis. PLoS ONE 11:e0151724
122. Borisov SE, D'Ambrosio L, Centis R, Tiberi S, Dheda K, Alffenaar J-W, Amale R, Belilowski E, Bruchfeld J, Canneto B, Denholm J, Duarte R, Esmail A, Filippov A, Davies Forsman L, Gaga M, Ganatra S, Igorevna GA, Lazaro Mastrapa B, Manfrin V, Manga S, Maryandyshev A, Massard G, González Montaner P, Mullerpattan J, Palmero DJ, Pontarelli A, Papavasileiou A, Pontali E, Romero Leyet R, Spanevello A, Udwadia ZF, Viggiani P, Visca D, Sotgiu G, Migliori GB (2019) Outcomes of patients with drug-resistant-tuberculosis treated with bedaquiline-containing regimens and undergoing adjunctive surgery. J Infect 78:35–39

123. Alffenaar JWC, van der Laan T, Simons S, van der Werf TS, van de Kasteele PJ, de Neeling H, van Soolingen D (2011) Susceptibility of clinical *Mycobacterium tuberculosis* isolates to a potentially less toxic derivate of Linezolid, PNU-100480. Antimicrob Agents Chemother 55:1287–1289
124. Bolhuis MS, van der Werf TS, Akkerman OW (2020) Treatment of highly drug-resistant pulmonary tuberculosis. N Engl J Med 382:2376–2377
125. Van Kampenhout E, Bolhuis MS, Alffenaar J-WC, Oswald LMA, Kerstjens HAM, de Lange WCM, van der Werf TS, Akkerman OW (2017) Pharmacokinetics of moxifloxacin and linezolid during and after pregnancy in a patient with multidrug-resistant tuberculosis. Eur Respir J 49:1601724



Tjip S. van der Werf trained as a tropical doctor and worked in Ghana, where he combined clinical work with research in tuberculosis and Buruli ulcer. After completing his Ph.D., he specialized in Pulmonary Medicine and worked for 13 years in Intensive Care but continued research in tuberculosis and Buruli ulcers after completing his Ph.D. He has been a full professor in Infectious Disease since 2006 and supervised most of his (38+) Ph.D. students on Buruli ulcer and Tuberculosis research. He published over 388 papers in PubMed, over 20 book chapters, and contributed to Buruli ulcer in UpToDate. His H-factor is 47; he retired from clinical work in 2020 while he continued his research activities.

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Important Targets and Inhibitors of *Mycobacterium tuberculosis*

21

Sisir Nandi, Mridula Saxena, and Anil Kumar Saxena

All truths are easy to understand once they are discovered; the point is to discover them.

Galileo Galilei

Summary

Tuberculosis (TB) is a global health issue. Millions of TB-infected patients remain undiagnosed, untreated, or improperly treated, leading to recurrence, re-infection, and resistance. Exploration of disease virulence via target analyses may provide the design of specific anti-TB therapeutics. Studies and research are ongoing to investigate different potential target enzymes of *Mycobacterium tuberculosis* necessary for its growth and survival. The identification of targets throws light of hope to design inhibitors that will impede the functioning of the target enzyme. TB treatment includes two months of an initial course of isoniazid, rifampin, pyrazinamide, and ethambutol, termed as first-line drugs, along with four months of additional course on isoniazid and rifampin. The necessity to continue studies of different targets and inhibitors is based on the fact of resistance of the drugs to different targets. Multidrug resistance (MDR-TB) arises due to non-compliance of the treatment in terms of missing of any one of the above-mentioned dosages thereby causing failure of the mycobacteria to respond to anti TB drugs and the XDR-TB results from infection of *Mycobacterium avium* complex when the patients also suffer from

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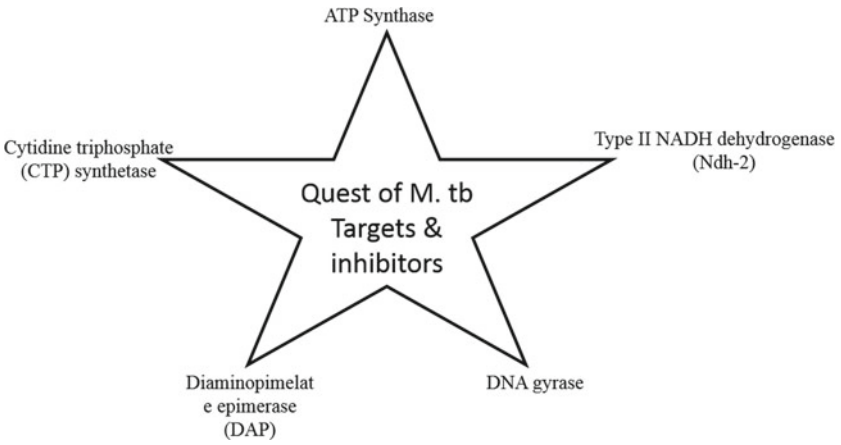
infectious HIV. This necessitates the use of second-line drugs, which include 6-fluoroquinolone (6-FQ) followed by amikacin or kanamycin. There is a need to develop new lead compounds with the least side effects to overcome the resistance problem to several drugs. Therefore, the knowledge of several drugs is of utmost importance in designing new inhibitors that fill the treatment gap to minimize drug resistance.

Graphical Abstract



Mycobacterium tuberculosis (*M. tb*) targets and their potential inhibitors

Keywords

ATP synthase · ClpC1 · Cytidine triphosphate synthetase · Diaminopimelate epimerase · DNA gyrase · IdeR · LipY · Lysine ε-aminotransferase · *M. tb* · MraY · NADH dehydrogenase

1 Introduction

Tuberculosis (TB) is an infectious disease due to *Mycobacterium tuberculosis* (*M. tb*) which attacks the lungs and other parts such as the kidney, brain, or spine. It is spread through respiratory droplets with 0.5–5.0 μm in diameter, floating in the air. TB-infected patients suffer from high fever, cough, fatigue, chest pain, weakness, loss of weight, and sweating at night. In 2018, the World Health Organization (WHO) reported that about ten million people, including about six million men, three million women, and one million children, contracted TB, and 1.5 million died. There were 205,000 TB-associated deaths in children, including children

co-infected with the human immune deficiency virus (HIV) and TB. TB attacks immune-compromised patients badly [1].

TB-infected individuals cough or sneeze, and through micro respiratory droplets, the healthy fellow who comes is in the vicinity of this micro density region of infected droplets becomes a victim. The contaminated droplets reach the alveolar sacs and enter alveolar macrophages to assault and multiply. *M. tb* is recognized as non-self and therefore undergoes phagocytosis, a process in which the macrophages encase *M. tb* and store it in a membrane-bound vesicle, known as a phagosome. Phagolysosome is, then, formed as the result of attachment between the phagosomes and the lysosomes to promote reactive oxygen species (ROS) and acid production that help to eradicate the *M. tb* [2].

TB is a curable disease treated with a WHO-recommended directly observed treatment short (DOTS) course comprising of:

- an initial phase, patients are given a two-month supply of a combination of four antimicrobials, e.g., isoniazid, rifampicin, pyrazinamide, and ethambutol; and
- a continuation phase, patients continue the treatment for four months with the first-line medicines: isoniazid and rifampicin [3].

Intentional or unintentional missing doses may develop multidrug-resistant tuberculosis (MDR-TB), a public health issue [4]. Second-line chemotherapeutics might help treat MDR-TB, e.g., as 6-fluoroquinolones (6-FQ) and one injectable medicine, e.g., kanamycin, capreomycin, or amikacin [5]. TB has created an emergency burden to India, with 79,000 deaths out of 24 million infection cases last year, followed by China, Indonesia, Pakistan, Nigeria, Bangladesh, and South Africa [1]. The most dreadful strains include *M. tuberculosis*, *M. tuberculosis hominis*, *M. tuberculosis bovis* (bovine strain), whereas the less virulent common strains *include M. microti, M. pinnipedii* (TB in wild and domesticated animals), *and M. canettii.* The non-pathogenic strains are *M. africanum* and *M. smegmatis* [2]. So, these strains have many new targets responsible for the disease virulence. New tuberculosis targets should be explored for their specific inhibitors to unlock the emergent trends of disease. Potential *M. tb* targets, e.g., InhA, ATPase, MmpL3, DprE1, QcrB, and MenA, along with their inhibitors, have been described recently [6, 7]. Genetic combinations of InhA and KasA play a role in the mycobacterial cell wall formation as parts of fatty acid components, which can be inhibited by isoniazid, triclosan, pyridomycin, NITD-916, PT70, GSK625, and GSK693. The MmpL3 is an inner membrane lipid transporter essential for *M. tb to* become more resistant, nodulate, and divide. Potential MmpL3 inhibitors are AU1235, THPP1, SQ109, Rimonabant, and BM212. The DprE1 representing decaprenylphospho-beta-D-ribofuranose 2-oxidase synthesizes arabinogalactan of the *M. tb* cell wall. The DprE1 inhibitors are BTZ043, PBTZ169, TCA1, Ty38c, and CT325. The mitochondrial enzyme QcrB helps in the electron transport chain (ETC), which can be inhibited by AX-35 and Q203. Menaquinone (MenA) is also an important component of the mycobacterial electron transport system. Inhibition of MenA

enzyme by the potent compound NM4 blocks the production of ATP [6]. Apart from the targets mentioned above, the more important *M. tb* targets are discussed in this chapter.

2 New *M. tb* Targets and Their Potential Inhibitors

New *M. tb* targets like ATP synthase, type II NADH dehydrogenase, DNA gyrase, diaminopimelate epimerase, triacylglycerol lipase (LipY), cytidine triphosphate synthetase, transcription factor IdeR, lysine ε-aminotransferase, ClpC1, and phospho-MurNAc-pentapeptide translocase (MraY) along with their potential inhibitors have been considered to develop potential chemotherapy medicines for MDR and XDR-TB.

2.1 ATP Synthase and Its Potential Inhibitors

ATP synthase is the final component of the electron transport chain that leads to the production of adenosine triphosphate (ATP), the energy input of the cell needed by the organisms for their survival. The process occurs via oxidative phosphorylation, which leads to oxygen production. It has a complex structure consisting of two components, F0 and F1. The F0 being an integral membrane protein, is hydrophobic and is responsible for proton motive force generation, while F1, a hydrophilic component projecting into the cytosol, plays a major role in producing ATP from ADP and inorganic phosphates [8]. The ATP is generated by protons passing through the ATPase channel that drives the rotation of the c subunit of F0, which acts as the rotor, and its movement is the cause of rotation of the gamma subunit of F1, but both move opposite to each other [9]. The ATP synthesis, thus, maintains the viability and energy metabolism of mycobacteria. To target inhibition of ATP synthase, diarylquinolines (DARQs) have been shown as a promising template for the generation of anti-TB drugs [10].

R207319, R126470, and R207910 could act as promising DARQ compounds. The most potent is R207910 or TMC207, with a 0.06 μg/ml MIC. The most active DARQ is TMC207, also known as Bedaquiline [11], a member of naphthalenes with a quinoline ring [12]. It blocks both the c subunit [13] and the ε subunit of ATP synthase [14], which ultimately hampers ATP production. It bears amine and alcohol side chains of the central quinoline ring, which contributes to its excellent antimicrobial activity and also shows its profound inhibitory effect against MDR and XDR strain of mycobacteria [15, 16]. Bedaquiline is an FDA-approved drug and is clinically used. The most potent and recently developed ATP synthase inhibitors have been given in Fig. 1.

An important congeneric DARQs compound, TBAJ-876, having a 3,5-dialkoxy-4-pyridyl group, has been developed as a very promising drug. It causes a reduction in the production of ATP by blocking the activity of ATP synthase. The

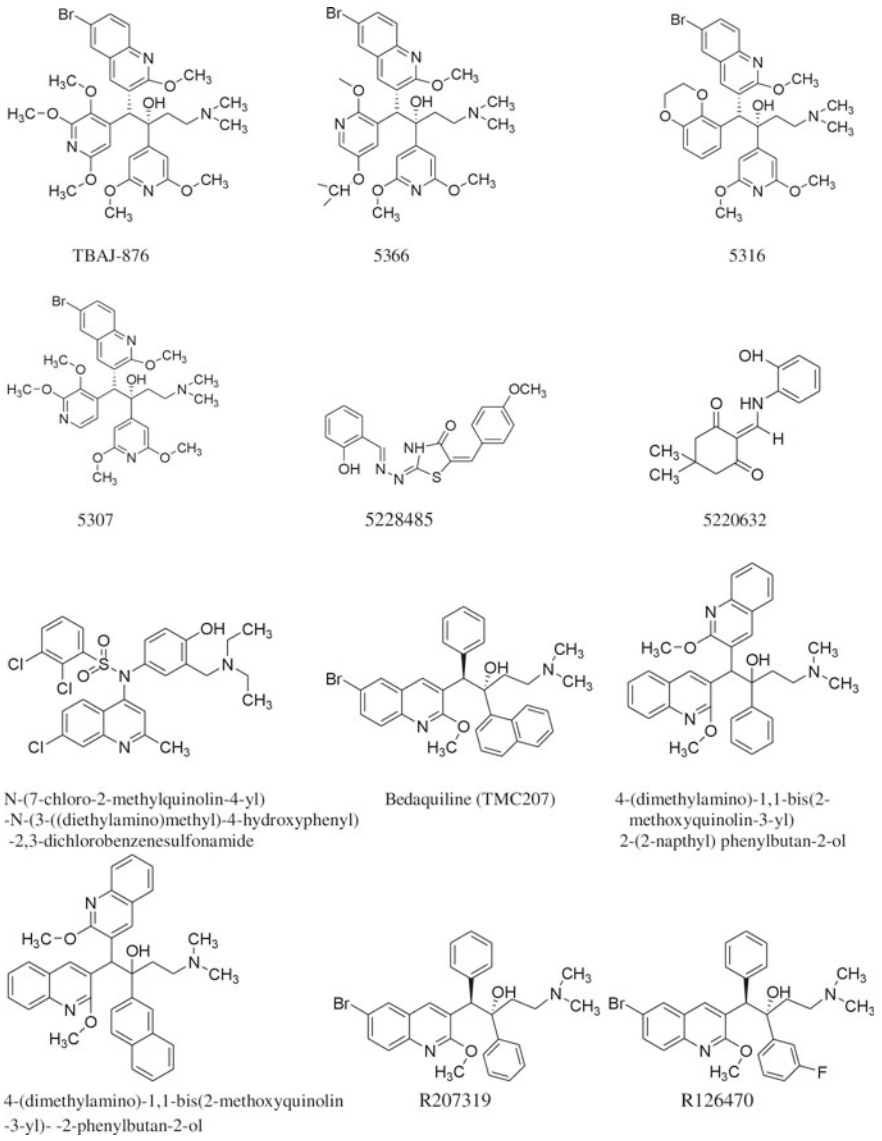


Fig. 1 Most potent ATP synthase inhibitors recently developed to combat *M. tb*

characteristics of high clearance rate, low lipophilic nature, shortening of the time of treatment [17–21], and a good IC50 value of 0.031–0.2 nM [19] enabled the designing of several analogs of the drugs like TBAJ-5366, 5316, and 5307 [22] having inhibitory activities of *M. tb* and *M. smegmatis*.

Because the existence of a quinoline ring in the compound has better bactericidal properties, screening of 700 bisquinoline analogs was done, which led to the

generation of two compounds, 5,228,485 and 5,220,632, that belong to the class thiazolidine and cyclohexanedione, respectively [23]. The compounds have MIC of 0.5 and 2.0 μg/ml respectively against *M. tb* [7] and were formed by substituting the phenyl moiety with the quinoline group.

Different chloroquinoline compounds were synthesized against *M. tb*, of which N-(7-chloro-2-methylquinolin-4-yl)-N-(3-((diethylamino)methyl)-4-hydroxyphenyl)-2,3-dichlorobenzene sulfonamide showed equivalent activity against non-replicating bacteria in a hypoxic environment. It was designed by altering the 2,3 dichlorophenyl group of the parent molecule to inhibit the ATP synthase enzyme. Its MIC and IC_{50} values are 3.12 μg/mL and 0.51 ± 0.030 μM [24]. Its CC_{50} value of >300 μg/mL shows its non-toxic nature to the Vero cell line. The mode of binding of this inhibitor toward ATP synthase has been explored by Singh et al. [25].

Kalia et al. designed many bisquinoline compounds by replacing the phenyl group with a quinoline moiety of the parent compound, TMC207. Among them, 4-(dimethylamino)-1,1-bis(2- methoxyquinoline-3-yl)-2-phenylbutan-2-ol and 4-(dimethylamino)-1,1-bis(2- methoxyquinoline-3-yl)-2-(2-naphthyl) phenylbutan-2-ol lowered intracellular colony forming units (CFUs) of *M. tb* to about 90% and 91%, respectively, which shows its potency to be a highly active drug. The respective ATPase IC_{50} values of the two compounds are 0.07 and 0.03 μM, and both fit into the enzyme's binding pocket as proved by molecular docking studies [26].

Saxena et al. built a model describing the variation in ATP synthase inhibitory activity in substituted quinolones with respect to the physicochemical parameters in terms of hydrophobicity, electronic and steric factors. According to the model, called quantitative structure-activity relationship (QSAR), the major influencing parameters are the positively contributing molar refractivity while negatively contributing lipophilicity and Connolly Molecular Area and also in the external data set. It was also concluded that the racemic compounds might also be used in the analysis by using the mean values of the computed 3D parameters of the enantiomers. Further, the observed similar dependence of antitubercular activity in whole *M. tb* H37Rv on the same parameters as for ATP synthase inhibition unambiguously proves that the target enzyme for the antitubercular activity in these molecules is ATP synthase [27].

2.2 Type II NADH Dehydrogenase and Its Potential Inhibitors

Type II NADH dehydrogenase (Ndh-2) is a 50 kDa weight membrane-bound protein of *M. tb* whose role is necessary for generating ATP by oxidative phosphorylation [28, 29]. Mycobacteria also possess both type I NADH dehydrogenase and type II NADH dehydrogenases, where unlike the former, the latter is an indispensable enzyme and thus has emerged to be an essential target. There are two copies of the enzyme: Ndh And NdhA, whose individual functions remain unknown, but the enzyme catalyzes the oxidation of NADH to NAD+ combining with menaquinone reduction as the major path for entry of electrons in the electron transport chain [30].

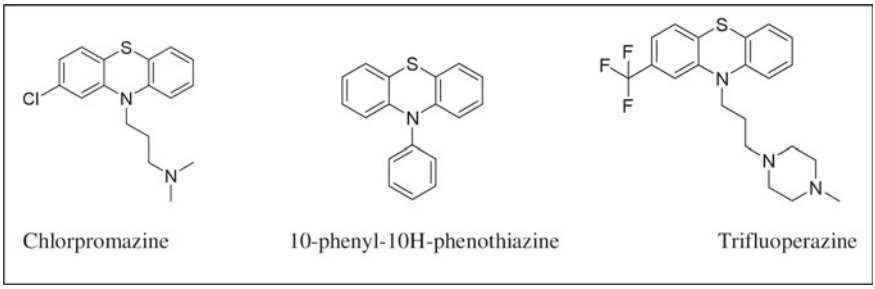


Fig. 2 Potent phenothiazines as Ndh-2 inhibitors

Different phenothiazine derivatives have been explored to find potential Ndh-2 inhibitors (Fig. 2). Among them, chlorpromazine has been found to inhibit the growth of intracellular *M. tb* by targeting Ndh-2 [31]. It also boosts the activity of many drugs against mycobacteria residing intracellularly as it functions mostly against intracellular bacteria than against extracellular ones [32]. It has CC_{50} and MIC_{90} value of 8 mg/mL and 22 mg/mL, respectively [31].

Several phenothiazine derivatives were designed and synthesized in the hope of finding a lead molecule with enhanced activity. The compound 10-phenyl-10H-phenothiazine, having a contrasting form and structural moiety, unlike other antimycobacterial drugs, showed enhanced activity against *M. tb* with greater potency compared to the parent compound chlorpromazine on account of its excellent MIC_{90} value of 4 mg/mL and $CC_{50} > 32$ mg/mL towards HepG2 cell lines [31].

Trifluoperazine, another member of phenothiazines, damages ATP homeostasis by effectively targeting the *M. tb* Ndh-2 enzyme. Additionally, it also inhibits malonyl coenzyme A-acyl-carrier-protein transacylase responsible for synthesizing fatty acid. Its major potential is to cause a reduction of bacterial load accumulating in the macrophage since phenothiazine shows its inhibitory action by assembling in the macrophage for inhibiting the growth of both replicating as well as dormant bacteria. The in vitro and ex vivo studies show that it can suppress and inhibit *M. tb* JAL2287 and *M. tb* 1934, two MDR isolates with MIC of 7.5 and 2.5 μg/ml, respectively, thus proving its efficiency to hinder the growth of MDR-TB [33].

Biological screening of a library of acetamide compounds led to the identification of 2-mercapto-quinazolinones, of which 2-(3,4-dihydro-4-oxoquinazolin-2-ylthio)-N-cyclohexylacetamide and 2-(3,4-dihydro-4-oxoquinazolin-2-ylthio)-N-benzylacetamide (Fig. 3) have been chosen to inhibit type II NADH dehydrogenase, the first complex of the electron transport chain. Both the compounds had good hepatic microsomal stability of 2.3 mL/min/g and 1.8 mL/min/g in mice, respectively, with average kinetic stability. The compound 2-(3,4-dihydro-4-oxoquinazolin-2-ylthio)-N-cyclohexylacetamide with potent ligand-lipophilicity efficiency (LLE) drug-likeness profile has exquisitely good human microsomal stability, and there was no cytotoxic effect in the HepG2 cell line [34]. Several group substitutions and modifications have been reported for finding a better lead with improved activity.

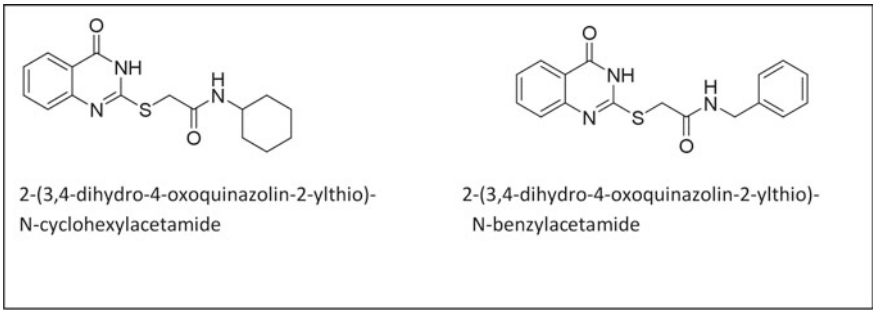


Fig. 3 Potent acetamides as Ndh-2 inhibitors

Clofazimine (CFZ) (Fig. 4), a derivative of riminophenazine [35–37], is another candidate drug against MDR-TB [38] known to inhibit Ndh-2. Its mechanism of action is based on a cycle of reduction and oxidation and has the unique ability of inhibition by generating reactive oxygen species (ROS). The hydrophobic nature of the drug proves its interaction with NADH-2 dehydrogenase, which is responsible for its reduction. Its reduced form is further oxidized by O_2, leading to ROS production, killing the mycobacteria. The inability of succinate dehydrogenase, the second complex of ETC, to reduce CFZ makes Ndh-2 reduction. It is ineffective against gram-negative bacteria but is sensitive against gram-positive bacteria such as *Staphylococcus*, *Streptococcus*, *Enterococcus*, and *Listeria species* and mycobacteria like *M. tb* [39].

CBR-1825 and CBR-4032 which are chemically known as 2-(3,4,5,6,7,8-hexahydro-4-oxoquinazolin-2-ylthio)-N-cyclohexylacetamide) having thioquinazoline core and (5-((5- chlorothiophene-3-yl)methylamino)-1-allyl-4,5,6,7-tetrahydro-1H-indazol-3-yl)(thiomorpholine)methanone possessing tetrahydroindazole scaffold have MIC_{50} values of 0.43 μM and 6.6 μM, respectively. These compounds stop the synthesis of ATP by blocking Ndh-2. Both the compounds show negligible cytotoxicity of less than 0.50 μM towards mammalian cell lines. Several structural analogs of thioquinazoline have been generated. The fused cyclohexyl has been replaced by phenyl moiety to produce CBR-5992 which is

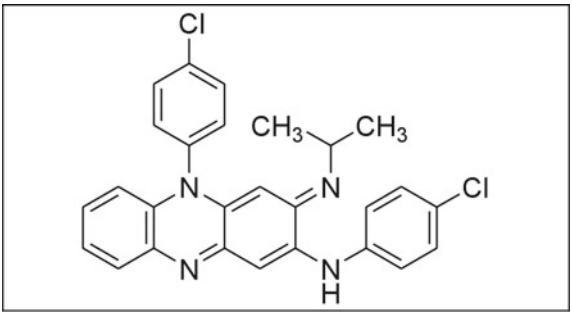


Fig. 4 Clofazimine as Ndh-2 inhibitor

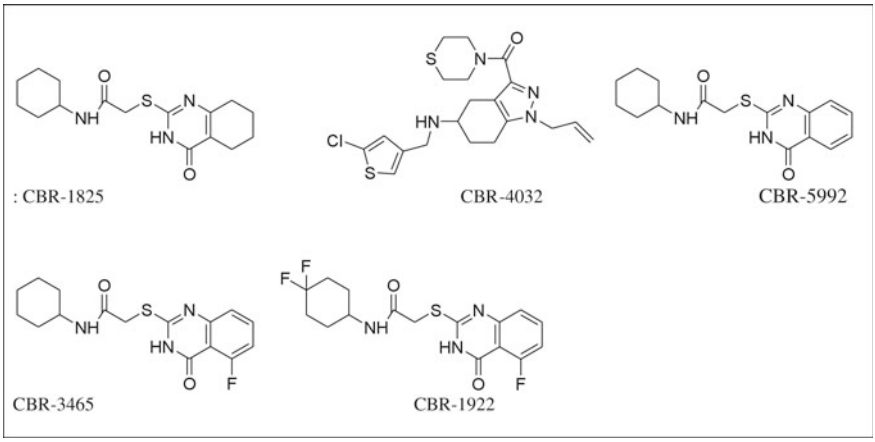


Fig. 5 More potent Ndh-2 inhibitors

twice as active as the initial hit CBR-1825 as it has a MIC of 0.67 μM. Further, the modification was done on the fused phenyl ring by introducing fluoro moiety at the fifth position to generate CBR3465 having MIC of 0.16 μM. The compound CBR-1922, chemically known as (5-fluoro-3,4-dihydro-4-oxoquinazolin-2-ylthio)-N-(4,4-difluorocyclohexyl)acetamide, has been formed by inserting a difluoro moiety in the acetamidocyclohexyl ring is far more active and potent than the original compound having MIC of 0.09 μM against *M. tb* [30] (Fig. 5).

2.3 DNA Gyrase and Its Potential Inhibitors

M. tb DNA gyrase is a type II topoisomerase enzyme that catalyzes an important reaction in DNA replication [40]. The unfolding of the double-helical strand of DNA produces strain during replication. The DNA gyrase enzyme plays a major role in releasing and relieving the strain to aid in replication. It also participates in introducing negative supercoils in the DNA by hydrolyzing ATP. The structure of this enzyme comprises two subunits: GyrA and GyrB [41]. The mode of action of the enzyme is that firstly DNA binds to the enzyme subunit in its active pocket, the tyrosine residue in GyrA cleaves the DNA, causing a double-stranded break, and another part of the segment is allowed to pass through the created break, which is then sealed again. This reaction is favored by ATP hydrolysis that takes place in the GyrB subunit. Without this enzyme, the topological state of DNA will be hampered [42].

Fluoroquinolones are a novel group of antibiotics that target DNA gyrase and inhibit the activity of the enzyme, thereby hampering the process of replication [43]. Ciprofloxacin, moxifloxacin, gatifloxacin, sparfloxacin, ofloxacin, trovafloxacin, and Isothiazolinone are some of the fluoroquinolone compounds (Fig. 6)

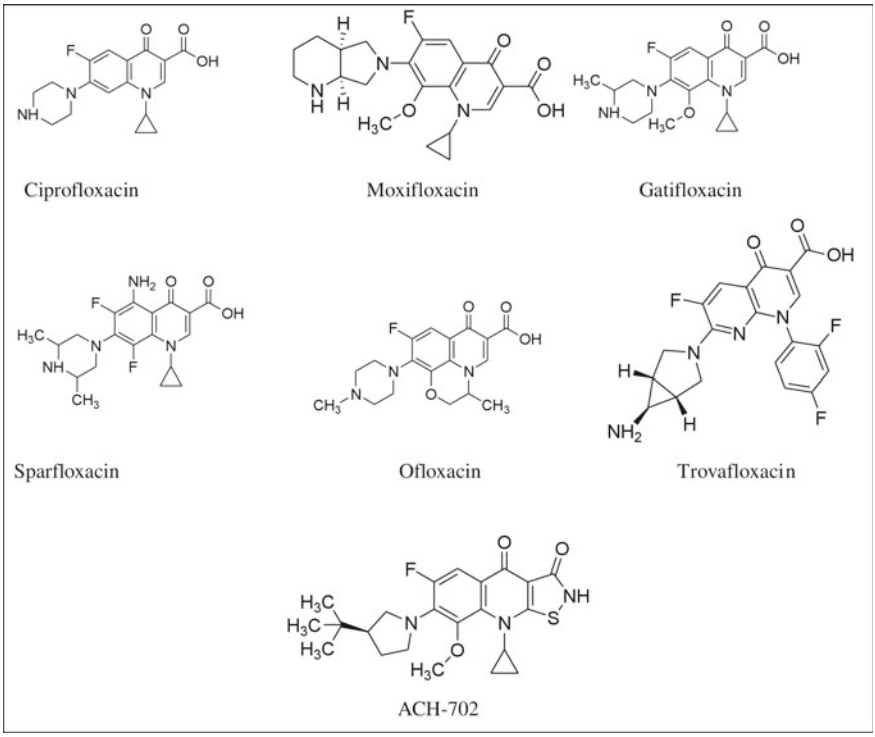


Fig. 6 Potent antitubercular 6-FQs

described here that are effective in killing *M. tb* by targeting DNA gyrase. It increases DNA breaks, thus altering the enzymatic activity and killing the mycobacteria [43].

The SAR and pharmacophore studies on 6-FQ show that a ternary stacking complex is formed with the mycobacterial DNA gyrase by the aromatic FQ ring and the N1 substituents where the hydrogen bond is formed between DNA and C-4 carbonyl and C-3 carboxylate group while the C-7 and 6-fluoro substituent along with carboxylate group interact at the enzyme binding site which makes the complex stable and responsible for the biological activity of FQs [44, 45]. From the pharmacophore model developed by Nandi et al., the substituent hydrophobicity at R7, R8 position, and hydrogen bond acceptance of the substituent at R8 position are very important for the prediction of DNA gyrase inhibition. Further, tertiary nitrogen atoms present in the heterocyclic rings at the R7 position are favorable. They may provide the positive ionization to interact with the negative heads of the mycobacterial DNA gyrase [46].

Ciprofloxacin belongs to the second-generation fluoroquinolone class of compounds that exert antibacterial action against both gram-positive and gram-negative bacteria but shows increased efficacies against gram-negative bacteria. The

existence of functional groups (cyclopropyl, carboxylic, fluoro, and piperazine-1-yl at positions 1, 3, 6, and 7, respectively) is an important property for its bactericidal effect [47]. It generally inhibits the activity of DNA gyrase by binding to it and is also known to inhibit the cytochrome P450 system of enzymes [43].

Moxifloxacin is a synthesized 8-methoxy fluoroquinolone [48], showing its bactericidal activity against gram-negative and gram-positive bacteria [43]. It inhibits the DNA replication process by targeting DNA gyrase. The good anaerobic property of this drug on account of having a methoxy group at position 8 is an important property of this fluoroquinolone group of compounds [43]. It also possesses excellent sterilizing properties and bactericidal activity better than isoniazid [49]. Studies have demonstrated that a combination of rifampicin and pyrazinamide with moxifloxacin has better activity than the standard DOTS regimen. Therefore, this drug is predicted to lower the duration of treatment [50]. Gatifloxacin, like moxifloxacin, is also an 8-methoxy fluoroquinolone compound that exerts its anti-bactericidal activity by inhibiting the action of the DNA gyrase enzyme [51]. It also belongs to a fourth-generation compound similar to moxifloxacin and has strong sterilizing activity [52–54]. But unlike moxifloxacin, this drug had been abandoned from the market on account of the high risk of dysglycaemia, which has been reported after using it [50]. Sparfloxacin is a member of the third-generation fluoroquinolone family. It acts by blocking the bacterial DNA gyrase enzyme [43]. Despite the activity, it has been withdrawn from the market on account of its side effects of causing elongation of the QTc cycle [55]. Ofloxacin is also a second-generation fluoroquinolone like ciprofloxacin. Nalidixic acid is the parent compound from which it has been derived synthetically [56]. It functions by hampering DNA gyrase's catalytic activity, which is implicated in DNA supercoiling. The third ring of ofloxacin contains a methyl group that adds to the antibacterial ability of the drug [43].

Trovafloxacin is a newer broad-spectrum drug that also displays increased antibacterial activity against anaerobes [43] and gram-negative and gram-positive bacteria. It may also be used in case of resistance to drugs in TB [57]. It acts on DNA gyrase and blocks the activity, thereby preventing replication. It possesses a typical bicyclic ring complex at position 7 [43]. It has been reported to cause liver toxicity and damage, which led to its withdrawal from the market [58].

Isothiazoquinolone is another class of compounds similar to quinolones based on structure, exhibiting bactericidal effects against bacteria, including those that show resistance to fluoroquinolones [59, 60]. By substituting the carboxylic acid moiety in the C-3 ring of fluoroquinolone by isothiazole, being more acidic than carboxylic acid, it becomes more potent against the enzyme, which interacts by hydrogen bonding with it and stopping its function [61]. Among the Isothiazoquinolone library consisting of different compounds, the lead is ACH-702 due to its excellent protein binding, inhibitory role, and anti-bactericidal effect [62].

2.4 Diaminopimelate (DAP) Epimerase and Its Potential Inhibitors

Diaminopimelate epimerase is an enzyme of the racemase family, which catalyzes the conversion of LL-2,6-diaminoheptanedioate to meso-diaminoheptanedioate. It is also involved in the synthesis of lysine [63, 64]. These two products, i.e., meso-DAP and lysine, participate in synthesizing the cell wall peptidoglycan layer of *M. tb* by cross-linking [65]. Both the gram-positive and gram-negative bacteria possess this enzyme for forming the cell wall, which maintains the shape and structure of the entire cell. Mammals are devoid of this enzyme [66].

The NRB05197, chemically known as 6,6-di(benzylthio)hexane-1,2,3,4,5-pentaol (Fig. 7), is a derivative of pentanol and a very active inhibitor that binds strongly with the target enzyme to stop the synthesis of a peptidoglycan layer. Based on the MABA assay (Micro Plate Alamar Blue Assay), it has been established that this compound causes inhibition of growth of *M. tb* to about 22% at 50 μM and 19.2% at 25 μM concentration, thus proving its excellent antibacterial ability.

The BTB13883, also known as 2,5-dioxotetrahydro-1H-pyrrol-1-yl laurate (Fig. 7), is another potent inhibitor of diaminopimelate epimerase, which, like NRB05197, was also selected on the basis of MABA assay among five compounds. It inhibits the growth of *M. tb* by about 13.5% at 25 μM and 17.5% at 50 μM concentration. Like the other three compounds, these two drugs also possess a good fitting into the active binding cavity of the enzyme with the free energy of −5.9 to 7.5 kcal/mol and H-bond interactions with the amino acid residues of the target. Additionally, the non-cytotoxic nature of the drugs toward the Vero cell line and their significant inhibitory role made these two compounds a promising candidate for future drug development.

2.5 LipY and Its Potential Inhibitors

LipY represents the mycobacterial Rv3097c-encoded lipase. A triacylglycerol lipase (LipY), an enzyme belonging to the lipase family encoded by the lipY gene, is the unique protein possessing enzymatic property in *M. tb* [67]. The role of the

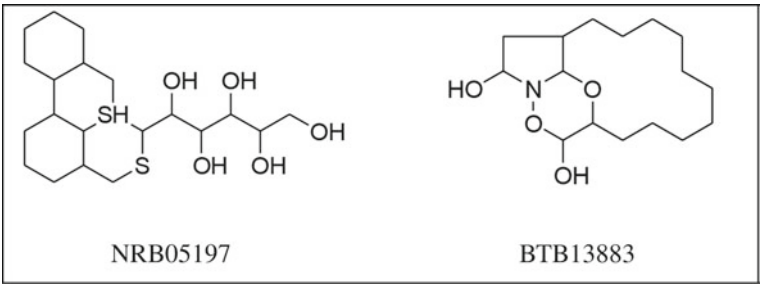


Fig. 7 Potent inhibitors of diaminopimelate epimerase

enzyme is hydrolysis and conversion of triacylglycerol (TAG), the major energy source of the mycobacteria, to diacylglycerol for the survivability of the dormant stage bacteria [7]. The expression of LipY lowers the amount of TAG for mycobacteria's survival [67] and is found ineffective in aerobic bacteria, which show its selectiveness and specificity for hypoxic grown mycobacteria only [68].

A library of active compounds (Fig. 8) against recombinant lipase enzyme by an in vitro, in-house screening of a group of compounds led to the generation of a new inhibitor (E)-7-chloro-6-fluoro-4-hydroxy-N-(3-phenylpropylidene)quinoline-3-carboxamide (LipY inh 1) belonging to the acyl-imine class having an IC_{50} value of 25 μM and CC_{50} less than 500 μg/mL [68]. Additional screening amalgamates 11 favorable series of compounds having hydrazine group attached to the acyl moiety with greater activity against LipY. Among the 11 different compounds, four compounds with IC_{50} values of 7.75 ± 0.21 (LipY inh 2), 9.25 ± 0.17 (LipY inh 3), 8.25 ± 0.43 (LipY inh 4), 9.25 ± 0.21 μM (LipY inh 5) respectively have powerful inhibition potential against the enzyme, but compound (E)-N′-(3,5-dichloro-2-hydroxybenzylidene)-7-chloro-6-fluoro-4-hydroxyquinoline-3-carbohydrazide (LipY inh 6) showed the best LipY inhibitory effects with IC_{50} value of 5.13 μM which is five times better result in inhibiting the enzymatic role of lipase as compared to LipY inh 1 with moderate inhibition potential [68]. According to the Resazurin Assay [69], these compounds do not show any cytotoxic effect on the Vero cell line, thus proving that hydrazine moiety worked better than the acyl imine group [68].

2.6 Cytidine Triphosphate Synthetase and Its Potential Inhibitors

Cytidine triphosphate (CTP) synthetase, an enzyme encoded by the gene pyrG in *M. tb*, is responsible for synthesizing pyrimidine, CTP (cytidine triphosphate). It is

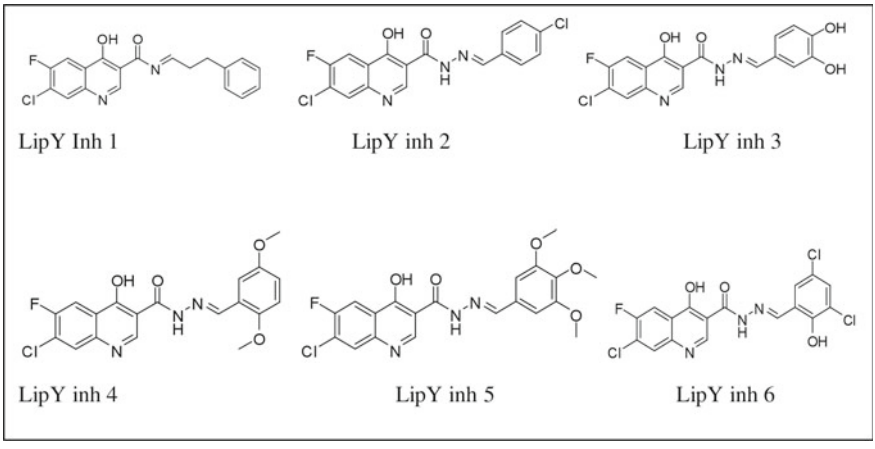


Fig. 8 Potent LipY inhibitors

an ATP-dependent process and causes the conversion of UTP into CTP by utilizing either ammonia or glutamine as a nitrogenous source [70]. This enzyme has been selected as the major drug target for causing inhibition of the growth of the pathogenic bacteria in the body.

Among 594 compounds undergone phenotypic screening, the two compounds-7947882 (5-methyl-*N*-(4-nitrophenyl)thiophene-2-carboxamide) and 7,904,688 (3-phenyl-*N*-[(4-piperidin-1-ylphenyl)carbamothioyl]propanamide) (Fig. 9) [71] showed increased bactericidal activity against intracellular, replicating, and non-replicating *M. tb* [71]. At a concentration lower than 40 μg/ml, both the compounds did not show any cytotoxicity towards HepG2, A549, Raw, and Huh7 cell lines and produced a MIC value of 0.5 μg/ml [70]. These two inhibitors behave like prodrugs that cause inhibition of target before its own activation, similar to isoniazid, pyrazinamide, and ethionamide. The EthA, a FAD containing monooxygenase, which was earlier well-known for activating ethionamide [72], is an efficient activator of 7,947,882 and 7,904,688. Their activated form binds to the enzyme and inhibits it [71].

The requirement for activation of 7,947,882 and 7,904,688 prior to the exhibition of their inhibitory effect necessitates the identification or synthesis of the drug that can function independently. The compound 11,426,026 (Fig. 9) has been derived from 7,947,882, and it is the S-dioxide derivative of 7,947,882. It is more active and does not require EthA activation. It has a MIC value of 1 μg/ml against the mycobacterial PyrG enzyme. It showed an IC50 of 0.035 mM against wild-type *M. tb;* it competes with ATP upon binding with the active pocket of the enzyme.

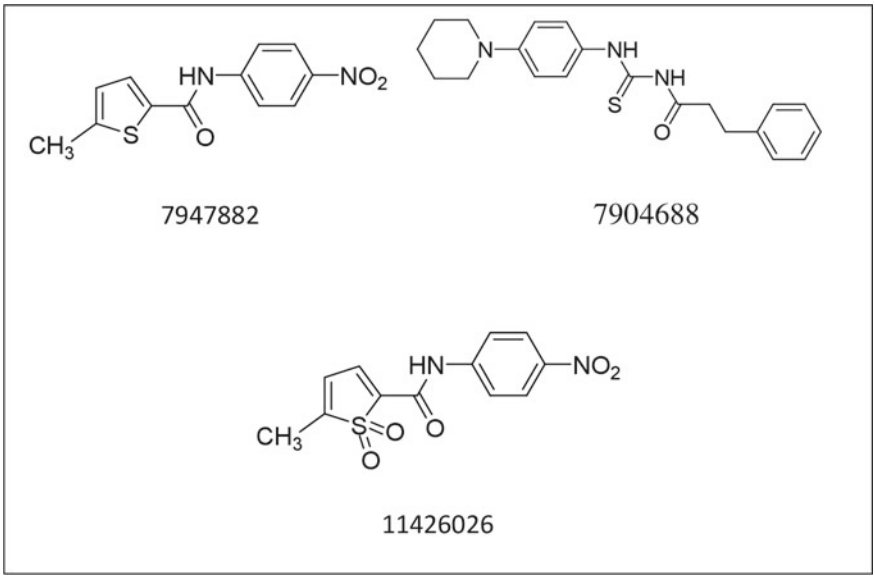


Fig. 9 Potent carboxamide compounds of cytidine triphosphate synthetase inhibitors

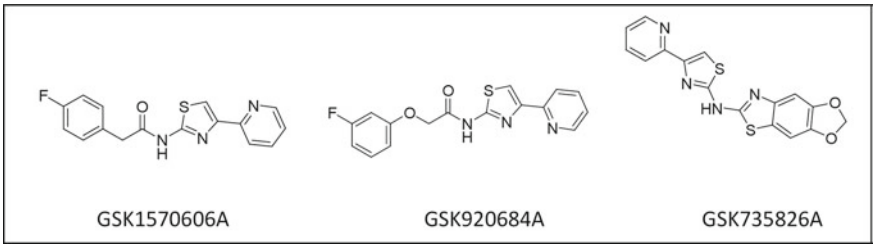


Fig. 10 Potent cytidine triphosphate synthetase inhibitors developed by GlaxoSmithKline

Among the library of 117 compounds obtained from GlaxoSmithKline compound set against *M. tb* [73], three antimycobacterial drugs such as GSK1570606A, GSK920684A, and GSK735826A (Fig. 10) were found to have good inhibitory activity with MIC value of 16 μM, 7.6 μM, and 1.4 μM, respectively. The result of enzyme assays also revealed their inhibition of PyrG as −90.8%, 79.2%, and 74.3%, respectively [74], which was higher than the rest of the other compounds. These compounds showed limited cytotoxicity to human cell lines [75, 76] and inhibited PyrG by binding competitively to the ATP binding pocket of the enzyme, where their phenyl moiety showed pi-stacking interactions with the Arg223 residue of the target.

Under the collaborative drug discovery research [77–79], the virtual screening of the database identified very effective inhibitors of mycobacterial CTP synthetase. Among the four identified compounds such as CDD-815202 (3-iodo-4-methyl-N-(2-methyl-4-nitrophenyl)benzamide), CDD-934506 (2-((5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)sulfanyl)-N-(4-nitrophenyl)acetamide), CDD-823953 (N-(2-benzoyl-4-nitrophenyl)-2-(4-benzylpiperazine-1-yl)acetamide), and CDD-833850 (5-chloro-2-hydroxy-N-(2-methoxy-4-nitrophenyl)benzamide), tested against the target, PyrG, the CDD-823953 (Fig. 11) was found to be the most effective by virtue of its 90% inhibition on PyrG at 200 μM concentration level. The MIC_{50} value of the potent compound is 4.392 μg/ml. The steady-state kinetics study also demonstrated the compound being not a strong competitive inhibitor of the ATP binding region of the active compound, thus competing weakly with ATP upon binding with the target enzyme.

2.7 Transcription Factor IdeR and Its Potential Inhibitors

Iron-dependent regulator (IdeR) is a mycobacterial transcription factor [80] that belongs to the member of the DtxR (diphtheria toxin repressor) family [81]. The protein has the property of interacting with both DNA and metal iron [82–84]. The IdeR-iron complex binds in the specific position of DNA in the promoter region and controls the transcription rate. For survival in the host's body, there is an urgent need to maintain and regulate the level of iron in *M. tb*, which is required for the functioning of enzymes and for performing redox reaction as the human serum is

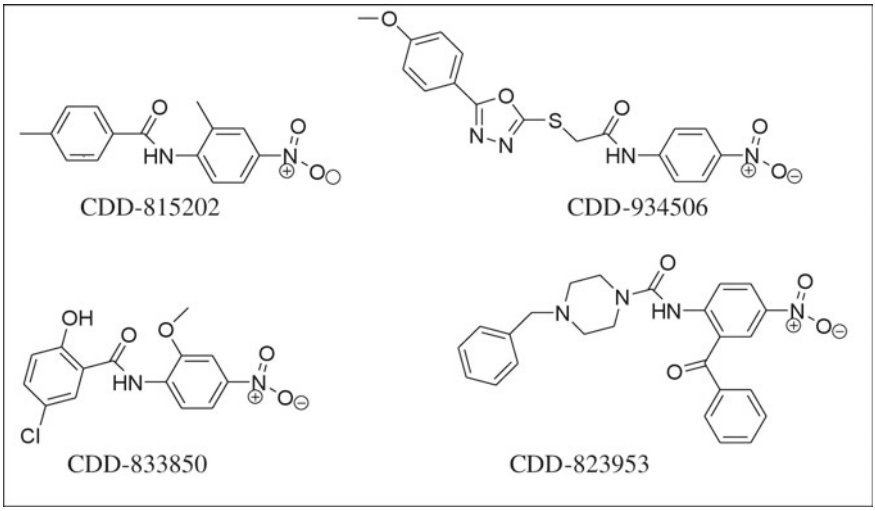


Fig. 11 The most efficient mycobacterial CTP synthetase inhibitor

tuberculostatic [85, 86]. In the absence of iron, IdeR becomes nonfunctional, and mycobacteria manufacture mycobactin, which acts as iron chelators [87] and binds with iron from the host proteins (transferrin) and transports it to the cytoplasm via transporters for its utilization, whereas the presence of iron makes IdeR functional which stops the synthesis of mycobactin, but activates iron storage protein production [80].

Virtual screening and EMSA assay of a series of compounds obtained from NCI library screening gave evidence of two potent compounds (Fig. 12):

- the compound 2-(3,4-dihydroxybenzyl)thiazolidine-2-carboxylic acid (I-20 or NSC 281,033) with IC_{50} values of 2.4 μg/ml, bearing thiazolidine-benzyl group; and
- benzyl-naphthalenyl with IC_{50} values of 1 μg/ml bearing compound (I-42 or NSC 12,453).

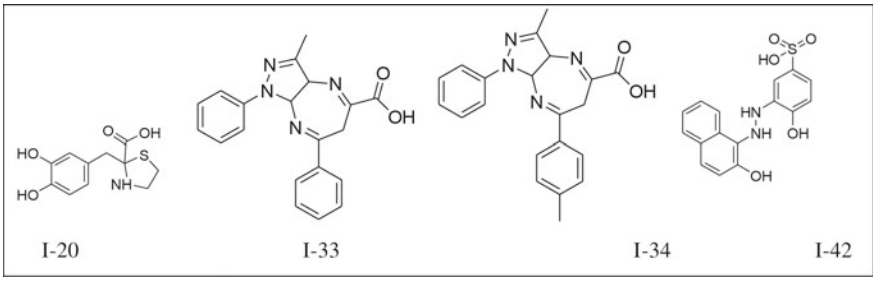


Fig. 12 Potential inhibitors of IdeR

These compounds interfere with the interaction of IdeR and DNA. The I-33 and I-34 having a common dihydropyrazolo-diazepine structure, are also considered as potent on account of an IC_{50} value of 21.7 μg/ml and 6.1 μg/ml, respectively towards *M. tb* inhibition with their negligible cytotoxicity towards HepG2 (human liver cancer cell line), MDCK (Madine Darby Canine Kidney cell line), THP-1 (human monocytic macrophage cell line), and HEK (Human Embryonic Kidney cell line) respectively [88].

2.8 Lysine ε-aminotransferase (LAT) and Its Potential Inhibitors

LAT is an important enzyme that helps *M. tb* persist and resist inside the host body, and therefore it is the causative agent of latent TB infection in the host [89]. Being a member of the aminotransferase family, requiring pyridoxal 5′-phosphate as a cofactor [3], it catalyzes reversible transamination reaction. The end product of the reaction is piperidine-6-carboxylic acid and glutamate formed after the transfer of the amino group of lysine to a-ketoglutarate [90]. Therefore, LAT has been considered a potential *M. tb* target for drug discovery (Fig. 13).

The LAT inhibitor is 2-(benzo[d]thiazol-2-yl)-3-(4-hydroxyphenyl) acrylonitrile) that binds to the active site of the enzyme. It has an IC_{50} value of 10.38 μM. In search of more potent inhibitors with greater efficacies, attempts were made by several moderations and changes in the compound at the R position by inserting phenyl and heterocycles, modifying aromatic ring, and keeping the benzothiazole derivative unchanged. This led to the synthesis of a set of 22 compounds. Out of

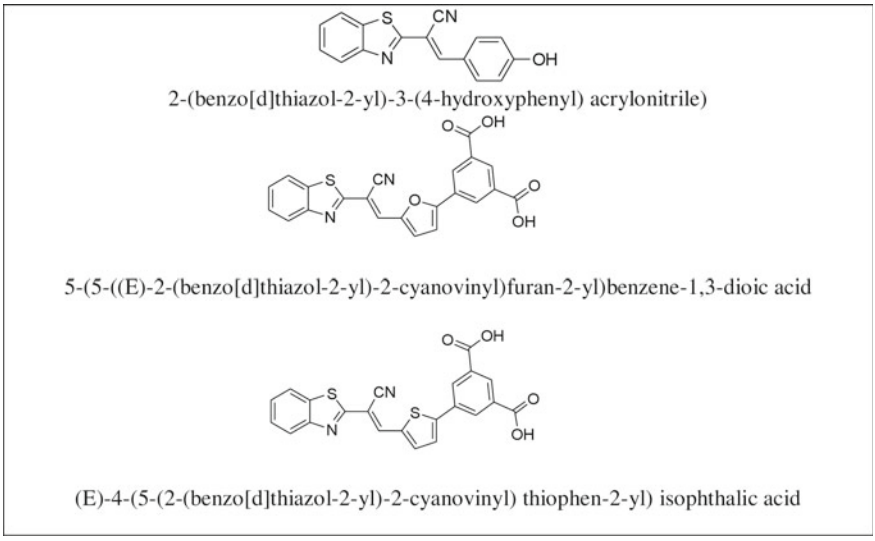


Fig. 13 Potent LAT inhibitors

those synthesized compounds, eight were found to have greater activity than the lead one. Amidst it, 5-(5-((E)-2-(benzo[d]thiazol-2-yl)-2-cyanovinyl)furan-2-yl) benzene-1,3-dioic acid showed the highest inhibition with tenfold lower IC50 1.15 ± 0.27 μM in comparison to the lead molecule. The 2.8-fold bacterial log reduction also suggested that this compound exhibits a better mode of inhibition than the first-line drugs like isoniazid (1.2 log fold). Another compound, ((E)-4-(5-(2-(benzo[d]thiazol-2-yl)-2-cyanovinyl) thiophen-2-yl) isophthalic acid) with IC_{50} of 2.62 μM has an inert role against active *M. tb* but shows its effectual activity against nutrient starving and biofilm-forming mycobacteria. This compound is the model for discovering new promising lead compounds [91].

The in silico high throughput screening has been carried out to design, synthesize, and evaluate potent LAT inhibitors utilizing structure and ligand-based approaches. The structure-based drug design screened a compound 4-methoxy-2-(pyridin-4-yl) thiazole-5-carboxylic acid (Fig. 14), which inhibited LAT with a good IC_{50} value of 1.22 ± 0.85 mM. It also has a significant bacterial log reduction value of 2.8-fold against nutrient-starved bacteria and showed a CC_{50} value of 24.87 ± 0.09 μM towards HEK 293 cell lines at a 50 μM concentration level which denotes a limited cytotoxicity level [89].

2.9 ClpC1 and Its Potential Inhibitors

The ClpC1 is a monomeric protein in *M. tb* comprising 848 amino acids. It belongs to the family of heat shock proteins (HSP100), having ATPase activity responsible for ATP hydrolysis. It consists of the N terminal domain and D1 and D2 large and small domains. The main function is to prevent mycobacterial nucleic acid accumulation followed by complete degradation. It unfolds the protein and transports those unfolded proteins to the ClpP protease for degradation. The whole process is driven by ATP hydrolysis and without the requirement of adaptor protein [92, 93]. The ClpC1 inhibitors are lassomycin, cyclomarin A, acyldepsipeptides, and ecumicin (Fig. 15).

Lassomycin is a ribosomally synthesized 16-amino acid cyclic peptide, basic in nature and naturally obtained from an extract of organism *Lentzeakentuckyensis*

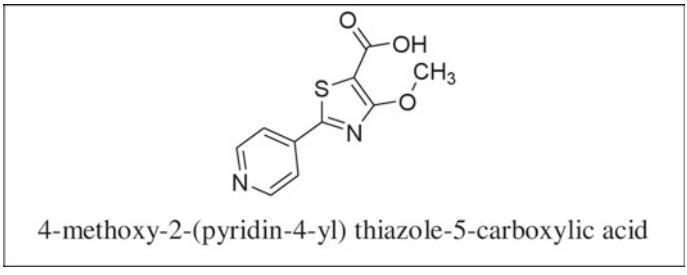


Fig. 14 In silico HTS of Potent LAT inhibitors

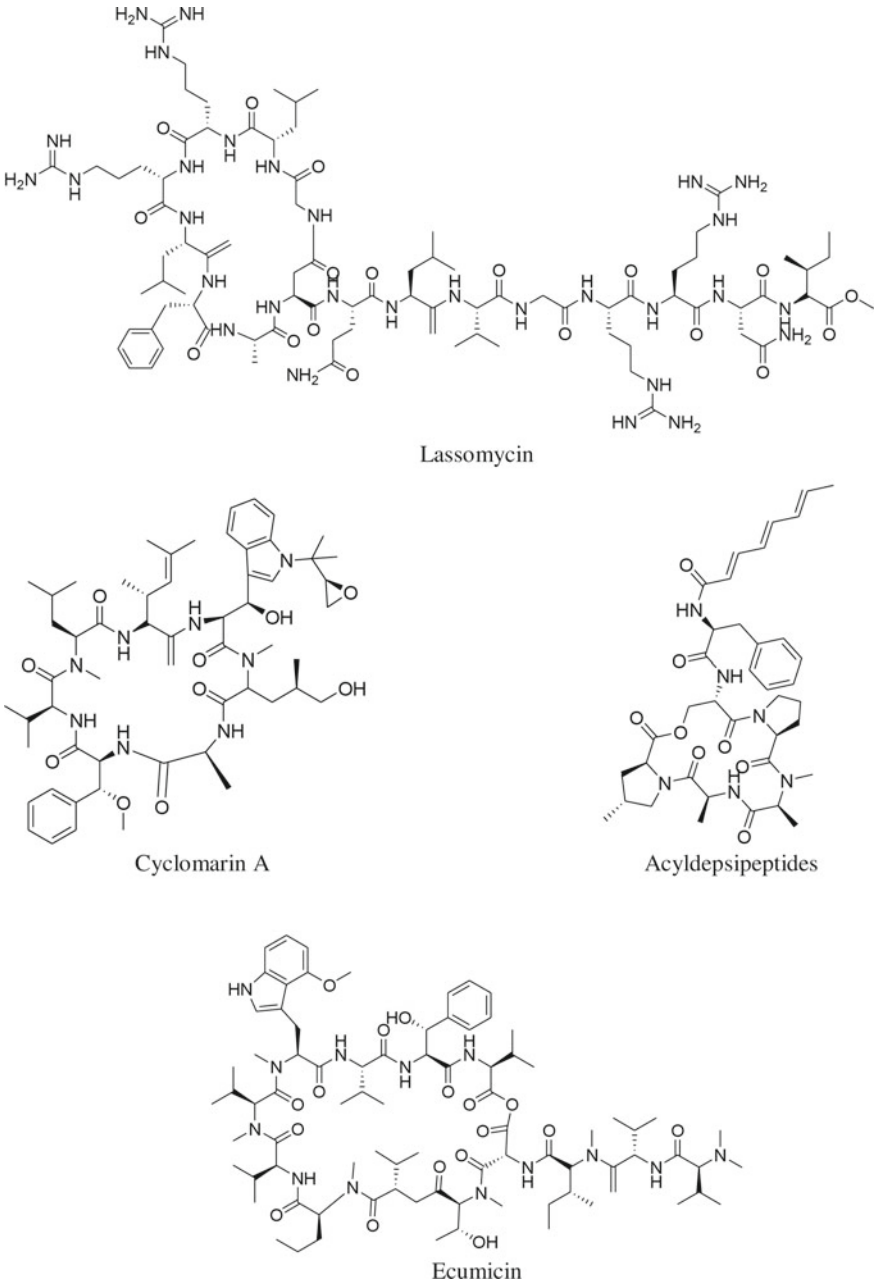


Fig. 15 ClpC1 inhibitors

species. It shows antibacterial activity with a minimum inhibitory concentration of 0.8–3 μg/ml, killing *M. tb* by targeting ClpC1 ATPase, thereby hampering its protein degradation function. The poor ability of the compound to penetrate the mammalian cells contributes to its low cytotoxicity of IC_{50} value of 350 μg/ml against human NIH 3T3 and HepG2 cells, and it does not destroy red blood cells. Unlike other antibiotics, it has an exceptional property of activating the target enzyme. It functions by stimulating ATPase activity by binding to the target but dissociating it from the complex ClpP1P2 and eventually lowers the proteolytic activity of the complex [94].

Cyclomarin A, a natural compound obtained from marine *Streptomyces species* [95], is a heptapeptide containing two common and the remaining five unusual amino acids [96]. It is a cyclic peptide that shows its activity against both growing and dormant non-replicating *M. tb* and MDR *M. tb* by binding to the N terminal domain of ClpC1 without interfering with the ATP binding domain and increasing the rate of proteolysis inside the cell [97]. The resistance of the compound lies in the fact that NTD is mutated, which denotes the importance of this domain. This antibiotic is ineffective against gram-positive and gram-negative bacteria despite containing CplC [97].

Acyldepsipeptide (ADEP) is a naturally obtained eight closely related compounds isolated from the fermentation broth of *Streptococcus hawaiiensis*. It functions either by preventing the association of ClpP with its regulatory ATPase or by splitting the previously associated ClpP/Clp-ATPase complexes [98]. The binding of ADEP to ClpP causes its conversion from a regulated state to an unregulated form resulting in the degradation of partially or fully unfolded proteins or polypeptides in the absence of Clp-ATPase [98, 99]. Apart from targeting ClpP, ADEP combines with ClpP causing atrophy of another bacterial protein, the FtsZ, which is involved in cell division, thereby preventing the occurrence of the vital process of division of the cell [100].

Ecumicin is a cyclic peptide composed of 13 amino acids obtained from the strain MJM5123 of *Nonomuraea species*. It is highly selective against both the MDR and XDR strains of *M. tb*, on which it exerts its antibacterial activity. Simultaneously, it shows its potency against *M. tb*, including both replicating as well as non-replicating bacteria in vitro and in vivo. It effectively inhibits the growth of the mycobacteria in the lungs of mice. The drug administration into the mice was done by encapsulating it in the form of micelles on account of its insoluble nature in water, thus concentrating in the region of the lung tissue as shown by its detrimental action of killing residing bacteria. Ecumicin targets ClpC1 by binding to the cavity of protein other than the ATPase domain. Studies showed that ClpC1 has ATPase activity as well as proteolytic activity; the latter is performed in complex with ClpP1/ClpP2. In the presence of the antibiotic, ClpC1 undergoes a conformational change resulting in the upregulation of the ATPase activity, which, in turn, causes an alteration in the functioning of the ClpC1/ClpP1/ClpP2 complex by repressing its proteolytic activity [101].

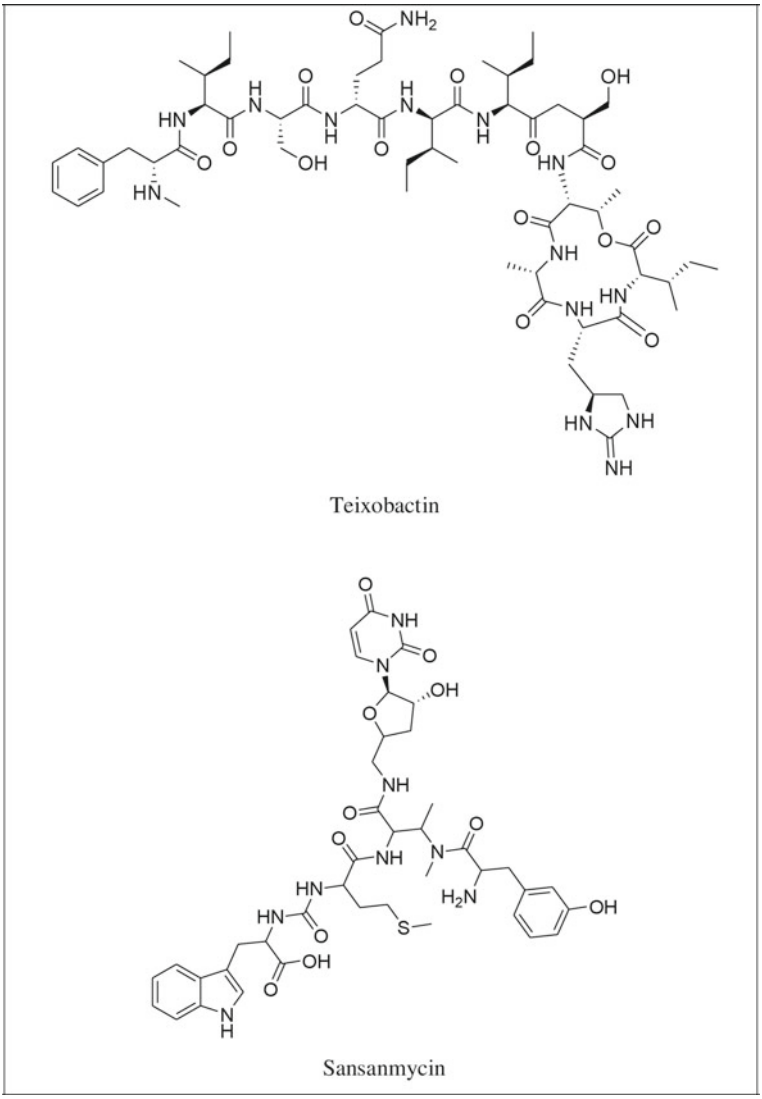


Fig. 16 MraY mediated *M. tb* peptidoglycan synthesis inhibitors

2.9.1 MraY and Its Potential Inhibitors

MraY translocase, also known as a phospho-MurNAc-pentapeptide translocase, is an essential, integral membrane protein of *M. tb* consisting of ten transmembrane segments, five cytoplasmic domains, and six periplasmic domains, including the N- and C-terminal ends. It is responsible for catalyzing the first step of peptidoglycan biosynthesis. The reversible reaction product, undecaprenyl-pyrophosphoryl-N-acetylmuramoyl-pentapeptide, is the result of the relocation of

phospho-MurNAc-pentapeptide from UDP-MurNAc-pentapeptide to undecaprenyl phosphate [102, 103]. Therefore, to inhibit *M. tb* peptidoglycan, MraY inhibitors (Fig. 16) have been explored. The two major drugs, teixobactin and sansanmycin, were shown to inhibit MraY.

Teixobactin belongs to antimicrobial peptides that contains 11 amino acids. Its natural source is *Eleftheria terrae, from which soil bacteria are isolated using iChip* [104–106]. *It* does not show any resistance to *M. tb* H37Rv in action. By adhering to the two lipid cell wall precursors, peptidoglycan and teichoic acid, it inhibits cell wall production [104, 107].

Sansanmycin, which is derived from *Streptomyces spices* [108], interferes with *M. tb* activities in terms of cell wall synthesis, cell division, and survival [109, 110]. Some anti-MDR-TB effects are attributable to this antibiotic–an uridyl peptide that targets the translocase I (MraY) and prevents peptidoglycan formation [111].

3 Conclusion

To tackle the worst situation of antitubercular drug resistance, the 6-FQ along with kanamycin/capreomycin/amikacin and the newly introduced bedaquiline may be useful in increasing the success of the tuberculosis treatment. The potential targets of *M. tb*, e.g., ATP synthase, type II NADH dehydrogenase, DNA gyrase, diaminopimelate epimerase, LipY, cytidine triphosphate synthetase, transcription factor IdeR, *lysine* ε-*aminotransferase*, ClpC1, and MraY, are of interest for further design and discovery of novel antitubercular drugs.

Core Messages

- *M. tb* causes dreadful TB.
- DOTS drug regimen is helpful to treat TB.
- MDR-TB and XDR-TB are complicated to control.
- New *M. tb* targets may help control MDR and XDR TB.

References

1. World Health Organization (2020) https://www.who.int/news-room/fact-sheets/detail/tuberculosis. Accessed 15 Aug 2020
2. Delogu G, Sali M, Fadda G (2013) The biology of *mycobacterium tuberculosis* infection. Mediterr J Hematol Infect Dis 5(1):e2013070
3. Tripathi KD (2006) Essentials of medical pharmacology. 6th edn. Jaypee Brothers Medical Publishers (P) Ltd., New Delhi, pp 745–750

4. Liu CH, Li L, Chen Z, Wang Q, Hu YL, Zhu B, Woo PCY (2011) Characteristics and treatment outcomes of patients with MDR and XDR tuberculosis in a TB referral hospital in Beijing: a 13-year experience. PLoS ONE 6(4):e19399
5. Nuermberger EL, Spigelman MK, Yew WW (2010) Current development and future prospects in chemotherapy of tuberculosis. Respirology 15(5):764–778
6. Dey R, Nandi S, Samadder A, Saxena A, Saxena AK (2020) Exploring the potential inhibition of candidate drug molecules for clinical investigation based on their docking or crystallographic analyses against *M. tuberculosis* enzyme targets. Curr Topics Med Chem 20 (29):2662–2680
7. Saxena AK, Singh A (2019) *Mycobacterial tuberculosis* enzyme targets and their inhibitors. Curr Top Med Chem 19(5):337–355
8. Yasuda R, Noji H, Yoshida M, Kinosita K, Itoh H (2001) Resolution of distinct rotational substeps by submillisecond kinetic analysis of F1-ATPase. Nature 410:898–904
9. Fillingame RH, Angevine CM, Dmitriev OY (2003) Mechanics of coupling proton movements to c-ring rotation in ATP synthase. FEBS Lett 555(1):29–34
10. de Jonge MR, Koymans LHM, Guillemont JEG, Koul A, Andries K (2007) A computational model of the inhibition of *Mycobacterium tuberculosis* ATPase by a new drug candidate R207910. Proteins 67(4):971–980
11. Hongmanee P, Rukseree K, Buabut B, Somsri B, Palittapongarnpim P (2007) In vitro activities of Cloxyquin (5-chloroquinolin-8-ol) against *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 51(3):1105–1106
12. Worley MV, Estrada SJ (2014) Bedaquiline: a novel antitubercular agent for the treatment of multidrug-resistant tuberculosis. Pharmacotherapy 34(11):1187–1197
13. Diacon AH, Lounis N, Dannemann B (2014) Multidrug-resistant tuberculosis and bedaquiline. N Engl J Med 371(25):2435–2436
14. Kundu S, Biukovic G, Grüber G, Dick T (2016) Bedaquiline targets the ε subunit of mycobacterial F-ATP synthase. Antimicrob Agents Chemother 60(11):6977–6979
15. Preiss L, Langer JD, Yildiz O, Eckhardt-Strelau L, Guillemont, JEG, Koul A, Meier T (2015) Structure of the mycobacterial ATP synthase Fo rotor ring in complex with the anti-TB drug bedaquiline. Sci Adv 1(4):e1500106
16. Huitric E, Verhasselt P, Andries K, Hoffner SE (2007) In vitro antimycobacterial spectrum of a diarylquinoline ATP synthase inhibitor. Antimicrob Agents Chemother 51(11):4202–4204
17. Tong AST, Choi PJ, Blaser A, Sutherland HS, Tsang SKY, Guillemont J, Motte M, Cooper CB, Andries K, den Broeck WV, Franzblau SG, Upton A, Denny WA, Palmer BD, Conole D (2017) 6-cyano analogues of bedaquiline as less lipophilic and potentially safer diarylquinolines for tuberculosis. ACS Med Chem Lett 8(10):1019–1024
18. Choi PJ, Sutherland HS, Tong AST, Blaser A, Franzblau SG, Lotlikar MU, Upton AM, Guillemont J, Motte M, Queguiner L, Andries K, den Broeck WV, Denny WA, Palmer BD (2017) Synthesis and evaluation of analogues of the tuberculosis drug bedaquiline containing heterocyclic B-ring units. Bioorg Med Chem Lett 27(23):5190–5196
19. Sutherland HS, Tong AST, Choi PJ, Conole D, Blaser A, Franzblau SG, Cooper CB, Upton AM, Lotlikar MU, Denny WA, Palmer BD (2018) Structure-activity relationships for analogs of the tuberculosis drug bedaquiline with the naphthalene unit replaced by bicyclic heterocycles. Bioorg Med Chem 26(8):1797–1809
20. Sutherland HS, Tong AST, Choi PJ, Blaser A, Conole D, Franzblau SG, Lotlikar MU, Cooper CB, Upton AM, Denny WA, Palmer BD (2019) 3,5-dialkoxypyridine analogues of bedaquiline are potent anti-tuberculosis agents with minimal inhibition of the hERG channel. Bioorg Med Chem 27(7):1292–1307
21. Blaser A, Sutherland HS, Tong AST, Choi PJ, Conole D, Franzblau SG, Cooper CB, Upton AM, Lotlikar M, Denny WA, Palmer BD (2019) Structure-activity relationships for unit C pyridyl analogues of the tuberculosis drug bedaquiline. Bioorg Med Chem 27 (7):1283–1291

22. Sarathy JP, Ragunathan P, Shin J, Cooper CB, Upton AM, Grüber G, Dick T (2019) TBAJ-876 retains bedaquiline's activity against subunits c and ε of *Mycobacterium tuberculosis* F-ATP synthase. Antimicrob Agents Chemother 63(10):e01191–e1219
23. Candéa AL, de Ferreira ML, Pais KC, Cardoso LN, Kaiser CR, Henriques Md, Lourenço MC, Bezerra FA, de Souza MV (2009) Synthesis and antitubercular activity of 7-chloro-4-quinolinylhydrazones derivatives. Bioorg Med Chem Lett 19(22):6272–6274
24. Khan SR, Singh S, Roy KK, Akhtar MS, Saxena AK, Krishnan MY (2013) Biological evaluation of novel substituted chloroquinolines targeting mycobacterial ATP synthase. Int J Antimicrob Agents 41(1):41–46
25. Singh S, Roy KK, Khan SR, Kashyap VK, Sharma A, Jaiswal S, Sharma SK, Krishnan MY, Chaturvedi V, Lal J, Sinha S, Dasgupta A, Srivastava R, Saxena AK (2015) Novel, potent, orally bioavailable and selective mycobacterial ATP synthase inhibitors that demonstrated activity against both replicating and non-replicating *M. tuberculosis*. Bioorg Med Chem 23 (4):742–752
26. Kalia D, Kumar A, Meenaa G, Sethi KP, Sharma R, Trivedi P, Khan SR, Verma AS, Singh S, Sharma S, Roy KK, Kant R, Krishnan MY, Singh BN, Sinha S, Chaturvedi V, Saxena AK, Dikshit DK (2015) Synthesis and antitubercular activity of conformationally-constrained and bisquinoline analogs of TMC207. Med Chem Commun 6(8):1554–1563
27. Saxena AK, Alam M (2020) ATP synthase inhibitors as antitubercular agents: QSAR studies in novel substituted quinolines. Curr Topics Med Chem 20(29):2723–2734
28. Weinstein EA, Yano T, Li L-S, Avarbock D, Avarbock A, Helm D, McColm AA, Duncan K, Lonsdale JT, Rubin H (2005) Inhibitors of type II NADH: menaquinone oxidoreductase represent a class of antitubercular drugs. PNAS 102(12):4548–4553
29. Teh JS, Yano T, Rubin H (2007) Type II NADH: menaquinone oxidoreductase of *Mycobacterium tuberculosis*. Infect Disord Drug Targets 7(2):169–181
30. Harbut MB, Yang B, Liu R, Yano T, Vilchèze C, Cheng B, Lockner J, Guo H, Yu C, Franzblau SG, Petrassi HM, Jacobs WR Jr, Rubin H, Chatterjee AK, Wang F (2018) Small molecules targeting *Mycobacterium tuberculosis* type II NADH dehydrogenase exhibit antimycobacterial activity. Angew Chem Int Ed Engl 57(13):3478–3482
31. He C-X, Meng H, Zhang X, Cui H-Q, Yin D-L (2015) Synthesis and bio-evaluation of phenothiazine derivatives as new anti-tuberculosis agents. Chin Chem Lett 26(8):951–954
32. Crowle AJ, Douvas GS, May MH (1992) Chlorpromazine: a drug potentially useful for treating mycobacterial infections. Chemotherapy 38(6):410–419
33. Advani MJ, Siddiqui I, Sharma P, Reddy H (2012) Activity of trifluoperazine against replicating, non-replicating and drug-resistant *M. tuberculosis*. PLoS ONE 7(8):e44245
34. Murugesan D, Ray PC, Bayliss T, Prosser GA, Harrison JR, Green K, de Melo CS, Feng T-S, Street LJ, Chibale K, Warner DF, Mizrahi V, Epemolu O, Scullion P, Ellis L, Riley J, Shishikura Y, Ferguson L, Osuna-Cabello M, Read KD, Green SR, Lamprecht DA, Finin PM, Steyn AJC, Ioerger TR, Sacchettini J, Rhee KY, Arora K, Barry CE III, Wyatt PG, Boshoff HIM (2018) 2-mercapto-quinazolinones as inhibitors of type II NADH dehydrogenase and *Mycobacterium tuberculosis*: structure-activity relationships, mechanism of action and absorption, distribution, metabolism, and excretion characterization. ACS Infect Dis 4(6):954–969
35. Barry VC, Belton JG, Conalty ML, Denneny JM, Edward DW, O'Sullivan JF, Twomey D, Winder F (1957) A new series of phenazines (rimino-compounds) with high anti-tuberculosis activity. Nature 179(4568):1013–1015
36. O'Connor R, O'Sullivan JF, O'Kennedy R (1995) The pharmacology, metabolism, and chemistry of clofazimine. Drug Metab Rev 27(4):591–614
37. Reddy VM, O'Sullivan JF, Gangadharam PRJ (1999) Antimycobacterial activities of riminophenazines. J Antimicrob Chemother 43(5):615–623
38. Bald D, Villellas C, Lu P, Koul A (2017) Targeting energy metabolism in *Mycobacterium tuberculosis*, a new paradigm in antimycobacterial drug discovery. mBio 8(2):e00272-17

39. Van Rensburg CE, Jooné GK, O'Sullivan JF, Anderson R (1992) Antimicrobial activities of clofazimine and B669 are mediated by lysophospholipids. Antimicrob Agents Chemother 36 (12):2729–2735
40. Nöllmann M, Crisona NJ, Arimondo PB (2007) Thirty years of *Escherichia coli* DNA gyrase: from in vivo function to single-molecule mechanism. Biochimie 89(4):490–499
41. Reece RJ, Maxwell A (1991) DNA gyrase: structure and function. Crit Rev Biochem Mol Biol 26(3–4):335–375
42. Karkare S, Yousafzai F, Mitchenall LA, Maxwell A (2012) The role of Ca^{2+} in the activity of *Mycobacterium tuberculosis* DNA gyrase. Nucleic Acids Res 40(19):9774–9787
43. Sharma PC, Jain A, Jain S (2009) Fluoroquinolone antibacterials: a review on chemistry, microbiology and therapeutic prospects. Acta Pol Pharm 66(6):587–604
44. Patrick GL (2003) Antibacterial agents. An introduction to medicinal chemistry. Oxford University Press, Oxford, UK, pp 379–435
45. Foroumadi A, Emami S, Hassanzadeh A, Rajaee M, Sokhanavr K, Moshafi MH, Shafiee A (2005) Synthesis and antibacterial activity of N-(5-benzylthio-1,3,4-thiadiazol-2-yl) and N-(5-benzylsulfonyl-1,3,4-thiadiazol-2-yl)piperazinyl quinolone derivatives. Bioorg Med Chem Lett 15(20):4488–4492
46. Dipiksha SM, Nandi S (2017) QSAR and pharmacophore modeling of antitubercular 6-fluoroquinolone compounds utilizing calculated structural descriptors. Med Chem Res 26:1903–1914
47. Berning SE (2001) The role of fluoroquinolones in tuberculosis today. Drugs 61(1):9–18
48. Gillespie SH (2016) The role of moxifloxacin in tuberculosis therapy. Eur Respir Rev 25 (139):19–28
49. Ji B, Lounis N, Maslo C, Truffot-Pernot C, Bonnafous P, Grosset J (1998) In vitro and in vivo activities of moxifloxacin and clinafloxacin against *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 42(8):2066–2069
50. O'Brien RJ, Da MM, Spigelman MD (2005) New drugs for tuberculosis: current status and future prospects. Clin Chest Med 26(2):327–340
51. Chiang C-Y, Van Deun A, Rieder HL (2016) Perspective Gatifloxacin for short, effective treatment of multidrug-resistant tuberculosis. Int J Tuberc Lung Dis 20(9):1143–1147
52. Veziris N, Truffot-Pernot C, Aubry A, Jarlier V, Lounis N (2003) Fluoroquinolone-containing third-line regimen against *Mycobacterium tuberculosis* in vivo. Antimicrob Agents Chemother 47(10):3117–3122
53. Ahmad Z, Tyagi S, Minkowski A, Peloquin CA, Grosset JH, Nuermbergeret EL (2013) Contribution of moxifloxacin or levofloxacin in second-line regimens with or without continuation of pyrazinamide in murine tuberculosis. Am J Respir Crit Care Med 188(1):97–102
54. Hu Y, Coates ARM, Mitchison DA (2003) Sterilizing activities of fluoroquinolones against rifampin-tolerant populations of *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 47(2):653–657
55. Ahmad S, Mokaddas E (2012) Role of fluoroquinolones in the treatment of tuberculosis. Rev Health Care 3(1):17–31
56. Goss WA, Deitz WH, Cook TM (1965) Mechanism of action of nalidixic acid on *Escherichia coli* II. inhibition of deoxyribonucleic acid synthesis. J Bacteriol 89(4):1068–1074
57. Pranger AD, Alffenaar JW, Aarnoutse RE (2011) Fluoroquinolones, the cornerstone of treatment of drug-resistant tuberculosis: a pharmacokinetic and pharmacodynamic approach. Curr Pharm Des 17:2900–2930
58. Pannu HK, Gottlieb L, Fishman EK (2001) Acute liver failure due to trovafloxacin: CT findings. Emerg Radiol 8(2):108–110
59. Pucci MJ, Cheng J, Podos SD, Thoma CL, Thanassi JA, Buechter DD, Mushtaq G, Vigliotti GA Jr, Bradbury BJ, Deshpande M (2007) In vitro and in vivo antibacterial activities of heteroaryl isothiazolones against resistant gram-positive pathogens. Antimicrob Agents Chemother 51(4):1259–1267

60. Wang Q, Lucien E, Hashimoto A, Pais GCG, Nelson DM, Song Y, Thanassi JA, Marlor CW, Thoma CL, Cheng J, Podos SD, Ou Y, Deshpande M, Pucci MJ, Buechter DD, Bradbury BJ, Wiles JA (2007) Isothiazoloquinolones with enhanced antistaphylococcal activities against multidrug-resistant strains: effects of structural modifications at the 6-, 7-, and 8-positions. J Med Chem 50(2):199–210
61. Nandi S, Ahmed S, Saxena AK (2018) Combinatorial design and virtual screening of potent antitubercular fluoroquinolone and isothiazoloquinolone compounds utilizing QSAR and pharmacophore modelling. SAR QSAR Environ Res 29(2):151–170
62. Pucci MJ, Podos SD, Thanassi JA, Leggio MJ, Bradbury BJ, Deshpande M (2011) In vitro and in vivo profiles of ACH-702, an isothiazoloquinolone, against bacterial pathogens. Antimicrob Agents Chemother 55(6):2860–2871
63. Hutton CA, Southwood TJ, Turner JJ (2003) Inhibitors of lysine biosynthesis as antibacterial agents. Mini Rev Med Chem 3(2):115–127
64. Dogovski C, Atkinson SC, Dommaraju SR, Downton M, Hor L, Moore S, Paxman JJ, Peverelli MG, Qiu TW, ReumannM, Siddiqui T, Taylor NL, Wagner J, Wubben JM, Perugini MA (2012) Enzymology of bacterial lysine biosynthesis. Biochemistry 225–262
65. Blanchard JS, Born TL (1999) Structure/function studies on enzymes in the diaminopimelate pathway of bacterial cell wall biosynthesis. Curr Opin Chem Biol 3(5):607–613
66. Cox RJ, Sutherland A, Vederas JC (2000) Bacterial diaminopimelate metabolism as a target for antibiotic design. Bioorg Med Chem 8(5):843–871
67. Mishra KC, de Chastellier C, Narayana Y, Bifani P, Brown AK, Besra GS, Katoch VM, Joshi B, Balaji KN, Kremer L (2008) Functional role of the PE domain and immunogenicity of the *Mycobacterium tuberculosis* triacylglycerol hydrolase LipY. Infect Immun 76(1):127–140
68. Saxena AK, Roy KK, Singh S, Vishnoi SP, Kumar A, Kashyap VK, Kremer L, Srivastava R, Srivastava BS (2013) Identification and characterisation of small-molecule inhibitors of Rv3097c-encoded lipase (LipY) of *Mycobacterium tuberculosis* that selectively inhibit growth of bacilli in hypoxia. Int J Antimicrob Agents 42(1):27–35
69. Anoopkumar-Dukie S, Carey JB, Conere T, O'Sullivan E, van Pelt FN, Allshire A (2005) Resazurin assay of radiation response in cultured cells. Br J Radiol 78(934):945–947
70. Long CW, Pardee AB (1967) Cytidine triphosphate synthetase of *Escherichia coli* B. J Biol Chem 242(20):4715–5721
71. Mori G, Chiarelli LR, Esposito M, Makarov V, Bellinzoni M, Hartkoorn RC, Degiacomi G, Boldrin F, Ekins S, de Jesus Lopes Ribeiro AL, Marino LB, Centárová I, Svetlíková Z, Blaško J, Kazakova E, Lepioshkin A, Barilone N, Zanoni G, Porta A, Fondi M, Fani R, Baulard AR, Mikušová K, Alzari PM, Manganelli R, de Carvalho LPS, Riccardi G, Cole ST, Pasca MR (2015) Thiophenecarboxamide derivatives activated by EthA Kill *Mycobacterium tuberculosis* by Inhibiting the CTP synthetase PyrG. Chem Biol 22(7):917–927
72. Dover LG, Alahari A, Gratraud P, Gomes JM, Bhowruth V, Reynolds RC, Besra GS, KremerL (2007) EthA, a common activator of thiocarbamide-containing drugs acting on different mycobacterial targets. Antimicrob Agents Chemother 51(3):1055–1063
73. Bento AP, Gaulton A, Hersey A, Bellis LJ, Chambers J, Davies M, Krüger FA, Light Y, Mak L, McGlinchey S, Nowotka M, Papadatos G, Santos R, Overington JP (2014) The ChEMBL bioactivity database: an update. Nucl Acids Res 42:D1083–D1090
74. Esposito M, Szadocka S, Degiacomi G, Orena BS, Mori G, Piano V, BoldrinF, Zemanová J, Huszár S, Barros D, Ekins S, LelièvreJ, Manganelli R, Mattevi A, Pasca MR, Riccardi G, Ballell L, Mikušová K, Chiarelli LR (2017) A phenotypic based target screening approach delivers new antitubercular CTP synthetase inhibitors. ACS Infect Dis 3(6):428–437
75. Ballell L, Bates RH, Young RJ,Alvarez-Gomez D, Alvarez-Ruiz E, Barroso V, Blanco D, Crespo B, Escribano J, González R, Lozano S, Huss S, Santos-Villarejo A, Martín-Plaza JJ, Mendoza A, Rebollo-Lopez MJ, Remuiñan-Blanco M, Lavandera JL,Pérez-Herran E, Gamo-Benito FJ, García-Bustos JF, Barros D, Castro JP, Cammack N (2013) Fueling open-source drug discovery: 177 small-molecule leads against tuberculosis. ChemMedChem 8(2):313–321

76. Rebollo-Lopez MJ, Lelièvre J, Alvarez-Gomez D, Castro-Pichel J, Martínez-Jiménez F, Papadatos G, Kumar V, Colmenarejo G, Mugumbate G, Hurle M, Barroso V, Young RJ, Martinez-Hoyos M, del Río RG, Bates RH, Lopez-Roman EM, Mendoza-Losana A, Brown JR, Alvarez-Ruiz E, Marti-Renom MA, Overington JP, Cammack N,Ballell L, Barros-Aguire D (2015) Release of 50 new, drug-like compounds and their computational target predictions for open source anti-tubercular drug discovery. PLoS ONE 10(12): e0142293
77. Ananthan S, Faaleolea ER, Goldman RC, Hobrath JV, Kwong CD, Laughon BE, Maddry JA, Mehta A, Rasmussen L, Reynolds RC, Secrist JA III, Shindo N, Showe DN, Sosa MI, Suling WJ, White EL (2009) High-throughput screening for inhibitors of *Mycobacterium tuberculosis* H37Rv. Tuberculosis (Edinb) 89(5):334–353
78. Ekins S, Bunin BA (2013) The collaborative drug discovery (CDD) database. Methods Mol Biol 993:139–154
79. Ekins S, Freundlich JS, Hobrath JV, White EL, Reynolds RC (2014) Combining computational methods for hit to lead optimization in *Mycobacterium tuberculosis* drug discovery. Pharm Res 31(2):414–435
80. Gold B, Marcela Rodriguez G, Salvatore AE, Pentecost M, Smith I (2001) The *Mycobacterium tuberculosis* IdeR is a dual functional regulator that controls transcription of genes involved in iron acquisition, iron storage and survival in macrophages. Mol Microbiol 42(3):851–865
81. Rodriguez GM, Voskuil MI, Gold B, Schoolnik GK, Smith I (2002) ideR, an essential gene in *Mycobacterium tuberculosis*: role of IdeR in iron-dependent gene expression, iron metabolism, and oxidative stress response. Infect Immun 70(7):3371–3381
82. Dussurget O, Rodriguez GM, Smith I (1996) An ideR mutant of Mycobacterium smegmatis has a derepressed siderophore production and an altered oxidative-stress response. Mol Microbiol 22(3):535–544
83. Pohl E, Holmes RK, Hol WGJ (1999) Crystal structure of the iron-dependent regulator (IdeR) from *Mycobacterium tuberculosis* shows both metal binding sites fully occupied. J Mol Biol 285(3):1145–1156
84. Schmitt MP, Predich M, Doukhan L, Smith I, Holmes RK (1995) Characterization of an iron-dependent regulatory protein (IdeR) of *Mycobacterium tuberculosis* as a functional homolog of the diphtheria toxin repressor (DtxR) from corynebacterium diphtheriae. InfectImmun 63(11):4284–4289
85. Kochan I (1971) Mechanisms of tuberculostasis in mammalian serum. Role of transferrin in human serum tuberculostasis. J Infect Dis 119:11–18
86. Sauton B (1912) Sur la nutrition minerale du bacilletuberculeux. C R Hebd Seances Acad Sci 155:860–861
87. Snow GA (1970) Mycobactins: iron-chelating growth factors from mycobacteria. Bacteriol Rev 34(2):99–125
88. Rohilla A, Khare G, Tyagi AK (2017) Virtual screening, pharmacophore development and structure based similarity search to identify inhibitors against IdeR, a transcription factor of *Mycobacterium tuberculosis*. Sci Rep 7(1):4653
89. Devi PB, Sridevi JP, Kakan SS, Saxena S, Jeankumar VU, Soni V, Anantaraju HS, Yogeeswari P, Sriram D (2015) Discovery of novel lysine ε-aminotransferase inhibitors: an intriguing potential target for latent tuberculosis. Tuberculosis (Edinb) 95(6):786–794
90. Duan X, Li Y, Du Q, Huang Q, Guo S, Xu M, Lin Y, Liu Z, Xie J (2016) Mycobacterium lysine ε-aminotransferase is a novel alarmone metabolism related persister gene via dysregulating the intracellular amino acid level. Sci Rep 6:19695
91. Reshma RS, Jeankumar VU, Kapoor N, Saxena S, Bobesh KA Vachaspathy AR, Kolattukudy PE Sriram D (2017) *Mycobacterium tuberculosis* lysine-e-aminotransferase a potential target in dormancy: benzothiazole based inhibitors. Bioorg Med Chem 25 (10):2761–2771

92. NarayaniP SKD, Rath P, Choudhary RK, Batra JK (2008) *Mycobacterium tuberculosis* ClpC1Characterization and role of the N-terminal domain in its function. FEBS J 275 (24):6149–6158
93. Marsee JD, Ridings A, Yu T, Miller JM(2018) *Mycobacterium tuberculosis* ClpC1 N-terminal domain is dispensable for adaptor protein-dependent allosteric regulation. Int J Mol Sci 19(11):3651
94. Gavrish E, Sit SC, Cao S, Kandror O Spoering A, Peoples A, Ling L, Fetterman A, Hughes D, Bissell A, Torrey H, Akopian T, Mueller A, Epstein S, Goldberg A, Clardy J, Lewis K (2014) Lassomycin, a ribosomally synthesized cyclic peptide, kills *Mycobacterium tuberculosis* by targeting the ATP-dependent protease ClpC1P1P2. Chem Biol 21(4):509–518
95. Renner MK, Shen Y, Cheng X, Jensen PR (1999) Cyclomarins A-C, new antiinflammatory cyclic peptides produced by a marine bacterium (*Streptomyces* sp.). J Am Chem Soc 121 (49):11273–11276
96. Vasudevan D, Rao SP, Noble CG (2013) Structural basis of mycobacterial inhibition by cyclomarin A. J Biol Chem 288(43):30883–30891
97. Schmit EK, Riwanto M, Sambandamurthy V, Roggo S, Miault C, Zwingelstein C, Krastel P, Noble C, Beer D, Rao SPS, Au M, Niyomrattanakit P, Lim V, Zheng J, Jeffery D, Pethe K, Camacho LR (2011) The natural product cyclomarin kills *Mycobacterium tuberculosis* by targeting the ClpC1 subunit of the caseinolyticprotease. Angew Chem Int Ed Engl 50 (26):5889–5891
98. Kirstein J, Hoffmann A, Lilie H, Schmidt R, Rübsamen-Waigmann H, Brötz-Oesterhelt H, Mogk A, Turgay K (2009) The antibiotic ADEP reprogrammes ClpP, switching it from a regulated to an uncontrolled protease. EMBO Mol Med 1(1):37–49
99. Brötz-Oesterhelt H, Beyer D, Kroll HP, Endermann R, Ladel C, Schroeder W, Hinzen B, Raddatz S, Paulsen H, Henninger K, Bandow JE, Sahl H-G, Labischinski H (2005) Dysregulation of bacterial proteolytic machinery by a new class of antibiotics. Nat Med 11 (10):1082–108718
100. Sass P, Josten M, Famulla K, Schiffer G, Sahl H-G, Hamoen L, Brötz-Oesterhelt H (2011) Antibiotic acyldepsipeptides activate ClpP peptidase to degrade the cell division protein FtsZ. Proc Natl Acad Sci USA 108(42):17474–17479
101. Gao W, Kim JY, Anderson JR, Akopian T, Hong S, Jin Y-Y, Kandror O, Kim J-W, Lee I-A, Lee S-Y, McAlpine JB, Mulugeta S, Sunoqrot S, Wang Y, Yang S-H, Yoon T-M, Goldberg AL, Pauli GF, Suh J-W, Franzblau S-G, Cho S (2015) The cyclic peptide ecumicin targeting ClpC1 is active against *Mycobacterium tuberculosis* in vivo. Antimicrob Agents Chemother 59(2):880–889
102. Bouhss A, Crouvoisier M, Blanot D, Mengin-Lecreulx D (2004) Purification and characterization of the bacterial MraY translocase catalyzing the first membrane step of peptidoglycan biosynthesis. J Biol Chem 279(29):29974–29980
103. Bugg TD, Lloyd AJ, Roper DI (2006) Phospho-MurNAc-pentapeptide translocase (MraY) as a target for antibacterial agents and antibacterial proteins. Infect Disord Drug Targets 6 (2):85–106
104. Ling LL, Schneider T, Peoples AJ, Spoering AL, Engels I, Conlon BP, Mueller A, Schäberle TF, Hughes DE, Epstein S, Jones M, Lazarides L, Steadman VA, Cohen DR, Felix CR, Fetterman KA, Millett WP, Nitti AG, Zullo AM, Chen C, Lewis K (2015) A new antibiotic kills pathogen without detectable resistance. Nature 517(7535):455–459
105. Piddock LJ (2015) Teixobactin, the first of a new class of antibiotics discovered by iChip technology? J Antimicrob Chemother 70(10):2679–2680
106. von Nussbaum F, Süssmuth RD (2015) Multiple attack on bacteria by the new antibiotic teixobactin. Angew Chem Int Ed Engl 54(23):6684–6686
107. Homma T, Nuxoll A, Gandt AB, Ebner P, Engels I, Schneider T, Götz F, Lewis K, Conlon BP (2016) Dual targeting of cell wall precursors by teixobactin leads to cell lysis. Antimicrob Agents Chemother 60(11):6510–6517

108. Xie Y, Xu H, Si S, Sun C, Chen R (2008) Sansanmycins B and C, new components of sansanmycins. J Antibiot 61(4):237–240
109. Li Y-B, Xie Y-Y, Du N-N, Lu Y, Xu H-Z, Wang B, Yu Y, Liu Y-X, Song D-Q, Chenet R-X (2011) Synthesis and in vitro antitubercular evaluation of novel sansanmycin derivatives. Bioorg Med Chem Lett 21(22):6804–6807
110. Xie Y, Chen R, Si S, Sun C, Xu H (2007) New nucleosidyl-peptide antibiotic, sansanmycin. J Antibiot 60(2):158–161
111. Winn M, Goss RJM, Kimura K, Bugg TDH (2010) Antimicrobial nucleoside antibiotics targeting cell wall assembly: recent advances in structure-function studies and nucleoside biosynthesis. Nat Prod Rep 27(2):279–304

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P-Type ATPases: A Relevant Component in *Mycobacterium tuberculosis* Viability

22

Paola Santos, Milena Maya-Hoyos, Marcela López-R, Cristian Rosales, Vanessa Vásquez, Andrés Varón, Bibiana Chavarro-Portillo, Nelson Enrique Arenas, and Carlos Y. Soto

Even if we all worked on different sides of the same problem, there were never problems of interfering in each others subjects, or about priority.

Jens Christian Skou (The winner of Nobel prize in chemistry for the discovery of the Na^+/K^+ -ATPase.)

Summary

Tuberculosis (TB) is an infectious disease that represents an important cause of worldwide death. Despite the treatments used to combat infections caused by *Mycobacterium tuberculosis* (*M. tb*), the increase of multidrug and extensively resistant (MDR and XDR) strains hinder TB control. *M. tb* faces different stress conditions inside macrophages during the infection process, including increased

Paola Santos and Milena Maya-Hoyos are contributed equally to this manuscript.

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concentrations of metal ions, hypoxia, and the production of reactive oxygen and nitrogen species (ROS/RNS). The *M. tb* genome contains 12 open reading frames encoding putative alkali/alkaline earth metal (CtpE, CtpF, CtpH, and CtpI) and heavy metal (CtpA, CtpB, CtpC, CtpD, CtpG, CtpJ, and CtpV) P-type ATPase transporters and one homolog of the potassium transporter KdpB. The transcriptional response of most of these genes, specially *ctpF*, is activated by one or more stress conditions inside macrophages. The inhibition of these cationic transporters promotes cation efflux imbalance across the mycobacterial plasma membrane and reduces the virulence and viability of tubercle bacilli. Therefore, mycobacterial P-type ATPases have the main role in ion regulation, detoxification, and virulence. These functions have attracted recent interest in studying these membrane transporters as potential drug targets or attenuation biomarkers. This chapter describes the structural and functional features of P-type ATPases of *M. tb* and their role in viability and virulence.

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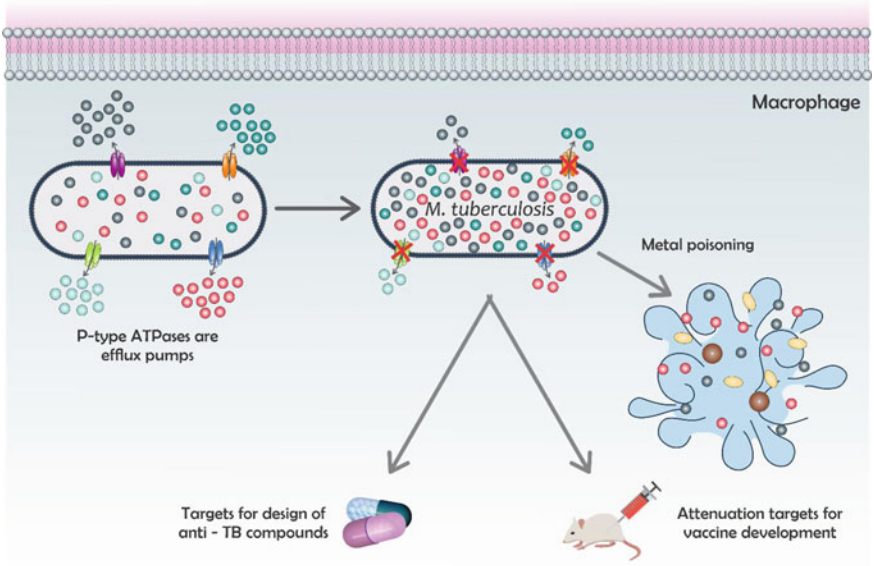
Graphical Abstract



P-type ATPases as targeted therapy for tuberculosis. When *M. tb* is phagocytosed by alveolar macrophages, it faces an arsenal of toxic substances and increased metal cations. P-type ATPases are pumps that support ion homeostasis. Altering the function of these pumps causes metal poisoning, which leads to decreased survival and attenuation of bacterial virulence. Thereby, P-type ATPases could be targeted for the design of new compounds and the development of vaccines

Keywords

Anti-TB targets · Attenuation · CtpF · *Mycobacterium tuberculosis* · Plasma membrane transporters · P-type ATPases

1 Introduction

Tuberculosis (TB) is a major global cause of human mortality, posing a challenge for public health authorities due to increased disease incidence in immunocompromised patients and the expansion of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains. Despite the progress made by the World Health Organization (WHO) regarding TB program indicators, such as reduction of TB cases and deaths, improved TB prevention and patient care, and increased funding, TB incidence remains approximately ten million new cases annually worldwide [1]. Globally, an estimated 1.7 billion people infected with *Mycobacterium tuberculosis*

(*M. tb*) will develop active disease. TB can affect anyone from any region, yet this disease frequently affects young adults (about 90%), leading to individual disability in the economically productive population. While effective treatment regimens have been developed since 1950, TB eradication challenges persist to date, including the accessibility of TB patients to early diagnosis and the directly observed treatment short-course (DOTS) to prevent disease spread. Treatment of MDR and XDR strains requires using second and third-line drugs that are more expensive and toxic. Failure to identify drug resistance (DR) may generate suboptimal therapeutic outcomes, amplify DR, and increase mortality [2, 3].

Taxonomically, mycobacteria belong to the order Actinobacteria and family Mycobacteriaceae, including only the genus *Mycobacterium* comprising more than 170 species [4]. Many mycobacteria are prominent pathogens, especially members of the *M. tb* complex, such as *M. bovis*, *M. leprae* and *M. ulcerans*. Furthermore, more than 150 species of environmental mycobacteria, known as nontuberculous mycobacteria (NTM), display different levels of pathogenicity and virulence [5].

Mycobacteria are strictly aerobic or microaerophilic gram-indeterminate bacteria [6] that display slow growth rates. Indeed, while the duplication time of *Enterobacteriaceae* is approximately 20 min, it exceeds 15 h in mycobacteria. All mycobacterial species can be cultivated, except *M. leprae*, although most require complex culture media [5]. Despite some differences among species, the morphology of mycobacteria is usually homogeneous, observed as long and slender acid-fast rods ranging from one to five μm in length and 0.2–0.6 μm in diameter. Although coccobacillus forms of mycobacteria are rarely found in pathological samples, these are commonly observed in laboratory preparations. *Mycobacterium* displays pleomorphism characterized by filamentous growth, which is sometimes branched. In addition, mycobacteria are non-motile and often non-sporogenic, except for some species [7].

ATPases are a family of transporters widely distributed across all life domains and divided into four superfamilies P-, F-, V-type, and ATP-binding cassette (ABC) transporters. ATPases are ubiquitous in biological membranes, transporting substrates against the concentration gradient using adenosine triphosphate (ATP) energy sources [8]. ATPases are also involved in cell proliferation, viability, energy production for metabolic processes, and cell volume control [9]. F- and V-type ATPases are proton transporters against the electrochemical gradient, whereas ABC transporters translocate other substrates, such as carbohydrates, amino acids, lipids, and drugs [10]. Specifically, P-type ATPases are a large group of membrane transporters of cations and lipids, which are essential to maintain gradients and convert metabolic energy into electrochemical gradients for cell signaling. P-type ATPases also mediate metal detoxification and supply metalloenzyme cofactors, providing an appropriate metal balance for cell survival [11]. Compared to the other ATPase families, P-types display a distinctive and highly conserved aspartate (D) residue that is phosphorylated in each catalytic cycle [10, 11]. Overall, P-type ATPases can be promising anti-TB drug targets given their essential roles in metabolic processes and stress response.

Table 1 The main functions, distribution among organisms, specificity, and number of TMH of the different subfamilies (P1–P5) of P-type ATPases

Subfamilies	Organisms	Specificity	H-TM	Function
P1A	Prokaryotes	K^+	7	Turgor pressure, pH homeostasis
P1B	Prokaryotes and eukaryotes	Cu^+, Ag^+, Cd^{2+}, Zn^{2+}, Pb^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}	6–8	Detoxification, protein metalation
P2A	Prokaryotes and eukaryotes	Ca^{2+}, Mn^{2+}, (SERCA)	10	Signaling and Ca^{2+} homeostasis
P2B	Prokaryotes and eukaryotes	Ca^{2+}, (PMCA)	10	
P2C	Prokaryotes and eukaryotes	Na^+–K^+/H^+–K^+	10	Membrane potential; acidification
P2D	Eukaryotes (fungi and protozoa)	Na^+	10	Counteract osmotic shocks and basic pH
P3A	Prokaryotes and eukaryotes (plants, fungi)	H^+	10	Membrane potential; pH homeostasis
P3B	Prokaryotes	Mg^{+2}	10	Mg^{2+} homeostasis
P4	Eukaryotes	Fosfolipids	10–12	Lipid bilayer asymmetry and vesicle formation
P5	Eukaryotes	Unknown	10–12	Mn^{2+} homeostasis in the endoplasmic reticulum

Prepared with data from [14–18]

2 Structure, Classification, and Function of P-Type ATPases

In addition to ion transport (Na^+, K^+, H^+, Ca^{2+}, Mg^{2+}, Cu^+, Cd^{2+}, Ag^+, Cu^{2+}, Co^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+}), P-type ATPases facilitate the translocation of amino-phospholipids across biological membranes [11, 12] (Table 1). This type of enzyme contains three cytoplasmic domains: activator or energy transduction (A), nucleotide-binding (N), and phosphorylation (P), and two transmembrane domains: transport (T) and specific support (S) [13] (Fig. 1).

P-type ATPases contain six to ten transmembrane segments (TMS), constituting a highly conserved structural core shared among archaea, prokaryotes, and eukaryotes. Although the A and N domains are not structurally homologous to other proteins, the P domain is homologous to the haloacid dehalogenase-like hydrolase domain [14].

Each domain of P-type ATPases has determined roles in the catalytic cycle. The N domain (kinase) initially makes a nucleophilic attack on the ATP (specifically in the γ-phosphate). Then, the conserved D residue of the P domain receives the phosphoryl group from ATP, generating a high-energy intermediate molecule (phosphoryl aspartate). During this process, a glutamate (G) residue within the A

domain (phosphatase) obtains energy from the phosphoryl aspartate group (P domain). The cytoplasmic domains are linked through transmembrane regions that define functional areas for ion transport across the cell membrane (Fig. 1) [14].

P-type ATPases are grouped into subfamilies (P1-P5) according to their ion specificity and membrane topology (Table 1). Each subfamily is also divided into subgroups that share the same catalytic mechanism of action and show similar protein structures. This classification was defined by the phylogenetic comparison of 159 proteins from different species; the main difference is the affinity for the transported substrate [11, 15].

P1-type ATPases comprise subclass P1A that includes the KdpB potassium pump and is involved in osmoregulation and pH homeostasis [19]. Moreover, subclass P1B catalyzes the translocation of heavy metals (Cu^{+}, Cu^{2+}, Ag^{+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Co^{2+}, Fe^{2+}, and Ni^{2+}) for ion detoxification and protein metalation (Table 1). P1B is classified into seven subclasses based on substrate specificity defined by invariant motifs located in the last three TMS and cytoplasmic metal-binding domains, displaying regulatory function [20].

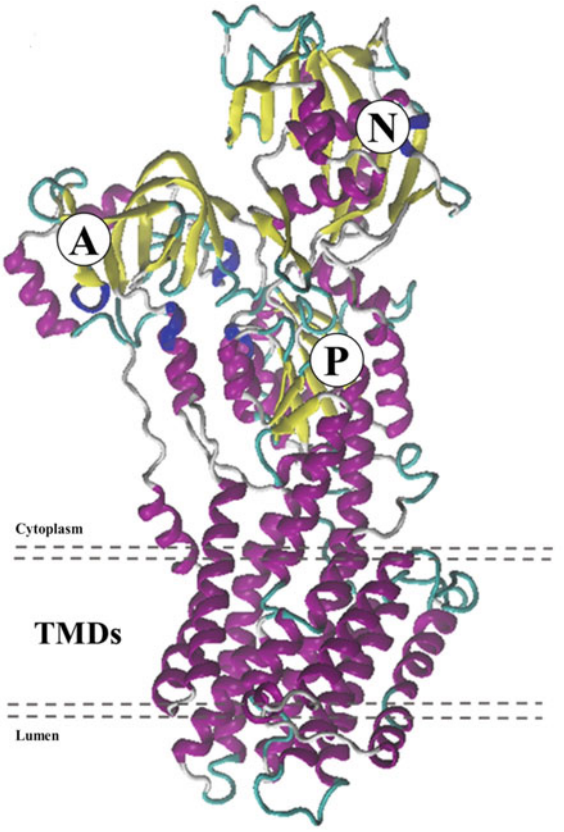


Fig. 1 Structural model predicted by homology modeling of *M. tb* CtpF. The cytoplasmic domains are represented: (N) nucleotide-binding, (P) phosphorylation, (A) actuator, and (TMDs) transmembrane domains. Reproduced with permission from [13], License Number 4904220999980

P2 is the most diverse subfamily of alkali/alkaline earth metals transporting P-type ATPases. This subfamily is divided into four subclasses:

i. P2A, it includes the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) involved in maintaining the gradient of Ca^{2+} concentration across the plasma membrane of certain organelles and cells [21];
ii. P2B, it contains the plasma membrane Ca^{2+}-ATPase (PMCA) transporter, which restores levels of Ca^{2+} in response to cellular signaling [11];
iii. P2C, P2C-type transporters comprise Na^+/K^+, and K^+/H^+ ATPases maintain the electrochemical gradient of Na^+ and K^+ that generate the membrane potential across the plasma membrane and gastric acid secretion through the electroneutral exchange of H^+ for K^+ [22]; and
iv. P2D, P2D-type are eukaryotic Na^+ ATPases and play a central role in Na^+ tolerance and basic pH (Table 1) [23].

The P3 subfamily is subdivided into subclass P3A, which are H^+ ATPases responsible for maintaining the membrane potential in fungi, bacteria, and plants, and P3B is involved in Mg^{2+} transport in prokaryotes [17] (Table 1). In contrast, the P4-type subfamily comprises ATPases involved in lipid bilayer maintenance and the formation of transport vesicles for endocytosis and secretion routes (Table 1) [11].

Regarding eukaryotic P5-type ATPases, the ion specificity was unknown until experimental evidence suggested that these enzymes are involved in Mn^{2+} homeostasis in the endoplasmic reticulum and might be associated with neurological disorders [18, 24] (Table 1). In the last decade, we studied ion transport mediated by P-type ATPases in mycobacteria and their possible role in *M. tb* attenuation. Using different approaches (e.g., bioinformatics, molecular biology, and biochemistry), we have studied the ion specificity of distinct P-type ATPases that may be relevant for the viability of tubercle bacilli.

3 *M. tb* P-Type ATPases

Twelve P-type ATPases have been identified in the *M. tb* proteome and are classified according to their ion specificity. Among these, seven are P1B-type or heavy metal transporters (CtpA, CtpB, CtpC, CtpD, CtpG, CtpJ, and CtpV); four are P2-type or alkali/alkaline earth metal transporters (CtpE, CtpF, CtpH, and CtpI); and one is P1A-type (KdpB) and corresponds to a potassium transporter (Fig. 2) [9, 25–27].

P-type ATPases are essential for *M. tb* survival by preserving the concentration of metal ions at the nutrient level for adequate cell function. Excessive cation accumulation inside mycobacteria is toxic because it replaces essential ions or functional groups, affecting the conformation of biomolecules and enzyme activities [28]. The number of P-type ATPases encoded in the *M. tb* genome (12 P-ATPases) suggests the importance of these transporters in tubercle bacilli virulence [25].

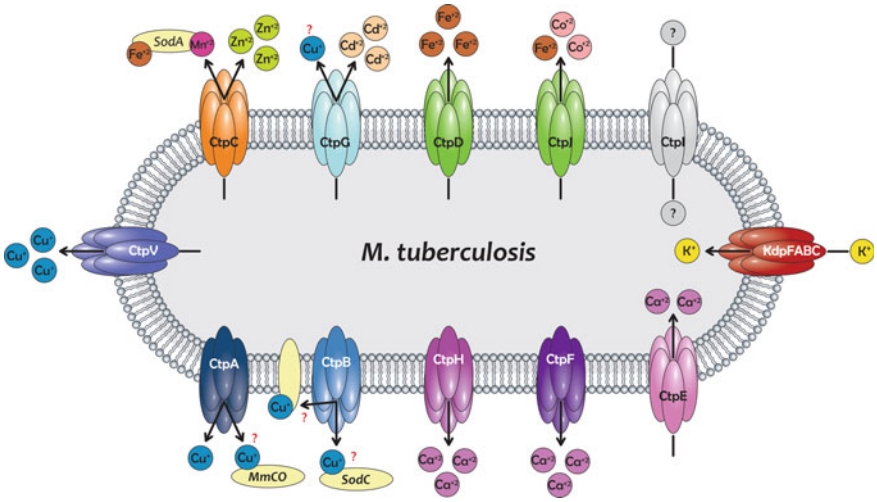


Fig. 2 Representative metal-cation transport of *M. tb* P-type ATPases. Arrows indicate the direction of ion transport across the mycobacterial plasma membrane. Potential metalation substrates by copper-transporting P-type ATPases are shown in beige

A meta-analysis of the transcriptional response of *M. tb* P-type ATPases in different conditions, such as hypoxia, oxidative stress, starvation, intoxication by chemical agents, and in vitro and in vivo infection processes, indicated that these transporters respond to different stress conditions that mycobacteria face inside macrophages [29]. Particularly, most mycobacterial P-type ATPase genes (e.g., *ctpF, ctpG, ctpC, ctpH*, and *ctpV*) are overexpressed during infection in human macrophages, suggesting that these pumps play a fundamental role in cell proliferation and even their inhibition alter the virulence of *M. tb* [29]. Thus, the deletion of several heavy metal P-type ATPase transporters impairs *M. tb* survival in human macrophages and animal models [27, 30, 31].

3.1 Heavy Metal P-Type ATPases of *M. tb*

3.1.1 Cu^+ transporters

Copper is an essential micronutrient in bacteria, given the role of this heavy metal in redox reactions involved in different metabolic processes [32, 33]. Therefore, copper misregulation can produce oxidative damage and cell death in bacteria [34]. Specifically, high intracellular concentrations of copper induce ROS production via Fenton reaction, protein denaturation, and displacement of metal cofactors in iron-sulfur cluster proteins [35].

Particularly, copper concentration increases between one to 24 h post-infection in phagosomes containing mycobacteria [36]. Therefore, to survive under host-mediated copper stress, *M. tb* activates resistance pathways mainly exerted by CtpV

P1B-type ATPase to maintain copper ion homeostasis (Fig. 2) [30]. Previous studies demonstrated that knock-out of the *ctpV* gene promotes copper sensitivity in *M. tb* and reduces mycobacterial growth rates during early infection in guinea pigs. Accordingly, *ctpV* activity has been associated with resistance to copper poisoning during in vivo infection [30].

Based on in silico predictions, *ctpA* and *ctpB* genes are homologous to *ctpV* in *M. tb* so that they may be involved in Cu^{+} transport and detoxification [25]. Nevertheless, further experimental validation showed that Cu^{+} transport mediated by CtpA and CtpB might be involved in physiological functions besides detoxification, such as metalation of extra-cytoplasmic cuproenzymes in response to oxidative stress conditions [26, 37]. Cuproenzymes, such as multicopper oxidase (MmcO), superoxide dismutase A (SodA), and cytochrome c oxidase, use copper as a cofactor [38] and are required for *M. tb* survival [39, 40].

Therefore, MmcO and SodA could be metalation substrates of CtpA and/or CtpB [26, 37] (Fig. 2). Overall, these findings suggest that Cu^{+} efflux via P-type ATPases is essential in metal detoxification, protein metalation, and response to oxidative stress in mycobacteria, as proposed in other cellular models [41–44].

3.1.2 Zn^{2+} Transporters

High levels of Zn^{2+} can displace iron from sulfhydryl residues of bacterial enzymes and interrupt manganese absorption, leading to decreased bacterial tolerance to free radicals, as described in several bacterial models [45]. In response to Zn^{2+} accumulation [46], *M. tb* activates efflux mechanisms associated with CtpC P-type ATPase to prevent Zn^{2+} intoxication inside the intraphagosomal environment [31]. Although CtpC lacks the conserved residues involved in Zn^{2+} coordination and the cytoplasmic N-terminal metal-binding domains (N-MBD), which is ubiquitous in Cu^{+}/Zn^{2+} ATPases [27], the role of CtpC in zinc detoxification has not been discarded.

Upstream *ctpC*, *M. tb* encodes a putative metallochaperone (Rv3269) that may confer zinc specificity to CtpC and be essential for Zn (II) efflux detoxification in vivo [47]. Therefore, CtpC may constitute the main strategy for *M. tb* resistance to zinc toxicity and survival in eukaryotic phagocytes [31].

3.1.3 Mn^{2+} Transporters

Manganese crucially contributes to the viability and virulence of bacterial pathogens [48]. This cation is a cofactor of enzymes involved in response to host oxidative stress in *M. tb* [47]. Mn^{2+} bioavailability is limited under infection conditions, probably restricting bacterial pathogen growth and resistance to ROS [49]. The *M. tb* CtpC P-type ATPase has also been associated with Mn^{2+}-efflux and, mainly, with the assembly of Fe^{+2}/Mn^{+2} superoxide dismutase A (SodA) [27] (Fig. 2). Besides regulating intracellular Mn^{2+} concentration, CtpC -Mn^{2+} transport could be used to metalate enzymes involved in redox responses during infection, constituting a key element in *M. tb* virulence.

3.1.4 Fe^{2+}, Co^{2+}, and Ni^{2+} Transporters

Iron, nickel, and cobalt are crucial for many biological processes due to their redox properties [50]; however, like other metal transitions, the excess of these elements is toxic to the cell. For instance, Fe^{2+} and Ni^{2+} generate toxic oxygen intermediates [51, 52], Co^{2+} inhibits the electron transport chain activity in bacteria and mitochondria [53], and Fe^{2+} causes mismetallation of non-iron metalloproteins [51–53]. The efflux of these metal cations is not completely understood in *M. tb* [54, 55]. The distinct roles of mycobacterial CtpJ and CtpD P1B-ATPases in the homeostasis of Co^{2+} and Fe^{2+} were recently reported [55, 56]. Patel et al. propose that Fe^{2+} efflux mediated by CtpD is implicated in response to iron dyshomeostasis produced by redox stress [55], suggesting that CtpD is required for *M. tb* virulence [57]. Moreover, CtpJ is responsible for maintaining Co^{2+} and Fe^{2+} cytoplasmic levels [55, 56]. These observations demonstrate the versatility of P1B-type ATPase-mediated transport and highlight a novel and possibly central role of these proteins in Fe^{+2} efflux; however, iron homeostasis has been little explored in *M. tb*.

3.1.5 Cd^{2+} Transporters

Cadmium has no specific function in the cell, yet it displays high toxicity and strong adverse effects in different cell types [58]. Cd^{2+} can affect the transport chain [53] and cation homeostasis [59], interact with nucleic acids, and displace cofactor for proteins making cells susceptible to oxidative stress [60]. *M. tb* contains a CtpG P-type ATPase that is located in an operon together with the Cd^{2+}/Pb^{2+}-sensing regulator CmtR (Rv1994c) and a metallochaperone (Rv1993c) [61], suggesting that these proteins could be involved in cadmium detoxification [61, 62]. The ATPase activity mediated by CtpG is activated by Cu^{2+} in the mycobacterial plasma membrane [62], and this transporter could be an alternative copper detoxification mechanism to CtpV [30]. However, experimental evidence shows that CtpG preferentially transports Cd^{2+} compared with other divalent cations across the mycobacterial plasma membrane [62].

3.2 Alkali/Alkaline Earth Cation P-Type ATPases of *M. tb*

3.2.1 K^+ and Na^+/K^+ Transporters

Na^+/K^+-ATPases (P2C subclass) maintain the electrochemical gradients of Na^+/K^+ ions by moving three Na^+ ions across the membrane and concurrently importing two K^+ ions through an ATP-dependent process [63]. These gradient concentrations are important for stabilizing the membrane potential, maintaining osmotic equilibrium and cell volume, energizing secondary transport processes, and promoting cellular signal transduction [22]. The increases in intracellular Na^+ concentration are related to inhibition of Na^+/K^+-ATPase activity in the cell and changes in intracellular pH due to the Na^+/H^+ exchange system, as well as altered intracellular Ca^{2+} concentrations by the action of the Na^+/Ca^{2+} exchange system. Despite the

essential roles of Na^+/K^+-ATPases in cell physiology and signaling responses, these pumps have not been characterized in *M. tb* [64].

Similarly, potassium ion homeostasis systems have a critical role in regulating osmotic pressure, pH, cell shape, and turgor. In *M. tb*, different systems for K^+ homeostasis, such as TrK and Kdp, have been described [65]. In the micromolar range, the *kdp* operon, which encodes the potassium pump, KdpFABC, is responsible for transporting K+ inside cells. This membrane complex is formed by four subunits, the KdpA, a channel-like subunit from the potassium transporters superfamily, the KdpB subunit of the superfamily of P-type ATPases, and two accessory subunits called KdpC and KdpF [19].

KdpB contains the specific functional domains of P-type ATPases in which the ATP hydrolysis occurs to energize ion transport. However, this subunit does not contain ion-binding motifs, so K^+ ions are actually transported through the KdpA subunit [66]. The KdpC subunit provides stability to the complex and mediates ATP hydrolysis of the KdpB subunit and ion transport through the KdpA and KdpF subunits [19]. *M. tb* encodes a KdpB subunit homolog (e.g., P9WPU3), which shares the highest sequence identity (63% identity) with KdpB from *E. coli* (Uniprot: P03960) and shows induced expression at low potassium concentrations in *M. tb* [67]. As a complex, the subunits of the KdpFABC system have been associated with the persistence of *M. tb* bacilli within the host; furthermore, the expression of this complex is promoted when other K^+ transport systems have been inactivated [65].

3.2.2 Ca^{2+} Transporters

Calcium homeostasis is relevant for various cellular processes, such as cell growth, proliferation, cellular motility, and development [68–70]. In bacteria, different experimental approaches have demonstrated calcium gradients across the cell membrane, although the reason for maintaining these gradients is not completely understood [71]. Calcium homeostasis requires proteins that regulate cytosolic concentrations, usually calcium transporters or proteins with calcium-binding domains [68]. In bacteria, passive [72, 73] and active [74] calcium transporters, as well as proteins with calcium-binding domains (CABDs) [75, 76], have been reported. One important CABD is the Repeat-in-Toxin (RTX) domain, which undergoes a structural change to a β-roll upon calcium binding and is associated with protein translocation across the membrane [68]. An RTX-related family of proteins is exclusively encoded by nearly 100 genes in mycobacteria, including *M. tb* [77]. These proteins have been associated with the capacity of pathogenic mycobacteria to survive within hostile conditions of the macrophage during infection [78].

The *M. tb* genome encodes proteins with CABDs, suggesting the existence of specialized machinery for calcium handling and regulation of cytosolic calcium concentration. In this context, the search for proteins that allow calcium import and export across the membrane in *M. tb* cells has become relevant. There is growing evidence that reveals the features of possible calcium active transporters encoded in the *M. tb* genome. One of the first attempts to identify potential calcium transporters

in the *M. tb* genome was the classification of CtpH, CtpI, CtpE, and CtpF as P2-type ATPases based on their hydrophobicity profiles and conserved structural motifs. These *M. tb* ATPases contain the four-residue motif PEGL associated with binding to alkali/alkaline-earth metals [79], except for CtpH, which contains a variation in the last residue (PEGM) that has not been previously described [25].

Experimental evidence generated by our research group showed that CtpH has a calcium-dependent transport activity, and *M. tb* cells defective in *ctpH* accumulate more calcium than the wild-type strain (unpublished results). Conversely, no evidence confirms the ion specificity of CtpI. Regarding CtpE, it has been characterized as a calcium uptake transporter in *M. tb* [80]; in this sense, P-type ATPases involved in cation uptake from the extracellular environment had not been previously described excepting KdpB. CtpE may be important to maintain calcium concentrations in low-Ca^{2+} environments similar to those experienced by bacilli inside the macrophage [81, 82]. The expression of *ctpE* as part of a negatively calcium-regulated operon leads to questioning how *M. tb* handles high external calcium concentrations, for example, in the lungs and mucous membranes [70]. One explanation is the use of a Ca^{2+}-ATPase that reestablishes the cytosol calcium concentration after induced surges of calcium, which is comparable to the function of SERCA in eukaryotic cells.

CtpF is the closest SERCA homolog in the *M. tb* genome, sharing 33% of identity and eight out of ten residues involved in calcium-binding, suggesting a possible calcium-dependent transport activity. We conducted a study to confirm the ion of specificity and direction of transport by CtpF, finding an ATP-dependent calcium transport preferentially towards the outside of the cell [83]. The sequence identity shared between CtpF and SERCA1a is relevant enough to allow for the use of cyclopiazonic acid (CPA) as a model for designing drugs targeted to CtpF that show in vitro antimycobacterial activity [13]. Cyclopiazonic acid is a well-characterized inhibitor of calcium-binding activity in SERCA1a. In CtpF, calcium-dependent transport is inhibited by this compound and reduces mycobacterial viability; therefore, it may be important for *M. tb* survival. Moreover, *M. tb* cells defective in *ctpF* accumulate calcium and show impaired viability when exposed to oxidative and nitrosative stress (see Section "CtpF as a Pivotal Target for *M. tb* Viability") [83]. Overall, CtpF is a promising drug and attenuation target.

4 CtpF as a Pivotal Target for *M. tb* Viability

4.1 CtpF as a Therapeutic Target

The rapid evolution of *M. tb* to generate resistance to the currently used anti-TB drugs underlines an urgent need to develop new compounds to treat sensitive and resistant TB, as well as identify alternative therapeutic targets [84]. To address this, different research groups focus on developing new anti-TB drugs by identifying targets in the *M. tb* cell membrane. For example, a novel anti-TB drug called

bedaquiline targets the membrane protein F1F0 ATP synthase and affects proton pumping across the *M. tb* membrane; furthermore, this drug was approved for controlled use in XDR-TB patients [85]. Similarly, N-Geranyl-N′-(2-adamantyl) ethane-1,2-diamine (SQ-109), a small molecule active currently in phase II clinical trials, blocks the trehalose monomycolate transporter, MmpL3, responsible for transporting trehalose monomycolate, an essential precursor in the biosynthesis of mycobacterial mycolic acids [86, 87]. Potential antimicrobial agents may target these plasma membrane proteins without having to penetrate the cell membrane [88]. In the light of this, P-type ATPases are attractive drug targets for designing new anti-TB compounds; for instance, these proteins are targets for successful antimicrobial agents, such as the antimalarial artemisinin, which inhibits Ca^{2+}-ATPase of *Plasmodium falciparum* (PfATP6) [89, 90].

CtpF (Rv1997) is a P-type Ca^{2+}-ATPase of *M. tb* associated with response to oxidative stress [83] and is an important protein that responds under infection conditions. During phagocytosis, *M. tb* faces high concentrations of Ca^{2+}, partially offset by CtpF to maintain normal intracellular concentrations of this metal [91]. In a previous study, we evidenced that the Ca^{2+}-ATPase activity mediated by CtpF was inhibited by cyclopiazonic acid, at concentrations similar to those reported for SERCA and PfATP6 activity in eukaryotes. In addition, cyclopiazonic acid inhibits mycobacterial growth, suggesting a relationship between Ca^{2+}-ATPase activity of CtpF and the *M. tb* viability [13]. A strategy including the 3D homology modeling of CtpF-*M. tb* was able to identify the pharmacophoric characteristics of the CtpF-CPA complex that were selected as a pharmacophoric model for searching inhibitory compounds of the Ca^{2+}ATPase activity of *M. tb*. This selected pharmacophoric model has carried out a pharmacophore-based virtual screening (PBVS) within the ZINC database that contains 22,723,923 compounds and identifies candidate molecules as CtpF inhibitors. Docking-based virtual screening (DBVS) and molecular mechanics/generalized Born surface area (MM/GBSA) re-scoring of the putative CtpF inhibitors allowed the identification of three compounds (ZINC63908257, ZINC55090623, and ZINC45605493) to be evaluated in vitro.

These compounds showed a minimum inhibitory concentration (MIC) between 50–100 μg/mL and reduced Ca^{2+}-ATPase activity in *M. tb* membrane vesicles with a half-maximal inhibitory concentration (IC50) ranging from 4.1 to 35.8 μM (Fig. 3a). Particularly, ZINC63908257 (MIC of 50 μg/mL) decreased Ca^{2+}-ATPase activity of the mycobacterial plasma membrane by 45% (IC50 = 4.4 μM). Preliminary results also showed that ZINC63908257 inhibited the intracellular replication of *M. tb* H37Rv in a murine macrophage model of infection on mouse alveolar macrophages (MH-S), displaying a low cytotoxic effect (Fig. 3b). Thus, the inhibition of Ca^{2+}-ATPase activity mediated by CtpF could affect the intracellular replication of *M. tb*.

A detailed analysis of the binding site and mode for the candidate anti-TB molecule ZINC63908257 shows that it binds to the transmembrane domain of CtpF in a pocket formed between TMS segments M1-M4 (Fig. 4). Particularly, this binding mode is similar to that of CPA to SERCA1a. In addition, a region between

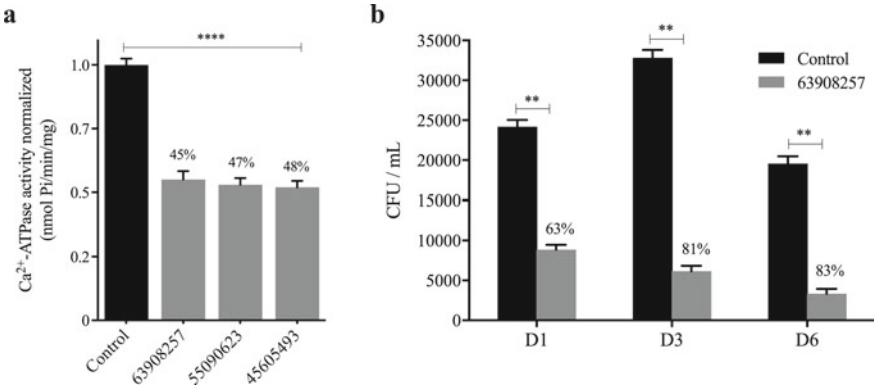


Fig. 3 Activity of compounds targeting CtpF selected by virtual screening: **a** Effect of compounds on normalized Ca^{2+}-ATPase activity of *M. tb* at a concentration of 100 µM. Ca^{2+}-ATPase activity in the absence of compounds was used as control. **b** Effect of ZINC63908257 on the intracellular viability of *M. tb* H37Rv strain. CFU/mL of *M. tb* H37Rv in the presence of the compound relative to the control without stimulation on days D1, D3, and D6 after phagocytosis are indicated. The values represent the mean ± SD (n = 3). Significant differences are marked with asterisks as $^{**}P < 0.001$; $^{****}p < 0.0001$. Reproduced with permission from [13], License Number 4904220999980

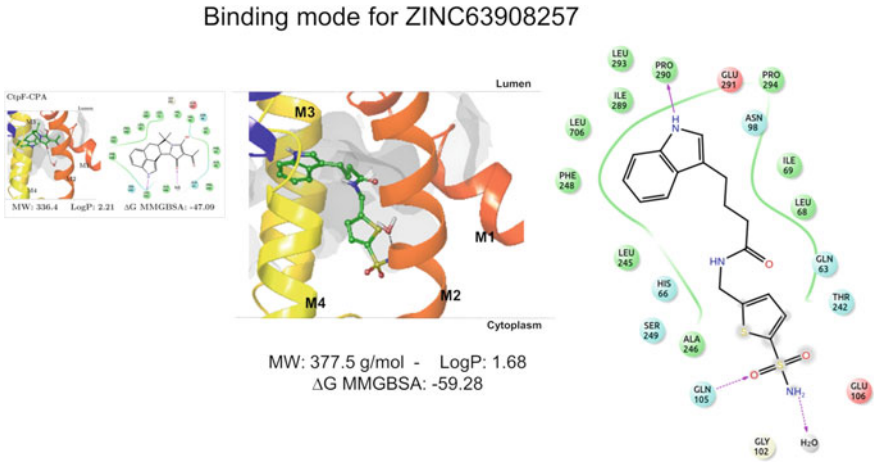


Fig. 4 3D and 2D representations of the binding modes of ZINC63908257 to CtpF. The small box on the left indicates the mode of CPA binding to CtpF. Residues at 4 Å of the ligand are shown, and TMDs M1 to M4 are indicated. This compound occupies the calcium access channel, similar to CPA, and makes short contacts with residues of M1 (Gln63, His66, and Ile69), M2 (Gly102, hydrogen bonding with Gln105 and Glu106), M3 (Thr242, Leu245, and Ala246), and M4 (Ile289, hydrogen bonding with Pro290, Glu291, Leu293, Pro294). Reproduced with permission from [13], License Number 4904220999980

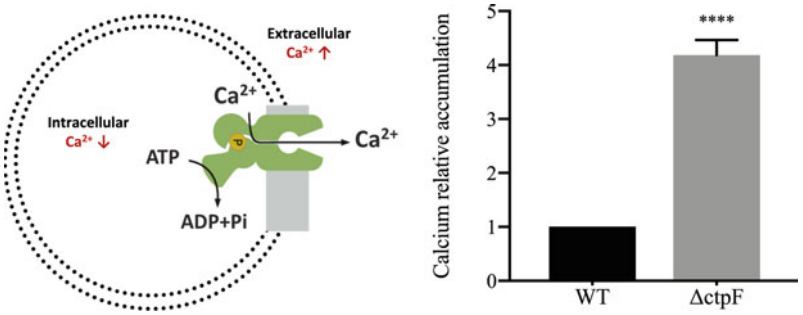


Fig. 5 Calcium accumulation in *M. tb* cells. The amount of calcium accumulated was internally normalized against the amount of calcium measured in the dry mass pellet from the WT strain. The data shown are representative of three independent experiments. Unpaired two-tailed t-test, ****$P < 0.0001$. Reproduced with permission from [83], License number 4903930171288

TMS M1, M2, M3, and M4 towards the cytoplasmic portion was identified as a target site to design potent inhibitors of Ca^{2+}-P-type ATPase mediated by CtpF. This combined strategy suggested that *M. tb* P-type ATPases, such as CtpF, are key molecular targets for designing new anti-TB compounds.

4.2 CtpF as a Target for *M. tb* Attenuation

The transcriptional profile of *M. tb* under different stress conditions, which simulate the adverse conditions faced by tubercle bacilli in phagosomes [29], shows that *ctpF* is the most responsive P-type ATPase to several stress conditions. This is especially observed under the presence of ROS/RNS and hypoxia [29], suggesting that CtpF is involved in strategies used by *M. tb* to face the hostile conditions inside phagosomes during infection processes.

These findings led us to focus our efforts on constructing a defective mutant of the *ctpF* gene in *M. tb* (*M. tb* Δ*ctpF*) by homologous recombination [92]. Functional analyses on the *M. tb* Δ*ctpF* strain showed that CtpF contributes to Ca^{2+} detoxification in environments with high concentrations of this metal by preventing calcium accumulation in the mycobacterial cytosol (Fig. 5) [83].

The *ctpF* gene is among 50 genes controlled by the global latency regulon (DosR) involved in tubercle bacillus adaptation to the anaerobic environment inside macrophages, entry to latency, and conservation of redox balance [93, 94]. For this reason, we assessed the relationship between the transcriptional response of ctpF and redox stress by evaluating the susceptibility of *M. tb* wild type (WT) and *M. tb* Δ*ctpF* strains against oxidizing agents, such as hydrogen peroxide (H_2O_2) and sodium nitroprusside (SNP). *M. tb* Δ*ctpF* was hypersensitive to H_2O_2 and SNP, suggesting a link between calcium transport and the mechanisms used by *M. tb* to neutralize ROS/RNS. Calcium is an intracellular messenger in bacteria that induces diverse mechanisms, including defense against oxidative stress (Fig. 6) [68–70].

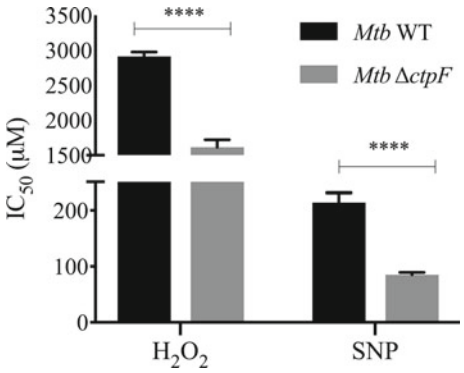


Fig. 6 Response of *M. tb* WT and *M. tb* ΔctpF to oxidative and nitrosative stress. Bacteria were grown in 7H9-OAD media supplemented with varying concentrations of H_2O_2 and SNP. OD_{600} cultures in the absence of oxidant agents are considered 100%. Values represent the IC50. Data are mean ± SEM from three independent experiments. Unpaired two-tailed t-test, $^{****}P < 0.0001$. Reproduced with permission from [83], License number 4903930171288

The increased intracellular Ca^{2+} concentration in mycobacteria in the phagosomal environment must be transitory to avoid bacterial toxicity; thus, the efflux function of CtpF is essential to maintain the ionic homeostasis of Ca^{2+} [83].

The activation of *ctpF* in response to redox stress (i.e., a condition faced by tubercle bacilli in the phagosomal environment) suggests that this transporter could play an important role in the course of infection processes. Accordingly, we evaluated the effect of *ctpF* deletion in *M. tb* virulence. Preliminary results show that *ctpF* is required for *M. tb* growth in MH-S cells; however, the mutant strain preserves the immunogenic capacity. In addition, mice infected with the *M. tb* Δ*ctpF* strain showed a higher survival rate than animals infected with *M. tb* WT strain in a lung TB model in BALB/c mice.[1] Therefore, mutations that affect nutrient uptake and detoxification systems can also affect the virulence and persistence of *M. tb* during infection processes. Overall, these findings demonstrate interesting targets for attenuation and provide a starting point for the rational design of live attenuated strains with vaccine potential.

5 Conclusion

The capacity to face changes in the environmental cation concentration is fundamental for mycobacterial viability; thence, the variety of P-type ATPase transporters in the *M. tb* genome suggests that these ATPases are essential for mycobacterial survival. *M. tb* must overcome multiple environments, such as the host cell's cytosol, early endosomes, phagolysosomes, early granuloma, and

[1] Unpublished results.

necrotic/caseous granuloma, leading tubercle bacilli to acquire several defense mechanisms to succeed as an intracellular pathogen. Furthermore, *M. tb* has many P-type ATPases that contribute to the detoxification of heavy metal ions, such as Cd^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, among others, which provide tubercle bacilli an evolutionary advantage over host immune cells that naturally increase cation concentrations to prevent the proliferation of invading pathogens. In particular, *M. tb* CtpF Ca^{2+} transporter is a P-type ATPase that faces and responds to the most stressful conditions, representing a biomarker of special interest as a virulence factor or drug target, as demonstrated through different experimental approaches.

Core Messages

- P-type ATPases are essential for *M. tb* survival by preserving the metal balance required for proper cell function.
- The relation between P-type ATPases' activity and mycobacterial survival points to these proteins as new anti-TB targets.
- CtpF is essential during infectious processes since its deletion leads to attenuation of the virulence in *M. tb.*

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References

1. World Health Organization (2019) Global tuberculosis report 2019
2. Shin SS, Keshavjee S, Gelmanova IY, Atwood S, Franke MF, Mishustin SP, Strelis AK, Andreev YG, Pasechnikov AD, Barnashov A, Tonkel TP, Cohen T (2010) Development of extensively drug-resistant tuberculosis during multidrug-resistant tuberculosis treatment. Am J Respir Crit Care Med 182:426–432
3. Ragonnet R, Trauer JM, Denholm JT, Marais BJ, McBryde ES (2017) High rates of multidrug-resistant and rifampicin-resistant tuberculosis among re-treatment cases: where do they come from? BMC Infect Dis 17
4. Fedrizzi T, Meehan CJ, Grottola A, Giacobazzi E, Fregni Serpini G, Tagliazucchi S, Fabio A, Bettua C, Bertorelli R, De Sanctis V, Rumpianesi F, Pecorari M, Jousson O, Tortoli E, Segata N (2017) Genomic characterization of *Nontuberculous mycobacteria*. Sci Rep 7
5. Pfyffer GE (2015) *Mycobacterium*: general characteristics, laboratory detection, and staining procedures. In: Manual of clinical microbiology, 11th edn, pp 536–569
6. Grange JM (1996) The biology of the genus *Mycobacterium*. J Appl Microbiol Symp Suppl 81
7. Ghosh J, Larsson P, Singh B, Pettersson BMF, Islam NM, Sarkar SN, Dasgupta S, Kirsebom LA (2009) Sporulation in mycobacteria. Proc Natl Acad Sci U S A 106:10781–10786

8. Axelsen KB, Palmgren MG (1998) Evolution of substrate specificities in the P-type ATPase superfamily. J Mol Evol 46:84–101
9. Agranoff D, Krishna S (2004) Metal ion transport and regulation in *Mycobacterium tuberculosis*. Front Biosci 9(1–3):2996–3006
10. Bublitz M, Morth JP, Nissen P (2012) P-type ATPases at a glance. J Cell Sci 124:2515–2519. https://doi.org/10.1242/jcs.102921
11. Palmgren MG, Nissen P (2011) P-type ATPases. Annu Rev Biophys 40:243–266
12. Agranoff DD, Krishna S (1998) Metal ion homeostasis and intracellular parasitism. Mol Microbiol 28:403–412
13. Santos P, Lopez-Vallejo F, Ramírez D, Caballero J, Mata Espinosa D, Hernández-Pando R, Soto CY (2020) Identification of *Mycobacterium tuberculosis* CtpF as a target for designing new antituberculous compounds. Bioorganic Med Chem 2
14. Bublitz M, Poulsen H, Morth JP, Nissen P (2010) In and out of the cation pumps: P-type ATPase structure revisited. Curr Opin Struct Biol 20:431–439
15. Dyla M, Kjærgaard M, Poulsen H, Nissen P (2020) Structure and mechanism of P-type ATPase ion pumps. Annu Rev Biochem 89:583–603
16. Chourasia M, Sastry GN (2012) The nucleotide, inhibitor, and cation binding sites of P-type II ATPases. Chem Biol Drug Des 79:617–627
17. Subramani S, Perdreau-Dahl H, Morth JP (2016) The magnesium transporter A is activated by cardiolipin and is highly sensitive to free magnesium in vitro. Elife 5
18. Cohen Y, Megyeri M, Chen OCW, Condomitti G, Riezman I, Loizides-Mangold U, Abdul-Sada A, Rimon N, Riezman H, Platt FM, Futerman AH, Schuldiner M (2013) The yeast P5 type ATPase, Spf1, regulates manganese transport into the endoplasmic reticulum. PLoS ONE 8
19. Huang CS, Pedersen BP, Stokes DL (2017) Crystal structure of the potassium-importing KdpFABC membrane complex. Nature 546:681–685
20. Smith AT, Smith KP, Rosenzweig AC (2014) Diversity of the metal-transporting P1B-type ATPases. J Biol Inorg Chem 19:947–960
21. Primeau JO, Armanious GP, Fisher MLE, Young HS (2018) The sarcoendoplasmic reticulum calcium ATPase. In: Subcellular biochemistry, pp 229–258
22. Jorgensen PL, Håkansson KO, Karlish SJD (2003) Structure and mechanism of Na, K-ATPase: functional sites and their interactions. Annu Rev Physiol 65:817–849
23. Fraile-Escanciano A, Garciadeblás B, Rodríguez-Navarro A, Benito B (2009) Role of ENA ATPase in Na^+ efflux at high pH in bryophytes. Plant Mol Biol 71:599–608
24. Di Fonzo A, Chien HF, Socal M, Giraudo S, Tassorelli C, Iliceto G, Fabbrini G, Marconi R, Fincati E, Abbruzzese G, Marini P, Squitieri F, Horstink MW, Montagna P, Libera AD, Stocchi F, Goldwurm S, Ferreira JJ, Meco G, Martignoni E, Lopiano L, Jardim LB, Oostra BA, Barbosa ER, Bonifati V, Bonifati V, Vanacore N, Meco G, Fabrizio E, Locuratolo N, Scoppetta C, Manfredi M, Berardelli A, Lopiano L, Giraudo S, Bergamasco B, Pacchetti C, Nappi G, Antonini A, Pezzoli G, Riboldazzi G, Bono G, Raudino F, Manfredi M, Fincati E, Tinazzi M, Bonizzato A, Ferracci C, Dalla Libera A, Abbruzzese G, Marchese R, Montagna P, Marini P, Massaro F, Guidi M, Minardi C, Rasi F, Onofrj M, Thomas A, Stocchi F, Vacca L, De Pandis F, De Mari M, Diroma C, Iliceto G, Lamberti P, Toni V, Trianni G, Mauro A, De Gaetano A, Rizzo M, Cossu G, Rieder CRM, Saraiva-Pereira ML (2007) ATP13A2 missense mutations in juvenile parkinsonism and young onset Parkinson disease. Neurology 68:1557–1562
25. Novoa-Aponte L, León-Torres A, Patiño-Ruiz M, Cuesta-Bernal J, Salazar LM, Landsman D, Mariño-Ramírez L, Soto CY (2012) In silico identification and characterization of the ion transport specificity for P-type ATPases in the *Mycobacterium tuberculosis* complex. BMC Struct Biol 12:25
26. León-Torres A, Arango E, Castillo E, Soto CY (2020) CtpB is a plasma membrane copper (I) transporting P-type ATPase of *Mycobacterium tuberculosis*. Biol Res 53(1)

27. Padilla-Benavides T, Long JE, Raimunda D, Sassetti CM, Argüello JM (2013) A novel P (1B)-type Mn^{2+}-transporting ATPase is required for secreted protein metallation in mycobacteria. J Biol Chem 288:11334–11347
28. Rathnayake IVN, Megharaj M, Bolan N, Naidu R (2010) Tolerance of heavy metals by gram positive soil bacteria. Int J Environ Eng 2:191–195
29. Novoa-Aponte L, Soto Ospina CY (2014) *Mycobacterium tuberculosis* P-type ATPases: possible targets for drug or vaccine development. Biomed Res Int 296
30. Ward SK, Abomoelak B, Hoye EA, Steinberg H, Talaat AM (2010) CtpV: a putative copper exporter required for full virulence of *Mycobacterium tuberculosis*. Mol Microbiol 77:1096–1110
31. Botella H, Peyron P, Levillain F, Poincloux R, Poquet Y, Brandli I, Wang C, Tailleux L, Tilleul S, Charrire GM, Waddell SJ, Foti M, Lugo-Villarino G, Gao Q, Maridonneau-Parini I, Butcher PD, Castagnoli PR, Gicquel B, De Chastellier C, Neyrolles O (2011) Mycobacterial P1-type ATPases mediate resistance to Zinc poisoning in human macrophages. Cell Host Microbe 10:248–259
32. Ma Z, Jacobsen FE, Giedroc DP (2009) Coordination chemistry of bacterial metal transport and sensing. Chem Rev 109:4644–4681
33. Ladomersky E, Petris MJ (2015) Copper tolerance and virulence in bacteria. Metallomics 7:957–964
34. Giachino A, Waldron KJ (2020) Copper tolerance in bacteria requires the activation of multiple accessory pathways. Mol Microbiol
35. Solioz M (2018) Copper and bacteria: evolution, homeostasis and toxicity. Springer International Publishing
36. Wagner D, Maser J, Lai B, Cai ZH, Barry CE, Bentrup KHZ, Russell DG, Bermudez LE (2005) Elemental analysis of *Mycobacterium avium*-, *Mycobacterium tuberculosis*-, and *Mycobacterium smegmatis*-containing phagosomes indicates pathogen-induced microenvironments within the host cell's endosomal system. J Immunol 174:1491–1500
37. León-Torres A, Novoa-Aponte L, Soto C-Y (2015) CtpA, a putative *Mycobacterium tuberculosis* P-type ATPase, is stimulated by copper (I) in the mycobacterial plasma membrane. BioMetals 28:713–724
38. Spagnolo L, Töro I, D'Orazio M, O'Neil P, Pedersen JZ, Carugo O, Rotilio G, Battistoni A, Djinović-Carugo K (2004) Unique features of the sodC-encoded superoxide dismutase from *Mycobacterium tuberculosis*, a fully functional copper-containing enzyme lacking zinc in the active site. J Biol Chem 279:33447–33455
39. Piddington DL, Fang FC, Laessig T, Cooper AM, Orme IM, Buchmeier NA (2001) Cu, Zn superoxide dismutase of *Mycobacterium tuberculosis* contributes to survival in activated macrophages that are generating an oxidative burst. Infect Immun 69:4980–4987
40. Rowland JL, Niederweis M (2013) A multicopper oxidase is required for copper resistance in *Mycobacterium tuberculosis*. J Bacteriol 195:3724–3733
41. Osman D, Patterson CJ, Bailey K, Fisher K, Robinson NJ, Rigby SEJ, Cavet JS (2013) The copper supply pathway to a Salmonella Cu, Zn-superoxide dismutase (SodCII) involves P1B-type ATPase copper efflux and periplasmic CueP. Mol Microbiol 87:466–477
42. Lee J, Dennison C (2019) Cytosolic copper binding by a bacterial storage protein and interplay with copper efflux. Int J Mol Sci 20(17):4144
43. Alquethamy SF, Khorvash M, Pederick VG, Whittall JJ, Paton JC, Paulsen IT, Hassan KA, McDevitt CA, Eijkelkamp BA (2019) The role of the copA copper efflux system in *Acinetobacter baumannii* virulence. Int J Mol Sci 20(3):575
44. Patel SJ, Padilla-Benavides T, Collins JM, Argüello JM (2014) Functional diversity of five homologous Cu^{+}-ATPases present in *Sinorhizobium meliloti*. Microbiol (UK) 160:1237–1251
45. Eijkelkamp BA, Morey JR, Ween MP, Ong CLY, McEwan AG, Paton JC, McDevitt CA (2014) Extracellular zinc competitively inhibits manganese uptake and compromises oxidative stress management in *Streptococcus pneumoniae*. PLoS ONE 9(2):e89427

46. Pyle CJ, Azad AK, Papp AC, Sadee W, Knoell DL, Schlesinger LS (2017) Elemental ingredients in the macrophage cocktail: role of zip8 in host response to *Mycobacterium tuberculosis*. Int J Mol Sci 18
47. Neyrolles O, Wolschendorf F, Mitra A, Niederweis M (2015) Mycobacteria, metals, and the macrophage. Immunol Rev 264:249–263
48. Lisher JP, Giedroc DP (2013) Manganese acquisition and homeostasis at the host-pathogen interface. Front Cell Infect Microbiol 3
49. Zondervan NA, Van Dam JCJ, Schaap PJ, Dos Santos VAPM, Suarez-Diez M (2018) Regulation of three virulence strategies of *Mycobacterium tuberculosis*: a success story. Int J Mol Sci 19
50. Wang C, Zhang R, Wei X, Lv M, Jiang Z (2020) Metalloimmunology: the metal ion-controlled immunity. In: Advances in immunology, pp 187–241
51. Schaible UE, Kaufmann SHE (2004) Iron and microbial infection. Nat Rev Microbiol 2:946–953
52. Das KK, Reddy RC, Bagoji IB, Das S, Bagali S, Mullur L, Khodnapur JP, Biradar MS (2019) Primary concept of nickel toxicity—an overview. J Basic Clin Physiol Pharmacol 30:141–152
53. Beard SJ, Hughes MN, Poole RK (1995) Inhibition of the cytochrome bd-terminated NADH oxidase system in *Escherichia coli* K–12 by divalent metal cations. FEMS Microbiol Lett 131:205–210
54. Campbell DR, Chapman KE, Waldron KJ, Tottey S, Kendall S, Cavallaro G, Andreini C, Hinds J, Stoker NG, Robinson NJ, Cavet JS (2007) Mycobacterial cells have dual nickel-cobalt sensors: sequence relationships and metal sites of metal-responsive repressors are not congruent. J Biol Chem 282:32298–32310
55. Patel SJ, Lewis BE, Long JE, Nambi S, Sassetti CM, Stemmler TL, Argüello JM (2016) Fine-tuning of substrate affinity leads to alternative roles of *Mycobacterium tuberculosis* Fe^{2+}-ATPases. J Biol Chem 291:11529–11539
56. Raimunda D, Long JE, Padilla-benavides T, Sassetti CM, Argüello JM, Hughes H, Chase C (2014) Differential roles for the Co^{2+}/Ni^{2+} transporting ATPases, CtpD and CtpJ, in *Mycobacterium tuberculosis* virulence. Mol Microbiol 91:185–197
57. Sassetti CM, Rubin EJ (2003) Genetic requirements for mycobacterial survival during infection. Proc Natl Acad Sci U S A 100:12989–12994
58. Rani A, Kumar A, Lal A, Pant M (2014) Cellular mechanisms of cadmium-induced toxicity: a review. Int J Environ Health Res 24:378–399
59. Begg SL, Eijkelkamp BA, Luo Z, Couñago RM, Morey JR, Maher MJ, Ong CLY, McEwan AG, Kobe B, O'Mara ML, Paton JC, McDevitt CA (2015) Dysregulation of transition metal ion homeostasis is the molecular basis for cadmium toxicity in *Streptococcus pneumoniae*. Nat Commun 6(1)
60. Xu FF, Imlay JA (2012) Silver(I), mercury(II), cadmium(II), and zinc(II) target exposed enzymic iron-sulfur clusters when they toxify *Escherichia coli*. Appl Environ Microbiol 78:3614–3621
61. Chauhan S, Kumar A, Singhal A, Tyagi JS, Prasad HK (2009) CmtR, a cadmium-sensing ArsR-SmtB repressor, cooperatively interacts with multiple operator sites to autorepress its transcription in *Mycobacterium tuberculosis*. FEBS J 276:3428–3439
62. López M, Quitian LV, Calderón MN, Soto CY (2018) The P-type ATPase CtpG preferentially transports Cd^{2+} across the *Mycobacterium tuberculosis* plasma membrane. Arch Microbiol 200:483–492
63. Pirahanchi Y, Aeddula NR (2019. Physiology, sodium potassium pump (Na^+ K^+ pump)
64. Kaplan JH (2002) Biochemistry of Na,K-ATPase. Annu Rev Biochem 71:511–535
65. Cholo MC, Van Rensburg EJ, Osman AG, Anderson R (2015) Expression of the genes encoding the Trk and Kdp potassium transport systems of *Mycobacterium tuberculosis* during growth in vitro. Biomed Res Int

66. Bin HuG, Rice WJ, Dröse S, Altendorf K, Stokes DL (2008) Three-dimensional structure of the KdpFABC complex of *Escherichia coli* by electron tomography of two-dimensional crystals. J Struct Biol 161:411–418
67. Steyn AJC, Joseph J, Bloom BR (2003) Interaction of the sensor module of *Mycobacterium tuberculosis* H37Rv KdpD with members of the Lpr family. Mol Microbiol 47:1075–1089
68. Domínguez DC, Guragain M, Patrauchan M (2015) Calcium binding proteins and calcium signaling in prokaryotes. Cell Calcium 57:151–165
69. Görlach A, Bertram K, Hudecova S, Krizanova O (2015) Calcium and ROS: a mutual interplay. Redox Biol 6:260–271
70. Rosch JW, Sublett J, Gao G, Wang YD, Tuomanen EI (2008) Calcium efflux is essential for bacterial survival in the eukaryotic host. Mol Microbiol 70:435–444
71. Campbell AK (2014) Intracellular calcium, 1st edn. Wiley
72. Nazarenko LV, Andreev IM, Lyukevich AA, Pisareva TV, Los DA (2003) Calcium release from synechocystis cells induced by depolarization of the plasma membrane: MscL as an outward Ca^{2+} channel. Microbiology 149:1147–1153
73. Chang Y, Bruni R, Kloss B, Assur Z, Kloppmann E, Rost B, Hendrickson WA, Liu Q (2014) Structural basis for a pH-sensitive calcium leak across membranes. Science 344(80–):1131–1135
74. Faxén K, Andersen JL, Gourdon P, Fedosova N, Morth JP, Nissen P, Møller JV (2011) Characterization of a Listeria monocytogenes Ca^{2+} pump: a SERCA-type ATPase with only one Ca^{2+}-binding site. J Biol Chem 286:1609–1617
75. Tossavainen H, Permi P, Annila A, Kilpeläinen I, Drakenberg T (2003) NMR solution structure of calerythrin, an EF-hand calcium-binding protein from Saccharopolyspora erythraea. Eur J Biochem 270:2505–2512
76. Xi C, Schoeters E, Vanderleyden J, Michiels J (2000) Symbiosis-specific expression of Rhizobium etli casA encoding a secreted calmodulin-related protein. Proc Natl Acad Sci U S A 97:11114–11119
77. Brennan MJ, Delogu G (2002) The PE multigene family: a "molecular mantra" for mycobacteria. Trends Microbiol 10:246–249
78. Meena LS (2015) An overview to understand the role of PE-PGRS family proteins in *Mycobacterium tuberculosis* H37Rv and their potential as new drug targets. Biotechnol Appl Biochem 62:145–153
79. Thever MD, Saier MH (2009) Bioinformatic characterization of P-type ATPases encoded within the fully sequenced genomes of 26 eukaryotes. J Membr Biol 229:115–130
80. Gupta HK, Shrivastava S, Sharma R (2017) A novel calcium uptake transporter of uncharacterized P-type ATPase family supplies calcium for cell surface integrity in *Mycobacterium smegmatis*. MBio 8:1–14
81. Soldati T, Neyrolles O (2012) Mycobacteria and the intraphagosomal environment: take it with a pinch of salt(s)! Traffic 13:1042–1052
82. Wagner D, Maser J, Moric I, Boechat N, Vogt S, Gicquel B, Lai B, Reyrat JM, Bermudez L (2005) Changes of the phagosomal elemental concentrations by *Mycobacterium tuberculosis* Mramp. Microbiology 151:323–332
83. Maya-Hoyos M, Rosales C, Novoa-Aponte L, Castillo E, Soto CY (2019) The P-type ATPase CtpF is a plasma membrane transporter mediating calcium efflux in *Mycobacterium tuberculosis* cells. Heliyon 5:e02852
84. Hofman S, Segers MM, Ghimire S, Bolhuis MS, Sturkenboom MGG, Van Soolingen D, Alffenaar JWC (2016) Emerging drugs and alternative possibilities in the treatment of tuberculosis. Expert Opin Emerg Drugs 21:103–116
85. Dupont C, Viljoen A, Thomas S, Roquet-Banères F, Herrmann JL, Pethe K, Kremer L (2017) Bedaquiline inhibits the ATP synthase in *Mycobacterium abscessus* and is effective in infected zebrafish. Antimicrob Agents Chemother 61:1
86. Vasava MS, Bhoi MN, Rathwa SK, Borad MA, Nair SG, Patel HD (2017) Drug development against tuberculosis: past, present and future. Indian J Tuberc 64:252–275

87. Tahlan K, Wilson R, Kastrinsky DB, Arora K, Nair V, Fischer E, Whitney Barnes S, Walker JR, Alland D, Barry CE, Boshoff HI (2012) SQ109 targets MmpL3, a membrane transporter of trehalose monomycolate involved in mycolic acid donation to the cell wall core of *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 56:1797–1809
88. Yatime L, Buch-Pedersen MJ, Musgaard M, Morth JP, Winther AML, Pedersen BP, Olesen C, Andersen JP, Vilsen B, Schiøtt B, Palmgren MG, Møller JV, Nissen P, Fedosova N (2009) P-type ATPases as drug targets: tools for medicine and science. Biochim Biophys Acta Bioenerg 1787:207–220
89. Uhlemann A-C, Cameron A, Eckstein-Ludwig U, Fischbarg J, Iserovich P, Zuniga F a, East M, Lee A, Brady L, Haynes RK, Krishna S (2005) A single amino acid residue can determine the sensitivity of SERCAs to artemisinins. Nat Struct Mol Biol 12:628–629
90. Krishna S, Pulcini S, Fatih F, Staines H (2010) Artemisinins and the biological basis for the PfATP6/SERCA hypothesis. Trends Parasitol 26:517–523
91. Sharma S, Meena LS (2017) Potential of Ca^{2+} in *Mycobacterium tuberculosis* H 37 Rv Pathogenesis and Survival. Appl Biochem Biotechnol 181:762–771
92. van Kessel JC, Hatfull GF (2007) Recombineering in *Mycobacterium tuberculosis*. Nat Methods 4:147–152
93. Peddireddy V, Doddam SN, Ahmed N (2017) Mycobacterial dormancy systems and host responses in tuberculosis. Front Immunol 8
94. Pulido PA, Novoa-Aponte L, Villamil N, Soto CY (2014) The DosR dormancy regulator of *Mycobacterium tuberculosis* stimulates the Na(+)/K (+) and Ca (2+) ATPase activities in plasma membrane vesicles of mycobacteria. Curr Microbiol 69:604–610

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The Challenges of Antitubercular Drug Discovery

23

João Lucas Bruno Prates, Guilherme Felipe dos Santos Fernandes, Cristhian N. Rodríguez-Silva, and Jean Leandro dos Santos

The biggest disease today is not leprosy or tuberculosis, but rather the feeling of being unwanted.

Mother Teresa

Summary

Although extensive efforts have been undertaken to eradicate tuberculosis (TB), it remains one of the deadliest infections. The rising number of drug-resistant strains contributing to high mortality rates has become a global concern, highlighting the need for suitable treatments to eliminate such strains. However, the limitations of current therapies are long treatment duration, adverse side effects, interactions with other drugs, toxicity, and poor efficacy against latent strains. Thus, the search for safe and effective anti-TB drugs is urgent. This chapter addresses the advances and challenges in drug discovery for treating TB by focusing on the recently approved drugs and the most promising candidates that can contribute to current therapies.

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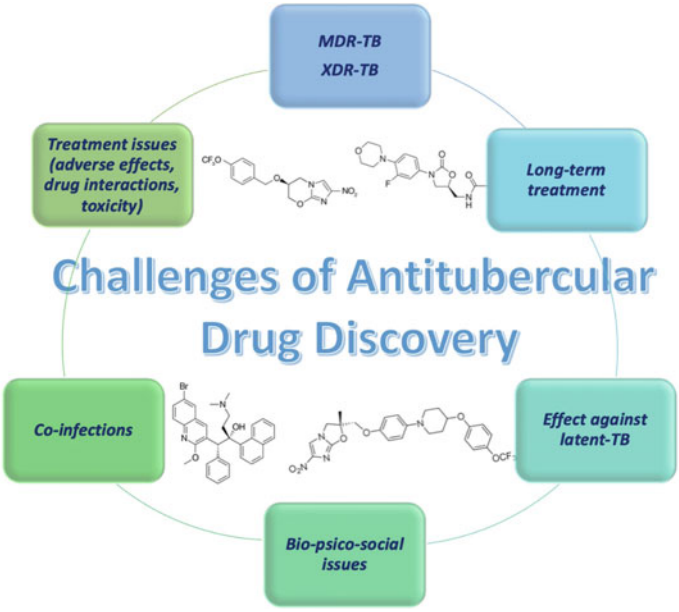
Graphical Abstract



Antitubercular drug discovery: challenges

Keywords

Antitubercular drugs · Drug design · *Mycobacterium tuberculosis* · New drugs · Tuberculosis

1 Introduction

Even after centuries, tuberculosis (TB) remains one of the deadliest infectious diseases inflicting humankind. The latest reports published by the World Health Organization (WHO) showed that TB was the main cause of death due to infections and even more lethal than HIV infection. In 2018, around ten million new cases of TB were reported, with 1.2 million deaths of non-HIV co-infected patients and up to 251,000 deaths of HIV co-infected patients.

The main infectious agent responsible for TB is the *Mycobacterium tuberculosis (M. tb)*. The emergence of resistant strains has lighted up concerns to the treatment of multidrug-resistant (MDR)-TB and extensively drug-resistant (XDR)-TB. According to a WHO report of 2018, the number of MDR-TB-infected patients reached up to 484,000, with an estimated number of deaths of 284,000 [1].

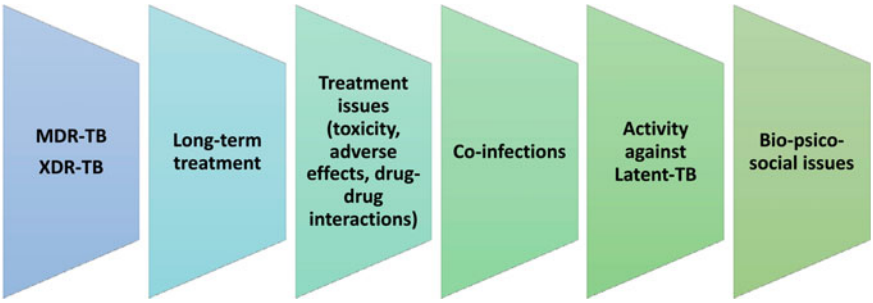


Fig. 1 Some challenges of antitubercular drug discovery

In general, the most common treatment for newly diagnosed pulmonary TB (PTB) includes a combination of isoniazid (INH), rifampin (RIF), ethambutol (EMB), and pyrazinamide (PZA) for two months, followed by four months of maintenance therapy using RIF and INH. However, for MDR-TB strains, the treatment regimen is long-term and more complex, mainly because of the lack of new therapeutic options [2].

Limitations of the current therapy include long treatment duration, high rates of treatment discontinuation, drug-drug interactions, high toxicity, and adverse effects induced by drugs (Fig. 1). Moreover, concerns about the lack of drugs for treating latent TB infection (LTBI), which can be detected in one-third of the worldwide population, show the immediate necessity for discovering new, safe, and effective drugs [3, 4].

A few advances in TB treatment were made by the end of 2012. One of the most significant improvements was developing a short-course directly observed treatment (DOTS) approach, which represents a patient-centered treatment strategy. This strategy increased treatment adherence and reduced treatment failure, mainly in developing countries [5]. In 2012, bedaquiline was approved by FDA to treat MDR-TB infection after a gap of 50 years since the discovery of rifampin in 1965 [6, 7]. Since then, efforts involving international partnerships focusing on the discovery of anti-TB drugs active against resistant strains have been intensified.

This chapter presents the advances and challenges in drug discovery for anti-TB agents by focusing on the recently approved drugs and the most promising drug candidates that can contribute to existing therapies.

2 Approved Drugs

Drug designing is a multidisciplinary process that requires multiple preclinical and clinical evaluations to guarantee the safety and effectiveness of a new drug. Some studies have estimated that the cost to develop a new drug could reach up to US$ 1.2 billion after spending around ten years of launching a new drug candidate in the

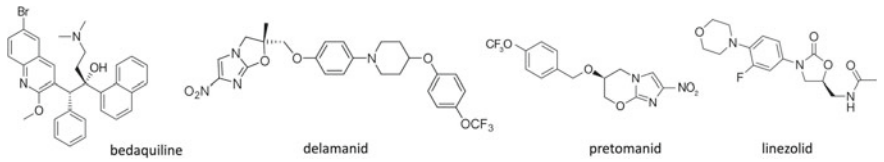


Fig. 2 Chemical structures of antitubercular approved drugs

market [8]. To optimize the drug development process to treat critical and urgent medical issues such as MDR-TB infections, regulatory agencies have established successful approaches for making such drugs available as soon as possible. Among these strategies, fast-track and accelerated approval procedures have been established to launch new anti-TB drugs in the market. Fast-track approval aims to contribute to drug development by expediting the assessment of new drugs from public interests against life-threatening diseases. The condition, named accelerated approval, aims to allow allows certain drugs to be administered under serious unmet medical conditions based on some accurate endpoints that guarantee their effectiveness. In the past years, using these approaches, some drugs have been approved for MDR-TB treatment, including bedaquiline, delamanid, pretomanid, and linezolid (Fig. 2). This section will discuss the drug discovery and clinical aspects of these drugs.

2.1 Bedaquiline

Bedaquiline, also known as TCM207, was developed by Johnson & Johnson by performing high-throughput screening (HTS) of a library containing more than 70,000 compounds. The high mortality rates caused by MDR-TB infection motivated the search for new drugs that could become an alternative to treating resistant infections [9].

After HTS, compounds belonging to the class of diarylquinolines showed promising activity against sensitive, mono-resistant strains, and clinical isolates. These drugs showed a distinct mode of action compared to those used in first- and second-line treatments [2, 9].

To comprehend the mechanism of action of bedaquiline, studies were performed using drug-resistant (DR) mutant strains of *M. tb* and *M. smegmatis*. Data from these studies demonstrated that TCM207 could inhibit the proton pump of ATP synthase. Specifically, the compound binds to the glutamic acid residue (Glu61) in the C subunit of the enzyme, interrupting the synthesis of adenosine 5-triphosphate (ATP). A study on the bedaquiline-induced *M. tb* resistance mechanism revealed changes at positions 63 and 66. In position 63, alanine was replaced by proline, while methionine was substituted by a leucine residue in position 66, found in atpE of subunit C, which prevents bedaquiline from accessing the Glu61 residue in the ATP synthase subunit cavity [6, 10].

TMC207 exhibited in vitro inhibitory activity with minimum inhibitory concentration (MIC) ranging from 0.002 to 0.06 μg/mL in pharmacodynamic studies against sensitive and resistant strains. In addition, TMC207 could inhibit dormant mycobacteria, acting through sterilizing effects with activity superior to that of rifampicin. Preclinical studies on efficacy using infected mice revealed that bedaquiline could eliminate *M. tb* from primary and secondary granulomas within six weeks of therapy. Monotherapy assays showed promising activity, even superior to the standard regimen using first-line drugs. Among the different combination therapies (RIF, INH, EMB, PZA, Bedaquiline), the combination of PZA and Bedaquiline was the most potent [9].

Phase I trials aiming to determine the preliminary pharmacokinetic profile in healthy patients using both single and multiple doses of bedaquiline revealed that the maximum plasma concentration was reached after five hours of administering a single dose (at a dosage of 10 mg, the plasma concentration was 0.07 μg/mL and at 700 mg it was 0.9 μg/mL). The half-life in humans was 173 h, allowing for its intermittent use [9].

The clinical trial subjects developed mild to moderate side effects after bedaquiline administration; the most common symptoms were nausea, dizziness, and body pain. However, more serious effects related to long QT syndrome were also observed. In a study that enrolled 1293 patients undergoing treatment with bedaquiline, 44 of them had to discontinue the treatment because they developed long QT syndrome, while eight others developed more serious cardiac complications that led to discontinuation. In 2017, WHO warned about bedaquiline risks after ten patients died during a phase II trial [7].

Despite these side effects, due to lack of better therapeutic options, bedaquiline was approved by the Food and Drug Administration (FDA) in 2012 to treat MDR-TB and XDR-TB [7, 11].

2.2 Pretomanid and Delamanid

Among the nitroimidazole derivative, both delamanid (Deltyba®; Otsuka Pharmaceutical) and pretomanid (TB Alliance) were approved by regulatory agencies to treat MDR-TB in 2014 and 2019, respectively. This heterocycle class has been used to treat infectious diseases, including TB. Pretomanid was the first nitroimidazole approved to treat TB. Both pretomanid and delamanid were active against *M. tb* in active or latent stages [12]. For pretomanid, a potent activity was found with MIC values ranging from 0.015 to 0.5 μM against resistant strains [13]. Nevertheless, this drug is distinguished from other anti-TB (first and second line) drugs by its activity against non-replicating mycobacteria [14]. Likewise, delamanid exhibited potent anti-*M.tb* effects with MIC values against resistant strains found at concentrations of 0.006 to 0.024 μg/mL. Furthermore, both drugs have been considered not harmful to humans since they have no genotoxicity, mutagenicity, or carcinogenicity [15].

These nitroimidazole drugs act through combined effects against genes responsible for respiratory chain and cell wall synthesis. The latter is thought to result from the bioreductive activation of the nitroimidazole subunit by the enzyme deazaflavin-dependent nitroreductase (Ddn) from *M. tb* [12, 16, 17]. Depletion of ketoymycolates and buildup of hydroxymycolates are the mechanisms by which this group of chemicals affects the biosynthesis of mycolic acid. In addition, these bicyclic compounds also act in non-replicating forms of *M. tb*. Recently, a new mechanism has been identified by which this class of compounds can act. Metabolomics studies have shown that pretomanid may induce the accumulation of the toxic compound methylglyoxal by interfering in the pentose phosphate pathway [18].

2.3 Linezolid

Drug repurposing is a well-established approach that allows discovering new medical uses for known therapeutic drugs. Drug repurposing has several advantages over traditional approaches for identifying new drugs, including low research risks, reduced development costs, and relatively less time required for commercialization. All these advantages allow fast approval of drugs for critical diseases such as MDR-TB infection. Therefore, the oxazolidinone derivative linezolid is widely used as an antimicrobial agent to treat infectious diseases caused by gram-positive microorganisms. Linezolid was included in group A by WHO to treat MDR-TB and XDR-TB infections. Several studies have demonstrated the efficacy of linezolid against resistant *Mycobacterium* strains. The use of linezolid in combination with other first and second-line anti-TB drugs has resulted in high recovery rates, low treatment failure, and high sputum culture conversion in 24 months; however, these desirable endpoints are counterbalanced by the adverse effects caused by the drug, including low red blood cell count, nerve damage, nausea, and vomiting. Some authors suggested that these adverse effects are prone to augment the rate of treatment discontinuation [19].

3 Drug Candidates in Clinical Trials

Although an increasing number of resistant strains have challenged conventional TB treatment options, the search for new anti-TB drugs has increased since bedaquiline was approved in 2012, bringing in a better long-term perspective. Among the several drug candidates being evaluated in human trials, some of the promising candidates include oxazolidinone, 1,2-ethylene diamine, imidazopyridine, oxaborole, riminophenazine derivatives, and DprE1 inhibitors spanning multiple chemical classes (Fig. 3; Table 1).

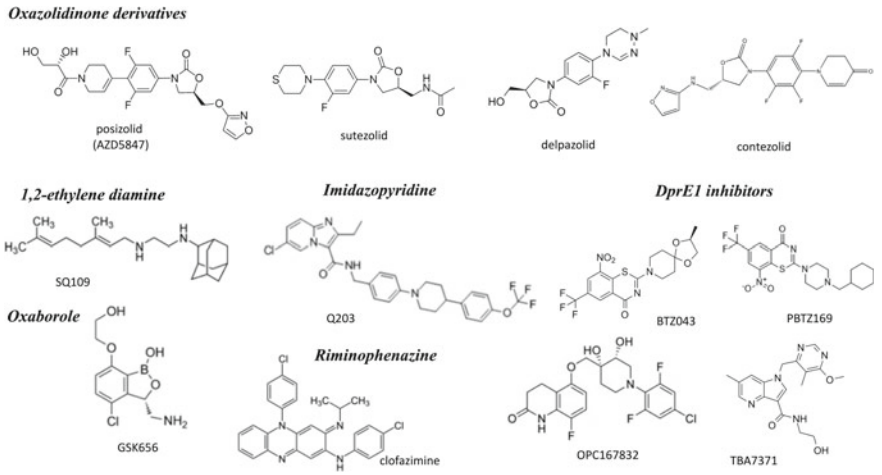


Fig. 3 Chemical structures of antitubercular drugs in clinical trials

Table 1 Drug candidates in clinical trials

Phase 1	Phase 2	Phase 3
BTZ043	Telacebec (Q203)	OPC-167832
TBI166	Macozinone (PBTZ169)	Clofazimine
Posazolid (AZD5847)	GSK656	
	SQ109	
	Sutezolid	
	Delpazolid (LCB01-0371)	
	Contezolid (MRX-I)	
	TBA7371	

3.1 Oxazolidinone Derivatives

To overcome toxicity caused by linezolid, novel oxazolidinone derivatives such as posizolid (AZD5847), sutezolid, delpazolid, and contezolid were designed and evaluated for treating MDR-TB infection. Despite the superior anti-TB effect of posizolid compared to linezolid, a phase I trial revealed its limited efficacy and serious adverse effects, including neurotoxicity, and hepatic and hematological disorders, leading to the discontinuation of the trial [7, 20].

Oxazolidinone derivatives inhibit protein synthesis by binding to the 23S ribosome. This distinct mode of action of novel anti-TB drugs is interesting because it reduces cross-resistance to existing drugs. Sutezolid, a thiomorpholine analog of linezolid, exhibits superior activity and less toxicity than its parental drug linezolid [21]. Infected mice treated with sutezolid have demonstrated a reduction in symptoms by one month compared to linezolid treatment. A phase I trial showed

that sutezolid did not cause neuropathy or abnormal hematological findings after healthy volunteers were treated with the drug for 28 days at a dosage of 600 mg twice daily, suggesting a superior safety profile compared to linezolid [21].

Delpazolid, also known as LCB01–0371, is another oxazolidinone derivative that exhibits similar efficacy against MDR-TB and XDR-TB as linezolid and can also act against linezolid-resistant strains [22]. Phase I clinical trials have shown that delpazolid is well-tolerated after administering multiple doses (1200 mg, twice daily) for 21 days in healthy male volunteers without inducing hematological toxicity [23].

Preclinical studies on contezolid, also known as MRX-I, revealed that this oxazolidinone derivative is active against MDR-TB clinical isolates, and its efficacy is comparable to that of linezolid. Infected mice treated with contezolid exhibited a reduction in mycobacterial load in the lungs compared to control [24]. In humans, the drug candidate exhibited a low safety and toxicity profile compared to linezolid [25].

Phase II trials are being conducted to evaluate the effectiveness of sutezolid, delpazolid, and contezolid against MDR-TB infection.

3.2 1,2-Ethylene Diamine

Another anti-TB drug candidate SQ109, whose 1,2-ethylene diamine subunit is similar to ethambutol, inhibits cell wall synthesis by interacting with the mycobacterial membrane protein large 3 (Mmpl3). This protein is a lipid transporter that binds to trehalose monomycolate and phosphatidylethanolamine across the membrane for cell wall synthesis [26, 27]. Preclinical studies have shown that SQ109 is active against MDR-TB, XDR-TB, and other mycobacteria such as *M. bovis,* BCG, and *M. fortuitum*. SQ109 also exhibited bactericidal action by acting synergistically with first- and second-line anti-TB drugs. Infected mice showed reduced levels of colony-forming unit (CFU) in the lungs after SQ109 treatment. Combining bedaquiline and pyrazinamide cured the infected animals in a reduced treatment duration of three to six months. In humans, phase I trials demonstrated the safety of SQ109 without causing serious adverse effects [28]. Unexpectedly, the results of a phase II trial of SQ109 did not meet the required remission criteria, and the study was discontinued [29].

3.3 Imidazopyridine

Telacebec (Q203), an imidazopyridine derivative, is an anti-TB drug candidate that exhibited a novel mechanism of action by hampering cellular energy production by inhibiting the mycobacterial cytochrome bc1 complex [30].

The antimycobacterial effect of the DR-H37RV strains provided an astonishing MIC value of 0.0027 μM, while that against MDR-TB and XDR-TB strains produced values of < 0.00043 μM. Infected mice treated with Q203 (dose, 10 mg/kg) showed > 99.9% reduction in CFU values in the lungs after four weeks of

treatment. These experiments confirmed the data obtained from in vitro studies against *M. tb* H37Rv, MDR-TB, and XDR-TB clinical isolates. Moreover, using mice, Q203 has demonstrated an appropriate pharmacokinetic profile and safety in acute toxicity models [31, 32].

All these promising preclinical results motivated further studies in humans. The safety profile of this drug in a phase I trial[1] led to the commencement of phase II of the clinical trial. To characterize the proof-of-concept for Telacebec, a randomized, prospective, open-label trial was performed that enrolled 61 patients.[2] These patients received the drug for 14 days at doses ranging from 100 to 300 mg alone or combined with first-line drugs. Telacebec was effective in RIF and INH-susceptible PTB, exhibiting tolerable adverse effects [33].

3.4 Oxaborole

From discovering oxaborole in 1808, boron-containing compounds have been neglected because of concerns about possible toxic effects. However, special features such as water solubility and the ability to perform reversible covalent interactions elicited an interest in its use in drug design [34]. The drug candidate (S)-3-(aminomethyl)-4-chloro-7-(2-hydroxyethoxy)benzo[c] [1, 2] oxaborol-1(3H)-ole, also known as GSK656, inhibits the enzyme leucyl-tRNA synthetase in *M. tb*, leading to a reduction in protein synthesis. This compound exhibited a remarkable in vitro effect against MTB H37Rv strains with a MIC value of 0.08 mM and IC50 value of 0.2 mM, demonstrating high selectivity for synthetase. Boron promotes adduct formation on the terminal nucleotide of tRNA, causing its inhibition. Preclinical assays using infected mice revealed that at 10 mg/kg concentration of the drug, the reduction in CFU of lungs was around 2.1 log10. These values were similar to that of linezolid used at 100 mg/kg. In animals, the compound was well-tolerated and exhibited an appropriate pharmacokinetic profile after oral administration [35]. Phase I study has shown that GSK656 did not induce serious adverse effects, being well-tolerated after both single and multiple doses in humans [36].

3.5 Decaprenylphosphoryl-β-D-Ribose-20-Epimerase (DprE1) Inhibitors

An important step during the synthesis of the mycobacterial cell wall is the conversion of decaprenyl-phosphoryl-β-D-ribose to decaprenylphosphoryl-β-D-arabinose performed by the flavoenzyme decaprenylphosphoryl-β-D-ribose-20-epimerase, also known as DprE1. This heterodimeric enzyme is constituted by two distinct proteins, DprE1 and DprE2. They act on the biosynthesis of decaprenylphosphoryl-β-D-arabinose and are located in the periplasmic space.

[1] ClinicalTrials.gov number, NCT02858973.
[2] ClinicalTrials.gov number, NCT03563599.

Several inhibitors exhibiting chemical structural diversity have been described in the literature. The inhibitors that progressed to clinical evaluation are BTZ043, PBTZ169, OPC167832, and TBA7371.

The compound BTZ043 is a potent benzothiazinone derivative with a MIC value of 0.001 μg/mL against *M. tb* H37Rv [37]. Several studies have described that BTZ043 forms a covalent bond with an amino acid residue Cys387 present in the active site of DprE1. The benzothiazinone derivative was also active against MDR-TB and XDR-TB, presenting an additive effect when combined with other anti-TB drugs [38]. However, their low solubility has interfered with the pharmacokinetic profile, reducing the activity when studied on acute and chronic infected murine models [39].

To solve this issue, researchers have explored the structure-activity relationship of benzothiazinone derivatives, which enabled the discovery of the compound PBTZ169 and further named macozinone. Although the activity of macozinone (MIC: 0.062 μg/mL) was inferior to that of BRTZ043, the drug candidate had a pronounced effect against MDR-TB with MIC values ranging from 0.116 to 0.232 μg/mL [40]. In addition, this compound was more stable with a high affinity toward DprE1, maybe because of the presence of cyclohexyl that can prevent the bioconversion performed by nitroreductase. In combination with other anti-TB drugs, an additive effect was observed, except for bedaquiline, with which combination became synergic. Preclinical assays on infected mice administered with macozinone in combination with pyrazinamide and bedaquiline showed better anti-*M. tb* effects than the first-line treatment (INH, RIF, and PZA) [39]. A phase I trial demonstrated an appropriate pharmacokinetic profile (linear PK), good tolerability, and safety. The compound is currently under phase II trial [41].

Using phenotypic screening to optimize carbostyril derivatives led to the discovery of the DprE1 inhibitor named OPC-167832. This 3, 4-dihydrocarbostyril derivative demonstrated astonishing MIC values ranging from 0.00024 to 0.002 μg/mL against *M. tb*, exhibiting bactericidal effect on growing and intracellular bacilli. During treatment of chronically infected mice, the same amount of bactericidal effect of the drug was observed at a dose of 0.625 mg/kg of body weight. The combined treatment using bedaquiline, linezolid, or moxifloxacin reduced mycobacterial burden at levels superior to standard treatment. The compound is being investigated in trials [42].

The 1, 4-azaindole derivative named TBA7371 was described as a noncovalent inhibitor of DprE1 with a promising effect against *M. tb* H37Rv and resistant clinical isolates (MDR-TB and XDR-TB). Preclinical studies demonstrated the effect against infected rodents without any serious adverse effects. Phase I trials have described visual disturbance as one of the main reported adverse effects. In addition, the pharmacokinetic study showed its impact on food intake. The drug candidate is under evaluation in phase II[3] [43].

[3] ClinicalTrials.gov, Identifier: NCT04176250.

3.6 Riminophenazine

Considering the emergence of MDR-TB and XDR-TB, one of the fast ways to find out newly available drugs is using a drug repurposing approach. Thus, researchers have found that clofazimine, a riminophenazine derivative used to treat *M. leprae* infections, is active against the resistant clinical isolates of *M. tb*. Therefore, it was included in the list of second-line drugs (group 5) to treat MDR-TB and XDR-TB without first-line therapeutic options. The mode of action is not completely comprehended, but adverse effects and undesirable physicochemical properties (i.e., high lipophilicity) promote its accumulation in fat tissues and prolong its half-life. Therefore, several analogs were synthesized and evaluated to improve these properties, which led to the discovery of TBI-166. Interestingly, for this riminophenazine derivative, the MIC value against MDR-TB and XDR-TB were in the range of 0.027–0.095 μg/mL, which suggested its superior activity over clofazimine [44]. Preliminary studies suggested that the mode of action of TBI-166 could be related to ion transport and cell respiration. Moreover, in vivo experiments using infected BALB/c and C3HeB/FeJNju mice revealed that regimens containing TBI-166 alone or in combination with other drugs (bedaquiline; pyrazinamide; bedaquiline + linezolid; bedaquiline + linezolid + pretomanid; and bedaquiline + pretomanid) for four to eight weeks are more potent than that of the control treatment (INH + RIF + PZA). After eight weeks of treatment in the groups using TBI-166, the lung log CFU counts of bacilli were undetectable, as in the group (TBI-166 + bedaquiline + linezolid) showed maximum activity for both the lines of mice [45]. Phase I trial on TBI-166 was approved by the China Food and Drug Administration (ChiCTR1800018780) [46].

4 Future Challenges

Ambitious plans to eradicate TB worldwide proposed by 'Stop TB Partnership' until 2030 are still far from concretize, mainly because of the inferior investments in TB research compared to those recommended by the agency [47]. Despite that, several compounds at the stage of discovery and lead optimization have been described in high numbers in past years, showing new fruitful research in this field [20, 48].

Future challenges are the development of:

- new regimens active against resistant strains;
- regimens shorter than the current treatment;
- active drugs against LTBI effective on all patients, including those co-infected with HIV; and
- new therapies focusing on the host targets (host-directed therapy) that increase the immune response against the *M. tb*

The long duration and adverse effects of the current therapy are responsible for relapse and failure in the treatment of resistant cases. Therefore, new regimens aiming to reduce the treatment period are desirable for MDR-TB. In 2016, WHO recommended a shorter and standardized regimen (nine to 12 months) for the treatment of pulmonary MDR-TB. It consisted of four to six months of high doses of isoniazid (10 mg/kg, up to the maximum 600 mg daily), ethambutol, pyrazinamide, moxifloxacin, ethionamide or prothionamide, clofazimine, and an injectable aminoglycoside (kanamycin or amikacin), followed by five months of continuation using pyrazinamide, ethambutol, moxifloxacin and clofazimine [49]. A similar protocol named Bangladesh regimen successfully reduced the MDR-TB treatment long duration to nine months. The main difference between the two protocols is the replacement of moxifloxacin in the WHO protocol by gatifloxacin in the Bangladesh regimen [50].

Within the new drugs, approved alternative regimens containing bedaquiline (trial NCT02409290), pretomanid, and linezolid (NiX-TB trial, NCT02333799) have contributed to improving efficacy and reducing treatment duration.

The WHO has estimated that around one-third of the worldwide population has LTBI. Alternatives to the high doses of isoniazid for nine months or more should be researched urgently. Some studies suggested that the combination of rifapentine and INH given for three months exhibits similar efficacy to that of INH individually, administered for nine months [51].

Another perspective in TB treatment is based on host-direct therapy. The strengthening of immune systems allows greater infection control by reducing treatment duration, preventing relapse, and controlling inappropriate immune responses due to the presence of bacilli. Among the strategies used, it is notorious for highlighting the inflammatory processes control in the lungs that prevent tissue damage. Moreover, improvement in host immunity is associated with eliminating bacilli at the early stages of infection without any complications. The importance of strengthening immunity is notorious mainly in patients co-infected with HIV, whose hampering of immune response increases mortality [52].

5 Conclusion

The emergence of MDR-TB and XDR-TB has brought new challenges for humankind worldwide. After a gap of 50 years without a new drug, the bedaquiline approval in 2012 opened new perspectives to finding new therapeutic options. The bio-psycho-social issues, including the stigma of the disease, the presence of co-infections, and social-economical-environmental difficulties in accessing the treatment, are still the same issues to overcome concomitantly. The past few years were fruitful in anti-TB drug discovery because of novel drugs (and drug candidates in clinical trials) exhibiting distinct modes of action against resistant and latent forms. The association of new drugs has shortened the treatment period and

diminished adverse effects. Despite these challenges, the scenario for anti-TB drug discovery has undergone a profound transformation in the past few years, bringing bright future perspectives to mitigate the disease.

Core Messages

- The chapter discusses the main challenges for TB elimination, including barriers to the current treatment.
- The chapter provides a state-of-art on antitubercular drug discovery involving different medicinal chemistry approaches.
- The chapter reviews advances in anti-TB drug discovery, highlighting the current stage of each drug candidate.
- The chapter includes the main results of drug candidates in clinical trials, highlighting some perspectives for investigating the field.

References

1. World Health Organization (2019) Global tuberculosis report. https://www.who.int/tb/publications/global_report/en/. Accessed 14 Aug 2020
2. Zumla A, Nahid P, Cole T (2013) Advances in the development of new tuberculosis drugs and treatment regimens. Nat Rev Drug Discov 12(5):388–404
3. Yee D, Valiquette C, Pelletier M, Parisien I, Rocher I, Menzies D (2003) Incidence of serious side effects from first-line antituberculosis drugs among patients treated for active tuberculosis. Am J Respir Crit Care Med 167(11):1472–1477
4. Fernandes GFDS, de Souza PC, Marino LB, Chegaev K, Guglielmo S, Lazzarato L, Fruttero R, Chung MC, Pavan FR, Dos Santos JL (2016) Synthesis and biological activity of furoxan derivatives against *Mycobacterium tuberculosis*. Eur J Med Chem 123:523–531
5. Müller AM, Osório CS, Silva DR, Sbruzzi G, de Tarso P, Dalcin R (2018) Interventions to improve adherence to tuberculosis treatment: systematic review and meta-analysis. Int J Tuberc Lung Dis 22(7):731–740
6. Andries K, Verhasselt P, Guillemont J, Göhlmann HWH, Neefs JM, Winkler H, Van Gestel J, Timmerman P, Zhu M, Lee E (2005) A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. Science 307(5707):223–227
7. Tiberi S, du Plessis N, Walzl G, Vjecha MJ, Rao M, Ntoumi F, Mfinanga S, Kapata N, Mwaba P, McHugh TD (2018) Tuberculosis: progress and advances in development of new drugs, treatment regimens, and host-directed therapies. Lancet Infect Dis 18(7):83–198
8. DiMasi JA, Grabowski HG, Hansen RW (2016) Innovation in the pharmaceutical industry: new estimates of R&D costs. J Health Econ 47:20–33
9. Matteelli A, Carvalho ACC, Dooley KE, Kritski A (2010) TCM207: the first compound of a new class of potent anti-tuberculosis drugs. Fut Micro 5(6):849–858
10. Jonge MR, Koymans LHM, Guillemont JEG, Koul A, Andries K (2007) A computational model of the inhibition of *Mycobacterium tuberculosis* ATPase by a new drug candidate R207910. Proteins 67(4):971–980
11. Cox E, Laessig K (2014) FDA approval of bedaquiline—the benefit-risk balance for drug-resistant tuberculosis. N Engl J Med 371:689–691
12. Denny WA, Palmer BD (2010) The nitroimidazooxazines (PA-824 and analogs): structure–activity relationship and mechanistic studies. Future Med Chem 2(8):1295–1304

13. Stover CK, Warrener P, VanDevanter DR, Sherman DR, Arain TM, Langhorne MH, Anderson SW, Towell JA, Yuan Y, McMurray DN (2000) A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. Nature 405(6789):962–966
14. Hu Y, Coates ARM, Mitchison DA (2007) Comparison of the sterilizing activities of the nitroimidazopyran PA-824 and moxifloxacin against persisting *Mycobacterium tuberculosis*. Int J Tuberc Lung Dis 12(1):69–73
15. Liu Y, Matsumoto M, Ishida H, Ohguro K, Yoshitake M, Gupta R, Geiter R, Hafkin J (2018) Delamanid: from discovery to its use for pulmonary multidrug-resistant tuberculosis (MDR-TB). Tuberculosis 111:20–30
16. Cellitti SE, Shaffer J, Jones DH, Mukherjee T, Gurumurthy M, Bursulaya B, Boshoff HI, Choi I, Nayyar A, Lee YS (2012) Structure of Ddn, the deazaflavin-dependent nitroreductase from *Mycobacterium tuberculosis* involved in bioreductive activation of PA-824. Structure 20 (1):101–112
17. Singh M, Sasi P, Rai G, Gupta V (2012) Studies on toxicity of antitubercular drugs namely isoniazid, rifampicin, and pyrazinamide in an in vitro model of HepG2 cell line. Med Chem Res 20(9):1611–1615
18. Baptista R, Fazakerley DM, Beckmann M, Baillie L, Mur LA (2018) Untargeted metabolomics reveals a new mode of action of pretomanid (PA-824). Sci Rep 8(1):5084
19. Singh B, Cocker D, Ryan H, Sloan DJ (2019) Linezolid for drug-resistant pulmonary tuberculosis. Cochrane Database Syst Rev. 3(3):CD012836
20. Fernandes GFDS, Chin MC, Dos Santos JL (2017) Advances in drug discovery of new antitubercular multidrug-resistant compounds. Pharmaceuticals 10(2):51
21. Wallis RS, Dawson R, Friedrich SO, Venter A, Paige D, Zhu T, Silvia A, Gobey J, Ellery C, Zhang Y (2014) Mycobactericidal activity of sutezolid (PNU-100480) in sputum (EBA) and blood (WBA) of patients with pulmonary tuberculosis. PLoS One. Published: 14 Apr 2014
22. Zong Z, Jing W, Shi J, Wen S, Zhang T, Huo F, Shang Y, Liang Q, Huang H, Pang Y (2018) Comparison of in vitro activity and mic distributions between the novel oxazolidinone delpazolid and linezolid against multidrug-resistant and extensively drug-resistant *Mycobacterium tuberculosis* in China. Antimicrob Agents Chemother. Published online 2018 Jul 27
23. Choi Y, Lee SW, Kim A, Jang K, Nam H, Cho YL, Yu KS, Jang IJ, Chung JY (2018) Safety, tolerability and pharmacokinetics of 21 day multiple oral administration of a new oxazolidinone antibiotic, LCB01-0371, in healthy male subjects. J Antimicrob Chemother 73(1):183–190
24. Shoen C, DeStefano M, Hafkin B, Cynamon M (2018) In Vitro and in vivo activities of contezolid (MRX-I) against *Mycobacterium tuberculosis*. Antimicrob Agents Chemother. Published online 2018 Jul 27
25. Wu J, Wu H, Wang Y, Chen Y, Guo B, Cao G, Wu X, Yu J, Wu J, Zhu D (2019) Tolerability and pharmacokinetics of contezolid at therapeutic and supratherapeutic doses in healthy Chinese subjects, and assessment of contezolid dosing regimens based on pharmacokinetic/pharmacodynamic analysis. Clin Ther 41:1164–1174
26. Tahlan K, Wilson R, Kastrinsky DB, Arora K, Nair V, Fischer E, Barnes W, Walker JR, Alland D, Barry CE (2012) SQ109 targets MmpL3, a membrane transporter of trehalose monomycolate involved in mycolic acid donation to the cell wall core of *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 56(4):1797–1809
27. Su CC, Klenotic PA, Bolla JR, Purdy GE, Robinson CV, Yu EW (2019) MmpL3 is a lipid transporter that binds trehalose monomycolate and phosphatidylethanolamine. Proc Natl Acad Sci 116(23):11241–11246
28. Sacksteder KA, Protopopova M, Barry CE, Andries K, Nacy CA (2012) Discovery and development of SQ109: a new antitubercular drug with a novel mechanism of action. Future Microbiol 7(7):823–837
29. Boeree MJ, Heinrich N, Aarnoutse R, Diacon AH, Dawson R, Rehal S (2017) High-dose rifampicin, moxifloxacin, and SQ109 for treating tuberculosis: a multi-arm, multi-stage randomised controlled trial. Lancet Infect Dis 17(1):39–49

30. Pethe K, Bifani P, Jang J, Kang S, Park S, Ahn S, Jiricek J, Jung J, Jeon HK, Cechetto J (2013) Discovery of Q203, a potent clinical candidate for the treatment of tuberculosis. Nat Med 19(9):1157–1160
31. Abrahams KA, Cox JA, Spivey VL, Loman NJ, Pallen NJ, Constantinidou C, Fernández R, Alemparte C, Remuiñán MJ, Barros D (2012) Identification of novel imidazo[1,2-a]pyridine inhibitors targeting *M. tuberculosis* QcrB. PLoS One. Published: 31 Dec 2012
32. Kang S, Kim RY, Seo MJ, Lee S, Kim YM, Seo M, Seo JJ, Ko Y, Choi I, Jang J (2014) Lead optimization of a novel series of imidazo[1,2-a]pyridine amides leading to a clinical candidate (Q203) as a multi- and extensively-drug-resistant anti-tuberculosis agent. J Med Chem 57 (12):5293–5305
33. de Jager VR, Dawson R, van Niekerk C, Hutchings J, Kim J, Vanker N, van der Merwe L, Choi J, Nam K, Diacon AH (2020) Telacebec (Q203), a new antituberculosis agent. N Engl J Med 382(13):1280–1281
34. Fernandes GFS, Denny WA, Dos Santos JL (2019) Boron in drug design: recent advances in the development of new therapeutic agents. Eur J Med Chem 179:791–804
35. Li X, Hernandez V, Rock FL, Choi W, Mak YSL, Mohan M, Mao W, Zhou Y, Easom EE, Plattner JJ (2017) Discovery of a potent and specific *M. tuberculosis* Leucyl-tRNA synthetase inhibitor: (S)-3-(Aminomethyl)-4-chloro-7-(2-hydroxyethoxy)benzo[c][1,2]oxaborol-1(3H)-ol (GSK656). J Med Chem 60(19):8011–8026
36. Tenero D, Derimanov G, Carlton A, Tonkyn J, Davies M, Cozens S, Gresham S, Gaudion A, Puri A, Muliaditan M (2019) First-time-in-human study and prediction of early bactericidal activity for GSK3036656, a potent Leucyl-tRNA synthetase inhibitor for tuberculosis treatment. Antimicrob Agents Chemother 63(8):00240–00319
37. Makarov V, Manina G, Mikusova K, Möllmann U, Ryabova O, Saint-Joanis B, Dhar N, Pasca MR, Buroni S, Lucarelli AP (2009) Benzothiazinones kill *Mycobacterium tuberculosis* by blocking arabinan synthesis. Science 324:801–804
38. Lechartier B, Hartkoorn RC, Cole ST (2012) In vitro combination studies of benzothiazinone lead compound BTZ043 against *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 56(11):5790–5793
39. Makarov V, Lechartier B, Zhang M, Neres J, Sar AM, Raadseen SA, Hartkoorn RC, Ryabova OB, Vocat A, Decosterd LA (2014) Towards a new combination therapy for tuberculosis with next generation benzothiazinones. EMBO Mol Med 6:372–383
40. Li L, Lv K, Yang Y, Sun J, Tao Z, Wang A, Wang B, Wang H, Geng Y, Liu M (2018) Identification of N-benzyl 3,5-dinitrobenzamides derived from PBTZ169 as antitubercular agents. ACS Med Chem Lett 9:741–745
41. Mariandyshev AO, Khokhlov AL, Smerdin SV, Shcherbakova VS, Igumnova OV, Ozerova IV, Bolgarina AA, Nikitina NA (2020) The main results of clinical trials of the efficacy, safety and pharmacokinetics of the perspective anti-tuberculosis drug makozinone (PBTZ169). Ter Arkh 92:61–72
42. Hariguchi N, Chen X, Hayashi Y, Kawano Y, Fujiwara M, Matsuba M, Shimizu H, Ohba Y, Nakamura I, Kitamoto R (2020) OPC-167832, a novel carbostyril derivative with potent antituberculosis activity as a DprE1 inhibitor. Antimicrob Agents Chemother 64(6): e02020-e2119
43. Gawad J, Bonde C (2018) Decaprenyl-phosphoryl-ribose 2'-epimerase (DprE1): challenging target for antitubercular drug discovery. Chem Cent J 12(1):72
44. Zhang D, Lu Y, Kai L, Liu B, Wang J, Zhang G, Zhang H, Liu Y, Wang B, Zheng M (2012) Identification of less lipophilic riminophenazine derivatives for the treatment of drug-resistant tuberculosis. J Med Chem 55:8409–8417
45. Zhang Y, Zhu H, Fu L, Wang B, Guo S, Chen X, Liu Z, Huang H, Yang T, Lu Y (2019) Identifying regimens containing TBI-166, a new drug candidate against mycobacterium tuberculosis in vitro and in vivo. Antimicrob Agents Chemother 63(7):e02496-e2518
46. Kumar D, Negi B, Rawat DS (2015) The anti-tuberculosis agents under development and the challenges ahead. Future Med Chem 7(15):1981–2003

47. Stop TB Partnership (2020) The paradigm shift 2016–2020: global plan to end TB; Geneva, 2015. http://www.stoptb.org. Accessed 14 Aug 2020
48. Dos Santos Fernandes GF, Hartmann JD, de Souza PC, Man CC, Rogerio PF, Dos Santos JL (2015) Current advances in antitubercular drug discovery: potent prototypes and new targets. Curr Med Chem 22:3133–3161
49. Piubello A, Harouna SH, Souleymane MB, Boukary I, Morou S, Daouda M, Hanki Y, Van Deun A (2014) High cure rate with standardised short-course multidrug-resistant tuberculosis treatment in Niger: no relapses. Int J Tuberc Lung Dis 18(10):1188–1194
50. Aung KJ, Van Deun A, Declercq E, Sarker MR, Das PK, Hossain MA, Rieder HL (2014) Successful "9-month Bangladesh regimen" for multidrug-resistant tuberculosis among over 500 consecutive patients. Int J Tuberc Lung Dis 18(10):1180–1187
51. Sterling TR, Villarino ME, Borisov AS, Shang N, Gordin F, Bliven-Sizemore E, Hackman J, Hamilton CD, Menzies D, Kerrigan A (2011) Three months of rifapentine and isoniazid for latent tuberculosis infection. N Engl J Med 365:2155–2166
52. Zumla A, Maeurer M, Chacaya J, Hoelscher M, Ntoumi F, Rustomjee R, Vilaplana C, Yeboah-Manu D, Rasolof V, Munderi P (2015) Towards host-directed therapies for tuberculosis. Nat Rev Drug Discov 14(8):511–512

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Exploring Decaprenylphosphoryl-β-*D*-Ribose 2′-Epimerase 1 (DprE1): A Target for Anti-tubercular Drugs

24

Mange Ram Yadav, Prashant R. Murumkar, Rahul B. Ghuge, Rahul R. Barot, and Monica Chauhan

The biggest disease today is not leprosy or tuberculosis, but rather the feeling of being unwanted.

Mother Teresa

Summary

Decaprenylphosphoryl-β-*D*-ribose 2′-epimerase 1(DprE1) is a new and competent target that could be exploited for drug discovery to tackle the problem of drug-resistant tuberculosis (TB). It is a flavoprotein that essentially contributes to mycobacterial cell wall biosynthesis. The enzyme is involved in the synthesis of Araf molecules, which are the building blocks in the synthesis of lipoarabinomannans and arabinogalactans. Benzothiazinones were the first molecules to be reported as DprE1 inhibitors. Since then, a number of new and novel compounds have been reported as DprE1 inhibitors. These inhibitors exhibit either covalent or non-covalent binding to the enzyme. Four DprE1 inhibitors, namely BTZ-043, Macozinone, OPC-167832, and TBA-7371, are currently in clinical trials. This chapter attempts to discuss DprE1 as a potential druggable target and its inhibitors for the discovery of anti-TB agents.

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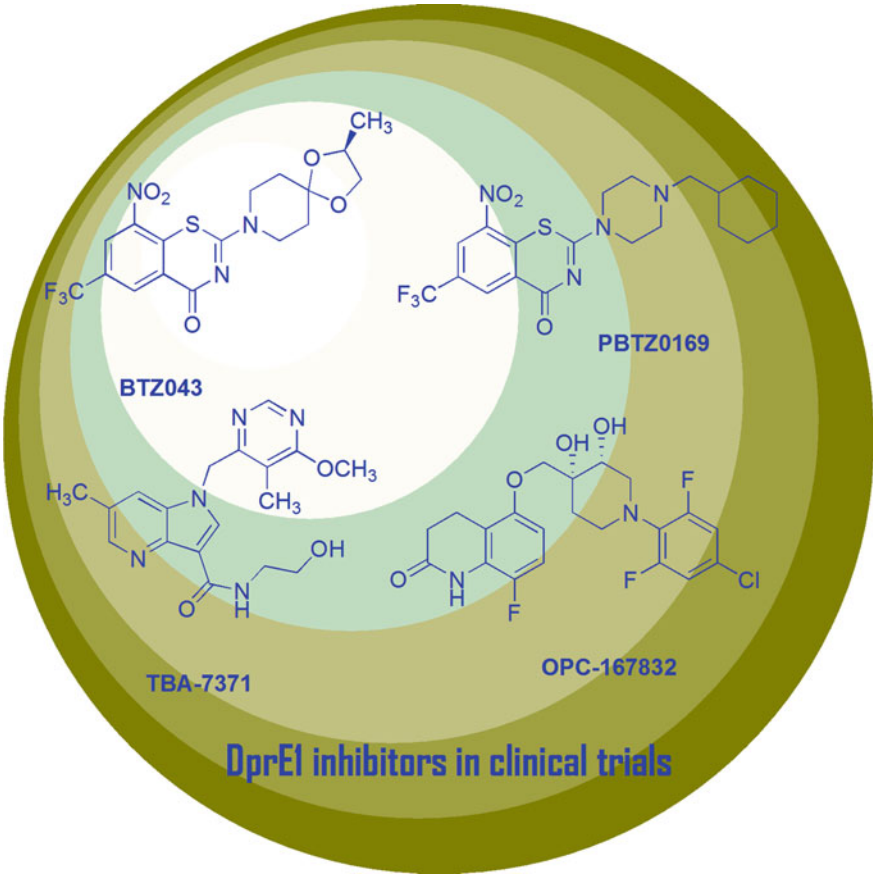
Graphical Abstract



DprE1 inhibitors in clinical trials

Keywords

DprE1 · DprE1 inhibitors · *Mycobacterium tuberculosis* · Tuberculosis

1 Introduction

Target identification serves as the commencing step of any drug discovery program. Medicinal chemists worldwide have been actively involved in unraveling novel tuberculosis (TB) targets and their promising inhibitors. Such a target should possess three essential characteristics:

(i) significance for the growth and persistence of bacteria;
(ii) selectivity for the bacteria over the host; and
(iii) drug approachability, i.e., the absence of structural barriers in the bacteria which would block the approach of the drug to the target.

Numerous first-line and second-line anti-TB drugs (Fig. 1) have been used clinically to treat TB for a long time, but the real fact is that the exact targets for many of them have not yet been recognized [1]. There are several targets identified to date for the inhibition of the active, replicating, and dormant forms of TB. Figure 2 depicts various targets and some promising TB inhibitors [2]. To date, a number of novel techniques, strategies, and programs have been undertaken as part of the TB drug discovery process. The overall picture is such that new drug discovery for the management of TB has become a daunting task for the researchers as the biggest culprit is *Mycobacterium tuberculosis (M. tb)* bacterium itself which exists in replicating as well as in dormant forms [3–5].

It is needed that novel anti-TB agents, either individually or in combination, have a shorter duration of treatment and manage drug resistance (DR) cases effectively with minimum or no toxicity. Recently, the decaprenylphosphoryl-β-D-ribose 2′-epimerase 1 (DprE1) enzyme possessing all the desirable requirements has emerged as a prospective novel target for the discovery of new anti-TB drugs [6]. DprE1, a flavoprotein present in the periplasm of the *M. tb* cell wall, is indispensable for cell wall synthesis. The significance of DprE1 as a potential druggable target for the discovery of anti-TB agents has been thoroughly discussed in the following sections of this chapter.

2 Special Features of the Enzyme Decaprenylphosphoryl-β-D-Ribose 2′-Epimerase 1 Which Make It a Target

To combat DR in mycobacterial strains, it is an urgent need to develop a drug that would be able to decrease the duration of treatment and kill the mycobacteria both in replicating and dormant forms completely. This is possible only when a valid target, which is essential for the growth of bacteria and its survival, is identified for the drug. Though many targets have been recognized and validated and a number of new anti-TB agents with potential anti-TB activity, the biggest threat of DR-TB still keeps on challenging the medicinal chemists' fraternity [2].

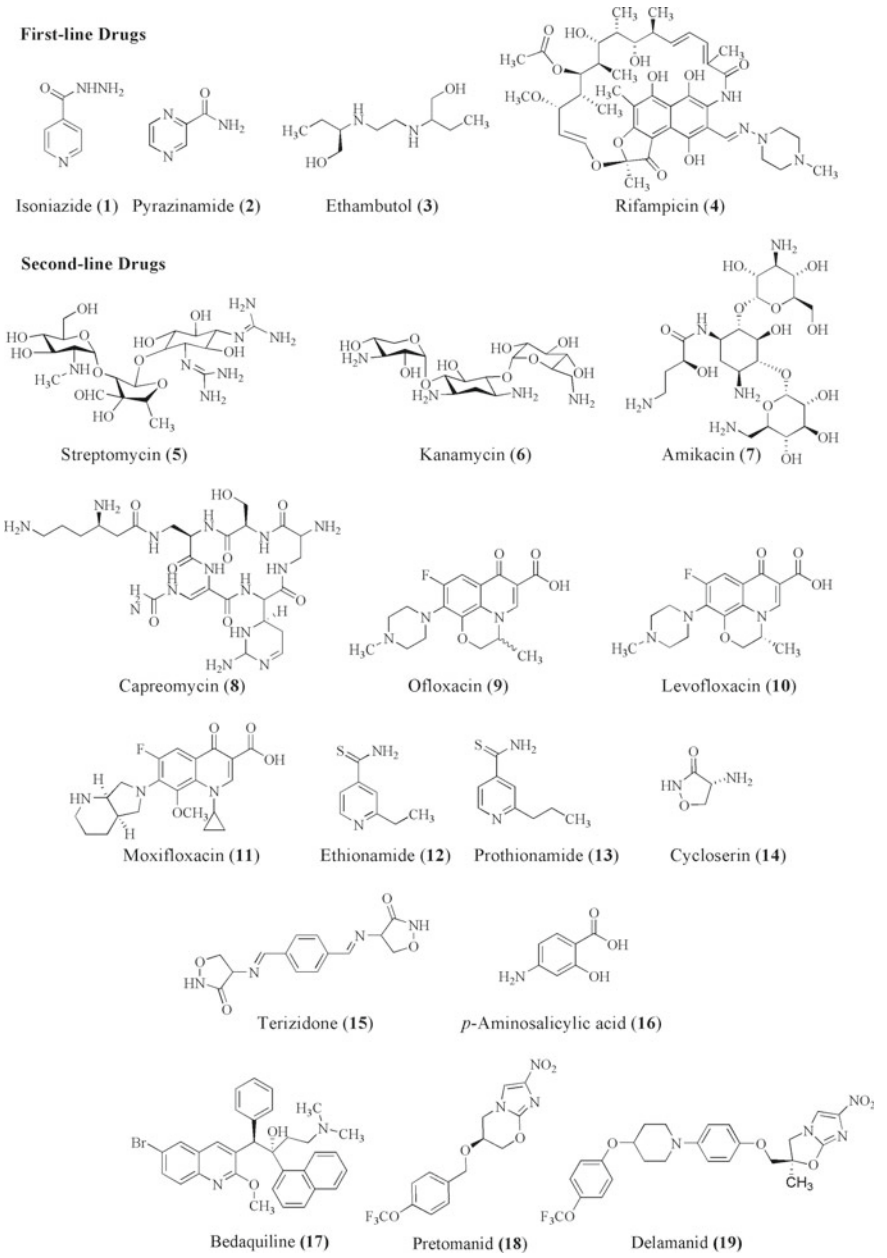


Fig. 1 Chemical structures of first (**1–4**) and second (**5–19**)-line anti-TB drugs

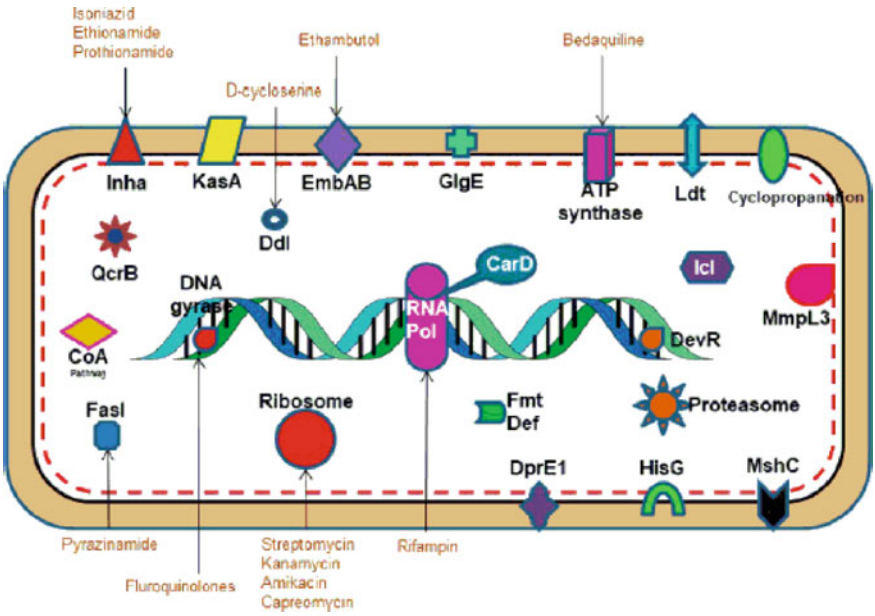


Fig. 2 Diagrammatic representation of *M. tb* cell demonstrating various possible targets within the cell structure [2]. Reprinted with permission from [2] Copyright© 2018 American Chemical Society

DprE1 has evolved as a new competent target that could be exploited for anti-TB drug discovery to tackle the problem of DR. It was postulated a few decades ago that blocking the biosynthetic process of mycobacterial cell wall would be the best way to win the battle against TB. DprE1 is a vital enzyme in the *M. tb* cell wall synthesis. In fact, inhibition of DprE1 causes cessation of generation of DPA required for the formation of Araf residues. Reduced levels of Araf residues hamper the supply of these essential building blocks of the mycobacterial cell wall, i.e., AG and LAM, which subsequently affect the biosynthesis of the *M. tb* cell wall. Thus, it clearly demonstrates that inhibiting DprE1 could hamper the growth as well as the survival of *M. tb*. Moreover, DprE1 is an ideal target as it is present only in mycobacteria and not in humans, which certainly underlines its importance as an anti-TB drug target for designing, developing, and discovering novel anti-TB agents. These special features of the DprE1 enzyme significantly make it a valuable drug target that could be utilized effectively for the discovery of novel anti-TB agents with enhanced biological potential and minimum toxicity [1, 7, 8].

2.1 Location and Role

Considering its function to provide overall strength to the cell and protect it from virulence and pathogenicity, the cell wall is the most important component of a

bacterial cell [9]. Therefore, cell wall biosynthesis has been considered the most promising target for most drugs, including antibiotics. Biosynthesis of the cell wall in *M. tb* consists of a number of processes that are ideal drug targets for discovering anti-TB drugs [10]. Several anti-TB agents, such as isoniazid (**1**) and ethambutol (**3**) of the first-line category, along with other second-line agents, actually interfere in the cell wall biosynthesis during different stages (Fig. 1) [11, 12]. With this strategy in mind to block the biosynthesis of the cell wall, various novel anti-TB targets, as shown in Fig. 2, have been recognized, which could be exploited further to develop novel anti-TB drugs.

The composition of the *M. tb* cell wall is quite complex as it is built of two unique complexes known as peptidoglycan-arabinogalactan-mycolic acid (PAM) complex or mAGP complex (mycolyl-arabinogalactan–peptidoglycan) and lipoarabinomannan (LAM) [13]. PAM complex is mainly composed of three layers:

(i) a highly impermeable lining of mycolic acid;
(ii) arabinogalactan polysaccharide (AG); and
(iii) peptidoglycan (PG), posing from outer side to inner side of the cell.

PG is covalently bound to AG through a phosphodiester linkage that gets attached to the mycolic acid, forming the PAM complex [14]. The second element, LAM, is a non-covalently bound lipopolysaccharide comprising D-arabinofuranose (Araf) and mannopyranosyl residues. Both the components (PAM and LAM) are a prerequisite to maintaining cell wall integrity and impart a crucial role in the *M. tb* virulence and pathogenesis [12, 15, 16].

Synthesis of Araf residues, essential building blocks of AG and LAM, is a crucial biosynthetic step. Biosynthesis of AG and LAM involves the addition of Araf residues to the galactan and mannan domains, respectively, catalyzed by a specific enzyme known as arabinosyltransferase. The arabinosyltransferase enzyme uses the sugar decaprenylphosphoryl-β-D-arabinose (DPA) generated by the epimerization of decaprenylphosphoryl-β-D-ribose (DPR). DPA is the only source for the Araf residues in *M. tb*. Without DPA, it is difficult for the bacteria to survive in latent as well as virulent forms as the cell wall synthesis would be ceased completely [12, 15, 17].

A heterodimeric enzyme, i.e., DprE, consists of two enzymes–DprE1 and DprE2–the key proteins involved in the biosynthesis of DPA. DprE1 is a FAD-dependent enzyme that converts DPR to decaprenylphosphoryl-2-keto-β-D-*erythro*-pentofuranose (DPX) by oxidation, and DPX is then further reduced to DPA in the presence of decaprenylphosphoryl-*D*-2-keto-*erythro*-pentose reductase (DprE2). DprE1 is required for the growth and survival of *M. tb*. Hence, blockade of DPA synthesis by inhibition of the DprE1 enzyme would be a key strategy to stop the biosynthesis of the *M. tb* cell wall [6, 18].

2.2 Mechanism of Action

Biosynthesis of DPA involves oxidation of DPR to DPX in the first step and reduction of DPX to DPA in the second step (Fig. 3). Oxidation of DPR is catalyzed by a flavoenzyme, DprE1, using flavin adenine dinucleotide (FAD) as an oxidant which gets reduced to $FADH_2$. Now, to start a new cycle of oxidation of DPR, $FADH_2$ has to be re-oxidized to its oxidizing form, i.e., FAD. Despite being an oxidase enzyme, DprE1 showed comparatively low reactivity with oxygen. Actually, DprE1 uses a natural membrane-embedded electron acceptor, menaquinone, present in *M. tb* to re-oxidize $FADH_2$ to FAD. In the second step, the reduction of intermediate DPX to DPA is catalyzed by DprE2 in the presence of cofactor NADH. Considering the above fact, DprE1 could be considered an oxidoreductase enzyme rather than a true oxidase. Therefore, the DprE1, DprE2, or DprE1-DprE2 complex could be exploited as potential TB targets to design and develop small molecule therapeutics [10, 19, 20].

2.3 Crystal Structure

The discovery of the crystal structure of DprE1 shed more light on the identification of the active sites of the enzyme and the possible mechanism of action of its inhibitors which proved beneficial for the medicinal chemists to design and develop novel anti-TB drugs with improved clinical potential. There have been several reports wherein crystal structures of DprE1 from *M. smegmatis and M. tb* co-crystallized with or without covalent/non-covalent inhibitors [7]. Neres et al. [19] and Batt et al. [20] were the first groups to report the crystal structure of the DprE1 enzyme in the year 2012. Thereafter, approximately 35 crystal structures of DprE1 have been reported to date, which are available in the protein data bank (PDB).[1] Piton et al. [7] and Chikhale et al. [2] have enlisted 23 of the available structures of DprE1 systematically according to their date of release, PDB IDs, resolution, and source of Mycobacterium species [2, 7]. A summary of the remaining structures of DprE1 from 2018 onwards has been presented in Table 1.

The crystal structure of DprE1 contains several active sites for binding inhibitors. The enzyme DprE1 (PDB code 4P8L; Fig. 4) consists of two active binding

[1] https://www.rcsb.org/search?request=%7B%22query%22%3A%7B%22type%22%3A%22group%22%2C%22nodes%22%3A%5B%7B%22type%22%3A%22group%22%2C%22nodes%22%3A%5B%7B%22type%22%3A%22group%22%2C%22nodes%22%3A%5B%7B%22type%22%3A%22terminal%22%2C%22service%22%3A%22full_text%22%2C%22parameters%22%3A%7B%22value%22%3A%22dpre1%22%7D%7D%5D%2C%22logical_operator%22%3A%22and%22%7D%5D%2C%22logical_operator%22%3A%22and%22%2C%22label%22%3A%22full_text%22%7D%5D%2C%22logical_operator%22%3A%22and%22%7D%2C%22return_type%22%3A%22entry%22%2C%22request_options%22%3A%7B%22pager%22%3A%7B%22start%22%3A0%2C%22rows%22%3A25%7D%2C%22scoring_strategy%22%3A%22combined%22%2C%22sort%22%3A%5B%7B%22sort_by%22%3A%22score%22%2C%22direction%22%3A%22desc%22%7D%5D%7D%2C%22request_info%22%3A%7B%22query_id%22%3A%22cdfdc2c113a57e3c8e2b8beba4d0b327%22%7D%7D.

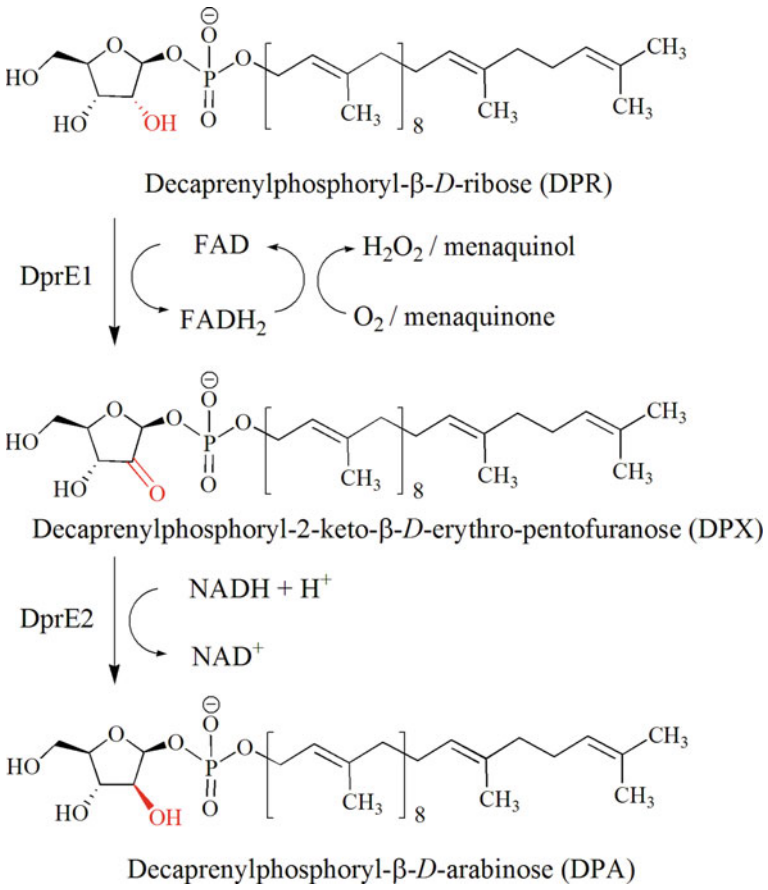


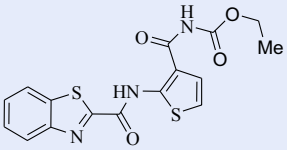
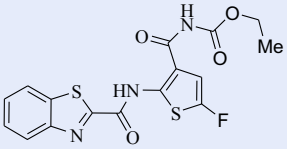
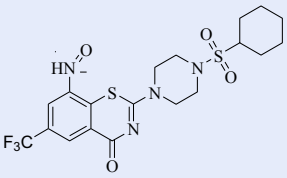
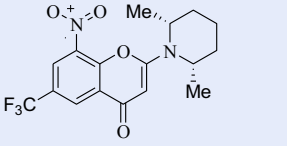
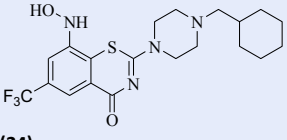
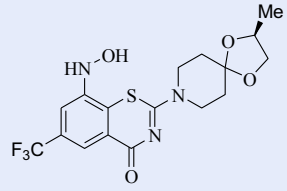
Fig. 3 Epimerization of 2′-OH group (highlighted in red color) of DPR by DprE1 and DprE2 to generate DPA [2]. Reprinted with permission from Ref. [2] Copyright© 2018 American Chemical Society

domains, similar to the other oxidoreductases such as vanillyl alcohol oxidase. These two active binding domains include:

(i) a FAD-binding domain with residues 7–196, 413–461; and
(ii) a substrate-binding domain with residues 197–412 (Fig. 4).

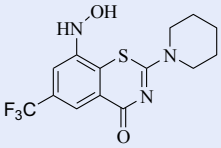
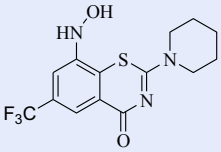
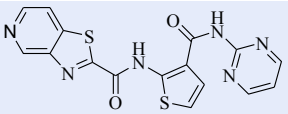
The cofactor FAD is located deep inside the FAD-binding domain of the enzyme, with the isoalloxazine ring of FAD lying at the interface of the substrate-binding domain. Two disordered loops are present above the substrate-binding domain, confirmed by the electron density map obtained in all of the crystal structures of DprE1 [7]. Interestingly, these two loops, disordered loop I with amino acid residues 269–303 and disordered loop II with amino acid residues 316–330, might be interacting with the cell membrane, with certain proteins

Table 1 Crystal structures of DprE1 in PDB along with their year of release, resolution, inhibitor, and mechanism of inhibition

S. No	Year (release date)	PDB ID	Co-crystal ligand	Resolution (Å)	*Mycobacterium* species
1	2018	5OEL	 **(20)**	2.20	*Mycobacterium tuberculosis* CDC1551
2	2018	5OEP	 **(21)**	2.35	*Mycobacterium tuberculosis* H37Rv
3	2018	6G83	 **(22)**	2.40	*Mycobacterium tuberculosis* CDC1551
4	2018	6HF0	 **(23)**	2.38	*Mycobacterium tuberculosis*
5	2018	6HF3	 **(24)**	2.20	*Mycobacterium tuberculosis*
6	2018	6HEZ	 **(25)**	2.30	*Mycobacterium tuberculosis*

(continued)

Table 1 (continued)

S. No	Year (release date)	PDB ID	Co-crystal ligand	Resolution (Å)	*Mycobacterium* species
7	2018	6HFW	 **(26)**	2.47	*Mycobacterium tuberculosis H37Rv*
8	2018	6HFV	 **(27)**	2.05	*Mycobacterium tuberculosis* H37Rv
9	2018	5OEQ	 **(28)**	2.25	*Mycobacterium tuberculosis* H37Rv

Prepared with data from Refs. [6, 21–23]

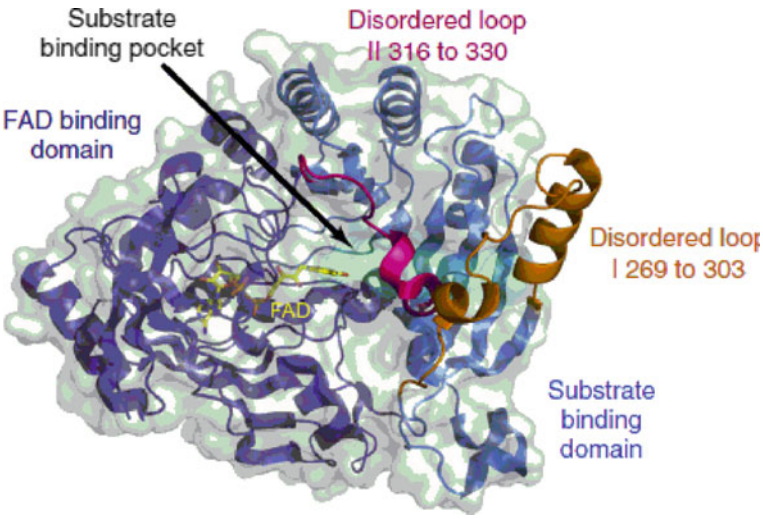


Fig. 4 Structure of DprE1 (PDB code 4P8L). A deep blue color represents the FAD-binding domain, whereas light blue color depicts the substrate-binding domain. Substrate-binding pocket is highlighted on the lime green surface. Orange and hot pink depict the two disordered loops [7]. Reprinted with permission from Ref. [7] Copyright © 2017 Elsevier

involved in the biosynthesis of DPA, a substrate for DprE1 or with DPR [20]. These two disordered loops actually keep the substrate-binding domain wide open, facilitating the accommodation of the substrate in the domain. Thus, these loops could be considered as the entrance gate for the substrate approaching the substrate-binding domain [7]. Recent developments of various DprE1 inhibitors have been discussed in the following sections of this chapter.

3 Insight into DprE1 Inhibitors

Previously in the year 2018, we have published from our lab an extensive review on DprE1 and its inhibitors as an anti-TB target [2]. Some important developments in discovering DprE1 inhibitors as potential anti-TB drugs have been discussed here.

The discovery of the co-crystal structure of DprE1 bound to benzothiazinone (BTZ) offered an important insight into the mechanistic view of DprE1 inhibitors that helped develop newer anti-TB agents. DprE1 inhibitors can be categorized or differentiated based on their binding interactions, viz. covalent or non-covalent binding to the enzyme. Here, DprE1 inhibitors are classified as covalent and non-covalent binding and miscellaneous inhibitors.

3.1 Covalent Binding Inhibitors

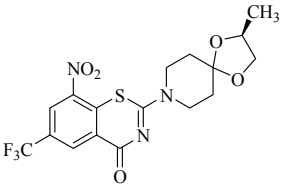
3.1.1 Benzothiazinones as the Leading Inhibitors of DprE1

Benzothiazinones (BTZs) evolved as a new class of anti-TB agents in 2009. They offered hope for the design and development of newer and effective drug candidates for the treatment of DR-TB [24]. BTZ scaffold demonstrated sub-micro molar MIC value against *M. tb*. BTZs have been proved to inhibit the DprE1 enzyme covalently. In a successful attempt to improvise the pharmacological properties of BTZs, their piperazine-containing analogs (PBTZ) were synthesized.

In the biological systems, the nitro functional group gets reduced primarily to the corresponding nitroso group, then to the hydroxylamine, and finally to the amine [25]. It is hypothesized that benzothiazinone-nitrosoarene derivatives bind with Cys387 residue of the enzyme via interaction with the thiol group of the residue, thereby forming a covalent adduct and, thus, behaving suicide inhibitors [26]. Later in 2013, Tiwari et al. [27] proposed another plausible mechanism behind the formation of the nitroso derivative. They proposed that the redox reaction responsible for converting the nitro into the nitroso derivative is facilitated by the thiol group of cysteine residue present in the enzyme. It was also hypothesized that this conversion does not require $FADH_2$ as the catalyst. Further, the cine addition of thiolate would initiate the redox reaction that generates the nitroso derivative.

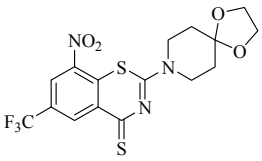
For the first time, BTZs were reported as nitro benzothiazinones, the active molecules that could inhibit DprE1 from Mollmann lab, Germany; Makarov lab, Moscow; and Cole lab at the Global Health Institute, Ecole Polytechnique,

Switzerland [16, 28]. BTZ043 (**29**) was found to be the most potent compound, exhibiting antimycobacterial activity with a minimum inhibitory concentration (MIC) value of 1 ng/mL, whereas MIC values for Isoniazid and ethambutol (EMB) were found in the range of 0.02–0.2 mg/mL and 1–5 mg/mL respectively. In order to determine the site of action of BTZ, radiolabeled studies were performed. These results demonstrated that BTZ targeted the biosynthesis of arabinogalactan, which in turn is an important component in the covalent linking of mycolic acid and peptidoglycan layer, thus inhibiting the biosynthesis of the *M. tb* cell wall.

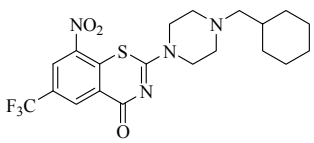


BTZ043 (**29**)

Gao et al. [29] attempted to establish the structure-activity relationship (SAR) of BTZs. In order to do so, they prepared a series of *N*-alkyl and heterocycle substituted BTZ compounds. These derivatives showed potent inhibition against the *M. tb* strains. It was noted that increasing the bulk or substituting bulky groups in the BTZ motif amplified the inhibitory activity. Also, the trifluoro substituent played a pivotal role in deciding the activity of the piperazine or piperidine analogs. Some compounds with spiro-piperidine moiety exhibited activity comparable to that of BTZ043. This indicated that the presence of sulfur in the azaspiro ring increased the activity. These compounds also demonstrated good bioavailability. Further, an attempt was made to substitute the oxygen of the BTZ043 carbonyl group with a sulfur atom, generating SKLB-TB1001 (**30**). Compound (**30**) showed promising in vitro activity and good ADMET properties, and it was found to be efficient in treating acute infection in a mouse model. Its MIC value was found to be 0.02 μg/ml. Subsequent work resulted in the design and synthesis of substituted 2-piperazine-benzothiazinone (PBTZ) (**31**) derivatives with enhanced lipophilicity [30]. SAR studies for this series indicated that the presence of hydrophilic groups such as secondary or tertiary amines, alcohols, etc., on the N-4 piperazine ring led to compounds with diminished or complete loss of activity.

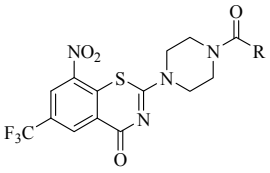


SKLB-TB1001 (**30**)



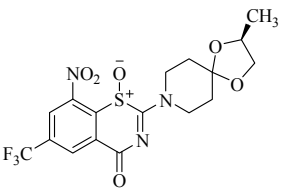
PBTZ0169 (**31**)

Benzothiazinones BTZ043 (**29**) and PBTZ0169 (**31**) were explored substantially due to their imposing DprE1 inhibition. To enhance the activity and pharmacokinetic parameters, Peng et al. [31] synthesized 1,3-benzothiazin-4-one derivatives with 4-carbonylpiperazine (**32**). MIC value of this most active compound was found to be 0.0131 μM.

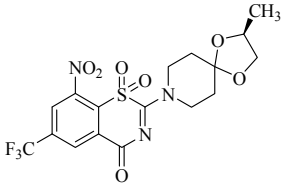


(32)

BTZ-SO (**33**) and BTZ-SO_2 (**34**) were prepared to study the effect of oxidation of sulfur on the biological activity of BTZ [32]. BTZ-SO was found to produce impressive antimycobacterial activity, whereas BTZ-SO_2 failed to exhibit anti-TB activity.



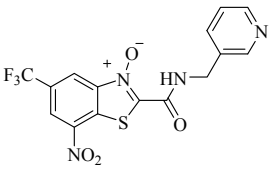
BTZ-SO (**33**)



BTZ-SO_2 (**34**)

3.1.2 Benzothiazole Containing DprE1 Inhibitors

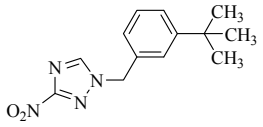
Langde el at. [33] discovered some new molecules containing benzothiazole, possessing antimycobacterial activity by high throughput screening of database from AstraZeneca Pharmaceuticals drug library, containing more than 100,000 compounds. Initial searching gave benzothiazole *N*-oxide as the lead molecule (8-BTO) (**35**). The most potent compound was found to possess a MIC value of 1 μg/mL. It was further taken up to optimize and develop a new series of derivatives. Benzothiazole oxide and benzothiazole were found to show good potency, but this potency came along with the side effect of mutagenicity. It was noted that substitution of the nitro group rendered the compound inactive. Furthermore, the authors tried to sterically hinder the nitro group so as to prevent the generation of reactive intermediate derivatives responsible for mutagenic properties. The IC_{50} value for 8-BTO (**35**) was found to be 0.026 μM.



(35)

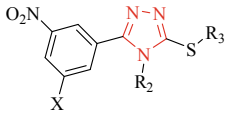
3.1.3 Triazole Scaffold Containing DprE1 Inhibitors

Stanley et al. [34] performed a cell-based HTS assay and reported various novel inhibitors. In this study, screening of the literature reported 20,000 antibacterial agents against *M. tb*. Additionally, a dataset of 341,808 compounds was also screened against *M. tb* using a 7H12 medium. 1-(4-(*tert.*Butyl)benzyl)-3-nitro-1*H*-1,2,4-triazole (**36**) came out as an initial hit showing an IC_{90} value of 0.5 μM. It was observed that compounds having nitro groups showed good activity, whereas compounds devoid of the nitro group exhibited reduced activity. Also, compounds with nitro substitution were bound covalently with the enzyme. These observations suggest the importance of the nitro group, which in turn gets reduced to some reactive species to provide interaction to the target.



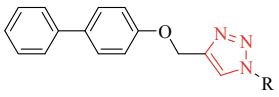
(36)

Karabanovich et al. [35] prepared various 3,5-dinitrophenyl-1,2,4-triazole containing compounds exhibiting magnificent and selective antimycobacterial activity. Among the 23 compounds, it was found that *S*-substituted 4-alkyl-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole-3-thiols (**37**) and their 3-nitro-5-(trifluoromethyl)phenyl (**38**) analogs exhibited the highest in vitro activity against *M. tb* H37Rv.



(37, 38)

37, X = -NO_2,
38, X = -CF_3



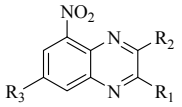
(39)

Ali et al. [36] synthesized seventeen novel 1,2,3-triazole derivatives (**39**) using 'click chemistry' methodology and evaluated them in vitro for their inhibitory

activity against *M. tb* H37Rv strain. Among the synthesized derivatives, six compounds were found to have significant activity with MIC values ranging from 3.12 to 0.78 μM with nil or negligible cytotoxicity against mouse bone marrow-derived macrophages. These six compounds possessed MIC values lesser than 6.25 μg/ mL along with a high affinity for the active site of DprE1.

3.1.4 Quinoxalines as DprE1 Inhibitors

Magnet et al. [37], while performing an experiment of screening a collection of kinase inhibitors containing 12,000 compounds against *M. tb*, reported the quinoxaline scaffold active against *M. tb*. Three compounds were obtained as initial hits having activity lesser than 10 μM. During the studies, it was observed that these compounds were non-mutagenic and non-toxic. All three hits contained quinoxaline as a basic scaffold (**40**) and were reported as specific inhibitors of DprE1. The mechanism of action or binding mode of these molecules is essentially the same as BTZs.



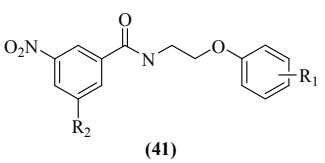
(40)

Comp.	R_1	R_2	R_3	MIC (μM)
40a	-CH_3	Phenyl	Br	3.1
40b	CH_3	Phenyl	CF_3	0.75
40c	Phenyl	CH_3	CF_3	6.25

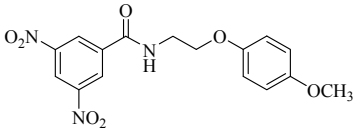
3.1.5 Nitrobenzamide-Based DprE1 Inhibitors

Nitrobenzamide derivatives depict another example of the application of high throughput screening (HTS) in drug discovery. Christophe et al. [38] performed screening of a collection of 56,984 molecules for checking drug-likeness by applying Lipinski's rule of five, and then the short-listed molecules were screened for their anti-TB activity at a single dose concentration. Four hundred eighty-six molecules from the selected molecules were checked using the serial dilution method. About 8% of these molecules showed MIC value comparable to isoniazid. Cluster analysis indicated that 69 compounds had a similar structure as isoniazid, and out of these, 24 compounds had benzamide as the common structural feature (**41**). These derivatives were further exploited to produce a series of compounds with improved activity [39]. SAR study was also undertaken to optimize the benzamide derivatives. It was noted that nitro groups at positions 3 and 5 were essential for the potency of the compounds as reduction of nitro to the corresponding hydroxylamine derivatives with no activity. An enhancement in the activity profile was observed when the amide nitrogen was substituted with benzyloxy or phenoxymethyl moieties. Cyclic benzamides demonstrated MIC values as low as 80 nM, but they lacked potency during the intracellular assay. Compound (**42**) was screened for antimycobacterial activity on replicating as well as non-replicating strains. Unfortunately, the results demonstrated that it was effective

only in the replicating cultures and was inactive against the non-replicating strains. These results concluded that compound (**42**) demonstrated activity by interacting with the Cys387 residue of the DprE1.

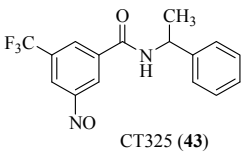


(**41**)

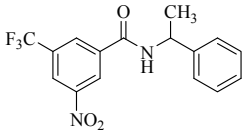


(**42**)

Furthermore, two more benzamide derivatives, CT325 (**43**) and CT319 (**44**), were synthesized by taking BTZ structure into consideration, which was found to bind to the active site of the DprE1 enzyme [18]. Both of them showed good inhibitory activity with specificity towards DprE1. It was seen that compound (**43**) interacted covalently with the enzyme acting as an irreversible inhibitor, whereas compound (**44**) formed a non-covalent bond with the enzyme.



CT325 (**43**)

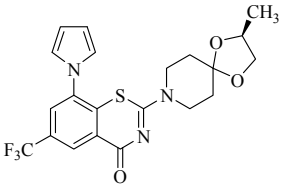


CT319 (**44**)

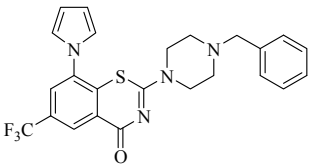
3.2 Non-covalent Inhibitors

3.2.1 Benzothiazinone Containing DprE1 Inhibitors

Earlier, benzothiazinones were known to interact only covalently with the enzyme. But the scenario changed when Makarov et al. [40] tried to replace the nitro group of the BTZ with a pyrrole ring. These efforts resulted in the discovery of active pyrrole-BTZ compounds (**45** and **46**) having a MIC value of 0.16 μg/mL. The IC_{50} values were as low as < 8 μM with appreciable ADMET and in vivopharmacokinetic parameters. Unfortunately, they failed to impress the animal models. Molecular docking studies revealed that pyrrole-BTZs bind to the same cavity of DprE1 as BTZ, with pyrrole rings located close to Cys387 residue. Surprisingly, any covalent interaction with the enzyme was absent, indicating that Pyr-BTZ compounds act as non-covalent inhibitors.



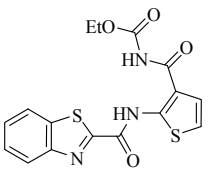
PyrBTZ01 (**45**)



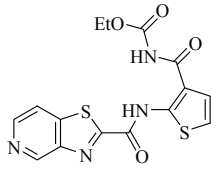
PyrBTZ02 (**46**)

3.2.2 Benzothiazole Based DprE1 Inhibitors

Benzothiazoles have been considered a boon for designing and developing DprE1 inhibitors. Wang et al. [41] performed cell-based phenotype screening and reported a small molecule TCA1 (**47**) as a DprE1 inhibitor. The Discovery of TCA1 (**47**) was serendipitous, as it was obtained during the screening of a collection of 70,000 molecules for their inhibitory activity against replicating and non-replicating *M. tb* strains. Compound (**47**) showed promising activity both in vitro and in vivo. Initially, it was observed that the compound (**47**) exhibited antimycobacterial action by downregulation of persistent genes and cell wall inhibition via interfering with mycolic acid synthesis. The discovery of TCA1 (**47**) offered a pathway for further developing DprE1 inhibitors by serving as a template molecule. Liu et al. [21] performed synthesis, molecular docking, and pharmacological evaluation along with SAR studies to optimize the benzothiazoles as DprE1 inhibitors. Three positions were modified, i.e., thiophene moiety, benzothiazole core, and carbamate group. It was observed that these structural changes were indeed beneficial for inhibitory activity, but they also caused CYP2C9 inhibition. Subsequently, to develop DprE1 inhibitors with no CYC2C9 inhibition, the authors carried out further studies using compound TCA007 (**48**) as a template molecule.

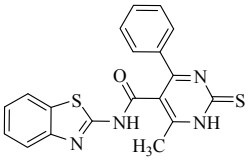


TCA1 (**47**)



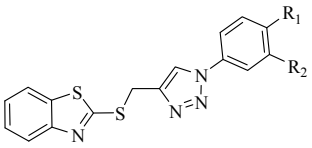
TCA007 (**48**)

In another study conducted by Chikhale et al. [42], a series of benzothiazolyl pyrimidine carboxamides was reported. This series provided information that compounds having para-substituents on the phenyl ring of the compound demonstrate favorable activity though the unsubstituted compound (**49**) offered the best results in terms of MIC value of 0.08 and MBC of 7.7 μM.



(49)

Fauzia et al. [43] reported anti-TB activity of benzothiazole and 1,2,3-triazole based *bis*-heterocycles against *M. tb*. Three compounds (50–52) showed good activity among the series. Furthermore, a molecular docking study was conducted to investigate the binding interactions between the compounds and DprE1. It was found that compound (**51**) possessed potent inhibitory properties owing to hydrogen bonding and hydrophobic interactions. It was seen that compound (**51**) interacted with Tyr60, Gly117, Ala375, Ser378, Asn385, Lys418, and Trp437 residues via H-bond.

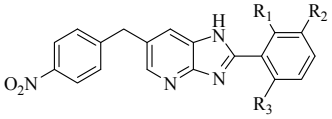


50, R_1= H, R_2= Cl
51, R_1= NO_2, R_2= H
52, R_1= H, R_2= NO_2

(50-52)

3.2.3 Imidazopyridine Based DprE1 Inhibitors

Gawad et al. [44] reported 6-(4-nitrophenoxy)-2-substituted-1*H*-imidazo[4,5-*b*] pyridine derivatives to explore the potential of 1*H*-imidazo[4,5-*b*]pyridine nucleus. In this study, the nitro group was intentionally substituted at the sixth position because of its proven binding with Cys387 residue of the DprE1 enzyme. Some of the derivatives have shown good anti-TB activity. The most potent compounds were found to have MIC values ranging from 0.5 to 0.8 μM. Interestingly, docking studies of these compounds yielded excellent docking scores. Binding interactions shown by these compounds were similar to that of the lead molecule TCA1 (**47**). Earlier it was reported that the nitro group got reduced and interacted with Cys387, but no such interaction was seen here. Information obtained from the docking studies and the in vitro studies indicated that further structural modifications could help develop better compounds as DprE1 inhibitors.

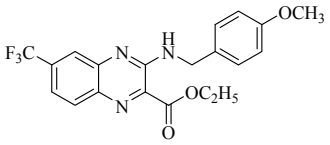


(53)

Comp.	R_1	R_2	R_3
53a	-OH	-OH	H
53b	$-OCH_3$	H	$-OCH_3$
53c	H	$-NO_2$	H
53d	H	Br	H

3.2.4 Quinoxalines as DprE1 Inhibitors

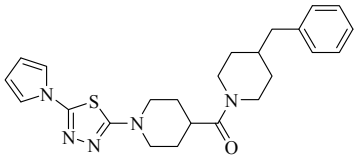
Neres et al. [45] performed phenotype screening of a collection of 266 compounds against *M. tb*. This resulted in the discovery of novel quinoxaline derivatives with promising bactericidal activity. The lead compound (**54**) was highly effective against *M. tb,* having an IC_{50} value of 6.1 μM. SAR studies disclosed that the absence of the 6-trifluoromethyl group rendered the compounds inactive, and its presence at the para-position of the C3 benzyl amine moiety affected the DprE1 inhibition significantly. Substituents like methoxyl and halogens on the phenyl ring of the benzylamino group yielded compounds with moderate activity. All the derivatives were active against both replicating and non-replicating strains of *M. tb*.



(54)

3.2.5 Thiadiazole Containing Inhibitors

Batt et al. [46] simultaneously utilized phenotype screening and target-based drug design strategy to report a series of novel DprE1 inhibitors. This study was performed using a library of 177 compounds with known *M. tb* inhibitory activity. These compounds were then tested for their enzymatic assay for DprE1 selectivity. Compound (**55**) was found to be the most potent derivative with an IC_{50} value of 0.054 μM, and it demonstrated the greatest binding affinity (Kd of 0.25 μM) for the enzyme.

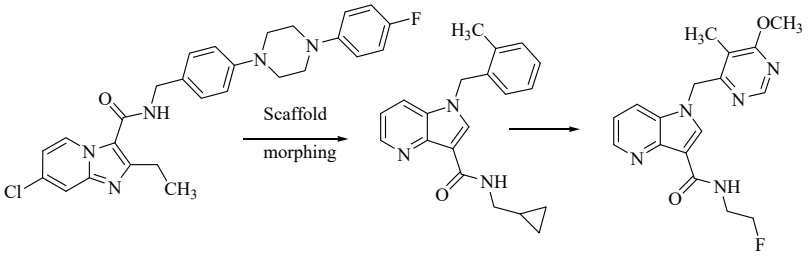


(55)

3.2.6 Azaindoles as DprE1 Inhibitors

Earlier various imidazopyridine-based compounds were reported as antimycobacterials, but these possessed very mild activity [44, 47, 48]. The imidazopyridine scaffold (56) was morphed into 1,4-azaindoles (**57** and **58**) to explore and enhance the activity. Shirude et al. [49, 50] attempted to improve 1,4-azaindoles wherein

they synthesized 23 compounds. The authors claimed that this novel class of inhibitors exhibited cellular activity via non-covalent inhibition of DprE1. The most potent derivatives exhibited MIC in the range of 0.39–0.78 μM. These derivatives were found to be better than the already reported DprE1 inhibitors. Yet they exhibited a couple of pitfalls, like inhibiting the PDE6 protein complex, which plays an essential role in the proper functioning of the human eyes and has shown not-so-good pharmacokinetic properties. In continuation, to optimize the lead (**58**), Shirude et al. [51] further reported other 27 compounds to overcome the pitfalls of the earlier compounds. These newer derivatives offered an optimal pharmacokinetic profile. Moreover, these were devoid of any inhibition of PDE6. Further, SAR was developed for the azaindole series (Fig. 5). It was noted that three essential structural features were necessary for the activity. The core 1,4-azaindole with a substituent on the sixth position was the minimum requirement for the activity. The amide chain was a requirement for optimal potency and binding affinity. It also influenced the physicochemical parameters. Small substituents were necessary for cellular potency. The hydrophobic pocket of the enzyme gets filled with an aromatic core at the N-1 position during its binding with the enzyme; thus, this aromatic core was found to be one of the requirements. One of the most potent compounds, TBA-7371 (**60**), from the azaindole series is under clinical trials.



3.2.7 Benzimidazole as DprE1 Inhibitors

Manjunatha et al. [52] used a scaffold morphing approach to modify the already known DprE1 inhibitors. They undertook azaindole TBA7371 (**60**) core as the template molecule and performed scaffold morphing to report benzimidazole derivatives (**61** and **62**). They demonstrated potent DprE1 inhibition with improved aqueous solubility and increased plasma function.

The benzimidazole derivative (**61**) and TBA7371 (**60**) were docked with the *M. tb* DprE1 enzyme (PDB ID 4KW5 binding site). One of the plausible binding modes for compound (**61**) from unconstrained docking is shown in Fig. 6a. It was seen that carbonyl oxygen was involved in H-bonding with Ser228, whereas amidic NH was bound with FAD carbonyl oxygen. The benzimidazole core generated CH $-\pi$ contacts with Trp230 and Tyr314 residues. The molecule was also found to have hydrophobic interactions with various amino acid residues. The binding mode of the overlaid azaindoles showed a similar binding mode for the amide group (Fig. 6b).

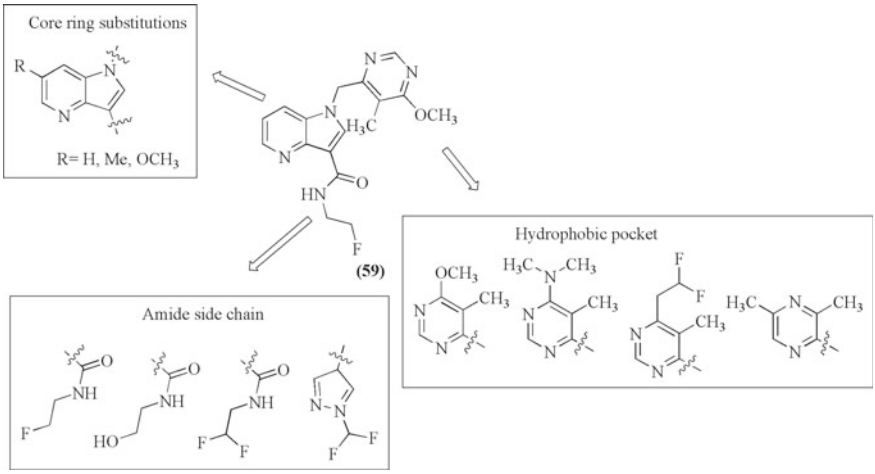


Fig. 5 SAR and SPR of 1,4-azaindoles. Adapted with permission from Ref. [51]

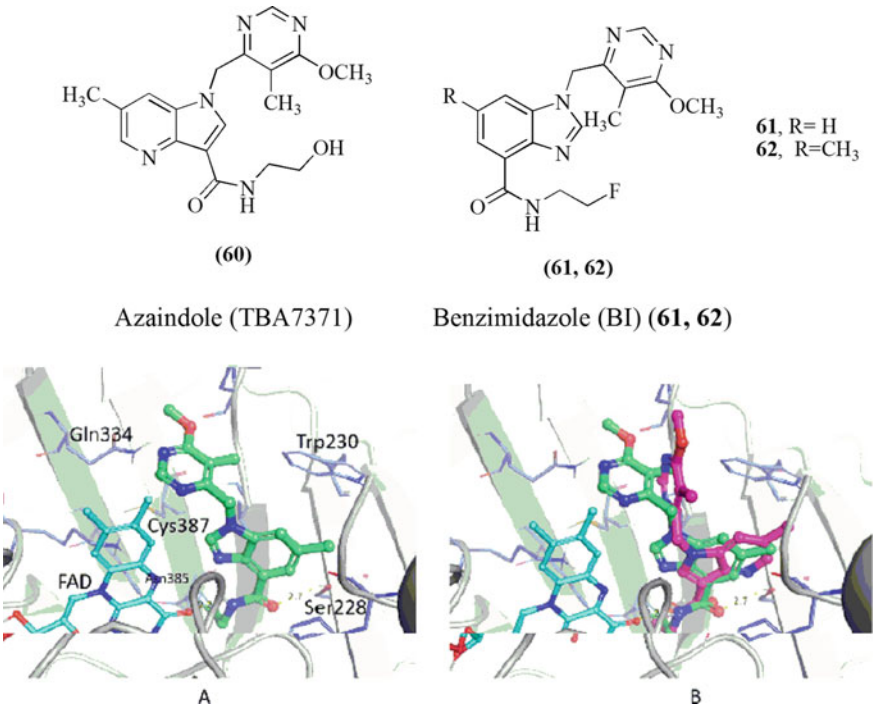
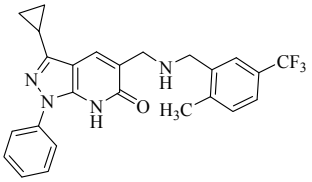


Fig. 6 **a** Binding mode of compound **61** in the DprE1 active site. **b** Docked poses of **60** (magenta) and **61** (green). Reprinted with permission from Ref. [52] Copyright© 2019 American Chemical Society

3.2.8 Pyrazolopyridine Based DprE1 Inhibitors

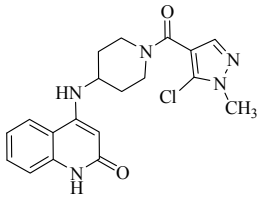
Panda et al. [53] performed a whole-cell screening assay against *M. tb* strains and reported a new series of pyrazolopyridones as the active scaffold against *M. tb*. In order to determine the selectivity of the compounds over DprE1, an overexpression assay was performed wherein gene *Rv3790* was overexpressed for MIC study. These compounds showed higher MIC values than the earlier reported compounds. These derivatives were found to be interacting non-covalently with DprE1 and were effective against both replicating and non-replicating strains. The IC_{50} value for compound (**63**) was reported to be 0.04 μM.



(63)

3.2.9 Aminoquinolone Scaffold Containing DprE1 Inhibitors

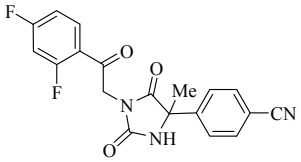
Naik et al. [54] reported 4-aminoquinoline piperidine amides as novel DprE1 inhibitors based on the whole-cell assay. AstraZeneca corporate collections of approximately 320,000 compounds were screened to yield a compound as the lead molecule (**64**). It was found to be reasonably active against DprE1. Based on the lead molecule (**64**), various other 4-aminoquinoline piperidine amides were prepared and evaluated for pharmacological activity. Various studies, such as mass spectrometry and enzyme kinetic studies, concluded that these derivatives exhibited non-covalent and reversible inhibition of the enzyme. Analogs were found to possess excellent cidal properties in vitro against both replicating and non-replicating *M. tb* strains.



(64)

3.2.10 Hydantoins as DprE1 Inhibitors

A target-based HTS study conducted by GlaxoSmithKline (GSK) led to the emergence and identification of a novel hydantoin-based hit motif as a DprE1 inhibitor. This report offered a totally different scaffold from the other known DprE1 inhibitors. In 2018, Rogacki et al. [55] explored the report results and optimized the hits while developing a SAR for the series. Compound (**65**) was taken as the starting point for hit-to-lead optimization as it had exhibited good DprE1 enzyme inhibitory activity (pIC_{50} = 7.0). Additionally, it was characterized by good solubility, lack of cytotoxicity, and acceptable lipophilicity.



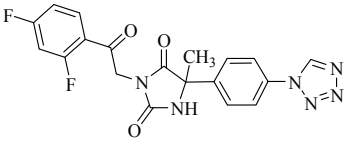
(65)

The authors carried out SAR studies to explore and optimize the hit (**65**) and divided the structure into five sub-structures, i.e., acetyl linker, substituents at C-5 of the hydantoin ring, phenyl ring at C-5, hydantoin ring substituents on the nitrogen (N-3) of the hydantoin ring, and the hydantoin moiety itself. The acetyl linker was seen as a potential liability because aromatic ketones are reactive groups, which may lead to enhanced metabolic instability of the compound. An attempt was made to modulate the linker by altering its length, removal of carbonyl group, or its substitution with known bioisosteric groups. It was observed that while most of the modulations resulted in the loss of activity, consistent low toxicity for the compounds throughout the series was worth noticing. Both methylations of the methylene group and bioisosteric replacement of the carbonyl moiety resulted in activity loss. These observations proved the importance of acetyl linker in the hit molecule (**65**).

C-5 of hydantoin had two substituents. The carbonitrile moiety has been known to react with cysteine and/or serine residues, leading to possible off-target covalent binding. Exploring this position indicated that the nitrile group was unnecessary for activity and could be exchanged for more active derivatives. Alteration of the methyl substituent resulted in either reduced or loss of activity. These results suggested that this part of the scaffold has a limited scope of modification. Modifications around position one by placing substituents on the nitrogen or exchanging it with carbon led to a decrease in enzymatic activity. Further, N-1 acyl substitution was tried, but the resulting derivatives lacked biological activity. This data suggested the importance of unsubstituted N_1-nitrogen atoms for binding to the enzyme. All the changes made at N-3 resulted in activity loss, indicating a lack of scope for modification at this position.

The authors acknowledged the possibility of hydantoin scaffold giving some undesired effects like inhibition of hERG potassium channels, the fatal hydantoin syndrome, and cardiovascular risks. This served as the liability for further development, so the authors planned to replace the hydantoin core. Structurally similar rings such as succinimide, imidazolidin-2-one, imidazole, and pyrazole were considered as alternatives to the hydantoin core since they differ in aspect of one or more hydrogen bond donors/acceptors while at the same time they have the same geometry as the hydantoin motif. Unfortunately, none of the core replacements served the purpose as these derivatives were found to be inactive. These results indicated the importance of hydantoin core. The authors suggested that the hydantoin core could also interact with the protein. It was also reported that the lead compound (**65**) exhibited reversible binding to the enzyme.

Recently, in 2020, Balabon et al. [56] expanded the exploration of SAR for the hydantoin family through 80 new compounds. Based on the results of their earlier studies, they tried to optimize the phenyl ring wherein they replaced cyano with different substituents. But the results were disappointing for most of the substituents. The only notable exceptions were the methyl ester and the fused bicyclic analog. The most potent compounds were (**66** and **67**), having tetrazole and sulphonamide moieties as the substituents on the phenyl ring.



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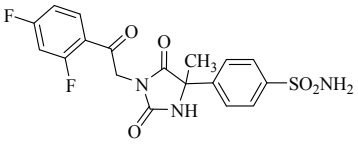
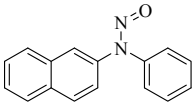


(67)

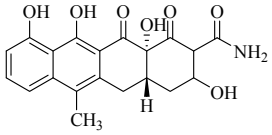
In vivo studies for the two most potent compounds (**66** and **67**) were carried out, and the efficacy of these derivatives was examined in a C57BL/6 J mouse model. No sign of adverse reaction was observed in any of the animals. The bioavailability of compound (**67**) was lower, and it exhibited significant blood exposure with a C_{max} value of 6380 ng/mL and an AUC value of 31,400 h * ng/mL. Also, it depicted the greatest reduction of Log_{10} CFUs (0.5). Although the value is lesser than that of moxifloxacin, it still indicates the capability of hydantoin derivatives to reach the lungs of the animals after oral administration. These results indicated that this chemical family displays no appreciable cytotoxicity or cardiotoxicity (hERG), an appropriate physiological profile, and satisfactory metabolic stability. Although the results are encouraging, these compounds need further research to improve in vivo efficacy.

3.3 Miscellaneous Inhibitors

Wisely et al. [57] conducted virtual screening of a dataset consisting of 4.1 million compounds against the enzyme DrpE1. For the screening purpose, the co-crystal structure of DprE1 CT319 (PDB ID:4FDO) was taken. Initially, 500 hits were identified, out of which 41 compounds were isolated based on the structural diversity, binding affinity, and binding conformation. Compounds (**68** and **69**) were obtained as the most potent compounds. Molecular docking studies depicted that –NH of the amide group formed H-bond with adjacent Tyr 60 via the hydroxyl group and with the phenylalanine 320 residue through the carbonyl group. Leu317 formed a hydrogen bond with the amide of the carbonyl group. The planar orientation of the inhibitor allowed it to fit well into the active cavity.



(68)



(69)

A series consisting of some novel 11α-substituted bile acid derivatives along with N-alkyl and N-acyl derivatives of C-11 amino bile acid esters were reported as anti-TB agents by Vandana et al. [58]. Among the reported series, four compounds (**70–73**) showed significant activity against the *M. tb* H37Ra strain. Docking studies revealed that the docking scores for these compounds varied from −9.951 to −4.995, while the reference compound showed a docking score of −7.953. It was found that compound (**71**) was stabilized via various interactions such as van der Waals and electrostatic interactions with amino acids. Interestingly a prominent pi–pi interaction was seen between the triazole ring of compound (**71**) and the imidazole ring in His132 residue. Additionally, some H-bonding is also observed within the active site. The authors also predicted ADME properties of these active compounds (Table 2) using in silicotechniques. These compounds demonstrated acceptable oral bioavailability along with low susceptibility towards acid hydrolysis.

Table 2 ADME (in silico) prediction data

Comp	Mol Wt	% Human oral absorption	Caco-2 cell permeability	MDCK cell permeability
70	655.87	75.74	110.36	45.68
71	621.77	72.10	150.47	63.86
72	706.87	51.46	60.23	29.82
73	714.93	64.83	151.99	64.56

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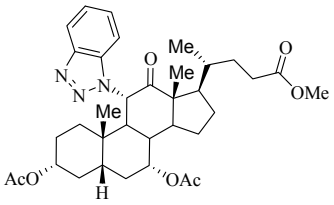

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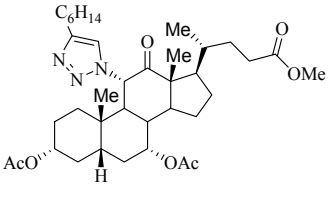

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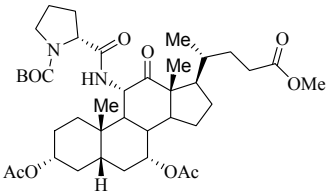

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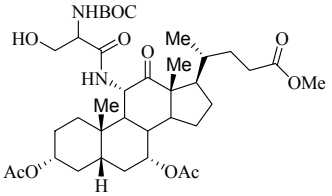
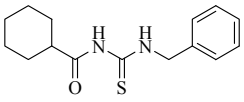


(73)

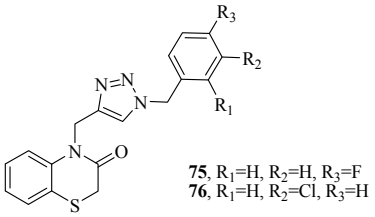
Jebiti et al. [59] performed molecular docking studies for some acyl thiourea derivatives against the DprE1 enzyme (PDB id: 4FDO). The results revealed that these thiourea derivatives interacted with the enzyme in a similar fashion as the co-crystallized ligand. The docking score for the most active compound (**74**) was −8.13, while the score was −6.77 for the co-crystallized ligand.



(74)

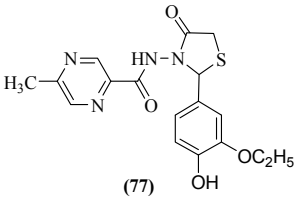
Shaikh et al. [60] reported novel triazole-based benzothiazinone derivatives as anti-TB agents. They carried out docking of the most potent compounds (**75** and **76**) to examine the binding interactions with the enzyme. The results from docking studies depicted that these derivatives interacted with the active site via van der Waals and electrostatic interactions. The benzothiazinone moiety and *m*-chloro group of the phenyl ring participated in van der Waals interactions, whereas the triazole ring was involved in interaction with Lys 418, Cys 317, and Ile386 residues. Additionally, H-bonding interactions facilitated steric and electrostatic interactions by anchoring the 3D-position of the compound within the active site.

Moreover, the stability of the compound (**76**) in the active site is facilitated by pi-pi stacking interactions.



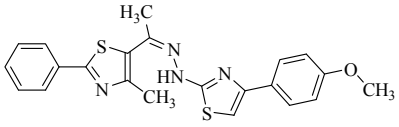
(**75,76**)

Chitre et al. [61] synthesized novel agents by hybridizing pyrazine and thiazolidine derivatives (**77**). These derivatives exhibited MIC values in the micro molar range. Molecular docking studies were reported for the novel hybrid molecule (**77**). The result showed that N-(4-oxo-2 substituted thiazolidin-3yl)pyrazine-2-carbohydrazide derivative fitted well in the active site and was located near the native ligand having a similar orientation. Their docking scores were in the range of −7.83 to −6.00 (native ligand: −7.953). These compounds fitted well in the active site via bonded and non-bonded interactions. They were found to be involved in electrostatic as well as van der Waals interactions with the amino acid residues. Thiazolidinone ring was found to be interacting with Lys418, Gly117, and Pro116 residues, whereas 3-ethoxy-4-hydroxyphenyl ring was involved in binding with Leu363 and Asp389 residues. The compound was further stabilized by pi-pi stacking between pyrazine ring and His132 residue.



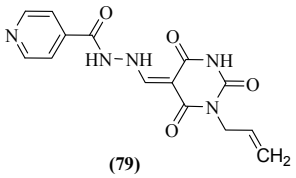
(**77**)

Bhalerao et al. [62] performed the docking study of some thiazole-based compounds (PDB code:4FDO). The docking study revealed that the compounds fitted snugly into the active site by acquiring a similar orientation as the native ligand. The docking score for the test compounds ranged between −7.31 and −6.00, and it was −7.95 for the active compound. The highest docking score was −7.84 for the compound (**78**).



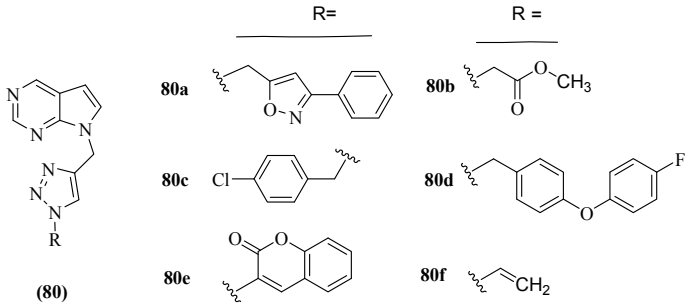
(78)

Yagao et al. [63] performed virtual screening of 6.2 million small molecules against DprE1 (PDB ID: 4FDO) using ICM 3.8.2 modeling software. Based on whether the compounds are occupying the binding pocket, many compounds were excluded. Further, the next filter used was Lipinski's rule of five for drug-likeness. Based on these two filters, 63 compounds came out of the whole database. Further, molecular docking was carried out that resulted in the finding of the compound (**79**). Docking studies illustrated that the ligand-binding pocket of the enzyme is in a zig-zag shape, and the compounds also bind in a zig-zag manner in this cavity by indulging in hydrophobic interactions with the amino acid residues.



(79)

Kasa et al. [64] reported the interaction studies of *M. tb* protein DprE1 (PDB ID: 4P8C) with various triazole-based pyrrole-pyrimidine analogs (**80**) by performing molecular docking. The compound (**80a**) came out with the highest Mol Dock score of −157.926 and was demonstrated to interact via hydrogen bonding and pi-lone pair interactions with the enzyme. It was noted that the results from in silico studies of the active compounds supported the activity data, indicating the importance of the triazole ring for exhibiting anti-TB activity.



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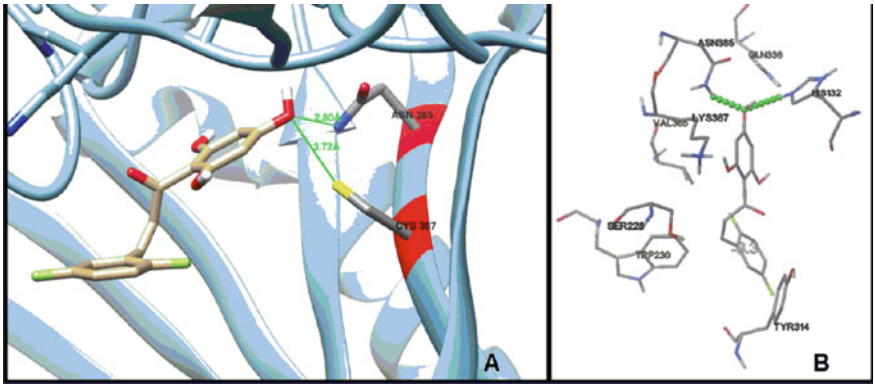
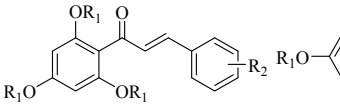
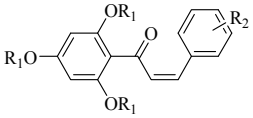


Fig. 7 Molecular docking results for **82 g**. **a** Analysis with Chimera. **b** Analysis with Autodock Tools. Reprinted with permission from Ref. [65] Copyright© 2018 Elsevier

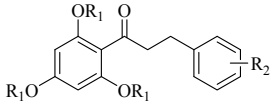
Yalcin et al. [65] performed molecular docking to evaluate the potential of fluoro-substituted chalcone derivatives (**81–83**) as DprE1 inhibitors. They synthesized fluoro and non-fluoro chalcone derivatives and evaluated their antiproliferative and anti-TB activity. The crystal structure used for the study was PDB ID: 4P8H. The synthesized chalcone derivatives had both *cis* and *trans* isomers. Among the synthesized compounds, (**82g**) was found to have the best binding affinity and inhibitory constant of −8.3 kcal/mol and 0.812 mM, respectively. The binding features of the compound (**82g**) are shown in Fig. 7. It can be seen that hydroxyl groups present in the structure form hydrogen bonds with the residues in the active site. The other phenyl moiety is involved in π-σ interaction with the Trp 230 residue.



(81)
(*trans*-chalcone)



(82)
(*cis*-chalcone)

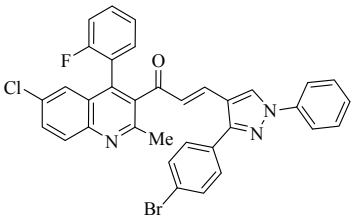


(83)

Comp.	R_1	R_2
81a, 82a, 83a	CH_3	H
81b, 82b, 83b	CH_3	2-F
81c, 82c, 83c	CH_3	3-F
81d, 82d, 83d	CH_3	2,5-diF
81e, 82e, 83e	CH_3	4-F
81f, 82f, 83f	H	2-F
81g, 82g, 83g	H	2,5-diF

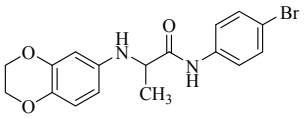
Docking results of these test compounds revealed that compound (**82g**) generated superior binding properties than the (**81g**) molecule. This may indicate that *trans*-configuration could be crucial for enhancing the binding properties of the molecules. However, no such relation was found on the other pair of isomers.

Recently in 2020, Kumar et al. [66] used a hybrid approach to synthesize some novel pyrazole-quinoline chalcones and pyrazole-coumarin chalcones and evaluated their anti-TB activity. Docking these compounds with the DprE1 enzyme demonstrated that the active compounds showed good binding to the target, having docking scores from −7.047 to −9.353 kcal/mol. Based on the molecular docking results, the authors proposed that the anti-TB activity of these could be due to inhibition of the DprE1 enzyme. The MIC value for the most potent compound (**84**) was observed to be 3.125 μg/ml.



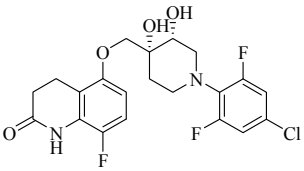
(84)

GlaxoSmithKline (GSK) conducted an HTS campaign to identify potential DprE1 inhibitors. This campaign led to the emergence of a novel series of 2-((2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)amino)-*N*-phenylpropanamides. Surprisingly, the structure of this series is unrelated to the already known inhibitors. Compound (**85**) represents the initial HTS hit that demonstrated DprE1 inhibition with a pIC_{50} value of 7.2. Whitehurst et al. [67] extracted some compound analogs (**85**) using similarity-based clusters of HTS hit. Further, these analogs and sub-structures of GSK compound collection were analyzed to gain early SAR information. For defining SAR, compound (**85**) was divided into three main components: the central alanine linker, left-hand side aminobenzodioxane, and right-side C-terminal alanine. SAR studies revealed that the compound (85) showed the maximum activity. Some derivatives were indeed found with equal activity. However, further research is required to assess the risk associated with the compound (**85**) and develop this new structure into a viable lead.



(85)

Hariguchi et al. [68] applied a phenotypic screening method to identify and optimize compounds with anti-TB activity containing carbostyril as the core. Carbostyril core has been chosen because it has been reported to have good ADMET properties and has been used in numerous drugs as the core moiety. As a result of these efforts, OPC-167832 (**86**) came into the lime light, which has potent in vitro and in vivo activities. Subsequently, the authors mapped DprE1 inhibition as the mode of action. They also reported preclinical data, including in vivoefficacy of the regimens composed of this compound.



(86)

3.4 Covalent vs. Non-covalent Binding Inhibitors

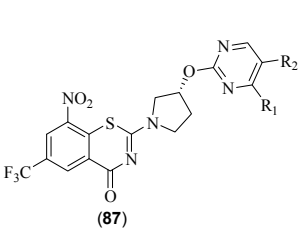
In the above three subsections, it is observed that anti-TB activity can be achieved by blocking DprE1 both covalently and non-covalently. To date, researchers have reported both covalent and non-covalent inhibitors with high potency and minimum toxicity. So, an obvious question arises, which type of inhibition is better. To answer this, we must understand the difference in their mechanism of action. Covalent inhibitors work by binding to the Cys387 residue of the enzyme. The binding is irreversible; thus, the inhibitors act as suicide substrates. Some reports also revealed that mutation in the Cys387 residue might develop resistance towards covalent inhibitors [69].

On the other hand, non-covalent inhibitors bind reversibly to the enzyme, leading to inefficient inhibition or development of resistance by incrementing the bacterial load [70]. However, compounds from both categories have found a place

in clinical trials. So, we consider the future of both the types of inhibitors, covalent and non-covalent, as bright in the direction of our search for new anti-TB drugs.

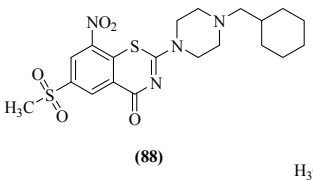
4 Patented DprE1 Inhibitors

During the last decade, many compounds have been patented as DprE1 inhibitors. The very first patent on DprE1 inhibitor was obtained on the benzothiazinone derivative BTZ043 (**29**) [71]. Subsequently, many patent applications were filed for various benzothiazinones such as PBTZ169 (**31**), BTZ-SO (**33**), and compounds (**87a** and **87b**) [72–74]. Recently patent applications for the two benzothiazinone derivatives (**88, 89**) have been filed [75, 76], claiming almost equal potency to PBTZ169 (**31**) but with a lower cLogP value. Compounds (**88, 89**) exhibited MIC values of 0.005 μM and 0.022 μM respectively. Another benzothiazinone derivative (**90**) yielded a MIC value lower than 0.000063 μg/ml against resistant *M. tb* strains [77].



(87)

87a: R_1=-CH_3, R_2=-H
87b: R_1=-H, R_2=-F



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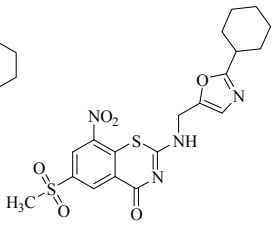

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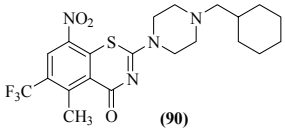
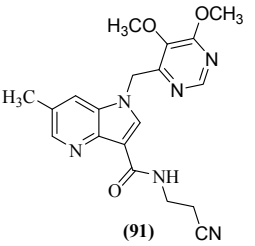


(90)

A carbostyril derivative, OPC-167832 (**86**), has also been patented as a DprE1 inhibitor. The claims mentioned in the application illustrate that the compound is specifically active against mycobacteria and is orally active with no gastrointestinal disturbances [78]. Benzothiazole derivative, TCA1 (**47**), and the azaindole derivative, TBA-7371 (**60**), have also been patented as DprE1 inhibitors [79, 80]. TBA-7371 (**60**) has been mentioned as pathogen-specific for *M. tb* and *M. smegmatis*. Recently, another azaindole amide derivative (**91**) has been reported under publication [81]. Compound (**91**) showed a MIC value of 0.1953 μg/ml against *M. tb*. Another invention claimed arylamide-substituted thiophenimide (**92**) esters as

DprE1 inhibitors. The compound (**92**) exhibited excellent in vivo activity with $Log_{10}CFU = 4.42$ [82]. Another invention reported nitrofuran derivatives (**93**) as DprE1 inhibitors with a MIC value of 1 μM [83]. Yet another patented compound (**94**) has been claimed to have excellent activity against *M. tb* with a MIC value less than 0.0625 μM [84].



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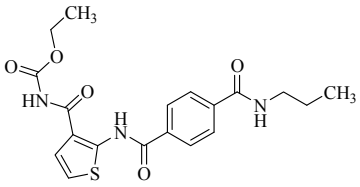

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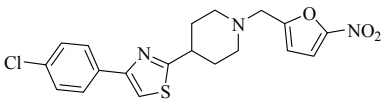

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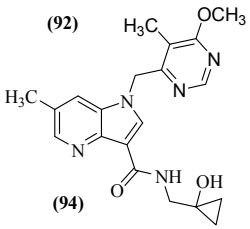
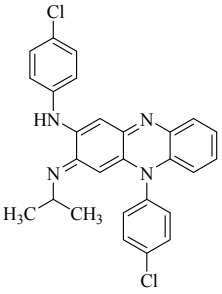


(94)

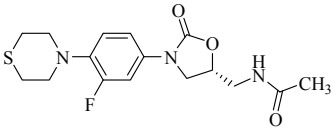
5 DprE1 Inhibitors in Clinical Trials

The discovery of benzothiazinones has marked the onward journey of DprE1 inhibitors in our quest for discovering novel anti-TB agents [24]. Two benzothiazinone derivatives, BTZ043 (**29**) and PBTZ-169 (**31**), are currently in phase 1 clinical trial. Macozinone (MCZ, PBTZ-169) (**31**), a piperazino benzothiazinone derivative, is obtained as a result of optimization of benzothiazinone lead molecule BTZ043 (**29**). PBTZ-169 (**31**) was found to have many merits over the lead BTZ-043, amongst which better pharmacodynamics, easier method of synthesis, and absence of chiral centers in its structure are some of them. The drug has additive effects with other anti-TB agents, both marketed and underdeveloped, while showing harmonious effects with bedaquiline (**17**) and clofazimine (**95**) in the preclinical stage [85]. Currently, it is in the second phase in Russia, whereas in Europe, it is in phase 1 [86, 87]. Another agent, TBA-7371 (**60**), is in phase 2 clinical trials [88]. TBA-7371, along with sutezolid (**96**), entered phase 1 clinical trials. It is developed by AstraZeneca in collaboration with TB Alliance. It is believed that TBA-7371 (**60**) does have the potential to be used for the treatment of

resistant cases of TB because it is devoid of any pre-existing resistance or cross-resistance with other drugs [89, 90]. OPC-167832 (**86**) is also reported to be in phase 1 clinical trials [68].



Clofazimine (**95**)



Sutezolid (**96**)

6 Microbial Resistance and DprE1 Inhibitors

The main issue with the already existing anti-TB drugs is the development of microbial resistance. First-line agents like isoniazid, pyrazinamide, etc., are vulnerable to the development of resistance. A decade ago, when BTZs came into the light, they attracted researchers worldwide due to the new target and the sub-micromolar MIC values they exhibited. Since then, continuous research has been going on DprE1 inhibitors. But it is important to evaluate whether this target is also vulnerable to resistance and, if yes, to what extent. To meet this particular requirement, Foo et al. [69] reported DprE1-mediated BTZ's resistance in *M. tb*. Results of the study revealed that the C387 residue of the enzyme served as the site of mutation, leading to the development of resistance towards BTZ. Additionally, it was proved that five mutations on the C387 residue were responsible for developing resistance. These mutations were caused by substituting different amino acids such as glycine, alanine, arginine, serine, and threonine. It was also observed that mutations with C387T, C387A, and C387S had a greater impact than C387N and C387G. The authors also claimed that the decreased potency of covalent inhibitors results from a mutation at C387 residue. While mutations at Ty38C residue resulted in resistance towards non-covalent inhibitors.

Warrier et al. [91] also studied the development of microbial resistance by overexpressing some genes, i.e., *rv0560c, rv0558*, and *rv0559c*. The authors claimed that *rv0560c* is a gene responsible for *S*-adenosyl-L-methionine-dependent methyltransferase, an enzyme that methylates the inhibitors and reduces their activity.

7 Conclusion

Since its discovery in 2009, DprE1 has been perceived as the best druggable target to combat TB [2, 92].The discovery of BTZ043 served as the starting point for researching novel covalent DprE1 inhibitors. The revelation of the mechanism of action of BTZ as covalent inhibitors was a breakthrough in the field of DprE1 inhibitors. Since then, continuous research has been carried out by researchers worldwide. Both covalent and non-covalent inhibitors have been looked out as potential anti-TB agents. Two such candidates, i.e., Macozinone and TBA-7371, are already in clinical trials. We keep our fingers crossed and hope to emerge some novel DprE1 inhibitors as efficient anti-TB agents.

Mother Teresa once said, "*The biggest disease today is not leprosy or tuberculosis, but rather the feeling of being unwanted.*" At present, the world needs new and effective drugs for eradicating an infectious disease like TB as it is the need of the hour to prevent and address TB patients' sufferings, a suffering due to ineffective treatment due to development of drug resistance, poor drug compliance by the patients, the cost of treatment, and the *feeling of unwanted by the society*.

Core Messages

- DprE1 has evolved as a new competent target that could be exploited for anti-TB drug discovery.
- Covalent and non-covalent inhibitors have been looked out as potential anti-TB agents.
- Four DprE1 inhibitors, namely BTZ-043, Macozinone, OPC-167832, and TBA-7371, are currently in clinical trials.

References

1. Manina G, Pasca MR, Buroni S, De Rossi E, Riccardi G (2010) Decaprenylphosphoryl-β-D-ribose 2′-epimerase from *Mycobacterium tuberculosis* is a magic drug target. Curr Med Chem 17(27):3099–3108
2. Chikhale RV, Barmade MA, Murumkar PR, Yadav MR (2018) Overview of the development of DprE1 inhibitors for combating the menace of tuberculosis. J Med Chem 61(19):8563–8593
3. Yuan T, Sampson NS (2018) Hit generation in TB drug discovery: from genome to granuloma. Chem Rev 118(4):1887–1916
4. Campanico A, Moreira R, Lopes F (2018) Drug discovery in tuberculosis. New drug targets and antimycobacterial agents. Eur J Med Chem 150:525–545
5. Wellington S, Hung DT (2018) The expanding diversity of *Mycobacterium tuberculosis* drug targets. ACS Infect Dis 4(5):696–714
6. Richter A, Rudolph I, Mollmann U, Voigt K, Chung CW, Singh OM, Rees M, Mendoza-Losana A, Bates R, Ballell L, Batt S (2018) Novel insight into the reaction of

nitro, nitroso and hydroxylamino benzothiazinones and of benzoxacinones with *Mycobacterium tuberculosis* DprE1. Sci Rep 8(1):1–12
7. Piton J, Foo CSY, Cole ST (2017) Structural studies of *Mycobacterium tuberculosis* DprE1 interacting with its inhibitors. Drug Discov Today 22(3):526–533
8. Riccardi G, Pasca MR (2014) Trends in discovery of new drugs for tuberculosis therapy. J Antibiot 67(9):655–659
9. Brennan PJ (2003) Structure, function, and biogenesis of the cell wall of *Mycobacterium tuberculosis*. Tuberculosis 83(1–3):91–97
10. Riccardi G, Pasca MR, Chiarelli LR, Manina G, Mattevi A, Binda C (2013) The DprE1 enzyme, one of the most vulnerable targets of *Mycobacterium tuberculosis*. Appl Microbiol Biotechnol 97(20):8841–8848
11. Rombouts Y, Brust B, Ojha AK, Maes E, Coddeville B, Elass-Rochard E, Kremer L, Guerardel Y (2012) Exposure of mycobacteria to cell wall-inhibitory drugs decreases production of arabinoglycerolipid related to mycolyl-arabinogalactan-peptidoglycan metabolism. J Bio Chem 287(14):11060–11069
12. Wolucka BA (2008) Biosynthesis of D-arabinose in mycobacteria—a novel bacterial pathway with implications for antimycobacterial therapy. FEBS J 275(11):2691–2711
13. Bhutani I, Loharch S, Gupta P, Madathil R, Parkesh R (2015) Structure, dynamics, and interaction of *Mycobacterium tuberculosis* (Mtb) DprE1 and DprE2 examined by molecular modeling, simulation, and electrostatic studies. PLoS ONE 10(3):e0119771
14. Alderwick LJ, Birch HL, Mishra AK, Eggeling L, Besra GS (2007) Structure, function and biosynthesis of the *Mycobacterium tuberculosis* cell wall: arabinogalactan and lipoarabinomannan assembly with a view to discovering new drug targets. Biochem Soc Trans 35 (5):1325–1328
15. Meniche X, de Sousa-d'Auria C (2008) Partial redundancy in the synthesis of the D-arabinose incorporated in the cell wall arabinan of Corynebacterineae. Microbiology (Reading) 154 (8):2315–2326
16. Makarov V, Manina G, Mikusova K, Möllmann U, Ryabova O, Saint-Joanis B, Dhar N, Pasca MR, Buroni S, Lucarelli AP, Milano A (2009) Benzothiazinones kill *Mycobacterium tuberculosis* by blocking arabinan synthesis. Science 324(5928):801–804
17. Mikusova K, Huang H, Yagi T, Holsters M, Vereecke D, D'Haeze W, Scherman MS, Brennan PJ, McNeil MR, Crick DC (2005) Decaprenylphosphoryl arabinofuranose, the donor of the D-arabinofuranosyl residues of mycobacterial arabinan, is formed via a two-step epimerization of decaprenylphosphoryl ribose. J Bacteriol 187(23):8020–8025
18. Abdel-Magid AF (2015) Decaprenylphosphoryl-β-D-ribose 2′-epimerase 1 (DprE1): a novel therapeutic target for the treatment of tuberculosis. ACS Med Chem Lett 6:373–374
19. Neres J, Pojer F, Molteni E, Chiarelli LR, Dhar N, Boy-Rottger S, Buroni S, Fullam E, Degiacomi G, Lucarelli AP, Read RJ, Giuseppe Z, Edmondson DE, Rossi ED, Pasca MR, McKinney JD, Dyson PJ, Riccardi G, Mattevi A, Cole ST, Binda C (2012) Structural basis for benzothiazinone-mediated killing of *Mycobacterium tuberculosis*. Sci Trans Med 4 (150):150ra121–150ra121
20. Batt SM, Jabeen T, Bhowruth V, Quill L, Lund PA, Eggeling L, Alderwick LJ, Futterer K, Besra GS (2012) Structural basis of inhibition of *Mycobacterium tuberculosis* DprE1 by benzothiazinone inhibitors. Proc Natl Acad Sci 109(28):11354–11359
21. Liu R, Lyu X, Batt SM, Hsu MH, Harbut MB, Vilcheze C, Cheng B, Ajayi K, Yang B, Yang Y, Guo H (2017) Determinants of the inhibition of DprE1 and CYP2C9 by Antitubercular thiophenes. Angew Chem Int Ed Engl 56(42):13011–13015
22. Piton J, Vocat A, Lupien A, Foo CS, Riabova O, Makarov V, Cole ST (2018) Structure-based drug design and characterization of sulfonyl-piperazine benzothiazinone inhibitors of DprE1 from *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 62(10):e00681-e718
23. Manina G, Bellinzoni M, Pasca MR, Neres J, Milano A, de Jesus Lopes Ribeiro AL, Buroni S, Skovierova H, Dianiskova P, Mikusova K, Marak J (2010) Biological and structural

characterization of the Mycobacterium smegmatis nitroreductase NfnB, and its role in benzothiazinone resistance. Mol Microbiol 77(5):1172–1185
24. Ribeiro AL, Degiacomi G, Ewann F, Buroni S, Incandela ML, Chiarelli LR, Mori G, Kim J, Contreras-Dominguez M, Park YS, Han SJ (2011) Analogous mechanisms of resistance to benzothiazinones and dinitrobenzamides in Mycobacterium smegmatis. PLoS ONE 6(11): e26675
25. Spain JC (1995) Biodegradation of nitroaromatic compounds. Annu Rev Microbiol 49 (1):523–555
26. Trefzer C, Rengifo-Gonzalez M, Hinner MJ, Schneider P, Makarov V, Cole ST, Johnsson K (2010) Benzothiazinones: prodrugs that covalently modify the decaprenylphosphoryl-β-D-ribose 2′-epimerase DprE1 of *Mycobacterium tuberculosis*. J Am Chem Soc 132(39):13663–13665
27. Tiwari R, Moraski GC, Krchnnak V, Miller PA, Colon-Martinez M, Herrero E, Oliver AG, Miller MJ (2013) Thiolates chemically induce redox activation of BTZ043 and related potent nitroaromatic anti-tuberculosis agents. J Am Chem Soc 135(9):3539–3549
28. Stewart CT (2010) New benzothiazinone derivatives and their use as antibacterial agents. EP2029583B1, 09 July 2010
29. Gao C, Ye TH, Wang NY, Zeng XX, Zhang LD, Xiong Y, You XY, Xia Y, Xu Y, Peng CT, Zuo WQ (2013) Synthesis and structure–activity relationships evaluation of benzothiazinone derivatives as potential anti-tubercular agents. Bioorg Med Chem Lett 23(17):4919–4922
30. Makarov V, Lechartier B, Zhang M, Neres J, van der Sar AM, Raadsen SA, Hartkoorn RC, Ryabova OB, Vocat A, Decosterd LA, Widmer N (2014) Towards a new combination therapy for tuberculosis with next generation benzothiazinones. EMBO Mol Med 6(3):372–383
31. Peng CT, Gao C, Wang NY, You XY, Zhang LD, Zhu YX, Xv Y, Zuo WQ, Ran K, Deng HX, Lei Q (2015) Synthesis and anti-tubercular evaluation of 4-carbonyl piperazine substituted 1,3-benzothiazin-4-one derivatives. Bioorg Med Chem Lett 25(7):1373–1376
32. Tiwari R, Miller PA, Cho S, Franzblau SG, Miller MJ (2015) Syntheses and antituberculosis activity of 1, 3-benzothiazinone sulfoxide and sulfone derived from BTZ043. ACS Med Chem Lett 6(2):128–133
33. Landge S, Mullick AB, Nagalapur K, Neres J, Subbulakshmi V, Murugan K, Ghosh A, Sadler C, Fellows MD, Humnabadkar V, Mahadevaswamy J (2015) Discovery of benzothiazoles as antimycobacterial agents: synthesis, structure–activity relationships and binding studies with *Mycobacterium tuberculosis* decaprenylphosphoryl-β-D-ribose 2′-oxidase. Bioorg Med Chem Lett 23(24):7694–7710
34. Stanley SA, Grant SS, Kawate T, Iwase N, Shimizu M, Wivagg C, Silvis M, Kazyanskaya E, Aquadro J, Golas A, Fitzgerald M (2012) Identification of novel inhibitors of M. tuberculosis growth using whole cell based high-throughput screening. ACS Chem Biol 7(8):1377–1384
35. Karabanovich G, Dusek J, Savkova K, Pavlis O, Pavkova I, Korabecny J, Kucera T, Koccovaa Vlcckovaa H, Huszar S, Konyarikova Z, Konecna K (2019) Development of 3, 5-dinitrophenyl-containing 1, 2, 4-triazoles and their trifluoromethyl analogues as highly efficient anti-tubercular agents inhibiting decaprenylphosphoryl-β-D-ribofuranose 2′-oxidase. J Med Chem 62(17):8115–8139
36. Ali AA, Gogoi D, Chaliha AK, Buragohain AK, Trivedi P, Saikia PJ, Gehlot PS, Kumar A, Chaturvedi V, Sarma D (2017) Synthesis and biological evaluation of novel 1, 2, 3-triazole derivatives as anti-tubercular agents. Bioorg Med Chem Lett 27(16):3698–3703
37. Magnet S, Hartkoorn RC, Szekely R, Pato J, Triccas JA, Schneider P, Szantai-Kis C, Orfi L, Chambon M, Banfi D, Bueno M (2010) Leads for anti-tubercular compounds from kinase inhibitor library screens. Tuberculosis 90(6):354–360
38. Christophe T, Jackson M, Jeon HK, Fenistein D, Contreras-Dominguez M, Kim J, Genovesio A, Carralot JP, Ewann F, Kim EH, Lee SY (2009) High content screening identifies decaprenyl-phosphoribose 2′ epimerase as a target for intracellular antimycobacterial inhibitors. PLoS Pathog 5(10):e1000645
39. Brodin PR (2011) Anti-infective compounds. WO2011113606 A1, 22 Sept 2011

40. Makarov V, Neres J, Hartkoorn RC, Ryabova OB, Kazakova E, Šarkan M, Huszár S, Piton J, Kolly GS, Vocat A, Conroy TM (2015) The 8-pyrrole-benzothiazinones are non-covalent inhibitors of DprE1 from *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 59 (8):4446–4452
41. Wang F, Sambandan D, Halder R, Wang J, Batt SM, Weinrick B, Ahmad I, Yang P, Zhang Y, Kim J, Hassani M (2013) Identification of a small molecule with activity against drug-resistant and persistent tuberculosis. Proc Natl Acad Sci 110(27):E2510–E2517
42. Chikhale R, Menghani S, Babu R, Bansode R, Bhargavi G, Karodia N, Rajasekharan MV, Paradkar A, Khedekar P (2015) Development of selective DprE1 inhibitors: design, synthesis, crystal structure and anti-tubercular activity of benzothiazolylpyrimidine-5-carboxamides. Eur J Med Chem 96:30–46
43. Mir F, Shafi S, Zaman MS, Kalia NP, Rajput VS, Mulakayala C, Mulakayala N, Khan IA, Alam MS (2014) Sulfur rich 2-mercaptobenzothiazole and 1, 2, 3-triazole conjugates as novel anti-tubercular agents. Eur J Med Chem 76:274–283
44. Gawad J, Bonde C (2018) Synthesis, biological evaluation and molecular docking studies of 6-(4-nitrophenoxy)-1*H*-imidazo[4,5-*b*]pyridine derivatives as novel anti-tubercular agents: future DprE1 inhibitors. Chem Cent J 12(1):1–11
45. Neres J, Hartkoorn RC, Chiarelli LR, Gadupudi R, Pasca MR, Mori G, Venturelli A, Savina S, Makarov V, Kolly GS, Molteni E (2014) 2-Carboxyquinoxalines kill *Mycobacterium tuberculosis* through non-covalent inhibition of DprE1. ACS Chem Biol 10:705–714
46. Batt SM, Cacho Izquierdo M, Castro Pichel J, Stubbs CJ, Vela-Glez Del Peral L, Pérez-Herrán E, Dhar N, Mouzon B, Rees M, Hutchinson JP, Young RJ (2015) Whole cell target engagement identifies novel inhibitors of *Mycobacterium tuberculosis* decaprenylphosphoryl-β-D-ribose oxidase. ACS Infect Dis 1(12):615–626
47. Brodin P (2010) Anti-infective compounds. WO2010003533A2, 11 Nov 2010
48. Brodin P (2011) Anti-infective pyrido(1,2-*a*)Pyrimidines. WO2011085990A1, 21 July 2011
49. Shirude PS, Shandil R, Sadler C, Naik M, Hosagrahara V, Hameed S, Shinde V, Bathula C, Humnabadkar V, Kumar N, Reddy J (2013) Azaindoles: non-covalent DprE1 inhibitors from scaffold morphing efforts, kill *Mycobacterium tuberculosis* and are efficacious in vivo. J Med Chem 56(23):9701–9708
50. Chatterji M, Shandil R, Manjunatha MR, Solapure S, Ramachandran V, Kumar N, Saralaya R, Panduga V, Reddy J, Prabhakar KR, Sharma S (2014) 1, 4-Azaindole, a potential drug candidate for treatment of tuberculosis. Antimicrob Agents Chemother 58(9):5325–5331
51. Shirude PS, Shandil RK, Manjunatha MR, Sadler C, Panda M, Panduga V, Reddy J, Saralaya R, Nanduri R, Ambady A, Ravishankar S (2014) Lead optimization of 1, 4-azaindoles as antimycobacterial agents. J Med Chem 57(13):5728–5737
52. Manjunatha MR, Shandil R, Panda M, Sadler C, Ambady A, Panduga V, Kumar N, Mahadevaswamy J, Sreenivasaiah M, Narayan A, Guptha S (2019) Scaffold morphing to identify novel DprE1 inhibitors with antimycobacterial activity. ACS Med Chem Lett 10 (10):1480–1485
53. Panda M, Ramachandran S, Ramachandran V, Shirude PS, Humnabadkar V, Nagalapur K, Sharma S, Kaur P, Guptha S, Narayan A, Mahadevaswamy J (2014) Discovery of pyrazolopyridones as a novel class of non-covalent DprE1 inhibitor with potent antimycobacterial activity. J Med Chem 57(11):4761–4771
54. Naik M, Humnabadkar V, Tantry SJ, Panda M, Narayan A, Guptha S, Panduga V, Manjrekar P, Jena LK, Koushik K, Shanbhag G (2014) 4-aminoquinolone piperidine amides: non-covalent inhibitors of DprE1 with long residence time and potent antimycobacterial activity. J Med Chem 57(12):5419–5434
55. Rogacki MK, Pitta E, Balabon O, Huss S, Lopez-Roman EM, Argyrou A, Blanco-Ruano D, Cacho M, Vande Velde CM, Augustyns K, Ballell L (2018) Identification and profiling of hydantoins—a novel class of potent antimycobacterial DprE1 inhibitors. J Med Chem 61 (24):11221–11249

56. Balabon O, Pitta E, Rogacki MK, Meiler E, Casanueva R, Guijarro L, Huss S, Lopez-Roman EM, Santos-Villarejo A, Augustyns K, Ballell L (2020) Optimization of hydantoins as potent antimycobacterial decaprenylphosphoryl-β-D-ribose oxidase (DprE1) inhibitors. J Med Chem 63(10):5367–5386
57. Wilsey C, Gurka J, Toth D, Franco J (2013) A large scale virtual screen of DprE1. Comput Biol Chem 47:121–125
58. Pore VS, Divse JM, Charolkar CR, Nawale LU, Khedkar VM, Sarkar D (2015) Design and synthesis of 11α-substituted bile acid derivatives as potential anti-tuberculosis agents. Bioorg Med Chem Lett 25(19):4185–4190
59. Haribabu J, Subhashree GR, Saranya S, Gomathi K, Karvembu R, Gayathri D (2015) Synthesis, crystal structure, and in vitro and in silico molecular docking of novel acyl thiourea derivatives. J Mol Struct 1094:281–291
60. Shaikh MH, Subhedar DD, Arkile M, Khedkar VM, Jadhav N, Sarkar D, Shingate BB (2016) Synthesis and bioactivity of novel triazole incorporated benzothiazinone derivatives as anti-tubercular and antioxidant agent. Bioorg Med Chem Lett 26(2):561–569
61. Chitre TS, Asgaonkar KD, Miniyar PB, Dharme AB, Arkile MA, Yeware A, Sarkar D, Khedkar VM, Jha PC (2016) Synthesis and docking studies of pyrazine–thiazolidinone hybrid scaffold targeting dormant tuberculosis. Bioorg Med Chem Lett 26(9):2224–2228
62. Bhalerao MB, Dhumal ST, Deshmukh AR, Nawale LU, Khedkar V, Sarkar D, Mane RA (2017) New bithiazolyl hydrazones: novel synthesis, characterization and anti-tubercular evaluation. Bioorg Med Chem Lett 27(2):288–294
63. Gao Y, Xie J, Tang R, Yang K, Zhang Y, Chen L, Li H (2019) Identification of a pyrimidinetrione derivative as the potent DprE1 inhibitor by structure-based virtual ligand screening. Bioorg Chem 85:168–178
64. Raju KS, AnkiReddy S, Sabitha G, Krishna VS, Sriram D, Reddy KB, Sagurthi SR (2019) Synthesis and biological evaluation of 1*H*-pyrrolo[2,3-*d*]pyrimidine-1,2,3-triazole derivatives as novel anti-tubercular agents. Bioorg Med Chem Lett 29(2):284–290
65. Yalcin G, Burmaoglu S, Yildiz I, Algul O (2018) Molecular docking studies on fluoro-substituted chalcones as potential DprE1 enzyme inhibitors. J Mol Struct 1164:50–56
66. Kumar G, Siva Krishna V, Sriram D, Jachak SM (2020) Pyrazole–coumarin and pyrazole–quinoline chalcones as potential antitubercular agents. Arch Pharm 353(8):e2000077
67. Whitehurst BC, Young RJ, Burley GA, Cacho M, Torres P, del Peral LV (2020) Identification of 2-((2,3-dihydrobenzo [*b*][1,4]dioxin-6-yl) amino)-N-phenylpropanamides as a novel class of potent DprE1 inhibitors. Bioorg Med Chem Lett 30(12):127192. https://doi.org/10.1016/j.bmcl.2020.127192
68. Hariguchi N, Chen X, Hayashi Y, Kawano Y, Fujiwara M, Matsuba M, Shimizu H, Ohba Y, Nakamura I, Kitamoto R, Shinohara T (2020) OPC-167832, a novel carbostyril derivative with potent antituberculosis activity as a DprE1 inhibitor. Antimicrob Agents Chemother 64 (6):e02020-e2119. https://doi.org/10.1128/AAC.02020-19
69. Foo CS, Lechartier B, Kolly GS, Boy-Röttger S, Neres J, Rybniker J, Lupien A, Sala C, Piton J, Cole ST (2016) Characterization of DprE1-mediated benzothiazinone resistance in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 60(11):6451–6459
70. Singh J, Petter RC, Baillie TA, Whitty A (2011) The resurgence of covalent drugs. Nat Rev Drug Discov 10:307–317
71. Makarov V (2011) Benzothiazinone derivatives and their use as antibacterial agents. US7863268B2, 4 Jan 2011
72. Makarov V (2014) 2-piperazin-1-yl-4*H*-1,3-benzothiazin-4-one derivatives and their use for the treatment of mammalian infections. US8796264B2, 5 Aug 2014
73. Milller MJ (2016) 1,3-Benzothiazinone sulfoxide and sulfone compounds. US9481683B2, 1 Nov 2016
74. Chao G (2018) Benzothiazine derivative, a preparation method, and uses thereof. CN108456204A, 28 Aug 2018

75. Chunhua Q (2020) Benzothiazinone derivatives, preparation method thereof, and application as anti-tuberculosis drugs. CN111303075A, 19 Jun 2020
76. Chunhua Q (2020) Benzothiazinone compound, preparation method thereof, and application as anti-tuberculosis medicine. CN111269197A, 12 Jun 2020
77. Florian K (2019) New anti-microbial compounds, their use for the treatment of mammalian infections and a new metabolic mechanism. EP3515920A1, 31 Jul 2019
78. Shimizu H (2018) Heterobicyclic compounds and their use for the treatment of tuberculsosis. US10053446B2, 21 Aug 2018
79. Chatterjee AK (2016) Compounds for treatment of drug resistant and persistent tuberculosis. US20160194299A1, 7 Jul 2016
80. Shirude PS (2015) Azaindole compounds, synthesis thereof, and methods of using the same. US9163020B2, 20 Oct 2015
81. Changlun A (2020) Azaindole amide compounds and preparation method and application thereof. CN111393435A, 10 Jul 2020
82. Haihong H (2020) 2-Arylamino-substituted thienylimide ester compound and preparation method and application thereof. CN110759889A, 7 Feb 2020
83. Lin D (2018) Nitrofuran antituberculous component. CN108558858A, 21 Sep 2018
84. Desai R (2019) Condensed azaheteroaryl compounds having antibacterial activity against tuberculosis bacteria. WO2019239382A1, 19 Dec 2019
85. https://www.newtbdrugs.org/pipeline/compound/macozinone-mcz-pbtz-169. Accessed on 09 Oct 2020
86. https://clinicaltrials.gov/ct2/show/record/NCT03334734. Accessed on 09 Oct 2020
87. http://im4tb.org/our-pipeline/#:~:text=PBTZ169%20(macozinone)%20%E2%80%93%20currently%20in,to%20treat%20multidrug%2Dresistant%20tuberculosis. Accessed on 09 Oct 2020
88. https://www.tballiance.org/portfolio/compound/tba-7371-dpre1-inhibitor. Accessed on 09 Oct 2020
89. https://www.tballiance.org/news/tb-alliance-moves-two-novel-tuberculosis-drugs-human-trials. Accessed on 09 Oct 2020
90. Degiacomi G, Belardinelli JM, Pasca MR, Rossi ED, Riccardi G, Chiarelli LR (2020) Promiscuous targets for antitubercular drug discovery: the paradigm of DprE1 and MmpL3. Appl Sci 10(2):623
91. Warrier T, Kapilashrami K, Argyrou A, Ioerger TR, Little D, Murphy KC, Nandakumar M, Park S, Gold B, Mi J, Zhang T (2016) N-methylation of a bactericidal compound as a resistance mechanism in *Mycobacterium tuberculosis*. Proc Natl Acad Sci 113(31):E4523–E4530
92. Murumkar PR, Sharma MK, Gupta P, Patel NM, Yadav MR (2022) Selection of suitable protein structure from Protein Data Bank: An important step in Structure based Drug Design Studies. Mini Rev Med Chem. https://doi.org/10.2174/1389557522666220512151454

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Energy Pathways in *Mycobacterium Tuberculosis*

25

Ankit Ganeshpurkar, Ravi Singh, Meenakshi Singh, Ashok Kumar, and Sushil Kumar Singh

Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less.

Marie Curie

Summary

Mycobacterium tuberculosis (M. tb) is responsible for the infectious illness tuberculosis (TB), which has a very high mortality rate of around 1.5 million people worldwide annually. The rise of antibiotic resistance, primarily due to *M. tb* strain mutation, is the biggest concern of the day. The *M. tb* energetics that involve both substrate and oxidative phosphorylations are essential for the survival of the bacilli during extreme conditions. The combination of dehydrogenases and oxidases constitutes the electron transport chain (ETC).

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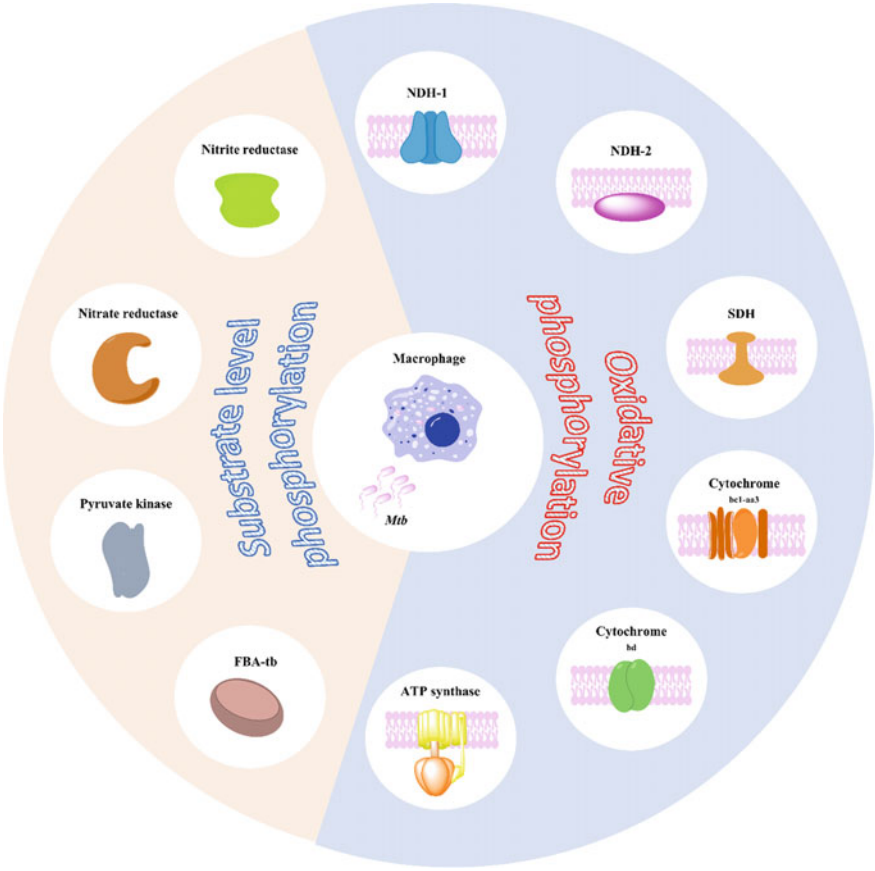
In this chapter, we have expounded on the significance of different enzymes involved in energy pathways. Further, the machinery involved in oxidative phosphorylation as a therapeutic target has been explored. The major classes of inhibitors targeting oxidative phosphorylation are also discussed.

Graphical Abstract



Various enzymes involved in the oxidative and substrate-level oxidation in *M. tb.*

Keywords

Dehydrogenase · *Mycobacterium tuberculosis* · Oxidative phosphorylation · Tuberculosis

1 Introduction

Mycobacterium tuberculosis (*M. tb*) and related species are causative organisms for tuberculosis (TB), an infectious disease. The global tuberculosis report 2019, released by the World Health Organisation (WHO), declared ten million new cases in the year of 2018 across the globe with approximately 1.2 million and 0.25 million deaths of patients without and with human immune-deficiency virus (HIV) co-infection, respectively. The burden of the disease is most in Southeast Asia (44%), followed by Africa (24%) and West Pacific (18%). Further, only a small number of cases are reported in America, Europe, and the Mediterranean region. *M. tb* primarily affects the lungs but may also affect bones, intestine, and central nervous system. It may be latent TB infection (LTBI), i.e., the subject might not have an active infection but is at the risk of getting sick in case of a weakened immune system [1]. Pulmonary TB (PTB) has typical symptoms, including cough lasting for more than three weeks, chest pain, sputum with blood, weakness, weight loss, sweating at night, chills and fever. It was postulated that the domestication of the cattle was the primary cause for the development of human-specific *M. tb* from its precursor *Mycobacterium bovis* (*M. bovis*) [2]. However, single nucleotide polymorphisms (SNPs) analysis showed that *M. tb* and *M. bovis* originated independently at the same time and with the same precursor, i.e., *Mycobacterium canetti* (*M. canetti*) [3, 4]. Robert Koch concluded that TB, a bacterial disease, was caused due to tubercle bacillus [5]. An experiment on the rabbit showed that not just the bacterium but the conditions like malnutrition and improper sanitation together were also responsible for the spread of the disease [6].

Schatz and Waksman discovered Streptomycin in the 1940s, the first anti-TB antibiotic. A combination of five antimicrobial drugs that includes Isoniazid, Streptomycin, Ethambutol, Pyrazinamide, and Rifampicin is available as the primary line of drug therapy. A directly observed treatment short-course (DOTS) strategy has been suggested by WHO for the control of TB. However, multidrug-resistant (MDR) cases have shown Isoniazid and Rifampicin resistance resulting in failure of DOTS [7]. The availability of the second line of anti-TB drugs provides a glimpse of hope. The second-line drugs include fluoroquinolones, injectable Amikacin, Kanamycin, Capreomycin, Cycloserine, Carbapenems, Para-amino salicylic acid, and Amoxicillin. But, these drugs have higher toxicity and lower efficacy, as compared to first-line drugs [8]. Further, patients with MDR- and extensive drug resistance (XDR)-TB require substantial use of the second-line drugs and also extended treatment regimens. Although, the availability of treatment makes it a curable disease, but TB is yet to be eliminated [9]. Bacillus Calmette-Guerin (BCG) vaccine comprises of an attenuated virulent strain of *M. bovis*. It was developed by Calmette and Guerin in the 1920s. The vaccine is recommended for neonates soon after birth and protects children from *M. tb* infection, including TB meningitis. However, the protection seems to be limited for 10–20 years of age. There is an increase in the population of patients from young and adult age groups after the protection period is over. Thus, the search for the vaccine should involve the development of a new

booster vaccine that reinforces the falling BCG-induced immunity [10]. The geriatric population is more prone to TB due to malnutrition, physiological changes leading to attenuated microbial clearance, chronic diseases, fall in cell-mediated immunity, etc. The symptoms are atypical, often shadowed by age-related diseases, and diagnosis is often overlooked [11].

TB-HIV co-infection is a syndemic that magnifies the burden of TB and has a substantial contribution toward the mortality figures. BCG vaccination should not be given to people with symptomatic HIV illness, including children, according to the WHO. The risk associated with the incidence of disease due to immunization increases with the degree of immunosuppression. When a patient with LTBI acquires HIV, progression of TB is accelerated, and its relapse is a common phenomenon in such patients [12, 13]. Hence, a better understanding of the pathophysiology and etiology of the disease is important for the development of future therapeutics.

2 Energy Pathways in *Mycobacterium Tuberculosis*

M. tb is an obligate aerobe and enters the lungs through aerosolized droplets from the contaminated person. It resides in the macrophages, alveolar epithelial type II pneumocytes, dendritic cells (DCs), and neutrophils [14]. *M. tb* primarily grows in pneumocytes in the initial stage and enters DCs, which act as antigen-presenting cells. It is proposed that infected cell recruits antigen-specific T lymphocytes and phagocytes [15, 16]. T lymphocytes produce an immunological response, which leads to a slowdown of bacterial multiplication and controls the infection. However, TB is a chronic infection and, in later stages, leads to granuloma lesions development. The granular lesion consists of cellular necrotic debris surrounded by phagocytes and lymphocytes [17]. The cellular debris contains *M. tb*, which is internalized into phagocytic cells [18].

Substrate level and oxidative phosphorylations are the two primary pathways that govern the energy requirement of *M. tb*. The substrate level phosphorylation employs decomposition of high energy level substrates and formation of adenosine triphosphate (ATP) simultaneously without any electron transfer. The process is less efficient but is critical for the survival of the organism [19]. Oxidative phosphorylation is a significant process for energy production in *M. tb*. During the infection cycle, the bacilli obtain reductive energy as electrons from carbohydrates and fatty acids, which are harvested from central metabolic pathways using nicotinamide adenine dinucleotide hydrogen (reduced) (NADH)/nicotinamide adenine dinucleotide (NAD^+) and/or flavin adenine dinucleotide (reduced) ($FADH_2$)/flavin adenine dinucleotide (FAD) redox pairs [20, 21]. The electrons enter into the electron transport chain (ETC) through the reduction of menaquinone. There are several dehydrogenase enzymes that feed electrons into the ETC. NAD^+, which is a primary electron sink, gets converted into NADH through NADH dehydrogenase [22]. The other primary dehydrogenases, i.e., succinate dehydrogenase, proline dehydrogenase, and L-lactate cytochrome c oxidoreductase, directly

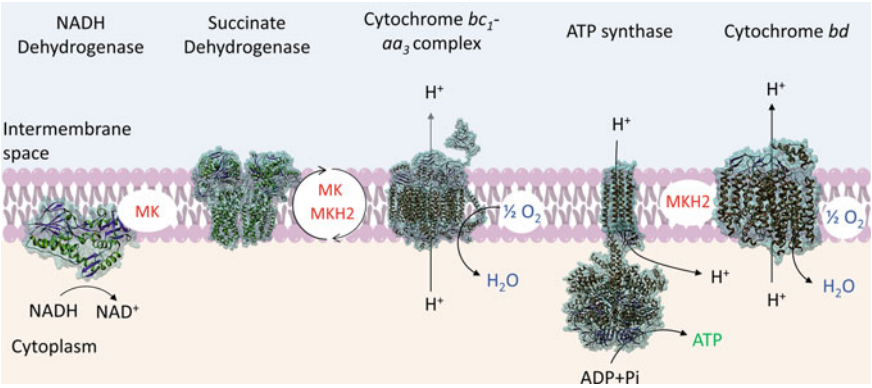


Fig. 1 Schematic representation of major enzymes in the ETC of *M. tb*. Electrons received from NADH are fuelled to the ETC using NDH-2 (PDB id. 5WED), resulting in the reduction of menaquinone (MK) to menaquinol (MKH_2). Menaquinone is the only carrier of electrons across the ETC in *M. tb*. SDH (PDB id. 6LUM) and also helps in increasing the MKH_2 pool. The electron is transferred from the MK pool to the terminal oxidases such as cytochrome bc_1 complex. The cytochrome bc_1 complex forms a super complex with cytochrome aa_3-terminal oxidase (PDB id. 6ADQ), which transfers the electrons to oxygen. Cytochrome bd oxidase (PDB id. 6RKO) also catalyzes the reduction of oxygen to water; it accepts the electrons directly from the menaquinone pool. The protons are pumped across the membrane leading to a PMF, which fuels the ATP production via F_0-F_1 ATP synthase. The ATP synthase consists of two parts: hydrophobic integral membrane region F_0 (PDB id. 4V1F) and hydrophilic region F_1 (6FOC)

pump electrons into the ETC. Subsequently, the quinones are re-oxidized by terminal oxidase, either cytochrome bc_1-aa_3 or cytochrome *bd* complex that is responsible for the electron transfer to oxygen (Fig. 1).

It is estimated that about one-third of the world's population has LTBI. During this state, bacteria survive in the body but do not cause any infection as well as damage. The survival of *M. tb* in the latent phase is due to the metabolic rewiring of its system to ensure carbon and energy supply using alternate sources and metabolic pathways [23]. The non-replicating bacterial state during dormancy faces low oxygen supply, as observed in autopsy samples of patients in knock-out and genomic studies. This results in the use of host-derived fatty acid through gluconeogenesis [24]. The other survival approaches allow the use of alternative electron acceptors, such as nitrite, in the absence of oxygen. The following section deals with the various enzymes and their role in energy production.

3 Enzymes Involved in Oxidative Phosphorylation

3.1 Type-I NADH Dehydrogenase

In the mycobacterial respiratory chain, primary NADH dehydrogenases play a critical function. Type-I NADH dehydrogenase (complex I, NDH-1), a membrane-

bound NADH dehydrogenase complex, utilizes menaquinone as an electron acceptor to convert NADH to NAD^+. It is composed of NDH-1, which contains 14 subunits (NuoA-NuoN, *Rv3145-Rv3158*) [25]. Although NDH-1 dehydrogenase is non-essential for *M. tb* survival, evidence shows reduced virulence in the *Nuo* gene-silenced mycobacterial species. It is a proton pump-based dehydrogenase that generates proton motive force (PMF) by pumping protons from the cytoplasm into the periplasmic site. NuoG and other subunits of the dehydrogenase are anti-apoptotic factors that could be used in vaccine development [26].

3.2 Type-II NADH Dehydrogenase

NADH regeneration is an important process for the survival of all life forms. The quinone oxidoreductases and dehydrogenases are the respiratory enzymes that perform the NADH regeneration at the cellular level in the ETC [27]. Type-II NADH: quinone reductase (NDH-2), which is also referred to as NADH dehydrogenase-2, is responsible for the oxidation of NADH and reduction of quinones. NADH is a key feature of the respiratory chain of human pathogens such as *M. tb, Staphylococcus aureus,* and *Plasmodium falciparum* but is absent in animals [28]. Due to the absence of NDH-2 in higher animals, it is an appropriate therapeutic drug target for the design of inhibitors. The enzyme is peripherally connected to the inner surface of the cytoplasmic membrane and is responsible for respiratory chain-linked NADH turnover involved in the synthesis of ATP and the maintenance of [NADH]/[NAD^+] balance [29]. NDH-2 is not a proton pump and contributes to membrane potential. Whereas NDH-1, a proton pump, is responsible for proton gradient [30].

The *M. tb* gene encodes two copies of NDH-2 proteins, i.e., Ndh and NdhA. Biochemical analysis revealed that NDH-2 is majorly involved in the oxidation of NADH in *M. tb* [31]. NDH-2 mediated NADH oxidation leads to greater metabolic flux and higher flow of carbon into bioenergetics pathways leading to increased ATP production with lower energetic efficacy of the ETC [32]. NDH-2 (EC 1.6.99.3) is a 45–60 kDa single-polypeptide that belongs to the two-dinucleotide binding domains flavoproteins superfamily. For the binding of dinucleotides, the enzyme possesses two structurally identical domains and adopts a Rossmann fold. The domain located near the N-terminal contains two GXGXXG motifs, of which one binds with FAD, and the other interacts with NADH. NAD-2 is also a monotopic protein since it has a membrane interaction domain near the C-terminus [33].

Two catalytic mechanisms, i.e., ternary complex and two-site ping-pong kinetics, are proposed for the NDH-2 and depend on the concentration of reactants and products present along with the enzyme dissociation complex [28]. In the ping-pong mechanism, the substrate binds, reacts, and dissociates either on the same (one site) or different binding sites (two-site) in a sequential manner. The two substrates are found to be co-bound in this mechanism. Further, NAD^+ and the reduced $FADH_2$ form a charge-transfer complex which is dissociated by the

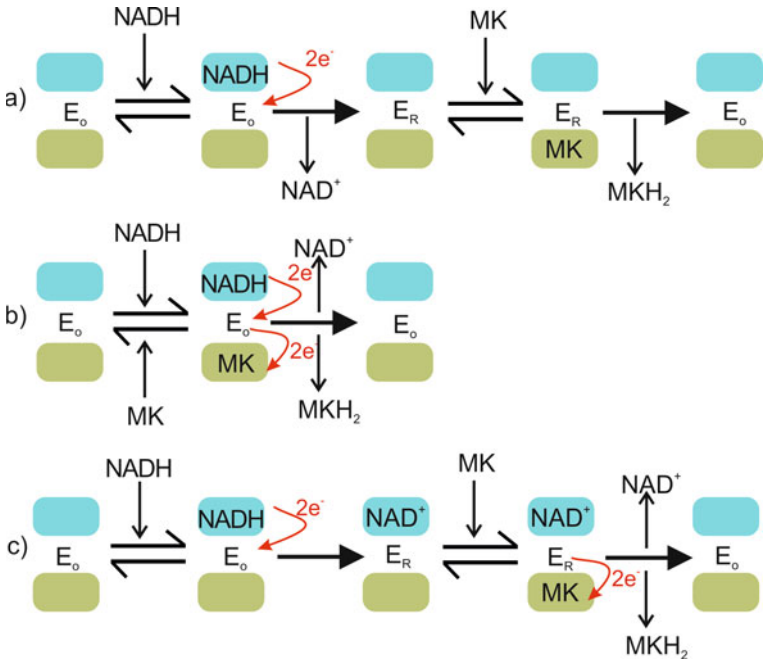


Fig. 2 Catalytic mechanism of NDH-2 enzyme. The binding sites for menaquinol (MKH_2)/menaquinones(MK) (green) and binding sites for nucleotide (blue): **a** Ping-pong mechanism—NADH and MK bind with the enzyme in a sequential manner, and they are never attached to the enzyme simultaneously; **b** Classical ternary mechanism—nucleotide and MK binds with the enzyme to form a ternary complex. Upon the completion of the reaction, the complex dissociates; **c** Atypical ternary mechanism—nucleotide binds and reacts with the enzyme but does not dissociate, then MK binds and reacts with the ternary complex and forms an enzyme-product complex

quinone [34]. On the contrary, both substrates are bonded together, and the products are released simultaneously in the traditional ternary complex mechanism. There is also another atypical ternary complex formation mechanism proposed in which NADH binds to the enzyme and reacts to form NAD^+. The quinone binds and reacts with the enzyme-product complex in the presence of NAD^+ in a ternary complex, which is followed by its reduction within the complex (Fig. 2).

The reaction follows a ternary-type mechanism during enzyme turnover with NADH and quinone, in which NAD^+ binds strongly to the reduced flavin following NADH oxidation and only dissociates on the oxidation of flavin by quinone. On the other hand, the slow enzyme turnover with NADH and quinone follows a ping-pong kinetics pattern. Since NAD^+ does not bind strongly to the reduced flavin and dissociates rapidly; hence, quinone is more likely to react with free $FADH_2$ [28].

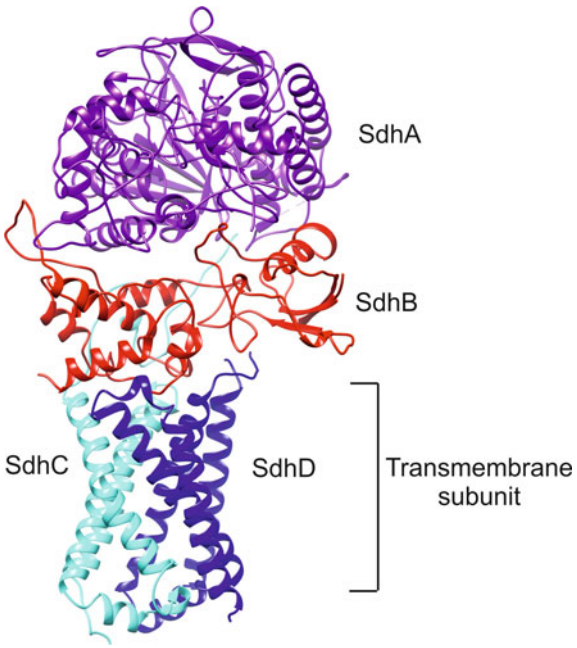


Fig. 3 Crystal structure of SDH-2 enzyme of *Mycobacterium* species representing the subunits

3.3 Succinate Dehydrogenase

Succinate dehydrogenase (SDH), also referred to as succinate/menaquinone oxidoreductase (complex II), catalyzes the conversion of succinate to fumarate through oxidation in the cytoplasm, and simultaneously menaquinone is reduced to menaquinol in the cell membrane. Under anaerobic conditions, fumarate reductase performs the opposite process, with fumarate serving as a terminal electron acceptor. SDH is a key component of the tricarboxylic acid (TCA) cycle, acting as a direct link between ETC and TCA cycle [35]. *Mycobacterial* species have two genes for SDH, i.e., SDH1 and SDH2, which are encoded by *sdh1 (Rv0247c-Rv0249c*) and *sdh2 (Rv3316-Rv3319*) genes, respectively. SDH1 acts during aerobic respiration when nutrients are abundantly present, while SDH2 takes over during hypoxic conditions and in nutrient scarcity. The deletion of SDH1 resulted in impaired respiratory rate through the ETC and reduced cell viability; hence, it is vital for survival and growth [36]. SDH1 of *M. tb* has high sequence similarity with SDH of other species. It is encoded by a group of four genes, i.e., *sdh A-D*. The genes *sdh A* and *sdh B* encode the cytoplasmic component of the enzyme involved in the conversion of succinate to fumarate (*sdh A*), and electrons are transitioned via three iron-sulfur centers (*sdh B*) to the transmembrane subunits. Further, the trans-membranous subunits are encoded by *sdh C* and *sdh D,* which catalyze the electron transfer to menaquinone (Fig. 3) [11].

Mutation studies were performed on SDH1 and SDH2 to understand the role of these isoforms under hypoxic adaptations. The results showed that the survival of

SDH1 mutants was impaired in the stationary phase, whereas no effect was observed in the survival of SDH2 mutants. Furthermore, depletion of SDH1 resulted in higher levels of menaquinol and a faster rate of respiration, suggesting that SDH1 is a regulator of respiration [37]. The role of SDH1 as the catalytic center during aerobic conditions was demonstrated using stable isotope labeling and mass spectrometry. *M. tb* cells use the reverse citric acid cycle to store and release succinate into the culture medium under hypoxic circumstances [38, 39]. SDH activity is essential for metabolic adaptation to hypoxia, membrane potential maintenance, and ATP production. Despite the significant role of the SDH enzyme, there has been a reluctance to develop its inhibitor due to the availability of its mammalian counterpart and isotypes [40].

3.4 Cytochrome bc_1-aa_3 Super Complex

A PMF over a proton-impermeable membrane aids in energy conservation during aerobic respiration. The membrane-bound components of ETC are asymmetrically distributed across the membrane to accomplish net cytoplasmic proton consumption and proton release outside the cell [41]. In a mechanism associated with proton translocation across the membrane, the cytochrome bc_1 transfers an electron from menaquinol to the cytochrome *c* oxidase.

In *M. tb,* the two terminal oxidases, i.e., cytochrome bc_1 and aa_3-type cytochrome *c* oxidase supercomplex and cytochrome *bd* oxidase, catalyze the conversion of oxygen to water by four-electron reduction [42]. The pathway involving cytochrome bc_1-aa_3 supercomplex is most energetically favored in *Mycobacterium* species and acts as a principal respiratory pathway under aerobic conditions. The cytochrome *c* pathway, which consists of a menaquinol-cytochrome *c* oxidoreductase called cytochrome bc_1 (complex III) and an aa_3-type cytochrome *c* oxidase (complex IV), is found in all *Mycobacterium* species [43]. The complex III and IV are encoded by *qcrCAB* and *ctaBCDE* operons, respectively, and both belong to the heme-copper respiratory oxidase family [44].

The bc_1 complex is made up of redox groups that include a 2Fe-2S center on Rieske protein (QcrA), as well as a heme from cytochrome c_1 (QcrC) and two *b*-type hemes (low and high potential) on a single polypeptide chain (QcrB). The transfer of every two electrons from quinol to cytochrome *c* results in the release of four protons from cytochrome bc_1 into the periplasmic space of the membrane [42]. The cytochrome *c* oxidase is considered essential, while the attempts to delete *qcrCAB* remained unsuccessful, indicating its importance for the survival of *Mycobacterium* [45]. The 2Fe-2S center of the complex has three transmembrane helices and characteristic motifs, i.e., CSHLGC and CPCH, similar to Rieske iron-sulfur proteins [46]. An electron is transferred directly from menaquinol to oxygen without the need for a separate cytochrome *c* electron shuttle. The ETC is a significant source of intracellular reactive oxidative species (ROS) that can cause cellular damage, which in turn is protected by the ROS scavenger superoxide dismutase (SOD) [47].

Two heme-binding motifs for c-type cytochromes are found in the QcrC subunit, CVSCH, and CASCH, indicating a covalent di-heme structure. The genome of *Mycobacterium* does not contain a gene for either a membrane-bound or soluble cytochrome *c*. To enable electron transfer and operate as a supercomplex, the bc_1 complex interacts directly with aa_3-type cytochrome c oxidase [48]. The *ctaE* gene of aa_3-type cytochrome *c* oxidase occurs just upstream of the *qcrCAB* operon, while the other genes are scattered across the chromosome. The aa_3-type cytochrome *c* oxidase consists of four subunits: CtaB (cytochrome *c* oxidase assembly factor), CtaC (cytochrome *c* oxidase, subunit II containing Cu_A), CtaD (cytochrome *c* oxidase subunit I containing heme *a*, heme a_3 and Cu_B), and CtaE (subunit III). The CtaD and CtaC are the catalytic subunits. The principal electron acceptors from the bc_1 complex are Cu_A and heme, whereas the a_3-Cu_B unit is an oxygen-reducing element. There is also evidence of subunits CtaI and CtaJ in *M. smegmatis* [44]. The subunit arrangements of the cytochrome bc_1-aa_3 supercomplex have been explained in Fig. 4.

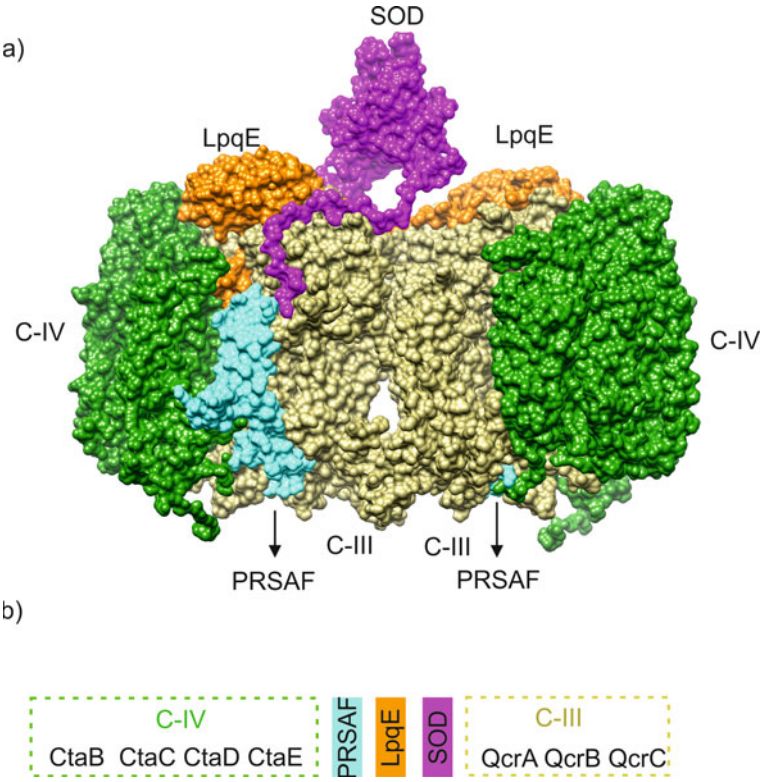


Fig. 4 **a** structure of mycobacterial cytochrome bc_1-aa_3 supercomplex: complex-IV(C-IV) (green), complex -III (C-III) (yellow), Prokaryotic respiratory supercomplex association factor (PRSAF) (cyan), LpqE (orange), and SOD (violet) (PDB id. 6ADQ); **b** linear representation of bc_1-aa_3 supercomplex along with their subunit compositions

The thioredoxin, *CcsX*, is essential for the insertion of the heme group into membrane-bound heme-containing proteins. *M. tb* with deleted *CcsX* displayed deficient heme insertion in the QcrC, leading to remarkable growth defect. However, the mutation still allows the growth and multiplication of *M. tb,* suggesting that the inactivation of the cytochrome *c* branch may be viable. It was also found that, in the absence of the *CcsX* gene, *M. tb* showed upregulation of cytochrome *bd* oxidase. The upregulated enzyme performed the function of an alternate terminal oxidase during the dysregulation of the cytochrome *c* branch [49].

3.5 Cytochrome *bd*

Cytochrome *bd* is a quinol: O_2 oxidoreductase enzyme involved in the ETC of prokaryotes and is absent in eukaryotic mitochondria. It is an oxygen reductase or terminal oxidase present at the end of the ETC and involved in the reduction of O_2 to water. CydA and CydB are the two membranous subunit proteins that form cytochrome *bd* [50]. The crystal structure, as well as the biochemistry of *M. tb* cytochrome *bd*, is still not available. The phylogenetic analysis also indicated that *M. tb* has two subunits in the cytochrome *bd* enzyme similar to other bacteria [51]. However, a recent elucidation of crystal structure for *Geobacillus thermodenitrificans* displayed that both the subunits of cytochrome *bd* have a nine helical trans-membranous structure. Hence, in light of the recent evidence, it could be hypothesized that *M. tb* cytochrome *bd* might have a similar structure [52]. Further, the subunit CydA has a quinol binding site along with three heme groups. It reduces the oxygen and releases the proton from the quinol substrate in the periplasmic space. This PMF is responsible for the transportation of the proton to the cytoplasm to produce water molecules [53].

M. tb and *M. smegmatis* do not require cytochrome *bd* to grow in typical aerobic circumstances [54, 55]. The *cydAB* gene is responsible for coding the two subunits of the enzyme [54]. Further, the *cydAB*-knocked out *M. tb* strain displayed no effect on its growth during acute infection in the mice model. However, the chronic phase of the infection displayed attenuated growth of the mutated bacterial strain [56]. Under stressed conditions, such as hypoxia and nitric oxide exposure, the upregulation in the expression of *cydAB* genes has been reported in various in vitro studies on *M. tb* [56–60]. Impairment of the action of another terminal oxidase cytochrome *bcc/aa*$_3$ due to hydrogen peroxide stress also induces expression of the cytochrome *bd* [48]. These studies suggest that the cytochrome *bd* comes in action under stressed conditions as well as under non-functional cytochrome bcc/aa$_3$, and this may be due to its affinity towards oxygen and quinol peroxidase type enzymatic activity.

A study on the *Mycobacterium marinum,* using a fluorescence-based reporter designed to investigate the expression of the cytochrome *bd*, evaluated the effect of various antibiotics. The probe identified that the expression of cytochrome *bd* was significantly higher in mice as well as in zebrafish models in comparison to that in vitroculture. Further, inhibitors of oxidative phosphorylation induce higher expression of the *cydA* gene than other anti-mycobacterial agents [60]. In another

genetic study on *M. smegmatis*, attenuation of subunit *b* of cytochrome *bcc* due to *qcrB* gene knock-out resulted in the upregulation of expression of *cydAB* operon. Interestingly, the growth of the mutant strain did not differ much from that of the control strain [45]. This indicated that in case of inhibition of cytochrome *bcc*, cytochrome *bd* takes its functional role. Telacebec, an imidazopyridine compound and inhibitor of the cytochrome *bd*, was evaluated in the clinical trials. The genetically altered strain *M. tb bd*-KO, a cytochrome *bd* knock-out strain, displayed complete elimination of *M. tb* on treatment with Telacebec, indicating absolute inhibition of respiratory chain [61, 62]. Further, the co-treatment of Aurachin D, a cytochrome *bd* inhibitor, along with Telacebec, showed the complete killing of the *M. tb* and *M. smegmatis* strains [62]. The in vitro treatment of *M. tb* with NDH-2 inhibitors viz. Chlorpromazine and Clofazimine led to the higher expression of *cydAB* genes hence, increased production of cytochrome *bd* [61, 62]. In another study on *M. smegmatis,* simultaneous administration of Clofazimine and inactivation of *cydA* gene increased the bactericidal activity. In contrast, treatment with only Chlorpromazine on the same mutant strain of *M. smegmatis* showed no improvement in the activity [63].

Bedaquiline, an ATP synthase inhibitor, is presently used in the treatment of MDR- and XDR-TB. It interacts with its target leading to depletion of *the M. tb* ATP pool [64]. The treatment of *M. tb* and *M. smegmatis* with the drug increased the expression of *cypAB* genes [65]. However, the killing efficiency of the drug on a mutant strain *M. tb cydA*-KO was found in the initial phase of treatment only [55]. In the case of *M. smegmatis* with deleted *cydA* gene, similar efficacy was observed [54]. On the contrary, Moosa et al. reported no enhancement in the killing action of Bedaquiline on *cydA*- or *cydAB*-deleted *M. tb* strains [66].

The administration of Amoxicillin and clavulanic acid combination showed an interesting role of the cytochrome *bd* [67]. Inhibition of *M. tb* cell wall synthesis during the treatment resulted in increased $NADH/NAD^+$ ratio, which might be due to cytochrome *bd*. The ratio $NADH/NAD^+$ was altered due to disruption of the cell wall [68]. Knock-out of alanine dehydrogenase and cytochrome *bd* resulted in the increase in the effect of Chlorpromazine, an NDH-2 inhibitor, on mutated *M. tb* [69, 70].

3.6 ATP Synthase

ATP synthase is a critical enzyme in the energy metabolism of all living cells and is also vital for the optimal growth of *M. tb*. ATP is synthesized via oxidative and substrate-level phosphorylation using membrane-bound F_1-F_0 ATP synthase. It utilizes the energy stored as the transmembrane electrochemical potential gradient of a coupling ion for ATP production [71]. As an ATPase, it hydrolyses ATP and transports protons from the cytoplasm to the extracellular space in the presence of high intracellular ATP concentrations and low PMF [72]. Under energetically unfavorable circumstances, some bacteria can employ ATPase to maintain PMF, whereas, in others, this function is prevented. In *M. tb*, there is pronounced suppression in ATP hydrolysis activity under normal conditions [32].

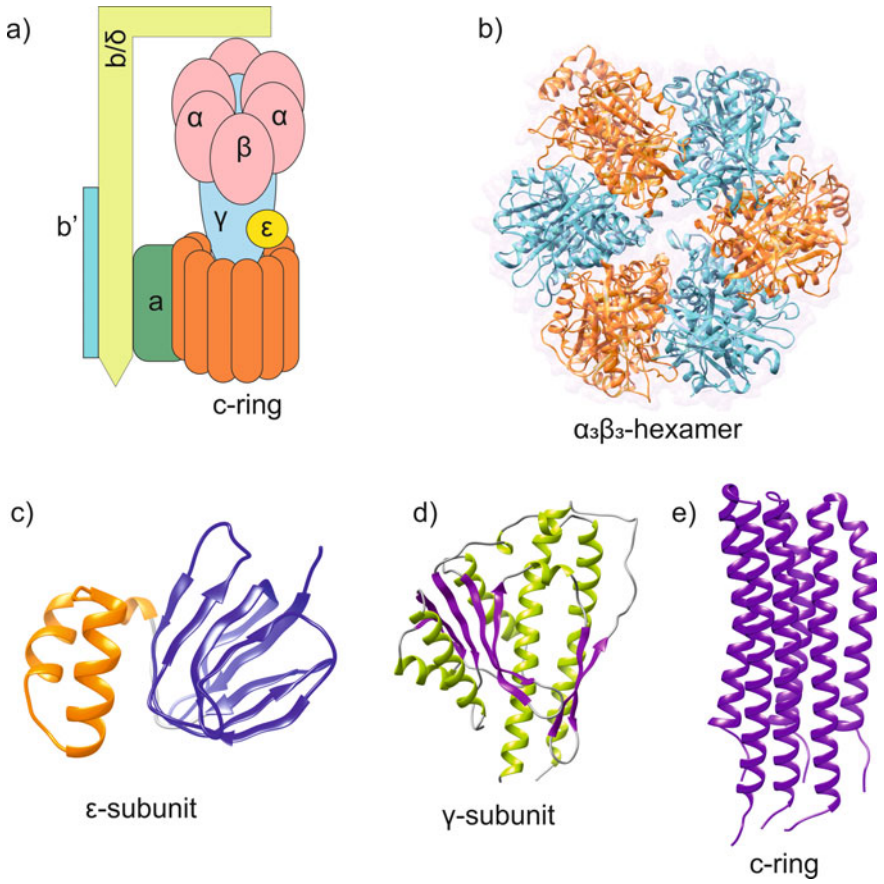


Fig. 5 **a** Subunit composition of mycobacterial ATP synthase; **b** crystal structure $\alpha_3\beta_3$ hexamer. α-subunit (orange) and β-subunit (turquoise) (PDB id. 6FOC); **c** crystal structure of ε-subunit. N-terminal is represented in blue and C-terminal (orange) (PDB id. 5YIO); **d** crystal subunit of γ-subunit (PDB id. 5ZWL); **e** crystal structure of mycobacterial ATP synthase c-ring (PDB id. 4V1F)

The ATP synthase of *M. tb* consists of two functional domains, i.e., a membrane-embedded F_0 unit and an external hydrophilic F_1 domain with subunit compositions of $a_1b_2c_{10\text{-}15}$ and $\alpha_3\beta_3\gamma\delta\varepsilon$, respectively. The b and δ subunits of F_0 and F_1 domains, respectively, are fused, and the size of the b-subunit in the stator stalk is shorter in *M. tb* as compared to other bacteria. The c-ring is composed of more than ten monomer units (Fig. 5a) [64].

The rotation of the c-ring is triggered by the passage of ions (mainly protons) via the F_0 subunit, which is associated with the rotation of the γ and ε-subunit. The ATP production is driven by the rotation of the γ subunit within the $\alpha_3\beta_3$ hexamer of the F_1 domain (Fig. 5b). Although, the structure and rotary catalytic

mechanism of ATP synthase are conserved across all the life forms. However, ATP synthesis is exceptionally challenging in pathogenic bacteria, as they have to deal with limited oxygen and nutrient supply. *M. tb* may survive for years in human cells and require acclimatization in the energy metabolism to give enough ATP and build a PMF that allows it to survive in the host [73]. A PMF of −110 mV is needed for the ATP synthase to generate ATP, which is equally contributed by the membrane potential and the pH in *M. tb*. In contrast, a PMF of around −220 mV is required in other bacteria. Hence, the low PMF requirement represents an adaptation of *M. tb* cells towards deficiency of nutrient and electron acceptor availability [74].

Mycobacterial ATP synthase can reversibly be separated into F_1 and F_0 parts. The $\alpha_3\beta_3$ hexamer is considered the catalytic core. In both *E. coli* and human mitochondria, the sequences of α and β, which include the nucleotide-binding sites, are conserved. The α subunit of *M. tb* shares 55% and 52% sequence similarity with the human mitochondrion and *E. coli*, respectively. The β-subunit shows 61% and 59% sequence similarity with the corresponding organisms. The functional motifs present in F_1-ATPases are the P-loop for nucleotide-binding (G171-T178). Arginine fingers are involved in the stabilization of ATP hydrolysis transition state (R376). Further, the acidic residue (E183) acts as a nucleophile for the ATP hydrolysis reaction, while the sequence DISLEED (D403-D409) is responsible for torque transmission is conserved in *M. tb* [75].

Several mechanisms contribute to the suppression of ATP hydrolysis, including subunit inhibition, Mg-ADP binding to the catalytic site, and binding of inhibitory protein subunit ζ [76]. The ADP produced at the active site gets entrapped in the nucleotide-binding site and does not dissociate from the enzyme. This inhibition can be relieved by PMF, which moves the γ subunit away from its position and allows ATP binding to the non-catalytic site located mainly in α-subunit [71].

The ε-subunit is the major regulator of the ATP synthase complex. It has a C-terminal helical domain and an N-terminal β-barrel domain (Fig. 5c). The helical domain of the C-terminal can adopt a hairpin state or an extended configuration. The two conformation states are reversible and are a crucial element for the regulation of ATP hydrolysis. The extended conformation suppresses the ATP hydrolase activity, and the hairpin conformation favors its hydrolysis [77]. The cellular energy status facilitates the transition between the two conformations as ATP favors the hairpin state.

The c-subunit of the F_0 domain is the proton-translocating unit of ATP synthase. It is an intrinsic membrane protein assembled in an oligomeric ring consisting of two transmembrane helices, i.e., the N-terminal helix is at the center of the oligomeric ring, whereas the C-terminal helix is on the periphery (Fig. 5e). Protein from the periplasmic side of the membrane can bind to an acidic amino acid residue in the membrane-spanning region of the carboxylate side chain of the C-terminal helix. This allows rotation of the c-ring of the hydrophobic core of the membrane, resulting in the release of the protons in the cytoplasm [77]. The polymorphic amino acids present in the membrane-spanning part of the c-subunit act as a probable drug-binding pocket. The presence of bulkier amino acids in human mitochondrial ATP synthase imposes steric hindrance on drug binding [77]. The

c-ring of γ-subunit of the F_1 domain is responsible for the connection of the F_0 rotary motor to the nucleotide-binding site present in the $\alpha_3\beta_3$ hexamer. The γ-subunit contains an extended coil composed of helical regions of N and C-terminal, which penetrates into the $\alpha_3\beta_3$ hexamer's cavity (Fig. 5d). It also has a globular mixed sheet/helical domain near the c-ring. *M. tb* γ-subunit shows a 25% sequence similarity with human mitochondrion homolog [77].

The non-rotating parts of F_0 and F_1, i.e., a subunit and an α3β3 hexamer, respectively, must be tightly attached by stator stalk in order to avoid unnecessary rotation of the ATP synthase complex during catalysis. The stator stalk is a hydrophilic structure that sits on the ATP synthase complex's periphery. It consists of δ subunit, located above α3β3 hexamer, fused with one of the subunits that have C-terminal of the dimeric b subunit. The C-terminal and N-terminal of b-subunit and δ subunit, respectively, are linked together by a 110 amino acids long linker. This fusion might improve power transmission inside the ATP synthase complex by stiffening the stator stalk. The existence of two forms of b-subunit improves mutual contact between the two subunits [78].

4 Enzymes Involved in Substrate-Level Phosphorylation

4.1 Fructose-1, 6-bisphosphate Aldolase

Fructose-1,6-bisphosphate aldolase class II (FBA-tb, E.C. 4.1.2.13) is a metalloenzyme produced by *M. tb* that plays a crucial role in glycolysis as well as gluconeogenesis [79, 80]. It is responsible for the production of glyceraldehyde 3-phosphate (G3P) in glycolysis and fructose 1, 6-bisphosphate (FBP) for gluconeogenesis. It catalyzes the formation of FBP from dihydroxyacetone phosphate (DHAP) and G3P through aldol condensation, which is reversible in nature [81]. It is observed that the class I FBA is expressed in *M. tb* during aerated aerobic conditions. However, hypoxia results in the expression of only FBA-tb, whether the carbon source is glucose or fatty acid [79, 82, 83]. Class II FBA is not expressed in humans, and thus it makes it an excellent anti-TB target with better selectivity [84].

FBA-tb is a homo-tetramer that consists of 334 amino acid residues and a molecular weight of 144 kDa [85]. The *fda* gene with an open frame of 1035 base pairs encodes FBA-tb protein. The enzyme was found in the cell membrane, cell wall, and cytoplasm in an immunoblot investigation employing polyclonal anti-FBA-tb antibodies. Further, it demonstrated that it moved across the compartments inside the bacilli cell [86]. The enzyme has $(\beta\alpha)_8$-barrel fold, which is observed in various class II aldolases [87]. It consists of two zinc and one sodium ions in each monomer. The zinc ion present on the active site is bound with two amino acid residues, i.e., His334 and His436, along with a water molecule. A 3_{10}-helix is observed between β2 strand and α3 helix and an additional α2α helix between α2 helix and β2 strand. In *M. tb,* for FBA-tb, four α helices are present in addition to the α0 helix, which blocks the entrance of the barrel. Two additional

α8b and α8c helices are also present near the arm formed by α8 and α8a [88]. The two adjacent monomers in the tetramer assembly interact with each other through hydrogen bonds, hydrophobic packing, and salt bridges [89]. The total interaction surface area among dimer of dimers interface of FBA-tb is about 1220 $Å^2$. The two dimers form a tetramer displayed interactions through residues beginning from Tyr281 present on α8a helix to Asp302 located beyond α8b helix. The key interactions between the two dimers are Phe292 and Tyr295 (hydrophobic interaction), Leu299 and Val301 (hydrophobic interaction), Tyr295 and Arg285 (hydrogen bonding), Arg285 and Tyr281 (hydrogen bonding), Arg285 and Asp302 (hydrogen bonding), and Lys300 and Asp302 (hydrogen bonding).

Dihydroxyacetone phosphate (DHAP), which is the substrate of the FBA-tb, has sp_3 hybridized C1 carbon with a tetrahedral geometry that undergoes a planer configuration between C1 and C2 carbon bond upon binding with the enzyme. Further, the hydroxyl group and enolate oxygen present on the first and second carbon of the DHAP form a coordinate bond with the zinc present on the active site. The C2 carbon DHAP also forms a hydrogen bond with the Gly253 amide backbone. While G3P, the other substrate, displays interaction with the enzyme. The hydrogens of the phosphate group interact with Ser53 and Arg314 through hydrogen bonding. The C3 hydroxyl group of FBP product shows interaction with catalytic zinc ion along with C2 ketone and C4 hydroxyl group.

4.2 Pyruvate Kinase

The enzyme *M. tb* pyruvate kinase (PK) is involved in the production of pyruvate from phosphoenolpyruvate (PEP) along with the production of ATP. It is the final step of glycolysis. It controls and regulates the TCA cycle as the pyruvate formed is the starting material [90]. It also acts as a crucial source of starting material for the process of gluconeogenesis in many organisms. PK shows allosteric activation from adenosine monophosphate and glucose 6-phosphate. The absence of the PK results in the inability to exploit other fermentable carbon sources such as glycerol for energy in *M. bovis* [91]. However, the introduction of the *pykA* gene in *M. bovis* obtained from *M. tb* H27Rv results in the utilization of glycerol as a carbon source [92]. In a mice model, it was observed that gluconeogenesis was essential for the survival of *M. tb* mediated by pyruvate carboxylase and phosphoenolpyruvate carboxykinase. It was established that *M. tb* is unable to draw a glycolytic substrate from the host organism [24].

The gene *pykA* (Rv1617) having 1419 bp is responsible for encoding the pyruvate kinase. The deletion of the gene resulted in inhibition of *M. tb* growth in the presence of fermentable as well as non-fermentable carbon substrates. Further, growth inhibition was also observed in fatty acid-containing media, indicating *pykA* deletion has a detrimental effect on gluconeogenesis. The absence of *M. tb* PK results in allosteric inhibition of isocitrate dehydrogenase due to the accumulation of the PEP, citrate, and aconitate [93].

4.3 Nitrate Reductase

Various bacteria can substitute nitrate as a final electron acceptor for molecular oxygen to sustain the proton motive gradient for ATP production. *M. tb* has metabolic machinery involved in the reduction of nitrate as a nitrate reductase enzyme. The activity of the enzyme is quite low in the aerobic condition whereas, in the hypoxic condition, the cellular *M. tb* nitrate reductase activity is substantially increased [94]. The deletion of gene *narGHJI* in *M. bovis* blocked nitrate reductase activity, and the mice infected with such mutant had no presentation of TB after 200 days [95]. This indicated that nitrate reduction served as an alternate pathway for energy production under stressed conditions. The nitrate reductase of *M. tb* displayed similarity with that of *E. coli* because it was inhibited by tungstate as well as azide. This established that it was a membrane-bound molybdenum-containing complex consisting of four subunits [96]. Nar-G, -H, -J, and -I are the four subunits, of which Nar-J is responsible for enzyme assembly. Nar-G has a catalytic site, which consists of 1232 amino acid residues. The Nar-G mutant strain of *M. bovis* displayed lower virulence and pulmonary damage in various mice models [97]. Due to the similarity with other nitrate reductases, it might have [4Fe-4S] cluster and molybdopterin guanine dinucleotide (Mo-bis). The increase of nitrate reductase activity in hypoxic conditions helps in cell survival, but does not support its growth and multiplication. Further, it helps the cell for redox balancing besides providing energy during the non-replicating phase [98].

4.4 Nitrite Reductase

The nitrate serves as an electron acceptor in *M. tb* and is converted to nitrite by the NarGHJI enzyme during the dormant phase [98]. The nitrite reductase NirBD converts nitrite to ammonia through reduction. In the absence of other nitrogen sources, *M. tb* uses nitrites, indicating its assimilatory role. The addition of asparagine to the growth media of *M. tb* decreased the nitrite reduction, thus indicating the utilization of asparagine as a nitrogen source over nitrite. This might be because of the requirement of six electrons for the reduction of nitrite, which is quite expensive, and hence, asparagine is a preferred nitrogen source. In hypoxic conditions, the viability of the NirBD mutant *M. tb* strain is reduced in the macrophage cell culture system than in the wild-type strain. This might be responsible for maintaining the $NADH/NAD^+$ balance during dormancy in hypoxic conditions. Another role of NirBd is to neutralize nitrite and nitric oxide and convert them into ammonia under aerobic conditions. However, during the hypoxic condition, it is exported outside the cell. The reduction of the nitrite yields ammonia, which reduces the acidity of the phagosome environment and helps in the survival of *M. tb* [99]. Further, the secreted ammonia may cease the fusion of the lysosome with the phagosome. Hence, NirBD is one of the essential enzymes responsible for virulence during the dormant phase. NirD is the subunit of the NirBD complex, which has a

double β-sandwich fold with no Fe–S cluster for electron transfer. It has cysteine residue present on the surface and may be responsible for interaction with NirB subunit [100].

5 Major Classes of Inhibitors Targeting *Mycobacterium Tuberculosis* Energetics

Various enzymes of oxidative phosphorylation are responsible for driving PMF across the membrane leading to ATP generation (Fig. 6). The inhibition of these enzymes leads to bacteriostatic as well as bactericidal actions on *M. tb*.

Clofazimine (CFZ) is chemically a riminophenazine derivative, which has shown anti-*M. tb* effect in various studies [101, 102]. It was initially used as an anti-leprotic drug. WHO has also recommended it in multibacillary disease in combination with other drugs [103]. It was suggested that Clofazimine, a redox compound, undergoes reduction as well as oxidation in *M. tb*, and this might be due to its conjugation in the respiratory chain [101]. Further, *M. tb* with catalase deficiency increased the activity of Clofazimine due to its ability to generate reactive oxidation species (ROS). Another study also supplemented that Clofazimine increased ROS production in *M. smegmatis,* leading to cell death [104]. The ROS production was mediated by NDH-2-reduced Clofazimine. The mice model showed complete elimination of *M. tb* on treatment with Clofazimine in five months [104, 105]. The drug is currently used as the second-line therapy for the treatment for TB.

Thioridazine has been a neuroleptic drug in use for over 60 years and has fewer side effects than other phenothiazines. The in vitro study on *M. tb* and its drug-resistant strains indicated that Thioridazine was effective with a minimum inhibitory concentration (MIC) range of 4–32 μg/ml [106, 107]. It was observed that the administration of Thioridazine reduced the resistance of the first-line drugs [108]. Thioridazine acts through various targets, including oxidoreductase, NDH-2, efflux protein, and fatty acid metabolism [69, 109, 110]. NDH-2 is indispensable for the growth of *M. tb* and is involved in anaerobic respiration [74, 111].

Telacebec, targeting the respiratory cytochrome bc_1 complex, is an imidazopyridine derivative. It showed potent anti-*M. tb* activity with MIC of 2.7 and 0.28 nM in broth culture medium and in the macrophage, respectively [112]. It has 90%

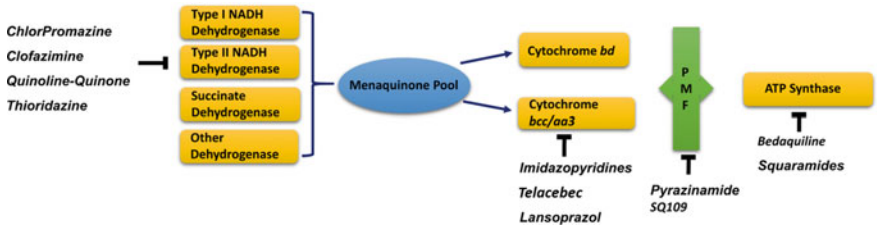


Fig. 6 Various classes of compounds that target mycobacterial energetics

bioavailability with a half-life of 23.4 h and low systemic clearance (4.03 ml min^{-1} kg^{-1}). It displayed two- to three-fold higher pulmonary concentration in comparison to the serum, along with the distribution volume of about 5.21 L/kg of body weight. The drug produced a significant reduction in *M. tb* load after four weeks of treatment in mice with a decrease in the number of pulmonary granulomas. Further, co-administration with isoniazid showed a reduction in the size of granulomas. The intracellular ATP levels were also reduced significantly due to cytochrome bc_1 inhibition. The phase I trial has established the safety and its pharmacokinetic profile. Currently, a phase II clinical trial on pulmonary TB patients is underway to evaluate the bactericidal activity of Telacebec [112].

Bedaquiline (BDQ) is a diarylquinoline derivative that is approved to target energy metabolism [113]. It acts as an ATP synthase inhibitor, and the co-crystal of bedaquiline bound to ATP synthase showed that it binds with a membrane-embedded rotor (c-ring) found on the F_0 subunit [64]. Bedaquiline is highly selective towards *M. tb* and acts on both active as well as dormant cells [114]. It has a half-life of five hours along with a triphasic elimination, but the terminal half-life is quite long (173 h in humans). It is oxidized by CYP3A4 to produce an active N-desmethyl metabolite. In a pre-clinical study to determine the early bactericidal activity, it was observed that there was an absence of bactericidal activity during the initial two to four days because the depletion of the ATP pool required three to four days. The clinical trial also showed efficacy of BDQ over placebo. The clinical study established the safety and tolerance for Bedaquiline at 400 mg daily dose. However, in a study on MDR-TB patients, a higher death rate was observed for the treatment group than that of placebo. Some strains of *M. tb* and *M. smegmatis* produced mutation and resistance for Bedaquiline. Gene *atpE* responsible for encoding subunit c of ATP synthase displayed mutation at 28^{th} and 63^{rd} positions, i.e., E28P and A63V, which prevented Bedaquiline binding [115, 116].

Pyrazinamide is a pyrazine derivative, which was discovered as an anti-TB drug through serendipity. It is a first-line drug and reduces the therapy duration from 12 months to six months [117]. It acts by targeting the post-translation process, energy production as well as pantothenate coenzyme [118]. Initially, the pro-drug is converted to pyrazinoic acid by the action of the pyrazinamidase enzyme present in the granuloma [119]. The pyrazinoic acid diffuses inside the cell as a neutral molecule if the pH of the granuloma environment is acidic. The unionized acid molecule transports and releases the protons inside the cell, increasing the proton concentration. This hampers the proton gradient and potential in the cell that adversely affects PMF and ATP generation [118]. Trans-translational modifications due to the addition of tmRNA on toxic and damaged proteins help its recognition and clearance by proteinase, especially under stress conditions, and support the survival of *M. tb* from damaged proteins, but pyrazinoic acid inhibits the process. Further, it also inhibits aspartate decarboxylase leading to inhibition of synthesis of pantothenate and coenzyme A (Fig. 7).

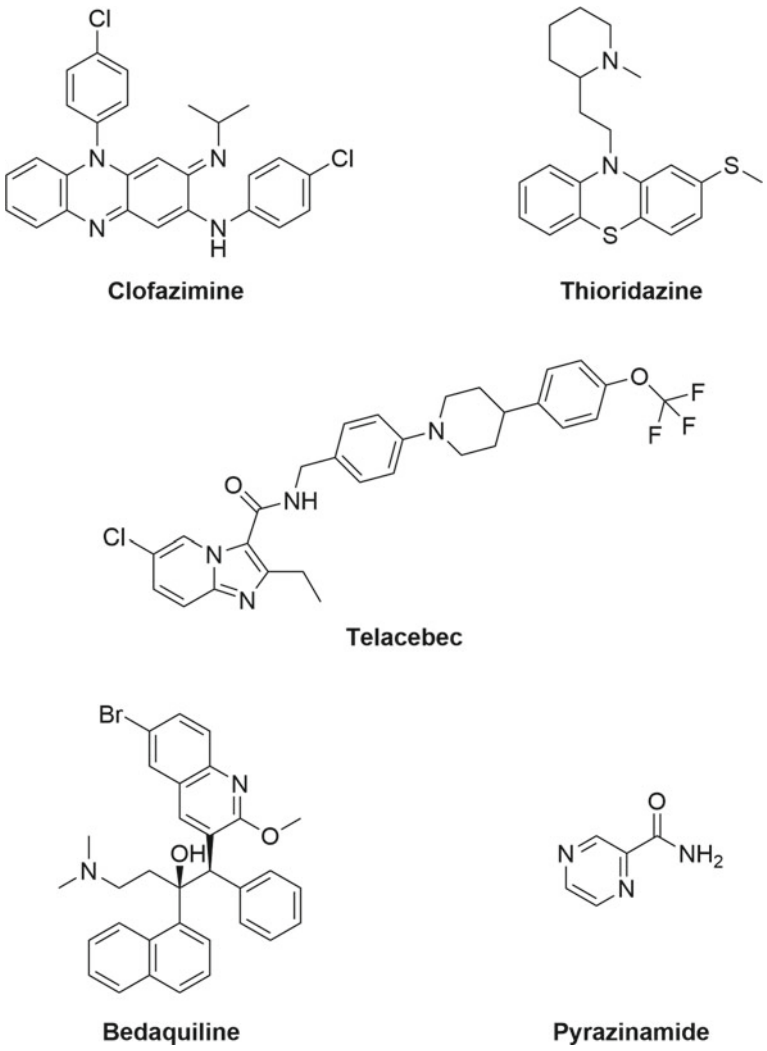


Fig. 7 Structure of anti-mycobacterial compounds

6 Conclusion

M. tb is a pathogenic bacterium and causes TB with a very high mortality rate. Unlike many bacteria that rely only on substrate-level phosphorylation for survival, *M. tb* relies on oxidative phosphorylation. During normal aerobic conditions, the breakdown of the carbon substrate releases the energy in the form of NADH, which is then funneled through the ETC to generate ATP. Electrons are transferred to the menaquinone pools of the cell directly from NADH by several dehydrogenases

found in *M. tb*, including NDH-2 and SDH. Further, the presence of two-terminal oxidase complex oxidases process menaquinone, resulting in the pumping of the protons outside the cell. Cytochrome *bcc/aa*$_3$ is the primary terminal oxidase, which functions in normal aerobic conditions. The periplasmic protons create PMF that results in the generation of ATP through ATP synthase. Another terminal oxidase, i.e., cytochrome *bd,* comes into action under extreme hypoxic conditions; however, that is less efficient to conserve energy.

The activated macrophages limit the availability of nutrients to restrict intracellular replication of *M. tb*. The adaptation of *M. tb* to host immunity entails the substitution of fatty acids for sugars as a carbon and energy source. The prototrophic bacteria use cholesterol and fatty acids as a source of carbon for their energy requirements and sustain their central metabolism via gluconeogenic conversion of lipid and amino acid-derived intermediates via gluconeogenic enzymes like PK and FBA-tb. Activated macrophages also release nitrate, which is antimicrobial and is reduced by *M. tb* to nitrite by multi-subunit nitrate reductase, NarGHJI. The enzyme also helps in nullifying the reactive nitrogen in a hypoxic environment. Further, the nitrite reductase converts the generated nitrite to ammonia, reduces the acidic environment of phagosomes of the host macrophagic cells, and helps in its survival. Since the past decade, numerous components of these pathways have been discovered as druggable molecular targets. Small-molecule-based oxidative phosphorylation inhibitors have been found to be effective against DR-TB. A number of drugs have shown their potential to treat *M. tb* infection in pre-clinical and clinical trials.

Core Messages

- Oxidative phosphorylation is an essential energy-efficient pathway for normal physiological processes and growth of *M. tb.*
- *M. tb* possesses the two-terminal oxidase cytochrome *bcc-aa*$_3$ and Cytochrome *bd*.
- Cytochrome *bc*$_1$*-aa*$_3$ and *bd* complexes are favored under aerobic and hypoxic conditions, respectively, for ATP production.
- Substrate level phosphorylation plays a vital role in *M. tb* survival in the dormant state inside the macrophages.
- The identification of various targets from ETC has acquired significance in anti-TB drug research.

References

1. Gutti G, Arya K, Singh SK (2019) Latent tuberculosis infection (LTBI) and its potential targets: an investigation into dormant phase pathogens. Mini Rev Med Chem 19(19):1627–1642

2. Stead WW (1997) The origin and erratic global spread of tuberculosis: how the past explains the present and is the key to the future. Clin Chest Med 18(1):65–77
3. Sreevatsan S, Pan X, Stockbauer KE, Connell ND, Kreiswirth BN, Whittam TS, Musser JM (1997) Restricted structural gene polymorphism in the *Mycobacterium tuberculosis* complex indicates evolutionarily recent global dissemination. Proc Natl Acad Sci 94(18):9869–9874
4. Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, Eiglmeier K, Garnier T, Gutierrez C, Hewinson G, Kremer K (2002) A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. Proc Natl Acad Sci 99(6):3684–3689
5. Koch R (1882) Die Aetiologie der Tuberkulose, Berl. klin. Wschr., 19, 221.-(1901). Trans Brit Congr Tuberc, London 1:23
6. Collins F (1998) Tuberculosis research in a cold climate. Tuber Lung Dis 78(2):99–107
7. Park S, Kim C, Song S (1998) Outcome of chemotherapy in 107 patients with pulmonary tuberculosis resistant to isoniazid and rifampin. Int J Tuberc Lung Dis 2(11):877–884
8. Rendon A, Tiberi S, Scardigli A, D'Ambrosio L, Centis R, Caminero JA, Migliori GB (2016) Classification of drugs to treat multidrug-resistant tuberculosis (MDR-TB): evidence and perspectives. J Thorac Dis 8(10):2666–2671. https://doi.org/10.21037/jtd.2016.10.14
9. Wainwright M (1991) Streptomycin: discovery and resultant controversy. Hist Phil Life Sci 97–124
10. Andersen P, Doherty TM (2005) The success and failure of BCG—implications for a novel tuberculosis vaccine. Nat Rev Microbiol 3(8):656–662. https://doi.org/10.1038/nrmicro1211
11. Rajagopalan S, Yoshikawa TT (2000) Tuberculosis in the elderly. Z Gerontol Geriatr 33 (5):374–380. https://doi.org/10.1007/s003910070034
12. McShane H (2005) Co-infection with HIV and TB: double trouble. Int J STD AIDS 16 (2):95–101
13. Kwan CK, Ernst JD (2011) HIV and tuberculosis: a deadly human syndemic. Clin Microbiol Rev 24(2):351–376
14. Cohen SB, Gern BH, Delahaye JL, Adams KN, Plumlee CR, Winkler JK, Sherman DR, Gerner MY, Urdahl KB (2018) Alveolar macrophages provide an early *Mycobacterium tuberculosis* niche and initiate dissemination. Cell Host Microbe 24(3):439-446.e434. https://doi.org/10.1016/j.chom.2018.08.001
15. Norris BA, Ernst JD (2018) Mononuclear cell dynamics in M. tuberculosis infection provide opportunities for therapeutic intervention. PLoS Pathog 14(10):e1007154
16. O'Garra A, Redford PS, McNab FW, Bloom CI, Wilkinson RJ, Berry MP (2013) The immune response in tuberculosis. Annu Rev Immunol 31:475–527
17. Martin CJ, Carey AF, Fortune SM (2016) A bug's life in the granuloma. Semi Immunopathol 38(2):213–220. https://doi.org/10.1007/s00281-015-0533-1
18. McClean CM, Tobin DM (2016) Macrophage form, function, and phenotype in mycobacterial infection: lessons from tuberculosis and other diseases. Pathog Dis 74(7). https://doi.org/10.1093/femspd/ftw068
19. Coleman JP, Smith CJ (2014) Microbial metabolism☆. In: Reference module in biomedical sciences. Elsevier
20. Boshoff HI, Barry CE 3rd (2005) Tuberculosis—metabolism and respiration in the absence of growth. Nat Rev Microbiol 3(1):70–80. https://doi.org/10.1038/nrmicro1065
21. Russell DG, VanderVen BC, Lee W, Abramovitch RB, Kim M-j, Homolka S, Niemann S, Rohde KH (2010) Mycobacterium tuberculosis wears what it eats. Cell Host Microbe 8 (1):68–76. https://doi.org/10.1016/j.chom.2010.06.002
22. Kerscher S, Dröse S, Zickermann V, Brandt U (2008) The three families of respiratory NADH dehydrogenases. Results Probl Cell Differ 45:185–222. https://doi.org/10.1007/400_2007_028
23. Barry CE 3rd, Boshoff HI, Dartois V, Dick T, Ehrt S, Flynn J, Schnappinger D, Wilkinson RJ, Young D (2009) The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. Nat Rev Microbiol 7(12):845–855. https://doi.org/10.1038/nrmicro2236

24. Marrero J, Rhee KY, Schnappinger D, Pethe K, Ehrt S (2010) Gluconeogenic carbon flow of tricarboxylic acid cycle intermediates is critical for Mycobacterium tuberculosis to establish and maintain infection. Proc Natl Acad Sci USA 107(21):9819–9824. https://doi.org/10.1073/pnas.1000715107
25. Vilchèze C, Weinrick B, Leung LW, Jacobs WR (2018) Plasticity of *Mycobacterium tuberculosis* NADH dehydrogenases and their role in virulence. Proc Natl Acad Sci 115 (7):1599. https://doi.org/10.1073/pnas.1721545115
26. Brazier B, McShane H (2020) Towards new TB vaccines. Semi Immunopathol 42(3):315–331. https://doi.org/10.1007/s00281-020-00794-0
27. Godoy-Hernandez A, Tate DJ, McMillan DGG (2019) Revealing the membrane-bound catalytic oxidation of NADH by the drug target type-II NADH dehydrogenase. Biochemistry 58(42):4272–4275. https://doi.org/10.1021/acs.biochem.9b00752
28. Blaza JN, Bridges HR, Aragão D, Dunn EA, Heikal A, Cook GM, Nakatani Y, Hirst J (2017) The mechanism of catalysis by type-II NADH:quinone oxidoreductases. Sci Rep 7 (1):40165. https://doi.org/10.1038/srep40165
29. Melo AMP, Bandeiras TM, Teixeira M (2004) New insights into type II NAD(P)H: quinone oxidoreductases. Microbiol Mol Biol Rev 68(4):603. https://doi.org/10.1128/MMBR.68.4.603-616.2004
30. Sellamuthu S, Singh M, Kumar A, Singh SK (2017) Type-II NADH dehydrogenase (NDH-2): a promising therapeutic target for antitubercular and antibacterial drug discovery. Expert Opin Ther Targets 21(6):559–570
31. Shirude PS, Paul B, Roy Choudhury N, Kedari C, Bandodkar B, Ugarkar BG (2012) Quinolinyl pyrimidines: potent inhibitors of NDH-2 as a novel class of anti-TB agents. ACS Med Chem Lett 3(9):736–740. https://doi.org/10.1021/ml300134b
32. Heikal A, Nakatani Y, Dunn E, Weimar MR, Day CL, Baker EN, Lott JS, Sazanov LA, Cook GM (2014) Structure of the bacterial type II NADH dehydrogenase: a monotopic membrane protein with an essential role in energy generation. Mol Microbiol 91(5):950–964. https://doi.org/10.1111/mmi.12507
33. Marreiros BC, Calisto F, Castro PJ, Duarte AM, Sena FV, Silva AF, Sousa FM, Teixeira M, Refojo PN, Pereira MM (2016) Exploring membrane respiratory chains. Biochim Biophys Acta (BBA)—Bioenerg 1857(8):1039–1067. https://doi.org/10.1016/j.bbabio.2016.03.028
34. Sena FV, Batista AP, Catarino T, Brito JA, Archer M, Viertler M, Madl T, Cabrita EJ, Pereira MM (2015) Type-II NADH:quinone oxidoreductase from *Staphylococcus aureus* has two distinct binding sites and is rate limited by quinone reduction. Mol Microbiol 98 (2):272–288. https://doi.org/10.1111/mmi.13120
35. Pecsi I, Hards K, Ekanayaka N, Berney M, Hartman T, Jacobs WR, Cook GM (2014) Essentiality of succinate dehydrogenase in *Mycobacterium smegmatis* and its role in the generation of the membrane potential under hypoxia. mBio 5(4):e01093–01014. https://doi.org/10.1128/mBio.01093-14
36. Hartman T, Weinrick B, Vilchèze C, Berney M, Tufariello J, Cook GM, Jacobs Jr WRJPP (2014) Succinate dehydrogenase is the regulator of respiration in *Mycobacterium tuberculosis*. 10(11):e1004510
37. Hartman T, Weinrick B, Vilchèze C, Berney M, Tufariello J, Cook GM, Jacobs WR Jr (2014) Succinate dehydrogenase is the regulator of respiration in *Mycobacterium tuberculosis*. PLoS Pathog 10(11):e1004510. https://doi.org/10.1371/journal.ppat.1004510
38. Maklashina E, Cecchini G, Dikanov SA (2013) Defining a direction: electron transfer and catalysis in *Escherichia coli* complex II enzymes. Biochim Biophys Acta (BBA)—Bioenerg 1827(5):668–678
39. Eoh H, Rhee KY (2013) Multifunctional essentiality of succinate metabolism in adaptation to hypoxia in *Mycobacterium tuberculosis*. Proc Natl Acad Sci 110(16):6554–6559
40. Cimen H, Han M-J, Yang Y, Tong Q, Koc H, Koc EC (2010) Regulation of succinate dehydrogenase activity by SIRT3 in mammalian mitochondria. Biochemistry 49(2):304–311. https://doi.org/10.1021/bi901627u

41. Cook GM, Hards K, Vilchèze C, Hartman T, Berney M (2014) Energetics of respiration and oxidative phosphorylation in mycobacteria pp 389–409
42. Foo CS, Pethe K, Lupien A (2020) Oxidative phosphorylation—an update on a new, essential target space for drug discovery in *Mycobacterium tuberculosis*. Appl Sci 10(7). https://doi.org/10.3390/app10072339
43. Cook GM, Hards K, Dunn E, Heikal A, Nakatani Y, Greening C, Crick DC, Fontes FL, Pethe K, Hasenoehrl E, Berney M (2017) Oxidative phosphorylation as a target space for tuberculosis: success, caution, and future directions. Microbiol Spectr 5(3). https://doi.org/10.1128/microbiolspec.TBTB2-0014-2016
44. Gong H, Li J, Xu A, Tang Y, Ji W, Gao R, Wang S, Yu L, Tian C, Li J, Yen H-Y, Man Lam S, Shui G, Yang X, Sun Y, Li X, Jia M, Yang C, Jiang B, Lou Z, Robinson CV, Wong L-L, Guddat LW, Sun F, Wang Q, Rao Z (2018) An electron transfer path connects subunits of a mycobacterial respiratory supercomplex. Science 362 (6418):eaat8923. https://doi.org/10.1126/science.aat8923
45. Matsoso LG, Kana BD, Crellin PK, Lea-Smith DJ, Pelosi A, Powell D, Dawes SS, Rubin H, Coppel RL, Mizrahi V (2005) Function of the cytochrome bc1-aa3 branch of the respiratory network in mycobacteria and network adaptation occurring in response to its disruption. J Bacteriol 187(18):6300–6308
46. Niebisch A, Bott M (2001) Molecular analysis of the cytochrome bc_1-aa_3 branch of the *Corynebacterium glutamicum* respiratory chain containing an unusual diheme cytochrome c1. Arch Microbiol 175(4):282–294. https://doi.org/10.1007/s002030100262
47. Zhao X, Drlica K (2014) Reactive oxygen species and the bacterial response to lethal stress. Curr Opin Microbiol 21:1–6. https://doi.org/10.1016/j.mib.2014.06.008
48. Small JL, Park SW, Kana BD, Ioerger TR, Sacchettini JC, Ehrt S (2013) Perturbation of cytochrome c maturation reveals adaptability of the respiratory chain in *Mycobacterium tuberculosis*. MBio 4(5)
49. Small JL, Park SW, Kana BD, Ioerger TR, Sacchettini JC, Ehrt S (2013) Perturbation of Cytochrome Maturation Reveals Adaptability of the Respiratory Chain in Mycobacterium tuberculosis. mBio 4(5):e00475–00413. https://doi.org/10.1128/mBio.00475-13
50. Borisov VB, Gennis RB, Hemp J, Verkhovsky MI (2011) The cytochrome bd respiratory oxygen reductases. Biochim Biophys Acta (BBA)—Bioenerg 1807(11):1398–1413. https://doi.org/10.1016/j.bbabio.2011.06.016
51. Allen RJ, Brenner EP, VanOrsdel CE, Hobson JJ, Hearn DJ, Hemm MR (2014) Conservation analysis of the CydX protein yields insights into small protein identification and evolution. BMC Genomics 15(1):946
52. Safarian S, Rajendran C, Müller H, Preu J, Langer JD, Ovchinnikov S, Hirose T, Kusumoto T, Sakamoto J, Michel H (2016) Structure of a bd oxidase indicates similar mechanisms for membrane-integrated oxygen reductases. Science 352(6285):583–586
53. Kita K, Konishi K, Anraku Y (1984) Terminal oxidases of *Escherichia coli* aerobic respiratory chain. II. Purification and properties of cytochrome b558-d complex from cells grown with limited oxygen and evidence of branched electron-carrying systems. J Biol Chem 259(5):3375–3381
54. Kana BD, Weinstein EA, Avarbock D, Dawes SS, Rubin H, Mizrahi V (2001) Characterization of thecydAB-encoded cytochrome bd oxidase from *Mycobacterium smegmatis*. J Bacteriol 183(24):7076–7086
55. Berney M, Hartman TE, Jacobs WR (2014) A *Mycobacterium tuberculosis* cytochrome bd oxidase mutant is hypersensitive to bedaquiline. MBio 5(4)
56. Shi L, Sohaskey CD, Kana BD, Dawes S, North RJ, Mizrahi V, Gennaro ML (2005) Changes in energy metabolism of *Mycobacterium tuberculosis* in mouse lung and under in vitro conditions affecting aerobic respiration. Proc Natl Acad Sci 102(43):15629–15634
57. Berney M, Cook GM (2010) Unique flexibility in energy metabolism allows mycobacteria to combat starvation and hypoxia. PLoS ONE 5(1):e8614

58. Gopinath V, Raghunandanan S, Gomez RL, Jose L, Surendran A, Ramachandran R, Pushparajan AR, Mundayoor S, Jaleel A, Kumar RA (2015) Profiling the proteome of *Mycobacterium tuberculosis* during dormancy and reactivation. Mol Cell Proteomics 14 (8):2160–2176
59. Cortes T, Schubert OT, Banaei-Esfahani A, Collins BC, Aebersold R, Young DB (2017) Delayed effects of transcriptional responses in *Mycobacterium tuberculosis* exposed to nitric oxide suggest other mechanisms involved in survival. Sci Rep 7(1):1–9
60. Boot M, Jim KK, Liu T, Commandeur S, Lu P, Verboom T, Lill H, Bitter W, Bald D (2017) A fluorescence-based reporter for monitoring expression of mycobacterial cytochrome bd in response to antibacterials and during infection. Sci Rep 7(1):1–10
61. Kalia NP, Hasenoehrl EJ, Ab Rahman NB, Koh VH, Ang ML, Sajorda DR, Hards K, Grüber G, Alonso S, Cook GM (2017) Exploiting the synthetic lethality between terminal respiratory oxidases to kill *Mycobacterium tuberculosis* and clear host infection. Proc Natl Acad Sci 114(28):7426–7431
62. Lu P, Asseri AH, Kremer M, Maaskant J, Ummels R, Lill H, Bald D (2018) The anti-mycobacterial activity of the cytochrome bcc inhibitor Q203 can be enhanced by small-molecule inhibition of cytochrome bd. Sci Rep 8(1):2625. https://doi.org/10.1038/s41598-018-20989-8
63. Lu P, Heineke MH, Koul A, Andries K, Cook GM, Lill H, Van Spanning R, Bald D (2015) The cytochrome bd-type quinol oxidase is important for survival of *Mycobacterium smegmatis* under peroxide and antibiotic-induced stress. Sci Rep 5(1):1–10
64. Preiss L, Langer JD, Yildiz Ö, Eckhardt-Strelau L, Guillemont JEG, Koul A, Meier T (2015) Structure of the mycobacterial ATP synthase F_o rotor ring in complex with the anti-TB drug bedaquiline. Sci Adv 1(4):e1500106. https://doi.org/10.1126/sciadv.1500106
65. Koul A, Vranckx L, Dhar N, Göhlmann HW, Özdemir E, Neefs J-M, Schulz M, Lu P, Mørtz E, McKinney JD (2014) Delayed bactericidal response of *Mycobacterium tuberculosis* to bedaquiline involves remodelling of bacterial metabolism. Nat Commun 5(1):1–10
66. Moosa A, Lamprecht DA, Arora K, Barry CE, Boshoff HI, Ioerger TR, Steyn AJ, Mizrahi V, Warner DF (2017) Susceptibility of *Mycobacterium tuberculosis* cytochrome bd oxidase mutants to compounds targeting the terminal respiratory oxidase, cytochrome c. Antimicrob Agents Chemother 61(10)
67. Demitto FO, do Amaral RCR, Maltempe FG, Siqueira VLD, Scodro RBL, Lopes MA, Caleffi-Ferracioli KR, Canezin PH, Cardoso RF (2015) In vitro activity of rifampicin and verapamil combination in multidrug-resistant *Mycobacterium tuberculosis*. PloS One 10(2): e0116545
68. Mishra S, Shukla P, Bhaskar A, Anand K, Baloni P, Jha RK, Mohan A, Rajmani RS, Nagaraja V, Chandra N (2017) Efficacy of β-lactam/β-lactamase inhibitor combination is linked to WhiB4-mediated changes in redox physiology of *Mycobacterium tuberculosis*. Elife 6:e25624
69. Boshoff HI, Myers TG, Copp BR, McNeil MR, Wilson MA, Barry CE (2004) The transcriptional responses of *Mycobacterium tuberculosis* to inhibitors of metabolism novel insights into drug mechanisms of action. J Biol Chem 279(38):40174–40184
70. Jeong J-A, Park SW, Yoon D, Kim S, Kang H-Y, Oh J-I (2018) Roles of alanine dehydrogenase and induction of its gene in *Mycobacterium smegmatis* under respiration-inhibitory conditions. J Bacteriol 200(14)
71. Lu P, Lill H, Bald D (2014) ATP synthase in mycobacteria: special features and implications for a function as drug target. Biochim Biophys Acta (BBA)—Bioenerg 1837(7):1208–1218. https://doi.org/10.1016/j.bbabio.2014.01.022
72. von Ballmoos C, Cook GM, Dimroth P (2008) Unique rotary ATP synthase and its biological diversity. Annu Rev Biophys 37(1):43–64. https://doi.org/10.1146/annurev.biophys.37.032807.130018
73. Saw W-G, Wu M-L, Ragunathan P, Biuković G, Lau A-M, Shin J, Harikishore A, Cheung C-Y, Hards K, Sarathy JP, Bates RW, Cook GM, Dick T, Grüber G (2019) Disrupting

coupling within mycobacterial F-ATP synthases subunit ε causes dysregulated energy production and cell wall biosynthesis. Sci Rep 9(1):16759. https://doi.org/10.1038/s41598-019-53107-3
74. Rao SPS, Alonso S, Rand L, Dick T, Pethe K (2008) The protonmotive force is required for maintaining ATP homeostasis and viability of hypoxic, non-replicating *Mycobacterium tuberculosis*. Proc Natl Acad Sci USA 105(33):11945–11950. https://doi.org/10.1073/pnas.0711697105
75. Leone V, Faraldo-Gómez JD (2016) Structure and mechanism of the ATP synthase membrane motor inferred from quantitative integrative modeling. J Gen Physiol 148(6):441–457. https://doi.org/10.1085/jgp.201611679%JJournalofGeneralPhysiology
76. Dautant A, Velours J, Giraud M-F (2010) Crystal structure of the Mg·ADP-inhibited state of the Yeast F1c10-ATP synthase. J Biol Chem 285(38):29502–29510. https://doi.org/10.1074/jbc.M110.124529
77. Feniouk BA, Kato-Yamada Y, Yoshida M, Suzuki T (2010) Conformational transitions of subunit ε in ATP synthase from thermophilic Bacillus PS3. Biophys J 98(3):434–442. https://doi.org/10.1016/j.bpj.2009.10.023
78. Del Rizzo PA, Bi Y, Dunn SD (2006) ATP synthase b subunit dimerization domain: a right-handed coiled coil with offset helices. J Mol Biol 364(4):735–746. https://doi.org/10.1016/j.jmb.2006.09.028
79. Bai NJ, Pai MR, Murthy PS, Venkitasubramanian TA (1974) Effect of oxygen tension on the aldolases of *Mycobacterium tuberculosis* H37Rv. FEBS Lett 45(1):68–70. https://doi.org/10.1016/0014-5793(74)80812-4
80. Bai NJ, Pai MR, Murthy PS, Venkitasubramanian TA (1982) Fructose-bisphosphate aldolases from mycobacteria. Methods in enzymology 90 Pt Elsevier, pp 241–250. https://doi.org/10.1016/s0076-6879(82)90133-1
81. Rutter WJ (1964) Evolution of aldolase. Fed Proc 23:1248–1257
82. Rosenkrands I, Slayden RA, Crawford J, Aagaard C, Barry CE 3rd, Andersen P (2002) Hypoxic response of *Mycobacterium tuberculosis* studied by metabolic labeling and proteome analysis of cellular and extracellular proteins. J Bacteriol 184(13):3485–3491. https://doi.org/10.1128/jb.184.13.3485-3491.2002
83. Wayne LG, Hayes LG (1996) An in vitro model for sequential study of shiftdown of *Mycobacterium tuberculosis* through two stages of non-replicating persistence. Infect Immun 64(6):2062–2069. https://doi.org/10.1128/iai.64.6.2062-2069.1996
84. Zhang Y (2005) The magic bullets and tuberculosis drug targets. Annu Rev Pharmacol Toxicol 45:529–564. https://doi.org/10.1146/annurev.pharmtox.45.120403.100120
85. Ramsaywak PC, Labbé G, Siemann S, Dmitrienko GI, Guillemette JG (2004) Molecular cloning, expression, purification, and characterization of fructose 1,6-bisphosphate aldolase from *Mycobacterium tuberculosis*—a novel Class II A tetramer. Protein Expr Purif 37(1):220–228. https://doi.org/10.1016/j.pep.2004.05.011
86. Ramsaywak PC, Labbé G, Siemann S, Dmitrienko GI, Guillemette JG (2004) Molecular cloning, expression, purification, and characterization of fructose 1, 6-bisphosphate aldolase from Mycobacterium tuberculosis—a novel Class II A tetramer. Protein Expr Purif 37(1):220–228
87. Fonvielle M, Coinçon M, Daher R, Desbenoit N, Kosieradzka K, Barilone N, Gicquel B, Sygusch J, Jackson M, Therisod M (2008) Synthesis and biochemical evaluation of selective inhibitors of class II fructose bisphosphate aldolases: towards new synthetic antibiotics. Chemistry (Weinheim an der Bergstrasse, Germany) 14(28):8521–8529. https://doi.org/10.1002/chem.200800857
88. Pegan SD, Rukseree K, Franzblau SG, Mesecar AD (2009) Structural basis for catalysis of a tetrameric class IIa fructose 1,6-bisphosphate aldolase from *Mycobacterium tuberculosis*. J Mol Biol 386(4):1038–1053. https://doi.org/10.1016/j.jmb.2009.01.003
89. Cooper SJ, Leonard GA, McSweeney SM, Thompson AW, Naismith JH, Qamar S, Plater A, Berry A, Hunter WN (1996) The crystal structure of a class II fructose-1,6-bisphosphate

aldolase shows a novel binuclear metal-binding active site embedded in a familiar fold. Structure (London, England : 1993) 4(11):1303–1315. https://doi.org/10.1016/s0969-2126(96)00138-4
90. Boyer PD, Krebs EG (1986) The enzymes. Academic Press
91. Chavadi S, Wooff E, Coldham NG, Sritharan M, Hewinson RG, Gordon SV, Wheeler PR (2009) Global effects of inactivation of the pyruvate kinase gene in the *Mycobacterium tuberculosis* complex. J Bacteriol 191(24):7545–7553. https://doi.org/10.1128/jb.00619-09
92. Keating LA, Wheeler PR, Mansoor H, Inwald JK, Dale J, Hewinson RG, Gordon SV (2005) The pyruvate requirement of some members of the *Mycobacterium tuberculosis* complex is due to an inactive pyruvate kinase: implications for in vivo growth. Mol Microbiol 56(1):163–174. https://doi.org/10.1111/j.1365-2958.2005.04524.x
93. Noy T, Vergnolle O, Hartman TE, Rhee KY, Jacobs WR Jr, Berney M, Blanchard JS (2016) Central role of pyruvate kinase in carbon co-catabolism of *Mycobacterium tuberculosis*. J Biol Chem 291(13):7060–7069. https://doi.org/10.1074/jbc.M115.707430
94. Wayne LG, Hayes LG (1998) Nitrate reduction as a marker for hypoxic shiftdown of *Mycobacterium tuberculosis*. Tuber Lung Dis 79(2):127–132. https://doi.org/10.1054/tuld.1998.0015
95. Weber I, Fritz C, Ruttkowski S, Kreft A, Bange FC (2000) Anaerobic nitrate reductase (narGHJI) activity of *Mycobacterium bovis* BCG in vitro and its contribution to virulence in immunodeficient mice. Mol Microbiol 35(5):1017–1025. https://doi.org/10.1046/j.1365-2958.2000.01794.x
96. Stewart V (1988) Nitrate respiration in relation to facultative metabolism in enterobacteria. Microbiol Rev 52(2):190–232
97. Fritz C, Maass S, Kreft A, Bange F-C (2002) Dependence of *Mycobacterium bovis* BCG on anaerobic nitrate reductase for persistence is tissue specific. Infect Immun 70(1):286–291
98. Sohaskey CD, Wayne LG (2003) Role of narK2X and narGHJI in hypoxic upregulation of nitrate reduction by *Mycobacterium tuberculosis*. J Bacteriol 185(24):7247–7256. https://doi.org/10.1128/jb.185.24.7247-7256.2003
99. Akhtar S, Khan A, Sohaskey CD, Jagannath C, Sarkar D (2013) Nitrite reductase NirBD is induced and plays an important role during in vitro dormancy of *Mycobacterium tuberculosis*. J Bacteriol 195(20):4592–4599
100. Izumi A, Schnell R, Schneider G (2012) Crystal structure of NirD, the small subunit of the nitrite reductase NirbD from *Mycobacterium tuberculosis* at 2.0 Å resolution. Proteins: Struct Funct Bioinf 80(12):2799–2803. https://doi.org/10.1002/prot.24177
101. Barry VC, Belton J, Conalty ML, Denneny JM, Edward DW, O'sullivan J, Twomey D, Winder F (1957) A new series of phenazines (rimino-compounds) with high anti-tuberculosis activity. Nature 179(4568):1013–1015
102. O'connor R, O'sullivan J, O'kennedy R (1995) The pharmacology, metabolism, and chemistry of Clofazimine. Drug Metab Rev 27(4):591–614
103. Reddy VM, O'Sullivan JF, Gangadharam PR (1999) Antimycobacterial activities of riminophenazines. J Antimicrob Chemother 43(5):615–623
104. Yano T, Kassovska-Bratinova S, Teh JS, Winkler J, Sullivan K, Isaacs A, Schechter NM, Rubin H (2011) Reduction of clofazimine by mycobacterial type 2 NADH: quinone oxidoreductase a pathway for the generation of bactericidal levels of reactive oxygen species. J Biol Chem 286(12):10276–10287
105. Grosset JH, Tyagi S, Almeida DV, Converse PJ, Li S-Y, Ammerman NC, Bishai WR, Enarson D, Trébucq A (2013) Assessment of clofazimine activity in a second-line regimen for tuberculosis in mice. Am J Respir Crit Care Med 188(5):608–612
106. Amaral L, Kristiansen JE, Abebe LS, Millett W (1996) Inhibition of the respiration of multi-drug resistant clinical isolates of *Mycobacterium tuberculosis* by thioridazine: potential use for initial therapy of freshly diagnosed tuberculosis. J Antimicrob Chemother 38(6):1049–1053. https://doi.org/10.1093/jac/38.6.1049

107. Amaral L, Kristiansen JE, Viveiros M, Atouguia J (2001) Activity of phenothiazines against antibiotic-resistant *Mycobacterium tuberculosis*: a review supporting further studies that may elucidate the potential use of thioridazine as anti-tuberculosis therapy. J Antimicrob Chemother 47(5):505–511. https://doi.org/10.1093/jac/47.5.505
108. Viveiros M, Amaral L (2001) Enhancement of antibiotic activity against poly-drug resistant *Mycobacterium tuberculosis* by phenothiazines. Int J Antimicrob Agents 17(3):225–228
109. Dutta NK, Mehra S, Kaushal D (2010) A *Mycobacterium tuberculosis* sigma factor network responds to cell-envelope damage by the promising anti-mycobacterial thioridazine. PLoS ONE 5(4):e10069. https://doi.org/10.1371/journal.pone.0010069
110. Yano T, Li L-S, Weinstein E, Teh J-S, Rubin H (2006) Steady-state kinetics and inhibitory action of antitubercular phenothiazines on *Mycobacterium tuberculosis* type-II NADH-menaquinone oxidoreductase (NDH-2). J Biol Chem 281(17):11456–11463
111. Sellamuthu S, Bhat MF, Kumar A, Singh SK (2018) Phenothiazine: a better scaffold against tuberculosis. Mini Rev Med Chem 18(17):1442–1451
112. Pethe K, Bifani P, Jang J, Kang S, Park S, Ahn S, Jiricek J, Jung J, Jeon HK, Cechetto J (2013) Discovery of Q203, a potent clinical candidate for the treatment of tuberculosis. Nat Med 19(9):1157–1160
113. Andries K, Verhasselt P, Guillemont J, Göhlmann HW, Neefs J-M, Winkler H, Van Gestel J, Timmerman P, Zhu M, Lee E (2005) A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. Science 307(5707):223–227
114. Haagsma AC, Abdillahi-Ibrahim R, Wagner MJ, Krab K, Vergauwen K, Guillemont J, Andries K, Lill H, Koul A, Bald D (2009) Selectivity of TMC207 towards mycobacterial ATP synthase compared with that towards the eukaryotic homologue. Antimicrob Agents Chemother 53(3):1290–1292
115. Lakshmanan M, Xavier AS (2013) Bedaquiline-The first ATP synthase inhibitor against multi drug resistant tuberculosis. J Young Pharm 5(4):112–115
116. de Jonge MR, Koymans LHM, Guillemont JEG, Koul A, Andries K (2007) A computational model of the inhibition of *Mycobacterium tuberculosis* ATPase by a new drug candidate R207910. Proteins: Struct Funct Bioinform 67(4):971–980. https://doi.org/10.1002/prot.21376
117. British Thoracic Association (1982) A controlled trial of six months chemotherapy in pulmonary tuberculosis. Second report: results during the 24 months after the end of chemotherapy. Am Rev Respir Dis 126(3):460–462. https://doi.org/10.1164/arrd.1982.126.3.460
118. Zhang Y, Shi W, Zhang W, Mitchison D (2013) Mechanisms of pyrazinamide action and resistance. Microbiol Spectr 2(4):1–12. https://doi.org/10.1128/microbiolspec.MGM2-0023-2013
119. Whitfield MG, Soeters HM, Warren RM, York T, Sampson SL, Streicher EM, van Helden PD, van Rie A (2015) A global perspective on pyrazinamide resistance: systematic review and meta-analysis. PLoS ONE 10(7):e0133869. https://doi.org/10.1371/journal.pone.0133869

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Drug Discovery for Non-tuberculous Mycobacteria: Recent Updates

26

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Twenty years from now you will be more disappointed by the things that you didn't do than by the ones you did do.

Mark Twain

Summary

Mycobacterium is a genus that includes several pathogenic species. Species causing tuberculosis (TB) and leprosy are well-known, whereas species responsible for causing diseases other than TB and leprosy remain largely neglected. The latter coming under non-tuberculous mycobacteria (NTM) can cause high morbidity and mortality due to their drug resistance mechanisms against many antibiotics. An alarming rise in NTM diseases has been evident in several reports. Meanwhile, the population of vulnerable people, i.e., patients with diabetes, cancer, immunocompromised conditions, AIDS, chronic obstructive pulmonary disease, etc., is also rising. Yet, NTM is a neglected health issue in most healthcare systems. Additional challenges are the pathophysiological

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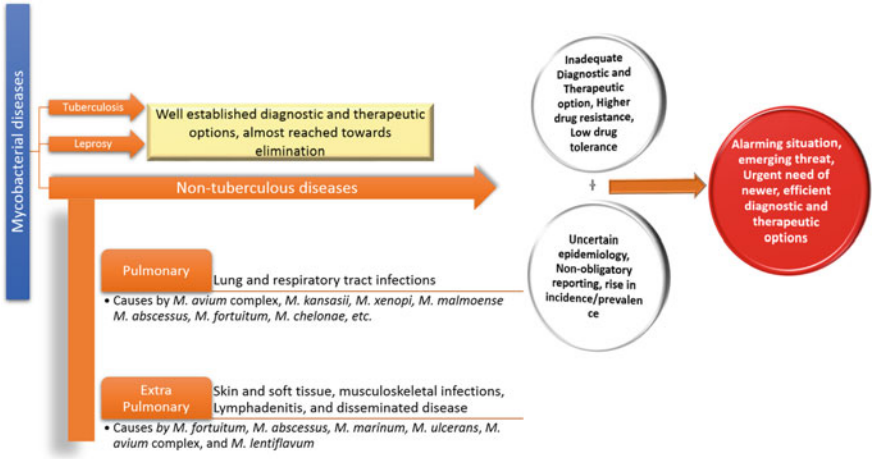
and microbiological similarity of NTM diseases with TB and the absence of well-developed diagnostics for NTM diseases. Hence, we urgently need efficient diagnostic and therapeutic options to manage NTM diseases.

Graphical Abstract



Non-tuberculosis mycobacterium (NTM): emerging threat and challenges

Keywords

Antibiotic · Drug resistance · Infectious disease · Mycobacterium · Non-tuberculous mycobacteria · Neglected disease · NTM

1 Introduction

The genus *Mycobacterium* is an extremely diverse class that includes over 200 bacterial species, characterized by the mycolic acid-rich, thick cell wall, branched filamentous growth, and high guanine and cytosine content. Approximately 95% of Mycobacteria are true saprophytes, ubiquitously present in many niches such as soil, water, and air in the free-living state. Only a few species are known to be pathogenic to humans and animals; they can be a strict or opportunistic pathogen, having the ability to cause pulmonary or extrapulmonary diseases [1, 2]. Tuberculosis (TB) and leprosy have affected humankind for centuries, as described in different ancient texts and scriptures. Additionally, DNA sequences belonging to the *Mycobacterium tuberculosis (M. tb)* complex (MTBC) isolated from fossils of ancient humans date back to 3000 years, giving scientific evidence to this assumption that our battle with

these mycobacterial pathogens is not new. However, the association of these mycobacterial pathogens with two notable diseases, i.e., TB and leprosy, was only established in the nineteenth century [2–4]. Non-tuberculous mycobacteria (NTM) are mycobacterial pathogens associated with diseases other than TB and leprosy [1].

From the historical perspective, the first description of NTM can be traced back to the nineteenth century–almost when Mtb was discovered. However, the burden of NTM diseases was not well-documented, their clinical impact was considered negligible, and they got neglected due to the broader prevalence of TB and leprosy. Indeed, almost all major diagnostic and therapeutic development was made for TB and leprosy [2, 3, 5], which is while the clinical relevance of NTM pathogens has dramatically increased after the acquired immunodeficiency syndrome (AIDS) epidemic, as reflected in the increased incidence of pulmonary diseases due to *Mycobacterium avium* complex (MAC) and others. This awareness of the comorbid situation of AIDS and NTM disease led to some significant development in the new diagnostics and therapeutics for NTM. Keeping in mind that the NTM-related morbidity and mortality are especially high in immunodeficient people, but also they are rising in immunocompetent patients, and given that besides people with AIDS, the number of people with diabetes, chronic obstructive pulmonary diseases (COPD), and cystic fibrosis (CF), who are vulnerable to NTM infections, too, is increasing, there is a need for more focused studies of NTM as emerging pathogens [6].

Diagnostics and therapeutics options for mycobacterial diseases are mainly limited to TB and leprosy, with very few efficient therapeutics options are available for infections due to NTM. This often leads to misdiagnosis of NTM infections. It usually bears symptomatic similarity with TB, and lack of potent sterilizing therapeutic options makes the infections often untreatable, leading to high morbidity and mortality [7–9].

2 Classification of Mycobacteria

There are three broad categories of the mycobacterial pathogens [2, 5, 10, 11]:

1. MTBC includes mycobacterial species associated with TB: *M.tuberculosis, M. bovis, M. africanum, M. microti,* and *M. canetti*;
2. NTM includes all other pathogenic mycobacteria that cause diseases other than TB and leprosy. They have been further classified into four different groups based on their cultural characteristics, such as pigmentation and growth rate (as suggested by Runyon) [11];

3. *M. leprae and M. lepromatosis* are two species responsible for causing leprosy; they are strictly parasitic and have undergone extreme genome reduction during evolution and lost their essential genes required to grow independently under laboratory conditions.

NTM, in turn, includes two major subgroups:

1. slowly-growing mycobacteria (SGM) species take more than one week to produce visible colonies on solid media; they belong to Runyon class I, II, and III based on their pigmentation pattern:
   - photochromogenic or Runyon I SGM species produce pigment upon the exposure of light: *M. kansasii, M. marinum, M. simiae, M. asiaticum*;
   - scotochromogenic or Runyon II species are SGMs whose pigmentation does not depend on the presence or absence of light: *M. szulgai, M. xenopi, M. lentiflavum*;
   - nonchromogenic slow growers or Runyon III species do not produce any pigmentation, or their pigmentation is almost negligible; they take more than one week to make a visible colony on solid media: *M. avium, M. intracellulare, M. haemophilum, M. celatum, M. bronderi, M. shimoide*; and
2. rapidly-growing mycobacteria (RGM) or Runyon IV species show no pigmentation or negligible pigmentation that produce visible colony on solid media in less than one week: *M. abscessus, M. chelonae, M. fortuitum, M. peregrinum, M. mucogenum*, etc. (Table 1).

3 Disease Burden

In the absence of any obligatory guidelines related to reporting NTM infections in most healthcare systems worldwide, the burden of these infections cannot be accurately estimated [10]. Recently, several reports suggest a rise in clinical cases of NTM infections [3–10, 12–14].

In the last two decades, a significant rise in the prevalence of pulmonary NTM diseases was seen as evident from the study by Marass et al., indicating a surge of prevalence rate from 9.1 to 14.1 NTM cases per one lakh population per year in North America between 1997 and 2003 [15]. Similarly, there was a rise in the

Table 1 Characteristic differences of slow growing mycobacteria and rapid growing mycobacteria

Characteristic	Slow growers	Fast growers
Doubling time	>12 h	<6 h
Visible colony on agar plates	More than a week	Less than 7 days
Pigmentation of colony	Can be pigmented or non-pigmented	Non-pigmented or negligible pigment

Table 2 Increasing prevalence of pulmonary NTM diseases across the different geographical conditions/population

Population	Earlier prevalence		Increased prevalence		References
	Prevalence/100,000 population	During year	Prevalence/100,000 population	During year	
Canada	9.1	1997	14.1	2003	[15]
United States	19.6	1994–1996	26.7	2004–2006	[16]
Australia	5.5	1999	10.2	2005	[17]
Asia	1.3	2000	7.9	2008	[17, 18]
Taiwan	2.7	2000	10.2	2008	[18]
Europe	0.9	1995	2.9	2006	[19, 20]

prevalence of pulmonary NTM disease in the elderly population aged over 60 years from 19.6 cases per 100,000 population in a year during 1994–1996 to 26.7 cases per 100,000 population in a year during 2004–2006 [16]. There have been reports of a similar increase in prevalence from other geographical areas, as summarized in Table 2.

However, most of these reports are based mainly on a specific geographical area [8, 9, 12–14]. A bird's eye view of the literature reveals differential clinical relevance of different NTM species varying greatly with the patient's geographical area, comorbid conditions, and age [14]. Several studies indicate MAC as the most prevalent NTM. MAC causes pulmonary diseases, particularly in AIDS and COPD patients [9, 12]. A global survey led by "Nontuberculous Mycobacteria Network European Trials Group" (NTM-NET) showed the wider prevalence of SGM species in sputum samples around the world. Around ~84% of NTM isolates were SGM, and the rest 16% isolates were RGM species. Overall, MAC was the most prevalent species (47% of total isolates), and almost half of NTM infections are caused by MAC species. After MAC, other prevalent SGM species were *M. gordonae, M. xenopi,* and *M. kansasii.* However, *M. gordonae* is considered a non-pathogenic, true-environmental mycobacterium most often isolated in sputum samples. Table 3 summarizes the outcome of the global survey by NTM-NET, showing continent-wise variation in the prevalence of different NTM species [19].

Most NTM diseases are associated with environmental exposure to pathogens rather than person-to-person transmission; their prevalence, thus, depends greatly on geographical conditions [5, 6]. However, *M. abscessus* has shown person-to-person transmission among patients with cystic fibrosis (CF). This is especially scary as *M. abscessus* is considered a nightmare of the healthcare system due to its intrinsic resistance to most approved drugs [14, 21].

Together the literature hints about an alarming rise in the occurrence and prevalence of NTM diseases in relation to various hazard factors such as immunosuppression, age, ethnicity, and history of other pulmonary diseases.

Table 3 Continent-wise variation in the distribution of different NTM isolates among the patients of pulmonary NTM diseases

Name of continent	%MAC among NTM isolates	% of slow growers isolates other than MAC					% of rapid growers isolates
		M. gordonae	*M. xenopi*	*M. kansasii*	*M. malmoense*	Other slow growers	
Asia	54	6	NA	3	NA	6	31
Australia	71	2	NA	4	NA	8	15
Europe	37	17	14	5	1	10	16
South Africa	50	5	1	3	NA	33	7
South America	31	17	NA	20	NA	11	21
North America	52	12	12	1	NA	3	20
Overall	47	11	8	3	1	14	16

4 Present Guidelines Regarding NTM Diseases

In 2007, the first official guidelines regarding diagnosis, treatment, and prevention of NTM diseases were jointly published by the American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA) [1]. Afterward, the British Thoracic Society (BTS) also published its guidelines for NTM diseases in 2017, based mainly on ATS/IDSA guidelines [3, 22].

4.1 Diagnosis of NTM Infections

As per ATS/IDSA guidelines [1], diagnostic criteria of pulmonary NTM disease are:

- chest X-ray that shows cavitation as a sign of necrosis or abscess. If no cavitation is seen in the chest X-ray, chest high-resolution computed tomography (HRCT) is indicated;
- the presence of acid-fast bacilli (AFB) in three or more sputum specimen analyses; and
- exclusion of TB and other diseases.

All clinical, microbiological, and radiographic criteria are equally important and must be met to diagnose pulmonary NTM infection. Multifocal bronchiectasis and multiple small nodules in HRCT scans and opacities, nodulation, and cavitation in chest X-ray are characteristic features of NTM lung diseases due to infection with MAC, *M. kansasii,* and *M. abscessus*. However, little is known about the pathological features of other respiratory NTM infections, so the diagnostic criteria outlined above cannot be applied to all pulmonary NTM diseases. Therefore, species-level identification by high-performance liquid chromatography (HPLC) is recommended for NTM diseases caused by RGM species such as *M. abscessus, M. fortuitum,* and *M. chelonae,* along with routine susceptibility testing for amikacin, tobramycin, doxycycline, fluoroquinolones, sulphonamides, cefoxitin, imipenem, linezolid and clarithromycin [1, 3, 22].

4.2 Therapeutics for NTM Infections

4.2.1 Pulmonary NTM Diseases

The ATS/IDSA and BTS guidelines for managing pulmonary NTM diseases recommend a combination of macrolides with anti-TB drugs like isoniazid, rifampicin, ethambutol, and injectable drugs, e.g., amikacin or streptomycin, until culture-negative on therapy after one year. The common NTM species associated with pulmonary infections are MAC, *M. kansasii, M. malmoense, M. xenopi*, *M. abscessus, M. fortuitum,* and *M. chelonae.* Therapeutic recommendations depend on NTM pathogen and type of diseases [1, 3, 22].

4.2.2 MAC

It is the most common species frequently isolated from patients with pulmonary NTM diseases [3, 12]. Before initiating the treatment regimen, susceptibility testing of isolates for amikacin and clarithromycin should be considered as well as the severity of the disease and the patient's tolerance of therapeutic indications [1, 22]. If a patient fails to respond to the macrolide treatment regimen and MAC reappears after culture conversion, susceptibility testing for a broader panel of macrolides is recommended.

Therapeutic recommendations for clarithromycin-sensitive MAC isolates include rifampin and ethambutol along with clarithromycin or azithromycin daily or intermittently three times a week. The regimen should be given until one-year sputum negative on therapy [1, 22]. On the other hand, an intermittent treatment regimen should not be used for someone who has severe MAC pulmonary diseases or a history of treatment failure. Furthermore, macrolide monotherapy or a dual therapy regimen containing macrolides and quinolones should not be used for MAC pulmonary diseases [1, 22].

4.2.3 *M. kansasii*

The treatment regimen for pulmonary NTM diseases caused by *M. kansasii* infection contains a daily dose of isoniazid, rifampin, ethambutol, or a macrolide (azithromycin or clarithromycin) up to one year from culture-negative on therapy [1]. However, the susceptibility of isolates for rifampin should be tested before initiating the regimen, and resistant strains should be tested for a broader panel of antibiotics to guide the most suitable therapeutic recommendation [1, 3, 22]. The treatment regimen for resistant strains must include a combination of three drugs based on susceptibility isolates [1, 22].

4.2.4 *M. abscessus*

In the case of *M. abscessus* pulmonary disease, no treatment regimen has any proven or predictable efficacy. A multidrug treatment regimen containing clarithromycin may relieve symptoms and somewhat reduce the severity of the disease. Still, surgical resection of affected parts in case of localized disease and multidrug therapy containing clarithromycin offer the best chances for cure [1, 3].

4.2.5 *M. malmoense*

The treatment regimen contains a daily oral dose of rifampin, ethambutol, and azithromycin or clarithromycin [1, 3, 10].

4.2.6 *M. xenopi*

A four-drug treatment regimen containing rifampin, ethambutol, and a macrolide (azithromycin or clarithromycin) along with isoniazid or a fluoroquinolone (ciprofloxacin or moxifloxacin) [1, 22] is recommended.

4.3 Treatment for Extrapulmonary NTM Disease

Several studies indicate that 20–30% of NTM diseases are extrapulmonary, including skin and soft tissue, lymphadenitis, musculoskeletal, and disseminated disease [3, 23–26].

4.3.1 Skin and Soft Tissues Disease

In most cases, these diseases are associated with surgical or cosmetic procedures and traumatic injuries [3, 24]. *M. fortuitum* is the most common RGM species associated with skin and soft tissue infections resulting from traumatic injuries and invasive surgical procedures. Thankfully, *M. fortuitum* is susceptible to a broader range of antibiotics. The treatment regimen for *M. fortuitum* infections should include at least two drugs for four to six months depending on the severity of the disease; however, a monotherapeutic regimen containing tetracyclines is also efficient to treat those infections by *M. fortuitum* [1, 22, 25].

In the case of infections by *M. ulcerans* (Buruli ulcer), surgery is the ultimate cure for the disease. However, an eight-week therapeutic regimen containing rifampin and a quinolone or a macrolide was promising for treating Buruli ulcers. Mild, localized cutaneous diseases caused by *M. marinum* can be treated with a combination of clarithromycin, rifampin, or ethambutol for one to two months after lesions resolve. However, surgical resection is necessary for deep tissue infections [1, 22, 26].

4.3.2 Lymphadenitis

NTM infections of the lymph node are commonly caused by MAC. However, *M. lentiflavum* is an emerging etiological agent causing lymph node infections [3, 27–29]. The therapeutic regimen is similar to other NTM diseases, containing a macrolide or fluoroquinolone. Still, surgical intervention is more effective and curative in most cases, even without antibiotics [1, 28, 29].

4.3.3 Disseminated Infection

Earlier, this type of infection by MAC was common among patients with end-stage HIV infection. Fortunately, a sharp decline in disseminated NTM diseases has been seen after introducing a HAART-based treatment regimen among AIDS patients; however, some disseminated infection cases still occur, mainly associated with the intravascular catheter and implants [3, 30, 31].

5 Therapeutic Options for NTM Diseases

Treatment regimens approved for NTM infections are discussed below, with their minimum inhibitory concentrations (MIC) in Table 4.

Table 4 Minimum inhibitory concentration (MIC) of different drugs (presently approved and repurposed) along with their categorical description

Drug name	Description	MIC (μg/ml)					References
		M. avium complex		Rapid growers			
		M. intracellulare	*M. avium*	*M. fortuitum*	*M. abscessus*	*M. chelonae*	
Azithromycin	Macrolide	0.03	4	2	0.03	0.5	[32]
Clarithromycin		0.12	0.5	1	0.03	0.06	
Amikacin	Aminoglycoside	0.06	2	0.25	1	4	
Kanamycin		0.5	1	4	2	16	
Tobramycin		0.5	0.5	16	8	2	
Streptomycin		0.25	2	32	16	32	
Ciprofloxacin	Fluoroquinolone	0.03	2	0.03	2	4	
Moxifloxacin		0.06	0.5	0.03	1	4	
Ofloxacin		0.03	2	0.12	4	32	
Levofloxacin		0.25	1	0.03	1	16	
Rifampin	First-line anti-TB drug	0.03	0.25	128	64	>256	
Ethambutol		0.25	8	256	32	128	
Isoniazid		0.5	16	64	>256	>256	
Clofazimine	Antileprotic drug	0.12	16	0.03	0.06	0.25	
Thioacetazone	Antitubercular agent	0.25	257	256	16	>256	
Dapsone	Antileprotic drug	0.25	257	8	64	32	
Delamanid	Newer antitubercular drugs	>32	0.25	>32	>32	>32	[33]
Pretomanid		>100	>100	>100	>100	>100	[34]
Bedaquiline		<0.008	<0.008	0.5	0.5	2	[33]
Linezolid	Oxazolidinone	0.06	32	8	4	8	[32]
Tedizolid		>32	>32	2	8	2	[35]

(continued)

Table 4 (continued)

Drug name	Description	MIC (μg/ml)					References
		M. avium complex		Rapid growers			
		M. intracellulare	*M. avium*	*M. fortuitum*	*M. abscessus*	*M. chelonae*	
SPR719/720	Pipeline drug for NTM diseases	2	2	1	4	4	[36]
Minocycline	Newer tetracyclines	0.5	32	8	2	64	[32]
Doxycycline		0.25	257	0.03	32	32	
Omadacycline				0.03–1	0.06–8	0.015–0.25	[37]
Tigecycline		0.25	64	16	128	32	[32]
Meropenem	β-lactam antibiotics	0.5	257	4	64	>256	
Cefoxitin		0.5	257	32	32	128	
Cefepime		4	257	>256	64	256	
Cefoperazone		8	128	>256	>256	>256	
Cefmetazole		1	257	4	32	0.25	

5.1 Macrolides

They are the first choice to treat most NTM infections [1, 3, 22]. Macrolides are a potent inhibitor of bacterial protein synthesis by preventing bacterial peptidyl transferase activity by reversible binding at the p site of the 50s subunit of the bacterial ribosome [38]. Azithromycin (250–500 mg/day) and clarithromycin (500–1000 mg/day) are two very potent drugs belonging to macrolides that are recommended for the treatment of several NTM diseases in combination with other drugs or as a part of monotherapeutic regimen [1, 22].

5.2 Aminoglycosides

Aminoglycosides inhibit bacterial protein biosynthesis by disturbing bacterial peptide elongation after binding at the 30s subunit of the ribosome [10]. Aminoglycosides are naturally derived compounds containing an amino-modified glycoside residue in their structure [39]. Streptomycin was the first aminoglycoside antibiotic and first anti-TB agent. Injectable aminoglycosides such as amikacin and streptomycin are recommended for use in combination with other drugs to treat NTM diseases [1, 3, 10, 22, 39].

5.3 Fluoroquinolones

Fluoroquinolones are a group of broad-spectrum antibacterial compounds that inhibit bacterial DNA replication by inhibiting DNA gyrase, type II topoisomerase, or topoisomerase IV activity, leading to double-strand or single-strand breaks in the genome and therefore, causing cell death [40]. ATS/IDSA and BTS guidelines recommend fluoroquinolone (oral moxifloxacin 400 mg/daily) combined with other drugs like amikacin, rifampin, and ethambutol for treatment of severe to mild cases of macrolides-resistant as well as susceptible cases of NTM diseases [1, 3, 10, 22].

5.4 Anti-TB Drugs

Pathological and microbiological features of many pulmonary and extrapulmonary NTM diseases share similarities with TB, and anti-TB drugs are also effective against NTM infections. The treatment regimen containing first-line anti-TB agents, e.g., rifampin, ethambutol, isoniazid, macrolides, and aminoglycosides, is recommended in ATS/IDSA and BTS guidelines for the management of NTM diseases [1, 3, 10, 22].

5.5 Surgical Intervention

In case of localized infection or infections which not respond to any treatment regimen (especially in case of *M. abscessus*), surgical resection of the affected part or organ along with a multidrug therapeutic regimen containing azithromycin or clarithromycin is recommended. Similarly, in Buruli ulcers caused by *M. ulcerans* and localized cutaneous disease caused by *M. marinum,* especially in deep tissues, surgical intervention along with a multidrug therapeutic regimen is required for the ultimate cure of disease [1, 22].

5.6 New Drugs Against NTM

The following section lists newly approved drugs that are active against Mtb and possess anti-NTM activity.

5.6.1 Delamanid

It is a new anti-TB drug that got approval for the treatment of MDR-TB in 2012. It is a nitroimidazole that inhibits mycobacterial cell wall biosynthesis. It is moderately active against certain slow-growing NTMs, giving some promise towards the utilization of delamanid to manage certain NTM infections [33]. However, in a few other studies, delamanid has demonstrated high MIC value against all other NTM species except *M. kansasii* [41, 42].

5.6.2 Pretomanid

It is a pro-drug with a bicyclic nitroimidazole scaffold, which exerts anti-TB activity by inhibiting mycolic acid biosynthesis and generating reactive nitrogen species (RNS) [43, 44]. Pro-drug pretomanid gets activated by the deazaflavin-dependent nitroreductase (Ddn) enzyme present in Mtb and some NTM species such as *M. kansasii*. However, pretomanid is ineffective against other NTM pathogens such as *M. avium, M. chelonae, M. fortuitum,* etc. They lack the Ddn enzyme required to activate pretomanid [34, 42–45].

5.6.3 Bedaquiline

It is a new class of oral anti-TB drug that belongs to the diarylquinolines class. Its target is the mycobacterial ATP synthesis pathway [34, 41]. Bedaquiline has highly promising in vitro activity with a potent MIC against most NTM species. In summary, it is effective against *M. abscessus, M. avium complex, M. kansasii,* and macrolide-resistant NTM strains, which suggests the potentiality of bedaquiline for being used in the clinical management of NTM diseases. It, however, lacks bactericidal activity against *M. abscessus* and MAC [1, 3, 10, 34, 41].

Table 5 Representation of some promising novel compounds showing activity against NTMs

Compound	Comments	Activity against NTM species	MIC range	References
Indolecarboxamide analogs	Series of indolecarboxamide analogs targeting mycolic acid transport, structure activity relationship (SAR) study of those analogs	*M. abscessus* *M. chelonae* *M. xenopi* *M. bolletii* *M. massiliense* *M. avium* complex	0.06 to > 32 μg/ml	[46, 47]
TP-271	Tetracycline related compounds, belong to novel fluorocycline class of antimicrobial, shown in vitro activity against NTM isolates	*M. fortuitum* *M. abscessus*	0.5 μg/ml (M.ab.) 0.06 μg/ml (M.f.)	[48]
Capuramycin analogs	Nucleoside analogs targeting synthesis of peptidoglycan and inhibiting cell wall, showed activity against NTM in vitro	*M. avium complex* *M. abscessus* *M. smegmatis* *M. ulcerans* *M. kansasii (M.k.)* *M. paratuberculosis*	1–32 μg/ml (M.k.) 0.12–8 μg/ml (M.p.) 0.06 to > 32 μg/ml (MAC)	[49, 50]
CyCs	Analogs of cyclophostin and cyclipostins having in vitro and within macrophage activity against NTM. Possibly inhibits enzymes of lipid metabolism and/or cell wall	*M. marinum* *M. smegmatis* *M. abscessus*	0.18–26.2 μg/ml	[51, 52]
PIPD1	Piperidinol-based molecule acting on mycolic acid transport	*M. abscessus*	0.12 μg/ml	[53]
Salicylanilide esters, carbamates and benzoates	De novo synthesised molecules with in vitro efficacy against *M. abscessus*; ability to block various bacterial enzymes and serve as proton shuttles, breaking a cell proton gradient that destroys bacteria	*M. avium* *M. kansasii* *M. abscessus*	1–8 μM (*M. avium*) 0.5–4 μM (*M. kansasii*)	[54, 55]
ACH-702	Isothiazoloquinolones, quinolone-related analogues targeting bacterial replication; in vitro action against NTM	*M. avium* complex *M. fortuitum*	0.06 μg/ml (M.f.) 4 μg/ml (MAC)	[56]
IAPs	Imidazo [1,2-a]pyridine-3-carboxamide; in vitro and in vivo efficacy against MAC	*M. avium* complex	0.31–27.5 μM	[57]

5.7 Other Anti-mycobacterial at the Preclinical Stage

Several other compounds having anti-mycobacterial activity are summarized in Table 5.

5.7.1 Repurposed Drugs

The following section lists repurposed drugs that are active against NTM.

5.7.2 Clofazimine

It belongs to the riminophenazine class and is a well-known anti-leprotic agent. Clofazimine has been recently repurposed to treat patients with multidrug-resistant (MDR)-TB and extensively drug-resistant (XDR)-TB, but several reports also suggest the potentiality of clofazimine to manage NTM diseases [3, 10, 34, 58]. The efficacy of clofazimine has been studied in an observational cohort study in pediatric and adult CF patients with pulmonary or extrapulmonary NTM infections and non-CF patients having pulmonary or extrapulmonary NTM diseases. In this study, clofazimine was well tolerated, effective upon oral administration, and appeared safe [58]. Clofazimine demonstrates very potent anti-mycobacterial activity against *M. kansasii* and MAC. Several studies have also reported promising clinical efficacy of clofazimine in treating pulmonary disease caused by MAC [59, 60]. Clofazimine can be utilized as an alternative therapeutic intervention for managing MAC infections in patients who are intolerant or non-responsive to regular therapy. The efficacy of clofazimine against *M. kansasii* does not change with the addition of the reserpine, an efflux pump inhibitor.

Clofazimine demonstrates very potent activity against SGM, and it synergizes with amikacin, clarithromycin, and tigecycline in vitro [3, 10, 34]. In an experimental study, clofazimine was able to prevent the regrowth of *M. abscessus,* which had been previously exposed to amikacin and clarithromycin. Clinical use of clofazimine is increasing to treat pulmonary NTM disease caused by *M. abscessus*. Outcomes from a recent study on 42 patients with pulmonary NTM infection of *M. abscessus* have shown promising treatment results with a clofazimine-containing regimen on microbiological, radiological, and clinical parameters. In this study, clofazimine was moderately successful [3, 61]. However, further research is required for clinical efficacy, tolerability, and possible adverse effects of long-term clofazimine-containing regimens. Clofazimine, along with two or three additional oral antibiotics, plus daily oral macrolide and inhaled amikacin, is recommended by the European Cystic Fibrosis Society and U.S. Cystic Fibrosis Foundation for pulmonary *M. abscessus* disease among CF patients [3, 10].

Clofazimine is also helpful in shortening the treatment duration of Buruli ulcers when combined with a more potent drug. For example, high-dose rifamycins with clofazimine have better effectiveness in treating Buruli ulcers than a regimen containing rifamycin with macrolides (azithromycin or clarithromycin). Combined rifamycin and clofazimine regimen prevented the relapse of Buruli ulcer despite a shorter treatment regimen than rifamycin with macrolides [1, 3, 10, 58–63].

5.7.3 Linezolid

It is a broad-spectrum antibiotic that can treat infectious diseases caused by gram-positive bacteria resistant to other antibacterials. Linezolid belongs to the oxazolidinone class and acts by inhibiting bacterial protein synthesis. Linezolid has shown excellent activity against a panel of RGM and SGM species, including clinical and reference strains of *M. fortuitum, M. abscessus, M. chelonae, M. avium complex, M. simiae,* etc. [64, 65].

5.7.4 Tedizolid

It is a second-generation oxazolidinone, approved by the US Food and Drug Administration (USFDA) in 2014 to treat acute bacterial skin infections. It showed better activity against most NTM strains in comparison to linezolid. It has a significantly low MIC than linezolid against *M. fortuitum, M. abscessus,* and *M. chelonae* [35].

5.7.5 SPR719/720

It is a novel benzimidazole developed by Spero Therapeutics, Inc. USA to treat DR-TB and other bacterial diseases. It is currently in phase II clinical trials to treat NTM diseases. It inhibits bacterial gyrase B and topoisomerase IV (GyrB/ParE). It exhibits very potent MIC 0.03–5.48 μg/mL against both drug-susceptible and MDR-Mtb and MIC 0.1–2 μg/mL against different NTM strains, including MAC, *M. abscessus,* and *M. kansasii* [36].

5.7.6 Omadacycline

It is a recently approved broad-spectrum tetracycline used to treat community-acquired pneumonia and bacterial soft tissue and skin infections [3, 10]. It has shown promising activity in managing lower respiratory tract infections associated with *M. abscessus* and other NTM infections. A recent study showed clinical improvement of pulmonary NTM infection with a treatment regimen containing omadacycline combined with amikacin and aztreonam. Omadacycline is formulated for oral dosing. It offers a better pharmacokinetic profile than tigecycline. It maintains a high and stable concentration in plasma, epithelial lining fluid, and lung alveoli [37, 66–68].

5.7.7 Tigecycline

It belongs to glycylcyclines, a derivative of the tetracycline class. Tigecycline is used to treat complicated soft tissue and skin infections but not complicated intra-abdominal and diabetic foot infections [3]. It shows reliable in vitro activity against *M. abscessus, M. fortuitum,* and *M. chelonae,* but it is not effective against *M. avium* complex, *M. marinum,* and *M. kansasii*. The tigecycline-containing treatment regimen for *M. abscessus* could improve the condition in both radiological and clinical terms. However, the unacceptable cultural conversion rate, low patient tolerance, and lack of oral formulation are significant drawbacks. Before initiating the tigecycline-containing treatment regimen, a careful patient evaluation is required, along with monitoring adverse drug reactions [69–71].

5.7.8 β-lactams

An interest in β-lactam antibiotics has reignited after discovering that β-lactamase of Mtb and *M. abscessus* can be inhibited by clavulanic acid and avibactam [3, 72]. Combinations of ceftazidime and ceftaroline or ceftazidime and imipenem have exhibited very potent in vitro activity against *M. abscessus*. These combinations inhibited *M. abscessus* growth at submicromolar concentration and also showed bactericidal kinetics. These dual β-lactam combinations are effective in THP-1 infection models of *M. abscessus* [73]. In other studies, β-lactam antibiotics appeared effective in monotherapy and showed powerful synergy with rifampin and clarithromycin to treat Buruli ulcers caused by *M. ulcerans*. The activity was further improved when β-lactamase inhibitor (clavulanic acid) was included. These lines suggest the potentiality of amoxicillin/clavulanate for treating Buruli ulcers as a single drug treatment regimen or in combination with clarithromycin and rifampin [74].

5.7.9 Mefloquine

Mefloquine is a derivative of 4-quinolinemethanol generally used for prophylactic and therapeutic purposes against parasitic infections, especially for *Plasmodium falciparum*. However, different studies have shown that mefloquine has good in vitro efficacy against different clarithromycin susceptible and resistant strains of MAC (MIC 16 μg/ml). Similar efficacy of mefloquine is also reflected in the murine disease model of MAC infections [10, 75].

Interestingly, mefloquine has bactericidal activity against both clarithromycin susceptible and resistant strains, and it synergizes with moxifloxacin and ethambutol against MAC [75]. The combination of these three drugs (40 mg/kg dose of mefloquine, 100 mg/kg moxifloxacin, and ethambutol at 100 mg/kg) has shown approximately one $\log_{10}$ CFU reduction in one week and more than 2 $\log_{10}$ CFU reduction of clarithromycin resistant-MAC in liver and spleen in the murine disease model of MAC infection [75]. Mefloquine is a racemic mixture of four different stereoisomers of α-2-piperidinyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol, among those four isomers, (+)-Erythro-Mefloquine has shown a significantly greater reduction of MAC in liver and spleen of C57BL/6bg+/bg+ mice as compared with control [76].

5.7.10 Thioridazine

Thioridazine is an antipsychotic drug and exhibits anti-mycobacterial activity via inhibiting the electron transport chain in Mtb [10, 77]. It showed very excellent in vitro efficacy against MAC (MIC 25 μg/ml), the same activity reflected in the hollow-fibre system (HFS) lung disease model of MAC (HFS-MAC). In this disease model, thioridazine, in combination with moxifloxacin, has shown very rapid killing of MAC (> 5 $\log_{10}$ reductions of CFU/mL in seven days) [77, 78].

5.7.11 Disulfiram

It is a de-addiction medicine used to manage chronic alcoholism [3]. A study from our lab has indicated disulfiram has very potent in vitro activity against RGM NTM pathogen; its activity was better than β-lactam antibiotics (Table 6). Disulfiram

Table 6 MIC of disulfiram against rapid-growing NTM along with MIC of standard drugs

Drug	MIC (μg/ml)		
	M. fortuitum ATCC 6841	*M. chelonae* ATCC 35752	*M. abscessus* ATCC 19977
Disulfiram	32	32	32
Amikacin	2	2	16
Ceftazidime	>64	>64	>64
Ceftriaxone	>64	>64	>64
Meropenem	32	32	>64
Levofloxacin	0.03	0.12	2

demonstrated concentration and time-dependent bactericidal activity, as evident by its time-kill kinetic against *M. fortuitum* and *M. abscessus*. It showed synergistic interaction with fluoroquinolones (moxifloxacin, ciprofloxacin), aminoglycoside (amikacin), β-lactam (meropenem), oxazolidinone (linezolid), and glycopeptide antibiotics (vancomycin and teicoplanin) against *M. fortuitum* and *M. abscessus*. In the J774 macrophage cell line, disulfiram showed better intracellular killing activity against *M. fortuitum* and *M. abscessus* than amikacin ($\sim$1.8 $\log_{10}$ CFU reduction by disulfiram, $\sim$ 1 $\log_{10}$ CFU reduction by amikacin in comparison with untreated cells). We have further tested in vivo efficacy of disulfiram in the murine disease model of *M. fortuitum* and found a significant reduction ($\sim$1.4 $\log_{10}$ CFU) of the bacterial load in kidneys of animals treated with a 50 mg/kg dose of disulfiram in comparison with animal treated with placebo [79, 80].

5.7.12 Sildenafil

It is used to treat erectile dysfunction by inhibiting phosphodiesterase V, which influences the nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) pathway. Pulmonary NTM diseases correlate with lower NO levels and reduced ciliary beat frequency (CBF) in respiratory epithelial cells. NO donors are known to increase mucociliary activity. Patients with pulmonary NTM disease treated with sildenafil exhibit increased ciliary beat frequency. Pulmonary NTM diseases are chronic diseases and most often life-long. Hence, a medication that can relieve symptoms of diseases by altering CBF, sputum production, etc., can be used for palliative care of patients suffering from non-curable NTM diseases. A clinical trial (NCT01853540) on sildenafil by the National Institute of Allergy and Infectious Diseases (NIAID), USA, aimed at this goal. Sildenafil could relieve patients' symptoms, as assessed using the St. George's Respiratory Questionnaire (SGRQ), and enhance CBF; however, these changes were not significant in reducing the clinical severity of disease [81].

5.8 Alternative Approaches for the Treatment of NTM Infections

5.8.1 Antimicrobial Peptides (AMPs)

AMPs include a diverse range of small peptide molecules that possess antibacterial properties against many microbial pathogens, including viruses, bacteria, fungi, and protozoans. AMPs are commonly found in nature, i.e., they can be isolated from almost all living species through the process of bioprospecting, as these AMPs are an integral part of the innate defense system of most living creatures. There is a huge diversity among different AMPs. Every living organism has to cope with a range of different enemies (pathogens), so AMPs from different species vary in length, peptide sequence, and spectrum of activity. Due to such a large heterogeneity among different AMPs, a well-established understanding of the mechanism of actions and a possible common target of these AMP is lacking [10]. Some may agree that they interfere with cell membranes of pathogens, which may contribute to cell death; these AMPs can also act intracellularly by interfering in the normal functioning of crucial biomolecules, i.e., DNA, RNA, or proteins. Besides that, some AMPs also possess immunomodulatory properties. For these reasons, AMPs are also referred to as host defense peptides. These versatilities increase the effectiveness of AMPs, but above all, it allows them to avoid possible microbial resistance. Other benefits of AMPs over antibiotics are their equipotent activity against metabolically active and inactive pathogens.

Certain AMPs have been identified as active against mycobacteria, either by direct killing or immunomodulation. Most notable is the human peptide cathelicidin LL-37, which exerts an immunomodulatory effect on the vitamin D signaling pathway and induces autophagy and phagosome maturation of macrophages infected with mycobacteria. Some studies have shown that mycobacterial infections upregulate the development of cathelicidin and certain defensins such as human neutrophil peptides (HNP) and β-defensins. Several studies have indicated the potential bactericidal or bacteriostatic effect of certain AMPs against *M. avium, M. abscessus, M. chelonae, M. marinum, M. massiliense,* and *M. kansasii*. As we are seeing a growing number of scientific literature on discovering various AMPs active against NTM pathogens, we are optimistic about the therapeutic utilization of these small peptides in the battle against mycobacterial diseases. Some promising AMPs active against NTMs are summarized in Table 7.

5.8.2 Bacteriophages

The rise of DR bacteria has alarmed the scientific community to look towards some non-conventional approaches, such as using bacteriophages to deal with infections caused by NTMs [10]. The use of bacteriophage has many possible benefits as an alternative to antimicrobial drugs and working with them in synergy. Bacteriophages do not infiltrate cells other than bacterial targets, producing no damage to host cells and no possible detrimental consequences on the patient. Bacteriophages can be administered at very low dosages as they multiply within the pathogenic bacterial host [10, 94]. Moreover, they act more quickly than antibacterial drugs,

Table 7 Some potential antimicrobial peptides (AMPs) showing activity against NTMs

AMPs	Origin	NTM spectrum	Activity exhibited in	References
LL-37	Human cathelicidin	*M. avium*	Macrophage	[82]
Lacticin 3147	*Lactococcus lactis*	*M. avium* *M. kansasii*	Axenic	[83]
Nisin	*Lactococcus lactis*	*M. paratuberculosis*	Axenic	[84]
Nisin A, S, T, and V	*Lactococcus lactis*	*M. kansasii* *M. avium*	Axenic	[85]
Ecumicin	*Actinomycetes* extracts	*M. avium* *M. kansasii* *M. chelonae* *M. abscessus* *M. marinum*	Axenic	[86]
Lassomycin	*Actinomycetes* extracts	*M. avium*	Axenic	[87]
NDBP-5.5	Scorpion (*Hadrurus gertschi*)	*M. abscessus*	Anexic, macrophagic and in vivo	[88]
Polydim-I	Wasp (*Polybia dimorpha*)	*M. abscessus*	Anexic, macrophagic and in vivo	[89]
Polybia-MPII	Wasp's Mastoparans (*Pseudopolybia vespiceps*)	*M. abscessus sp. massiliense*	Axenic and Macrophagic	[90]
Mcdef	Manila clams (*Ruditapes philippinarum*)	*M. fortuitum*	Axenic	[91]
LFcin17-30 and variants	Bovine lactoferricin	*M. avium*	Axenic and macrophagic	[92, 93]

and their antibacterial activity is independent of the metabolic state of the bacterium. However, eukaryotic cell membranes are nearly impermeable for bacteriophages; thus, assisted delivery of bacteriophages inside the intracellular space of infected cells is required in the case of NTM pathogens that reside within host cells. Encapsulation of TM4 mycobacteriophage in a giant liposomal body has been successfully used to deliver phages inside the THP-1 monocytic cells infected with *Mycobacterium* [95]. Subsequent studies have shown the in vitro and in vivo efficacy of TM4 mycobacteriophage against Mtb and *M. avium*; it substantially reduces the bacterial burden [96]. Out of more than 4200 known mycobacteriophages, this TM4 is just a single representation of bacteriophage as anti-mycobacterial agents. Further studies on mycobacteriophages against various NTM pathogens are needed to elaborate our understanding of these phages and help us rationally use a specific mycobacteriophage to treat infection caused by a particular NTM pathogen [10, 94–97].

5.8.3 Host Directed Therapeutics

The charm of antibiotics as "magic bullets" has faded after the emergence of DR, mainly due to antibiotic-induced selection pressure and a very high evolution rate of pathogens, which eventually enables pathogens to modify those crucial targets on which antibiotics act and to develop DR. We are seeing growing interest in the host factors, which are essential for the survival or replication of pathogens inside the host. Fortunately, by targeting certain important host factors that are crucial for a pathogen to establish infection and cause disease, we can prevent the growth of pathogens and control the progression of the disease without inducing DR. Meanwhile, these host-directed approaches can also enhance the effectiveness of regular antibiotics [98].

The importance of interferon-gamma (IFN-γ) produced from T-cells is well known for controlling infections caused by mycobacterial pathogens. Several reports have shown the efficacy of immunomodulation in controlling mycobacterial infection in murine disease models [10].

Studies on immunocompetent Balb/c mice and CD-4 depleted immunocompromised SCID mice show that administration of exogenously produced recombinant IL-12 has stimulated the production of IFN-γ and protected mice against infections of *M. avium*. While injection of DNA sequences containing the gene for the synthesis of IL-18 resulted in substantial reduction of bacterial CFU in lungs and persistent IFN-γ production up to eight weeks in a murine infection model of *M. avium complex* using Balb/c mice [99, 100].

Another study has shown the synergistic effect of etanercept (an approved tumor necrosis factor-alpha (TNF-α) inhibitor drug) with regular anti-TB agents. The combination of etanercept with anti-TB drugs resulted in a higher reduction in bacterial burden in animals than when the drug was used alone in the lungs of mice infected with Mtb [101].

However, the negative implication of TNF-α inhibitors in subjects with chronic inflammatory disease has also been reported in numerous studies. A higher risk of TB reactivation and worsening NTM disease are serious concerns associated with the administration of TNF-α inhibitor drugs. These risk factors limit their clinical utilization to manage mycobacterial diseases [10, 98–102].

5.8.4 Ion Chelators

Iron and other critical ions are necessary at different stages of the bacterial life cycle. Hence, they play an important role in bacterial metabolism for the effective development of infections. Likewise, mycobacteria also need iron and other metal ions. They have developed very sophisticated techniques such as expressing high-affinity siderophores, including mycobactins and carboxymycobactins, to withdraw iron from the host cells [103]. These siderophores effectively obtain iron from transferrin and lactoferrin, two very important iron-binding proteins of host cells; Mtb can also use heme proteins as their source of iron [104].

The necessity of iron is not only limited to co-factors of various crucial enzymes involved in bacterial metabolisms. Iron is also crucial to the virulency of mycobacterial pathogens. The prevention of phagosome maturation in macrophage

cells infected with *M. avium* depends on the iron capturing ability of the bacteria [105]. The importance of iron in *M. smegmatis* ability to form a biofilm and a substantial reduction in the growth of *M. avium* inside the macrophages and in vivo conditions upon addition of iron chelators have also been reported in the literature. In another study, the addition of rhodamine residues over 3-hydroxy-4-pyridinone, a well-known iron-chelating moiety, has enhanced its anti-mycobacterial activity, possibly by increasing the concentration of ion-chelators inside the phagosome and reducing the intracellular availability of iron required by mycobacterial pathogens to prevent phagosome maturation. Synergistic interaction of ion chelators with ethambutol has been reported to reduce the growth of *M. avium* inside the macrophage [10, 106].

5.8.5 Natural Oils

Six natural oils (cinnamon oil, oregano oil, carvacrol, trans-cinnamaldehyde, 2,5-dihydroxybenzaldehyde, and 2-hydroxy-5-methoxybenzaldehyde) have shown potential activity in the inhibition of *M. avium* subspecies *paratuberculosis* [107]. These six natural oils act via leakage of phosphate ions in the extracellular environment in a concentration- and time-dependent manner, which may be possibly associated with disruption of the bacterial cell membrane [108]. These natural oils are major essential oils obtained from the *Labiatae family*. They are generally regarded as safe (GRAS) and are allowed for cooking purposes, and several reports indicate the various medicinal properties of these oils. Among these, carvacrol has shown potent in vitro activity against a range of NTM pathogens, including *M. fortuitum*, *M. abscessus, M. chelonae,* and *M. mucogenicum*, etc. (MIC 64 μg/mL against most of NTM). The vapor of carvacrol seems more potent (MIC 16 μg/ml) than its liquid. Carvacrol can also inhibit biofilm formation by NTM species [10, 107, 108].

5.8.6 Nitric Oxide

Inhalation of NO for the treatment of pulmonary *M. abscessus* disease seems promising. Exposure of 250 ppm NO alone for ten hours using NO exposure chamber has shown a five to six log reduction of clinical isolates of *M. abscessus* and reference strain ATCC 19977. The combination of clofazimine synergized with NO exposure caused an additional one $\log_{10}$ reduction of *M. abscessus* [109, 110].

6 Clinical Trials

In addition to the above-listed molecules, several other molecules and repurposed drugs are under investigation in clinical trials to treat NTM infections. A compiled list of those trials on NTM diseases can be retrieved from clinicaltrials.gov.

7 Conclusion

Infectious diseases pose a continuous threat to humanity, where the emergence of DR has outcompeted the rate of new drug development. DR infections cause a substantially heavy burden on the healthcare system worldwide. Previously, NTM were considered non-pathogenic, but now they are emerging as severe pathogens. There are still no specific, definite treatments for NTM infections. Guidelines related to the management of NTM diseases mainly recommend a combination of two or more drugs:

- a first-line anti-tubercular drug, e.g., rifampin with/without ethambutol and clarithromycin for the infections with SGM species; and
- a quinolone (moxifloxacin) and a macrolide (clarithromycin) for diseases caused by RGM species.

The re-emergence of difficult-to-control NTM pathogens (*M. chimaera* and *M. abscessus*) serves as a wake-up call to take these mycobacterial pathogens more seriously. In addition, we are concerned about *M. abscessus,* which causes highly difficult-to-treat infections–an abiding nightmare of the healthcare system.

For the reasons mentioned above, it is critically important to provide a correct and fast diagnosis of NTM pathogens since treatment varies considerably depending on the species. In addition, certain NTMs, especially RGM, need to be identified at the subspecies level, for which antimicrobial susceptibility testing is indicated to choose the best possible chemotherapy. Despite these uncertainties, recent information also gives the scope of encouragement. Nebulized amikacin has demonstrated strong efficacy in clinical trials and could be a major boom in the care of NTM patients. Novel medicines of tremendous promise have also been created in recent decades, but the lack of clinical safety and efficacy data restricts us from including them in the existing recommendations. The drug pipeline is also undergoing a shift, with new drugs exhibiting potent anti-NTM activity. Our understanding of epidemiology is growing, and the proper utilization of modern molecular biology tools such as whole-genome sequencing will give us a clearer picture of the transmission of these pathogens.

Core Messages

- NTM diseases remain highly neglected in most health care systems.
- NTM diseases cause a high degree of morbidity and mortality.
- NTM diseases are, due to pathophysiological and microbiological similarities with tuberculosis, largely misdiagnosed.
- NTM species possess intrinsic resistance mechanisms against different antibiotics, making their treatment challenging.
- The rise of NTM infections in vulnerable people is alarming.

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References

1. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F et al (2007) An official ATS/IDSA statement: diagnosis, treatment, and prevention of non-tuberculous mycobacterial diseases. Am J Respir Crit Care Med 175(4):367–416
2. Wolinsky E (1979) Non-tuberculous mycobacteria and associated diseases. Am Rev Respir Dis 119(1):107–159
3. Muñoz-Egea MC, Carrasco-Antón N, Esteban J (2020) State-of-the-art treatment strategies for non-tuberculous mycobacteria infections. Expert Opin Pharmacother 1–13
4. Rothschild BM, Martin LD, Lev G, Bercovier H, Bar-Gal GK, Greenblatt C et al (2001) Mycobacterium tuberculosis complex DNA from an extinct bison dated 17,000 years before the present. Clin Infect Dis 33(3):305–311
5. Prevots DR, Marras TK (2015) Epidemiology of human pulmonary infection with non-tuberculous mycobacteria: a review. Clin Chest Med 36(1):13–34
6. Adjemian J, Daniel-Wayman S, Ricotta E, Prevots DR (2018) Epidemiology of non-tuberculous mycobacteriosis. In: Seminars in respiratory and critical care medicine, vol 39, no 03. Thieme Medical Publishers, pp 325–335
7. Drummond WK, Kasperbauer SH (2019) Non-tuberculous mycobacteria: epidemiology and the impact on pulmonary and cardiac disease. Thorac Cardiovasc Surg 29(1):59–64
8. González SM, Cortés AC, Yoldi LAS, García JMG, Álvarez LMA, Gutiérrez JJP (2017) Non-tuberculous mycobacteria. An emerging threat? Arch Bronconeumol 53(10):554–560 (English Edition)
9. Falkinham JO (2016) Current epidemiologic trends of the non-tuberculous mycobacteria (NTM). Curr Environ Health Rep 3(2):161–167
10. Bento CM, Gomes MS, Silva T (2020) Looking beyond typical treatments for atypical mycobacteria. Antibiotics 9(1):18
11. Runyon EH (1959) Anonymous mycobacteria in pulmonary disease. Med Clin North Am 43 (1):273–290
12. Shah NM, Davidson JA, Anderson LF, Lalor MK, Kim J, Thomas HL et al (2016) Pulmonary mycobacterium avium-intracellulare is the main driver of the rise in non-tuberculous mycobacteria incidence in England, Wales and Northern Ireland, 2007–2012. BMC Infect Dis 16(1):1–6
13. Sharma SK, Sharma R, Singh BK, Upadhyay V, Mani I, Tripathi M, Kumar P (2019) A prospective study of non-tuberculous mycobacterial disease among tuberculosis suspects at a tertiary care centre in north India. Indian J Med Res 150(5):458
14. Baldwin SL, Larsen SE, Ordway D, Cassell G, Coler RN (2019) The complexities and challenges of preventing and treating non-tuberculous mycobacterial diseases. PLoS Negl Trop Dis 13(2):e0007083
15. Marras TK, Chedore P, Ying AM, Jamieson F (2007) Isolation prevalence of pulmonary non-tuberculous mycobacteria in Ontario, 1997–2003. Thorax 62(8):661–666
16. Prevots DR, Shaw PA, Strickland D, Jackson LA, Raebel MA, Blosky MA et al (2010) Non-tuberculous mycobacterial lung disease prevalence at four integrated health care delivery systems. Am J Respir Crit Care Med 182(7):970–976
17. Gopalaswamy R, Shanmugam S, Mondal R, Subbian S (2020) Of tuberculosis and non-tuberculous mycobacterial infections—a comparative analysis of epidemiology, diagnosis and treatment. J Biomed Sci 27(1):1–17
18. Lai CC, Tan CK, Chou CH, Hsu HL, Liao CH, Huang YT et al (2010) Increasing incidence of non-tuberculous mycobacteria, Taiwan, 2000–2008. Emerg Infect Dis 16(2):294

19. Hoefsloot W, Van Ingen J, Andrejak C, Ängeby K, Bauriaud R, Bemer P et al (2013) The geographic diversity of non-tuberculous mycobacteria isolated from pulmonary samples: an NTM-NET collaborative study. Eur Respir J 42(6):1604–1613
20. Moore JE, Kruijshaar ME, Ormerod LP, Drobniewski F, Abubakar I (2010) Increasing reports of non-tuberculous mycobacteria in England, Wales and Northern Ireland, 1995–2006. BMC Public Health 10(1):1–6
21. Koh WJ, Jeong BH, Kim SY, Jeon K, Park KU, Jhun BW et al (2017) Mycobacterial characteristics and treatment outcomes in Mycobacterium abscessus lung disease. Clin Infect Dis 64(3):309–316
22. Haworth CS, Floto RA (2017) Introducing the new BTS guideline: management of non-tuberculous mycobacterial pulmonary disease (NTM-PD)
23. Wentworth AB, Drage LA, Wengenack NL, Wilson JW, Lohse CM (2013) Increased incidence of cutaneous non-tuberculous mycobacterial infection, 1980 to 2009: a population-based study. In: Mayo clinic proceedings, vol 88, no 1. Elsevier, Amsterdam, pp 38–45
24. Holt MR, Kasperbauer S (2018) Management of extrapulmonary non-tuberculous mycobacterial infections. In: Seminars in respiratory and critical care medicine, vol 39, no 03. Thieme Medical Publishers, New York, pp 399–410
25. Winthrop KL, Albridge K, South D, Albrecht P, Abrams M, Samuel MC et al (2004) The clinical management and outcome of nail salon—acquired mycobacterium fortuitum skin infection. Clin Infect Dis 38(1):38–44
26. Friedman ND, Athan E, Walton AL et al (2016) Increasing experience with primary oral medical therapy for Mycobacterium ulcerans disease in an Australian cohort. Antimicrob Agents Chemother 60(5):2692–2695
27. Tortoli E (2009) Clinical manifestations of non-tuberculous mycobacteria infections. Clin Microbiol Infect 15(10):906–910
28. Jiménez-Montero B, Baquero-Artigao F, Saavedra-Lozano J, Tagarro-García A, Blázquez--Gamero D, Cilleruelo-Ortega MJ et al (2014) Comparison of Mycobacterium lentiflavum and Mycobacterium avium-intracellulare complex lymphadenitis. Pediatr Infect Dis J 33 (1):28–34
29. Miqueleiz-Zapatero A, Santa Olalla-Peralta C, Guerrero-Torres MD, Cardeñoso-Domingo L, Hernández-Milán B, Domingo-García D (2018) Mycobacterium lentiflavum as the main cause of lymphadenitis in pediatric population. Enfermedadesinfecciosas y microbiologia-clinica 36(10):640–643 (English ed.)
30. El Helou G, Viola GM, Hachem R, Han XY, Raad II (2013) Rapidly growing mycobacterial bloodstream infections. Lancet Infect Dis 13(2):166–174
31. El Helou G, Hachem R, Viola GM, El Zakhem A, Chaftari AM, Jiang Y et al (2013) Management of rapidly growing mycobacterial bacteremia in cancer patients. Clin Infect Dis 56(6):843–846
32. Li G, Pang H, Guo Q, Huang M, Tan Y, Li C et al (2017) Antimicrobial susceptibility and MIC distribution of 41 drugs against clinical isolates from China and reference strains of non-tuberculous mycobacteria. Int J Antimicrob Agents 49(3):364–374
33. Yu X, Gao X, Li C, Luo J, Wen S, Zhang T et al (2019) In vitro activities of bedaquiline and delamanid against non-tuberculous mycobacteria isolated in Beijing, China. Antimicrob Agents Chemother 63(8):e00031-19
34. Soni I, De Groote MA, Dasgupta A, Chopra S (2016) Challenges facing the drug discovery pipeline for non-tuberculous mycobacteria. J Med Microbiol 65(1):1–8
35. Brown-Elliott BA, Wallace RJ (2017) In vitro susceptibility testing of tedizolid against non-tuberculous mycobacteria. J Clin Microbiol 55(6):1747–1754
36. Brown-Elliott BA, Rubio A, Wallace RJ (2018) In vitro susceptibility testing of a novel benzimidazole, SPR719, against non-tuberculous mycobacteria. Antimicrob Agents Chemother 62(11)

37. Shoen C, Benaroch D, Sklaney M, Cynamon M (2019) In vitro activities of omadacycline against rapidly growing mycobacteria. Antimicrob Agents Chemother 63(5)
38. Vázquez-Laslop N, Mankin AS (2018) How macrolide antibiotics work. Trends Biochem Sci 43(9):668–684
39. Mingeot-Leclercq MP, Glupczynski Y, Tulkens PM (1999) Aminoglycosides: activity and resistance. Antimicrob Agents Chemother 43(4):727–737
40. Monique IA, Alasdair P, MacGowan AP (2003) Development of the quinolones. J Antimicrob Chemother 51(1):1–11
41. Kim DH, Jhun BW, Moon SM, Kim SY, Jeon K, Kwon OJ et al (2019) In vitro activity of bedaquiline and delamanid against non-tuberculous mycobacteria, including macrolide-resistant clinical isolates. Antimicrob Agents Chemother 63(8):e00665-19
42. Doi N, Disratthakit A (2006) Characteristic anti-mycobacterial spectra of the novel anti-TB drug candidates OPC-67683 and PA-824. Poster F1-1377a, 46th ICAAC
43. Lenaerts AJ, Gruppo V, Marietta KS, Johnson CM, Driscoll DK, Tompkins NM et al (2005) Preclinical testing of the nitroimidazopyran PA-824 for activity against Mycobacterium tuberculosis in a series of in vitro and in vivo models. Antimicrob Agents Chemother 49 (6):2294–2301
44. Singh R, Manjunatha U, Boshoff HI, Ha YH, Niyomrattanakit P, Ledwidge R et al (2008) PA-824 kills nonreplicating Mycobacterium tuberculosis by intracellular NO release. Science 322(5906):1392–1395
45. Stover CK, Warrener P, VanDevanter DR, Sherman DR, Arain TM, Langhorne MH et al (2000) A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. Nature 405(6789):962–966
46. Kozikowski AP, Onajole OK, Stec J, Dupont C, Viljoen A, Richard M, Chaira T, Lun S, Bishai W, Raj VS et al (2017) Targeting mycolic acid transport by indole-2-carboxamides for the treatment of Mycobacterium abscessus infections. J Med Chem 60:5876–5888
47. Franz ND, Belardinelli JM, Kaminski MA, Dunn LC, de Moura VCN, Blaha MA et al (2017) Design, synthesis and evaluation of indole-2-carboxamides with pan anti-mycobacterial activity. Bioorg Med Chem 25(14):3746–3755
48. Cynamon M, Jureller J, Desai B, Ramachandran K, Sklaney M, Grossman TH (2012) In vitro activity of TP-271 against Mycobacterium abscessus, Mycobacterium fortuitum, and Nocardia species. Antimicrob Agents Chemother 56(7):3986–3988
49. Dubuisson T, Bogatcheva E, Krishnan MY, Collins MT, Einck L, Nacy CA, Reddy VM (2010) In vitro antimicrobial activities of capuramycin analogues against non-tuberculous mycobacteria. J Antimicrob Chemother 65(12):2590–2597
50. Liu X, Jin Y, Cai W, Green KD, Goswami A, Garneau-Tsodikova S et al (2016) A biocatalytic approach to capuramycin analogues by exploiting a substrate permissive N-transacylase CapW. Org Biomol Chem 14(16):3956–3962
51. Madani A, Ridenour JN, Martin BP, Paudel RR, Abdul Basir A, Le Moigne V et al (2019) Cyclipostins and cyclophostin analogues as multitarget inhibitors that impair growth of Mycobacterium abscessus. ACS Infect Dis 5(9):1597–1608
52. Nguyen PC, Madani A, Santucci P, Martin BP, Paudel RR, Delattre S et al (2018) Cyclophostin and Cyclipostins analogues, new promising molecules to treat mycobacterial-related diseases. Int J Antimicrob Agents 51(4):651–654
53. Dupont C, Viljoen A, Dubar F, Blaise M, Bernut A, Pawlik A et al (2016) A new piperidinol derivative targeting mycolic acid transport in Mycobacterium abscessus. Mol Microbiol 101 (3):515–529
54. Baranyai Z, Krátký M, Vinšová J, Szabó N, Senoner Z, Horváti K et al (2015) Combating highly resistant emerging pathogen Mycobacterium abscessus and Mycobacterium tuberculosis with novel salicylanilide esters and carbamates. Eur J Med Chem 101:692–704
55. Krátký M, Bősze S, Baranyai Z, Szabó I, Stolaříková J, Paraskevopoulos G, Vinšová J (2015) Synthesis and in vitro biological evaluation of 2-(phenylcarbamoyl) phenyl 4-substituted benzoates. Bioorg Med Chem 23(4):868–875

56. Molina-Torres CA, Ocampo-Candiani J, Rendón A, Pucci MJ, Vera-Cabrera L (2010) In vitro activity of a new isothiazoloquinolone, ACH-702, against Mycobacterium tuberculosis and other mycobacteria. Antimicrob Agents Chemother 54(5):2188–2190
57. Moraski GC, Cheng Y, Cho S, Cramer JW, Godfrey A, Masquelin T et al (2016) Imidazo [1, 2-a] pyridine-3-carboxamides are active antimicrobial agents against Mycobacterium avium infection in vivo. Antimicrob Agents Chemother 60(8):5018–5022
58. Martiniano SL, Wagner BD, Levin A, Nick JA, Sagel SD, Daley CL (2017) Safety and effectiveness of clofazimine for primary and refractory non-tuberculous mycobacterial infection. Chest 152(4):800–809
59. Jarand J, Davis JP, Cowie RL, Field SK, Fisher DA (2016) Long-term follow-up of Mycobacterium avium complex lung disease in patients treated with regimens including clofazimine and/or rifampin. Chest 149(5):1285–1293
60. Srivastava S, Gumbo T (2018) Clofazimine for the treatment of Mycobacterium kansasii. Antimicrob Agents Chemother 62(8)
61. Ferro BE, Meletiadis J, Wattenberg M, De Jong A, van Soolingen D, Mouton JW, van Ingen J (2016) Clofazimine prevents the regrowth of Mycobacterium abscessus and Mycobacterium avium type strains exposed to amikacin and clarithromycin. Antimicrob Agents Chemother 60(2):1097–1105
62. van Ingen J, Totten SE, Helstrom NK, Heifets LB, Boeree MJ, Daley CL (2012) In vitro synergy between clofazimine and amikacin in treatment of non-tuberculous mycobacterial disease. Antimicrob Agents Chemother 56(12):6324–6327
63. Yang B, Jhun BW, Moon SM, Lee H, Park HY, Jeon K et al (2017) Clofazimine-containing regimen for the treatment of Mycobacterium abscessus lung disease. Antimicrob Agents Chemother 61(6)
64. Cavusoglu C, Soyler I, Akinci P (2007) Activities of linezolid against non-tuberculous mycobacteria. Microbiol Q J Microbiol Sci 30(4):411–414
65. Wallace RJ, Brown-Elliott BA, Ward SC, Crist CJ, Mann LB, Wilson RW (2001) Activities of linezolid against rapidly growing mycobacteria. Antimicrob Agents Chemother 45 (3):764–767
66. Bax HI, de Vogel CP, Mouton JW, de Steenwinkel JE (2019) Omadacycline as a promising new agent for the treatment of infections with Mycobacterium abscessus. J Antimicrob Chemother 74(10):2930–2933
67. Minhas R, Sharma S, Kundu S (2019) Utilizing the promise of omadacycline in a resistant, non-tubercular mycobacterial pulmonary infection. Cureus 11(7)
68. Kaushik A, Ammerman NC, Martins O, Parrish NM, Nuermberger EL (2019) In vitro activity of new tetracycline analogs omadacycline and eravacycline against drug-resistant clinical isolates of Mycobacterium abscessus. Antimicrob Agents Chemother 63(6)
69. Ferro BE, Srivastava S, Deshpande D, Pasipanodya JG, van Soolingen D, Mouton JW et al (2016) Tigecycline is highly efficacious against mycobacterium abscessus pulmonary disease. Antimicrob Agents Chemother 60(5):2895–2900
70. Kwon YS, Levin A, Kasperbauer SH, Huitt GA, Daley CL (2019) Efficacy and safety of tigecycline for Mycobacterium abscessus disease. Respir Med 158:89–91
71. Fernández-Roblas R, Martin-de-Hijas NZ, Fernandez-Martinez AI, García-Almeida D, Gadea I, Esteban J (2008) In vitro activities of tigecycline and 10 other antimicrobials against nonpigmented rapidly growing mycobacteria. Antimicrob Agents Chemother 52 (11):4184–4186
72. Edoo Z, Iannazzo L, Compain F, Li de la Sierra Gallay I, van Tilbeurgh H, Fonvielle M et al (2018) Synthesis of avibactam derivatives and activity on β-lactamases and peptidoglycan biosynthesis enzymes of mycobacteria. Chem Eur J 24(32):8081–8086
73. Pandey R, Chen L, Manca C, Jenkins S, Glaser L, Vinnard C et al (2019) Dual β-lactam combinations highly active against mycobacterium abscessus complex in vitro. MBio 10(1)

74. Arenaz-Callao MP, del Río RG, Quintana AL, Thompson CJ, Mendoza-Losana A, Ramón-García S (2019) Triple oral beta-lactam containing therapy for Buruli ulcer treatment shortening. PLoS Negl Trop Dis 13(1):e0007126
75. Bermudez LE, Kolonoski P, Petrofsky M, Wu M, Inderlied CB, Young LS (2003) Mefloquine, moxifloxacin, and ethambutol are a triple-drug alternative to macrolide-containing regimens for treatment of Mycobacterium avium disease. J Infect Dis 187(12):1977–1980
76. Bermudez LE, Inderlied CB, Kolonoski P, Chee CB, Aralar P, Petrofsky M et al (2012) Identification of (+)-erythro-mefloquine as an active enantiomer with greater efficacy than mefloquine against Mycobacterium avium infection in mice. Antimicrob Agents Chemother 56(8):4202–4206
77. Deshpande D, Srivastava S, Musuka S, Gumbo T (2016) Thioridazine as chemotherapy for Mycobacterium avium complex diseases. Antimicrob Agents Chemother 60(8):4652–4658
78. Srivastava S, Deshpande D, Sherman CM, Gumbo T (2017). A 'shock and awe' thioridazine and moxifloxacin combination-based regimen for pulmonary Mycobacterium avium—intracellulare complex disease. J Antimicrob Chemother 72(suppl_2):i43–i47
79. Leung JM, Olivier KN (2013) Non-tuberculous mycobacteria: the changing epidemiology and treatment challenges in cystic fibrosis. Curr Opin Pulm Med 19(6):662
80. Das S, Garg T, Chopra S, Dasgupta A (2019) Repurposing disulfiram to target infections caused by non-tuberculous mycobacteria. J Antimicrob Chemother 74(5):1317–1322
81. Fowler C, Wu UI, Shaffer R, Smith C, Barnhart L, Bryant C et al (2020) The effects of sildenafil on ciliary beat frequency in patients with pulmonary non-tuberculous mycobacteria disease: phase I/II trial. BMJ Open Respir Res 7(1):e000574
82. Cirone KM, Lahiri P, Holani R, Tan YL, Arrazuria R, De Buck J et al (2020) Synthetic cathelicidin LL-37 reduces Mycobacterium avium subsp. paratuberculosis internalization and pro-inflammatory cytokines in macrophages. Cell Tissue Res 379(1):207–217
83. Draper LA, O'Connor PM, Coffey A, O'Mahony J (2010) Comparison of the activities of the lantibiotics nisin and lacticin 3147 against clinically significant mycobacteria
84. Ali ZI, Saudi AM, Albrecht R, Talaat AM (2019) The inhibitory effect of nisin on Mycobacterium avium ssp. paratuberculosis and its effect on mycobacterial cell wall. J Dairy Sci 102(6):4935–4944
85. Carroll J, Field D, O'Connor PM, Cotter PD, Coffey A, Hill C, O'Mahony J (2010) The gene encoded antimicrobial peptides, a template for the design of novel anti-mycobacterial drugs. Bioengineered Bugs 1(6):408–412
86. Gao W, Kim JY, Anderson JR, Akopian T, Hong S, Jin YY et al (2015) The cyclic peptide ecumicin targeting ClpC1 is active against Mycobacterium tuberculosis in vivo. Antimicrob Agents Chemother 59(2):880–889
87. Gavrish E, Sit CS, Cao S, Kandror O, Spoering A, Peoples A et al (2014) Lassomycin, a ribosomally synthesized cyclic peptide, kills Mycobacterium tuberculosis by targeting the ATP-dependent protease ClpC1P1P2. Chem Biol 21(4):509–518
88. Trentini MM, das Neves RC, Santos BDPO, DaSilva RA, Souza AC, Mortari MR et al (2017) Non-disulfide-bridge peptide 5.5 from the scorpion hadrurus gertschi inhibits the growth of Mycobacterium abscessus subsp. massiliense. Front Microbiol 8:273
89. das Neves RC, Trentini MM, de Castro e Silva J, Simon KS, Bocca AL, Silva LP et al (2016) Anti-mycobacterial activity of a new peptide polydim-I isolated from neotropical social wasp polybia dimorpha. PLoS One 11(3):e0149729
90. Silva JC, Neto LM, Neves RC, Gonçalves JC, Trentini MM, Mucury-Filho R et al (2017) Evaluation of the antimicrobial activity of the mastoparan Polybia-MPII isolated from venom of the social wasp Pseudopolybia vespiceps testacea (Vespidae, Hymenoptera). Int J Antimicrob Agents 49(2):167–175
91. Adhya M, Jeung HD, Kang HS, Choi KS, Lee DS, Cho M (2012) Cloning and localization of MCdef, a defensin from Manila clams (Ruditapes philippinarum). Comp Biochem Physiol B Biochem Mol Biol 161(1):25–31

92. Silva T, Magalhães B, Maia S, Gomes P, Nazmi K, Bolscher JG et al (2014) Killing of Mycobacterium avium by lactoferricin peptides: improved activity of arginine-and D-amino-acid-containing molecules. Antimicrob Agents Chemother 58(6):3461–3467
93. Silva T, Moreira AC, Nazmi K, Moniz T, Vale N, Rangel M et al (2017) Lactoferricin peptides increase macrophages' capacity to kill mycobacterium avium. Msphere 2(4)
94. Azimi T, Mosadegh M, Nasiri MJ, Sabour S, Karimaei S, Nasser A (2019) Phage therapy as a renewed therapeutic approach to mycobacterial infections: a comprehensive review. Infect Drug Resist 12:2943
95. Nieth A, Verseux C, Barnert S, Süss R, Römer W (2015) A first step toward liposome-mediated intracellular bacteriophage therapy. Expert Opin Drug Deliv 12 (9):1411–1424
96. Broxmeyer L, Sosnowska D, Miltner E, Chacón O, Wagner D, McGarvey J et al (2002) Killing of Mycobacterium avium and Mycobacterium tuberculosis by a mycobacteriophage delivered by a nonvirulent mycobacterium: a model for phage therapy of intracellular bacterial pathogens. J Infect Dis 186(8):1155–1160
97. Danelishvili L, Young LS, Bermudez LE (2006) In vivo efficacy of phage therapy for Mycobacterium avium infection as delivered by a nonvirulent mycobacterium. Microb Drug Resist 12(1):1–6
98. Torfs E, Piller T, Cos P, Cappoen D (2019) Opportunities for overcoming mycobacterium tuberculosis drug resistance: emerging mycobacterial targets and host-directed therapy. Int J Mol Sci 20(12):2868
99. Silva RA, Pais TF, Appelberg R (1998) Evaluation of IL-12 in immunotherapy and vaccine design in experimental Mycobacterium avium infections. J Immunol 161(10):5578–5585
100. Kim SH, Cho D, Kim TS (2001) Induction of in vivo resistance to Mycobacterium avium infection by intramuscular injection with DNA encoding interleukin-18. Immunology 102 (2):234–241
101. Skerry C, Harper J, Klunk M, Bishai WR, Jain SK (2012) Adjunctive TNF inhibition with standard treatment enhances bacterial clearance in a murine model of necrotic TB granulomas. PLoS One 7(6):e39680
102. Yoo JW, Jo KW, Kang BH, Kim MY, Yoo B, Lee CK et al (2014) Mycobacterial diseases developed during anti-tumour necrosis factor-α therapy. Eur Respir J 44(5):1289–1295
103. Sritharan M (2016) Iron homeostasis in Mycobacterium tuberculosis: mechanistic insights into siderophore-mediated iron uptake. J Bacteriol 198(18):2399–2409
104. Jones CM, Niederweis M (2011) Mycobacterium tuberculosis can utilize heme as an iron source. J Bacteriol 193(7):1767–1770
105. Kelley VA, Schorey JS (2003) Mycobacterium's arrest of phagosome maturation in macrophages requires Rab5 activity and accessibility to iron. Mol Biol Cell 14(8):3366–3377
106. Moniz T, Silva D, Silva T, Gomes MS, Rangel M (2015) Anti-mycobacterial activity of rhodamine 3, 4-HPO iron chelators against Mycobacterium avium: analysis of the contribution of functional groups and of chelator's combination with ethambutol. MedChemComm 6(12):2194–2203
107. Nowotarska SW, Nowotarski K, Grant IR, Elliott CT, Friedman M, Situ C (2017) Mechanisms of antimicrobial action of cinnamon and oregano oils, cinnamaldehyde, carvacrol, 2, 5-dihydroxybenzaldehyde, and 2-hydroxy-5-methoxybenzaldehyde against Mycobacterium avium subsp. paratuberculosis (Map). Foods 6(9):72
108. Marini E, Di Giulio M, Ginestra G, Magi G, Di Lodovico S, Marino A et al (2019) Efficacy of carvacrol against resistant rapidly growing mycobacteria in the planktonic and biofilm growth mode. PLoS One 14(7):e0219038
109. Bentur L, Gur M, Ashkenazi M, Livnat-Levanon G, Mizrahi M, Tal A et al (2020) Pilot study to test inhaled nitric oxide in cystic fibrosis patients with refractory Mycobacterium abscessus lung infection. J Cyst Fibros 19(2):225–231

110. Yaacoby-Bianu K, Gur M, Toukan Y, Nir V, Hakim F, Geffen Y, Bentur L (2018) Compassionate nitric oxide adjuvant treatment of persistent Mycobacterium infection in cystic fibrosis patients. Pediatr Infect Dis J 37(4):336–338

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Challenges for Contact Tracing and Tuberculosis Preventive Therapy Scale-up

27

Anete Trajman

An ounce of prevention is worth a pound of cure.

Benjamin Franklin

Summary

Tuberculosis preventive therapy (TPT) is a key strategy to eliminate tuberculosis (TB) by 2050. However, less than one-fifth of those needing TPT complete it because of the many losses through the complex tuberculosis infection (TBI) cascade of care. The largest and higher-risk populations targeted for TPT are people living with HIV and contacts of patients with pulmonary TB. New tests to detect TBI and shorter and better-tolerated treatment regimens to treat TBI have been incorporated by several countries in recent years, but the public health impact of these advances is poor, as many other barriers are still pending. This chapter reviews the progress and bottlenecks for scaling up TPT to contacts worldwide. The perspective for new tests, treatments, and innovative approaches is also discussed.

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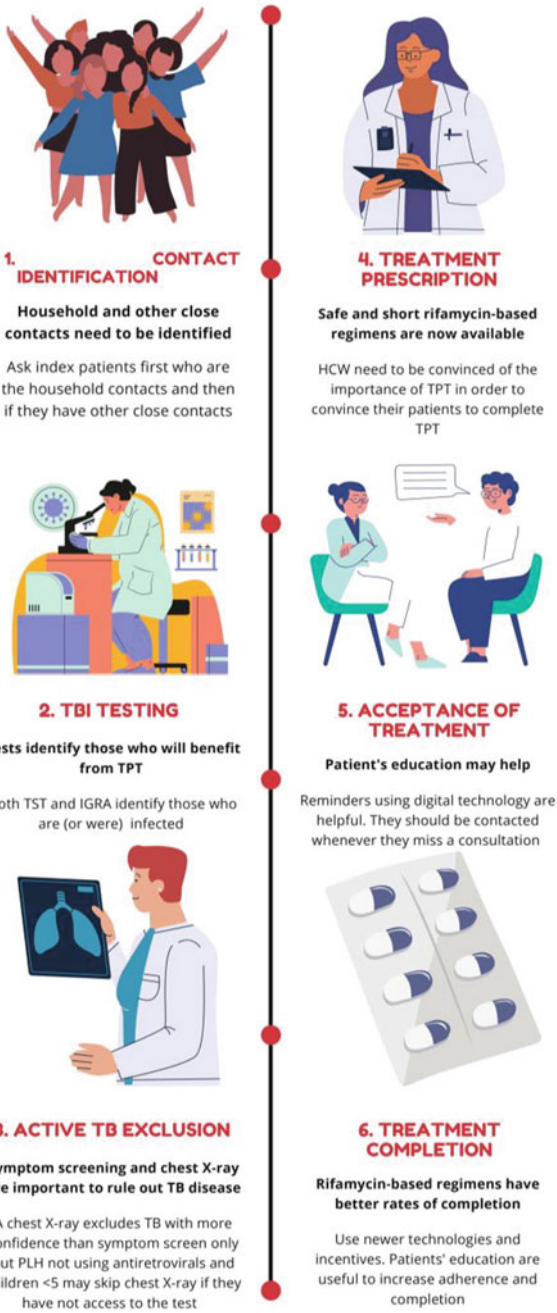
Graphical Abstract



Keywords

Cascade of care · Infection · Preventive therapy · Rifamycin · Tuberculosis

1 Introduction

One-fourth of the planet's population has tuberculosis infection (TBI) [2], and they constitute a major reservoir for new tuberculosis (TB) cases. Therefore, tuberculosis preventive therapy (TPT) is a key strategy to eliminate TB [3]. TPT effectively reduces by 90% the risk of progression to TB in people with TBI [4]. The main targeted populations for TPT are people living with HIV (PLHIV) and close contacts of patients with microbiologically confirmed pulmonary tuberculosis (PTB, index patients). In the United Nations High-Level Meeting held in 2018, representatives of more than 100 countries committed to provide, by 2022, 30 million TPT to PLHIV (six million), children under five years of age (four million), and other close contacts of index patients (20 million) [5]. However, poor progress has been made in TPT scale-up since 2018, especially among the latter [6]. The reasons for this include the complex TBI cascade of care [7] as well as patients' and health providers' fear of adverse events [8]. Additionally, TBI diagnosis has several limitations [9], and above all, most people with diagnosed TBI will never have the disease. Currently, no tests are available to detect the risk of progression to disease. This chapter will review the challenges and solutions for TBI management and TPT scale-up, particularly among contacts of index patients, and technological innovations underway to move forward towards a world free of TB.

2 Identification of the Targeted Population for Tuberculosis Preventive Therapy

Although the large population with TBI is the main source of new TB cases, most will never progress to active TB [10]. There are no biomarkers of risk of progression available for clinical purposes. Therefore, clinical and epidemiological markers are used to select the targeted population with TBI for TPT. Those include immunosuppression from any cause (HIV infection and use of immune modulators,[1] steroids, and chemotherapy) and those with recent TBI, i.e., those with a recent tuberculin skin test (TST) conversion or contacts of index patients with a positive TBI test (in whom recent infection is not sure but is likely) [11]. Other highly exposed populations, such as immigrants (refugees or others) and people living in prisons, also constitute large populations with a high risk of progression to the disease.

[1] These agents are increasingly prescribed for several autoimmune disorders.

The largest world population targeted for TPT are index case contacts of all ages. Active TB is found in 1.4% and TBI in 28% of contacts in high-income countries, while in low- and medium-income countries (LMIC), 2.1% have active disease, and 51% have TBI [12]. Thus, contact tracing has high yields of identification of TB in all its stages. Moreover, the absolute risk of progression to active TB in contacts is higher than in patients using immune modulators or transplanted patients [13]. Therefore, contacts constitute a large population with a high risk of progression to active TB when infected, which constitutes a high priority both from the individual (clinical) and from the populational (public health) perspective.

Because TBI is an asymptomatic condition, healthcare workers should actively look for TBI in contacts of index patients. The first step is to identify all households and other close contacts adequately. To this end, a careful history should be taken in a private and quiet space, and all contacts spending more than one hour per day for five days in the same room or one night per week in the same house of the index case (close contacts) in the previous three months should be identified, besides those living in the same house (household contacts). Efforts should be made to avoid stigma and breaches of confidentiality during this interview. Subsequent encounters with the index case should constitute new opportunities to identify more close contacts, as index patients gain confidence in the health team and have further time to recall her/his recent history.

3 Diagnosis of Tuberculosis Infection

Persons with a high probability of TBI and a high risk of progression to active disease should be tested and treated when positive. The main targeted populations for testing are thus PLHIV and contacts of index patients, although other exposed and immunosuppressed patients are also candidates. There is no standard gold test for TBI. The main currently available tests are TST and interferon-gamma release assays (IGRA).

TST has been used to diagnose TBI for more than a century. TST has good sensitivity and reasonable specificity in immunocompetent individuals. However, false-negative results may be observed in pregnant women, undernourished and immunosuppressed patients, and patients with acute infectious diseases or vaccination [14–16]. Conversely, patients with other mycobacterial diseases or with repeated or recent BCG vaccination may present false-positive results [17]. In addition, patients need to come back for reading, which results in more losses in the cascade of care. TST and IGRA do not distinguish recent from remote TBI and are not good markers of risk of disease. Finally, trained personnel are needed, and there has been a shortage of tuberculin in recent years. The main advantage of TST is its wide availability, as laboratory facilities are not necessary [9]. TST conversion is an excellent marker of recent TBI.

IGRA has been incorporated in many countries to overcome the TST limitations. IGRA uses ESAT-6 and CFP-10 antigens, specific to *Mycobacterium tuberculosis* (*M. tb*). However, despite the higher specificity of currently available IGRA tests [18], indeterminate results have been reported, and lower sensitivity in immunosuppressed patients remains an issue. The newer generation Quantiferon®-Gold-Plus (Qiagen, USA) was developed to increase sensitivity in PLHV, but studies show overlapping pooled sensitivity compared to the previous generation Quantiferon®-Gold-in-Tube [19]. Spontaneous conversions and reversions have been reported; thus, conversion of IGRA tests is not reliable proof of recent infection [20]. IGRA or TST are equally recommended by the World Health Organization (WHO) [11].

New skin tests using recombinant tuberculin based on the same antigens as IGRA have been developed and produced in high-burden countries [14–16]. They potentially have the same accuracy as IGRA, with the advantage of not depending on laboratory infrastructure. These tests are currently not commercially available in most countries.

The main drawback of all TBI tests is the same: most patients with a positive test will never progress to active TB [21, 22]. There is no evidence that IGRA is superior to TST or vice versa in predicting progression to active disease [23]. In other words, we need to treat a high number of patients with a positive TBI test to prevent one case of active TB, and this number decreases over time as the risk is higher in the first 24 months after infection. This reflects that TBI tests detect immunity to *M. tb*, not necessarily infection. New tests to detect viable and active *M. tb*, i.e., incipient TB, are being explored. Incipient TB carries a much higher risk of progression to disease. Currently, no markers of risk of progression are available. At least eight transcriptional signature tests distinguish TBI from incipient TB or progressors from non-progressors, but to date, no such test has attained the ideal 75% sensitivity and specificity [24] set by the WHO. The same drawback as those for TBI tests exists: the ability to predict progression to disease is low and reduces with time, although the number necessary to treat to prevent one case is half that with TBI tests [25].

Until accurate biomarkers of progression become available, testing for TBI is advisable in the targeted populations mentioned above, as the risk of progression to active TB is substantially higher in those with a positive test (either IGRA or TST) [13] and the benefit of TPT has been shown to be substantially higher for those with a positive test [26, 27]. However, the risk among young children (< five years of age) contacts and PLHIV is so high that the WHO recommends that these populations, in high-TB burden countries, treat TBI regardless of TBI tests [11]. These recommendations should be reserved for settings where tests may represent a bottleneck to TPT scale-up. Testing to select candidates for TPT will avoid overtreatment and unnecessary exposure to adverse events.

4 Exclusion of Active Tuberculosis

Once TBI is detected, active TB should be ruled out before TPT prescription. This includes symptom screening, medical evaluation, and chest radiograph (CXR) [11]. Screening for cough of any duration, fever, night sweats, and weight loss has an 80% sensitivity for active TB in PLHIV not using antiretroviral therapy, corresponding to 98% and 90% negative predictive values in a 5% and 20% prevalence population, respectively [28]. However, in PLHIV using antiretroviral therapy, the sensitivity of symptoms screening drops substantially to 51%, leading to very low negative predictive values [29]. Adding CXR to symptom screening increases sensitivity to 85% [29]. Because adding CXR may be unaffordable in poor-resource countries and increase losses in the cascade of care, with a modest gain in the post-test probability of active TB [30], WHO recommends that CXR should not be a barrier to TPT [11].

However, for contacts, mainly for those over five years of age—the largest targeted population for TPT—CXR before offering TPT is mandatory [11]. CXR will detect patients with subclinical TB, i.e., with radiological signs of active disease despite the absence of symptoms [31]. Although TPT may cure subclinical disease, it may also induce acquired resistance to anti-TB drugs in a small subset of patients [32, 33]. Despite the low risk of developing resistance with TPT [34, 35], CXR will further decrease this risk by ruling out subclinical TB.

In order to reduce the costs and delays of conventional CXR, computer-aided diagnostic tools coupled with portable CXR devices—developed to increase and speed active TB detection [36]—could be tested in different scenarios for ruling out active TB in the context of contact investigation [37]. Finally, efforts are underway to evaluate highly sensitive blood-based signature tests that would rule out active TB with confidence without needing CXR or other images [38–40].

5 Tuberculosis Preventive Therapy Regimen Options

For over 60 years, isoniazid for six or nine months has been the standard TPT regimen. Isoniazid reduces the risk of progression to active disease by 90% [4]. However, drug-induced liver injury—which can be fatal [41]—is a major concern and has reduced the acceptance of TPT both for providers and for patients. Thus, despite the high individual benefit of TPT, little public health benefit has been observed [6].

Rifamycin-based shorter regimens have been tested in the last decade. They are more acceptable (less minor intolerance) and safer (less severe adverse events) and have better completion rates. Also, they are non-inferior to nine months of isoniazid in terms of efficacy [32, 33, 42, 43]. These regimens include four months of rifampin (4R) daily [33, 42], three months of rifampin and isoniazid (3RH) daily [44], 12 weekly supervised doses of isoniazid and rifapentine (3HP) [32, 43], or one month of daily doses of isoniazid and rifapentine (1HP) [45]. All four have been

added to the list of regimens recommended by the WHO [11]. The selection of the best regimen depends on what patients, healthcare providers, and policymakers prefer.

The USA Centers for Disease Control and Prevention (CDC), for example, recommends the use of any rifamycin regimen: 3HP, 3RH, or 4R [46]. Although self-administered treatment with 3HP has similar rates of success to supervised treatment in North-American patients, adherence is low in LMIC countries, and this regimen may be less acceptable in these settings [47], as supervision increases the costs of TPT substantially. Yet, 3HP has been considered cost-effective both in high-income countries (HIC) and LMIC [48, 49], and rifapentine is being subsided by multilateral agreements to LMIC. The 1HP regimen has been shown to be safe and non-inferior to 9H in PLHIV > 12 years old. 1HP should not be used with Nevirapine, and its safety and efficacy in PLHIV using other antiretroviral drugs and in children are not established.

On the other hand, network meta-analyses show 4R—a drug used for more than half a century—is the safest regimen [50, 51]. However, no evidence exists regarding the head-to-head comparison of rifamycin-based regimens. 4R is also cost-effective in HIC and LMIC [52] and is readily available worldwide, provided by Ministries of Health, as it is part of the treatment for active TB. Another advantage of rifampicin is its syrup child-friendly formulation. Because safety is a major priority for preventive treatments, and rifampin is largely available, 4R could be preferred by clinicians and health policymakers. Finally, RH is easily available from Global Drug Facility in all high-burden countries as it is used for active TB treatment during the maintenance phase (four last months).

Studies are underway to evaluate new child-friendly water-dispersible formulations of 3HP (NCT03730181), regimens compatible with dolutegravir (NCT04272242), and other high-dose rifampicin shorter duration (two months) regimens (NCT03988933). Current regimens, doses, and main side effects are shown in Table 1.

TPT does not induce significant acquired drug resistance [34, 35]. There is no consensus regarding the treatment of contacts of index patients with known drug-resistant TB. 4R can be safely prescribed for contacts of isoniazid mono-resistant TB patients as long as multidrug-resistant (MDR) TB is ruled out. Conversely, isoniazid can be safely prescribed to contacts of patients with rifampicin mono-resistant TB. For contacts of MDR patients, the WHO recommends close follow-up [11], while a joint guideline published by the American CDC and medical societies recommends a six to 12 months later-generation fluoroquinolone regimen alone or with a second drug, according to the drug susceptibility profile of the index-case *M. tb* isolate. Pyrazinamide should not be the second drug because of toxicity and frequent discontinuation [54].

In summary, safer and shorter regimens have been widely available as an alternative for isoniazid preventive therapy in the last decade. Their use should be encouraged for contacts and other high-risk populations with TBI.

Table 1 Current options for TPT, doses, and main adverse events

Regimen	Dosage	Adverse events
Isoniazid alone for six to nine months	Adults, 5 mg/kg; children, 10 mg/kg (maximum, 300 mg)	Drug-induced liver injury, nausea, vomiting, abdominal pain, rash, peripheral neuropathy, dizziness, drowsiness, and seizure
Rifampicin alone for four months	Adults, 10 mg/kg; children, 10 mg/kg (maximum if < 45 kg, 450 mg; maximum if ≥ 45 kg, 600 mg)	Influenza-like syndrome, rash, drug-induced liver injury, anorexia, nausea, abdominal pain, neutropenia, thrombocytopenia, and renal reactions (e.g., acute tubular necrosis and interstitial nephritis)
Isoniazid plus rifampicin for three months	As above	As above
Weekly rifapentine plus isoniazid for three months	Adults and children: rifapentine, 15–30 mg/kg (maximum, 900 mg); isoniazid, 15 mg/kg (maximum, 900 mg)	Hypersensitivity reactions, petechial rash, drug-induced liver injury, anorexia, nausea, abdominal pain, and hypotensive reactions
Daily rifapentine plus isoniazid for one month	Adults and adolescents (> 12 years old): 300 mg daily for a weight of < 35 kg, 450 mg daily for a weight of 35–45 kg, and 600 mg for a weight of > 45 kg) plus isoniazid at a dose of 300 mg daily	Nausea, vomiting, drug-associated fever, anemia, neutropenia, elevated liver enzyme levels, peripheral neuropathy

Reproduced from [53]

6 Public Health Approach

Losses in the cascade of care of contacts have been quantified in several studies, summarized in a systematic review in 2017 [7]. The reasons for these losses, however, are poorly understood. Fear of severe adverse events and of drug resistance induction by healthcare providers, treatment intolerance with poor adherence, fear of stigma from index cases, shortage of tuberculin, unavailability of trained personnel to apply and read TST, costs of IGRA and of CXR, unavailability of radiologic services, delay for CXR diagnosis, have all been claimed to hamper the scale-up of TPT. Reasons may vary from setting to setting and even from clinic to clinic.

Several interventions to reduce the losses in the TBI cascade of care were reported, with varying effectiveness [55]. The most effective interventions were incentives, healthcare worker education, home visits, and digital solutions.

Based on these premises, a large international operational trial was carried out to understand the barriers and propose solutions to the specific barriers in each clinic [56]. Barriers were identified through questionnaires and interviews with key

players (index patients, contacts, and healthcare providers). Results of this phase were presented to the staff and managers and tailored implemented solutions. Solutions varied widely, including in-service training, educational materials to healthcare workers and patients, incentives to present to the clinics, meetings in churches, and the extension of opening hours of the clinics. This approach effectively increased the number of contacts starting TPT per 100 index cases in the intervention clinics in LMIC. The effect was also sustainable and cost-effective[2] and should be encouraged by national tuberculosis programs. Cost-effectiveness modeling has shown that the investment in scale-up of TBI treatment is outweighed mainly by savings from fewer active TB treatments in LMIC [53].

7 Conclusion

Contacts of index patients with TBI constitute the largest high-risk population for progression to active TB, thus a priority population for TPT. They should be actively found using any of the currently available tests and encouraged to complete a TPT course after exclusion of active TB. IGRA and TST have high sensitivity and specificity for diagnosing TBI, although their ability to predict progression is low. Active disease should be ruled out by symptoms screening and CXR where available. Rifamycin-based TPT regimens are safe and effective and increase completion rates, but shorter regimens are not sufficient to scale-up TPT, as this is the final step of a long cascade. Scale-up TPT for contacts of index patients is feasible but requires political will and a pragmatic approach. All steps of the cascade of care need to be locally analyzed, and specific solutions to overcome identified barriers should be implemented. Contact investigation and their appropriate management need urgent attention to attain the United Nations' goals.

Core Messages

- TPT is a key strategy to eliminate TB.
- Contacts of index patients are the largest population with a high risk of progression to active TB when infected, thus a priority for TPT.
- IGRA and TST accurately detect TB infection in contacts and predict benefits from TPT compared to those with a negative test.
- Clinical and radiological exclusion of active TB is recommended in contacts over five years of age without HIV infection.
- Rifamycin-based regimens are shorter, safer, and better tolerated and completed than isoniazid, with non-inferior efficacy.

[2] Oxlade et al., Lancet Public Health, in press; Bastos et al., Int J Tuberc Lung Dis, in press.

References

1. Fox GJ, Nguyen TA, Coleman M, Trajman A, Velen K, Marais BJ (2021) Implementing tuberculosis preventive treatment in high-prevalence settings. Int J Infect Dis 113:S13–S15. https://doi.org/10.1016/j.ijid.2021.02.094
2. Houben RMGJ, Dodd PJ (2016) The global burden of latent tuberculosis infection: a re-estimation using mathematical modelling. PLoS Med 13:e1002152. https://doi.org/10.1371/journal.pmed.1002152
3. Dye C, Glaziou P, Floyd K, Raviglione M (2013) Prospects for tuberculosis elimination. Annu Rev Public Health 34:271–286. https://doi.org/10.1146/annurev-publhealth-031912-114431
4. Smieja MJ, Marchetti CA, Cook DJ, Smaill FM (2000) Isoniazid for preventing tuberculosis in non-HIV infected persons. Cochrane Database Syst Rev CD001363. http://doi.org/10.1002/14651858.CD001363
5. United Nations (2018) Political declaration of the UN General Assembly high-level meeting. Resolution A/RES/73/
6. WHO (2021) Global tuberculosis report 2021. World Health Organization, Geneva, Switzerland. Licence: CC BY-NC-SA 3.0 IGO
7. Alsdurf H, Hill PC, Matteelli A, Getahun H, Menzies D (2016) The cascade of care in diagnosis and treatment of latent tuberculosis infection: a systematic review and meta-analysis. Lancet Infect Dis. https://doi.org/10.1016/S1473-3099(16)30216-X
8. Sumartojo E (1993) When tuberculosis treatment fails. A social behavioral account of patient adherence. Am Rev Respir Dis 147:1311–1320. https://doi.org/10.1164/ajrccm/147.5.1311
9. Trajman A, Steffen RE, Menzies D (2013) Interferon-gamma release assays versus tuberculin skin testing for the diagnosis of latent tuberculosis infection: an overview of the evidence. Pulm Med 2013:601737. https://doi.org/10.1155/2013/601737
10. Getahun H, Matteelli A, Chaisson RE, Raviglione M (2015) Latent Mycobacterium tuberculosis infection. N Engl J Med 372:2127–2135. https://doi.org/10.1056/NEJMra1405427
11. World Health Organization (2020) WHO consolidated guidelines on tuberculosis. Module 1, Prevention: tuberculosis preventive treatment
12. Fox GJ, Barry SE, Britton WJ, Marks GB (2013) Contact investigation for tuberculosis: a systematic review and meta-analysis. Eur Respir J 41:140–156. https://doi.org/10.1183/09031936.00070812
13. Campbell JR, Winters N, Menzies D (2020) Absolute risk of tuberculosis among untreated populations with a positive tuberculin skin test or interferon-gamma release assay result: systematic review and meta-analysis. BMJ 368. https://doi.org/10.1136/bmj.m549
14. Abubakar I, Jackson C, Rangaka MX (2017) C-Tb: a latent tuberculosis skin test for the 21st century? Lancet Respir Med 5:236–237. https://doi.org/10.1016/S2213-2600(17)30012-7
15. Zellweger JP, Sotgiu G, Corradi M, Durando P (2020) The diagnosis of latent tuberculosis infection (LTBI): currently available tests, future developments, and perspectives to eliminate tuberculosis (TB). Med Lav 111:170–183. http://doi.org/10.23749/mdl.v111i3.9983
16. Nikitina IY, Karpina NL, Kasimceva OV, Gergert VY, Ergeshov A, Lyadova IV (2019) Comparative performance of QuantiFERON-TB Gold versus skin test with tuberculosis recombinant allergen (Diaskintest) among patients with suspected pulmonary tuberculosis in Russia. Int J Infect Dis 86:18–24. https://doi.org/10.1016/j.ijid.2019.06.014
17. Menzies D (1999) Interpretation of repeated tuberculin tests. Boosting, conversion, and reversion. Am J Respir Crit Care Med 159:15–21. https://doi.org/10.1164/ajrccm.159.1.9801120
18. Zwerling A, van den Hof S, Scholten J, Cobelens F, Menzies D, Pai M (2011) Interferon-gamma release assays for tuberculosis screening of healthcare workers: a systematic review. Thorax. https://doi.org/10.1136/thx.2010.143180

19. Sotgiu G, Saderi L, Petruccioli E, Aliberti S, Piana A, Petrone L, Goletti D (2019) QuantiFERON TB Gold Plus for the diagnosis of tuberculosis: a systematic review and meta-analysis. J Infect 79:444–453. https://doi.org/10.1016/j.jinf.2019.08.018
20. Pai M, O'Brien R (2007) Serial testing for tuberculosis: can we make sense of T cell assay conversions and reversions? PLoS Med 4:e208. https://doi.org/10.1371/journal.pmed.0040208
21. Mandalakas AM, Detjen AK, Hesseling AC, Benedetti A, Menzies D (2011) Interferon-gamma release assays and childhood tuberculosis: systematic review and meta-analysis. Int J Tuberc Lung Dis 15:1018–1032. https://doi.org/10.5588/ijtld.10.0631
22. Diel R, Loddenkemper R, Nienhaus A (2012) Predictive value of interferon-γ release assays and tuberculin skin testing for progression from latent TB infection to disease state: a meta-analysis. Chest 142:63–75. https://doi.org/10.1378/chest.11-3157
23. Auguste P, Madan J, Tsertsvadze A, Court R, McCarthy N, Sutcliffe P, Taylor-Phillips S, Pink J, Clarke A (2019) Identifying latent tuberculosis in high-risk populations: systematic review and meta-analysis of test accuracy. Int J Tuberc Lung Dis 23:1178–1190. https://doi.org/10.5588/ijtld.18.0743
24. Gupta RK, Turner CT, Venturini C, Esmail H, Rangaka MX, Copas A, Lipman M, Abubakar I, Noursadeghi M (2020) Concise whole blood transcriptional signatures for incipient tuberculosis: a systematic review and patient-level pooled meta-analysis. Lancet Respir Med 8:395–406. https://doi.org/10.1016/S2213-2600(19)30282-6
25. Esmail H, Cobelens F, Goletti D (2020) Transcriptional biomarkers for predicting development of TB: progress and clinical considerations. Eur Respir J. https://doi.org/10.1183/13993003.01957-2019
26. Akolo C, Adetifa I, Shepperd S, Volmink J (2010) Treatment of latent tuberculosis infection in HIV infected persons. Cochrane Database Syst Rev CD000171. http://doi.org/10.1002/14651858.CD000171.pub3
27. Samandari T, Agizew TB, Nyirenda S, Tedla Z, Sibanda T, Shang N, Mosimaneotsile B, Motsamai OI, Bozeman L, Davis MK, Talbot EA, Moeti TL, Moffat HJ, Kilmarx PH, Castro KG, Wells CD (2011) 6-month versus 36-month isoniazid preventive treatment for tuberculosis in adults with HIV infection in Botswana: a randomised, double-blind, placebo-controlled trial. The Lancet 377:1588–1598. https://doi.org/10.1016/S0140-6736(11)60204-3
28. Getahun H, Kittikraisak W, Heilig CM, Corbett EL, Ayles H, Cain KP, Grant AD, Churchyard GJ, Kimerling M, Shah S, Lawn SD, Wood R, Maartens G, Granich R, Date AA, Varma JK (2011) Development of a standardized screening rule for tuberculosis in people living with HIV in resource-constrained settings: individual participant data meta-analysis of observational studies. PLoS Med 8:e1000391. https://doi.org/10.1371/journal.pmed.1000391
29. Hamada Y, Lujan J, Schenkel K, Ford N, Getahun H (2018) Sensitivity and specificity of WHO's recommended four-symptom screening rule for tuberculosis in people living with HIV: a systematic review and meta-analysis. Lancet HIV 5:e515–e523. https://doi.org/10.1016/S2352-3018(18)30137-1
30. Hanrahan C, Dowdy D (2018) Chest X-ray for tuberculosis preventive therapy: use caution. Lancet HIV 5:e478–e479. https://doi.org/10.1016/S2352-3018(18)30213-3
31. Drain PK, Bajema KL, Dowdy D, Dheda K, Naidoo K, Schumacher SG, Ma S, Meermeier E, Lewinsohn DM, Sherman DR (2018) Incipient and subclinical tuberculosis: a clinical review of early stages and progression of infection. Clin Microbiol Rev 31. https://doi.org/10.1128/CMR.00021-18
32. Sterling TR, Villarino ME, Borisov AS, Shang N, Gordin F, Bliven-Sizemore E, Hackman J, Hamilton CD, Menzies D, Kerrigan A, Weis SE, Weiner M, Wing D, Conde MB, Bozeman L, Horsburgh CR Jr, Chaisson RE, TB Trials Consortium PREVENT TB Study Team (2011) Three months of rifapentine and isoniazid for latent tuberculosis infection. N Engl J Med 365:2155–2166. https://doi.org/10.1056/NEJMoa1104875

33. Menzies D, Adjobimey M, Ruslami R, Trajman A, Sow O, Kim H, Obeng Baah J, Marks GB, Long R, Hoeppner V, Elwood K, Al-Jahdali H, Gninafon M, Apriani L, Koesoemadinata RC, Kritski A, Rolla V, Bah B, Camara A, Boakye I, Cook VJ, Goldberg H, Valiquette C, Hornby K, Dion M-J, Li P-Z, Hill PC, Schwartzman K, Benedetti A (2018) Four months of rifampin or nine months of isoniazid for latent tuberculosis in adults. N Engl J Med 379:440–453. https://doi.org/10.1056/NEJMoa1714283
34. Balcells ME, Thomas SL, Godfrey-Faussett P, Grant AD (2006) Isoniazid preventive therapy and risk for resistant tuberculosis. Emerging Infect Dis 12:744–751. https://doi.org/10.3201/eid1205.050681
35. den Boon S, Matteelli A, Getahun H (2016) Rifampicin resistance after treatment for latent tuberculous infection: a systematic review and meta-analysis. Int J Tuberc Lung Dis 20:1065–1071. https://doi.org/10.5588/ijtld.15.0908
36. Khan FA, Pande T, Tessema B, Song R, Benedetti A, Pai M, Lönnroth K, Denkinger CM (2017) Computer-aided reading of tuberculosis chest radiography: moving the research agenda forward to inform policy. Eur Respir J 50. https://doi.org/10.1183/13993003.00953-2017
37. WHO Technical Consultation on latent TB infection management: research in support of scale-up
38. Trajman A, Cordeiro-Santos M, Brito de Souza A, Esmail A, Lipman M, Santin M, Noguera-Julian A, Dheda K (2019) A novel blood-based triage test, ImmiPrint®-TB, to rule out active tuberculosis: a prospective multicentre study. Int J Tuberc Lung Dis S577
39. Warsinske HC, Rao AM, Moreira FMF, Santos PCP, Liu AB, Scott M, Malherbe ST, Ronacher K, Walzl G, Winter J, Sweeney TE, Croda J, Andrews JR, Khatri P (2018) Assessment of validity of a blood-based 3-gene signature score for progression and diagnosis of tuberculosis, disease severity, and treatment response. JAMA Netw Open 1:e183779. https://doi.org/10.1001/jamanetworkopen.2018.3779
40. Chegou NN, Sutherland JS, Malherbe S, Crampin AC, Corstjens PLAM, Geluk A, Mayanja-Kizza H, Loxton AG, van der Spuy G, Stanley K, Kotzé LA, van der Vyver M, Rosenkrands I, Kidd M, van Helden PD, Dockrell HM, Ottenhoff THM, Kaufmann SHE, Walzl G, AE-TBC Consortium (2016) Diagnostic performance of a seven-marker serum protein biosignature for the diagnosis of active TB disease in African primary healthcare clinic attendees with signs and symptoms suggestive of TB. Thorax 71:785–794. https://doi.org/10.1136/thoraxjnl-2015-207999
41. Kabbara WK, Sarkis AT, Saroufim PG (2016) Acute and fatal isoniazid-induced hepatotoxicity: a case report and review of the literature. Case Rep Infect Dis 2016:3617408. https://doi.org/10.1155/2016/3617408
42. Diallo T, Adjobimey M, Ruslami R, Trajman A, Sow O, Obeng Baah J, Marks GB, Long R, Elwood K, Zielinski D, Gninafon M, Wulandari DA, Apriani L, Valiquette C, Fregonese F, Hornby K, Li P-Z, Hill PC, Schwartzman K, Benedetti A, Menzies D (2018) Safety and side effects of rifampin versus isoniazid in children. N Engl J Med 379:454–463. https://doi.org/10.1056/NEJMoa1714284
43. Martinson NA, Barnes GL, Moulton LH, Msandiwa R, Hausler H, Ram M, McIntyre JA, Gray GE, Chaisson RE (2011) New regimens to prevent tuberculosis in adults with HIV infection. N Engl J Med 365:11–20. https://doi.org/10.1056/NEJMoa1005136
44. Ena J, Valls V (2005) Short-course therapy with rifampin plus isoniazid, compared with standard therapy with isoniazid, for latent tuberculosis infection: a meta-analysis. Clin Infect Dis 40:670–676. https://doi.org/10.1086/427802
45. Swindells S, Ramchandani R, Gupta A, Benson CA, Leon-Cruz J, Mwelase N, Jean Juste MA, Lama JR, Valencia J, Omoz-Oarhe A, Supparatpinyo K, Masheto G, Mohapi L, da Silva Escada RO, Mawlana S, Banda P, Severe P, Hakim J, Kanyama C, Langat D, Moran L, Andersen J, Fletcher CV, Nuermberger E, Chaisson RE, BRIEF TB/A5279 Study Team (2019) One month of rifapentine plus isoniazid to prevent HIV-related tuberculosis. N Engl J Med 380:1001–1011. https://doi.org/10.1056/NEJMoa1806808

46. Sterling TR (2020) Guidelines for the treatment of latent tuberculosis infection: recommendations from the National Tuberculosis Controllers Association and CDC. MMWR Recomm Rep 69. http://doi.org/10.15585/mmwr.rr6901a1
47. Belknap R, Holland D, Feng P-J, Millet J-P, Caylà JA, Martinson NA, Wright A, Chen MP, Moro RN, Scott NA, Arevalo B, Miró JM, Villarino ME, Weiner M, Borisov AS, TB Trials Consortium iAdhere Study Team (2017) Self-administered versus directly observed once-weekly isoniazid and rifapentine treatment of latent tuberculosis infection: a randomized trial. Ann Intern Med 167:689–697. https://doi.org/10.7326/M17-1150
48. Denholm JT, McBryde ES, Eisen D, Street A, Matchett E, Chen C, Shultz TR, Biggs B, Leder K (2017) SIRCLE: a randomised controlled cost comparison of self-administered short-course isoniazid and rifapentine for cost-effective latent tuberculosis eradication. Intern Med J 47:1433–1436. https://doi.org/10.1111/imj.13601
49. Johnson KT, Churchyard GJ, Sohn H, Dowdy DW (2018) Cost-effectiveness of preventive therapy for tuberculosis with isoniazid and rifapentine versus isoniazid alone in high-burden settings. Clin Infect Dis 67:1072–1078. https://doi.org/10.1093/cid/ciy230
50. Stagg HR, Zenner D, Harris RJ, Muñoz L, Lipman MC, Abubakar I (2014) Treatment of latent tuberculosis infection: a network meta-analysis. Ann Intern Med 161:419–428. https://doi.org/10.7326/M14-1019
51. Zenner D, Beer N, Harris RJ, Lipman MC, Stagg HR, van der Werf MJ (2017) Treatment of latent tuberculosis infection: an updated network meta-analysis. Ann Intern Med 167:248–255. https://doi.org/10.7326/M17-0609
52. Bastos ML, Campbell JR, Oxlade O, Adjobimey M, Trajman A, Ruslami R, Kim HJ, Baah JO, Toelle BG, Long R, Hoeppner V, Elwood K, Al-Jahdali H, Apriani L, Benedetti A, Schwartzman K, Menzies D (2020) Health system costs of treating latent tuberculosis infection with four months of rifampin versus nine months of isoniazid in different settings. Ann Intern Med 173:169–178. https://doi.org/10.7326/M19-3741
53. WHO (2020) Target product profiles for tuberculosis preventive treatment. World Health Organization, Geneva, Switzerland. Licence: CC BY-NC-SA 3.0 IGO
54. Nahid P, Mase SR, Migliori GB, Sotgiu G, Bothamley GH, Brozek JL, Cattamanchi A, Cegielski JP, Chen L, Daley CL, Dalton TL, Duarte R, Fregonese F, Horsburgh CR, Ahmad Khan F, Kheir F, Lan Z, Lardizabal A, Lauzardo M, Mangan JM, Marks SM, McKenna L, Menzies D, Mitnick CD, Nilsen DM, Parvez F, Peloquin CA, Raftery A, Schaaf HS, Shah NS, Starke JR, Wilson JW, Wortham JM, Chorba T, Seaworth B (2019) Treatment of drug-resistant tuberculosis. An official ATS/CDC/ERS/IDSA clinical practice guideline. Am J Respir Crit Care Med 200:e93–e142. https://doi.org/10.1164/rccm.201909-1874ST
55. Barss L, Moayedi-Nia S, Campbell JR, Oxlade O, Menzies D (2020) Interventions to reduce losses in the cascade of care for latent tuberculosis: a systematic review and meta-analysis. Int J Tuberc Lung Dis 24:100–109. https://doi.org/10.5588/ijtld.19.0185
56. Oxlade O, Trajman A, Benedetti A, Adjobimey M, Cook VJ, Fisher D, Fox GJ, Fregonese F, Hadisoemarto P, Hill PC, Johnston J, Long R, Obeng J, Ruslami R, Valiquette C, Menzies D (2019) Enhancing the public health impact of latent tuberculosis infection diagnosis and treatment (ACT4): protocol for a cluster randomised trial. BMJ Open 9:e025831. https://doi.org/10.1136/bmjopen-2018-025831

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Exploring Problematizations Underlying Tuberculosis Control Strategies: A Cross-Country Analysis of India and Kenya

28

G. K. Mini, Sapna Mishra, Jinbert Lordson, and Malu Mohan

Stopping TB requires a government program that functions every day of the year, and that's hard in certain parts of the world. And partly it's because of who tuberculosis affects: it tends to affect the poor and disenfranchised most.

Thomas. R. Frieden

Summary

This chapter explores the evolution of tuberculosis (TB) control strategies of two lower-middle-income economies—India and Kenya—which are counted among the leading thirty countries with the high-TB burden. We have also examined how the 'problem of TB' is characterized within the national policy documents of these nations using a specific approach to policy analysis, namely 'What's the Problem Represented to Be' (WPR). The results of our analysis indicate that the underlying problematization of TB control in the global initiatives appeared to

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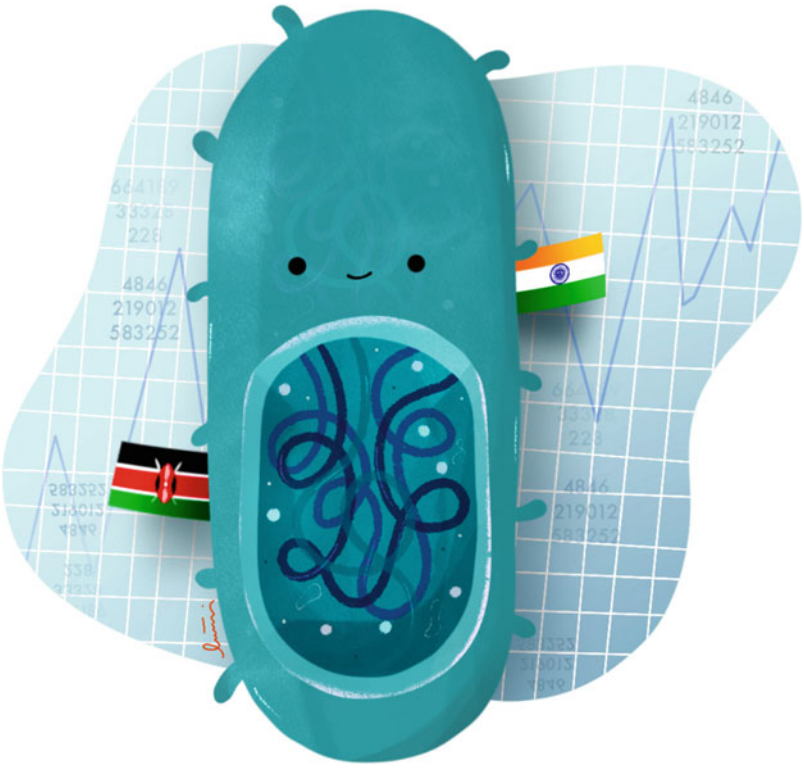
be largely techno-managerial in the beginning with a focus on health system strengthening, ensuring early case detection and cure. While a paradigm shift could be observed with the changing discourse around health propelled by the Commission of Social Determinants of Health (CSDH) report, the same was not reflected in the context of TB control. This could be because TB control was predominantly being problematized within a disease-centered paradigm instead of a health promotion one. Both nations have been committed to achieving the SDG goal of ending the TB epidemic by 2030. However, the strategies of both nations continue to rely heavily on biomedical approaches over initiatives focused on the social determinants of TB. High poverty level, under-nutrition, and inequalities persisting in both settings mandate a paradigmatic shift in problematization and approach, with a TB control strategy that is primarily driven by action on structural determinants influencing health with the techno-managerial solutions assuming only a supplementary role.

Graphical Abstract



Problematizations underlying tuberculosis control strategies in India and Kenya

Keywords

Biomedical · Problematization · Problem representation · Social determinants · Techno-managerial · Tuberculosis control

1 Introduction

Tuberculosis (TB) was declared a global emergency in 1993. Close to three decades later, it is steadily stationed among the leading ten causes of death and the major cause of deaths resulting from a sole infectious agent worldwide. According to the Global Tuberculosis Report (2019), in 2018, about 10 million people were affected, and the disease killed 1.2 million people. A series of interventions and initiatives have been undertaken over the past three decades by nations worldwide, under the aegis of international organizations and multilateral agencies, to tackle this age-old human scourge [1].

The declaration of TB as a global emergency had already catalyzed a coordinated surge in the disease control initiatives in many nations, propelled by the WHO-recommended cost-effective strategy called directly observed treatment, short-course (DOTS). However, the rising surge of the pandemic in the late 1990s triggered another series of global initiatives towards TB control. In 1998, the Stop TB initiative was launched. This was followed by the momentous *Amsterdam Declaration to Stop TB* (2001) that exhorted action from governmental delegations of 20 countries with the highest disease burden. The Stop TB initiative thus grew into an international partnership spearheading coordinated actions towards TB control. The vision of this partnership was to eliminate the disease as a public health concern. It had as its mission the resolve to safeguard that every patient has access to diagnosis, treatment, and cure, to stop disease transmission, to reduce unfair social and economic implications of the disease, and to develop new preventive, diagnostic, and therapeutic measures for elimination. The first Global Plan to Stop TB (2001–2005) focused on expanding DOTS coverage, adapting strategies to address the challenge of drug-resistant (DR) co-epidemic of TB and HIV-TB, strengthening the global partnership against TB and developing tools including new diagnostics, drugs, and vaccines. This period also witnessed an improvement in political commitment in the form of participation of country partners from both resource-poor and high-income settings in international initiatives, a core pillar of the DOTS strategy [2].

In 2005, the World Health Assembly passed a resolution towards "sustainable financing towards TB control and prevention," in which a commitment was made to strengthen the activities to achieve TB-related targets in Millennium Development Goals (MDGs) [3]. Following this, the WHO Stop TB strategy (2006–2015) was developed in 2006 to build on and enhance the TB-related MDGs and the Stop TB Partnership targets. This new strategy acknowledged that the rates of progress until 2005 were deficient in realizing the disease-related targets set under MDGs. The

Stop TB strategy focused on expanding and strengthening DOTS and addressing TB-HIV co-epidemic, other comorbidities, DR-TB, and the necessities of patient contacts and the disadvantaged and vulnerable social sections. Additionally, it also emphasized strengthening health systems, involving providers, empowering individuals and communities, and boosting research. The Stop TB strategy and the agreement of a resolution addressing multi and extensively drug-resistant forms have been instrumental in achieving the MDG target "to halt and begin to reverse the incidence of tuberculosis by 2015" [4].

The World Health Assembly, in 2014, proposed an "End TB strategy" for the prevention, care, and control of the disease in the post-MDG world. The strategy, which envisioned a disease-free world free of the epidemic, has three major components [5]:

1. unified, client-centered care and preventive measures;
2. emphatic policies and systems; and
3. enhanced research and innovations.

Though the Stop TB strategy had also incorporated addressing the needs of deprived and vulnerable communities and empowering patients and communities among its focus areas, the End TB strategy had a more pronounced equity focus. It identified as its focus areas such as enhancing access through universal health coverage policy, social and financial protection, and action on poverty along with other determinants of health [5].

The actions and initiatives over the past three decades have managed to bring about commendable progress in TB control. There has been an overall reduction of over 30% in the global incidence of TB and an average yearly decline of 1.6% between 2000 and 2018. However, the overall burden of the disease in absolute numbers remains very high, and there is considerable skepticism about the chances of meeting the Sustainable Development Goals (SDGs). The cumulative reduction in the global incidence of the disease between 2015 and 2018 was only 6.3%. This did not look promising in the context of the goal of 20% reduction by 2020 set by the End TB Strategy. The total reduction in TB-related deaths between 2000 and 2018 is about 43%. However, between 2015 and 2018, the global reduction in deaths has been about 11%, which is considerably shorter than the proposed milestone of 35% by 2020. This apparent slowdown encountered in our journey towards the realization of a TB-free world is a matter of grave concern, one which merits a nuanced analysis of the strategies adopted so far [1].

2 Challenges of Tuberculosis Control

Despite the commendable progress over the years, the situation of global TB control indicates a few major challenges which need to be explored and resolved as we gear forward to realize the dream of a TB-free world.

2.1 The Problem of 'Missing' Cases

Many TB cases are estimated to go 'missing' every year. Missing cases can be defined as the difference between the estimated number of people affected by TB in a year and the number notified to national TB programs [6]. The proportion of missed cases has remained almost steady during the past eight years, and every year about three million people with active TB stay undiagnosed. In 2018, among the ten million who were estimated to be suffering from the disease, about three million went undiagnosed [1]. Thus, many TB cases do not receive the care they need and deserve every year. The barriers faced in accessing services, especially for those who belong to hard-to-reach vulnerable populations such as migrants, refugees, children, and people living with HIV, constitute a major reason for under-reporting and consequently "missing" cases. In addition to death due to TB, many of these patients continue to infect others and, in the event of getting improper or incomplete treatment, could develop DR [7].

2.2 Drug-Resistant Tuberculosis

DR has become one of the most perplexing and tenacious challenges in the history of global TB control, threatening to reverse all the hard-earned achievements over the past decades. It is estimated that about 3.4% of new and 18% of already treated cases in 2018 had multidrug-resistant (MDR)/Rifampicin-resistant TB. The three countries which are largely bearing the brunt of MDR-TB and accounting for almost 50% of the total burden of cases are India (27%), China (14%), and Russian Federation (9%) [1]. In 2018, an average proportion of MDR-TB cases with XDR-TB was estimated to be 6.2% [1].

The treatment success rate of MDR-TB does not appear to be very encouraging, as recent data highlighted it to be 56% [1]. Chaotic treatment protocols leading to DR and amplification of DR patterns through repeated courses of DOTS continue to be a major driving force in several settings which grapple with limited resources for appropriate diagnosis or treatment of drug-resistant forms. In addition to which, there are risks of community and hospital-based transmission, especially in crowded facilities [8]. DR forms of TB are also much more expensive and difficult to treat, with the treatment protocol burdening both patients and health systems [9].

2.3 Gender Disparities

Gender disparities related to TB pose another major challenge. Worldwide, men are found to have a greater likelihood than women to contract and die from TB. However, it is still the leading infectious disease that kills women globally. Women are also more likely to be caregivers, which places them at a higher risk of exposure to caregiving situations. Due to inequalities in power and economic situations,

people of different genders are affected differently by TB in terms of varying levels of stigma and other barriers to access. Gender impacts levels of stigma and intensifies the risk of infection and severity of disease in many settings [10].

3 Inequalities in Tuberculosis Distribution

> The disease picked out and killed a few Princes and it carried off more than one bejewelled, tender-hearted courtesan; but it slaughtered the poor by the million.
>
> Dormandy

The earliest evidence of TB dates back to 8000 BCE. The disease has appeared in history in various contexts and forms, medical treatises and records, military and government records, political and social writings, and literature as a major reason for misery, poverty, and death. During the eighteenth and nineteenth centuries, the TB epidemic is known to have wreaked havoc in Europe, causing countless deaths, particularly among the deprived social sections. Poverty, overcrowding, and undernutrition, the classic triad, which predisposed the working poor of Europe to the disease during the industrial revolution, continues to tip the burden of disease disproportionately to the poor and vulnerable sections of the society till this date. These disparities were also reflected in the fact that wealthy patients could afford to travel to better climates and sought treatment in mountain sanatoria, while the poor had to continue suffering in the dark, poorly ventilated rooms and die of the disease [11].

In recent years, one of the key factors that appears to shift the discourse of TB control strategies among the international community has been the inequitable distribution of the disease across the world. Geographically, the greatest number of TB cases are reported from Southeast Asia (44%), Africa (24%), and the Western Pacific (18%). Eight countries of the world have accounted for about two-thirds of the global cases, and about 87% of all the cases have been accounted for by the 30 countries with a high-TB burden [1]. The assembling of cases among specific sections of the society, including the poor, the hungry, the ethnic minorities, the refugees, and the migrants, among many other vulnerable groups, has brought the role of social determinants of TB to stark focus. One of the reasons for this shift has been the growing international consciousness regarding social determinants of health and discussions regarding "the circumstances in which we grow, live, work, and age," which give rise to unequal, unfair distributions of population health. The global initiatives based on DOTS have evolved over time and expanded their scope to address the needs of vulnerable sections, universal health coverage, social and financial protection, and determinants of health. However, the larger focus of these initiatives appears to be case detection, treatment, cure, accurate reporting, and HIV/TB co-epidemic. Thus, the primary focus of global TB control initiatives appears to have been "disease control" as opposed to "health promotion." The way

these initiatives have been interpreted and adapted to national contexts, especially by resource-poor settings which are heavily dependent on technical and financial assistance from international sources, is also critical.

4 Conceptualization of the 'Tuberculosis Problem'

The challenges posed in the path to a TB-free world mandate a nuanced examination of the strategies through which TB control has been carried out. These strategies and initiatives could have conceptualized the TB control in a particular way, and in order to understand that particular conceptualization, we have used the 'What's the Problem Represented to Be' approach (WPR), introduced by Carol Bacchi. WPR is an approach to policy analysis that challenges the traditional view that policies are responses to problems that operate outside the policy process. It questions the assumption that these problems are waiting to be exposed and resolved. On the contrary, it attempts to critically examine how policies produce 'problems' as a peculiar kind of problems, which she labels as 'problematizations,' with important political implications. She argues that governing takes place through these problematizations. A 'WPR' analysis aims to discern how the 'problem' is represented within them and scrutinizes this problem representation through a series of six questions [12]:

1. What's the 'problem representation' in the policy proposal/document?
2. What assumptions underlie this problem representation?
3. How did this problem representation come about?
4. What is left unaddressed in this problem representation or what are the silences?
5. What are the consequences of this problem representation?
6. How and where is a representation created, circulated, and justified? If it has been challenged, how has it been challenged or questioned? (if it has not been challenged, then could it be challenged or questioned)

Through the WPR approach, we have attempted to explore the underlying problematization of TB control through various policy documents of two geographically distant settings relevant to the global TB story—India and Kenya. Both are lower-middle-income economies included in the list of high-TB burden countries (Figs. 1 and 2). Both have been striving hard for decades to control TB, have been actively engaged with the global initiatives to tackle the problem, managed to reduce their TB burden considerably, and now face similar challenges in the way forward. However, there are a few differences. For instance, compared to India, Kenya has made significant strides to reach the targets of the End TB strategy. Hence, Kenya is also globally recognized as a pathfinder for TB control.

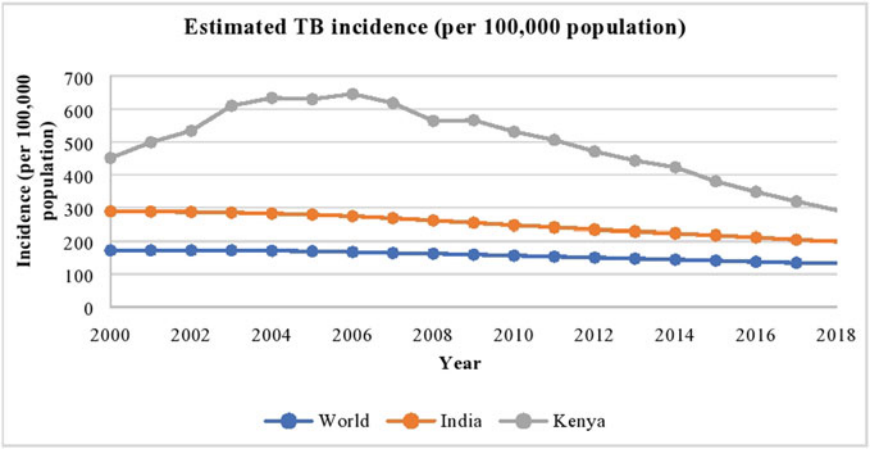


Fig. 1 Trend of estimated TB incidence per 100,000 population: World, India, and Kenya

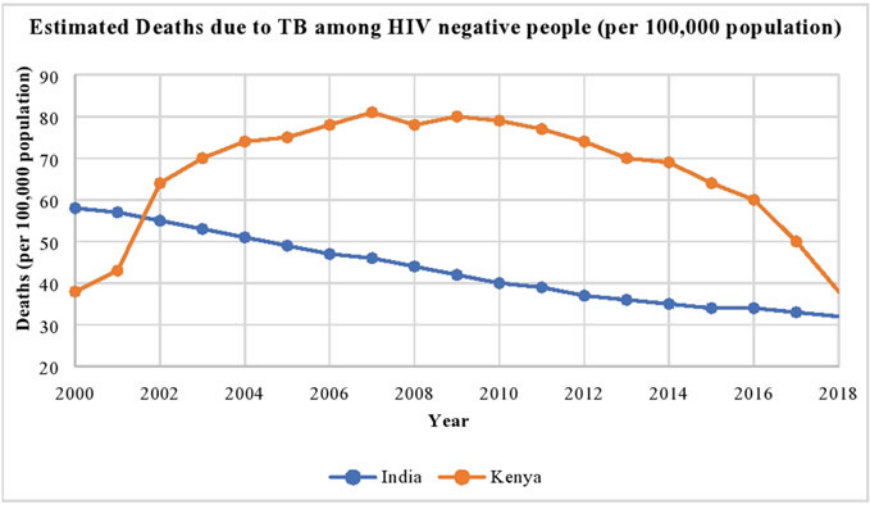


Fig. 2 Estimated deaths due to TB among HIV negative people (per 100,000 population): India and Kenya

5 Tuberculosis Control in India

5.1 Tuberculosis in India: An Overview

India bears the highest disease burden globally, and its tryst with TB is over a hundred years old. In addition to killing an estimated 480,000 Indians every year, it

is associated with significant socioeconomic burdens, including social stigmatization and impoverishment. The union government of independent India recognized the significance of TB control and in 1949 established a separate TB division within the Directorate General of Health Services of the Ministry of Health. The establishment of the TB Research Centre (1956), National TB Institute (1959), and pilot testing of the National Tuberculosis Control Programme (NTCP) in 1962 were major steps towards the goal of TB control [13].

Revised National Tuberculosis Control Program (RNTCP), which was renamed in 2020 as National Tuberculosis Elimination Programme (NTEP), was piloted in 1993 in five states with a population of 2.4 million. This declaration of TB as a global emergency by the WHO, the global acknowledgment of the HIV/AIDS epidemic, and the rise in drug resistance in India had also pushed the need for a new initiative towards TB control. After piloting, RNTCP was launched as a national program in 1997, intended to be scaled up in a phased manner [13, 14].

5.2 The Problematization of Tuberculosis Control: India (1997–2020)

In this section, we have critically examined the problematization of TB control and examined its evolution over time in four phases:

Phase I: 1997–2005
Phase II: 2006–2011
Phase III: 2012–2017
Phase IV: 2017 onwards

The major activities carried out during each phase have been thematically analyzed and summarized in Table 1. The major activities carried out to control TB between 2000 and 2018 are highlighted in Fig. 3.

5.2.1 Phase I: 1997–2005

Problem Representation

The evolution of TB control in India has been primarily need-based, relating to "problems of a technical, operational and managerial nature that arose over a period of time" (13).

The themes based on activities in the first phase of RNTCP reveal its focus on expansion with nationwide coverage and effective implementation through detailed planning and financial decentralization. The other major focus areas during this phase included integration of RNTCP into the health system, ensuring access to quality-assured diagnosis, formulation of a standardized treatment protocol, ensuring high treatment success rate, facilitated uninterrupted drug availability, the institution of a standardized reporting system, and supervision, monitoring, and feedback. The concern of TB-HIV co-epidemic was given due importance, and

Table 1 Major activities carried out over the four phases of TB control initiatives in India

Phase I (1997–2005)	Phase II (2006–2011)	Phase III (2012–2017)	Phase IV (2017 onwards)
• Programme expansion • Efficient programme implementation • Enhanced access to quality assured diagnosis • Establishing a standardized treatment protocol and ensuring a high treatment success rate • Integrating TB care under RNTCP with the general health care system • Ensuring uninterrupted drug availability • Establishment of a standardized recording and reporting system • Supervision, monitoring and feedback established at all levels • Co-ordinated action initiated between RNTCP and National AIDS Control Organization (NACO) • Development of technical policies and training modules for health personnel and their periodic revision • Collaborative efforts with multiple sectors—Non-governmental Organizations, medical colleges, private practitioners, other government departments and the corporate sector	• Multidrug resistant (MDR-TB) and extensively drug resistant (XDR)-TB emerged as priority areas and DOTS Plus rolled out • Developed the roadmap to honour the commitment to implementing the 2006 Global Strategy to Stop TB and reaching the TB related targets of the Millennium Development Goals by 2015 • Enhanced role of National Rural Health Mission in the programme with ASHAs trained to be DOTS providers in rural areas • TB-HIV collaborative activities scaled up all over the country • Implementation of quality improvement processes in the RNTCP Laboratory Network • Public Private Mix activities expanded and active efforts to engage all providers • Intensified efforts towards co-ordination with other sectors • Paediatric TB emerged as a focus area and patient wise drug boxes developed for paediatric TB cases	• Programmatic Management of Drug Resistant TB rolled out to combat DR-TB and achieved nation-wide coverage • Composite Indicators for monitoring of programme performance of RNTCP developed and rolled out • Executive Order for 'Notification of TB cases' wherein the healthcare providers shall notify every TB case to local authorities • Newer diagnostic approaches introduced • Web enabled patient management system and online notification system—NIKSHAY introduced and integrated with other platforms • First National Drug Resistance Survey initiated • Nationwide scale up of the Intensified TB-HIV package under the TB-HIV Collaborative Activities under RNTCP was achieved • Ban imposed on manufacture, sale, distribution, use and import of the	• Programme renamed as National Tuberculosis elimination programme • Active case finding focusing on clinically, socially and occupationally vulnerable populations • ICT based patient-friendly adherence monitoring • Social welfare schemes initiated for people affected with TB • Direct Benefit Transfer (DBT) mechanism for transfer of monetary support and incentives to patients • Expansion of Daily Regimen for treatment of TB across the country • Scale up of Bedaquiline Conditional Access Programme initiated under PMDT • Laboratory Information Management System (LIMS) was introduced • Expansion of Drug Susceptibility Testing laboratories

(continued)

Table 1 (continued)

Phase I (1997–2005)	Phase II (2006–2011)	Phase III (2012–2017)	Phase IV (2017 onwards)
• Drug resistance identified as a concern and DOTS-Plus guidelines developed for the treatment of multidrug-resistant TB	• Greater efforts to engage people with TB and other vulnerable communities (urban slum dwellers, prisoners, migrants) through Advocacy Communication and Social Mobilization (ACSM) • Thrust to build National Operational Research capacity • Vision and Targets for RNTCP (2012–17) developed for a "TB-free India"	Sero-diagnostic test kits for diagnosis of TB • Project Axshya, civil society-based initiative by the International Union against TB and lung diseases using The Global Fund was launched • Intensive focus on campaigns through mass media, social media and print media	• Laboratories supported to achieve the National Accreditation Board for Testing and Calibration Laboratories (NABL) accreditation • Further decentralization of Drug Resistant TB treatment to the district level • National Framework for Gender-Responsive approach to TB • TB Survivors to TB Champions' is an important strategy in engaging with TB affected communities

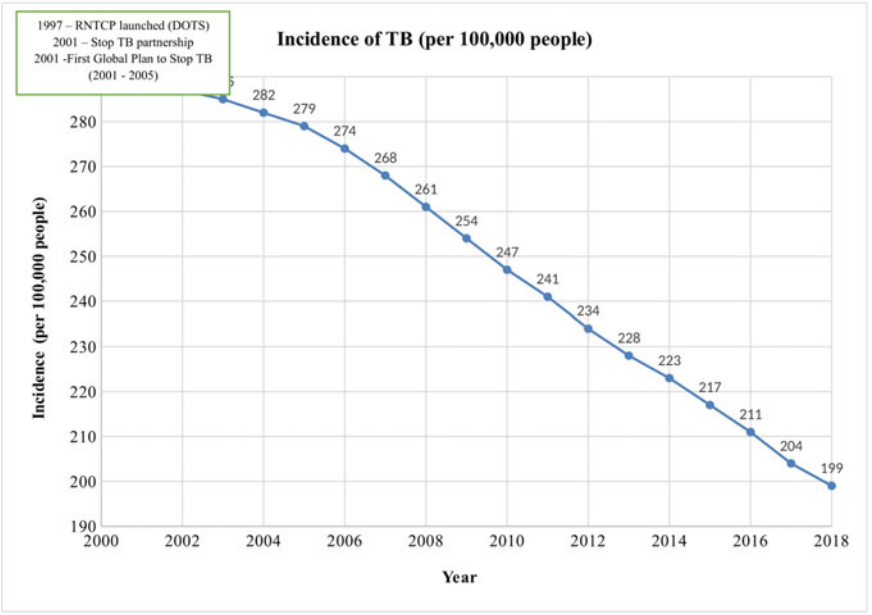


Fig. 3 Trends in TB incidence and major programmatic initiatives in India since 2000

there were efforts to institute collaborative activities between RNTCP and the *National AIDS Control Organization (NACO)* at various levels. There were also efforts to effectively integrate with other sectors like non-governmental organizations (NGOs), medical colleges, private practitioners, other government departments, and the corporate sector. From the program's focus areas in its first phase, RNTCP appears to have problematized the TB control initiative in India almost entirely within the biomedical paradigm advanced through a narrowly defined health system approach [15–19].

The actions under each of these initiatives are focused on maximally integrating all sectors to ensure that the program expands in coverage and there is greater access to diagnostic and treatment services to people residing all over the country. The links between TB, undernutrition, and poverty, the disproportionately greater burden and consequences of the disease on vulnerable sections, and the social burden of the disease (stigma, compromised child care, and lost school days of children of parents who suffer from TB) which women and children predominantly bear, remain part of the background and the activities continue to constitute a curative program. The stigma associated with the disease, which is particularly intensified in the case of HIV/AIDS patients with TB, is directed to be addressed as part of Joint IEC campaigns of NACO and RNTCP. Except this, even the IEC campaigns during this phase were predominantly devoted to establishing RNTCP and DOTS as the ultimate resolutions [15–19].

What Could Be the Possible Underlying Assumptions?

The activities imply that a well-implemented, nationwide program almost entirely focused on diagnosis, treatment, and cure is the right approach to effective TB control. Although DR has been identified as a concern, the problematization around it reflects the assumption that initiating a more refined program targeting DR and expanding it would be sufficient to address the concern.

What Could Be the Possible Silences?

Although mention of the larger social and economic determinants underlying the disease forms a part of the introduction of the documents, these aspects are almost completely neglected subsequently. The issue of DR has been identified in the documents, and the very first annual report (2001) stated forthrightly that.

> drug-resistant tuberculosis is a symptom of poor programme performance. [15]

However, despite this acknowledgment, there has not been any systematic inquiry into or discussion about the potential determinants of rising DR. The most significant policy silence during this phase, however, is with regard to primary healthcare and its significant role in TB prevention and control.

5.2.2 Phase II: 2006–2011

Problem Representation

RNTCP had achieved nationwide coverage in 2006, and the program's second phase was initiated. The same year, WHO initiated the Stop TB strategy with the aim of reducing the global burden by 2015. This was in accordance with the MDGs and the Second Global Plan to end TB (2006–2015). Beyond the focus on high-quality expansion and enhancement of the program, including the rolling out of DOTS-PLUS targeting drug resistance, there was a clear thrust on coordinated activities between NACO and RNTCP to address the TB-HIV synergy. Implementing quality improvement processes in the RNTCP Laboratory Network was also given focus [4].

The Stop TB strategy had emphasized the needs of the poor and vulnerable populations, due to which, for the first time, the higher exposure to the disease among residents of urban slums, the disadvantages faced by vulnerable sections like the tribal population and the gender-based inequities in access and utilization of TB care were emphasized in the national documents (4). This is reflected in the actions only as greater efforts to engage with these sections through advocacy communication and social mobilization (ACSM) campaign. The global initiative calls for a concerted effort to involve care providers, which has been echoed in the activities as an expansion of the public–private mix. One of the strategies proposed in the Stop TB initiative includes health system strengthening, which has been addressed under RNTCP as refinements in the health system to facilitate the execution and consolidation of the program [20–25].

What Could Be the Possible Underlying Assumptions?

The problematization of DR in this phase once again reiterates the techno-managerial assumptions underlying it. The approach to addressing the needs of vulnerable populations reflect the assumption that they can overcome their vulnerabilities by advocacy, providing them with accurate information about the disease and available services, and through social mobilization instead of tangible measures to address the life circumstances that predispose them to a higher risk of disease.

What Could Be the Possible Silences?

One major aspect neglected in the problematization of TB control in the first two phases is the two-way relationship between TB and malnutrition. Evidence suggests that poor nutritional status is a major risk factor for treatment failures and mortality among TB patients, including those with DR-TB [26, 27]. Critics have pointed out that considering the difference between the prevalence of undernutrition (15%) and HIV (0.2%), a much higher proportion of TB incidence could be attributed to undernutrition [28]. However, there have been no efforts towards a nationwide state-led program. Health system strengthening was one of the core strategies of the Stop TB strategy. Considering the prominent role of basic health determinants in the causation and transmission of TB, this presented an excellent opportunity to enhance the state funding to the country's public health sector and strengthen primary healthcare. However, the problematization of this component in India was limited to strengthening diagnostic and curative services.

5.2.3 Phase III: 2012–2017

Problem Representation

The strategic vision document (2012–2017) for a TB-free India stated:

> universal access to quality TB diagnosis and treatment for all TB patients in the community

as its central theme. The strategy involved maintaining the program's successes until then, finding unreached cases, and treating them effectively to avert the development of MDR-TB. The primary proposed action in this vision statement included early and enhanced detection through intensive case-finding efforts, especially among vulnerable social sections, by extending services to the maximum, including those accessing the private sector. The vision document identifies that to ensure treatment completion and prevent MDR-TB, all diagnosed TB cases must have access to patient-friendly, newer diagnostic modalities for accurate diagnosis and high-quality treatment. The strategies proposed entail "active case finding," which acknowledges the disproportionately heavy burden among specific sections of the society [25].

The highlights of this phase were the nationwide scale-up of programmatic management of DR-TB, the intensified TB-HIV package, and a major focus on social media, mass media, and print media campaigns. Other efforts included the development of composite indicators for effective program monitoring, the

implementation of web-enabled patient management, an online notification system (NIKSHAY), Project Axshya, and a civil society-based initiative focusing on the ACSM campaign [29–34].

What Could Be the Possible Underlying Assumptions?

The vision document and the activities during the phase reiterated the problem representation of TB control followed during the previous phases as a predominantly treatment-oriented program. The very theme of universal access to diagnosis and treatment implies that such access will naturally lead to the control of the disease. However, the vision only commits to universal access to care in the event of the disease. It does not commit to universal access to a TB-free existence. Such a commitment will need action on the primary determinants of the disease and primary healthcare rather than a purely curative approach based on aggressive case-finding and cure. As part of an initial appraisal of the program, the vision document identifies weak or delayed treatment-seeking behavior, the predominant use of the private sector in health as the first point of contact, low sensitivity diagnostic tools, and poor accountability in case notification and registration as the major challenges [29–34]. This reiterates the assumption that the challenges in TB control are related to the underutilization or underperformance of health systems.

What Could Be the Possible Silences?

The findings from the fourth round of the National Family Health Survey (2015–2016) indicate that the disease distribution was disproportionately high among the poor and illiterate and those belonging to historically disadvantaged social groups such as the other backward castes [35]. Another analysis using the data from NFHS-4 indicates that a range of risk factors associated with living conditions, e.g., smoking, cooking fuel used, lack of separate kitchen, type of floor, roofing and wall material, number of persons sharing a room and toilet, and potable water, was found to be strongly associated with the TB prevalence [36]. However, during this phase, the proposed and undertaken activities reiterate the techno-managerial problematization.

5.2.4 Phase IV (2017 Onwards)

Problem Representation

India, in 2017, renamed RNTCP as National Tuberculosis Elimination Programme and set the lofty goal of elimination of TB by 2025. In addition to reducing TB incidence, prevalence, and mortality, the program also set the achievement of completely avoiding catastrophic costs for affected families due to TB as one of its impact indicators. Thus, greater social and financial protection for those affected is integral to the program [34].

According to the National Strategic Plan for Tuberculosis Elimination (2017–2025) draft vision document, the program has been built on four strategic pillars of "Detect–Treat–Prevent–Build" (DTPB). This is a more intensified approach compared to its predecessors in the first two components of detecting and treating, the

proposed activities being scaling up of free, high sensitivity diagnostic tests and algorithms, encouragement of universal testing, systematic screening of high-risk populations, provision of free TB care to all patients, adequate patients support systems to prevent the loss of patients to care, universal daily drug regimen and speedy scale-up of short-course regimens for DR-TB types and use of drug susceptibility testing. The third component of "prevent" involves a new and comprehensive approach on fundamental determinants of the disease, and the proposed strategies include scaling up of airborne infection control measures, treatment of latent infection among contacts of cases, and addressing social determinants of TB among vulnerable communities. The final component of "build" focuses on building facilitating policies, institutions, and human resources with heightened capacities, which has always been a priority for RNTCP [34].

The activities in the fourth phase until 2020 included efforts for active case finding among social groups which are vulnerable not only clinically but also socially and occupationally. The activities also included patient-friendly adherence monitoring, social welfare schemes initiated for people affected with TB, and a direct benefit transfer (DBT) mechanism for the transfer of money and other incentives to patients. TB Survivors to TB Champions' is developed as a key strategy in engaging with TB-affected people, and capacity building for TB survivors has been initiated in this regard. Expansion of drug susceptibility testing laboratories and supporting them to achieve the esteemed accreditation from the National Accreditation Board for Testing and Calibration Laboratories (NABL) have been pursued. Expansion of new treatment regimens (daily regimen) and drugs (Bedaquiline Conditional Access Programme) has been undertaken [37–39].

Although TB notification rates are higher for men, the social and economic burden of the disease is experienced differently by women and transgender people. Despite the lower incidence, the number of TB-affected women in India represents a huge burden, while the incidence of the disease among transgender people is not known. Several studies have suggested that gender differences and inequalities play a significant role in the epidemiology of the disease (higher risk among pregnant and postpartum women), exposure, risk, and vulnerability to the disease (roles of social norms, indoor air pollution, and undernutrition), health-seeking behaviors, and treatment adherence [40]. Gender is an aspect axis of vulnerability that has not been adequately represented in the problem representation of TB control. In a promising move, a National Framework for Gender-Responsive approach to TB was released in 2019, which proposes to provide equitable and rights-based services for women, men, and transgender persons by implementing a gender-specific programmatic approach and to organize, empower, and engage persons of these three gender groups in the response at the health system and community levels within the DTPB framework.

What Could Be the Possible Underlying Assumptions?

The activities proposed by the strategic vision and undertaken so far reflect a commitment to the global End TB strategy, focusing on social and financial

protection. However, based on the activities undertaken so far, the program's overall focus remains on the disease, the patients, and its cure and control.

What Could Be the Possible Silences?

In addition to the persistent silences, the latest vision statement and activities have been silent about the practical approaches to achieving the "prevent" component. Introducing the "prevent" component in the DTPB framework is indeed promising, but there is no clear roadmap for prevention. The only exception to this was the release of the National Framework for Gender-Responsive approach to TB.

6 Tuberculosis Control in Kenya

6.1 Tuberculosis in Kenya: An Overview

Like India, Kenya also has a long history of TB control closely linked to its trajectory of leprosy control. The National Leprosy and Tuberculosis Program (NLTP) was introduced by the Government of Kenya in 1980, which combined the TB control activities undertaken since 1956 with the leprosy control measures initiated in the early seventies. The health sector reform in 1983, which entailed decentralization of provision of health services to the district level, further affected TB control. Decentralization was accompanied by rigorous training and orientation of district-level personnel. Kenya developed the finalized version of its national DOTS strategy in 1991. In 1993, short-course chemotherapy was introduced to reach all districts by 1997. The first National Health strategic plan was developed for 1999–2004, marked by major health sector reforms focusing on decentralization and an essential health package, and NLTP retained its central focus in these reforms. In 2005, Kenya started HIV testing and counseling of all TB patients as the HIV epidemic contributed to the TB epidemic [41]. NLTP had its first strategic plan (2006–2010) in line with the second National Health Strategic plan (2006–2010). In 2007, the program was elevated to a separate division in the health ministry in the preventive and promotive department as the Division of Leprosy, Tuberculosis and Lung Disease (DLTLD) [42]. Several strategic plans were adopted as well as prepared by the DTLD with the help of WHO and other donor agencies to control TB, such as second strategic plan for the period 2011–2015, stop TB partnership strategy plan for the period 2014–2018, and the third strategic plan for 2015–2018.

6.2 The Problematization of Tuberculosis Control: Kenya (1999–2018)

In this section, we have explored the problematization of TB control along with its evolution over time in the following four phases:

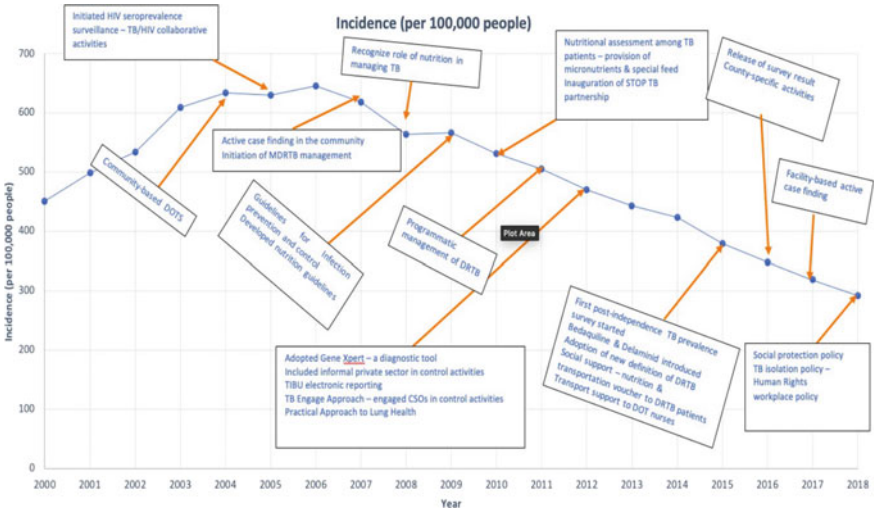


Fig. 4 Trends in TB incidence and major programmatic initiatives in Kenya since 2000

Phase I: 1999–2004
Phase II: 2005–2010
Phase III: 2011–2014
Phase IV: 2015–2018

The major activities carried out to control TB between 2000 and 2018 are highlighted in Fig. 4. The activities have also been detailed phase-wise in Table 2.

6.2.1 Phase I: 1999–2004

Problem Representation

The activities carried out in this phase of TB control appear to focus largely on the decentralization of planning activities, expansion of TB control activities, such as diagnosis and treatment to a larger number of healthcare facilities, development of human resources by training of healthcare workers and increased involvement of private sector [41]. These activities give an impression that the control of TB was largely problematized as a health system-centered problem through a biomedical lens. Further, TB itself is conceptualized predominantly as a curative problem. Hence, from the activities, one could make out that control of TB appears to be largely skewed towards curative care.

What Could Be the Possible Underlying Assumptions?

The construction of this problem seems to rest on the notion that strengthening the health system alone towards diagnosis, treatment, and cure can control TB.

Table 2 Major activities carried out over the four phases of TB control initiatives in Kenya

Phase I (1999–2004)	Phase II (2005–2010)	Phase III (2011–2014)	Phase IV (2015–2018)
• Integration of TB control activities in the district and provincial-level planning • Expansion of TB diagnosis and treatment services • Development of human resources • Funding from the national government and international donors to strengthen health infrastructure and availability of drugs	• Paradigm shift in case finding—from passive to active • Strengthening of laboratory services • Introduction of electronic data reporting • Strengthening of TB/HIV co-ordination activities • Engagement of stakeholders for provision of free treatment • Launch of Community based TB care (CB-DOTS) • Focus on nutrition support, assessment, and monitoring for registered TB patients • Decentralization of TB services to focus on drug-resistant TB • Institution of a Central Reference TB lab • Focus on Infection prevention and control (IPC) • Introduction of a Pharmaceutical unit to avoid interrupted supply of medicines • Development of a new strategic plan (2011–2015) with a focus on policy, planning, and research • Advocacy, communication, and social mobilization (ACSM) for TB prevention • Development of TB, poverty and gender policy document and sensitization of personnel • Establishment of supervision, monitoring, and feedback at all levels	• Labelling of management of DRTB as programmatic management of Drug Resistant TB (PMDT)—Provision of transport support along with the nutritional support to the MDR TB patients • One new activity in the form of provision of DOTS by family members was included • Introduction of LED fluorescent microscopy in high volume laboratories • Engaging small and informal providers in intensified case finding for TB; engaging selected providers in sputum collection from TB suspects • Introduction of gene expert machines • Introduction of Laboratory information management system (LIMS)	• Facility based active case finding approach—to screen all patients visiting a health facility • Intensified training of healthcare workers on childhood TB, Gene Xpert, Procurement of audiometers • GeneXpert designated as first line diagnostic technology—Xpert expansion plan (2011–2016) • Introduction of Delamanid, launch of child friendly TB medicines, launch of dolutegravir for adolescents and adults (for TB/HIV co-infection) • Launch of shorter-term regimen for rifampicin resistant TB patients • Engaging elected leaders in TB advocacy

(continued)

Table 2 (continued)

Phase I (1999–2004)	Phase II (2005–2010)	Phase III (2011–2014)	Phase IV (2015–2018)
		• Introduction of TIBU electronic reporting system • Practical Approach to Lung Health (PAL)—training of healthcare workers • Developed a four-year national strategic plan (2015–2018)	• Launch of STOP TB Partnership—Kenya strategic plan (2014–2018)—addressing the social determinants of health • Extension for community health outcomes project (ECHO) • Nutrition support—therapeutic food support, nutrition counselling, nutrition education, vitamin-A supplementation, and pyridoxine supplementation • Engaging TB advocates in advocacy—recognizes the strength of the voices of persons infected and affected by TB • Continuous decentralization of services to improve diagnosis and management of TB patients • Conducted and reported the result of the first post-independence TB prevalence survey

(continued)

Table 2 (continued)

Phase I (1999–2004)	Phase II (2005–2010)	Phase III (2011–2014)	Phase IV (2015–2018)
			• Integration of childhood TB into other maternal and child health services • Human Rights and Gender—launch of TB isolation policy • Workplace policy for TB was developed • Pharmacists included in the array of providers • Conducted a TB patient cost survey—leading to the development of Kenya social protection policy for tuberculosis and leprosy patients

What Could Be the Possible Silences?

It was evident for a long that TB was spreading across certain kinds of geographical areas as well as among certain groups of people such as the poor, mobile, refugees, HIV infected people, and prisoners; still, there were no specific strategies to engage with them to control TB.

6.2.2 Phase II: 2007–2010

Due to the unavailability of policy documents and/or annual reports for the years 2005 and 2006 (in the public domain), the activities of the years could not be documented or analyzed.

Problem Representation

This period marked a paradigm shift in case finding technique—moving from passive to active one. Kenya started testing and counseling all TB patients for HIV, recognizing the role of the HIV epidemic in propagating the TB epidemic. Though undernutrition was always recognized as the paramount risk, it was only since 2008 that the government started developing strategies to address it among TB patients in the form of counseling and provision of nutritional support to drug-susceptible TB patients and transportation support along with nutritional support to MDR-TB. Though a mention of the development of TB, poverty, and gender policy document could be observed in the reports, the activities carried out were limited to outreach activities, including intensive door to door campaigns, active case finding, referral of suspected cases, tracing of defaulter cases and community sensitization. The other major focus areas during this phase included ensuring access to quality-assured diagnosis (strengthening lab services, especially in case of MDR-TB); increased involvement of various healthcare providers (NGOs, faith-based organizations, private sector); institution of a standardized reporting system and supervision; emphasis on infection prevention and control in isolation facilities; facilitated uninterrupted drug availability; advocacy, communication, and social mobilization; monitoring and feedback [42–45].

The strategies during this period appear to have moved a little beyond the health system-centered approach (although it continues to be largely a health system-centered approach) and have included the community in the control of TB. The community's involvement appears again to be limited to facilitating the expansion of biomedical management of TB, which is the expansion of DOTS. Further, TB itself continues to be conceptualized predominantly as a curative problem and hence the control of TB appears to be largely skewed towards curative care.

What Could Be the Possible Underlying Assumptions?

The construction of the problem seems to assume that community involvement in expanding DOTS and provision of nutritional and transportation support to TB patients would be required in addition to health system factors to control TB. Contemplating a policy on TB, poverty, and gender gives an impression that poverty and gender could be hampering TB control, and hence it is imperative to address the same.

What Could Be the Possible Silences?

The reports did acknowledge that poverty, with its manifestations in unintended social structures and situations like slums, malnutrition, and poor sanitation, leads to an increase in TB cases. Though there is a mention of developing a policy for TB, poverty, and gender, no specific strategy has been proposed. Regarding malnutrition, the entire effort sums up to provide nutritional support to TB patients and not to the poor population in general who is bearing the brunt of undernutrition. The strategies appear to be silent on addressing the fundamental determinants of health.

In 2010, the Kenyan constitution underwent an amendment, consequent that health, food, social protection, and nutrition are committed as basic rights. The amendment also marked the replacement of provinces with a system of counties.

6.2.3 Phase III: 2011–2014

Problem Representation

The activities initiated in the early period continued to sustain the achievements made to date. A few new strategies were adopted; the management of DR-TB was brought under program mode, and engagement of various healthcare providers (informal private sector providers were included during this period) was increased. Greater emphasis was again given to the technical aspects of TB control, such as further strengthening of Lab services, the introduction of new technologies in the form of new medicines (Bedaquiline), gene expert machine (to facilitate diagnosis of MDRTB), and data management system (TIBU) to address the issue related to diagnosis of MDR-TB. Attention was also given to address other lung conditions by developing a practical approach to lung health (PAL); a gene expert machine was introduced. The Engage Approach was designed to integrate community-based activities through the enhanced engagement of civil society organizations. Kenya also formulated the STOP TB partnership (which mirrors of Global stop TB Partnership) and launched its strategic plan (2014–2018) in an attempt to address those determinants that go beyond the health sector [46–48].

Strategies during this period again appear to be largely geared towards enhancing the techno-managerial aspect of controlling TB, thereby giving the impression that the control of TB is predominantly conceptualized as a health system-centered problem using a biomedical lens. Further, the community's involvement through civil society organizations appears to facilitate the expansion of biomedical management of TB, which is the expansion of DOTS, active case finding, screening, etc. This phase again appears to continue with the curative rhetoric.

What Could Be the Possible Assumptions?

The construction of the problem again appears to assume that enhancing the techno-managerial aspect would help control TB, to the extent that even the role of community appears to supplement the same.

What Could Be the Possible Silences?
Though a document on TB, poverty, and gender is available, no specific strategies to address poverty and gender appeared during this period.

6.2.4 Phase IV: 2015–2018

Problem Representation
The National strategic plan (2015–2018) was developed in 2014, which has revolutionized how TB control was addressed until then. The plan envisaged country-specific strategies for TB control to focus the scarce resources on areas with a high burden of TB and HIV. The activities initiated in the early period continued to sustain the achievements made to date with the addition of new activities. On recognizing diabetes mellitus (DM) as a risk factor for TB, TB/DM collaborative strategies were also strengthened. Following active case-finding in the community almost for a decade, the case-finding approach was shifted to facility-based active case-finding. The techno-managerial aspect of TB control was further expanded along with the introduction of new medicines, the launch of child-friendly TB medicines, integration of childhood TB with other healthcare services. Under the STOP TB partnership, many advocates were given platforms for advocacy to profile TB and use their experiences to push for improved services; they also mobilized members of parliament to increase their commitment towards TB elimination in Kenya. A set of new policies was formulated during this period, including:

- TB isolation policy, it was framed after the court declared the confinement of TB patients (belonging to certain categories such as non-compliant patients and MDR-TB patients) in prison as unconstitutional. This policy was hailed for using human rights approach while dealing with TB patients in isolation facilities at healthcare centers;
- workplace policy; and
- Social protection policy for TB and leprosy patients includes cash transfers, food assistance, health insurance, and advocacy of social security legal frameworks that cover both formal and informal sectors to reduce the share of affected families who experiences catastrophic costs from TB and leprosy [49–52].

Strategies during this period again appear to be largely geared towards enhancing the techno-managerial aspect of controlling, giving the impression that the control of TB is predominantly conceptualized as a health system-centered problem using a biomedical lens. Further, the STOP TB partnership appears to problematize the control of TB as a multisectoral problem that goes beyond the

health sector. Still, no prominent activities were carried out other than mobilizing political leaders and creating awareness. A prevalence survey conducted in 2015–2016 reported the underdiagnosis of bacteriologically confirmed pulmonary TB and reported the TB incidence rate to be 348 as compared to the WHO estimated rate of 233 for 2016 [53]. The recommendations were made again on the techno-managerial line of TB control, such as replacing smear microscopy with gene expert technique, implementing chest X-ray for screening, increasing engagement of private healthcare providers, etc. Kenya has abandoned its policy of keeping non-compliant TB patients to prisons and has adopted a new policy of isolating non-compliant TB patients in healthcare facilities following human rights. Here again, the control of TB appears to be problematized as a health system-centered problem.

What Could Be the Possible Assumptions?

The construction of the problem again appears to assume that enhancing the techno-managerial aspect would help control TB.

What Could Be the Possible Silences?

The strategies appear to continue to remain silent on addressing the primary social determinants of health. Even the social protection policy appears to address the problem of those who have TB or leprosy.

7 Conclusion

The underlying problematization of TB control in the global initiatives, which started with the 44th World Health Assembly (1991) declaration of the disease as a global public health problem, appeared to be largely techno-managerial. The focus was on health system strengthening and systemic strategies to enhance case detection and cure. However, the changing discourse around health propelled by the Report of the Commission of Social Determinants of Health by the WHO (2005) has influenced the way people's health is being looked upon since the latter half of the first decade of the new year millennium. The health policy documents started incorporating concepts of social determinants of health and, consequently, higher exposure, vulnerability, and poorer access among specific social groups. However, in the context of TB control, these aspects were presented in the international policy documents without any clear strategy or roadmap to address them in real contexts. This is also because TB control was predominantly being problematized within a disease-centered paradigm, where any emphasis made on the underlying determinants was primarily to control the disease, as opposed to promoting health. Further, the conceptions and priorities of these initiatives (Global Plans of Action to End TB, Stop TB Strategy, and End TB Strategy) appear to have significantly shaped the underlying problematization of TB control strategies in India and Kenya, as well as several other nations across the world. Both nations have been

committed to achieving MDGs by 2015 and are now committed to achieving the SDG of ending the TB epidemic by 2030.

India and Kenya have a long history of the fight against TB, and its disproportionate burden among the poor and vulnerable sections of the society has also been documented in both settings. Yet, the national programs based on the DOTS strategy initiated in both countries during the last decade of the twentieth century with international organizations' technical and financial assistance appear to have problematized TB control almost entirely from a germ-centric view.

As the global initiatives evolved to address the disease from a social determinants approach, the national policy documents also acknowledged their role. However, the actions initiated to address these determinants have been largely limited to IEC strategies, advocacy, and social mobilization campaigns to engage the vulnerable sections of society. The underlying assumption behind this approach could be indicating that the higher exposure and risk among them is attributable to their lack of knowledge and disengagement from the program, rather than the disadvantages posed by lack of resources and poor living and working conditions.

The global initiatives have placed a significant thrust on the TB-HIV co-epidemic from the beginning, and this is also a key component of the national programs. However, undernutrition is another significant concern in both these settings, with its roots planted firmly in the poverty problem. With its relatively lower prevalence of HIV/AIDS compared to Kenya, India has invested considerable resources to address the co-epidemic. While Kenya addresses nutritional support and rehabilitation of TB patients and the TB-HIV co-epidemic in its national strategy, such initiatives are largely regional and sketchy in India. However, the fact also remains that even Kenya has addressed the nutrition problem only among TB patients and not as a primary determinant of health.

Despite the decline in TB-related morbidity and mortality over the past two decades, the disease burden remains high in both settings. A grave issue that threatens the reversal of achievements of the strategies implemented so far is the rise of DR-TB, again largely affecting the same poor and vulnerable population. However, the actions included in the national programs to address it are largely centered around expansion and refinement of the existing program, drug susceptibility testing, newer diagnostics, newer drugs, and active case finding, once again implying that the underlying determinants are largely techno-managerial. The critics of India's strategies of TB control argue that the emergence of DR is a clear indication of the failure of a long-running under-funded curative public program. There have also been efforts in both India and Kenya to actively engage and incorporate the private sector and generate a public–private mix in diagnosis and care. However, the rising burden of DR-TB (Figs. 5 and 6) raises a question on the validity of this approach, instead of stronger regulation on the private sector and closer monitoring of the prescribing practices of private practitioners. Rising comorbidities like DM and the high prevalence of other risk factors like air pollution have also potentially contributed to the spread of DR-TB, reiterating the need for building a holistic approach to health built on primary healthcare. Lastly, the

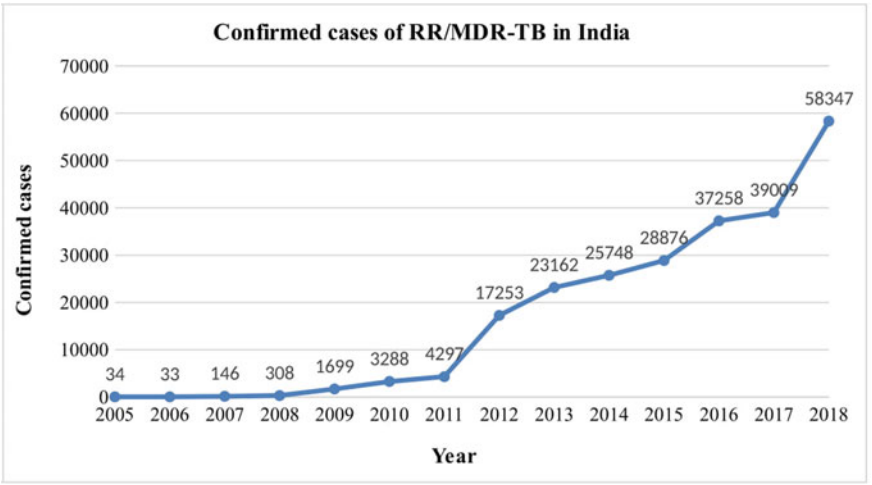


Fig. 5 Trends in confirmed cases of rifampicin resistant/MDR-TB in India since 2005

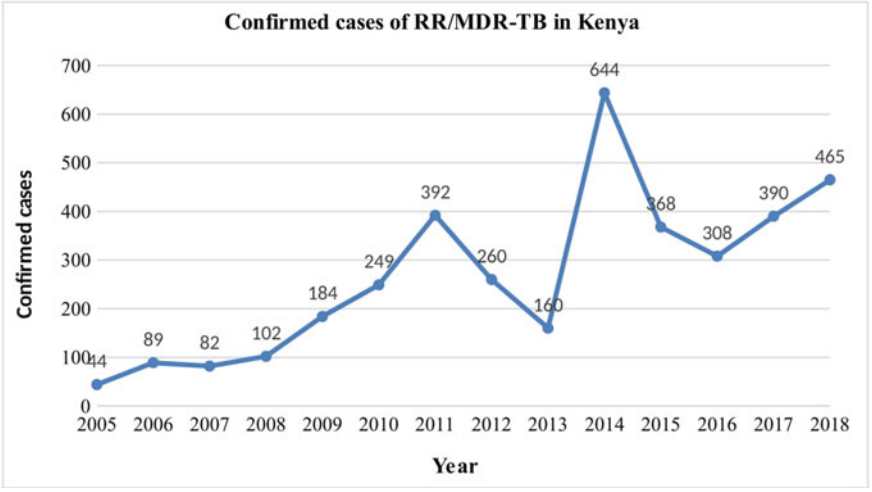


Fig. 6 Trends in confirmed cases of rifampicin resistant/MDR-TB in Kenya since 2005

poor state spending on the health sector clearly indicates the absence of a fundamental tenet of the DOTS strategy—a strong political will.

With the vision of a TB-free world on the horizon, it is critical to note that the post-2015 strategies of both nations are heavily focused on the initiation of newer drugs, regimes, diagnostic technologies, drug susceptibility testing, and web-based platforms for online notification, with hardly any creative approaches to tackle

poverty, unemployment, housing or nutrition. The high poverty level, undernutrition, and inequalities persisting in both these settings mandate a paradigmatic shift in problematization and approach, with TB control being primarily driven by action on structural determinants influencing health with the techno-managerial solutions assuming only a supplementary role.

You cannot get rid of this age-old epidemic, which has always had the dispossessed as its manifest, without eliminating the structural inequalities.

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Core Messages

- The biomedical approach drives the TB control strategies in both India and Kenya with little focus on social determinants of health.
- While DR-TB has emerged as a major challenge in both settings, nuanced exploration of underlying reasons seems absent.
- Need for a paradigmatic shift in the problematization of TB control from a disease-centric view to one of health promotion.

References

1. World Health Organization (2019) Global tuberculosis report 2019. World Health Organization, Geneva. https://www.who.int/tb/publications/global_report/en/. Accessed 5 Aug 2020
2. Stop TB Partnership (2001) Global plan to stop TB phase 1: 2001 to 2005. Stop TB Partnership, Geneva. http://www.stoptb.org/assets/documents/global/plan/GLOBAL_PLAN_TO_STOP_TB_2001_2005.pdf. Accessed 12 Aug 2020
3. World Health Organization (2005) Fifty-eighth World Health Assembly: sustainable financing for tuberculosis prevention and control (WHA58.14). World Health Organization, Geneva. https://apps.who.int/gb/ebwha/pdf_files/WHA58/WHA58_14-en.pdf?ua=1. Accessed 12 Aug 2020
4. World Health Organization (2006) The stop TB strategy. World Health Organization, Geneva. https://apps.who.int/iris/bitstream/handle/10665/69241/WHO_HTM_STB_2006.368_eng.pdf;jsessionid=FE00D107AED73B312B4703E91251ACAD?sequence=1. Accessed 7 Aug 2020
5. World Health Organization (2014) The end TB strategy: global strategy and targets for tuberculosis prevention, care and control after 2015. World Health Organization, Geneva. https://www.who.int/tb/strategy/End_TB_Strategy.pdf?ua=1. Accessed 7 Aug 2020
6. Herbert N, George A, Masham B et al (2014) World TB day 2014: finding the missing 3 million. Lancet 383(9922):1016–1018. http://doi.org/10.1016/S0140-6736(14)60422-0
7. Wandwalo E (2017) Make a global priority of finding missing cases of tuberculosis. The Global Fund. https://www.theglobalfund.org/en/blog/2017-10-10-make-a-global-priority-of-finding-missing-cases-of-tuberculosis/. Accessed 20 Aug 2020
8. Seung KJ, Keshavjee S, Rich ML (2015) Multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis. Cold Spring Harb Perspect Med 5:9. https://doi.org/10.1101/cshperspect.a017863

9. Hof SV, Collins D, Hafidz F, Beyene D, Tursynbayeva A, Tiemersma E (2016) The socioeconomic impact of multidrug resistant tuberculosis on patients: results from Ethiopia, Indonesia and Kazakhstan. BMC Infect Dis 16:1–470. https://doi.org/10.1186/s12879-016-1802-x
10. Stop TB Partnership (2019) The paradigm shift 2018–2022. Stop TB Partnership. http://www.stoptb.org/assets/documents/global/plan/GPR_2018-2022_Digital.pdf. Accessed 20 Aug 2020
11. Frith J (2014) History of tuberculosis: part 1—phthisis, consumption and the white plague. J Mil Vet Health 22(2):29–35
12. Bacchi C (2014) Analysing policy: what's the problem represented to be? 1st edn. Pearson Australia, Australia
13. Agarwal SP, Chauhan LS (2005) Tuberculosis control in India. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://pdfs.semanticscholar.org/0eaf/20ddbffe4140add6ed37ba913e27d196e7c0.pdf. Accessed 5 Aug 2020
14. World Health Organization (2010) A brief history of tuberculosis control in India. World Health Organization, Geneva. https://apps.who.int/iris/bitstream/handle/10665/44408/9789241500159_eng.pdf?sequence=1 Accessed 11 Aug 2020
15. Central TB Division (2001) TB India 2001 RNTCP status report. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://tbcindia.gov.in/showfile.php?lid=2913. Accessed 6 Aug 2020
16. Central TB Division (2002) TB India 2002 RNTCP status report. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://tbcindia.gov.in/showfile.php?lid=2914. Accessed 6 Aug 2020
17. Central TB Division (2003) TB India 2003 RNTCP status report. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://tbcindia.gov.in/showfile.php?lid=2915. Accessed 6 Aug 2020
18. Central TB Division (2004) TB India 2004 RNTCP status report. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://tbcindia.gov.in/showfile.php?lid=2916. Accessed 6 Aug 2020
19. Central TB Division (2005) TB India 2005 RNTCP status report. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://tbcindia.gov.in/showfile.php?lid=2917. Accessed 6 Aug 2020
20. Central TB Division (2006) TB India 2006 RNTCP status report. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://tbcindia.gov.in/showfile.php?lid=2918. Accessed 6 Aug 2020
21. Central TB Division (2007) TB India 2007 RNTCP status report. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://tbcindia.gov.in/showfile.php?lid=2919. Accessed 6 Aug 2020
22. Central TB Division (2008) TB India 2008 RNTCP status report. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://tbcindia.gov.in/showfile.php?lid=2920. Accessed 6 Aug 2020
23. Central TB Division (2009) TB India 2009 RNTCP status report. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://tbcindia.gov.in/showfile.php?lid=2921. Accessed 6 Aug 2020
24. Central TB Division (2010) TB India 2010 RNTCP status report. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://tbcindia.gov.in/showfile.php?lid=2922. Accessed 6 Aug 2020
25. Central TB Division (2011) TB India 2011 revised national TB control programme annual status report. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://tbcindia.gov.in/showfile.php?lid=3164. Accessed 6 Aug 2020
26. Lonnroth K, Williams BG, Cegielski P et al (2010) A consistent log-linear relationship between tuberculosis incidence and body mass index. Int J Epidemiol 39(1):149–155

27. Bhargava A, Sharma A, Oxlade O et al (2014) Undernutrition and the incidence of tuberculosis in India: national and subnational estimates of the population-attributable fraction related to undernutrition. Natl Med J India 27:128–133
28. Bhargava A (2015) TB control in India: mapping the gaps. In: Sethi H (ed) Eradicating TB in India: challenges, perspectives, solutions, 1st edn. Wiley-Blackwell, New Delhi, pp 20–27
29. Central TB Division (2012) TB India 2012 revised national TB control programme annual status report. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://tbcindia.gov.in/showfile.php?lid=3141. Accessed 6 Aug 2020
30. Central TB Division (2013) TB India 2013 revised national TB control programme annual status report. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://tbcindia.gov.in/showfile.php?lid=3163. Accessed 6 Aug 2020
31. Central TB Division (2014) TB India 2014 revised national TB control programme annual status report. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://tbcindia.gov.in/showfile.php?lid=3142. Accessed 6 Aug 2020
32. Central TB Division (2015) TB India 2015 revised national TB control programme annual status report. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://tbcindia.gov.in/showfile.php?lid=3166. Accessed 6 Aug 2020
33. Central TB Division (2016) TB India 2016 revised national TB control programme annual status report. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://tbcindia.gov.in/index1.php?lang=1&level=2&sublinkid=4569&lid=3174. Accessed 6 Aug 2020
34. Central TB Division (2017) TB India 2017 revised national TB control programme annual status report. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://tbcindia.gov.in/WriteReadData/TB%20India%202017.pdf. Accessed 6 Aug 2020
35. Mazumdar S, Satyanarayana S, Pai M (2019) Self-reported tuberculosis in India: evidence from NFHS-4. BMJ Glob Health. https://gh.bmj.com/content/bmjgh/4/3/e001371.full.pdf. Accessed 7 Aug 2020
36. Singh SK, Kashyap GC, Puri P (2018) Potential effect of household environment on prevalence of tuberculosis in India: evidence from the recent round of a cross-sectional survey. BMC Pulm Med 18:66 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5934826/pdf/12890_2018_Article_627.pdf. Accessed 18 Aug 2020
37. Central TB Division (2018) TB India 2018 revised national TB control programme annual status report. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://tbcindia.gov.in/showfile.php?lid=3314. Accessed 6 Aug 2020
38. Central TB Division (2019) TB India 2019 revised national TB control programme annual status report. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://tbcindia.gov.in/WriteReadData/India%20TB%20Report%202019.pdf. Accessed 6 Aug 2020
39. Central TB Division (2020) TB India 2020 revised national TB control programme annual status report. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://tbcindia.gov.in/showfile.php?lid=3538. Accessed 6 Aug 2020
40. Central TB Division (2019) National framework for a gender-responsive approach to TB in India. Directorate General of Health Services/Ministry of Health and Family Welfare, New Delhi. https://tbcindia.gov.in/WriteReadData/l892s/388838054811%20NTEP%20Gender%20Responsive%20Framework_311219.pdf. Accessed 7 Aug 2020
41. World Health Organization (2008) A brief history of tuberculosis control in Kenya. WHO. https://www.who.int/tb/publications/tb-kenya-report/en/. Accessed 10 Aug 2020
42. Division of Leprosy, Tuberculosis and Lung Disease (2007). Annual report 2007. Ministry of Public Health and Sanitation. https://www.nltp.co.ke/download/anual-report-2007/. Accessed 6 Aug 2020

43. Division of Leprosy, Tuberculosis and Lung Disease (2008) Annual report 2008. Ministry of Public Health and Sanitation. https://www.nltp.co.ke/download/anual-report-2008/. Accessed 6 Aug 2020
44. Division of Leprosy, Tuberculosis and Lung Disease (2009) Annual report 2009. Ministry of Public Health and Sanitation. https://www.nltp.co.ke/download/anual-report-2009/. Accessed 6 Aug 2020
45. Division of Leprosy, Tuberculosis and Lung Disease (2010) Annual report 2010. Ministry of Public Health and Sanitation. https://www.nltp.co.ke/download/anual-report-2010/. Accessed 6 Aug 2020
46. Division of Leprosy, Tuberculosis and Lung Disease (2011) Annual report 2011. Ministry of Public Health and Sanitation. https://www.nltp.co.ke/download/anual-report-2011/. Accessed 7 Aug 2020
47. Division of Leprosy, Tuberculosis and Lung Disease (2012) Annual report 2012. Ministry of Public Health and Sanitation. https://www.nltp.co.ke/download/anual-report-2012/. Accessed 7 Aug 2020
48. National Tuberculosis, Leprosy and Lung Disease Program (2014) Annual report 2014. Ministry of Health. https://www.nltp.co.ke/download/anual-report-2014/. Accessed 8 Aug 2020
49. National Tuberculosis, Leprosy and Lung Disease Program (2015) Annual report 2015. Ministry of Health. https://www.nltp.co.ke/download/annual-report-2015-2/. Accessed 8 Aug 2020
50. National Tuberculosis, Leprosy and Lung Disease Program (2016) Annual report 2016. Ministry of Health. https://www.nltp.co.ke/download/annual-report-2016/. Accessed 9 Aug 2020
51. National Tuberculosis, Leprosy and Lung Disease Program (2017) Annual report 2017. Ministry of Health. https://www.nltp.co.ke/download/annual-report-2017/. Accessed 9 Aug 2020
52. National Tuberculosis, Leprosy and Lung Disease Program (2018) Annual report 2018. Ministry of Health. https://www.nltp.co.ke/download/annual-report-2018/. Accessed 9 Aug 2020
53. Enos M, Sitienei J, Ong'ang'o J, Mungai B, Kamene M, Wambugu J, Kipruto H, Manduku V, Mburu J, Nyaboke D, Ngari F, Omesa E, Omale N, Mwirigi N, Okallo G, Njoroge J, Githiomi M, Mwangi M, Kirathe D, Kiplimo R, Ndombi A, Odeny L, Mailu E, Kandie T, Maina M, Kasera K, Mulama B, Mugi B, Weyenga H (2018) Kenya tuberculosis prevalence survey 2016: challenges and opportunities of ending TB in Kenya. PLoS One 13:12. https://doi.org/10.1371/journal.pone.0209098

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Challenges in Prevention and Management of Tuberculosis

29

Mohammed Assen Seid

The disease is still around, it's still contagious, and despite the fact that the vaccine costs approximately sixteen cents to produce, and $3.13 to buy, tuberculosis continues to ravage periphery countries. Millions of people die from tuberculosis every year - and it's totally treatable. This is a disease we can eradicate in our lifetime.

Jennifer Wright

Summary

Tuberculosis (TB) is a highly contagious and deadliest infectious disease that remains a threat to human health worldwide. Even though the World Health Organization (WHO) aims to eradicate TB by 2050, TB elimination rates are not as expected globally. The spread of drug-resistant (DR) TB and its association with HIV/AIDS make it difficult to control TB in low and middle-income countries. TB control strategies include finding TB cases, contact tracing, treating the case, and preventing transmission. Delay in diagnosis and poor infection control practices are still major challenges of TB control and prevention. The migrants from high-TB burden countries are considered a new epidemiological source of TB infection, resulting in a big challenge for TB care and control. Detention centers have also facilitated the transmission of TB and the development of DR-TB. Therefore, TB control actions should be conducted considering prisons. Another challenge in TB control is getting an effective vaccine. It is now clear that the Bacillus Calmette–Guérin (BCG)

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vaccine cannot fully arrest the global TB epidemic. A new vaccine is required to replace or boost the existing vaccine for the better control of TB. For the urgency of TB elimination, multiple stakeholders should also collaborate for the comprehensiveness and correctness of care for TB patients. Furthermore, a strong commitment of all administrative authorities to support the development of highly effective vaccines will be required to decrease the incidence and disease burden globally as per the WHO target.

Graphical Abstract

Challenges in prevention and management of tuberculosis

Keywords

Challenges · Management · Prevention · TB control · Treatment · Tuberculosis

1 Introduction

Tuberculosis (TB) is a highly contagious and the deadliest infectious disease that remains a global health threat. TB mainly affects the lung, but it also infects other body parts (extrapulmonary TB) [1]. TB is one of the main causes of death among infectious diseases globally. The evidence shows that there is very slow TB elimination rate worldwide. According to the world health organization

(WHO) reports, more than ten million people were affected with TB in 2018 [2, 3]. Although WHO targets to eradicate TB by 2050, it continues to be the main public health problem in the world [4, 5]. Though it seems challenging, some countries aim to control TB by 2035. This target will be realized through an intensive and large scope of case finding and treating latent TB infection (LTBI) [6, 7]. The spread of drug-resistant (DR) TB and its association with HIV/AIDS make it difficult to control TB in various countries [5, 8]. Early detection and diagnosis of TB is still a major challenge for its control and prevention [9].

The three pillars of TB control strategies are finding TB cases, treating those cases, and preventing new TB infection (including relapse and reinfection) [10]. It is considered that among the TB control strategies, the main attention is given to TB prevention. However, the distribution of crucial TB prevention services is very limited globally [11]. The major TB prevention strategies include treating latent TB infection in high-risk individuals, detecting and treating infectious TB, early anti-TB therapy (ATT) initiation, and providing vaccination for targeted individuals [10, 12]. Besides, successful TB prevention will be effective when they can identify children infected with TB [12]. It is depicted that targeting special populations, including children, will considerably reduce the burden of TB. The main prevention and treatment barriers for children include medication dosing errors, time taking and intensive pediatric formulation preparation, medication administration problems, poor adherence, and resistance to isoniazid preventive therapy (IPT) [13]. A comprehensive approach is needed to consider both the child and the family [14, 15]. To effectively control TB in children, new drugs, new diagnostic techniques, and vaccines that fit for them are required [10].

2 Challenges with Tuberculosis Control

It is clear that much effort has been made to control the global burden of TB over the past decades, but it faces major challenges. The uncontrolled TB burden negatively impacts socioeconomic development and increases DR [16]. The increasing trend of DR-TB is hastened by limited resources, poverty, and negligence [17].

Controlling TB and the spread of DR-TB has been an ongoing challenge. Especially migrants from high-TB burden countries are considered a new source of TB infection. Poor living conditions in the migration centers increase TB transmission. Strong motivation from different stakeholders and a political decision will be necessary to halt the transmission in these centers [5]. The detention centers are also one of the areas which result in the development of DR-TB [18]. Therefore, TB control actions should also consider prisons [19, 20].

Not only treating active TB but also contact tracing play a vital role in eliminating TB. Even if policies are recommended to investigate and provide preventive therapy for TB contacts, there are still gaps in ensuring accountability [21]. There are challenges concerning contact tracing, which include they do not want to travel to health facilities for evaluation, reluctance to accept to take medication while they

do not feel sick, and there are knowledge gaps among healthcare workers and patients. Besides, various countries do not have clear guidelines on managing individuals who have contact with DR-TB patients [21–23].

One of the main challenges in TB control is getting an effective vaccine. It is now clear that the BCG vaccine cannot fully arrest the global TB epidemic. The rapid increase of DR-TB worldwide is alarming that there is an urgent need for an effective vaccine that can control TB infection [1, 24]. Conversely, in recent years, the global TB vaccine development showed very slow progress than its necessity [25]. Without improved TB vaccines, TB will not be fully controlled. Therefore a new vaccine is required to replace or boost the existing vaccine for the better control of TB [7, 26].

Patient and facility-related conditions also have an immense impact on the control of TB. Patient-related factors including substance abuse, and HIV co-infection, which impose difficulty in TB treatment [27]. Patient-centered approaches that consider the social factors influencing their persistence on TB treatments are needed [28]. The main challenges that TB patients encountered while on their ATT include the treatment pill burden, economic impacts of ATT, healthcare provider communication problems, and the psychological toll of TB [27, 29]. Many patients said that when hospitalized in an isolated hospital setting, the discouraging hospital environment, including inadequate infrastructure, is one of the key factors influencing their motivation to stick to their protracted ATT phases [27, 28].

In general, to effectively control TB around the globe, the involvement of multiple stakeholders is very vital [19]. Furthermore, strong commitments of all government officials will be needed to support research projects targeted at developing new diagnostic tools and/or techniques, effective vaccines, and better ATT regimen options [8, 30].

3 Conclusion

TB remains to be a human health threat globally. Its eradication at the global level continues to face major challenges (Table 1.). The uncontrolled TB burden affects socioeconomic development and hastens the development of DR-TB migration from high-TB burden countries. Policy gaps in locating individuals who have had contact with TB patients, delays in TB diagnosis, poor infection control practice, the development of DR-TB, and the link of TB with HIV/AIDS are all difficulties for poor TB control. There is also evidence that the current BCG vaccine cannot halt the global TB epidemic. As a result, TB control necessitates multi-sectoral, personalized, and pragmatic methods for rapid diagnosis, stopping transmission,

Table 1 Examples of reported challenges that affect the global control of tuberculosis (TB) by specific countries

Challenges	Country	Reported by, year
• Low socioeconomic status • Low healthcare-seeking behavior • Centralized patient care • Negative provider-patient relationships	Ukraine [27]	Aibana et al. (2020)
• Forced mass displacement and violations of humanitarian law • Limited capacity to diagnose, contact tracing, and follow-up	Syria [31]	Abbara et al. (2020)
• TB patients have limited resources to visit health facilities • Some providers feel ambivalence to give preventive therapy	Peru [22]	Yuen et al. (2019)
• Diagnosis of TB meningitis is clinically challenging	Texas [32]	Varleva et al. (2019)
• The issues related with the effectiveness of BCG vaccine	UK [1]	Stylianou and Paul (2019)
• Most vaccine trials focus on adults, and there are many challenges with TB diagnosis in children	Multicenter study [14]	Reuter et al. (2019)
• There is a challenge to transit a nationally-funded TB control program	Bulgaria [33]	Doan (2019)
• The complex genetics of TB drug resistance	South Africa [35]	Koch et al. (2018)
• A huge number of migrants with LTBI • Poor migrant-focused latent TB screening • Migrants flooding from high TB burden countries	EU/EEA [5, 36]	Greenaway et al. (2018), Sotgiu et al. (2017)
• Lack of effective communication techniques between professionals and the public	Korea [37]	Go et al. (2018)
• HIV co-infection • Seasonal variation of TB epidemic • Insufficient level of decentralization • Inadequate health infrastructure • Higher cost of treatment	Ethiopia [38–40]	Seid et al. (2018), Gashu et al. (2018), Nooh (2019)
• TB patients delay seeking care behavior and want initial care from informal providers	Multicenter study [9]	Getnet et al. (2017)
• High prevalence of multi-drug resistant tuberculosis	Iran [41]	Bialvaei et al. (2017)
• Limited budget allocation for TB control program by the government • Weak TB control program implementation and management • Suboptimal quality of care in the private sector, Insufficient advocacy about TB	India [42]	Pai et al. (2016)

(continued)

Table 1 (continued)

Challenges	Country	Reported by, year
• Increasing of drug-resistant TB		
• Extensively drug-resistant TB (XDR-TB)	Multicenter study [17]	Kurz et al. (2016)
• Differences in the – organizational structure, culture, and – system of care in the country	Australia [6]	Degeling et al. (2020)
• A widespread of extensively drug-resistant (XDR) tuberculosis • Limited funding for TB control program • Lack of tailored recording and reporting tools	South Africa [10, 42]	Churchyard et al. (2014), Shah et al. (2017)
• Initiating TB treatment in a demoralizing hospital setting	Latvia [28]	Kielmann et al. (2018)

and halting DR-TB development. Besides, enhanced contact investigation to detect and treat latent TB infection will play an important role in decreasing TB incidence.

Core Messages

- The spread of DR-TB and its association with HIV/AIDS are the major challenges to controlling TB.
- Migrants from high-TB incidence countries and detention centers are the main source of TB transmission and increasing DR-TB.
- It is now clear that the current BCG vaccine has only limited protection and cannot stop the global TB epidemic.
- In some countries, political commitment is needed to control TB.

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References

1. Stylianou E, Paul MJ (2019) Mucosal delivery of tuberculosis vaccines: a review of current approaches and challenges. 18(12):1271–1284. http://doi.org/10.1080/14760584.2019.1692657
2. Global tuberculosis report 2019. World Health Organization, Geneva. Licence: CC BY-NC-SA 3.0 IGO
3. Harding E (2020) WHO global progress report on tuberculosis elimination. Lancet Respir Med 8(1):19

4. World Health Organization (WHO) (2014) Documentation for world health assembly 67, Geneva
5. Sotgiu G, Dara M, Centis R, Matteelli A, Solovic I, Gratziou C, Rendon A, Migliori GB (2017) Breaking the barriers: migrants and tuberculosis. La Presse Médicale 46(2):e5–e11
6. Degeling C, Carroll J, Denholm J, Marais B, Dawson A (2020) Ending TB in Australia: organizational challenges for regional tuberculosis programs. Health Policy 124(1):106–112
7. Dockrell HM (2016) Towards new TB vaccines: what are the challenges? Pathog Dis 74(4)
8. Martini M, Barberis I, Gazzaniga V, Icardi G (2020) The fight to end tuberculosis: a global challenge in strong partnership. J Prev Med Hyg 61(Suppl 1):E1
9. Getnet F, Demissie M, Assefa N, Mengistie B, Worku A (2017) Delay in diagnosis of pulmonary tuberculosis in low-and middle-income settings: systematic review and meta-analysis. BMC Pulm Med 17(1):202. https://doi.org/10.1186/s12890-017-0551-y
10. Churchyard G, Mametja L, Mvusi L, Ndjek N, Hesseling A, Reid A, Babatunde S (2014) Tuberculosis control in South Africa: successes, challenges and recommendations. S Afr Med J 104(3):244–248
11. World Health Organization (2015) Global status report on road safety 2015. World Health Organization
12. Fox GJ, Dobler CC, Marais BJ, Denholm JT (2017) Preventive therapy for latent tuberculosis infection—the promise and the challenges. Int J Infect Dis IJID 56:68–76. https://doi.org/10.1016/j.ijid.2016.11.006
13. Chiang S, Roche S, Contreras C, Del Castillo H, Canales P, Jimenez J, Tintaya K, Becerra M, Lecca L (2017) Barriers to the treatment of childhood tuberculous infection and tuberculosis disease: a qualitative study. Int J Tuberc Lung Dis 21(2):154–160
14. Reuter A, Hughes J, Furin J (2019) Challenges and controversies in childhood tuberculosis. The Lancet 394(10202):967–978
15. Taiwo BO, Pinelli EO, van Soolingen D, Rhodes SG, Reuter A, Seddon JA, Marais BJ, Furin J (2020) Preventing tuberculosis in children: a global health emergency. PLoS Negl Trop Dis. https://doi.org/10.1371/journal.pntd.000806910.1016/j.prrv.2020.02.004
16. Bhatia V, Srivastava R, Reddy KS, Sharma M, Mandal PP, Chhabra N, Jhalani S, Mandal S, Arinaminpathy N, Aditama TY, Sarkar S (2020) Ending TB in Southeast Asia: current resources are not enough. 5(3):e002073. http://doi.org/10.1136/bmjgh-2019-002073
17. Kurz SG, Furin JJ, Bark CM (2016) Drug-resistant tuberculosis: challenges and progress. Infect Dis Clin North Am 30(2):509–522. https://doi.org/10.1016/j.idc.2016.02.010
18. Abbara A, AlKabbani H, Al-Masri I, Sahloul Z, Sparrow A (2018) Populations under siege and in prison require investment from Syria's national tuberculosis programme. Lancet Respir Med 6(7):e34
19. Kritski A, Andrade KB, Galliez RM, Maciel ELN, Cordeiro-Santos M, Miranda SS, Villa TS, Ruffino Netto A, Arakaki-Sanchéz D, Croda J (2018) Tuberculosis: renewed challenge in Brazil. Rev Soc Bras Med Trop 51(1):2–6. https://doi.org/10.1590/0037-8682-0349-2017
20. Telisinghe L, Charalambous S, Topp SM, Herce ME, Hoffmann CJ, Barron P, Schouten EJ, Jahn A, Zachariah R, Harries AD, Beyrer C, Amon JJ (2016) HIV and tuberculosis in prisons in sub-Saharan Africa. Lancet (London, England) 388(10050):1215–1227. https://doi.org/10.1016/s0140-6736(16)30578-5
21. Rodriguez C, Sasse S, Yuengling K, Azzawi S, Becerra M, Yuen C (2017) A systematic review of national policies for the management of persons exposed to tuberculosis. Int J Tuberc Lung Dis 21(8):935–940
22. Yuen CM, Millones AK, Contreras CC, Lecca L, Becerra MC, Keshavjee S (2019) Tuberculosis household accompaniment to improve the contact management cascade: a prospective cohort study. PLoS One 14(5):e0217104
23. Szkwarko D (2017) Child contact management in high tuberculosis burden countries: a mixed-methods systematic review. PLoS One 12(8):e0182185. https://doi.org/10.1371/journal.pone.021272910.1371/journal.pone.0182185

24. Petersen E, Maeurer M, Marais B, Migliori GB, Mwaba P, Ntoumi F, Vilaplana C, Kim K, Schito M, Zumla A (2017) World TB day 2017: advances, challenges and opportunities in the "End-TB" Era. Int J Infect Dis 56:1–5
25. Voss G, Casimiro D, Neyrolles O, Williams A, Kaufmann SH, McShane H, Hatherill M, Fletcher HA (2018) Progress and challenges in TB vaccine development. F1000Research 7
26. Al-Humadi HW, Al-Saigh RJ, Al-Humadi AW (2017) Addressing the challenges of tuberculosis: a brief historical account. Front Pharmacol 8:689
27. Aibana O, Dauria E, Kiriazova T, Makarenko O, Bachmaha M, Rybak N, Flanigan TP, Petrenko V, Becker AE, Murray MB (2020) Patients' perspectives of tuberculosis treatment challenges and barriers to treatment adherence in Ukraine: a qualitative study. BMJ Open 10 (1)
28. Kielmann K, Vidal N, Riekstina V, Krutikov M, van der Werf MJ, Biraua E, Duric P, Moore DA (2018) "Treatment is of primary importance, and social assistance is secondary": a qualitative study on the organisation of tuberculosis (TB) care and patients' experience of starting and staying on TB treatment in Riga, Latvia. PLoS One 13(10):e0203937
29. Charyeva Z, Curtis S, Mullen S, Senik T, Zaliznyak O (2019) What works best for ensuring treatment adherence. Lessons from a social support program for people treated for tuberculosis in Ukraine. PLoS One 14(8):e0221688
30. Kaplan G (2020) Tuberculosis control in crisis-causes and solutions. Prog Biophys Mol Biol 1 (152):6–9
31. Abbara A, Almalla M, AlMasri I, AlKabbani H, Karah N, El-Amin W, Rajan L, Rahhal I, Alabbas M, Sahloul Z, Tarakji A, Sparrow A (2020) The challenges of tuberculosis control in protracted conflict: the case of Syria. PLoS Negl Trop Dis 90:53–59. https://doi.org/10.1371/journal.pntd.000708310.1016/j.ijid.2019.10.015
32. Varleva T, Zamfirova M, Tyufekchieva M, Keshelava A, Hristov K, Yaneva A, Gadzheva B, Zhang S, Irbe S, Ragonnet R, McBryde ES, Trauer JM, Nguyen DT (2019) Trends of tuberculosis meningitis and associated mortality in Texas, 2010–2017, a large population-based analysis. Epidemiol Infect 14(2):e0212729. https://doi.org/10.1017/s095026881900185710.1371/journal.pone.0212729
33. Doan TN (2019) Strategic investment in tuberculosis control in the Republic of Bulgaria. Respirology (Carlton, Vic) 147:e304. https://doi.org/10.1111/resp.1330310.1017/s0950268819001857
34. Koch A, Cox H, Mizrahi V (2018) Drug-resistant tuberculosis: challenges and opportunities for diagnosis and treatment. Curr Opin Pharmacol 42:7–15
35. Greenaway C, Pareek M, Abou Chakra CN, Walji M, Makarenko I, Alabdulkarim B et al. (2018) The effectiveness and cost-effectiveness of screening for active tuberculosis among migrants in the EU/EEA: a systematic review. Euro Surveil: bulletin Europeen sur les maladies transmissibles = Eur Commun Dis Bull 23(14). http://doi.org/10.2807/1560-7917.es.2018.23.14.17-00542
36. Go U, Park M, Kim UN, Lee S, Han S, Lee J et al. (2018) Tuberculosis prevention and care in Korea: evolution of policy and practice. J Clin Tuberc Other Mycobact Dis 11:28–36. https://doi.org/10.1016/j.jctube.2018.04.006
37. Seid MA, Ayalew MB, Muche EA, Gebreyohannes EA, Abegaz TM (2018) Drug-susceptible tuberculosis treatment success and associated factors in Ethiopia from 2005 to 2017: a systematic review and meta-analysis. BMJ Open 8(9):e022111
38. Gashu Z, Jerene D, Datiko DG, Hiruy N, Negash S, Melkieneh K et al. (2018) Seasonal patterns of tuberculosis case notification in the tropics of Africa: a six-year trend analysis in Ethiopia. PLoS One 13(11):e0207552. https://doi.org/10.1371/journal.pone.0207552
39. Nooh F (2019) The impact of pastoralist mobility on tuberculosis control in Ethiopia: a systematic review and meta-synthesis. F1000Research 8(1):73. http://doi.org/1186/s40249-019-0583-z
40. Bialvaei AZ, Asgharzadeh M, Aghazadeh M, Nourazarian M, Kafil HS (2017) Challenges of tuberculosis in Iran. Jundishapur J Microbiol 10(3):1

41. Pai M, Daftary A, Satyanarayana S (2016) TB control: challenges and opportunities for India. Trans R Soc Trop Med Hyg 110(3):158–160. https://doi.org/10.1093/trstmh/trw003
42. Shah NS, Auld SC, Brust JC, Mathema B, Ismail N, Moodley P et al. (2017) Transmission of extensively drug-resistant tuberculosis in South Africa. N Engl J Med 376(3):243–253

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Tuberculosis in Contacts and Healthcare Workers

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Jean-Pierre Zellweger

Je pense, il est vrai, qu'il y a beaucoup de maladies que nous ne savons ni prévenir ni guérir, au moins d'une manière certaine et incontestable. Il ne s'agit pas, ce me semble, de savoir si cela est triste; il s'agit de savoir si cela est vrai.

RTH Laennec, Treatise of auscultation, 1826

I think, it is true, that there are many diseases that we do not know how to prevent or cure, at least in a certain and unquestionable way. The question, it seems to me, is not whether this is sad but whether it is true.

Summary

Persons in close contact with tuberculosis (TB) who are at risk of transmission (mostly smear- or culture-positive pulmonary tuberculosis) and healthcare workers in contact with such patients before the initiation of an adequate treatment have a high risk of being infected. Some of them will later develop tuberculosis. The risk of being infected depends on the duration and intensity of contact; the risk of progression to TB depends on age and the quality of the immune defense mechanisms. Preventive treatment should be considered in contacts at the highest risk of developing TB. Screening of exposed contacts and preventive treatment of persons at risk are now part of the global End TB Strategy.

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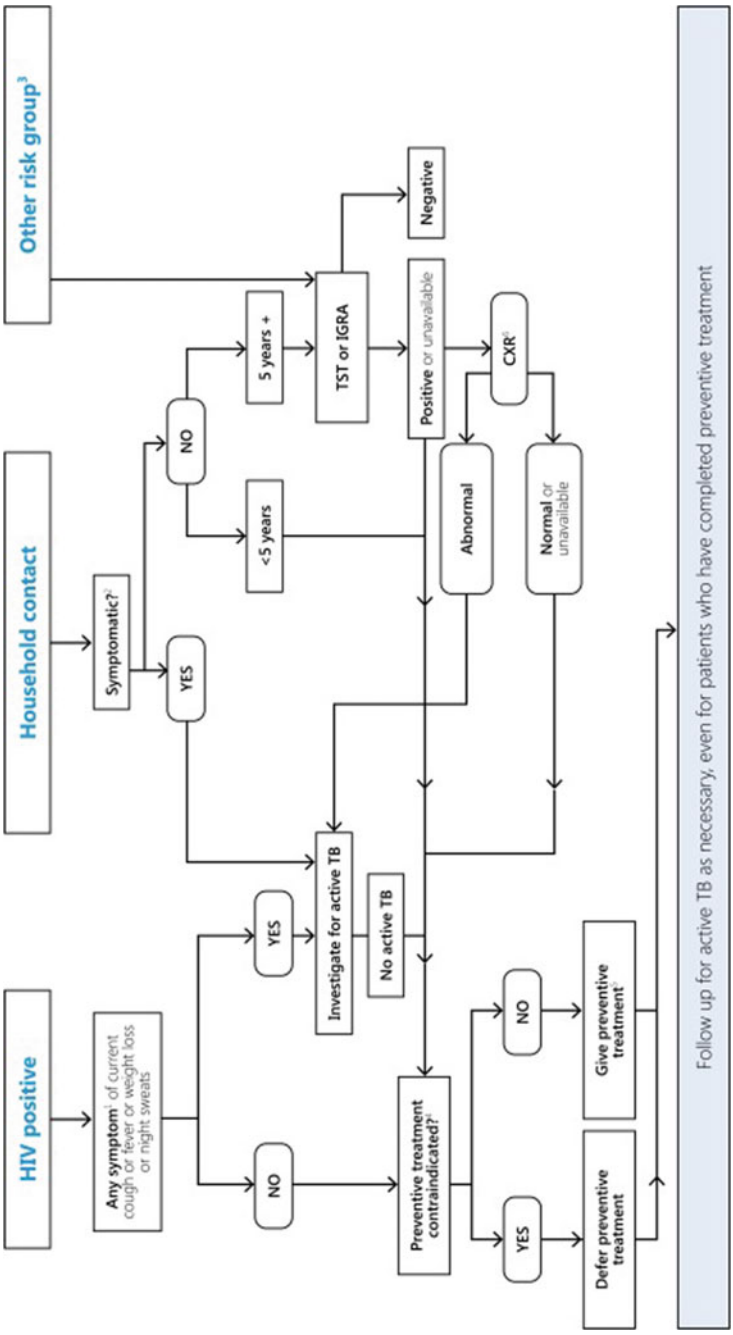
Graphical Abstract



Algorithm for LTBI testing and TB preventive treatment in individuals at risk

WHO consolidated guidelines on tuberculosis: tuberculosis preventive treatment: Module 1: prevention recommendations are as follows:

1. "If < 10 years, any one of current cough or fever or history of contact with TB or reported weight loss or confirmed weight loss > 5% since last visit or growth curve flattening or weight for age < 2 Z-scores.
   Asymptomatic infants < 1 year with HIV are only treated for LTBI if they are household contacts of TB. TST or IGRA may identify PLHIV who will benefit most from preventive treatment. Chest radiography (CXR) may be used in PLHIV on ART, before starting LTBI treatment.
2. Any one of cough or fever or night sweats or haemoptysis or weight loss or chest pain or shortness of breath or fatigue. In children < 5 years, they should also be free of anorexia, failure to thrive, not eating well, decreased activity or playfulness to be considered asymptomatic.
3. Including silicosis, dialysis, anti-TNF agent treatment, preparation for transplantation or other risks in national guidelines.
4. Including acute or chronic hepatitis; peripheral neuropathy (if isoniazid is used); regular and heavy alcohol consumption. Pregnancy or a previous history of TB are not contraindications.
5. Regimen chosen based on considerations of age, strain (drug susceptible or otherwise), risk of toxicity, availability and preferences.
6. CXR may have been carried out earlier on as part of intensified case finding". (*Adapted from* [1]*, copyright © World Health Organization 2020, Available under the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 IGO licence, CC BY-NC-SA 3.0 IGO;* https://creativecommons.org/licenses/by-nc-sa/3.0/igo)

Keywords

Contact investigation · Contacts · Healthcare workers · Latent tuberculosis infection · LTBI · Tuberculosis

1 Introduction

Tuberculosis (TB) is transmitted by aerial route from patients with pulmonary TB (PTB) to bystanders. Persons who live or work in close proximity to such patients are most likely to inhale infectious particles, be infected, and, in some cases, develop TB. Household contacts of patients with PTB have a risk between 25 and 50% of being infected [2] and between 1.9 and 3.1% to have already developed active TB at the time of diagnosis of the index case [3]. The risk of infection is influenced by the severity of the disease in the index case (in particular, the presence of cavities and a high concentration of mycobacteria in sputum) and the

proximity and duration of contact with the index case [4]. Infected contacts [those with signs of a positive immunological reaction like the tuberculin skin test (TST) or a positive interferon-gamma release assay (IGRA)] have a higher risk of developing TB than contacts with negative test results.

For close contacts during the first year following exposure to TB, the risk is estimated at 500 per 100,000 people in high-income countries and 1500 per 100,000 people in low-to-middle-income countries. Over one to two years following contact, it is at its maximum level; with time, it diminishes, but it remains greater than the local population [3]. The risk of developing TB in infected contacts is increased by a young age, low quality of the immune defense mechanisms, and individual factors like diabetes, smoking, and nutritional status. Over a 12-year period, 688 per 100,000 contacts in British Columbia, Canada, were diagnosed with TB [5]. A recent meta-analysis concluded that among untreated persons with latent tuberculosis infection (LTBI), the risk of incident TB within two years was 14.6% among recent child contacts below the age of 15 years, 3.7% among adult contacts, 4.1% among migrants, and 2.4% among people screened because of immune suppression [6]. Child contacts under the age of five had a far higher risk of contracting the disease than those between the ages of five and 14 years old (26.0% vs. 12.4%) and the development of TB was more rapid than in adults. The higher risk for exposed and infected children has been confirmed by another study [7].

2 Screening for Infection and Tuberculosis Among Contacts

Because those who have been in close contact with a person who has contagious TB are at the greatest risk of contracting the disease, it has long been customary to look for further cases of active TB among those people [8]. The benefit of contact investigations has been documented by randomized studies. For instance, a study conducted in Viet Nam showed that screening contacts by symptoms and chest radiography increased the proportion of notified TB cases by 2.5 times and the proportion of microbiologically confirmed TB by 6.4 times compared with passive screening only [9]. Searching for potentially contaminated but otherwise healthy individuals has also been suggested to offer preventive treatment to those contacts who are most likely to develop TB in the future, especially in locations and circumstances where infection prevention is well-established [10, 11]. The search for exposed contacts was mainly directed at small children and immunocompromised contacts until recently. In recent years, new guidelines issued by the World Health Organization (WHO) have extended the population categories for which the screening for LTBI is recommended, now including all contacts of patients with PTB, without an age limit [1, 12, 13].

The procedures for the identification of secondary cases of active TB and infected contacts have been defined in a European-specific consensus paper [14]. To locate infected contacts, a list of close friends and family members is made, and everybody is invited to have their symptoms assessed and take a TB screening test (TST or IGRA). One asks about prior exposure to TB or risk factors for progression, e.g., immunodeficiency, and then they have a chest radiograph to evaluate if there is evidence of incipient or prior TB. An infected person may be given a preventive medication for three to nine months (isoniazid, rifampicin, or a combination of rifampicin or rifapentine and isoniazid), according to the contacts' demographic characteristics and health risks, as well as the duration since the previous contact. This contact tracing may take a long time and is usually done by a member of the local healthcare team. Distant contacts are only evaluated if the initial circle of contacts displays a high proportion of disease.

3 Infection and Tuberculosis in Healthcare Workers

Another category of persons with a higher risk of being infected and developing TB are the healthcare workers caring for patients with undetected or inadequately treated TB. This concerns mainly persons active in hospital or health settings where new patients are examined, particularly patients belonging to risk groups with a high prevalence of TB like immigrants from high-incidence countries, immunocompromised patients, or underserved populations like homeless or users of injectable drugs. Historically, it has been accepted that most healthcare workers in contact with patients will acquire TB infection [15], with the risk being increased with age and duration of occupation [16, 17]. In some hospitals, the prevalence of TB among healthcare workers could be ten times higher than in the local population [18]. The decline in TB incidence in industrialized countries and the implementation of administrative and technical measures (early diagnosis, timely isolation until treatment implementation, and increased ventilation) has decreased healthcare workers' exposure to cases of contagious TB, and infection has decreased to a level comparable to that of the general population [19], at least in countries where TB is not highly prevalent. However, in nations where the incidence of TB is still elevated, the risk of TB among healthcare professionals remains significant. According to a recent meta-analysis, the prevalence of LTBI among healthcare professionals was found to be 37%, and the incidence rate of active TB was found to be 97 per 100,000, which is clearly higher than the rates in the general population in most countries [20]. Therefore, in most settings where patients with undetected or inadequately treated TB may be encountered, due attention to the possibility of TB and preventive measures should be implemented, particularly in resource-limited settings [21].

4 Problems and Controversies

4.1 Contact Investigation and Preventive Treatment

The investigation of contacts of an index case needs several conditions in order to be implemented in a systematic and efficient way. The policy has to be defined in a national guideline; a team of dedicated and trained healthcare personnel should be available, the costs should be covered by the state or by an institution and not by the individuals, and the procedure should be standardized. Many of these conditions may be absent, particularly in countries with limited resources. In settings with a high burden of TB, passive case finding may be the only available policy, and active case finding may not be considered as a priority, although studies have clearly demonstrated that this is cost-effective and beneficial.

Contact investigation was traditionally thought to be a "luxury" intervention only affordable in high-income nations with low TB burdens since the low TB burden allowed for extra public health efforts that were not mainly focused on treating patients with active illness. However, this has changed. Concerns have been raised about the impact of contact investigation activities on limited resources in countries with a high TB burden [22]. Recently, however, contact investigation has been demonstrated as an important component of the global strategy for TB elimination and is now recommended as a routine practice in all TB programs, with adaptations to the local conditions, whatever the prevalence of TB in the population [23].

One of the obstacles in practice may be the selection of contacts, which usually needs the cooperation of the index case. Assuming the patient has active TB, it may be difficult to inquire about the identities of all household contacts and much more difficult to locate and meet all of them for testing. Because of the likely reluctance of the contacts to pass a test and an examination, an expert nurse must be on hand to explain the distinction between exposure, infection, and TB illness in a clear and concise manner. Because distant or casual contacts outside of the near family circle have a reduced risk of infection, it makes sense to limit screening to the close family circle. The children of a TB-infected parent are also easy to meet when the whole family lives together.

A further obstacle is the selection of the screening procedure for the detection of contacts with infection and active TB. If the latter usually relies on the presence of symptoms of possible disease (cough, loss of weight, fever), the detection of infection, which is by definition symptom-free, has to be performed by an indirect immunological testing (TST or IGRA), which needs an intervention (skin test and dual visit for the TST, blood sampling and availability of a laboratory for the IGRA). The positive and negative predictive values of both tests are not equivalent. To put it simply, the TST is cheaper but associated with a high proportion of false-positive test results (mainly related to prior vaccination with BCG or exposure to environmental mycobacteria), meaning that a higher number of contacts may be selected for an unnecessary preventive treatment whereas the IGRA are more costly but more specific and allow a reduction in the number of contacts who might benefit from

preventive treatment [24]. The availability of point-of-care tests in the near future may modify the options and facilitate the selection of the screening test. It is still most cost-effective to prescribe a preventative medication without previous testing in children under the age of three or five since the risk of TB, especially in severe forms of TB, is so high in this age range that a simple procedure justifies the cost [25].

It only makes sense to conduct infection screenings on people who are most likely to develop active illness. It is of great importance for children and immunocompromised contacts but is now recommended for all recent contacts. Screening all children who have been exposed to TB and providing preventative therapy to those who have been infected between the ages of 5 and 14 years has been shown to potentially avoid 159,000 cases of TB and 108,000 fatalities in children under the age of 15 per year [26]. Many national guidelines still do not mention the preventive treatment or restrict its use to small children below the age of five and persons living with HIV (PLHIV). According to the latest TB reports from WHO, only a small proportion of persons eligible for preventive treatment receive it. In 2018 and 2020, the majority of PLHIV received preventive treatment, but only 29% of children contacts under the age of five years and only 1.6% of household contacts aged more than five years received it [27].

It has been argued that if eligible infected contacts do not take preventive medication, then screening for infection is pointless, which is one of the grounds against implementing contact investigation and preventive treatment for all TB contacts on a systematic basis [28]. In spite of some pessimistic reports [28], it has been demonstrated that a dedicated team working in a supportive environment can obtain satisfactory completion rates (up to 80%) of preventive treatment [29–31].

Contacts of TB patients and the at-risk population are screened using an algorithm detailed in the most current WHO guidelines (Graphical Abstract) [1, 13] (Table 1).

4.2 Healthcare Workers

As mentioned above, the risk of TB infection and disease in healthcare workers is very diverse. In settings where the risk is still higher than in the local population, preventive measures and surveillance are warranted. The most important is the improvement of the diagnostic procedures for the detection of unknown cases of TB, rapid detection of drug resistance (in order not to initiate an inadequate treatment in patients with undetected drug resistance), selected isolation of patients with potential TB until they benefit from an adequate treatment, and implementation of appropriate environmental measures, mainly ventilation [32]. The wide implementation of rapid diagnostic tests indicating the presence of rifampicin resistance (Xpert MTB/RIF) allows a decrease in the time lag between hospitalization and initiation of TB treatment [33]. Personal protection (wearing of masks or respirators) is traditional, although their usefulness during the care of patients under adequate anti-tuberculous treatment is questionable. Shortening the duration of hospitalization and switching from long inpatient treatment to outpatient

Table 1 Citations from the WHO guidelines for the detection and preventive treatment of persons exposed to TB

WHO guidelines 2012 [8]	WHO guidelines 2015 [11]	WHO guidelines 2018 [12]
Clinical evaluation of household and close contacts for active TB is recommended. Priority should be given to: – people of all ages with symptoms suggestive of TB; – children < 5 years of age; and – people with known or suspected immunocompromising conditions (especially PLHIV) and contacts of index cases with MDR-TB or XDR-TB (proven or suspected)	Systematic testing and treatment of LTBI should be performed in people living with HIV, adult and child contacts of pulmonary TB cases, patients initiating anti-tumor necrosis factor (TNF) treatment, patients receiving dialysis, patients preparing for organ or hematologic transplantation, and patients with silicosis	In countries with a low TB incidence, adults, adolescents and children who are household contacts of people with bacteriologically confirmed pulmonary TB should be systematically tested and treated for LTBI
PLHIV and children < 5 years of age who are household or close contacts of people with TB and who, after an appropriate clinical evaluation, are found not to have active TB should be treated for presumed LTBI as per WHO guidelines	For resource-limited countries and other middle-income countries that do not belong to the above category People living with HIV and children below 5 years of age who are household or close contacts of people with TB and who, after an appropriate clinical evaluation, are found not to have active TB but have LTBI should be treated	In countries with a high TB incidence, children aged $\geq$ 5 years, adolescents and adults who are household contacts of people with bacteriologically confirmed pulmonary TB who are found not to have active TB by an appropriate clinical evaluation or according to national guidelines may be given TB preventive treatment
Tests for LTBI, including the tuberculin skin test and interferon-gamma release assays, can be used to identify people at increased risk for developing active TB and who are therefore candidates for treatment of LTBI (other than children < 5 years of age and PLHIV, for whom isoniazid preventive treatment is recommended without testing for LTBI (1, 4), once active TB is excluded Unless a plan includes policies and procedures for treating LTBI, testing for LTBI should not be undertaken	Either TST or IGRA can be used to test for LTBI in high-income and upper middle-income countries with estimated TB incidence less than 100 per 100,000	Either a tuberculin skin test (TST) or interferon-gamma release assay (IGRA) can be used to test for LTBI LTBI testing by TST or IGRA is not a requirement for initiating preventive treatment in people living with HIV or child household contacts aged < 5 years The availability and affordability of the tests will determine which LTBI test will be chosen by clinicians and programme managers

Prepared with data from [8, 12, 13]

treatment as soon as possible also contributes to the reduction of exposure of healthcare workers. This may need the implementation of an appropriate team of outreach workers for the administration of TB treatment, also using the opportunities of remote control by electronic digital procedures [34]. Systematic surveillance of healthcare workers at regular intervals by clinical, radiological, or immunological tests (for instance, annual tuberculin skin test) has long been performed, but their cost-efficiency is dubious, controversial, and has not been demonstrated.

In case of documented exposure of healthcare workers (as well as other patients or visitors) to a patient with contagious TB, a contact investigation is mandatory, with the provision of preventive treatment if indicated. One of the problems in practice is the reluctance of some healthcare workers to the preventive treatment [35].

In countries where the risk of infection and TB among healthcare workers is low and similar to the risk in the general population, which is the case in most industrialized countries with a low incidence rate of TB, systematic surveillance of healthcare workers is now considered as obsolete, with the exception of initial screening at hiring for those workers who will be active in an environment with potential exposure to unknown TB cases, in order to detect a possible immunological change in case of documented exposure [36, 37].

5 Conclusion

Close contacts of patients with transmissible TB and healthcare workers exposed without protection to patients with untreated TB are population groups at high risk of infection and active TB. Therefore, screening exposed persons and preventive treatment for those at the highest risk of TB are now considered part of the global End TB Strategy and the management of patients with active TB.

Core Messages

- Persons in contact with a case of untreated PTB and exposed healthcare workers may be infected by *M. tb*.
- Some of them will develop TB; the risk depends on the intensity of contact and the quality of the defense mechanisms.
- Preventive treatment decreases the risk of TB in infected persons.
- Screening exposed persons for infection and preventive treatment of infected persons help control TB.

References

1. World Health Organization (2020) WHO consolidated guidelines on tuberculosis. Module 1: prevention—tuberculosis preventive treatment, 41. World Health Organization, Geneva
2. Zellweger JP, Sotgiu G, Block M, Dore S, Altet N, Blunschi R et al (2015) Risk assessment of tuberculosis in contacts by IFN-gamma release assays. A tuberculosis network European trials group study. Am J Respir Crit Care Med 191(10):1176–1184. http://doi.org/10.1164/rccm.201502-0232OC
3. Fox GJ, Barry SE, Britton WJ, Marks GB (2013) Contact investigation for tuberculosis: a systematic review and meta-analysis. Eur Respir J 41(1):140–156. https://doi.org/10.1183/09031936.00070812
4. Acuna-Villaorduna C, Jones-Lopez EC, Fregona G, Marques-Rodrigues P, Gaeddert M, Geadas C et al (2018) Intensity of exposure to pulmonary tuberculosis determines risk of tuberculosis infection and disease. Eur Respir J 51(1). http://doi.org/10.1183/13993003.01578-2017
5. Moran-Mendoza O, Marion SA, Elwood K, Patrick D, FitzGerald JM (2010) Risk factors for developing tuberculosis: a 12-year follow-up of contacts of tuberculosis cases. Int J Tuberc Lung Dis 14(9):1112–1119
6. Gupta RK, Calderwood CJ, Yavlinsky A, Krutikov M, Quartagno M, Aichelburg MC et al (2020) Discovery and validation of a personalized risk predictor for incident tuberculosis in low transmission settings. Nat Med. https://doi.org/10.1038/s41591-020-1076-0
7. Martinez L, Cords O, Horsburgh CR, Andrews JR, Pediatric TBCSC (2020) The risk of tuberculosis in children after close exposure: a systematic review and individual-participant meta-analysis. Lancet 395(10228):973–984. https://doi.org/10.1016/S0140-6736(20)30166-5
8. World Health Organization (2012) Recommendations for investigating contacts of persons with infectious tuberculosis in low- and middle-income countries, vol WHO/HTM/TB/2012.9, Geneva
9. Fox GJ, Nhung NV, Marks GB (2018) Household-contact investigation for detection of tuberculosis in Vietnam. N Engl J Med 378(22):2141. https://doi.org/10.1056/NEJMc1804977
10. American Thoracic Society (1976) Guidelines for the investigation and management of tuberculosis contacts. Am Rev Respir Dis 114:459-463
11. American Thoracic Society (2000) Targeted tuberculin testing and treatment of latent tuberculosis infection. Joint Statement of the American Thoracic Society (ATS) and the Centers for Disease Control and Prevention (CDC). Am J Respir Crit Care Med 161(4 Pt 2):S221–S247
12. World Health Organization (2015) Guidelines on the management of latent tuberculosis infection, vol WHO/HTM/TB/2015.01. WHO
13. World Health Organization (2018) Latent tuberculosis infection. Updated and consolidated guidelines for programmatic management. World Health Organization, Geneva
14. Erkens CG, Kamphorst M, Abubakar I, Bothamley GH, Chemtob D, Haas W et al (2010) Tuberculosis contact investigation in low prevalence countries: a European consensus. Eur Respir J 36(4):925–949. https://doi.org/10.1183/09031936.00201609
15. Sepkowitz KA (1994) Tuberculosis and the health care worker: a historical perspective. Ann Intern Med 120:71–79
16. Schablon A, Harling M, Diel R, Nienhaus A (2010) Risk of latent TB infection in individuals employed in the healthcare sector in Germany: a multicentre prevalence study. BMC Infect Dis 10(1):107. https://doi.org/10.1186/1471-2334-10-107
17. Bjartveit K (2003) Olaf Scheel and Johannes Heimbeck: their contribution to understanding the pathogenesis and prevention of tuberculosis. Int J Tuberc Lung Dis 7(4):306–311
18. Sotgiu G, Arbore AS, Cojocariu V, Piana A, Ferrara G, Cirillo DM et al (2008) High risk of tuberculosis in health care workers in Romania. Int J Tuberc Lung Dis 12(6):606–611
19. Youakim S (2016) The occupational risk of tuberculosis in a low-prevalence population. Occup Med (Lond) 66(6):466–470. https://doi.org/10.1093/occmed/kqw040

20. Uden L, Barber E, Ford N, Cooke GS (2017) Risk of tuberculosis infection and disease for health care workers: an updated meta-analysis. Open Forum Infect Dis 4(3):ofx137. http://doi.org/10.1093/ofid/ofx137
21. Furin J, Sotgiu G (2019) Protecting those who serve: are we doing enough to prevent tuberculosis in healthcare workers? Eur Respir J 53(4). http://doi.org/10.1183/13993003.00485-2019
22. Styblo K (1991) Preventive chemotherapy for tuberculosis control in developing countries. The case against preventive chemotherapy. Bull Int Union Tub Lung Dis 66(suppl. 1990/1991)
23. Fox GJ, Johnston JC, Nguyen TA, Majumdar SS, Denholm JT, Asldurf H et al (2021) Active case-finding in contacts of people with TB. Int J Tuberc Lung Dis 25(2):95–105. https://doi.org/10.5588/ijtld.20.0658
24. Erkens CG, Dinmohamed AG, Kamphorst M, Toumanian S, van Nispen-Dobrescu R, Alink M et al (2014) Added value of interferon-gamma release assays in screening for tuberculous infection in the Netherlands. Int J Tuberc Lung Dis 18(4):413–420. https://doi.org/10.5588/ijtld.13.0589
25. Mandalakas AM, Hesseling AC, Gie RP, Schaaf HS, Marais BJ, Sinanovic E (2013) Modelling the cost-effectiveness of strategies to prevent tuberculosis in child contacts in a high-burden setting. Thorax 68(3):247–255. https://doi.org/10.1136/thoraxjnl-2011-200933
26. Dodd PJ, Yuen CM, Becerra MC, Revill P, Jenkins HE, Seddon JA (2018) Potential effect of household contact management on childhood tuberculosis: a mathematical modelling study. Lancet Glob Health 6(12):e1329–e1338. https://doi.org/10.1016/S2214-109X(18)30401-7
27. World Health Organization (2021) Global tuberculosis report 2021, vol WHO/CDS/TB/2021. World Health Organization, Geneva
28. Alsdurf H, Hill PC, Matteelli A, Getahun H, Menzies D (2016) The cascade of care in diagnosis and treatment of latent tuberculosis infection: a systematic review and meta-analysis. Lancet Infect Dis 16(11):1269–1278. https://doi.org/10.1016/S1473-3099(16)30216-X
29. Adjobimey M, Masserey E, Adjonou C, Gbenagnon G, Schwoebel V, Anagonou S et al (2016) Implementation of isoniazid preventive therapy in children aged under 5 years exposed to tuberculosis in Benin. Int J Tuberc Lung Dis 20(8):1055–1059. https://doi.org/10.5588/ijtld.15.0493
30. Sarivalasis A, Bodenmann P, Langenskiold E, Lutchmaya-Flick C, Daher O, Zellweger JP (2013) High rate of completion of preventive therapy for latent tuberculosis infection among asylum seekers in a Swiss Canton. Swiss Med Wkly 143:w13860. https://doi.org/10.4414/smw.2013.13860
31. Gullon-Blanco JA, Rodrigo-Sanz T, Alvarez-Navascues F, Tabernero-Huguet E, Sabria-Mestres J, Garcia-Garcia JM (2021) Completion of treatment for latent TB infection in a low prevalence setting. Int J Tuberc Lung Dis 25(4):321–323. https://doi.org/10.5588/ijtld.20.0862
32. Migliori GB, Nardell E, Yedilbayev A, D'Ambrosio L, Centis R, Tadolini M et al (2019) Reducing tuberculosis transmission: a consensus document from the World Health Organization Regional Office for Europe. Eur Respir J 53(6). http://doi.org/10.1183/13993003.00391-2019
33. Zawedde-Muyanja S, Manabe YC, Sewankambo NK, Nakiyingi L, Nakanjako D (2018) Xpert MTB/RIF associated with improved treatment initiation among patients with smear-negative tuberculosis. Int J Tuberc Lung Dis 22(12):1475–1480. https://doi.org/10.5588/ijtld.17.0460
34. Falzon D, Migliori GB, Jaramillo E, Weyer K, Joos G, Raviglione M et al (2017) Digital health to end tuberculosis in the sustainable development goals era: achievements, evidence and future perspectives. Eur Respir J 50(5). http://doi.org/10.1183/13993003.01632-2017

35. Balmelli C, Zysset F, Pagnamenta A, Francioli P, Lazor-Blanchet C, Zanetti G et al (2014) Contact tracing investigation after professional exposure to tuberculosis in a Swiss hospital using both tuberculin skin test and IGRA. Swiss Med Wkly 144:w13988. https://doi.org/10.4414/smw.2014.13988
36. Dobler CC, Farah WH, Alsawas M, Mohammed K, Breeher LE, Murad MH et al (2018) Tuberculin skin test conversions and occupational exposure risk in US healthcare workers. Clin Infect Dis 66(5):706–711. https://doi.org/10.1093/cid/cix861
37. Sosa LE, Njie GJ, Lobato MN, Bamrah Morris S, Buchta W, Casey ML et al (2019) Tuberculosis screening, testing, and treatment of U.S. health care personnel: recommendations from the National Tuberculosis Controllers Association and CDC. MMWR Morb Mortal Wkly Rep 68(19):439–443. http://doi.org/10.15585/mmwr.mm6819a3

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Tuberculosis Among People Who Use Drugs: Multilevel Considerations for Prevention, Diagnosis, and Treatment

31

Ashly E. Jordan and David C. Perlman

In an era in which we have effective therapies, why has tuberculosis remained the leading infectious cause of young adult deaths in much of the world? […] We must explore not only the life experiences of those sick with tuberculosis, but also the larger social contexts in which they become infected, fall ill, and meet with a series of therapeutic misadventures leading to complications, ongoing transmission to others and, often enough, death. […] The experiences and commentaries of the sick and their providers must be embedded in broader analyses informed by history, political economy, epidemiology and a sociology of knowledge. Such an analysis brings into relief not only cultural specificity but also jarring similarities: living with both poverty and tuberculosis means poor outcomes whether you live in rural Haiti, urban Peru or the inner-city United States.

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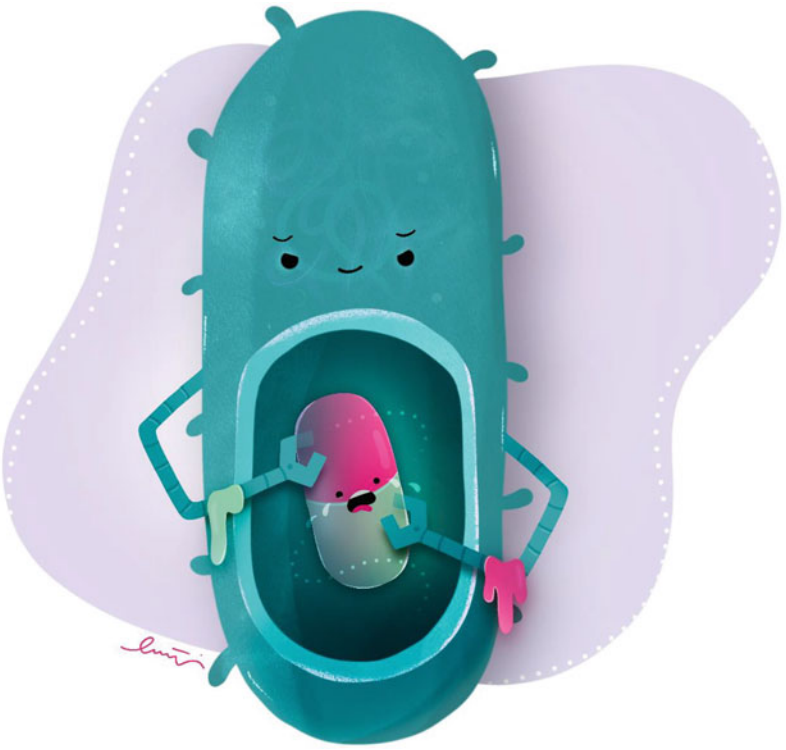
Summary

Tuberculosis (TB) and drug use have been integrally related for over a century. This relationship is driven by numerous factors that operate at the individual level (e.g., age, co-existing conditions, specific behaviors) and potently at the social and structural levels. The mutually reinforcing nature of multiple highly prevalent social and structural conditions (e.g., crowding, incarceration, poor housing, social and economic conditions) that disproportionality affects people who use drugs (PWUD) globally contributes to TB substantial transmission, morbidity, and mortality among PWUD. Yet, the social and structural contexts perpetuating TB among PWUD and others should be understood as modifiable through interventions and policy. Structural and policy interventions by localities, nations, regions, and international organizations are urgently needed to reduce the incidence and prevalence of TB infections and the morbidity and mortality of active TB among PWUD and to achieve enhanced TB control.

Graphical Abstract



Keywords

Drug use · Drug use disorders · Epidemiology · Multilevel risk factors · Mycobacterium tuberculosis · Opioids · People who use drugs · Structural vulnerability · Tuberculosis

1 Introduction

One-third of the human population is infected with *Mycobacterium tuberculosis (M. tb)* [2]. Despite the availability of efficacious treatment of both latent and active tuberculosis (TB) infection, TB causes an estimated 1.5 million deaths each year [2]. While *M. tb* is the pathogen causing infection and disease in the human host, it does so differentially in specific hosts and environments. Pathogen-based factors (e.g., virulence, susceptibility), host-based factors (e.g., age, co-existing conditions, behaviors), social and environmental conditions (e.g., poorly ventilated housing, crowding), and structural factors (e.g., socio-economic conditions, policies) drive TB epidemiology. The use of drugs that have been made illegal or which are marginalized by cultural norms is one critical factor linked to TB risk through these potent, mutually reinforcing, multilevel factors which contribute to ongoing TB transmission, morbidity, and mortality [3–5].

Understanding TB epidemiology, treating individuals and populations with TB, and controlling and eliminating TB at the population level require understanding TB as a complex disease inextricably linked to multilevel factors that create vulnerable populations and contexts through poverty, malnutrition, crowding, incarceration, HIV infection, and substance use. As a result, barriers to global, national, and local TB control include challenges in TB diagnosis and in the implementation of efficacious TB treatment as a result of both some specific behaviors people engage in (such as drug use) as well as a result of some specific social and structural contexts and conditions in which people live and in which TB transmission is perpetuated.

People who use drugs (PWUD) have had a high prevalence and incidence of TB infection and disease since the early 1900s [3–6]. Illicit drug use generally and injection drug use (IDU) in particular are important in global TB epidemiology and in TB control efforts [3, 7]. A key link between IDU and TB is through the immunological consequences human immunodeficiency virus (HIV) transmitted via the injection route. As HIV-related TB is covered in other chapters, this chapter will focus on the general relationship between drug use and TB. The national incidence of TB has decreased in most industrialized countries over the last several decades. However, in settings such as prisons and jails, and among populations such as PWUD and those unstably housed, TB transmission remains stable or is increasing [3, 8–11].

This chapter will examine the specific environmental, public health, and socio-economic contexts for TB risk among people who use drugs (PWUD) globally and in specific regions and countries. While the use of other substances, particularly alcohol, is linked to TB risk, there are unique considerations that relate to the connections between TB and drug use given drug use's criminalized and marginalized status [12–15]. The epidemiology of TB, drug use, their overlap among PWUD, and the evolving historical relationship between TB and drug use will be presented. Multilevel factors at the structural, social, environmental, and individual levels driving relationships between TB and drug use will be examined. Specific considerations for the diagnosis and treatment of TB among PWUD and opportunities for structural and policy intervention to reduce TB among PWUD and enhance TB responses will be discussed.

2 Global Epidemiology of Tuberculosis, and Drug Use, and Their Intersection

2.1 Global Tuberculosis Epidemiology

TB is a leading infectious cause of death and one of the ten most frequent causes of death worldwide [2]. In 2018, there were approximately ten million (range, 9.0–11.1 million) new cases of TB; expressed as a rate, this translates to 132 cases (range, 118–146) per 100,000 population [2].

The TB burden remains highest in the poorest economies, where the burden is concentrated among specific vulnerable populations, including PWUD [10, 16, 17] [18]. The low-, medium-, and high-TB burden geographic categories closely overlap the low-, middle-, and high-income World Bank country classifications [19]. Thirty "high TB burden countries" account for 87% of estimated incident global TB cases. In most high-income countries, there were fewer than ten TB cases per 100,000 population. In contrast, other countries, primarily low- and middle-income, had documented TB incident cases ranging from 150 to 400 to over 500 in the Central African Republic and the Philippines, respectively, for example [2]. Table 1 depicts global TB incidence in 2018; total new and relapse TB case notifications and incidence rates vary dramatically by WHO region, with a TB incidence of 28 compared with 220 cases per 100,000 persons, respectively in the European region and the South-East Asian region.

2.2 Global Substance Use Epidemiology

According to the United Nations Office on Drugs and Crime (UNODC), globally, in 2018, there were 35 million people with drug use disorder, and 11,260,000 (8,860,000–15,301,000) million people who inject drugs (PWID) (Table 1).

Table 1 Illicit drug use and tuberculosis (TB) statistics by World Health Organization (WHO) and United Nations Regions in 2018

		Opioids		Cocaine		Amphetamines and methamphetamine		People who inject drugs		Total new and relapse TB case notifications	TB incidence[d,e] (2018)	
		Number (thousands)[b]	Prevalence[c] (percentage)[b]	Number (thousands)[b]	Prevalence[c] (percentage)[b]	Number (thousands)[b]	Prevalence[c] (percentage)[b]	Number (thousands)[b]	Prevalence[c] (percentage)[b]		Number (thousands)[b]	Rate (per 100,000 population)
UNODC Region[a]	Africa	7440 (6190–11,800)	1.04 (0.87–1.66)	1900 (510–4140)	0.27 (0.07–0.58)	2930 (690–5810)	0.41 (0.10–0.82)	930 (560–2700)	0.13 (0.08–0.38)			
	Americas	12,470 (10,990–15,210)	1.86 (1.64–2.27)	9900 (9240–10,680)	1.49 (1.38–1.60)	8710 (8190–9460)	1.30 (1.22–1.41)	2380 (1910–2970)	0.36 (0.28–0.44)			
	Asia	33,550 (13,620–44,670)	1.11 (0.45–1.47)	1820 (1160–2620)	0.06 (0.04–0.09)	12,670 (11,430–13,690)	0.42 (0.38–0.45)	5220 (3900–6630)	0.17 (0.13–0.22)			
	Europe	3730 (3450–4020)	0.68 (0.63–0.74)	4870 (4670–5070)	0.89 (0.86–0.93)	2550 (2230–2870)	0.47 (0.41–0.53)	2630 (2400–2900)	0.48 (0.44–0.53)			
	Oceania	660 (580–740)	2.47 (2.17–2.78)	440 (410–440)	1.64 (1.56–1.67)	360 (310–380)	1.35 (1.16–1.41)	100 (100–110)	0.38 (0.37–0.41)			
WHO Region[e]	Africa									1373	2450 (2190–2730)	231 (206–257)
	The Americas									234	289 (268–310)	29 (27–31)
	Eastern Mediterranean									526	810 (639–1000)	115 (91–142)
	Europe									218	259 (225–296)	28 (24–32)
	South-East Asia									3183	4370 (3480–5370)	220 (175–271)
	Western Pacific									1417	1840 (1520–2180)	96 (79–114)

(continued)

Table 1 (continued)

	Opioids		Cocaine		Amphetamines and methamphetamine		People who inject drugs		Total new and relapse TB case notifications	TB incidence[d,e] (2018)	
	Number (thousands)[b]	Prevalence[c] (percentage)[b]	Number (thousands)[b]	Prevalence[c] (percentage)[b]	Number (thousands)[b]	Prevalence[c] (percentage)[b]	Number (thousands)[b]	Prevalence[c] (percentage)[b]		Number (thousands)[b]	Rate (per 100,000 population)
Global	57,850 (34,820–76,430)	1.16 (0.70–1.53)	19,020 (16,000–22,950)	0.38 (0.32–0.46)	27,220 (22,850–32,220)	0.55 (0.46–0.65)	11,260 (8860–15,310)	0.23 (0.18–0.31)	6951	10,000 (8990–11,100)	132 (118–146)

The countries included in the UNODC and WHO regions differ; hence, regional data for drug use and TB are presented separately

[a] Data derived from the United Nations 2020 World Drug Report

[b] Reflects the best estimate and the range reflects the lower and upper bounds

[c] Refers to the number of people who have consumed an illicit drug at least once over the prior 12 months

[d] The number of new TB cases per 100,000 population including upper and lower, 97.5th and 2.5th, centiles of rates

[e] Data derived from the WHO 2019 World TB Report

(*Prepared with data from the UN World Drug Report 2020 and the WHO 2019 World TB Report*)

In 2017, 271 million people (over 5% of the global population aged 15–64) had used drugs in the prior year. Some studies have identified higher rates of drug injection. A 2017 systematic review synthesizing international data from 1147 unique sources estimated that the global prevalence of drug injection was 15.6 million people (95% confidence interval (CI), 10.2–23.7 million) [9]. This review also found significant regional variation in the prevalence of IDU, ranging from 0.09% (95% CI, 0.07–0.11) in the South Asian region to 1.30% (95% CI 0.71–2.15) in the Eastern European region. The largest numbers of PWID were in Southeast Asia (4.0 million, 95% CI, 3.0–5.0 million), Eastern Europe (3.0 million, 95% CI, 1.7–5.0 million), and North America (2.6 million, 95% CI, 1.5–4.4 million) [9]. Table 1 depicts the UNODC 2018 data on the global annual prevalence of use (past year use of any route) of specific drugs, identifying the Asia region as having the largest number of opioid and amphetamine/methamphetamine users and the Americas region as having the largest number of cocaine users (Table 1).

2.3 Global Overlap of Tuberculosis and Substance Use Epidemiology

In the past two decades, there have been substantial efforts to improve and standardize national and international surveillance and reporting of both TB and drug use [20–22]. Of note, reporting of both TB and drug use data varies widely, subject to a range of limitations, including those due to stigma, resources, and political instability, as well as different methodologies used to generate country-level estimates [23]. These, as well as variability in the ways different international bodies aggregate the data, might affect, and in some cases might limit, the ability to compare TB and drug use data among nations and regions.

In regions of high-TB burden, PWUD often represent one of the populations with the highest incidence, prevalence, and mortality due to TB. PWUD often experience more food insecurity, malnutrition, unstable housing, crowding, incarceration, and poverty than non-drug using populations [24–26]. Each of these factors has been associated with increased TB risk, contributing to a vulnerable population of PWUD in poor areas of many countries [24, 25].

While national income and TB burden patterns among countries overlap closely, the overlap patterns between areas of high-TB incidence or prevalence and areas of high-IDU prevalence vary between and within countries and regions [2, 10, 18] (Table 1). The countries included in the UNODC regions differ from those included in the WHO regions. Hence, the 2018 regional drug use data derived from UNODC reports and 2018 TB data derived from WHO reports are presented separately in Table 1. There are regions in the world where there are high rates of TB but low rates of IDU; for example, 20–25% of new TB cases and more than 80% of TB deaths among people without HIV infection were in Africa in 2016, while under 0.2% of the adult population reported IDU [2, 27, 28]. In North America, there are high rates of IDU and low rates of TB [2, 9]. In Southeast Asia, there are generally very high rates of both IDU and TB infection [2, 27]. Further, there are variations of

rates of IDU and TB within countries [2, 27]; higher rates of both TB and IDU are often found in urban rather than rural areas [29].

3 Historical Context of Tuberculosis and Drug Use

3.1 Human History and TB

Both TB and the use of psychoactive drugs have been part of humanity's history for millennia. Humans have co-evolved with *Mycobacterial* species that evolved into *M. tb* [30]. The genus *Mycobacterium* has been traced back roughly 150 million years, and early progenitors of *M. tb* appear to have infected early hominins in East Africa three million years ago. *M. tb* emerged as a human pathogen some 70,000 years ago in Africa. *M. tb* then migrated as humans migrated, first across Africa and then diffused globally through successive waves of human migration, travel, trade, and resettlement [31]. There is clear paleontological and written evidence of human TB in the third millennium BC with both skeletal findings typical of Pott's disease and depictions of Pott's disease in early Egyptian art, and written documentation of TB in India and China in 1300 BC and 300 BC, respectively [32–36].

As a highly virulent human pathogen, *M. tb* appears to have evolved the key virulence trait of latency some 30,000–40,000 years ago, contributing substantively to its perpetuation [37]. It remained transmissible and virulent and yet, through latency, could allow sufficient reproduction of its host and then reactivate decades later in post-reproductive aged, or otherwise debilitated, hosts. These pathogen-specific factors (latency, virulence, and greater relative virulence in debilitated hosts) partly allow and underlie the associations between TB and drug use and the ways drug use is linked to TB exposure and host compromise facilitating established TB infection and TB disease.

3.2 The Human History of Substance Use

Humans have a long history of appropriating, manipulating, and distributing natural products, including opioids, for use to achieve pain relief and for psychotropic effects [38, 39]. The use of opioids and other psychotropic medications preceded the development of the germ theory and played roles in folk approaches to promoting and restoring health, as well as roles assigned both within the development of national and transnational trade and within evolving healthcare systems [40, 41]. Archeological evidence suggests that the domestication of the opium poppy began in the sixth millennium BC, that its anesthetic and hypnotic effects were known by at least the third millennium BC, that there was trade within the Mediterranean of a liquid opium preparation by the mid-second millennium BC. That use became increasingly globalized with the global extension of trade. Both human TB

infection and the use of psychoactive substances, including opioids, continued to spread globally as trade routes extended across continents and oceans, often along the same routes as each other and through which other developments and other infections spread as well [42].

Archeologic data suggest that the use of psychoactive substances in 'prehistoric' eras was primarily ceremonial by elites or priests, offering those with accessing the potential for 'other worldly' experiences [43]. The evidence for "use," however, does not necessarily imply prevalent use, nor are there data suggesting prevalent misuse, meaning use resulting in dependence or other adverse consequences of use. Evidence of dependence and adverse effects of psychoactive drug use emerges historically later than evidence of use [43]. However, it is unclear whether this is due to a low prevalence of adverse effects when access was limited, or that evidence of use can be derived from nonwritten archeological data, whereas evidence of adverse effects of use requires written evidence that was available only later in history. Throughout history, opioid use has been inextricably, directly and indirectly, linked to both improvements in health through relief of pain or other symptoms such as cough or diarrhea, the facilitation of surgery, as well as for psychoactive effects [38, 44, 45]. Psychotropic agents, including opioids, have played socially acceptable, even prominent, roles within various societies. Yet, they have also had socially unacceptable roles and have been variously assigned illegal status within specific societies [6, 38, 40]. In the 19th and early twentieth centuries, before the development of effective anti-TB agents, opioids were utilized to manage active TB for their cough suppressive activity and the sense of euphoria and well-being they induce. The use of opioids to treat TB was so prevalent as part of TB management that most patients with TB developed opioid dependence, this at a time when most opioid dependence was iatrogenic [38, 46].

4 People Who Use Drugs as A Population Construct and Historical Context of Tuberculosis and Drug Use Overlap

An important issue when examining the relationship between TB and PWUD is understanding that the population, PWUD, should be clearly defined in specific contexts, as associations vary by what are considered "drugs" and by how drug use is defined and measured; each of these has varied historically and by context. Broadly, the construct of PWUD as population refers to a heterogeneous group of people who use a diverse range of illicit substances by diverse routes, for diverse reasons, in diverse contexts for varying durations. Some individuals may personally identify with their drug use and hence identify as drug users, and others may not only deny drug use but may not self-identify as drug users despite using substances. Further, the use of some substances may be considered normative or acceptable in some settings and as non-normative or even deviant in others. Further, which drugs are illegal or otherwise marginalized (i.e., illicit) varies by country, culture, and

time [38, 40]. Yet, the construct of PWUD does not necessarily imply that PWUD are, in fact, socially connected or have a collective identity as drug users, although they may be.

In the nineteenth century, most opioid misuse and dependence, particularly in middle- and high-income countries, was iatrogenic, resulting from the use of over-the-counter legally marketed or prescribed opioids as treatment of many conditions, particularly TB [38]. The demographics of opioid use shifted substantially around the turn of the twentieth century as numerous countries established laws, regulations, and policies (e.g., in the United States (US) The Harrison Act of 1914) that categorized certain psychoactive substances as licit pharmaceuticals to be controlled by emerging states, evolving health systems and practitioners, and categorized other psychoactive substances as illegal, effectively criminalizing users as well as the drugs. The Harrison Act and analogous laws in other countries made individual non-prescribed use of opioids and other drugs a crime, resulting in dramatic changes in the demographics of what was or what had become illicit use. Different opioids increasingly came to be used as primary different marginalized groups, and as a result, drug use acquired new degrees of social stigmatization [40].

In 1961, the United Nations Convention Against Illicit Traffic in Narcotic Drugs formally prohibited the production and supply of specific drugs reflecting a significant shift towards a more prohibitionist approach to drugs [47]. This prohibition had disproportionate social and cultural effects on low- and middle-income countries where opium production and use had been historically embedded for centuries [48, 49]. The criminalization of drug use was temporally linked to a transition to a demographic pattern in which drug use came to predominate among poor, often urban, and socially marginalized populations. These populations commonly had a high prevalence of latent and active TB due to concomitant social-economic and structural factors such as poorly ventilated and crowded housing, poor nutrition, and incarceration [8, 10, 18, 24, 50, 51].

Treatment of drug use emerged in the mid-twentieth century (initially non-pharmacologic and later pharmacologic with the advent of methadone in the 1960s) as TB treatment advanced to be pharmacotherapy-based (from the 1940s forward) [38, 52]. The social construction of drug use treatment followed an organizational design similar to that of early TB treatment; with the increasing criminalization of drug use, systems of social control of PWUD increasingly relied on their physical separation from non-users of drugs, both through use of often geographically remote treatment facilities and in correctional facilities, the latter coming to predominate in the second half of the twentieth century [53–56]. For example, in the 1940s, the US created, as part of its Public Health Service, two national referral centers in Lexington, Kentucky and Fort Worth, Texas where those arrested throughout the US for illicit drug use were sent for incarceration and “drug treatment” (such as it was at the time) [45]. US Public Health Service physicians at these centers reported a very high prevalence of TB among the inmates [4, 45, 57]. This reflected the full establishment of the shift from predominant iatrogenic opioid dependence among those with TB to the converse, opioid dependence occurring on

a substrate of risk factors for TB exposure and progression to active TB disease and in the context of conditions accelerating latent TB reactivation, i.e., the general dynamics that are operative today.

5 Multilevel Conditions that Create Risk for Tuberculosis, Particularly Among People Who Use Drugs

Conditions operating at multiple levels produce risk for TB acquisition and transmission, specifically for PWUD [58]. To highlight structural factors which are often under-considered and are perhaps relatively understudied, we review key multilevel forces beginning with structural factors, followed by social and environmental factors, and lastly, review some selected individual-level factors.

5.1 Structural-Level Vulnerability

Unlike infections such as HIV and hepatitis C virus, where infection risk is directly linked to the act of drug use, TB risk among PWUD is not primarily linked directly to the act of drug use itself; but rather to the socio-economic conditions in which PWUD live and drug use occurs. TB rates in high-income countries decreased substantially and generally progressively in the twentieth century, due both to general public health improvements (such as better ventilated housing) before the anti-infective era and to wide-scale efforts and detection and treatment of active and latent TB as efficacious treatment and prevention became available and implemented [2, 8, 59].

However, several 'natural experiments' reveal that these public health improvements require continued efforts to be sustained. TB rates are very sensitive to degrees of public funding, resources, and infrastructure. Decreases in TB-specific financing can lead to resurgent TB epidemics even in contexts where TB rates have generally fallen [59–63]. Three examples include events in New York City (NYC) in the 1980-the 90s, in the nations of the former Soviet Union in the years after the Soviet Union's disintegration, and in more recently in Venezuela; in the first two, there were clear links between TB and drug use, and in the third actively ongoing process data are still emerging.

Trends in TB incidence and mortality can be mapped to important structural-level processes—what have been called "big events," such as social, political, and economic transformations which impact funding for public-health initiatives, including control of TB [64–66]. The construct of big events embodies how macro-level phenomena, such as environmental disasters, economic crises, and rapid political and social changes, can disrupt infrastructure and systems resulting in new risk environments that directly pose threats to public health [67]. Such big events have important consequences for epidemics, including TB, drug use, and HIV, with clear implications for TB [59, 64–66, 68]. Big events such as economic

crises and increases in economic inequality may lead to independent yet interconnected rises in drug use initiation, homeless among PWUD, disruptions in TB control programs, and reshaped HIV risk environments leading to rises in both drug use and TB [69–71].

After years of declining TB incidence and prevalence in the US and NYC specifically, in the 1970s, an NYC governmental task force recommended reductions in public health and TB-specific health spending, including a significant reduction in TB hospital beds [59, 70]. While the task force recommended increases in outpatient TB funding, only 10% of the recommended funding was allocated and spent in response to a fiscal crisis in NYC. Subsequently, between 1978 and 1989 rates of new TB cases in NYC increased from 17.2 per 100,000 to 36.0 per 100,000 [59]. Cases were concentrated among the poor, the homeless, those with alcoholism, and with the recently emerged HIV epidemic, particularly among those whose HIV risk factor was IDU [59, 72]. In a study examining NYC census block groups, rates of TB in 1984–1992 were associated with poverty [73].

A process with important similarities and differences emerged in the states of the former Soviet Union in the 1990s. IDU rose rapidly in several Eastern European countries at the time of the fall of the Soviet Union and during the political and market reforms of the 1990s [74]. In Estonia, for example, employment and the gross domestic product significantly declined, and there were marked reductions in spending on social services, including in TB-relevant services, mandated as part of loans from international lenders [75, 76]. These changes were followed by concomitant increases in TB [77, 78]. Ecologic studies have demonstrated a direct association between receipt of World Bank loans (mandating reductions in government social service and health expenditures) and increases in national TB rates [50, 51, 79].

In addition to these two well-characterized events, other data support associations between economic crises and rises in both TB and alcohol misuse (as occurred in South Korea in response to the East Asian Financial Crisis, which began in 1997) [80] and between economic crises and drug use and HIV (as occurred in response to the Greek economic crisis in which began in 2009) [64, 81].

The ongoing (as of this writing) situation in Venezuela is a more recent example of a big event leading to a resurgence of TB [82]. Since 2012, Venezuela's social, political, and economic changes have resulted in a massive humanitarian crisis with the convergence of collapsing public health infrastructure and widespread food shortages [83, 84]. In this context, TB incidence has risen dramatically to the highest in the past 40 years to 32.4 per 100,000 people [84, 85]. Many arrests and incarcerations are reportedly due to drug crimes, and many deaths among inmates in Venezuela appear to be due to TB [86]. This crisis is still evolving, and data on drug use trends and on links between drug use and TB in this setting are sparse; more research and better data are needed.

In addition, during 2011–19, HIV outbreaks occurred among people who inject drugs in multiple countries, with varying underlying TB prevalence, including Canada, Athens (Greece), Dublin (Ireland), Tel Aviv (Israel), Luxembourg, Bucharest (Romania), Glasgow (Scotland), and in parts of the US. Varying

combinations of structural factors, including economic stresses and rises in homelessness, as well as interruptions in public health services, were associated with these outbreaks, with clear implications for risks of resurgent TB outbreaks among PWUD [87, 88].

5.2 Social and Environmental Factors

Social networks and environmental settings are also key factors in the epidemiology of TB among PWUD. Compared to non-PWUD, PWUD are more likely to be part of case clusters of active TB due to shared *M. tb* genotypes [89]. In a low TB-incidence area, drug use and drug sharing were more prevalent among TB cases and infected contacts than those without latent TB infection [90]. Social network analyses have facilitated TB outbreak investigations, often finding links through settings in which drugs are consumed [91]. Similarly, in a community-based study of United Kingdom-born adults in England, TB risk was independently associated with homelessness, area-level deprivation, and drug use (especially injectable drugs) [92].

Poverty is a potent independent risk factor for TB. A US-based study identified that for each 10% increase in the percent of residents in an area with incomes below the poverty level was associated with a 33% increase in TB incidence [19, 93]. Further, neighborhoods with declining median household income have higher rates of TB infection than neighborhoods with increasing median household income [73].

Global demographic shifts and the rise of mega-cities on all continents, referred to as the "planet of slums," increase the overlaps of dense concentrations of TB and drug use [94–96]. Housing settings (e.g., public housing, shelters, favelas) where PWUD live are often characterized by various environmental hazards, unsanitary conditions, and poor air circulation leading to increased risk for TB transmission; additionally, crowding of housing units due to financial hardship can lead to increased risk [3, 8, 11, 59, 74]. Studies from many regions of the globe find that drug use is higher among urban slums than in other non-urban or non-slum areas in those regions [97–99]. Similarly, a systematic review examining the relationships between housing settings and risk of TB found an almost five-fold increase in active TB risk among those living in slum settings compared with non-slum settings [96].

Jails and prisons are also environmental contexts that drive links between drug use and TB. PWUD are subjected to high incarceration rates due to the criminalized nature of drug use in most countries [100, 101]. Increased time spent in this congregate setting contributes to higher rates of TB infection among PWUD [102, 103]. Data demonstrate that both the population incarcerated and the prison environments themselves contribute to TB transmission [17, 104–106]. Dolan et al., based on 2005–2015 data, estimated the prevalence of active TB among incarcerated persons worldwide as 2.8% (based on 46 studies from 25 countries) [107]. Correctional settings worldwide are characterized by crowding and poor ventilation, increasing the likelihood that any case of active TB can lead to transmission among inmates [25]. The high concentration of PWUD, people living with HIV

infection, and people with TB produce high-risk environments for TB transmission in both prison settings and housing settings due to poor ventilation and crowding [8, 104]. Dolan et al. also identified that of 189 countries, 21, two, and 43 countries, respectively, conducted TB screening in more than 50%, less than 50%, or in an unknown proportion of prisons (with no data available for 123 countries). Similarly, TB treatment was available in 20, four, and 38 countries in at least 50%, less than 50%, or in an unknown proportion of prisons (with data absent for 127 countries) [107]. Consequently, a downstream consequence of the criminalization of drug use in most, but not all, countries is an excess of TB risk among PWUD.

Settings in which drugs are consumed have historically been conducive to TB transmission. In addition to concentrating groups with a relatively high prevalence of TB disease and a high prevalence of relevant co-morbidities, drug use settings such as opium dens and shooting galleries have commonly been crowded and poorly ventilated environments increasing the likelihood of transmission from any active TB case present [5, 57].

5.3 Individual-Level Factors

Many drugs are used either primarily or occasionally by smoking, including marijuana, hashish, cocaine, opioids, and amphetamines. Drug smoking may exacerbate TB risk because inhaled smoke damages and causes inflammation of the respiratory airways, adversely affecting local host defenses and potentially increasing the risk of *M. tb* acquisition [108, 109]. One study found a higher odds of smear-positive active TB among PWUD than non-drug users; the risk of TB was particularly high among people who reported smoking crack [110]. TB has been reported among people who smoke marijuana, associated with sharing a water pipe and with "hotboxing," the practice of groups smoking drugs in an enclosed space to allow exhaled smoked drugs to be repeatedly inhaled [111, 112]. Similarly, a higher prevalence of TB infection has been reported among people who "shotgun" drugs, which is the practice of one person inhaling smoked drugs and then directly exhaling this smoke into the mouth of another. These practices have a clear potential for TB transmission if either the person inhaling exhaled smoked drugs or particularly if the person exhaling smoked drugs into another has active TB [113].

Other drugs may increase TB risk through direct effects inducing immunosuppression. For example, khat is a natural plant product that contains cathinone, a psychoactive drug with stimulant effects [114, 115]. It is used by an estimated 20 million people in Africa and the Arabian peninsula [115]. Cathinone has an immunomodulatory effect that appears to be mediated by causing elevated levels of resistin, a pro-inflammatory signal protein predominately secreted by human macrophages [114, 115]. Saudi Arabia has recently become a hot spot for TB, despite being well-resourced and having nationwide coverage of TB directly observed therapy programs, with the rise in cases concentrated among immigrants from countries in which khat use is prevalent [114]. One study found higher resistin levels in khat users and possibly higher levels in khat-using TB patients than

non-khat using TB patients, suggesting a need for further study [114]. A rise in TB associated with khat chewing has also been reported in Somaliland, where khat is usually consumed over prolonged periods in poorly ventilated rooms [116]. A study of HIV-infected persons in Ethiopia found khat chewing is independently associated with active TB [117]. Khat-chewing people with TB may experience more stigma than other TB patients [118]. Further, studies of TB patients in several countries, including Yemen, Ethiopia, and Saudi Arabia, have found khat chewing is associated with worse TB treatment outcomes [114, 119, 120].

The high prevalence of co-existing substance use and mental health disorders is also an important link between TB and substance use; mental health and substance use disorders often co-exist with each other and with TB [121, 122]. The co-occurring conditions are associated with delays in testing and care engagement and poor adherence to care [123]. Co-occurring mental health and substance use disorders may thus increase the chance of delayed TB diagnosis, prolonged TB transmission, and increased TB treatment failure and acquired drug resistance, consequently leading to greater morbidity, mortality, and community transmission [122].

6 Diagnosis of Tuberculosis Infection

The diagnosis of TB among PWUD is, in principle, the same as for other patient groups. The general challenges in TB control that relate to finding active cases and, where resources allow, to identifying latent TB infection apply to PWUD. However, there are often additional specific challenges to TB case finding among PWUD that relate to issues of stigmatization, marginalization, and criminalization and the overlapping conditions of poverty and unstable housing. PWUD are sometimes considered "hidden" or "difficult to reach" populations but are more accurately understood as historically, socially, and structurally excluded from traditional public health and clinical systems. Consequently, strategies are needed to meet populations of PWUD "where they are at" [124].

In addition to traditional healthcare and public health settings, some programs operating in "non-traditional" settings have been used successfully to expand the engagement of PWUD in TB services by approaching PWUD at sites they may already attend. These non-traditional TB settings have included sterile syringe service programs (SSPs), mobile vans, welfare or social service offices, drug treatment programs, and navigation and linkages services focused on persons released from correctional settings [125–127]. SSPs are valuable venues for TB screening of PWUD, and drug treatment programs are effective venues for delivering treatment for both latent and active TB [128]. For example, at a public methadone clinic in Dar es Salaam, Tanzania, in 2011, active TB case finding identified 11% with TB compatible symptoms and a 4% active TB prevalence (many folds above the national Tanzanian TB prevalence of 0.2%) [129].

There are important issues with regards to implementing the most effective and cost-effective modes of providing TB services to PWUD and when non-traditional settings are used regarding the best strategies to provide linkages from these novel settings to traditional TB care settings when needed. Low infrastructure non-traditional settings may not have the capacity for procedures such as chest radiography, but linkages to radiology facilities can be established, and adherence to linkage from these to higher infrastructure settings can, when needed, be enhanced by incentive strategies, which in some settings may be cost-saving from a societal perspective [128, 130]. Models for integrating TB care into drug treatment and HIV care settings have been developed and implemented [131–133]. Similarly, the US Centers for Disease Control (CDC), WHO, and UNODC have issued guidelines to enhance coordination of TB care among PWUD, with some calling for integrated (i.e., horizontal rather than vertical) care systems for PWUD [2, 132, 133].

There are specific issues relevant to TB contact tracing among populations of PWUD. Traditional TB case finding based on home, work, and school and based on named contacts may be incomplete in identifying either contacts of PWUD with TB or other contacts who are PWUD. PWUD may not have stable homes, may not have formal employment, may not be in school, and may not know their contacts by actual names. As TB is transmitted by prolonged exposure to air shared with a person with active pulmonary (or laryngeal) TB, relevant contacts include those who have been in shared spaces, and tracing contacts through the presence in such spaces has been called "context tracing" [4, 134]. Asking individuals to identify places where they spend time is valuable to traditional contact tracing, often revealing drug and alcohol consumption sites as relevant transmission sites [4, 134]. Strategies employed include traditional contact tracing, social network-based contact tracing, and context tracing. While both contact and context tracing have clear public health utility, they simultaneously pose potential privacy violations risks and discourage care-seeking among PWUD. Hence, they require careful further study and implementation with relevant privacy protections [135–137].

7 Treatment of Tuberculosis Infection

Pharmacotherapy for the treatment of both latent and active TB among PWUD is also, in general, the same as in other patients. However, as discussed for the diagnosis of TB, due to the stigmatization and criminalization of drug use, issues with promoting adherence of PWUD to long treatment courses due to competing individual considerations, and interruptions in care by transitions of PWUD into and out of criminal-legal settings, expanded implementation of TB services in non-tradition or other integrated care settings would be valuable [3, 126, 130, 138].

There are drug-drug interactions between opioids and rifamycins that are relevant to TB treatment practices among PWUD. Rifamycins are potent inducers of the cytochrome P450 enzyme systems that metabolize opioids [139–141]. Concomitant use of opioids and rifampin does not lead to lower rifampin concentrations

or lesser anti-tuberculosis treatment efficacy. However, rifampin can decrease methadone concentrations by 33–68%, and consequently, this interaction precipitates opioid withdrawal in patients on previously stable methadone doses to treat opioid use disorder. Rifabutin has a less potent effect on the catabolism of methadone, with rifapentine having an analogous but intermediate effect, suggesting attention to the possible need to increase methadone doses when either rifabutin or rifapentine are used with methadone [142, 143]. Similarly, rifampin can precipitate opioid withdrawal in a patient maintained on buprenorphine for opioid use disorder treatment, with rifabutin again having a less potent effect [144].

Further data are needed on the interaction of rifapentine and buprenorphine, but clinically monitoring for potential induction of withdrawal is warranted. These drug-drug interactions can contribute to poor adherence to TB treatment if unaddressed. However, these interactions can be anticipated, patients can be warned, and clinicians need to recognize that it is common to increase methadone doses to maintain efficacious opioid use disorder treatment and facilitate adherence to rifampin as TB treatment.

8 Potential Structural and Policy Responses to Address Tuberculosis Among People Who Use Drugs

The relationship between TB and drug use has evolved due to changes in social, environmental, and structural conditions and national and international policies regarding drug use, public health initiatives, and demographic changes, including immigration and urbanization [6, 38, 145]. TB has long been associated with transmission within populations in places characterized by poverty, marginalization, and crowding [146]. Studies of social networks and molecular epidemiology have demonstrated the importance of "place," and in particular of substance-using places, in TB transmission [134].

Despite the long duration during which drugs have been used in settings likely to facilitate TB transmission, the association between these settings and TB transmission should be understood as potentially modifiable. For example, wider implementation of structural interventions such as 'safer drug consumption facilities' could reshape environments, reducing risk and increasing health-promoting environments [147, 148]. Safer drug consumption facilities are structural interventions designed and utilized to reduce harms associated with drug use; such facilities could be further modified to reduce TB transmission in drug use settings and be more widely implemented to contribute to TB control [138, 149–154].

These considerations are related to the larger issues of reducing homelessness and crowded housing to their contribution to TB risk [122]. In addition, the expanded use of non-traditional settings, including SSPs and drug treatment programs, to engage PWUD in TB services and expanded implementation of integrated care settings for TB, HIV, and drug use disorder, could facilitate progress toward TB control.

Enhanced national and international data collection and surveillance of TB and drug use and their overlap, and greater coordination among international organizations monitoring and addressing either TB or drug use, could facilitate greater recognition of TB-drug use links and facilitate the expanded implementation of needed focused services. Expanded social network and context tracing into standard TB contact tracing initiatives would aid case detection and reduce transmission.

Policy interventions, such as drug decriminalization or legalization, could reduce the number of PWUD in congregate settings such as jails and prisons at risk for TB transmission. Interventions to improve the physical environments of jails and prisons could further reduce TB transmission both among PWUD and other prisoners. Expanded implementation of TB screening and latent and active TB treatment in jail and prisons could further reduce risk.

Greater recognition among policymakers that local, regional, and national TB public health funding remains essential to prevent TB resurgences and outbreaks, that responses to fiscal and economic crises which contemplate or implement cuts in TB services risk outbreaks, and that international loans which require cuts in TB services risk outbreaks and would be best avoided, and all are needed to ensure progress toward TB control.

9 Conclusion

TB and drug use have been integrally related for over a century. This relationship is driven by numerous factors that operate at the individual level (e.g., age, co-existing conditions, behaviors) and potently at the social and structural levels. The mutually reinforcing nature of multiple highly prevalent social and structural conditions (e.g., crowding, incarceration, poor housing, social and economic conditions) that disproportionally affect people who use drugs (PWUD) globally contributes to ongoing TB transmission and substantial TB morbidity and mortality among PWUD.

While such social and structural factors may lie beyond the scope of any individual clinician treating an individual patient, that these factors have evolved and developed over time and have been impacted for the better and the worse by events, laws, practices, and policies highlight that these social and structural forces are potentially modifiable by sound public health, political and economic policy and intervention. Greater recognition is needed that factors, such as the global growth of mega-cities, increases in poverty and economic inequality, increases in urban crowding and homelessness, the deterioration of existing housing stock, and increases in drug use in urban and other areas linked to big events are all inter-related multilevel forces that create risk for TB among PWUD (and others). Structural and policy interventions by localities, nations, regions, and international organizations are urgently needed to reduce TB incidence, prevalence, morbidity, and mortality among PWUD and generally contribute to enhanced TB control.

The historical and current convergence of tuberculosis and (illicit) drug use demonstrate that tuberculosis occurs in socially constructed contexts and that achieving TB control and the goal of TB elimination, will require addressing and changing global systems, policies and structures, and indeed, improving the world we live in.

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Core Messages

- Processes, policies, and structures operating overtime at multiple levels produce risk for TB among PWUD.
- Factors such as poverty, inequality, urban crowding, and homelessness are inter-related forces that create TB risk.
- The association between contexts and TB transmission should be understood as potentially modifiable.
- Policy interventions (e.g., drug decriminalization, improving prison environments) could reduce TB transmission.
- Expanded implementation of TB services in prisons and in places PWUD receive services could reduce the TB burden.

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References

1. Farmer PE (2000) The consumption of the poor: tuberculosis in the 21st century. Ethnography 1(2):183–216
2. World Health Organization (2019) Global tuberculosis report 2019, Geneva
3. Deiss RG, Rodwell TC, Garfein RS (2009) Tuberculosis and illicit drug use: review and update. Clin Infect Dis 48(1):72–82. https://doi.org/10.1086/594126
4. Perlman DC, Salomon N, Perkins MP, Yancovitz S, Paone D, Des Jarlais DC (1995) Tuberculosis in drug users. Clin Infect Dis 21(5):1253–1264
5. UNODC (1953) https://www.unodc.org/unodc/en/data-and-analysis/bulletin/bulletin_1953-01-01_2_page004.html.
6. Booth M (2013) Opium: a history. St. Martin's Griffin
7. Getahun H, Baddeley A, Raviglione M (2013) Managing tuberculosis in people who use and inject illicit drugs. Bull World Health Organ 91:154–156
8. Altice FL, Azbel L, Stone J, Brooks-Pollock E, Smyrnov P, Dvoriak S, Taxman FS, El-Bassel N, Martin NK, Booth R (2016) The perfect storm: incarceration and the high-risk environment perpetuating transmission of HIV, hepatitis C virus, and tuberculosis in Eastern Europe and Central Asia. The Lancet 388(10050):1228–1248

9. Degenhardt L, Peacock A, Colledge S, Leung J, Grebely J, Vickerman P, Stone J, Cunningham EB, Trickey A, Dumchev K (2017) Global prevalence of injecting drug use and sociodemographic characteristics and prevalence of HIV, HBV, and HCV in people who inject drugs: a multistage systematic review. Lancet Glob Health 5(12):e1192–e1207
10. Kyu HH, Maddison ER, Henry NJ, Mumford JE, Barber R, Shields C, Brown JC, Nguyen G, Carter A, Wolock TM (2018) The global burden of tuberculosis: results from the global burden of disease study 2015. Lancet Infect Dis 18(3):261–284
11. Prüss-Üstün A, Wolf J, Corvalán C, Bos R, Neira M (2016) Preventing disease through healthy environments: a global assessment of the burden of disease from environmental risks. World Health Organization
12. Imtiaz S, Shield KD, Roerecke M, Samokhvalov AV, Lönnroth K, Rehm J (2017) Alcohol consumption as a risk factor for tuberculosis: meta-analyses and burden of disease. Eur Respir J 50(1)
13. Lönnroth K, Williams BG, Stadlin S, Jaramillo E, Dye C (2008) Alcohol use as a risk factor for tuberculosis—a systematic review. BMC Public Health 8:289. https://doi.org/10.1186/1471-2458-8-289
14. Rehm J, Samokhvalov AV, Neuman MG, Room R, Parry C, Lönnroth K, Patra J, Poznyak V, Popova S (2009) The association between alcohol use, alcohol use disorders and tuberculosis (TB). A systematic review. BMC Public Health 9(1):450. https://doi.org/10.1186/1471-2458-9-450
15. Simou E, Britton J, Leonardi-Bee J (2018) Alcohol consumption and risk of tuberculosis: a systematic review and meta-analysis. Int J Tuberc Lung Dis 22(11):1277–1285
16. WHO, World Bank dataset incidence of tuberculosis (per 100,000 people). Global tuberculosis report. World Health Organization
17. Mabud TS, de Lourdes Delgado Alves M, Ko AI, Basu S, Walter KS, Cohen T, Mathema B, Colijn C, Lemos E, Croda J (2019) Evaluating strategies for control of tuberculosis in prisons and prevention of spillover into communities: an observational and modeling study from Brazil. PLoS Med 16(1):e1002737
18. Pescarini JM, Rodrigues LC, Gomes MGM, Waldman EA (2017) Migration to middle-income countries and tuberculosis-global policies for global economies. Glob Health 13(1):15–15. https://doi.org/10.1186/s12992-017-0236-6
19. Huddart S, MacLean E, Pai M (2016) Location, location, location: tuberculosis services in highest burden countries. Lancet Glob Health 4(12):e907–e908. https://doi.org/10.1016/s2214-109x(16)30248-0
20. Mor Z, Migliori GB, Althomsons SP, Loddenkemper R, Trnka L, Iademarco MF (2008) Comparison of tuberculosis surveillance systems in low-incidence industrialised countries. Eur Respir J 32(6):1616–1624
21. van der Heijden Y, Hughes J, Dowdy D, Streicher E, Chihota V, Jacobson K, Warren R, Theron G (2019) Overcoming limitations of tuberculosis information systems: researcher and clinician perspectives. Public Health Action 9(3):120–127
22. Centis R, D'Ambrosio L, Zumla A, Migliori GB (2017) Shifting from tuberculosis control to elimination: where are we? What are the variables and limitations? Is it achievable? Int J Infect Dis 56:30–33
23. Degenhardt L, Hall W (2012) Extent of illicit drug use and dependence, and their contribution to the global burden of disease. Lancet 379(9810):55–70
24. Feleke BE, Feleke TE, Biadglegne F (2019) Nutritional status of tuberculosis patients, a comparative cross-sectional study. BMC Pulm Med 19(1):182
25. World Health Organization (2019) WHO consolidated guidelines on drug-resistant tuberculosis treatment, vol WHO/CDS/TB/2019.7. World Health Organization
26. Pouget ER, Kershaw TS, Niccolai LM, Ickovics JR, Blankenship KM (2010) Associations of sex ratios and male incarceration rates with multiple opposite-sex partners: potential social determinants of HIV/STI transmission. Public Health Rep 125(Suppl 4):70

27. World Drug Report (2020) (United Nations publication, Sales No. E.20.XI.6). Available at: https://wdr.unodc.org/wdr2020/en/index2020.html
28. Technical Brief: Tuberculosis, Gender, and Human Rights (2017) The Global Fund. Geneva, Switzerland. Available at: https://www.theglobalfund.org/media/6349/core_tbhumanrightsgenderequality_technicalbrief_en.pdf
29. Millet J-P, Moreno A, Fina L, del Baño L, Orcau A, de Olalla PG, Caylà JA (2013) Factors that influence current tuberculosis epidemiology. Eur Spine J 22(Suppl 4):539–548. http://doi.org/10.1007/s00586-012-2334-8
30. Brites D, Gagneux S (2015) Co-evolution of Mycobacterium tuberculosis and Homo sapiens. Immunol Rev 264(1):6–24. https://doi.org/10.1111/imr.12264
31. Hershberg R, Lipatov M, Small PM, Sheffer H, Niemann S, Homolka S, Roach JC, Kremer K, Petrov DA, Feldman MW, Gagneux S (2008) High functional diversity in Mycobacterium tuberculosis driven by genetic drift and human demography. PLoS Biol 6 (12):e311. https://doi.org/10.1371/journal.pbio.0060311
32. Gutierrez MC, Brisse S, Brosch R, Fabre M, Omaïs B, Marmiesse M, Supply P, Vincent V (2005) Ancient origin and gene mosaicism of the progenitor of Mycobacterium tuberculosis. PLoS Pathog 1(1):e5
33. Kapur V, Whittam TS, Musser JM (1994) Is Mycobacterium tuberculosis 15,000 years old? J Infect Dis 170(5):1348–1349
34. Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, Eiglmeier K, Garnier T, Gutierrez C, Hewinson G, Kremer K (2002) A new evolutionary scenario for the Mycobacterium tuberculosis complex. Proc Natl Acad Sci 99(6):3684–3689
35. Zimmerman MR (1979) Pulmonary and osseous tuberculosis in an Egyptian mummy. Bull N Y Acad Med 55(6):604
36. Barberis I, Bragazzi NL, Galluzzo L, Martini M (2017) The history of tuberculosis: from the first historical records to the isolation of Koch's bacillus. J Prev Med Hyg 58(1):e9–e12
37. Gagneux S (2012) Host-pathogen coevolution in human tuberculosis. Philos Trans R Soc Lond B Biol Sci 367(1590):850–859. https://doi.org/10.1098/rstb.2011.0316
38. Courtwright DT, Courtwright DT (2009) Forces of habit: drugs and the making of the modern world. Harvard University Press, Cambridge
39. Des Jarlais DC (1995) Harm reduction—a framework for incorporating science into drug policy. Am J Public Health 85(1):10–12
40. Singer M (2007) Drugging the poor: legal and illegal drugs and social inequality. Waveland Press
41. Paley D (2014) Drug war capitalism. AK Press, Oakland CA
42. O'Neill MB, Shockey A, Zarley A, Aylward W, Eldholm V, Kitchen A, Pepperell CS (2019) Lineage specific histories of Mycobacterium tuberculosis dispersal in Africa and Eurasia. Mol Ecol 28(13):3241–3256
43. Guerra-Doce E (2015) Psychoactive substances in prehistoric times: examining the archaeological evidence. Time and Mind 8(1):91–112
44. Infections in intravenous drug abusers (1991). Oxford University Press, New York. Accessed from http://nla.gov.au/nla.cat-vn276537
45. Lange WR, Ball JC, Pfeiffer MB, Snyder FR, Cone EJ (1989) The Lexington addicts, 1971–1972: demographic characteristics, drug use patterns, and selected infectious disease experience. Int J Addict 24(7):609–626
46. Dubos RJ, Dubos J (1952) The white plague; tuberculosis, man and society. Little, Brown, Boston
47. Bewley-Taylor D. Fifty years of the 1961 single convention on narcotic drugs: a reinterpretation
48. Bewley-Taylor D, Jelsma M (2012) Regime change: re-visiting the 1961 single convention on narcotic drugs. Int J Drug Policy 23(1):72–81. https://doi.org/10.1016/j.drugpo.2011.08.003

49. McAllister WB (2004) The global political economy of scheduling: the international-historical context of the Controlled Substances Act. Drug Alcohol Depend 76(1):3–8. https://doi.org/10.1016/j.drugalcdep.2004.02.012
50. Austin KF, DeScisciolo C, Samuelsen L (2016) The failures of privatization: a comparative investigation of tuberculosis rates and the structure of healthcare in less-developed nations, 1995–2010. World Dev 78:450–460
51. Maynard G, Shircliff EJ, Restivo M (2012) IMF structural adjustment, public health spending, and tuberculosis: a longitudinal analysis of prevalence rates in poor countries. Int J Sociol 42(2):5–27
52. Murray JF, Schraufnagel DE, Hopewell PC (2015) Treatment of tuberculosis. A historical perspective. Ann Am Thorac Soc 12(12):1749–1759
53. Martini M, Gazzaniga V, Behzadifar M, Bragazzi NL, Barberis I (2018) The history of tuberculosis: the social role of sanatoria for the treatment of tuberculosis in Italy between the end of the 19(th) century and the middle of the 20(th). J Prev Med Hyg 59(4):E323–E327. https://doi.org/10.15167/2421-4248/jpmh2018.59.4.1103
54. Ott K (1996) Fevered lives: tuberculosis in American culture since 1870. Harvard University Press
55. Rotham SM (1995) Living in the shadow of death: tuberculosis and the social experience of illness in American history. Johns Hopkins U Press, Baltimore, Maryland, USA
56. Drucker E (2011) A plague of prisons: the epidemiology of mass incarceration in America. New Press
57. Courtwright D, Joseph H, Des Jarlais D (1989) Addicts who survived. University of Tennessee Press, Knoxville
58. Daley CL, Hahn JA, Moss AR, Hopewell PC, Schecter GF (1998) Incidence of tuberculosis in injection drug users in San Francisco: impact of anergy. Am J Respir Crit Care Med 157 (1):19–22
59. Brudney K, Dobkin J (1991) Resurgent tuberculosis in New York City. Human immunodeficiency virus, homelessness, and the decline of tuberculosis control programs. Am Rev Respir Dis 144(4):745–749. http://doi.org/10.1164/ajrccm/144.4.745
60. Podlekareva DN (2009) Mortality from HIV and TB coinfections is higher in Eastern Europe than in Western Europe and Argentina. AIDS 23(18):2485–2495. https://doi.org/10.1097/QAD.0b013e3283326879
61. Post FA, Grint D, Werlinrud AM, Panteleev A, Riekstina V, Malashenkov EA, Skrahina A, Duiculescu D, Podlekareva D, Karpov I, Bondarenko V, Chentsova N, Lundgren J, Mocroft A, Kirk O, Miro JM (2014) Multi-drug-resistant tuberculosis in HIV positive patients in Eastern Europe. J Infect 68(3):259–263. https://doi.org/10.1016/j.jinf.2013.09.034
62. Soodla P, Rajasaar H, Avi R, Zilmer K, Kink K, Novikova L, Huik K, Maimets M, Lutsar I (2015) Design and structure of the Estonian HIV cohort study (E-HIV). Infect Dis (London, England) 47(11):768–775. https://doi.org/10.3109/23744235.2015.1061203
63. Stuckler D, King LP, Basu S (2008) International monetary fund programs and tuberculosis outcomes in post-communist countries. PLoS Med 5(7):e143. https://doi.org/10.1371/journal.pmed.0050143
64. Nikolopoulos GK, Sypsa V, Bonovas S, Paraskevis D, Malliori-Minerva M, Hatzakis A, Friedman SR (2015) Big events in Greece and HIV infection among people who inject drugs. Subst Use Misuse 50(7):825–838. https://doi.org/10.3109/10826084.2015.978659
65. Friedman SR, Rossi D, Braine N (2009) Theorizing "big events" as a potential risk environment for drug use, drug-related harm and HIV epidemic outbreaks. Int J Drug Policy 20(3):283–291. https://doi.org/10.1016/j.drugpo.2008.10.006
66. Friedman SR, Pouget ER, Sandoval M, Nikolopoulos GK, Mateu-Gelabert P, Rossi D, Auerbach JD (2020) New measures for research on men who have sex with men and for at-risk heterosexuals: tools to study links between structural interventions or large-scale social change and HIV risk behaviors, service use, and infection. AIDS Behav 24(1):257–273. https://doi.org/10.1007/s10461-019-02582-w

67. O'Riordan M, Fitzpatrick F (2015) The impact of economic recession on infection prevention and control. J Hosp Infect 89(4):340–345. https://doi.org/10.1016/j.jhin.2014.11.020
68. Choi H, Chung H, Muntaner C (2019) Social selection in historical time: the case of tuberculosis in South Korea after the East Asian financial crisis. PLoS One 14(5):e0217055
69. Suk JE, Manissero D, Büscher G, Semenza JC (2009) Wealth inequality and tuberculosis elimination in Europe. Emerg Infect Dis 15(11):1812–1814. https://doi.org/10.3201/eid1511.090916
70. Coker R (1998) Lessons from New York's tuberculosis epidemic. Tuberculosis is a political as much as a medical problem-and so are the solutions. BMJ 317(7159):616. http://doi.org/10.1136/bmj.317.7159.616
71. Casal B, Rivera B, Currais L (2020) Economic crisis, unemployment and illegal drug consumption in Spain. Appl Econ Anal 28(83):153–170
72. American Thoracic Society, Centers for Disease Control and Prevention, and Infectious Diseases Society of America (2005) American thoracic society/centers for disease control and prevention/infectious diseases society of America: controlling tuberculosis in the United States. Am J Respir Crit Care Med 172(9):1169–1227
73. Barr RG, Diez-Roux AV, Knirsch CA, Pablos-Méndez A (2001) Neighborhood poverty and the resurgence of tuberculosis in New York City, 1984–1992. Am J Public Health 91(9):1487–1493
74. Lagerspetz M, Moskalewicz J (2002) Drugs in the postsocialist transitions of Estonia, Latvia, Lithuania and Poland. Eur Addict Res 8(4):177–183
75. Lauristin M, Pettai V (2011) Estonian human development report 2010/2011. Baltic way(s) of human development: twenty years on AS Eesti Ajalehed. Available at: https://hdr.undp.org/system/files/documents//2011nhdrestoniapdf.pdf
76. Staehr K (2004) Economic transition in Estonia. Background, reforms and results. In: Contemporary change in Estonia, pp 37–67
77. Uuskula A, Plank T, Lassus A, Bingham JS (2001) Sexually transmitted infections in Estonia—syndromic management of urethritis in a European country? Int J STD AIDS 12(8):493–498
78. Uuskula A, Silm H, Vessin T (1997) Sexually transmitted diseases in Estonia: past and present. Int J STD AIDS 8(7):446–450
79. Glewwe P, Hall G (1994) Poverty, inequality, and living standards during unorthodox adjustment: the case of Peru, 1985–1990. Econ Dev Cult Change 42(4):689–717
80. Khang Y-H, Lynch JW, Kaplan GA (2005) Impact of economic crisis on cause-specific mortality in South Korea. Int J Epidemiol 34(6):1291–1301. https://doi.org/10.1093/ije/dyi224
81. Kondilis E, Giannakopoulos S, Gavana M, Ierodiakonou I, Waitzkin H, Benos A (2013) Economic crisis, restrictive policies, and the population's health and health care: the Greek case. Am J Public Health 103(6):973–979. https://doi.org/10.2105/AJPH.2012.301126
82. Page KR, Doocy S, Reyna Ganteaume F, Castro JS, Spiegel P, Beyrer C (2019) Venezuela's public health crisis: a regional emergency. The Lancet 393(10177):1254–1260. https://doi.org/10.1016/s0140-6736(19)30344-7
83. Suárez JA, Carreño L, Paniz-Mondolfi AE, Marco-Canosa FJ, Freilij H, Riera JA, Risquez A, Rodriguez-Morales AG, Hernández-Rincón E, Alvarado-Socarras J (2019) Infectious diseases, social, economic and political crises, anthropogenic disasters and beyond: Venezuela 2019—implications for public health and travel medicine. Rev Panam Enferm Infecc 1(2):73–93
84. Espinosa L, Mirinaviciute G (2019) Health crisis in Venezuela: status of communicable diseases and implications for the European Union and European Economic Area, May 2019. Eurosurveillance 24(22):1900308
85. Panamerican Health Organization (2018) UNAIDS GBdV Plan maestro para el fortalecimiento de la respuesta al VIH, la tuberculosis y la malaria en la República Bolivariana de Venezuela desde una perspectiva de salud pública. Available at: https://www.paho.org/disasters/dmdocuments/Plan%20Maestro_VIH_TB%20MAL%202018%20VEN.PDF

86. InSight (2020) Coronavirus could worsen health crisis in Venezuela prisons
87. Des Jarlais DC, Sypsa V, Feelemyer J, Abagiu AO, Arendt V, Broz D, Chemtob D, Seguin-Devaux C, Duwve JM, Fitzgerald M, Goldberg DJ, Hatzakis A, Jipa RE, Katchman E, Keenan E, Khan I, Konrad S, McAuley A, Skinner S, Wiessing L (2020) HIV outbreaks among people who inject drugs in Europe, North America, and Israel. Lancet HIV 7(6):e434–e442. https://doi.org/10.1016/s2352-3018(20)30082-5
88. Proyecto: Monitoreo a la situacion de los centros de detencion preventiva en Venezuals (2019)
89. Oeltmann JE, Kammerer JS, Pevzner ES, Moonan PK (2009) Tuberculosis and substance abuse in the United States, 1997–2006. Arch Intern Med 169(2):189–197. https://doi.org/10.1001/archinternmed.2008.535
90. McElroy P, Rothenberg R, Varghese R, Woodruff R, Minns G, Muth S, Lambert L, Ridzon R (2003) A network-informed approach to investigating a tuberculosis outbreak: implications for enhancing contact investigations. Int J Tuberc Lung Dis 7(12):S486–S493
91. Fitzpatrick LK, Hardacker JA, Heirendt W, Agerton T, Streicher A, Melnyk H, Ridzon R, Valway S, Onorato I (2001) A preventable outbreak of tuberculosis investigated through an intricate social network. Clin Infect Dis 33(11):1801–1806
92. Nguipdop-Djomo P, Rodrigues LC, Smith PG, Abubakar I, Mangtani P (2020) Drug misuse, tobacco smoking, alcohol and other social determinants of tuberculosis in UK-born adults in England: a community-based case-control study. Sci Rep 10(1):5639. https://doi.org/10.1038/s41598-020-62667-8
93. Spence DP, Hotchkiss J, Williams CS, Davies PD (1993) Tuberculosis and poverty. BMJ 307(6907):759–761
94. Davis M (2006) Planet of slums. Verso
95. Banu S, Rahman MT, Uddin MKM, Khatun R, Ahmed T, Rahman MM, Husain MA, van Leth F (2013) Epidemiology of tuberculosis in an urban slum of Dhaka City, Bangladesh. PLoS One 8(10):e77721
96. Noykhovich E, Mookherji S, Roess A (2019) The risk of tuberculosis among populations living in slum settings: a systematic review and meta-analysis. J Urban Health 96(2):262–275. https://doi.org/10.1007/s11524-018-0319-6
97. Ghulam R, Verma K, Sharma P, Razdan M, Razdan RA (2016) Drug abuse in slum population. Indian J Psychiatry 58(1):83–86. https://doi.org/10.4103/0019-5545.174390
98. Swahn M, Haberlen M, Palmier JB (2014) Alcohol and drug use and other high-risk behaviors among youth in the slums of Kampala, Uganda: perceptions and contexts obtained through focus groups. Int J Alcohol Drug Res 3(4):289–295
99. Faiza B, Mehmood H, Naz S, Naz S (2019) Prevalence and determinants of substance abuse among slum dwellers in Islamabad-Pakistan. Int J Transl Med Res Public Health 3(2):107–113
100. Fazel S, Yoon IA, Hayes AJ (2017) Substance use disorders in prisoners: an updated systematic review and meta-regression analysis in recently incarcerated men and women. Addiction 112(10):1725–1739. https://doi.org/10.1111/add.13877
101. Mundt AP, Baranyi G, Gabrysch C, Fazel S (2018) Substance use during imprisonment in low-and middle-income countries. Epidemiol Rev 40(1):70–81
102. Bellin EY, Fletcher DD, Safyer SM (1993) Association of tuberculosis infection with increased time in or admission to the New York City jail system. JAMA 269(17):2228–2231
103. Guerra J, Mogollón D, González D, Sanchez R, Rueda ZV, Parra-López CA, Murcia MI (2019) Active and latent tuberculosis among inmates in La Esperanza prison in Guaduas, Colombia. PLoS One 14(1):e0209895. https://doi.org/10.1371/journal.pone.0209895
104. Rich JD, Beckwith CG, Macmadu A, Marshall BD, Brinkley-Rubinstein L, Amon JJ, Milloy M, King MR, Sanchez J, Atwoli L (2016) Clinical care of incarcerated people with HIV, viral hepatitis, or tuberculosis. The Lancet 388(10049):1103–1114
105. Dara M, Acosta CD, Melchers NVV, Al-Darraji HA, Chorgoliani D, Reyes H, Centis R, Sotgiu G, D'Ambrosio L, Chadha SS (2015) Tuberculosis control in prisons: current situation and research gaps. Int J Infect Dis 32:111–117

106. Baussano I, Williams BG, Nunn P, Beggiato M, Fedeli U, Scano F (2010) Tuberculosis incidence in prisons: a systematic review. PLoS Med 7(12):e1000381
107. Dolan K, Wirtz AL, Moazen B, Ndeffo-Mbah M, Galvani A, Kinner SA, Courtney R, McKee M, Amon JJ, Maher L (2016) Global burden of HIV, viral hepatitis, and tuberculosis in prisoners and detainees. The Lancet 388(10049):1089–1102
108. French CE, Coope CM, McGuinness LA, Beck CR, Newitt S, Ahyow L, Hickman M, Oliver I (2019) Cannabis use and the risk of tuberculosis: a systematic review. BMC Public Health 19(1):1006–1006. https://doi.org/10.1186/s12889-019-7127-0
109. Joshi M, Joshi A, Bartter T (2014) Marijuana and lung diseases. Curr Opin Pulm Med 20 (2):173–179. https://doi.org/10.1097/mcp.0000000000000026
110. Story A, Bothamley G, Hayward A (2008) Crack cocaine and infectious tuberculosis. Emerg Infect Dis 14(9):1466–1469. https://doi.org/10.3201/eid1409.070654
111. Oeltmann JE, Oren E, Haddad MB, Lake Lk, Harrington TA, Ijaz K, Narita M (2006) Tuberculosis outbreak in marijuana users, Seattle, Washington, 2004. Emerg Infect Dis 12 (7):1156–1159. http://doi.org/10.3201/eid1207.051436
112. Munckhof W, Konstantinos A, Wamsley M, Mortlock M, Gilpin C (2003) A cluster of tuberculosis associated with use of a marijuana water pipe. Int J Tuberc Lung Dis 7(9):860–865
113. Perlman DC, Perkins MP, Paone D, Kochems L, Salomon N, Friedmann P, Des Jarlais DC (1997) "Shotgunning" as an illicit drug smoking practice. J Subst Abuse Treat 14(1):3–9. https://doi.org/10.1016/s0740-5472(96)00182-1
114. Alvi A, Fatima N, Jerah AA, Rizwan M, Hobani YH, Al Sunosi R, Taha MMEH, Habiballah EM, Agarwal PK, Abdulwahab SI (2015) Correlation between resistin, tuberculosis and khat addiction: a study from south western province of Saudi Arabia. PLoS One 10(10):e0140245
115. NIDA. https://www.drugabuse.gov/drug-topics/commonly-used-drugs-charts#khat
116. Humanitarian TN (09/23/2010) Khat-chewing "contributes to rise in Burao TB patients"
117. Alemu YM, Awoke W, Wilder-Smith A (2016) Determinants for tuberculosis in HIV-infected adults in Northwest Ethiopia: a multicentre case-control study. BMJ Open 6 (4):e009058. https://doi.org/10.1136/bmjopen-2015-009058
118. Duko B, Bedaso A, Ayano G, Yohannis Z (2019) Perceived stigma and associated factors among patient with tuberculosis, Wolaita Sodo, Ethiopia: cross-sectional study. Tuberc Res Treat 2019:5917537. https://doi.org/10.1155/2019/5917537
119. Jaber AAS, Khan AH, Sulaiman SAS (2017) Evaluating treatment outcomes and durations among cases of smear-positive pulmonary tuberculosis in Yemen: a prospective follow-up study. J Pharm Policy Pract 10(1):36
120. Soboka M, Tolessa O, Tesfaye M, Adorjan K, Krahl W, Tesfaye E, Yitayih Y, Strobl R, Grill E (2020) Magnitude and predictors of khat use among patients with tuberculosis in Southwest Ethiopia: a longitudinal study. PLoS One 15(7):e0236154
121. Sweetland AC, Galea J, Shin SS, Driver C, Dlodlo RA, Karpati A, Wainberg ML (2019) Integrating tuberculosis and mental health services: global receptivity of national tuberculosis program directors. Int J Tuberc Lung Dis 23(5):600–605. https://doi.org/10.5588/ijtld.18.0530
122. Noppert GA, Malosh RE, Moran EB, Ahuja SD, Zelner J (2018) Contemporary social disparities in TB infection and disease in the USA: a review. Curr Epidemiol Rep 5(4):442–449
123. Storla DG, Yimer S, Bjune GA (2008) A systematic review of delay in the diagnosis and treatment of tuberculosis. BMC Public Health 8:15. https://doi.org/10.1186/1471-2458-8-15
124. Woolhouse S, Brown JB, Thind A (2011) 'Meeting people where they're at': experiences of family physicians engaging women who use illicit drugs. Ann Fam Med 9(3):244–249. https://doi.org/10.1370/afm.1225

125. Salomon N, Perlman DC, Friedmann P, Ziluck V, Des Jarlais DC (2000) Prevalence and risk factors for positive tuberculin skin tests among active drug users at a syringe exchange program. Int J Tuberc Lung Dis 4(1):47–54
126. Perlman DC, Perkins MP, Solomon N, Kochems L, Des Jarlais DC, Paone D (1997) Tuberculosis screening at a syringe exchange program. Am J Public Health 87(5):862–863
127. Binepal G, Agarwal P, Kaur N, Singh B, Bhagat V, Verma RP, Satyanarayana S, Oeltmann JE, Moonan PK (2015) Screening difficult-to-reach populations for tuberculosis using a mobile medical unit, Punjab India. Public Health Action 5(4):241–245. https://doi.org/10.5588/pha.15.0042
128. Paone D, Perlman DC, Perkins MP, Kochems LM, Salomon N, Des Jarlais DC (1998) Organizational issues in conducting tuberculosis screening at a syringe exchange program. J Subst Abuse Treat 15(3):229–234
129. Gupta A, Mbwambo J, Mteza I, Shenoi S, Lambdin B, Nyandindi C, Doula BI, Mfaume S, Bruce RD (2014) Active case finding for tuberculosis among people who inject drugs on methadone treatment in Dar es Salaam, Tanzania. Int J Tuberc Lung Dis 18(7):793–798. https://doi.org/10.5588/ijtld.13.0208
130. Perlman DC, Gourevitch MN, Trinh C, Salomon N, Horn L, Des Jarlais DC (2001) Cost-effectiveness of tuberculosis screening and observed preventive therapy for active drug injectors at a syringe-exchange program. J Urban Health 78(3):550–567. https://doi.org/10.1093/jurban/78.3.550
131. Bruce RD, Lambdin B, Chang O, Masao F, Mbwambo J, Mteza I, Nyandindi C, Zamudio-Haas S, Buma D, Dunbar MS, Kilonzo G (2014) Lessons from Tanzania on the integration of HIV and tuberculosis treatments into methadone assisted treatment. Int J Drug Policy 25(1):22–25. https://doi.org/10.1016/j.drugpo.2013.09.005
132. WHO (2008) Policy guidelines for collaborative TB and HIV services for injecting and other drug users—an integrated approach. Available at: http://whqlibdoc.who.int/publications/2008/9789241596930_eng.pdf
133. Integrated prevention services for HIV infection, viral hepatitis, sexually transmitted diseases, and tuberculosis for persons who use drugs illicitly: summary guidance from CDC and the U.S. Department of Health and Human Services (2012). MMWR Recomm Rep 61 (Rr-5):1–40
134. Klovdahl AS, Graviss EA, Yaganehdoost A, Ross M, Wanger A, Adams G, Musser JM (2001) Networks and tuberculosis: an undetected community outbreak involving public places. Soc Sci Med 52(5):681–694
135. Wilce M, Shrestha-Kuwahara R, Taylor Z, Qualls N, Marks S (2002) Tuberculosis contact investigation policies, practices, and challenges in 11 US communities. J Public Health Manag Pract JPHMP 8(6):69
136. National Tuberculosis Controllers Association, Centers for Disease Control and Prevention (2005) Recommendations from the National Tuberculosis Controllers Association and CDC. Guidelines for the investigation of contacts of persons with infectious tuberculosis. MMWR Recomm Rep 54:1–47
137. Jung G, Lee H, Kim A, Lee U (2020) Too much information: assessing privacy risks of contact trace data disclosure on people with COVID-19 in South Korea. Front Public Health 8
138. Jozaghi E, Reid AA, Andresen MA, Juneau A (2014) A cost-benefit/cost-effectiveness analysis of proposed supervised injection facilities in Ottawa, Canada. Subst Abuse Treat Prev Policy 9:31. https://doi.org/10.1186/1747-597X-9-31
139. Weschules DJ, Bain KT, Richeimer S (2008) Actual and potential drug interactions associated with methadone. Pain Med 9(3):315–344
140. McCance-Katz EF, Sullivan LE, Nallani S (2010) Drug interactions of clinical importance among the opioids, methadone and buprenorphine, and other frequently prescribed medications: a review. Am J Addict 19(1):4–16

141. Kreek MJ, Garfield JW, Gutjahr CL, Giusti LM (1976) Rifampin-induced methadone withdrawal. N Engl J Med 294(20):1104–1106
142. Borisov AS, Morris SB, Njie GJ, Winston CA, Burton D, Goldberg S, Woodruff RY, Allen L, LoBue P, Vernon A (2018) Update of recommendations for use of once-weekly isoniazid-rifapentine regimen to treat latent Mycobacterium tuberculosis infection. Morb Mortal Wkly Rep 67(25):723
143. Brown LS, Sawyer RC, Li R, Cobb MN, Colborn DC, Narang P (1996) Lack of a pharmacologic interaction between rifabutin and methadone in HIV-infected former injecting drug users. Drug Alcohol Depend 43(1–2):71–77
144. McCance-Katz EF, Moody DE, Prathikanti S, Friedland G, Rainey PM (2011) Rifampin, but not rifabutin, may produce opiate withdrawal in buprenorphine-maintained patients. Drug Alcohol Depend 118(2–3):326–334
145. Havens JR, Oser CB, Knudsen HK, Lofwall M, Stoops WW, Walsh SL, Leukefeld CG, Kral AH (2011) Individual and network factors associated with non-fatal overdose among rural Appalachian drug users. Drug Alcohol Depend 115(1–2):107–112. https://doi.org/10.1016/j.drugalcdep.2010.11.003
146. Farmer P (1999) Infections and inequalities: the modern plagues. University of California Press, Berkley, California
147. McGowan C, Viens A, Harris M, Rhodes T (2017) Risk environments and the ethics of reducing drug-related harms. Am J Bioeth 17(12):46–48
148. Blankenship KM, Friedman SR, Dworkin S, Mantell JE (2006) Structural interventions: concepts, challenges and opportunities for research. J Urban Health 83(1):59–72. https://doi.org/10.1007/s11524-005-9007-4[doi]
149. Harvard SSM, Hill WDP, Buxton JAMMF (2008) Harm reduction product distribution in British Columbia. Can J Public Health 99(6):446–450
150. Jozaghi E, Andresen MMA (2013) Should North America's first and only supervised injection facility (InSite) be expanded in British Columbia, Canada? Harm Reduc J 10(1). http://doi.org/10.1186/1477-7517-10-1
151. Small W, Moore D, Shoveller J, Wood E, Kerr T (2012) Perceptions of risk and safety within injection settings: injection drug users' reasons for attending a supervised injecting facility in Vancouver, Canada. Health Risk Soc 14(4):307–324. https://doi.org/10.1080/13698575.2012.680950
152. Wood E, Kerr T, Lloyd-Smith E, Buchner C, Marsh DC, Montaner JSG, Tyndall MW (2004) Methodology for evaluating Insite: Canada's first medically supervised safer injection facility for injection drug users. Harm Reduc J 1(9). http://doi.org/10.1186/1477-7517-1-9
153. Greenwald G (2009) Drug decriminalization in Portugal: lessons for creating fair and successful drug policies. Cato Institute whitepaper series
154. Wolfe D, Luhmann N, Harris M, Momenghalibaf A, Albers E, Byrne J, Swan T (2015) Human rights and access to hepatitis C treatment for people who inject drugs. Int J Drug Policy 26(11):1072–1080. https://doi.org/10.1016/j.drugpo.2015.05.007

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Anti-tumor Necrosis Factor-α Antagonists and Tuberculosis

32

Rachel K. Lim, Dina A. Fisher, and Stephen K. Field

Medicine is a science of uncertainty and an art of probability.

William Osler

Summary

Tumor necrosis factor-α inhibitors (TNFi) represent an important advance in the treatment of numerous autoimmune conditions. There are important differences between the five commercially available TNFis that will be discussed in this chapter. Immune suppression associated with their use increases the risk of a variety of infections, including tuberculosis (TB). In order to prevent incident TB, it is mandatory that people be screened and initiated on chemoprophylaxis for latent TB infection (LTBI) prior to initiating treatment with TNFi. If patients develop active TB while on treatment with a TNFi, it should be stopped, and TB treatment should be started immediately. Surveillance for TB should continue during and after treatment with TNFis because of the ongoing risk of disease.

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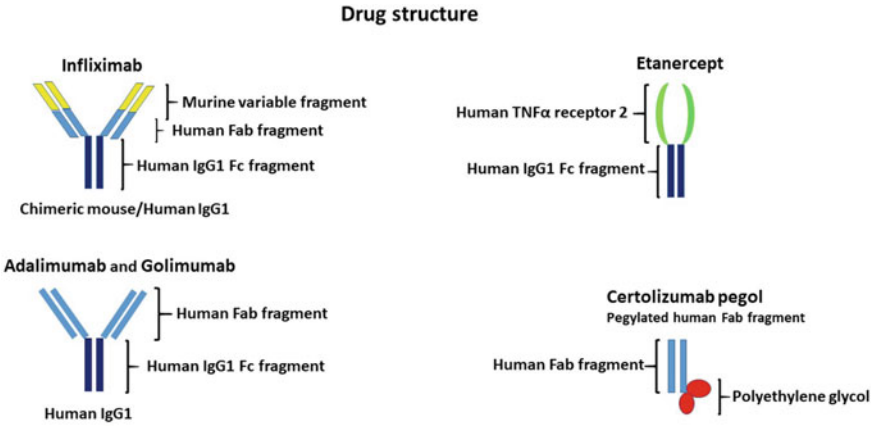
Graphical Abstract



Structures of the anti-tumor necrosis factor-α (TNFα) inhibitors. Fab, fragment antigen-binding region; Fc-fragment crystallizable region; IgG1, Immunoglobulin G1. This diagram is originally made by the authors by inspiration from [1, Fig. 1]

Keywords

Immune suppression · Interferon-γ · Tuberculosis · Tumor necrosis factor-α

1 Introduction

Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine that is critical for an effective immune response to *Mycobacterium tuberculosis (M. tb)*. TNF-α also contributes to the pathogenesis of many autoimmune diseases. Consequently, TNF-α inhibitors (TNFi) are widely used to treat patients with inflammatory bowel disease (IBD), rheumatoid arthritis (RA), ankylosing spondylitis (AS), as well as psoriasis and psoriatic arthritis (PsA). In 2001, the first reports of increased risk of tuberculosis (TB) disease with TNFi use were published, and much has since been discovered about the association of mycobacterial disease with this class of biologic pharmaceuticals [2].

1.1 TNF-α Biology

TNF-α is a pleiotropic cytokine involved in inflammatory, infectious, and neoplastic responses. It was named by Lloyd Old and colleagues after it was linked to tumor necrosis and regression in mice [3]. TNF-α exists in a precursor form at the

surface of various cells in a homotrimeric form [4]. This transmembrane protein is cleaved by membrane metalloproteinase, TNF-α converting enzyme (TACE), into the soluble form of TNF-α [5]. The active form of both transmembrane and soluble TNF-α is a trimer, which mediates its activity through two receptors, TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2). TNFR1 is constitutively expressed on almost all nucleated cells and binds either form of TNF-α, while TNFR2 is inducible and interacts mainly with transmembrane TNF-α [6]. TNF-α plays many roles:

- stimulating cytokine synthesis (interleukin (IL)-1, IL-6, chemokines);
- activation of macrophages, neutrophils, and eosinophils;
- phagosome maturation via interferon-gamma (IFN-γ);
- dual induction and inhibitory apoptotic action in macrophages and T cells;
- immune cell migration and adhesion; and
- granuloma formation and maintenance [7–17].

1.2 TNF-α-Medicated Immune Response to Tuberculosis

TNF-α plays a critical role in TB immunity (Fig. 1). Much of what is known about the functions of TNF-α and the pathogenesis of TB are derived from rodent models [18–21]. In TNF-α deficient mice infected with *M. tb*, microbial growth increases rapidly, followed by fatal TB progression [7]. During *M. tb* infection, an intact innate immune system responds initially by increasing TNF-α production, particularly by myeloid and lymphoid cells [22–27]. TNF-α promotes the recruitment of mononuclear cells such as T cells to the site of infection while synergizing with IFN-γ to prime macrophages for phagocytic action [28, 29]. TNF-α prevents excessive inflammation by the host and promotes mycobacterial clearance by inducing apoptosis in T cells and *M. tb*-infected macrophages, respectively [16, 30–32]. Importantly, TNF-α drives the formation of well-organized granulomas composed of lymphocytes, dendritic cells, and transformed macrophages (foamy macrophages, giant cells, and epithelioid macrophages) surrounding the inner core of *M. tb* containing macrophages [33].

TNF-α activity is necessary not only for granuloma formation but also for maintaining granuloma integrity through its anti-infective properties and immune cell recruitment [35]. The contributions made by either soluble or transmembrane TNF-α are not entirely clear, although transmembrane TNF-α may have greater importance in the initial immune response [36–40]. A mice study demonstrated that selectively neutralizing soluble TNF-α while sparing transmembrane TNF-α led to no significant increase in mycobacterial infections [41]. With reduced quantities or non-selective neutralization of TNF-α, granulomas become disintegrated and necrotic, allowing dissemination of previously dormant mycobacteria [42]. The dissolution of granulomas occurred as soon as nine days after TNFi were administered to rats [43].

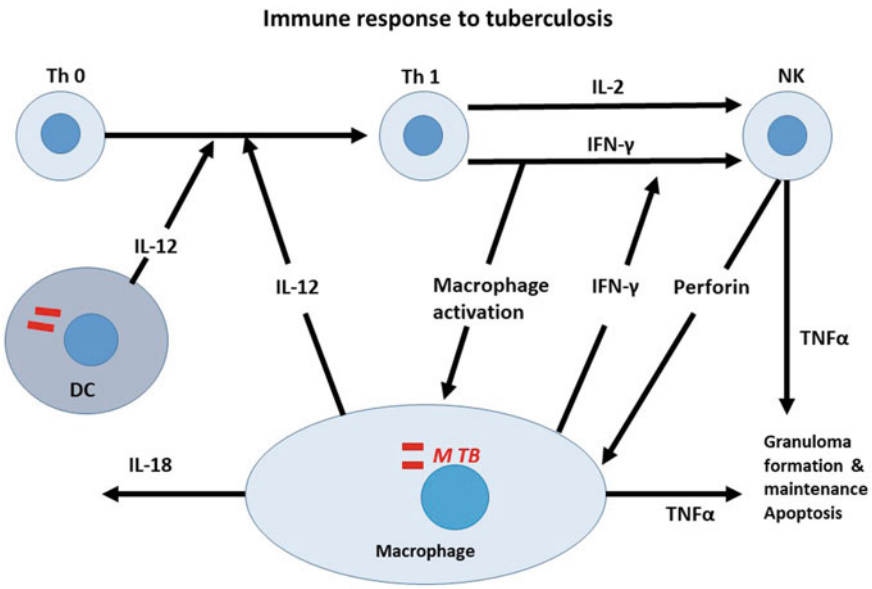


Fig. 1 Immune response to tuberculosis DC, dendritic cells; IFN-γ, interferon-gamma, IL-2, interleukin 2; IL-12, interleukin 12; IL-18, interleukin-18, *M tb*, *Mycobacterium tuberculosis*, NK, natural killer cell; Th, T helper lymphocyte; TNFα, tumor necrosis factor-alpha. This diagram is originally made by the authors by inspiration from [34, Fig. 3]

1.3 TNF-α Inhibitors: Structural and Pharmacologic Differences

Four monoclonal anti-TNF-α antibodies and one soluble TNF-α receptor are approved for clinical use, as well as multiple biosimilars. The first anti-TNF-α agent was infliximab, which is a chimeric human-murine monoclonal antibody (mAb) that has been approved to treat AS, RA, IBD [both Crohn's disease (CD) and ulcerative colitis (UC)], and psoriasis/PsA. Two further anti-TNF-α agents are fully humanized, adalimumab and golimumab (Graphical Abstract). The monoclonals bind both monomeric (inactive) and trimeric (active) TNF-α [44]. Infliximab and adalimumab can form stable immune complexes with up to two soluble or transmembrane TNF-α molecules at a time [45]. Additionally, a trimeric TNF-α molecule can bind up to three molecules of an IgG1 mAb. Adalimumab is approved for similar indications as well as hidradenitis suppurativa and non-infectious uveitis. Golimumab is approved for UC, RA, PsA, and AS.

Certolizumab pegol is the only TNFi that is composed of a fragment antigen-binding region (Fab) domain attached to a polyethylene glycol chain (PEG). It binds and neutralizes soluble TNF-α. Unlike other TNFi, certolizumab pegol does not contain the fragment crystallizable region (Fc), preventing it from forming multimeric immune complexes. The PEGylation leads to prolongation of

the half-life and increases the solubility of certolizumab pegol [46]. Certolizumab pegol is approved for CD, RA, psoriasis, PsA, and AS.

Etanercept is unique within its biologic class as it is a soluble TNF-α receptor fused with an Fc moiety of human IgG. It contains two TNFR-2 moieties that can only bind one trimeric TNF-α at a time [44]. Etanercept has a greater affinity for TNF-α molecules than infliximab and adalimumab. However, binding to soluble and transmembrane TNF-α is weaker and more transient than the mAbs, which may correlate with its neutralizing activity [45, 47]. Etanercept is the only anti-TNF-α agent that binds TNF-β, a closely related cytokine also possessing antimycobacterial functions [14, 48]. The serum half-life of etanercept is 3–5.5 days, which is noticeably shorter than that for infliximab, adalimumab, golimumab, and certolizumab that their half-time falls within the range of 8–20 days [49]. Etanercept is approved for rheumatoid, psoriatic, and juvenile idiopathic arthritis and AS. Unlike the mAbs, etanercept has not been shown to be effective in IBD.

Infliximab, adalimumab, and golimumab can bind to transmembrane TNF-α-bearing T cells leading to complement-mediated cytotoxicity and apoptosis, although golimumab has weaker cytotoxicity compared to infliximab and adalimumab [50]. Due to a lack of an Fc domain, certolizumab pegol does not cause complement or cell-mediated toxicity. Instead, certolizumab pegol causes direct non-apoptotic death of transmembrane TNF-α expressing cells [50]. Etanercept cannot activate complement component (C)3′ due to an absence of CH1 domain on its Fc fragment, rendering it ineffective at activating the classical complement pathway. Mitoma and colleagues confirmed that etanercept has lower complement-mediated cytotoxicity than infliximab and adalimumab [51]. The same study concluded that all three were relatively equivalent in exerting antibody-dependent cell-mediated cytotoxicity, but infliximab and adalimumab are also capable of direct outside-to-inside signaling leading to cell cycle arrest. Infliximab also affects adaptive immunity to *M. tb* by inducing apoptosis of granulysin expressing effector memory CD8+ T cells, which normally have a direct antimicrobial effect on *M. tb*-infected macrophages [52]. It has also been shown that infliximab and adalimumab inhibit T-cell activation and reduce the number of CD4+ cells, while etanercept does not [53]. Plessner and colleagues examined the effects of molecules modeled after infliximab and etanercept on each stage of TB infection in mice [54]. In mice with established latent TB infection (LTBI), exposure to the mAb (i.e., infliximab) led to fatal infection from a high bacterial burden shortly after injection. Evident was a loss of granuloma structure, compared to more organized granulomas among mice treated with the soluble receptor fusion molecule (i.e., etanercept). Fluorescent labeling of the molecules also demonstrated better penetration of mAbs into granulomas, which could also contribute to increased neutralization of TNF-α. Among the mice acutely infected with *M. tb*, both molecules reduced survival compared to control mice. Treated mice had similar burdens of bacteria and increased inflammation in the lungs. This increased risk of TB reactivation with infliximab compared to etanercept and similar risk of progression with new infection has also been demonstrated in humans [55].

Differences in stoichiometry, binding mechanics (particularly to transmembrane TNF-α), pharmacokinetics, and effects against granulomas and TNF-α expressing cells may explain differences in the degrees of therapeutic efficacy as well as the risk of granulomatous disease among anti-TNF-α agents. Etanercept has more transient binding to both forms of TNF-α, cannot crosslink TNF-α-bearing cells, and has reduced complement-dependent cytotoxicity than the mAbs. Etanercept also disrupts established granulomas to a lesser degree than the other TNFi. Theoretically, these differences may explain the absence of efficacy in granulomatous diseases like sarcoidosis and IBD, together with a lower risk of TB disease [56]. On the other hand, certolizumab pegol also differs by lacking complement-mediated cytotoxicity and crosslinking properties yet is effective in treating CD and reactivating TB [57, 58]. Overall, each TNFi alters the immune response resulting in an elevated risk of mycobacterial disease.

2 Risk of Tuberculosis Associated with TNF-α Inhibitors

The precise risk of developing active TB due to TNFi is difficult to define. There is a wide variation in TNFi-related TB disease incidence depending on factors such as the background population prevalence of TB, underlying immune-mediated disease, concomitant immunosuppressant use, and other host factors. Recommendations to screen for LTBI in patients being considered for treatment with TNFi were widely disseminated in 2002, leading to a decrease in TB cases within TNFi trials conducted afterward. Clinical trials consistently report low numbers of TB cases, but this risk is underestimated because of short follow-up times and strict screening and treatment of subjects with LTBI. Several meta-analyses have pooled randomized clinical trials (RCTs) to improve the confidence in the risk estimate of TB. A systematic review by Minozzi et al. identified 19 studies involving 8320 patients with RA, PsA, or AS treated with any of the five TNFi, concluding an odds ratio (OR) of 3.29 (95% CI 1.48–7.33) of developing active TB (32 cases all in the treatment groups compared to none in the placebo groups) [59]. Their analysis was underpowered to assess for differential risk between TNFis. Another meta-analysis of RCTs (including expanded indications of IBD, sarcoidosis, asthma, and graft-versus-host disease) was published in the same year, including 11,879 patients from 29 trials [60]. Compared to the control groups, those on TNFi had almost twice the risk of developing TB (OR 1.94, 95% CI 1.10–3.44). Similarly, the authors did not find a difference in risk between TNFis. Two older meta-analyses did not demonstrate an increased risk associated with TNFis within trials, concluding that RCTs have inadequate observation periods to capture all TB cases and are conducted in locations with low-TB incidence [61, 62].

Contrary to clinical trials, real-world observational data reveal a stronger signal of increased risk (Table 1). In the few years after infliximab was approved in the United States (US), the national adverse event reporting system documented 70 cases of TB among the approximately 147,000 persons exposed to infliximab [2].

Table 1 Summary of TB incidence according to the selected registry and post-marketing surveillance studies

Author Year [References]	Country or region	Years of data	TB case rate per 100,000 person-years No. TB cases/exposure years (y) or patients (pts)				
			Infliximab	Adalimumab	Etanercept	Golimumab	Certolizumab
Pettipher 2020 [72]	South Africa	1999–2017	2160 15/694 y	1625 48/2954 y	861 19/2207 y	1099 3/273 y	–
Wang 2019 [73]	Hong Kong	2006–2015	1855 34/760 pts	760 9/646 pts	435 10/959 pts	633 4/437 pts	0 0/38 pts
Sartori 2019 [74]	Brazil	2006–2016	– 3/623 pts	– 27/2980 pts	– 10/2543 pts	– 1/492 pts	– 2/164 pts
Rutherford 2018 [75]	UK	2002–2015	73 13/17670 y	83 24/28751 y	46 17/36663 y	–	89 2/2247 y
Arkema 2015 [76]	Sweden	2002–2011	51 5/9889 y	62 6/9635 y	12 2/16778 y	0 0/243 y	0 0/204 y
Winthrop 2013 [77]	USA	2000–2008	83 8/2786 pts	91 7/2338 pts	17 8/5328 pts	–	–
Atzeni 2012 [67]	Italy	1999–2012	259 6/837 pts	113 2/802 pts	39 1/1130 pts	–	–
Kim 2011 [78]	South Korea	2002–2009	540 2/366 y	308 1/204 y	0 0/1214 y	–	–
Tubach 2009 [65]	France	2004–2007	188 –	215 –	9 –	–	–
Favalli 2009 [69]	Italy	2002–2008	183 3/519 pts	191 1/303 pts	291 1/242 pts		
Carmona 2005 [79]	Spain	2000–2004	32 34/6328 y	0 0/122 y	0 0/1375 y	–	–
Listing 2005 [70]	Germany	2001–2003	310 1/346 pts	–	0 0/512 pts	–	–

Infliximab-treated RA patients in the US were four times as likely to develop TB (24.4 cases per 100,000 vs. a background rate of 6.2 cases per 100,000) [2]. In Europe, the overall incidence ranges from 123 to 716 cases per 100,000 population per year in patients prescribed infliximab [63–69]. For adalimumab-prescribed people, the incidence has been reported from 176 to 308 cases per 100,000 population per year [63–66]. The incidence rate among the etanercept-treated population is lower. Registry and post-surveillance data suggest a four-fold increased risk of TB in patients receiving infliximab and adalimumab compared to etanercept and a three-fold risk of etanercept-associated TB over the general population [63, 70, 71].

Due to the more recent introduction to the market and increased TB screening in studies with golimumab and certolizumab pegol, TB cases are fewer than the other TNFi. Golimumab is a mAb similar to infliximab and adalimumab; therefore, the intrinsic risk of TB could be assumed to be similar. However, safety data from five clinical trials spanning five years did not identify any increase in TB compared to placebo [80]. Most phase III trials with golimumab used a dual LTBI testing strategy with both Mantoux skin testing and interferon gamma-release assay (IGRA), which likely increased the detection and subsequent treatment of LTBI [77]. Interestingly, certolizumab pegol was found to have more cases of TB in clinical trials with open-label extensions but included more patients from high-TB incidence areas [81]. A systematic review of adverse outcomes found an overall relative risk (RR) of TB of 2.47 (95% CI 0.64–9.56; $p = 0.19$) in those treated with certolizumab pegol [82]. TB rates with certolizumab pegol are similar to other mAbs but fell after a stricter LTBI definition (Mantoux skin testing cut-off of induration changed to five mm or more), which was introduced into clinical trial protocols in 2007 [81]. A British biologics registry has shown that the rate of TB among TNFi-exposed people has fallen from 2002 to 2015 (783 cases per 100,000 patient-years (py) vs. 38 cases per 100,000 py of exposure) likely as a result of the detection and treatment of LTBI [83].

While registry and post-marketing surveillance databases span several countries and contain vast patient years of exposure, study flaws include a lack of a comparison group and detection bias. Additionally, registries have limited data on additional factors that can impact TB incidence rates, such as other TB risk factors and assessment and treatment of LTBI. The background prevalence of TB impacts the risk of TNFi-associated TB, and most literature is based on low-incidence countries. Larger risk estimates were derived from the South African Biologics Registry (SABIO), with incidence rates of TB highest with monoclonal TNFi (1683/100,000 py), compared to etanercept (871/100,000 py) and non-TNFi users (681/100,000 years) [84]. The incidence of TNFi-related TB in South Africa has also declined between 2011 and 2017, similar to the British data. Wang and colleagues conducted a population-based study in Hong Kong, which is considered an intermediate-TB incidence region [75]. They found that patients treated with TNFi had an incidence of 956/100,000 py compared to 71.5/100,000 py in the general population. Among specific disease populations, IBD patients had the highest incidence (1253/100,000 py) compared to those with RA (784/100,000 py) and cutaneous conditions (377/100,000 py), suggesting that disease may modify the risk

of TB independent of immunosuppressive therapy [75]. The risk was highest with infliximab with a standardized incidence ratio (SIR) of 25.95 (95% CI 17.23–34.67), followed by adalimumab. No cases of etanercept-associated TB were reported in studies of RA and AS patients in South Korea [72, 73, 76] (Table 1).

Immune-mediated diseases can also carry an inherent risk of TB without TNFi exposure. An analysis of a large hospital dataset found a significantly elevated risk of subsequent TB among those hospitalized for CD, psoriasis, AS, and RA, in addition to numerous other immune-mediated diseases [78]. In the pre-TNFi era, an IBD population was shown to be at increased risk (RR 2.36, 95% CI 1.17–4.74) for active TB, mainly driven by immunosuppressant use like corticosteroids [85]. As a result of immune dysregulation and non-biologic immunosuppressant use, patients with RA are more susceptible to mycobacterial infections [86]. A review of TB cases in Sweden between 1999 and 2004 concluded a 60% increased risk of TB in the RA population (RR 1.6, 95% CI 1.3–1.9) [67]. In South Korea, the risk of TB was nine-fold higher in the RA population and 30-fold higher in infliximab users compared to the general population [72]. Winthrop and colleagues found pooled TNFi associated rates of TB in California, US of 49 per 100,000 py, which is 5.6 and 17.5 times higher than unexposed RA patients and the general population, respectively [71]. In many of these disease states, concurrent immunosuppression with corticosteroids or disease-modifying therapies (DMARDs) is common. A systematic review found the risk of TB with combined therapy was 13-fold higher than with TNFi therapy alone (OR 13.3; 95% CI 3.7–100) [87].

In summary, all TNFi increase the risk of TB, but the risk is lowest with etanercept. The risk with the newer agents, golimumab and certolizumab pegol, has not yet been adequately established with real-world data but is likely similar to infliximab and adalimumab. Concomitant immunosuppression related to disease and medications, as well as having lived in regions with higher TB prevalence, will also increase the risk of active TB.

3 Screening for Latent Tuberculosis Infection

The heightened risk of developing TB disease and increased severity of TNFi-associated TB disease [63, 84] make it imperative that patients are screened and treated for LTBI (also known as chemoprophylaxis) before starting TNFi treatment. There are unique challenges to testing in this population, who are often medically immunosuppressed. The currently available tests are the tuberculin skin test (TST) and IGRA.

TST is performed by the Mantoux method, which involves intradermal injection of purified protein derived from *M. tb*. There is vast experience with this century-old test, and its advantages are the low cost, ease of use, and accessibility. However, accurate interpretation can be affected by numerous factors, including faulty administration, interpretation, and boosting phenomenon. False-positive results can be seen in those with prior Bacille Calmette-Guerin (BCG) vaccination, non-tuberculous

mycobacteria (NTM) sensitization, and incorrect interpretation of induration [88]. False-negative results can occur in those already on immunosuppressive therapies or with inflammatory conditions, which are common among candidates for TNFi therapy [89–92]. In immunosuppressed states, anergy can lead to T cells' absent or diminished recall response to previously exposed antigens, leading to a false-negative TST [90]. A minimum induration of five mm for TST positivity is recommended for improved sensitivity in immunocompromised states [93]. In RA, anergic responses may be facilitated by a reduction in circulating memory CD4 T cells as well as lower antigen-presenting capacity by monocytes [91, 94]. Often, patients with RA are already on non-biologic immunosuppressive treatments such as corticosteroids, methotrexate, leflunomide, and sulfasalazine, all of which have been associated with anergic responses in a dose-dependent fashion [95, 96]. Whenever possible, screening for LTBI should occur before the onset of immunosuppressive therapies, including DMARDs. Patients with psoriasis may also develop new psoriatic lesions at the site of BCG implantation due to the Koebner phenomenon [97].

IGRA is an in vitro test that quantifies the IFN-γ production from T cells, again relying on a recall response upon exposure of either whole blood or mononuclear cells to *M. tb* antigens. There are two commercially available IGRAs:

1. *QuantiFERON TB Gold In Tube (QFT-GIT; Cellestis Limited, Carnegie, Victoria, Australia)*
2. *T-SPOT.TB test (T-SPOT, manufactured by Oxford Immunotec Ltd, Abingdon, United Kingdom).*

These antigens (derived from ESAT-6 and CFP-10) are more specific for *M. tb*, and the lack of cross-reactivity to BCG and most NTM results in improved specificity of the IGRA compared to the TST. Anergic responses are often denoted by an "indeterminate" (due to a reduced mitogen response) or negative result. If anergy is suspected as the cause of an indeterminate or negative IGRA result, then the decision regarding LTBI treatment must be based on other patient factors or investigations. Rates of indeterminate results range from 0 to 33% among immunosuppressed patients, particularly those with more severe immunosuppression, as shown in the HIV population [98, 99].

Both tests have reduced sensitivity in immunosuppressed states. The observation that concordance is higher among patients with negative TST and IGRA compared to when either test is positive speaks to the likelihood of anergic responses leading to false-negative results [77, 98]. However, IGRA appears more sensitive than TST in immunosuppressed subjects, as the overall positivity rate is substantially higher among BCG-naïve patients [77, 98, 100–103]. The odds of a positive IGRA result are also correlated more strongly with the presence of LTBI risk factors than the TST [100, 104, 105]. In addition, corticosteroids and DMARDs appear to have less impact on IGRA positivity compared to TST positivity [100, 104].

Guidelines on LTBI testing vary considerably across disease states and jurisdictions [106]. Recommendations from many guidelines, including those by the American Thoracic Society, Infectious Diseases Society of America, and Center for

Disease Control, are that patients likely to be infected with *M. tb* and at high risk of reactivation, such as candidates for TNFi, should be screened with either test and if the test is positive, to pursue chemoprophylaxis [107–110]. In order to increase the sensitivity of detecting LTBI, one may also perform a second alternative test if the first test is negative. During phase III trials of golimumab in inflammatory arthritis, combination LTBI testing was used, and chemoprophylaxis was given if either test was positive. Among those given chemoprophylaxis, none had developed active TB disease at one year [77]. Guidelines from the American College of Rheumatology recommend either test in those starting biologics [111]. If the initial test is negative, a second test should be considered in RA patients with risk factors for LTBI. An IGRA may be preferred if there is a history of BCG vaccination, particularly after infancy or repeated vaccination [112, 113].

In individuals with negative baseline screening, there is clinical equipoise on the need for LTBI rescreening, methods of rescreening, and the significance of IGRA conversion during TNFi therapy. A prospective cohort study of rheumatic patients on TNF antagonist therapy found that conversion was common (up to 30%) when tested with either or both TST and IGRA one year after biologic therapy initiation [114]. The 2016 joint guideline by the American Thoracic Society, Infectious Diseases Society of America, and Centers for Disease Control and Prevention does not recommend serial testing with IGRA at this time, given that IFN-γ levels fluctuate and it is not yet clear how to differentiate biologic variability from new infection [107]. In contrast, other guidelines recommend annual rescreening if there are ongoing risks of TB exposure [112, 115, 116]. Regardless of retesting, patients should continue to be monitored for signs of active TB disease during and after TNFi therapy, even in cases where baseline screening was negative or in that chemoprophylaxis was completed.

3.1 Chemoprophylaxis

Patients with evidence of LTBI should start chemoprophylaxis before commencing TNFi therapy. Active TB must be ruled out first based on the patient history, physical examination, and chest radiograph, along with any necessary microbiological testing. The number of regimens for LTBI treatment has expanded in recent years, and solely isoniazid-based regimens are no longer preferred. Recommended regimens include four months of daily rifampin, twelve weeks of weekly rifapentine and isoniazid, three months of daily rifampin and isoniazid, and six to nine months of daily isoniazid.

The effectiveness of chemoprophylaxis in reducing the incidence of active TB disease during TNFi therapy has been shown in prior RCTs and registry data. After recommendations to prevent TB reactivation were implemented in 2002, a Spanish registry (BIODASER) of patients with rheumatic conditions being treated with infliximab, adalimumab, or etanercept found the rate of active TB decreased by 78% [117]. All trials for the newer TNFi (golimumab and certolizumab pegol) were required to evaluate and treat patients for LTBI. Overall, comprehensive TB

screening led to low rates of active TB disease after five years of golimumab trials, even in TB-endemic regions [80]. Applying a strict TST threshold of five mm induration compared to more liberal cut-offs led to more treatment for LTBI and subsequently decreased the incidence of active TB during certolizumab pegol clinical trials (from 0.51 per 100 py to 0.18 per 100 py) [79].

There is no definitive evidence for the optimal interval between LTBI treatment and TNFi initiation. Most experts and guidelines recommend a minimum of four weeks of LTBI treatment before starting the biologic. However, other periods from three weeks through the completion of LTBI treatment have also been suggested [106, 113].

4 Active Tuberculosis Among TNFi Users

4.1 Site of Tuberculosis

Among those diagnosed with TB while taking TNFi, a large proportion have disseminated and extrapulmonary TB (EPTB) disease. Studies related to TNFi usage in the US between 1999 and 2001 found more than half of the patients had EPTB, and one-quarter had disseminated TB [2]. An earlier epidemiology study in the US found 11.3% of cases were extrapulmonary [118]. The BSRBR and RATIO registries also reported similar proportions with EPTB (61–62%) [63, 64]. In a recently published Brazilian registry, 40% of TB cases involved extrapulmonary sites [119]. Pulmonary TB (PTB) occurred in 62%, EPTB in 38%, and disseminated TB in 7% of cases from the SABIO registry in South Africa [84]. Clinicians and patients must be vigilant in monitoring TB, including extrapulmonary and constitutional symptoms, even in the absence of pulmonary symptoms. Paradoxical sarcoid reactions, such as intrathoracic lymphadenopathy, are increasingly seen with TNFi, and this can be challenging to differentiate from TB lymphadenitis [74, 120–122]. The pathogenesis of this phenomenon is unclear.

4.2 Timing of Tuberculosis Diagnosis

Using data from the US's national adverse event reporting system, Wallis and colleagues characterized infectious complications from infliximab and etanercept [123]. Almost half of TB cases were within 90 days of initiating infliximab, compared to only 12.5% with etanercept ($p < 0.001$). The median time to TB diagnosis is three to six months with infliximab and adalimumab compared to longer than a year with etanercept [1]. Etanercept disrupts pre-existing granulomas to a lesser degree than infliximab and adalimumab. This is a possible explanation for the longer time to onset (and reduced overall risk of TB) with etanercept [54]. A significant proportion of TB cases are diagnosed after drug discontinuation, and monitoring for symptoms should continue for six to 12 months after a TNFi is

stopped [63, 71]. The time to onset of TB depends not only on the specific anti-TNFα agent but also on the degree of immunosuppression, stage of TB infection (reactivation of latent infection versus new infection), and the recency of LTBI treatment as the protective effects may wane with time [124].

4.3 Treatment of TNFi-Associated Tuberculosis

There is a higher risk of fulminant TB disease in those exposed to TNFi [63, 84]; therefore, diagnosis and treatment must be expedited as soon as possible if there is suspicion of TB. Expert consensus recommends that the TNFi should be discontinued at least temporarily [113]. However, the optimal time for reinitiating TNFi after TB treatment is unclear. Case series do not suggest worse outcomes with restarting TNFi during TB treatment, but sample sizes were small [125, 126].

Developing TB in the midst of TNF-α blockade increases the risk of a paradoxical reaction upon discontinuation of the TNFi, akin to the immune reconstitution inflammatory syndrome (IRIS) seen in the HIV population [127–129]. Risk factors for developing this paradoxical reaction are disseminated TB (OR 11.4, 95% CI 1.4–92.2; $P = 0.03$), corticosteroid use (OR 4.6, 95% CI 1.2–17.2; $P = 0.02$), and history of TB exposure (OR 12.7, 95% CI 1.6–103; $P = 0.02$) [130]. The onset of exaggerated inflammatory symptoms such as fever, pulmonary infiltrates, lymphadenopathy, or diarrhea will typically occur four to 20 weeks after the TNFi is discontinued, corresponding to the washout of TNF-α neutralization. In the case of a paradoxical reaction, the TNFi may be reinitiated sooner as long as the patient is established on active TB treatment. It is unknown whether corticosteroids are effective in dampening the paradoxical response, although their efficacy in HIV-related IRIS is proven [131].

5 Conclusion

An effective immune response to *M. tb* infection is dependent on TNF-α. TNFi use is increasing substantially; thus, clinicians ought to be cautious of the several ways in which the immune response to M. tb might be disrupted, increasing the risk of TB. There are also unique aspects in preventing, diagnosing, and managing TNFi-associated TB. TB disease is a serious complication of TNFi use that can largely be prevented with adequate screening and treating of patients with LTBI before starting biologic treatment.

Core Messages

- TNF-α is pivotal to initiating and maintaining an effective immune response to *M. tb* infection.

- All TNF-α inhibitors increase the risk of TB reactivation, albeit to varying degrees; etanercept confers the lowest risk.
- The risk of TB reactivation is mitigated by appropriate screening for LTBI before starting TNFi therapy.
- Standard LTBI treatment regimens are used, and a delay of TNFi initiation of at minimum one month should be considered.
- Management of TB disease includes suspension of the TNFi, although this can lead to paradoxical inflammatory reactions.

References

1. Godfrey MS, Friedman LN (2019) Tuberculosis and biologic therapies: anti-tumor necrosis factor-α and beyond. Clin Chest Med 40(4):721–739
2. Keane J, Gershon S, Wise RP et al (2001) Tuberculosis associated with infliximab, a tumor necrosis factor α–neutralizing agent. N Engl J Med 345(15):1098–1104
3. Carswell E, Old LJ, Kassel R et al (1975) An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci 72(9):3666–3670
4. Vassalli P (1992) The pathophysiology of tumor necrosis factors. Annu Rev Immunol 10 (1):411–452
5. Black RA, Rauch CT, Kozlosky CJ et al (1997) A metalloproteinase disintegrin that releases tumour-necrosis factor-α from cells. Nature 385(6618):729–733
6. Grell M, Douni E, Wajant H et al (1995) The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor. Cell 83(5):793–802
7. Roach DR, Bean AG, Demangel C et al (2002) TNF regulates chemokine induction essential for cell recruitment, granuloma formation, and clearance of mycobacterial infection. J Immunol 168(9):4620–4627
8. Mulligan MS, Vaporciyan AA, Miyasaka M et al (1993) Tumor necrosis factor alpha regulates in vivo intrapulmonary expression of ICAM-1. Am J Pathol 142(6):1739
9. Billmeier U, Dieterich W, Neurath MF et al (2016) Molecular mechanism of action of anti-tumor necrosis factor antibodies in inflammatory bowel diseases. World J Gastroenterol 22(42):9300
10. Ungar B, Kopylov U (2016) Advances in the development of new biologics in inflammatory bowel disease. Ann Gastroenterol 29(3):243
11. Chaabo K, Kirkham B (2015) Rheumatoid arthritis—anti-TNF. Int Immunopharmacol 27 (2):180–184
12. Yamanaka H (2015) TNF as a target of inflammation in rheumatoid arthritis. Endocr Metab Immune 15(2):129–134
13. Elyoussfi S, Thomas BJ, Ciurtin C (2016) Tailored treatment options for patients with psoriatic arthritis and psoriasis: review of established and new biologic and small molecule therapies. Rheumatol Int 36(5):603–612
14. Yamauchi PS, Bissonnette R, Teixeira HD et al (2016) Systematic review of efficacy of anti-tumor necrosis factor (TNF) therapy in patients with psoriasis previously treated with a different anti-TNF agent. J Am Acad Dermatol 75(3):612–618
15. Kindler V, Sappino A-P, Grau GE et al (1989) The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. Cell 56(5):731–740

16. Mohan VP, Scanga CA, Yu K et al (2001) Effects of tumor necrosis factor alpha on host immune response in chronic persistent tuberculosis: possible role for limiting pathology. Infect Immun 69(3):1847–1855
17. Ehlers S (2005) Tumor necrosis factor and its blockade in granulomatous infections: differential modes of action of infliximab and etanercept? Clin Infect Dis S199–S203
18. Champsi J, Young L, Bermudez L (1995) Production of TNF-alpha, IL-6 and TGF-beta, and expression of receptors for TNF-alpha and IL-6, during murine Mycobacterium avium infection. Immunology 84(4):549
19. Bean AG, Roach DR, Briscoe H et al (1999) Structural deficiencies in granuloma formation in TNF gene-targeted mice underlie the heightened susceptibility to aerosol Mycobacterium tuberculosis infection, which is not compensated for by lymphotoxin. J Immunol 162 (6):3504–3511
20. Chan J, Xing Y, Magliozzo RS et al (1992) Killing of virulent Mycobacterium tuberculosis by reactive nitrogen intermediates produced by activated murine macrophages. J Exp Med 175(4):1111–1122
21. Flesch I, Kaufmann S (1990) Activation of tuberculostatic macrophage functions by gamma interferon, interleukin-4, and tumor necrosis factor. Infect Immun 58(8):2675–2677
22. Valone SE, Rich EA, Wallis RS et al (1988) Expression of tumor necrosis factor in vitro by human mononuclear phagocytes stimulated with whole Mycobacterium bovis BCG and mycobacterial antigens. Infect Immun 56(12):3313–3315
23. Henderson RA, Watkins SC, Flynn J (1997) Activation of human dendritic cells following infection with Mycobacterium tuberculosis. J Immunol 159(2):635–643
24. Mayer-Barber K, Andrade D, Barber SH et al (2011) Innate and adaptive interferons suppress IL-1alpha and IL-1beta production by distinct pulmonary myeloid subsets during Mycobacterium tuberculosis infection. Immunity 35:1023–1034
25. Harari A, Rozot V, Enders FB et al (2011) Dominant TNF-α+ Mycobacterium tuberculosis–specific CD4+ T cell responses discriminate between latent infection and active disease. Nat Med 17(3):372–376
26. Barnes PF, Abrams J, Lu S et al (1993) Patterns of cytokine production by mycobacterium-reactive human T-cell clones. Infect Immun 61(1):197–203
27. Lang F, Peyrat MA, Constant P et al (1995) Early activation of human V gamma 9V delta 2 T cell broad cytotoxicity and TNF production by nonpeptidic mycobacterial ligands. J Immunol 154(11):5986–5994
28. Schaible UE, Sturgill-Koszycki S, Schlesinger PH et al (1998) Cytokine activation leads to acidification and increases maturation of Mycobacterium avium-containing phagosomes in murine macrophages. J Immunol 160(3):1290–1296
29. Via L, Fratti R, McFalone M et al (1998) Effects of cytokines on mycobacterial phagosome maturation. J Cell Sci 111(7):897–905
30. Keane J, Balcewicz-Sablinska MK, Remold HG et al (1997) Infection by Mycobacterium tuberculosis promotes human alveolar macrophage apoptosis. Infec Immun 65(1):298–304
31. Leemans JC, Juffermans NP, Florquin S et al (2001) Depletion of alveolar macrophages exerts protective effects in pulmonary tuberculosis in mice. J Immunol 166(7):4604–4611
32. Keane J, Shurtleff B, Kornfeld H (2002) TNF-dependent BALB/c murine macrophage apoptosis following Mycobacterium tuberculosis infection inhibits bacillary growth in an IFN-γ independent manner. Tuberculosis 82(2–3):55–61
33. Russell DG, Cardona P-J, Kim M-J et al (2009) Foamy macrophages and the progression of the human tuberculosis granuloma. Nat Immunol 10(9):943–948
34. Yasui K (2014) Immunity against Mycobacterium tuberculosis and the risk of biologic anti-TNF-α reagents. Pediatr Rheumatol 12(1):1–7
35. Egen JG, Rothfuchs AG, Feng CG et al (2008) Macrophage and T cell dynamics during the development and disintegration of mycobacterial granulomas. Immunity 28(2):271–284

36. Marino S, Sud D, Plessner H et al (2007) Differences in reactivation of tuberculosis induced from anti-TNF treatments are based on bioavailability in granulomatous tissue. PLoS Comput Biol 3(10):e194
37. Olleros ML, Guler R, Vesin D et al (2005) Contribution of transmembrane tumor necrosis factor to host defense against Mycobacterium bovis bacillus Calmette-guerin and Mycobacterium tuberculosis infections. Am J Pathol 166(4):1109–1120
38. Olleros ML, Guler R, Corazza N et al (2002) Transmembrane TNF induces an efficient cell-mediated immunity and resistance to Mycobacterium bovis bacillus Calmette-Guerin infection in the absence of secreted TNF and lymphotoxin-α. J Immunol 168(7):3394–3401
39. Saunders BM, Tran S, Ruuls S et al (2005) Transmembrane TNF is sufficient to initiate cell migration and granuloma formation and provide acute, but not long-term, control of Mycobacterium tuberculosis infection. J Immunol 174(8):4852–4859
40. Quesniaux VF, Jacobs M, Allie N et al (2010) TNF in host resistance to tuberculosis infection. TNF Pathophysiol 11:157–179
41. Olleros ML, Vesin D, Lambou AF et al (2009) Dominant-negative tumor necrosis factor protects from Mycobacterium bovis Bacillus Calmette-Guérin (BCG) and endotoxin-induced liver injury without compromising host immunity to BCG and Mycobacterium tuberculosis. J Infect Dis 199(7):1053–1063
42. Reece ST, Loddenkemper C, Askew DJ et al (2010) Serine protease activity contributes to control of Mycobacterium tuberculosis in hypoxic lung granulomas in mice. J Clin Invest 120(9):3365–3376
43. Chakravarty SD, Zhu G, Tsai MC et al (2008) Tumor necrosis factor blockade in chronic murine tuberculosis enhances granulomatous inflammation and disorganizes granulomas in the lungs. Infect Immun 76(3):916–926
44. Wallis RS (2008) Tumour necrosis factor antagonists: structure, function, and tuberculosis risks. Lancet Infect Dis 8(10):601–611
45. Scallon B, Cai A, Solowski N et al (2002) Binding and functional comparisons of two types of tumor necrosis factor antagonists. J Pharmacol Exp Ther 301(2):418–426
46. Pasut G (2014) Pegylation of biological molecules and potential benefits: pharmacological properties of certolizumab pegol. BioDrugs 28(1):15–23
47. Mitoma H, Horiuchi T, Tsukamoto H et al (2016) Molecular mechanisms of action of anti-TNF-a agents—comparison among therapeutic TNF-a antagonists. Cytokine 101:56–63
48. Ehlers S, Hölscher C, Scheu S et al (2003) The lymphotoxin β receptor is critically involved in controlling infections with the intracellular pathogens Mycobacterium tuberculosis and Listeria monocytogenes. J Immunol 170(10):5210–5218
49. Suzuki T, Ishii-Watabe A, Tada M et al (2010) Importance of neonatal FcR in regulating the serum half-life of therapeutic proteins containing the Fc domain of human IgG1: a comparative study of the affinity of monoclonal antibodies and Fc-fusion proteins to human neonatal FcR. J Immunol 184(4):1968–1976
50. Ueda N, Tsukamoto H, Mitoma H et al (2013) The cytotoxic effects of certolizumab pegol and golimumab mediated by transmembrane tumor necrosis factor α. Inflamm Bowel Dis 19 (6):1224–1231
51. Mitoma H, Horiuchi T, Tsukamoto H et al (2008) Mechanisms for cytotoxic effects of anti–tumor necrosis factor agents on transmembrane tumor necrosis factor α–expressing cells: comparison among infliximab, etanercept, and adalimumab. Arthritis Rheum 58(5):1248–1257
52. Bruns H, Meinken C, Schauenberg P et al (2009) Anti-TNF immunotherapy reduces CD8+ T cell–mediated antimicrobial activity against Mycobacterium tuberculosis in humans. J Clin Invest 119(5):1167–1177
53. Saliu OY, Sofer C, Stein DS et al (2006) Tumor-necrosis-factor blockers: differential effects on mycobacterial immunity. J Infect Dis 194(4):486–492

54. Plessner HL, Lin PL, Kohno T et al (2007) Neutralization of tumor necrosis factor (TNF) by antibody but not TNF receptor fusion molecule exacerbates chronic murine tuberculosis. J Infect Dis 195(11):1643–1650
55. Wallis RS (2008) Mathematical modeling of the cause of tuberculosis during tumor necrosis factor blockade. Arthritis Rheum 58(4):947–952
56. Utz JP, Limper AH, Kalra S et al (2003) Etanercept for the treatment of stage II and III progressive pulmonary sarcoidosis. Chest 124(1):177–185
57. Sandborn WJ, Feagan BG, Stoinov S et al (2007) Certolizumab pegol for the treatment of Crohn's disease. New Engl J Med 357(3):228–238
58. Da W, Zhu J, Wang L et al (2013) Efficacy and safety of certolizumab pegol for Crohn's disease: a systematic review and meta-analysis. Adv Ther 30(5):541–553
59. Minozzi S, Bonovas S, Lytras T et al (2016) Risk of infections using anti-TNF agents in rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis: a systematic review and meta-analysis. Expert Opin Drug Saf 15(sup1):11–34
60. Zhang Z, Fan W, Yang G et al (2017) Risk of tuberculosis in patients treated with TNF-α antagonists: a systematic review and meta-analysis of randomised controlled trials. BMJ Open 7(3):e012567
61. Souto A, Maneiro JR, Salgado E et al (2014) Risk of tuberculosis in patients with chronic immune-mediated inflammatory diseases treated with biologics and tofacitinib: a systematic review and meta-analysis of randomized controlled trials and long-term extension studies. Rheumatology 53(10):1872–1885
62. Ai J-W, Zhang S, Ruan Q-L et al (2015) The risk of tuberculosis in patients with rheumatoid arthritis treated with tumor necrosis factor-α antagonist: a metaanalysis of both randomized controlled trials and registry/cohort studies. J Rheum 42(12):2229–2237
63. Dixon W, Hyrich K, Watson K et al (2010) Drug-specific risk of tuberculosis in patients with rheumatoid arthritis treated with anti-TNF therapy: results from the British Society for Rheumatology Biologics Register (BSRBR). Ann Rheum Dis 69(3):522–528
64. Tubach F, Salmon D, Ravaud P et al (2009) Risk of tuberculosis is higher with anti-tumor necrosis factor monoclonal antibody therapy than with soluble tumor necrosis factor receptor therapy: the three-year prospective French research axed on tolerance of biotherapies registry. Arthritis Rheum 60(7):1884–1894
65. Gómez-Reino JJ, Carmona L, Ángel Descalzo M (2007) Risk of tuberculosis in patients treated with tumor necrosis factor antagonists due to incomplete prevention of reactivation of latent infection. Arthritis Care Res 57(5):756–761
66. Atzeni F, Sarzi-Puttini P, Botsios C et al (2012) Long-term anti-TNF therapy and the risk of serious infections in a cohort of patients with rheumatoid arthritis: comparison of adalimumab, etanercept and infliximab in the GISEA registry. Autoimmun Rev 12 (2):225–229
67. Askling J, Fored CM, Brandt L et al (2005) Risk and case characteristics of tuberculosis in rheumatoid arthritis associated with tumor necrosis factor antagonists in Sweden. Arthritis Rheum 52(7):1986–1992
68. Favalli EG, Desiati F, Atzeni F et al (2009) Serious infections during anti-TNFα treatment in rheumatoid arthritis patients. Autoimmun Rev 8(3):266–273
69. Listing J, Strangfeld A, Kary S et al (2005) Infections in patients with rheumatoid arthritis treated with biologic agents. Arthritis Rheum 52(11):3403–3412
70. Cantini F, Niccoli L, Goletti D (2014) Adalimumab, etanercept, infliximab, and the risk of tuberculosis: data from clinical trials, national registries, and post-marketing surveillance. J Rheum Supp 91:47–55
71. Winthrop K, Baxter R, Liu L et al (2013) Mycobacterial diseases and antitumour necrosis factor therapy in USA. Ann Rheum Dis 72(1):37–42
72. Seong S-S, Choi C-B, Woo J-H et al (2007) Incidence of tuberculosis in Korean patients with rheumatoid arthritis (RA): effects of RA itself and of tumor necrosis factor blockers. J Rheum 34(4):706–711

73. Kim E-M, Uhm W-S, Bae S-C et al (2011) Incidence of tuberculosis among Korean patients with ankylosing spondylitis who are taking tumor necrosis factor blockers. J Rheum 38 (10):2218–2223
74. Decock A, Van Assche G, Vermeire S et al (2017) Sarcoidosis-like lesions: another paradoxical reaction to anti-TNF therapy? J Crohns Colitis 11(3):378–383
75. Wang X, Wong SH, Wang X-S et al (2019) Risk of tuberculosis in patients with immune-mediated diseases on biological therapies: a population-based study in a tuberculosis endemic region. Rheumatology 58(5):803–810
76. Arkema EV, Jonsson J, Baecklund E et al (2015) Are patients with rheumatoid arthritis still at an increased risk of tuberculosis and what is the role of biological treatments? Ann Rheum Dis 74:1212–1217
77. Hsia EC, Schluger N, Cush JJ et al (2012) Interferon-γ release assay versus tuberculin skin test prior to treatment with golimumab, a human anti-tumor necrosis factor antibody, in patients with rheumatoid arthritis, psoriatic arthritis, or ankylosing spondylitis. Arthritis Rheum 64(7):2068–2077
78. Ramagopalan SV, Goldacre R, Skingsley A et al (2013) Associations between selected immune-mediated diseases and tuberculosis: record-linkage studies. BMC Med 11(1):97
79. Mariette X, Vencovsky J, Lortholary O et al (2015) The incidence of tuberculosis in patients treated with certolizumab pegol across indications: impact of baseline skin test results, more stringent screening criteria and geographic region. RMD Open 1(1)
80. Kay J, Fleischmann R, Keystone E et al (2016) Five-year safety data from 5 clinical trials of subcutaneous golimumab in patients with rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis. J Rheum 43(12):2120–2130
81. Bykerk VP, Cush J, Winthrop K et al (2015) Update on the safety profile of certolizumab pegol in rheumatoid arthritis: an integrated analysis from clinical trials. Ann Rheum Dis 74 (1):96–103
82. Sansone AC, Mantarro S, Tuccori M et al (2015) Safety profile of certolizumab pegol in patients with immune-mediated inflammatory diseases: a systematic review and meta-analysis. Drug Saf 38(10):869–888
83. Rutherford AI, Patarata E, Subesinghe S et al (2018) Opportunistic infections in rheumatoid arthritis patients exposed to biologic therapy: results from the British Society for Rheumatology Biologics Register for rheumatoid arthritis. Rheumatology 57(6):997–1001
84. Pettipher C, Benitha R (2020) Tuberculosis in biologic users for rheumatic diseases: results from the South African Biologics Registry (SABIO). Ann Rheum Dis 79(2):292–299
85. Aberra FN, Stettler N, Brensinger C et al (2007) Risk for active tuberculosis in inflammatory bowel disease patients. Clin Gastroenterol Hepatol 5(9):1070–1075
86. Carmona L, Hernández-García C, Vadillo C et al (2003) Increased risk of tuberculosis in patients with rheumatoid arthritis. J Rheum 30(7):1436–1439
87. Lorenzetti R, Zullo A, Ridola L et al (2014) Higher risk of tuberculosis reactivation when anti-TNF is combined with immunosuppressive agents: a systematic review of randomized controlled trials. Ann Med 46(7):547–554
88. Vassilopoulos D, Stamoulis N, Hadziyannis E et al (2008) Usefulness of enzyme-linked immunospot assay (Elispot) compared to tuberculin skin testing for latent tuberculosis screening in rheumatic patients scheduled for anti-tumor necrosis factor treatment. J Rheum 35(7) 1271–1276
89. Farhat M, Greenaway C, Pai M et al (2006) False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria? Int J Tuberc Lung Dis 10 (11):1192–1204
90. De León DP, Acevedo-Vasquez E, Sanchez-Torres A et al (2005) Attenuated response to purified protein derivative in patients with rheumatoid arthritis: study in a population with a high prevalence of tuberculosis. Ann Rheum Dis 64(9):1360–1361

91. Seitz M, Napierski I, Kirchner H (1988) Depressed PPD and tetanus toxoid presentation by monocytes to T lymphocytes in patients with rheumatoid arthritis: restoration by interferon gamma. Rheum Int 8(5):189–196
92. Sezer I, Kocabas H, Melikoglu MA et al (2009) Positiveness of purified protein derivatives in rheumatoid arthritis patients who are not receiving immunosuppressive therapy. Clin Rheum 28(1):53–57
93. Abubakar I, Drobniewski F, Southern J et al (2018) Prognostic value of interferon-γ release assays and tuberculin skin test in predicting the development of active tuberculosis (UK PREDICT TB): a prospective cohort study. Lancet Infect Dis 18(10):1077–1087
94. Verwilghen J, Vertessen S, Stevens EA et al (1990) Depressed T-cell reactivity to recall antigens in rheumatoid arthritis. J Clin Immunol 10(2):90–98
95. Tamborenea MN, Tate G, Mysler E et al (2010) Prevalence of positive PPD in a cohort of rheumatoid arthritis patients. Rheum Int 30(5):613–616
96. Bélard E, Semb S, Ruhwald M et al (2011) Prednisolone treatment affects the performance of the QuantiFERON Gold in-tube test and the tuberculin skin test in patients with autoimmune disorders screened for latent tuberculosis infection. Inflamm Bowel Dis 17 (11):2340–2349
97. Sivamani RK, Goodarzi H, Garcia MS et al (2013) Biologic therapies in the treatment of psoriasis: a comprehensive evidence-based basic science and clinical review and a practical guide to tuberculosis monitoring. Clin Rev Allerg Immunol 44(2):121–140
98. Mamishr S, Pourakbari B, Marjani M et al (2014) Diagnosis of latent tuberculosis infection among immunodeficient individuals: review of concordance between interferon-γ release assays and the tuberculin skin test. Brit J Biomed Sci 71(3):115–124
99. Luetkemeyer AF, Charlebois ED, Flores LL et al (2007) Comparison of an interferon-γ release assay with tuberculin skin testing in HIV-infected individuals. Am J Respir Crit Care Med 175(7):737–742
100. Matulis G, Jüni P, Villiger PM et al (2008) Detection of latent tuberculosis in immunosuppressed patients with autoimmune diseases: performance of a Mycobacterium tuberculosis antigen-specific interferon γ assay. Ann Rheum Dis 67(1):84–90
101. De Leon DP, Acevedo-Vasquez E, Alvizuri S et al (2008) Comparison of an interferon-gamma assay with tuberculin skin testing for detection of tuberculosis (TB) infection in patients with rheumatoid arthritis in a TB-endemic population. J Rheum 35 (5):776–781
102. Song GG, Bae SC, Lee YH (2013) Interferon-gamma release assays versus tuberculin skin testing in patients with rheumatoid arthritis. Int J Rheum Dis 16(3):279–283
103. Schoepfer AM, Flogerzi B, Fallegger S et al (2008) Comparison of interferon-gamma release assay versus tuberculin skin test for tuberculosis screening in inflammatory bowel disease. Am J Gastroenterol 103(11):2799–2806
104. Ruan Q, Zhang S, Ai J et al (2016) Screening of latent tuberculosis infection by interferon-γ release assays in rheumatic patients: a systemic review and meta-analysis. Clin Rheum 35 (2):417–425
105. Vassilopoulos D, Tsikrika S, Hatzara C et al (2011) Comparison of two gamma interferon release assays and tuberculin skin testing for tuberculosis screening in a cohort of patients with rheumatic diseases starting anti-tumor necrosis factor therapy. Clin Vaccine Immunol 18(12):2102–2108
106. Hasan T, Au E, Chen S et al (2018) Screening and prevention for latent tuberculosis in immunosuppressed patients at risk for tuberculosis: a systematic review of clinical practice guidelines. BMJ Open 8(9):e022445
107. Lewinsohn DM, Leonard MK, LoBue PA et al (2017) Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention clinical practice guidelines: diagnosis of tuberculosis in adults and children. Clin Infect Dis 64(2):e1–e33

108. Prignano F, Bartoloni A, Bartalesi F et al (2014) Latent tuberculosis infection in psoriasis and other dermatological immunomediated diseases: a combined approach by QuantiFERON-TB Gold and tuberculin skin tests. Int J Dermatol 53(8):e372-374
109. Kleinert S, Tony H, Krueger K et al (2012) Screening for latent tuberculosis infection: performance of tuberculin skin test and interferon-γ release assays under real-life conditions. Ann Rheum Dis 71(11):1791–1795
110. Mazurek GH, Jereb JA, Vernon A et al (2010) Updated guidelines for using interferon gamma release assays to detect Mycobacterium tuberculosis infection—US 2010. MMWR Recomm Rep 59(RR-5):1–25
111. Singh JA, Furst DE, Bharat A et al (2012) 2012 update of the 2008 American College of Rheumatology recommendations for the use of disease-modifying antirheumatic drugs and biologic agents in the treatment of rheumatoid arthritis. Arthrit Care Res 64(5):625–639
112. Singh JA, Saag KG, Bridges SL Jr et al (2016) 2015 American College of Rheumatology guideline for the treatment of rheumatoid arthritis. Arthritis Rheum 68(1):1–26
113. Solovic I, Sester M, Gomez-Reino JJ et al (2010) The risk of tuberculosis related to tumour necrosis factor antagonist therapies: a TBNET consensus statement: European Respiratory Society. Eur Respir J 36(5):1185–1206. https://doi.org/10.1183/09031936.00028510
114. Hatzara C, Hadziyannis E, Kandili A et al (2015) Frequent conversion of tuberculosis screening tests during anti-tumour necrosis factor therapy in patients with rheumatic diseases. Ann Rheum Dis 74(10):1848–1853
115. Cantini F, Nannini C, Niccoli L et al (2015) Guidance for the management of patients with latent tuberculosis infection requiring biologic therapy in rheumatology and dermatology clinical practice. Autoimmun Rev 14(6):503–509
116. Doherty SD, Van Voorhees A, Lebwohl MG et al (2008) National Psoriasis Foundation consensus statement on screening for latent tuberculosis infection in patients with psoriasis treated with systemic and biologic agents. J Am Acad Dermatol 59(2):209–217
117. Carmona L, Gómez-Reino JJ, Rodríguez-Valverde V et al (2005) Effectiveness of recommendations to prevent reactivation of latent tuberculosis infection in patients treated with tumor necrosis factor antagonists. Arthritis Rheum 52(6):1766–1772
118. Mehta JB, Dutt A, Harvill L et al (1991) Epidemiology of extrapulmonary tuberculosis: a comparative analysis with pre-AIDS era. Chest 99(5):1134–1138
119. Sartori NS, Picon P, Papke A et al (2019) A population-based study of tuberculosis incidence among rheumatic disease patients under anti-TNF treatment. PLoS One 14(12): e0224963
120. Cleynen I, Vermeire S (2012) Paradoxical inflammation induced by anti-TNF agents in patients with IBD. Nat Rev Gastroenterol Hepatol 9(9):496–503
121. Kanellopoulou T, Filiotou A, Kranidioti H et al (2011) Sarcoid-like granulomatosis in patients treated with anti-TNFα factors. A case report and review of the literature. Clin Rheum 30(4):581–583
122. Massara A, Cavazzini L, La Corte R et al (2010) Sarcoidosis appearing during anti-tumor necrosis factor α therapy: a new "class effect" paradoxical phenomenon. Two case reports and literature review. Semin Arthritis Rheum 39:313–319
123. Wallis R, Broder M, Wong J et al (2004) Granulomatous infectious diseases associated with tumor necrosis factor antagonists. Clin Infect Dis 38(9):1261–1265
124. Samandari T, Agizew TB, Nyirenda S et al (2015) Tuberculosis incidence after 36 months' isoniazid prophylaxis in HIV-infected adults in Botswana: a posttrial observational analysis. AIDS 29(3):351–359
125. Ozguler Y, Hatemi G, Ugurlu S et al (2016) Re-initiation of biologics after the development of tuberculosis under anti-TNF therapy. Rheum Int 36(12):1719–1725
126. Abreu C, Sarmento A, Magro F (2016) Reintroduction of anti-TNFα therapy after (or even during) anti-TNFα-associated tuberculosis in immune-mediated diseases. J Crohns Colitis 10 (1):120–121

127. Hsu DC, Faldetta KF, Pei L et al (2016) A paradoxical treatment for a paradoxical condition: infliximab use in three cases of mycobacterial IRIS. Clin Infect Dis 62(2):258–261
128. Gupta M, Jafri K, Sharim R et al (2015) Immune reconstitution inflammatory syndrome associated with biologic therapy. Curr Allergy Asthma Rep 15(2):499
129. Bell LC, Breen R, Miller RF et al (2015) Paradoxical reactions and immune reconstitution inflammatory syndrome in tuberculosis. Int J Infect Dis 32:39–45
130. Rivoisy C, Tubach F, Roy C et al (2016) Paradoxical anti-TNF-associated TB worsening: frequency and factors associated with IRIS. Joint Bone Spine 83(2):173–178
131. Meintjes G, Stek C, Blumenthal L et al (2018) Prednisone for the prevention of paradoxical tuberculosis-associated IRIS. New Engl J Med 379(20):1915–1925

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Breast Tuberculosis

33

Gianluca Quaglio, Damiano Pizzol, and Giovanni Putoto

There are young-old and old-young people, and in the last I place myself …; There are many physicians - and I am a physician - who do not understand or do not want to understand that health is bought, and that there are thousands and thousands of men and women who cannot buy health …; who do not want to understand, that the greater the poverty, the greater the diseases, and the greater the diseases, the greater the poverty ….

Salvador Guillermo Allende Gossens (1908–1973)

Summary

In this chapter, we share our experience with breast tuberculosis (TB) at the surgical department of Wolisso hospital, Ethiopia. It provides an overview of breast TB in women and men. In women, clinical features suggestive of breast

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TB are not well defined, and establishing a diagnosis can be problematic. Breast cancer and a pyogenic abscess may be mistaken for breast TB. The ensuing delay in diagnosis may extend many months, and patients may have to endure numerous ineffective therapies before they are finally identified properly. Axillary lymphadenitis and subsequent sinus or fistula are the most prevalent clinical signs in breast TB. Fine-needle aspiration cytology (FNAC) is the most often used diagnostic method, followed by a biopsy, acid-fast bacteria Ziehl–Neelsen stain (AFB), and culture. Most patients respond well to anti-tubercular treatment alone and have a favorable prognosis. Surgery, when necessary, most frequently consists of excision, followed by drainage, and then mastectomy. Men's breast TB is quite uncommon. Other disorders like gynecomastia and breast carcinoma might be mistaken for this one. It often manifests as a solitary breast lump, accompanied by fever and discomfort. FNAC is the most common diagnostic method. A standard anti-tuberculosis regimen is most commonly employed, in some cases involving incision and drainage.

Graphical Abstract

Wolisso hospital: health professionals at work, Saint Luke's Hospital, Wolisso, Ethiopia. (*Photography by Doctors with Africa-CUAMM*)

Keywords

Breast tuberculosis · Diagnosis · Extrapulmonary tuberculosis · Granulomatous mastitis · Risk factors · Treatment · Tuberculous mastitis

1 Introduction

Tuberculosis (TB) is the most lethal infectious illness in the world today. By far, the great majority of TB deaths (95% of all cases) are in low and middle-income countries (LMICs). Here, TB is one of the leading causes of mortality among women ages 15–44 [1]. TB may affect any part of the body. The breast is a rare extrapulmonary location of TB [2]. Cooper recorded the first case of female breast TB in 1829 [3], but Richet and Powers provided a detailed description of the disease at the end of the nineteenth century [4, 5].

In this chapter, we share our experience with breast TB at the surgical department of Wolisso hospital, Ethiopia. In addition, this chapter provides an overview of breast TB in women from epidemiological, clinical, diagnostic, and therapeutic aspects. A final section concerns breast TB in men.

2 Descriptions of 12 Cases of Breast Tuberculosis at Wolisso Hospital

Doctors with Africa CUAMM (DwA) is a non-governmental organization (NGO) that aims to enhance the health of African communities. DwA works in eightcountries in Sub-Saharan Africa.

The breast TB cases described here were collected at Wolisso hospital, a referral, non-profit facility located in Wolisso town, 115 km from Addis Ababa. Wolisso hospital has 200 beds, with a total staff of 350 people (230 of them qualified health personnel). The hospital carries our roughly 80,000 outpatient visits per year, 15,000 admissions per year, and about 4000 deliveries per year. All the medical records of patients with breast pathology seen in the surgical department of Wolisso hospital were retrospectively and consecutively reviewed for 18 months. During the study period, 2446 women accessing health services in the hospital and in the eight health centers within the catchment area were screened for breast cancer. Twelve women were diagnosed with TB mastitis (0.49% of the total breast pathology). The average age was 32 years (range 17–55 years). All patients were tested for HIV and were negative. Two were pregnant, and one was breastfeeding. One patient was treated for pulmonary TB (PTB) in the past. None of the other patients reported a history of known TB contact or active PTB symptoms. A patient had a history of neck lymphadenitis treated with antibiotics three years before showing current symptoms (potentially a misdiagnosis of TB lymphadenitis). Only one reported constitutional symptoms, specifically fever and occasional night sweating. The duration of symptoms varied from two weeks to more than two years (average 40 weeks). Six cases experienced the involvement of the left breast; five cases the right one; one bilateral. In the vast majority of cases ($N = 8$), the lesion appeared as a bump in the breast. Signs of surrounding inflammation were detected in two patients, and axillary's lymph nodes involvement was detected in five ones. A cold abscess was documented in two cases. The remaining four patients showed more advanced conditions like bilateral masses with sinus discharge and axillary's and

neck lymphadenitis in one case. Fungated masses with open wounds and discharge were discovered in the remaining three cases. Eight patients revealed epithelioid cell granulomas and necrosis using the fine-needle aspiration cytology (FNAC), which was the sole diagnostic technique during this time period. FNAC was not conclusive in four samples, and the diagnosis was essentially clinical. Anti-TB treatment (ATT) was prescribed with isoniazid, rifampicin, pyrazinamide, and ethambutol for two months, followed by a two-drug continuation phase with isoniazid and rifampicin for four months. Follow-up was available for six patients: five completed the treatment with complete resolution of the symptoms. After completing six months of treatment with good adherence, one patient was still symptomatic; an extended duration of ATT of three months, together with the repeated aspiration of the abscess, was successfully performed.

3 Epidemiology

In a systematic review of 1478 breast TB cases [6], the prevalence of breast TB in LMICs ranged from 0.2% [7] to 6.8% [8], with an average of 1.7%. In Western countries, breast TB prevalence is rare. Women of reproductive age are most likely to develop breast TB; however, older women may also be affected [9–11]. Notably, women under 18 are least unlikely to get breast TB [12–14].

4 Risk Factors

Multiparity, pregnancy, breastfeeding, HIV-positive status, and a history of TB have all been linked to an increased risk of breast TB. Pregnant and breastfeeding women with dilated breast ducts may be more susceptible to infection [15]. Pregnancy reduces the T-helper 1 pro-inflammatory response, increasing the risk of a new infection or TB reactivation [16]. In a recent systematic review [6], 67% of breast TB cases were aged 14–45; 4% were pregnant; 15% were lactating mothers; 70% had multiparity. These figures do not provide conclusive evidence that the aforementioned features are risk factors for breast TB. However, they suggest a prior pregnancy may have induced breast TB. Also, though breast TB is unusual to be a presenting sign of HIV, having HIV makes an individual prone to a new infection, reactivation, and further bouts of TB due to exogenous reinfection [17–21].

5 Clinical Presentation

The breast infection is frequently due to another infection, which may or may not be clinically obvious [22, 23]. When no demonstrable TB focus exists elsewhere, breast TB may be the primary site that can spread by lymphatic dissemination from the axillary lymph nodes [2].

Breast TB manifests with a range of clinical presentations, making it a difficult-to-diagnose condition. Breast cancer and a pyogenic abscess may be mistaken for breast TB [2, 9]. The ensuing delay in diagnosis may extend many months, and patients may have to endure numerous ineffective therapies before they are finally identified properly [24, 25]. A lump on the breast is the most frequent clinical manifestation, whether or not it is painful. The lump may seem like a cancerous tumor, as it is hard and firmly attached to the skin, muscles, or chest wall [9, 26]. Swelling of the breasts, edema, fistulization, sinuses, and skin ulcers are other possible manifestations [27–30].

In the largest review of breast TB in women [6], 75% of patients were presented with a breast lump, followed by 15% of cases with a breast abscess and a few cases with cold abscess and diffuse breast inflammation. The disease equally affected either breast, and bilateral localization was rare [6]. Axillary lymphadenitis accounted for 33% of all cases, whereas sinus or fistula formation accounted for 24%, skin ulceration 23%, and nipple retraction 17% [6]. It is noteworthy compared to patients with cancer who display gross nipple-areola involvement in eight to 12% of instances [31, 32]. Contrary to breast cancer, where localized pain is uncommon [33], the most common constitutional symptoms in breast TB were pain (42% of the cases) and fever (28%) [6]. Usually, the pain is noncyclical mastalgia that is not tied to the menstrual cycle (as it is in fibrocystic disease or breast abscess).

Before seeking medical attention, the length of symptoms differed widely across people and regions of the country, ranging from a few weeks in Europe [13, 34, 35] to several months in India and sub-Saharan Africa on average [27, 29, 36]. This is due to both patients and healthcare providers encountering various delays, including those related to the complexity of the diagnosis and socioeconomic disparities [37].

6 Diagnosis

The differential diagnoses to be considered are fibroadenoma [38, 39] and, to a lesser extent, cancer, inflammatory and infectious diseases [40–43], and fat necrosis [38, 44, 45]. Cancer and breast TB comorbidity is quite uncommon [46–48].

Both acid-fast bacteria Ziehl–Neelsen stain (AFB) and culture can make breast TB diagnosis definite by detecting *Mycobacterium tuberculosis* (*M. tb*). AFB is not sensitive enough for paucibacillary infections, while the cost of culture is prohibitive in low-resource settings [2, 9]. Polymerase chain reaction (PCR) may also be used to detect *M. tb* [30]. Fine needle aspiration cytology (FNAC) is more frequently preferred, but it has its own problems; it reveals the existence of epithelioid cell granulomas and necrosis, while its results remain not applicable to difficult to diagnose cases, e.g., granulomatous mastitis and sarcoidosis [49, 50]. On biopsy, pathological findings include persistent granulomatous inflammation, caseous necrosis, and Langhans-type giant cells [9]. Finally, imaging modalities

like ultrasound, mammography, computed tomography (CT), and magnetic resonance imaging (MRI) can be employed, though with limited application in LMICS and not for a clear diagnosis [7, 27, 30, 51–53].

Taken together, the two most common diagnostic methodologies appear to be FNAC and biopsy, being applied in 75% and 90% of cases, respectively. Biopsies have the greatest sensitivity but the worst specificity; FNACs have neither. In most instances, treatment response to ATT confirms the pathology-based diagnosis [6, 54]. Table 1 reports the diagnostic procedures in large cohorts of female breast TB.

7 Treatment

Overall, breast TB has a good prognosis. In most cases, anti-TB drugs are prescribed, either with or instead of surgery [2, 22]. Patients are often administered a standard ATT that includes two phases:

1. two months of isoniazid, rifampicin, pyrazinamide, and ethambutol; and
2. four months of isoniazid and rifampicin [2].

There is also a nine-month regimen that again takes place in two phases:

1. two months of isoniazid, rifampicin, pyrazinamide, and ethambutol; and
2. seven months of isoniazid and rifampicin [28, 59].

Some patients might develop multidrug-resistant TB (MDR-TB), necessitating a different ATT regimen [72, 73]. Quaglio et al. reported, through a systematic review of studies, that 70% of patients were given the conventional six-month ATT and 30% an ATT that was altered regarding the kind and length of time it was administered [6]. Most people showed full recovery [2, 6].

Often, patients who had surgery did so after months of visiting a doctor for their first symptoms when late presentation resulted in abscesses or sinuses requiring surgery [6]. Cold abscess aspiration, sinus formation, or necrotic tissue removal are usually sufficient; however, a complete or partial mastectomy may be necessary in certain cases [2].

8 Tuberculous Mastitis in Men

Breast TB in men is an extremely rare clinical entity, even in those LMICs where TB is common. As early as 1927 [74], the first incidence of male breast TB was documented. When World War II ended, there were only 21 documented instances of breast TB in males [74–77]. A study of around 800 men with breast mass by Lilleng et al. found no evidence of breast TB [78].

Table 1 Brest TB in women: list of the largest series described in the literature (20 or more cases)

Refs.	Firs author	Year	Country	No. of cases	Lump lesions[a]	Diagnosis[c]				
						X-ray[b]	FNAC	Biopsy	Culture	AFB
[55]	Kakkar	2000	India	160	160/160	U	118/160	31/31	U	6/28
[28]	Shinde	1995	India	100	85/100	2/100	21/60	61/61	6/9	21/100
[56]	Ben Hassouna	2005	Tunisia	65	55/65	1/65	2/8	65/65	U	0/0
[57]	Ramaema	2015	South Africa	65	17/65	15/47	18/65	61/65	0/0	7/65
[58]	Metha	2010	India	63	46/63	7/63	47/54	16/16	0/0	3/4
[27]	Khanna	2002	India	52	39/52	7/52	52/52	0/0	0/4	0/4
[59]	Jalali	2005	Pakistan	50	30/50	1/50	U	U	U	U
[60]	Khan	2014	Bangladesh	50	40/50	6/50	5/5	0/0	0/0	0/5
[61]	McGuire	2019	UK	47	41/47	6/47	13/23	12/36	15/36	2/33
[62]	Kilic	2016	Turkey	46	34/46	3/46	0/0	29/31	9/46	4/46
[63]	Puneet	2005	India	42	U	U	42/42	0/0	U	28/42
[36]	Harris	2006	India	38	33/38	5/38	28/28	9/9	1/22	2/22
[64]	Longman	2017	UK	33	20/33	U	4/16	10/17	U	U
[65]	Farrokh	2019	Iran	32	28/32	9/32	U	U	U	U
[22]	Tewari	2005	India	30	22/30	0/30	11/30	23/23	0/0	0/0
[14]	Afridi	2009	Pakistan	30	6/30	4/30	6/6	12/12	3/30	4/30
[66]	Gonzales Muro	2013	Peru	29	8/29	10/29	U	U	U	U
[67]	Pinto Paz	2013	Peru	28	6/28	0/28	0/0	28/28	2/28	2/28
[68]	Tanrikulu	2010	Turkey	27	11/27	U	2/27	22/27	1/27	0/0
[69]	Lin	2010	Taiwan	26	17/26	0/26	0/2	11/24	1/15	3/17

(continued)

Table 1 (continued)

Refs.	Firs author	Year	Country	No. of cases	Lump lesions[a]	Diagnosis[c]				
						X-ray[b]	FNAC	Biopsy	Culture	AFB
[50]	Gupta	1999	India	22	22/22	U	22/22	0/0	U	5/22
[70]	Tandon	2014	India	22	8/22	4/22	U	U	U	U
[71]	Khodabakhshi	2014	Iran	22	13/22	U	0/0	18/22	2/22	U
[51]	Meerkotter	2011	South Africa	21	7/21	U	21/21	0/0	13/21	2/21
[23]	Da Silva	2009	Brasil	20	5/20	0/1	0/0	19/19	U	1/20

[a] Lump lesions on the total of the case described
[b] Previous TB; *FNAC* Fine-needle aspiration cytology; *AFB* Acid-fast bacilli; *U* Unknown
[c] Positive on the total number of tests performed

Like female breast TB, male breast TB has no specific clinical symptoms, which may lead to it being misdiagnosed for other clinical disorders, such as gynecomastia and breast cancer [79]. A recent systematic review on TB mastitis in men found 26 cases of male breast TB. Around 90% of patients had an isolated breast lump, 28% had axillary lymphadenitis, and 33% had skin irritation. Pain (65%) and fever (35%) were the most prevalent constitutional symptoms. FNAC was the most often used diagnostic technique. Both incision and drainage and a conventional ATT were used alone or in combination [80]. Table 2 summarizes 30 case reports of breast TB in men.

Table 2 Table summary of 30 reported cases of breast TB in men

Refs.	First author	Year	Country	Age	Type of lesion (localization)	Diagnosis
[81]	Wilson	1990	USA	83	Disseminated (L)	X-ray−, culture +, AFB-
[81]	Wilson	1990	USA	66	Lump (L)	X-ray*, biopsy −, culture+, AFB−
[82]	Jaideep	1997	India	43	Lump (R)	X-ray−, FNAC +
[83]	Thompson	1997	USA	58	Lump (R)	X-ray−, FNAC +, culture+, AFB+
[84]	Reyes	1999	USA	68	Lump (R)	X-ray−, FNAC +, culture+, AFB+
[85]	Luna	2000	Spain	17	Lump (L)	Biopsy+, culture +, AFB−
[86]	Gupta	2003	India	25	Lump (R)	FNAC+
[30]	Bani-Hani	2005	Jordan	68	Lump (L)	X-ray*, biopsy+
[87]	Winzer	2005	Germany	53	Lump (R)	X-ray−, culture −, PCR+
[88]	Marie	2007	France	63	Lump (R)	Biopsy+, Culture+, AFB +, PCR+
[89]	Reyes	2007	USA	68	Disseminated (R)	FNAC+, AFB+, culture+
[89]	Reyes	2007	USA	59	Lump (U)	FNAC+, AFB+
[89]	Reyes	2007	USA	29	Lump (U)	FNAC+, AFB+
[90]	Ursavas	2007	Turkey	41	Lump (R)	X-ray−, FNAC +, culture+, AFB+
[11]	Luh	2007	China	92	Lump (R)	X-ray−, FNAC −, culture−, biopsy+

(continued)

Table 2 (continued)

Refs.	First author	Year	Country	Age	Type of lesion (localization)	Diagnosis
[11]	Luh	2007	China	80	Lump (L)	FNAC−, biopsy +
[91]	Rajagopala	2008	India	25	Lump (R)	X-ray+, FNAC +, AFB+
[92]	Moujahid	2011	Morocco	50	Lump and abscess (L)	X-ray−, biopsy +, culture+
[92]	Moujahid	2011	Morocco	50	Lump and abscess (L)	X-ray−, biopsy +, culture+
[93]	Cantisani	2013	Italy	28	Lump (R)	X-ray−, FNAC +, culture+, AFB+
[94]	Rabesalama	2013	Madagascar	23	Lump (R)	X-ray−, biopsy +
[95]	Mahajan	2014	India	20	Lump (L)	X-ray−, FNAC +, culture+, AFB+
[96]	El Hammoumi	2014	Morocco	55	Lump (L)	X-ray−, FNAC +, biopsy+, culture+
[97]	Prakash	2015	India	60	Lump (R)	FNAC+, biopsy +, AFB−
[98]	Khaparde	2015	India	60	Lump (R)	FNAC−, culture +, AFB+, biopsy+
[99]	Brown	2016	UK	44	Abscess (L)	X-ray+, culture +, AFB−, PCR+
[100]	Orerah	2016	Kenya	70	Lump (L)	PCR+
[101]	Mutcal	2018	Turkey	28	Not reported (L)	X-ray−, AFB+
[102]	Fatima	2019	Pakistan	62	Lump (L)	X-ray−, biopsy +, AFB+
[103]	Wembulua Shinga	2020	Senegal	33	Ulceration (R)	GeneXpert−, AFB−, biopsy+ for GM
[104]	Haddaoui	2020	Morocco	32	Lump (L)	GeneXpert+, culture−

L Left; *R* Right; *FNAC* Fine-needle aspiration cytology; *AFB* Acid-fast bacilli; *PCR* Polymerase chain reaction; *U* Unknown; X-ray*, previous TB; *GM* Granulomatous mastitis

9 Conclusion

From a clinical, diagnostic, and treatment point of view, breast TB is a poorly defined health condition. Western nations have a well-established technique for diagnosing breast lumps called the "triple evaluation" [105]. Unfortunately, in many LMICs, this process is not possible. The diagnosis of TB mastitis—especially in LMICs—is delayed, mainly because of the time taken between the onset of symptoms and first consultation. The health authorities must further implement educational campaigns designed to increase awareness of breast TB among health professionals and the general population, improving attitudes and perceptions. Breast TB data collection should be standardized for both routine monitoring and future research. Especially, data should be collected on risk factors, symptoms, and clinical presentations for diagnostic purposes.

Core Messages

- Breast TB has a protean clinical presentation.
- Establishing a diagnosis of breast TB is challenging.
- The time it takes to diagnose breast TB might be months.
- The most common clinical appearance of breast TB is a breast lump.
- The most common diagnostic methods of breast TB are FNAC and biopsy.

References

1. World Health Organisation (WHO) (2019) Global tuberculosis report
2. Baharoon S (2008) Tuberculosis of the breast. Ann Thorac Med 3:110–114
3. Cooper A (1829) Illustration of the diseases of the breast. Part I. Longmans. Orme, Brown and Green, London, United Kingdom
4. Richet M (1880) Tumeur rare du sein; sarcome kystique. Gaz Hop LIII:553
5. Powers C (1894) Tuberculosis of the breast. Ann Surg 20:159–164
6. Quaglio GL, Pizzol D, Isaakidis P et al (2019) Breast tuberculosis in women: a systematic review. Am J Trop Med Hyg 101(1):12–21
7. Sakr AA, Fawzy RK, Fadaly G, Baky MA (2004) Mammographic and sonographic features of tuberculous mastitis. Eur J Radiol 51:54–60
8. Goyal M, Sharma R, Sharma A, Chumber S, Sawhney S, Berry M (1998) Chest wall tuberculosis simulating breast carcinoma: imaging appearance. Australas Radiol 42:86–87
9. Marinopoulos S, Lourantou D, Gatzionis T, Dimitrakakis C, Papaspyrou I, Antsaklis A (2012) Breast tuberculosis: diagnosis, management and treatment. Int J Surg Case Rep 3:548–550
10. Zandrino F, Monetti F, Gandolfo N (2000) Primary tuberculosis of the breast. A case report. Acta Radiol 41:61–63
11. Luh SP, Hsu JD, Lai YS, Chen SW (2007) Primary tuberculous infection of breast: experiences of surgical resection for aged patients and review of literature. J Zhejiang Univ Sci B 8:580–583

12. Indumathi CK, Alladi A, Dinakar C, Rout PL (2007) Tuberculosis of the breast in an adolescent girl: a rare presentation. J Trop Pediatr 53:133–134
13. Green M, Millar E, Merai H, O'shea M, Dedicoat M, Inglea H (2013) Mammary tuberculosis in the young: a case report and literature review. Breast Dis 34:39–42
14. Afridi SP, Memon A, Rehman SU, Memon A, Baig N (2009) Spectrum of breast tuberculosis. J Coll Physicians Surg Pak 19:158–161
15. Walker M (2008) Conquering common breast-feeding problems. J Perinat Neonatal Nurs 22:267–274
16. Mathad JS, Gupta A (2012) Tuberculosis in pregnant and postpartum women: epidemiology, management, and research gaps. Clin Infect Dis 55:1532–1549
17. Sharma SK, Kadhiravan T, Banga A, Goyal T, Bhatia I, Saha PK (2004) Spectrum of clinical disease in a series of 135 hospitalised HIV-infected patients from north India. BMC Infect Dis 4:52
18. Corbett EL, Steketee RW, ter Kuile FO, Latif AS, Kamali A, Hayes RJ (2002) HIV-1/AIDS and the control of other infectious diseases in Africa. Lancet 359:2177–2187
19. Korenromp EL, Scano F, Williams BG, Dye C, Nunn P (2003) Effects of human immunodeficiency virus infection on recurrence of tuberculosis after rifampin-based treatment: an analytical review. Clin Infect Dis 37:101–112
20. Fred HL (1995) An enlarging breast mass in an HIV-seropositive woman. Hosp Pract 30:31–32
21. Hartstein M, Leaf HL (1992) Tuberculosis of the breast as a presenting manifestation of AIDS. Clin Infect Dis 15:692–693
22. Tewari M, Shukla HS (2005) Breast tuberculosis: diagnosis, clinical features and management. Indian J Med Res 122:103–110
23. Da Silva BB, Lopes-Costa PV, Pires CG, Pereira-Filho JD, Santos AR (2009) Tuberculosis of the breast: analysis of 20 cases and a literature review. Trans R Soc Trop Med Hyg 103:559–563
24. Da Silva BB, dos Santos LG, Costa PV, Pires CG, Borges AS (2005) Primary tuberculosis of the breast mimicking carcinoma. Am J Trop Med Hyg 73:975–976
25. Akçay MN, Saglam L, Polat P, Erdogan F, Albayrak Y, Povoskı SP (2007) Mammary tuberculosis—importance of recognition and differentiation from that of a breast malignancy: report of three cases and review of the literature. World J Surg Oncol 5:67
26. Jah A, Mulla R, Lawrence FD, Pittam M, Ravichandran D (2004) Tuberculosis of the breast: experience of a UK breast clinic serving an ethnically diverse population. Ann R Coll Surg Engl 86:416–419
27. Khanna R, Prasanna GV, Gupta P, Kumar M, Khanna S, Khanna AK (2002) Mammary tuberculosis: report on 52 cases. Postgrad Med J 78:422–424
28. Shinde SR, Chandawarkar RY, Deshmukh SP (1995) Tuberculosis of the breast masquerading as carcinoma: a study of 100 patients. World J Surg 19:379–381
29. Elsiddig KE, Khalil EA, Elhag IA, Elsafi ME, Suleiman GM, Elkhidir IM et al (2003) Granulomatous mammary disease: ten years' experience with fine needle aspiration cytology. Int J Tuberc Lung Dis 7:365–369
30. Bani-Hani KE, Yaghan RJ, Matalka II, Mazahreh TS (2005) Tuberculous mastitis: a disease not to be forgotten. Int J Tuberc Lung Dis 9:920–925
31. Laronga C, Kemp B, Johnston D, Robb GL, Singletary SE (1999) The incidence of occult nipple-areola complex involvement in breast cancer patients receiving a skin-sparing mastectomy. Ann Surg Oncol 6:609–613
32. Santini D, Taffurelli M, Gelli MC, Grassigli A, Giosa F, Marrano D et al (1989) Neoplastic involvement of nipple-areolar complex in invasive breast cancer. Am J Surg 158:399–403
33. Iddon J, Dixon JM (2013) Mastalgia. BMJ 347:3288
34. Meggiorini ML, Vitolo D, Russo A, Trinchieri V, De Felice C (2012) Breast tuberculosis: rare but still present in Italy. A case of mycobacterium breast infection. Breast Dis 33:177–182

35. Soto C, Vizcaıno I, Isarria S, Pastor MR (2008) Tuberculosis of the breast: imaging findings in two patients. Radiologia 50:518–521
36. Harris SH, Khan MA, Khan R, Haque F, Syed A, Ansari MM (2006) Mammary tuberculosis: analysis of thirty-eight patients. ANZ J Surg 76:234–237
37. Segagni Lusignani L, Quaglio GL, Atzori A et al (2013) Factors associated with patient and health care system delay in diagnosis for tuberculosis in the province of Luanda, Angola. BMC Infect Dis 13–168
38. Olu-Eddo AN, Ugiagbe EE (2011) Benign breast lesion in an African population. Niger Med J 52:211–216
39. Amin AL, Purdy AC, Mattingly JD, Kong AL, Termuhlen PM (2013) Benign breast disease. Surg Clin North Am 93:299–308
40. Seo HR, Na KY, Yim HE, Kim TH, Kang DK, Oh KK et al (2012) Differential diagnosis in idiopathic granulomatous mastitis and tuberculous mastitis. J Breast Cancer 15:111–118
41. Nicholson BT, Mills SE (2007) Sarcoidosis of the breast: an unusual presentation of a systemic disease. Breast J 13:99–100
42. Mathew M, Siwawa P, Misra S (2015) Idiopathic granulomatous mastitis: an inflammatory breast condition with review of the literature. BMJ Case Rep 2015
43. Korkut E, Akcay MN, Karadeniz E, Subasi ID, Gursan N (2015) Granulomatous mastitis: a ten-year experience at a university hospital. Eurasian J Med 47:165–173
44. Nemenqani D, Yaqoob N (2009) Fine needle aspiration cytology of inflammatory breast lesions. J Pak Med Assoc 59:167–170
45. Kataria SP, Sharma J, Singh G, Kumar S, Malik S, Kumar V (2016) Primary breast mucormycosis: FNAC diagnosis of a rare entity. Diagn Cytopathol 44:761–763
46. Alzaraa A, Dalal N (2008) Coexistence of carcinoma and tuberculosis in one breast. World J Surg Oncol 6:29
47. Tulasi NR, Raju PC, Damodaran V, Radhika TS (2006) A spectrum of coexistent tuberculosis and carcinoma in the breast and axillary lymph nodes: report of five cases. Breast 15:437–439
48. Akbulut S, Sogutcu N, Yagmur Y (2011) Coexistence of breast cancer and tuberculosis in axillary lymph nodes: a case report and literature review. Breast Cancer Res Treat 130:1037–1042
49. Martınez-Parra D, Nevado-Santos M, Melendez-Guerrero B, Garcıa-Solano J, Hierro-Guilmain CC, Perez-Guillermo M (1997) Utility of fine-needle aspiration in the diagnosis of granulomatous lesions of the breast. Diagn Cytopathol 17:108–114
50. Gupta D, Rajwanshi A, Gupta SK, Nijhawan R, Saran RK, Singh R (1999) Fine needle aspiration cytology in the diagnosis of tuberculous mastitis. Acta Cytol 43:191–194
51. Meerkotter D, Spiegel K, Page-Shipp LS (2011) Imaging of tuberculosis of the breast: 21 cases and a review of the literature. J Med Imaging Radiat Oncol 55:453–460
52. Makanjuola D, Murshid K, Al Sulaimani S, Al SM (1996) Mammographic features of breast tuberculosis: the skin bulge and sinus tract sign. Clin Radiol 51:354–358
53. Romero C, Carreira C, Cereceda C, Pinto J, Lopez R, Bolanos F (2000) Mammary tuberculosis: percutaneous treatment of a mammary tuberculous abscess. Eur Radiol 10:531–533
54. Ozol D (2006) Bacteriology or pathology for tuberculosis mastitis. Int J Tuberc Lung Dis 10:824
55. Kakkar S, Kapila K, Singh MK, Verma K (2000) Tuberculosis of the breast. A cytomorphologic study. Acta Cytol 44:292–296
56. Ben Hassouna J, Gamoudi A, Bouzaiene H, Dhiab T, Khomsi F, Chargui R, Sifi H, Mtaallah M, Makhlouf R, Chebbi A, Boussen H, Héchiche M, Rahal K (2005) Mammary tuberculosis: a retrospective study of 65 cases. Gynecol Obstet Fertil 33:870–876
57. Ramaema DP, Buccimazza I, Hift RJ (2015) Prevalence of breast tuberculosis: retrospective analysis of 65 patients attending a tertiary hospital in Durban, South Africa. S Afr Med J 105:866–869

58. Mehta G, Mittal A, Verma S (2010) Breast tuberculosis. Clinical spectrum and management. Indian J Surg 72:433–437
59. Jalali U, Rasul S, Khan A, Baig N, Khan A, Akhter R (2005) Tuberculous mastitis. J Coll Physicians Surg Pak 15:234–237
60. Khan MR, Barua A, Tarek N, Rouf A, Karim A, Bhiyan NH, Bhattacharjee T, Nizamuddin M (2014) Mammary tuberculosis: a clinical experience on 50 cases. Chattagram Maa-O-Shishu Hospital Medical College J 13:2
61. McGuire E, Carey L, Tiberi S, Rahman A, Jayasekera N, White V, Kunst H (2020) Breast tuberculosis in East London: a 13-year retrospective observational study. Breast J 26:235–239
62. Kilic MO, Sağlam C, Ağca FD, Terzioğlu SG (2016) Clinical, diagnostic and therapeutic management of patients with breast tuberculosis: analysis of 46 cases. Kaohsiung J Med Sci 32:27–31
63. Puneet MS, Tiwary SK, Ragini R, Singh S, Gupta SK, Shukla VK (2004) Breast tuberculosis: still common in India. Internet J Trop Med 2:1–4
64. Longman CF, Campion T, Butler B, Suaris TD, Khanam A, Kunst H, Tiberi S, O'Keeffe SA (2017) Imaging features and diagnosis of tuberculosis of the breast. Clin Radiol 72:217–222
65. Farrokh D, Alamdaran A, Feyzi Laeen A, Fallah Rastegar Y, Abbasi B (2019) Tuberculous mastitis: a review of 32 cases. Int J Infect Dis 87:135–142
66. Gonzales Muro DJ, Siccha GC, Gutiérrez RR (2013) Características clínicas de la tuberculosis mamaria en pacientes atendidas en un servicio de ginecoobstetricia, 2002–2011. Rev Peruana Ginecol Obstet 59:107–113
67. Pinto Paz ME, Piazze LR, Garcia FB, Santa Cruz E, Carrera Palao D (2014) Mastitis crónica granulomatosa tuberculosa. Diagnóstico y tratamiento en 28 casos. Rev Senol Patol Mamar 27:27–33
68. Tanrikulu AC, Abakay A, Abakay O, Kapan M (2010) Breast tuberculosis in Southeast Turkey: report of 27 cases. Breast Care (Basel) 5:154–157
69. Lin TL, Chi SY, Liu JW, Chou FF (2010) Tuberculosis of the breast: 10 years' experience in one institution. Int J Tuberc Lung Dis 14:758–763
70. Tandon M, Chintamani PP (2014) Breast tuberculosis at a tertiary care centre: a retrospective analysis of 22 cases. Breast Dis 34:127–130
71. Khodabakhshi B, Mehravar F (2014) Breast tuberculosis in northeast Iran: review of 22 cases. BMC Womens Health 14:72
72. Kumar P, Sharma N (2003) Primary MDR-TB of the breast. Indian J Chest Dis Allied Sci 45:63–65
73. Giri VP, Giri P, Kumawat P (2017) Primary multidrug-resistant tuberculosis of the breast—a rare presentation. Ann Med Health Sci Res 7:70–72
74. Dickinson AM (1927) Mammary tuberculosis: report of a case in the male. Am J Surg 3:595–597
75. Morgen M (1931) Tuberculosis of the breast. Surg Gynecol Obstet 53:593–605
76. Webster CS (1939) Tuberculosis of the breast. Am J Surg 45:557–6235
77. Crausman RI, Goldman ML (1945) Tuberculosis of the breast: a report of 9 cases including two cases of coexisting carcinoma and tuberculosis. Am J Surg 67:48–56
78. Lilleng R, Paksoy N, Vural G, Langmark F, Hagmar B (1995) Assessment of fine needle aspiration cytology and histopathology for diagnosing male breast masses. Acta Cytol 39:877–881
79. Janes SE, Lengyel JA, Singh S, Aluwihare N, Isgar B (2006) Needle core biopsy for the assessment of unilateral breast masses in men. Breast 15:273–275
80. Quaglio GL, Pizzol D, Bortolani A, Manenti F, Isaakidis P, Putoto G, Olliaro PL (2018) Breast tuberculosis in men: a systematic review. PLoS One 13(4):e0194766
81. Wilson JP, Chapman SW (1990) Tuberculous mastitis. Chest 98:1505–1509
82. Jaideep C, Kumar M, Khanna AK (1997) Male breast tuberculosis. Postgrad Med J 73:428–429

83. Thompson KS, Donzelli J, Jensen J, Pachucki C, Eng AM, Reyes CV (1997) Breast and cutaneous mycobacteriosis: diagnosis by fine-needle aspiration biopsy. Diagn Cytopathol 17:45–49
84. Reyes CV, Thompson KS, Jensen J (1999) Fine needle aspiration biopsy of mastitis secondary to empyema necessitatis. A report of two cases. Acta Cytol 43:873–876
85. Luna A, Julián JF, Mariscal A, Sopena N, Fernández-Llamazares J, Broggi M (2000) Breast tuberculosis in a man. Breast 9:58–59
86. Gupta PP, Gupta KB, Rohtas K, Yadav RK, Agarvval D (2003) Tuberculous mastitis: a review of seven consecutive cases. Ind J Tub 50:47–50
87. Winzer KJ, Menenakos C, Braumann C, Mueller JM, Guski H (2005) Breast mass due to pectoral muscle tuberculosis mimicking breast cancer in a male patient. Int J Infect Dis 9:176–177
88. Marie I, Hervé F, Robaday S, Levesque H (2007) Tuberculous myositis mimicking breast cancer. Quarterly J Med 100:59
89. Reyes CV (2007) Cutaneous tumefaction in empyema necessitatis. Int J Dermatol 46:1294–1297
90. Ursavas A, Ege E, Bilgen OF, Taşdelen I, Coskun F, Sönmez S et al (2007) Breast and osteoarticular tuberculosis in a male patient. Diagn Microbiol Infect Dis 58:477–479
91. Rajagopala S, Agarwal R (2008) Tubercular mastitis in men: case report and systematic review. Am J Med 121:539–544
92. Moujahid M, Ziadi T, Lamsiah T, Ouzzad O, Kechna H, Moudden A (2011) Male breast tuberculosis. Sante 21:57–60
93. Cantisani C, Lazic T, Salvi M, Richetta AG, Frascani F, De Gado F et al (2013) Male tuberculous mastitis: a rare entity. Clin Ter 164:e293–e295
94. Rabesalama SSEN, Randriamandrato TAV, Randrianirina A, Rakotoarijaona AH, Rakoto Ratsimba HN (2013) Brest lump in male: possibility of breast tuberculosis. Revue Tropicale de Chirurgie 7:10–11
95. Mahajan RK, Sharma S, Kumar P, Jangid K (2014) Breast tuberculosis in a immunocompetent adult male. Int J Curr Microbiol Appl Sci 3:686–689
96. El Hammoumi M, Ktaibi A, El Oueriachi F, Arsalane A, Kabiri EH (2015) Breast cancer-mimicking tuberculosis with pacchypleurite in male. Rev Pneumol Clin 71:249–251
97. Prakash V, Kumar V, Mishra A, Verma AK, Joshi A, Kant S (2015) A rare entity of tubercular mastitis with chest wall extension in a male. J Ass Chest Phys 3:57–59
98. Khaparde SH, Jain D (2015) Primary tuberculosis of the male breast. VIMS Health Sci J 2:23–25
99. Brown S, Thekkinkattil DK (2016) Tuberculous cold abscess of breast: an unusual presentation in a male patient. Gland Surg 5:361–365
100. Orerah GI, Wasike RW (2016) Tuberculosis of the breast. Clin Oncol 1:1068
101. Mutcal SI, Kaya A, Alkan M, Özdemir YE Kaya SY (2018) Paradoxical reaction in male breast tuberculosis. Clin Case Stud Rep. http://doi.org/10.15761/CCSR.1000107
102. Fatima K, Naz F (2019) Tuberculosis of male breast: a rare benign entity. Cureus 11(5): e4709
103. Wembulua Shinga B, Ndiaye M, Badiane AS, Belem AR, Andriateloasy S, Aïssatou Lakhe N, Dièye A, Cisse Diallo VMP, Diallo Mbaye K, Ka D, Déguénonvo LF, Seydi M (2020) Tuberculose mammaire primitive chez un sujet de sexe masculin: à propos d'un cas. Ann Biol Clin 78:177–180
104. Haddaoui H, Bouytse K, Bourkadi JE (2020) Pulmonary and breast tuberculosis in man: an unusual association. Pan African Med J 2. http://doi.org/10.11604/pamj-cm.2020.2.23.21097
105. National Institute for Health and Clinical Excellence (2009) Early and locally advanced breast cancer, diagnosis and treatment. National Institute for Health and Clinical Excellence, London

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Central Nervous System Tuberculosis: Pathogenesis, Diagnosis, and Management

34

Alexander E. Braley and Walter A. Hall

A Physician is obligated to consider more than a diseased organ, more than even the whole man, he must view the man in his world.

Harvey Cushing

Summary

Tuberculosis (TB) affects the central nervous system (CNS) in several distinct but interrelated ways. Tuberculous meningitis (TBM) is slow onset meningitis with non-specific origins that develops over days to weeks and yet can overwhelm and incapacitate patients with devastating consequences. Despite TBM representing the most common CNS manifestation of TB infection, the treatment is far from optimized. Therapy involves primarily medical treatment, which is largely derived from pulmonary TB therapy. The efficacy and even the CNS penetration of the antibiotics are poorly studied. TBM leads to several clinical sequelae such as hydrocephalus, arachnoiditis, and vasculitis, causing focal neurologic deficits, stroke, and death. Tuberculomas represent a mass lesion often confused for tumors or abscesses from other organisms and behave similarly to both. Treatment for all of these conditions

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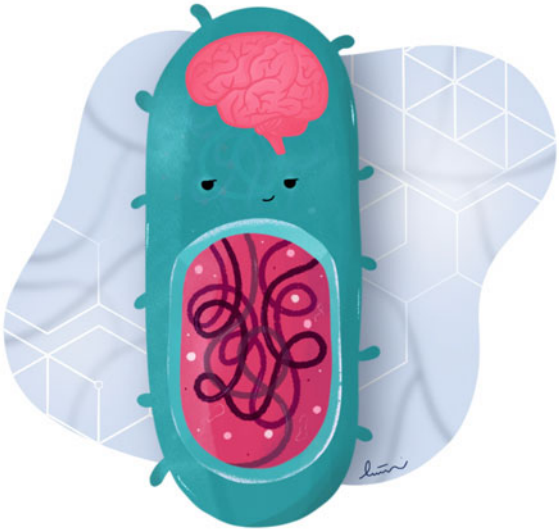
consists of anti-TB therapy; however, key surgical indications and adjunctive medications are important to maximize the clinical outcome of patients affected by CNS-TB.

Graphical Abstract



Tuberculosis of the central nervous system. Adapted with permission from the Association of Science and Art (ASA), Universal Scientific Education and Research Network (USERN); Made by Nastaran-Sadat Hosseini

Keywords

CNS tuberculosis · TBM · Tuberculoma · Tuberculous hydrocephalus · Tuberculous meningitis

1 Introduction

Tuberculosis of the central nervous system (CNS-TB) accounts for one of the least common manifestations of extrapulmonary tuberculosis infections (EPTB) but is the deadliest of the EPTB case types [1, 2]. The high morbidity and mortality of CNS-TB make identifying and managing this disease entity paramount. CNS-TB

can be broken down into several inter-related diseases, including but not limited to tuberculoma, tuberculous meningitis (TBM), tuberculous abscess or tuberculoma, tuberculous hydrocephalus (TBH), spinal tuberculous arachnoiditis, etc. The most common and most deadly of these is TBM.

TB remains a widespread problem that affects developing countries more commonly than developed nations. The most common source of infection in developed nations involves travelers and immigrants from endemic countries. Worldwide, cases have been dropping as identification and treatment modalities have improved. However, nearly ten million new cases are still identified each year globally, with between one and two million deaths occurring annually [1]. The United States has persistently shown declining cases of TB each year, yet still, nearly 10,000 cases are diagnosed per year with an incidence rate of 2.9 per 100,000 [1]. Pulmonary tuberculosis (PTB), as one can imagine, makes up the vast majority of cases, totaling nearly 70% of all cases. Combined PTB and EPTB make up around 10% of cases, and isolated cases of EPTB make up the remaining 20% of cases [1]. EPTB in regions of low incidence tends to be a manifestation of reactivation rather than a new primary infection. Cases involving the CNS make up to 1% of all TB cases and 5–10% of EPTB cases.

This chapter aims to orient the reader to the clinical manifestations of CNS-TB, highlight the importance of expeditious recognition of this entity, and guide the initial management and understanding of complications and sequelae in a practical and applicable way in developed countries and under-developed nations alike. This chapter aims to touch in detail on the most common forms of CNS-TB and briefly address fewer common forms.

2 Pathophysiology

Mycobacterium tuberculosis (*M. tb*) enters the CNS like all EPTB cases via hematogenous spread during the bacteremia phase of infection [1–4]. The different types of TB are interrelated in their etiology. As such, a brief description of the general CNS-TB will be described here. Still, one should recognize that the exact pathophysiology of CNS-TB is poorly understood, with some existing dogma and promising avenues for expansion of our current understanding.

M. tb is an obligate aerobe, perhaps the best-known example of an acid-fast bacterium. TB nearly always originates from a pulmonary source, where it invades and resides in type-II alveolar cells. *M. tb* enters the bloodstream either directly or via an infected leukocyte carrier. This hematogenous spread is critical to extrapulmonary spread (and even intra-pulmonary spread) of the disease [4]. This creates the opportunity for CNS invasion in an incompletely understood fashion. The blood–brain barrier (BBB) and blood-cerebrospinal fluid (CSF) barrier (BCB) are critical structures that prevent large molecules and organisms from entering the protected CNS. The thought has been proposed that the *M. tb* may enter the CNS through a carrier leukocyte; however, this is less likely true given that data shows

CD18-/- mice with leukocyte migration and invasion deficits still develop CNS-TB [3]. Additionally, leukocyte invasion into the CNS is minimal unless the CNS is already under considerable inflammation. Given the propensity for TB to cause CNS and systemic vasculitis, this raises the possibility that the vasculature contributes to the pathogenesis. The longstanding Rich focus origin theory fits neatly with a vascular-derived origin of CNS-TB. Arnold Rich and Howard McCordick postulated that a subcortical or meningeal focus of caseating TB can be created during the bacillemic phase of disseminated TB [5]. These Rich foci can become encapsulated granulomatous masses known as tuberculomas. Given extended periods of bacteremia, there is an increased chance that these Rich foci may deposit along the meninges or near to the ependyma. The rupture of these foci allows the bacteria to enter the subarachnoid space resulting in tuberculous meningitis (TBM). Both tuberculomas and TBM can cause communicating or non-communicating TBH. This illustrates how the three main manifestations of CNS-TB are interrelated, and understanding one disease process leads to a better understanding of the others.

3 Tuberculoma

As stated above, an encapsulated caseating granulomatous collection of tuberculous bacteria is known as a tuberculoma (Fig. 1). Tuberculomas are the most common manifestation of CNS-TB after TBM (Fig. 2). These lesions are often subcortical in location and, unlike a Rich focus, do not rupture to subsequently cause TBM. These lesions are asymptomatic and discovered incidentally on imaging; however, they can cause symptoms secondary to mass effects such as headache, nausea, vomiting, depressed consciousness, and seizures. Depending on the size and location of the lesion, manifestations may take the form of focal neurologic deficits and even hydrocephalus. Brain stem lesions tend to give rise to more cranial nerve palsies and long tract signs (Fig. 3) [6].

3.1 Diagnosis

Tuberculomas on imaging are difficult to differentiate from other mass lesions in the brain as they typically consist of a ring-enhancing lesion with surrounding vasogenic edema on both computed tomography (CT) and magnetic resonance imaging (MRI). The extent of encapsulation and maturity of a lesion can affect its imaging characteristics, with early-stage lesions appearing hypo- or isodense on CT with extensive edema secondary to a very thin abscess capsule. Likewise, mature well-encapsulated lesions appear hyperdense with less surrounding edema [7]. Lesions are usually solitary, but they may be multiple in up to one-third of the cases [7].

Additional advanced imaging modalities are useful in distinguishing tuberculoma from other cerebral mass lesions. Magnetic resonance spectroscopy

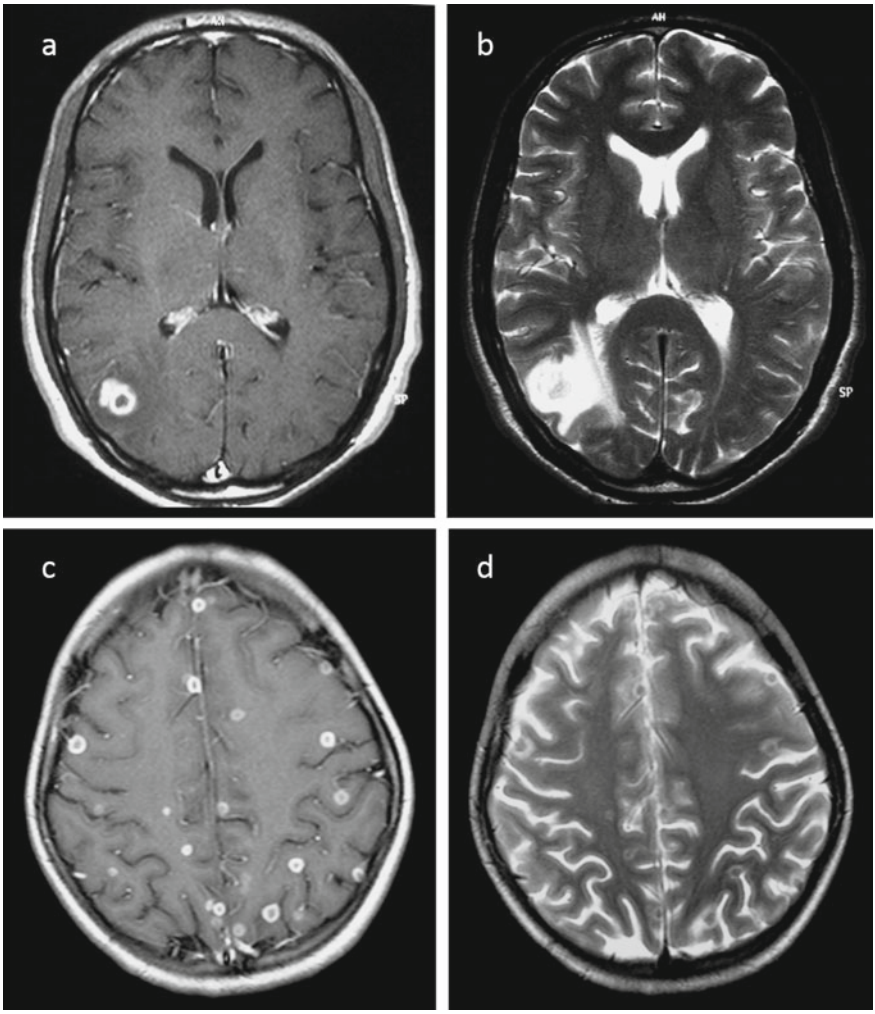


Fig. 1 Representative Magnetic Resonance Imaging (MRI) examples of the spectrum of presentation of CNS tuberculomas. Figures **a**, **b** represent T1 axial contrast-enhanced and T2 non-contrast slices (respectively) of a solitary right parietal tuberculoma with moderate edema and mild mass effect. In contrast, Figures **c**, **d** represent T1 axial contrast-enhanced and T2 non-contrast (respectively) images of multiple brain stem tuberculomas with minimal edema and no mass effect. Reproduced with permission from the courtesy of A. Akhaddar, M.D., Department of Neurosurgery, Avicenne Military Hospital of Marrakech, Mohammed V University in Rabat, Rabat, Morocco

demonstrates a characteristic peak at 3.8 ppm and will have a choline/creatinine ratio that exceeds one, which helps distinguish TB from neurocysticercosis [8]. Positive emission tomography with fluorodeoxyglucose (FDG-PET) will help rule

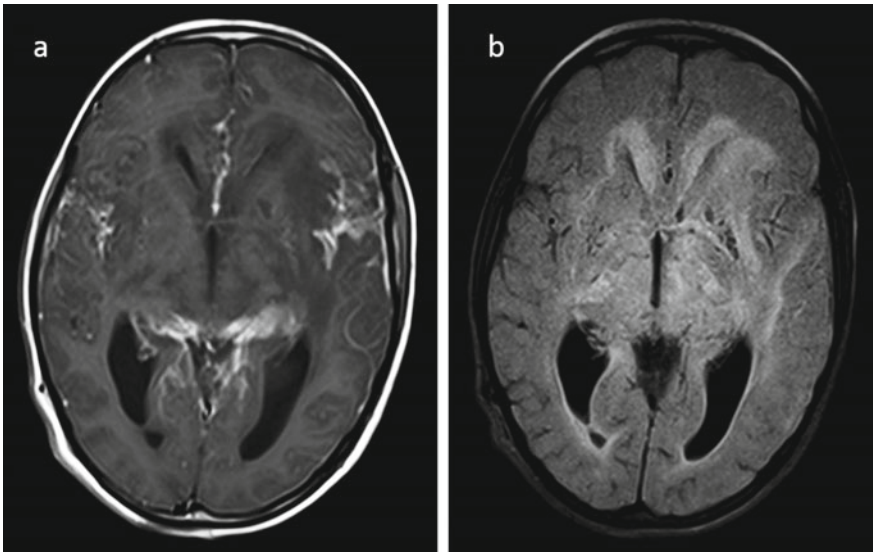


Fig. 2 MRI slices of a patient with tuberculosis meningoencephalitis. Figure **a** is an axial slice of a T1 contrast-enhanced MRI which demonstrates leptomeningeal enhancement in the bilateral Sylvian and interhemispheric fissure as well as the ambient cisterns. Figure **b** is a Non-contrast fluid-attenuated inversion recovery (FLAIR) sequence that demonstrates periventricular edema and left basal ganglia infarcts typical of hydrocephalus and tuberculosis vasculitis secondary to tuberculosis meningitis. Reproduced with permission from the courtesy of A. Akhaddar, M.D., Department of Neurosurgery, Avicenne Military Hospital of Marrakech, Mohammed V University in Rabat, Rabat, Morocco

out neoplasm if uptake is low; however, this is a non-specific finding as uptake may be low or elevated in brain tuberculomas [8].

Given significant encapsulation, *M. tb* may not be detected in the serum or CSF, and the CSF profile may even be normal, unlike in TBM. Pathology samples will demonstrate typical caseating granulomas with Langhans giant cells and epithelioid features. Tuberculomas may result in an aberration of endocrine functions, often resulting in hyponatremia or hypothalamic-pituitary axis disruption. The derangement may manifest as panhypopituitarism or may affect individual hormones, of which adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH), and prolactin are the most commonly affected in descending order [8].

3.2 Management

The management of cerebral tuberculoma consists primarily of medical treatment. Given the significant diagnostic dilemma created by tumor-like symptoms from a ring-enhancing lesion with a variable degree of cerebral edema, tuberculomas may require a definitive pathologic sample for diagnosis. It is important to note that

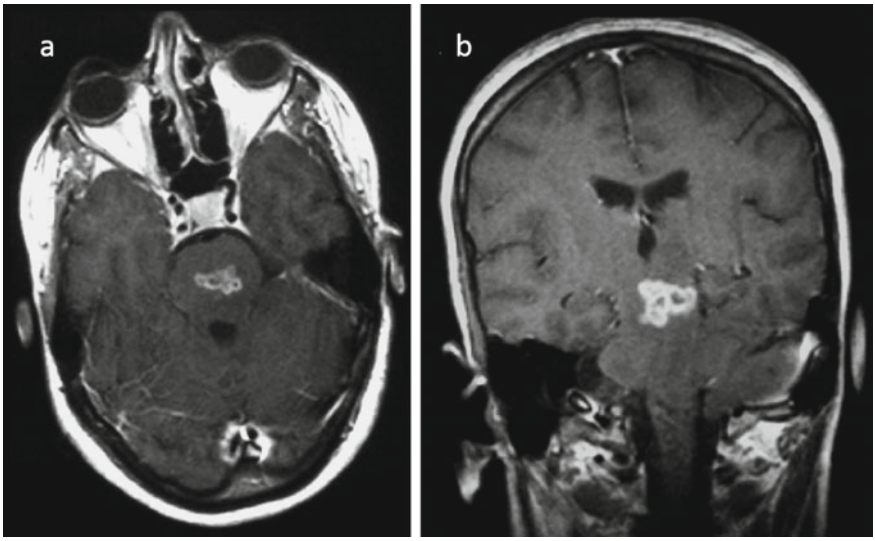


Fig. 3 Multilobulated brainstem tuberculoma as demonstrated by contrast-enhanced T1 axial (**a**) and coronal (**b**) projections. Reproduced with permission from the courtesy of A. Akhaddar, M. D., Department of Neurosurgery, Avicenne Military Hospital of Marrakech, Mohammed V University in Rabat, Rabat, Morocco

anti-tuberculosis therapy (ATT) should not be delayed, pending a complete workup or finalized culture results. Indeed, a low threshold for the initiation of treatment has been linked to improved survival [7]. In the case of well-established primary TB with or without extrapulmonary manifestations, a presumptive diagnosis of tuberculoma may be made based on typical tuberculoma imaging findings. Medical treatment should proceed, and close clinical and radiographic follow-up should be pursued. If imaging fails to show improvement or the patient clinically worsens, the presumptive tuberculoma diagnosis should be called into question, and a definitive biopsy is undertaken [8].

3.3 Surgical Technique

As indicated above, in the case of atypical tuberculous lesions or typical lesions that do not respond appropriately to medical therapy, the patient should undergo a formal brain biopsy. Additionally, scenarios may exist where a previous cancer history or the possibility of infection from other organisms (such as neurocysticercosis, histoplasmosis, etc.) coincides with a known extracranial TB infection. This may require a formal biopsy as it may be possible that the intracranial symptoms and signs are not related to extracranial TB. As such, both diagnoses require very different medical management, making an accurate definitive diagnosis paramount and empiric ATT not a viable option.

When an eloquently located lesion is the most accessible, it is reasonable to opt for a stereotactic needle biopsy or minimally invasive stereotactic microsurgical open biopsy. However, in the case of surface lesions or even non-surface lesions in a non-eloquent area, some advocate for full microsurgical resection even in the absence of mass effect or increased intracranial pressure, as this reduces the amount of remaining viable bacteria and resection of the capsule allows for better penetration of the ATT [6, 8]. Tuberculomas are typically removed with clean surgical planes due to encapsulation of the bacterium. It is important to realize that despite the "gross total" resection of a TB lesion, the duration of medical therapy should not be shortened as a result [6]. Special precautions are unnecessary, and additional personal protective equipment need not be used for suspected or confirmed cases of CNS-TB [6].

4 Tuberculous Meningitis

TBM (Fig. 4) is the most common and most devastating of the cranial manifestations of TB. TBM is characterized by typical meningitis symptoms: stiff neck, headache, fever, leukocytosis, and possible altered mental status. As the inflammation spreads to include the cerebral tissue itself, tuberculosis meningoencephalitis results in more focal neurologic deficits as a consequence. Similarly, irritation and inflammation of the spinal cord lead to tuberculous myelomeningitis. For this chapter, we will not distinguish between tuberculous meningitis and meningoencephalitis. The timeline of TBM is what sets it apart from other entities because the onset of symptoms is usually gradual over days to weeks versus the abrupt onset of symptoms seen with pyogenic meningitis. Leonard describes typical TBM as characterized by three distinct phases:

i. the first consists of two to three weeks of low-grade fever, lethargy, malaise, mild headache, or neck discomfort;
ii. the next phase consists of more typical meningitis symptoms such as severe headache, vomiting, neck pain, neck stiffness, and focal neurologic deficits; and
iii. the final stage involves the paralytic phase, with coma, stupor, seizures, and more profound focal neurologic deficits.

The process typically takes five to eight weeks from inception to mortality [1]. The insidious onset of symptoms may delay the diagnosis and even deceive the treating clinician into assuming that this disease is not as deadly as pyogenic bacterial meningitis, but this cannot be further from the truth. Patients who may have had smoldering meningitis can decompensate acutely, leading to fatality while tests and cultures are pending, which makes prompt recognition and the initiation of empiric anti-tuberculous antibiotics critical [4]. Mortality for TBM can approach 100% for the worst affected patient demographic, which is HIV co-infection with multi-drug resistant TB (MDR-TB). It is also important to note that exposing every

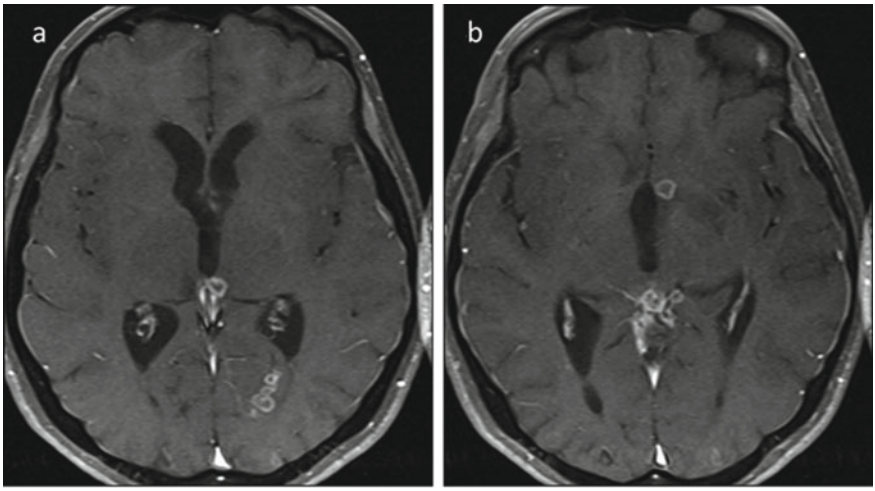


Fig. 4 T1 axial contrast-enhanced MR sequences demonstrating periventricular ring-enhancing tuberculoma lesions with associated tuberculosis hydrocephalus. Reproduced with permission from the courtesy of M. Turgut, M.D., Ph.D., Department of Neurosurgery, Aydin Adnan Menderes University School of Medicine, Aydin, Turkey

patient who theoretically could have TB to ATT medications would lead to a significant risk of toxicity. As such, the key to a good clinical outcome resides in identifying patients with a reasonable risk for a TB infection. Such patients include immigrants or visitors from endemic areas, those in close quarters such as prisons and hospitals, and people at risk for contracting the disease secondary to immune suppression (whether secondary to concomitant disease or advanced age) [4].

4.1 Pathogenesis

As alluded to above, the most well-recited theory on the pathogenesis of TBM dates back to 1933 when Rich and McCordick postulated that prolonged bacteremia seeds the CNS with small foci of *M. tb*, with varying degrees of encapsulation and granuloma formation. These small tubercles may progress to a tuberculoma or may rupture into the subarachnoid space, causing TBM. This Rich focus theory has predominated in the literature for nearly a century. The process by which the bacteria enter the CNS is not well understood and not fully explained by the Rich Focus theory. The most recent challenge to this theory highlights the importance of macrophage infiltration and dissemination into the CNS [4]. This itself is challenged by the evidence discussed above regarding leukocyte migration in immunodeficient mice that do go on to form CNS-TB, particularly TBM [3]. With CNS-TB, extensive vasculitis is seen, which leads to ischemia and infarcts, often in the deep grey matter structures known as the "tubercular zone" [3, 8, 9]. A potential

explanation for the CNS penetration by *M. tb* focuses on the vascular nature of TB. In vitro studies have shown endothelial invasion by *M. tb,* and as such, the initial anchor into the CNS may be represented by endothelial cells themselves.

TBM goes on to cause several sequelae; some will be expanded upon further in this chapter. The T-cell mediated inflammation caused in TBM results in high levels of tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) levels. These sequelae are largely secondary to the aforementioned inflammation. The inflammation results in vasogenic edema, which causes mass effect and focal neurologic deficits [3]. The inflammatory exudate formed blocks normal circulation of CSF and can cause obstructive and communicating hydrocephalus. Inflammation also leads to vasculitis, which, as mentioned above, leads to a potential stroke.

4.2 Diagnosis

Patients presenting with typical symptoms, as stated above, will undergo systemic TB testing but also should have CSF samples taken when safe. *M. tb* DNA or acid-fast bacilli from CSF samples yield around a 50% sensitivity, and as such adjunct tests are often needed [4]. The CSF profile, along with the clinical scenario, helps make the diagnosis of TBM. Duration of symptoms for over four days, age over 30, and CSF with <1000 TNC and >70% lymphocytes are very suggestive of TBM [4]. As with tuberculoma, TBM may result in endocrine dysfunction ranging from hyponatremia to panhypopituitarism. CSF markers such as ALOX-5, which is involved in the metabolic pathway of leukotriene B4 and lipoxin, show promise as a biomarker in the CSF and may play a role in the pathogenesis of TBM [8].

4.3 Management

TBM itself is primarily a disease for medical management. The best medical management largely consists of ATT. As with tuberculoma, there is a relative dearth of information regarding the most efficacious medical regimen, both in antibiotic choice and length of therapy. The treatment guidelines from the World Health Organization (WHO) for management for TBM were originally based on the efficacy of ATT for PTB. This consists of the typical RIPE therapy (Rifampin, Isoniazid, Pyrazinamide, and Ethambutol) for two months and another ten months of Rifampin and Isoniazid. This management is effective for PTB; however, this does not consider CSF/BBB penetration of these medications. For instance, Isoniazid and Pyrazinamide are very effective in crossing the BBB; however, Rifampin crosses weakly, and CSF levels only reach up to 20% of plasma levels. Some dose-ranging trials have demonstrated that typical dosing is too low to reach sufficient efficacy [9]. Ethambutol is very poor at crossing the BBB even during periods of heightened inflammation and may not be helpful during isolated TBM [9]. Davis et al. provide an excellent review of current medical strategies and future avenues and offer a great treatment algorithm for TBM [9].

Although TBM by itself is medically managed, the complications and sequelae of TBM often have surgical indications. As mentioned above, inflammation is key in the pathogenesis of TBM complications, and as such, steroids are extremely efficacious. Nearly all patients with TBM (with the notable exception of HIV co-infected patients) should be treated with steroids. In fact, steroid therapy with ATT and diuretics are often sufficient to delay or prevent the need for surgery, even for conditions such as hydrocephalus [9]. Some groups have advocated additional adjunctive treatment with aspirin to reduce ischemic risks in TBM; this is partially due to the anti-inflammatory and anti-platelet properties [9]. Management of endocrine dysfunction involves a workup to identify the derangement, such as checking for hormone levels and supplementing appropriately. Hyponatremia may be secondary to cerebral salt wasting or the syndrome of inappropriate anti-diuretic hormone (SIADH) secretion. Tuberculoma treatment mainly consists of hypertonic sodium administration or salt supplementation with the possible addition of fludrocortisone if the fluid restriction is not practical [8, 9]. It is important to note that TBM may cause increased intracranial pressure (ICP) secondary to diffuse inflammation and demyelination requiring ICP monitoring [4].

5 Tuberculous Hydrocephalus

Hydrocephalus is the clinical manifestation of neurologic compromise secondary to excessive CSF buildup in the ventricles or extra-axial spaces, resulting in higher than normal pressure on the surrounding cerebral structures. Hydrocephalus takes many forms and may present in numerous different ways. This can range from neonatal hydrocephalus with splayed sutures and enlarging head size to indolent small personality changes and balance difficulties with incontinence found in normal pressure hydrocephalus (NPH). Regardless of the etiology, hydrocephalus tends to be a disease principally managed with neurosurgical techniques ranging from CSF diversion to choroid plexus cauterization.

TBH is largely secondary to the inflammatory exudate that builds up in the basal cisterns' characteristic of TBM. This exudate causes what is described as communicating hydrocephalus, but there are sites of obstruction, so it should not be referred to as non-obstructive in the strictest sense. Although classical obstructive hydrocephalus typically consists of ventricular obstruction, perhaps at the cerebral aqueduct or the third ventricle, TBH in 80% of the cases is secondary to cisternal exudates causing an obstruction. Around 20% of cases will have fourth ventricular outlet obstruction causing hydrocephalus, with the remaining causes such as foramen of Monroe or aqueductal obstruction making up a small proportion [4].

5.1 Diagnosis

Hydrocephalus is measured and diagnosed regardless of the etiology with cranial imaging followed by ICP monitoring and trials of CSF diversion. Imaging characteristic for TBH consists of pan-ventricular dilation with obliteration of the basal cisterns, reduced subarachnoid space volume, and diffuse enhancement after the addition of contrast [4]. Lumbar punctures (LPs) are a key diagnostic procedure for obtaining a CSF sample for cultures and profiles and measuring the ICP to diagnose hydrocephalus. With TBH, LP will correctly diagnose elevated pressure in around 70% of cases, typically where the obstruction is at the basal cisterns [4]. However, as with other forms of obstructive hydrocephalus, LP may incorrectly identify a low opening CSF pressure in the case of obstruction proximal to the fourth ventricular outlets. Additionally, a large-volume LP may be dangerous in this setting, given the potential for inducing downward cerebral herniation. In this case, placing a ventriculostomy may appropriately diagnose the elevated ICP and serve to temporarily divert CSF. Developing countries with limited resources may utilize the Lorber technique wherein 5–8 cc of air is injected into the thecal sac via LP. An immediate upright lateral radiograph of the skull is taken: if the air is found in the ventricles and the cisterns, then the hydrocephalus is communicating; if it is just seen in the cisterns, then it is thought to be non-communicating hydrocephalus [4]. This technique is not necessary for developed countries where advanced imaging techniques (MRI, contrast, isotope, and CSF flow studies) are available to identify the causes of obstruction.

5.2 Management

TBH is quite often successfully treated without permanent CSF diversion. In up to one-third of patients with TBH and in up to 70% of communicating TBH patients, they may avoid the need for a permanent shunt with ATT and steroid treatment with the addition of mannitol, furosemide, and acetazolamide [4, 6]. This is a critical fact given the lack of dedicated neurosurgeons in regions endemic with TB, which can be a fatal scenario given the heightened risk of shunt failure with TBH. Additionally, TBM resulting in hydrocephalus confounds the clinical picture. The Vellore grading system is useful in patients with tuberculous meningitis hydrocephalus (TBMH) as it potentially identifies patients who will be responsive to shunt placement. Vellore grade I and II patients have no neurologic deficits and a normal sensorium or mild neurologic deficits with normal sensorium, respectively. Grade III/IV patients have altered sensorium ranging from lethargy to deep coma and have poor morbidity and mortality rates, with grade IV patients having up to 80% mortality rates [6, 7]. After adequate medical management, these patients with persistent hydrocephalus should undergo shunting and will likely respond, unlike grade III/IV patients [4, 6, 7]. Given the pan-arteritis found in TBM, much of the altered sensorium in higher grade TBMH is thought to be secondary to small infarcts in the thalamus and basal ganglia rather than due to secondary to

hydrocephalus [6]. In fact, only around 20% of grade III/IV patients will improve with shunting. TBMH patients with Vellore grade III/IV should undergo a trial of ventricular drainage, and if patients do not improve, they should not undergo CSF shunting [6].

For patients deemed appropriate to undergo CSF shunting, the choice of the surgical technique remains. TBH consists of CSF high in protein secondary to the inflammatory exudate. This complicates both traditional ventricular shunting procedures and attempts to perform a third ventriculostomy. The exudate leaves traditional ventricular shunting susceptible to frequent shunt failures from obstruction of the valve/catheters; as mentioned before, this is especially troublesome in regions where neurosurgeons are not readily available. Additional risks of shunting involve the risk of a secondary infection of the implanted foreign bodies. A reasonable concern is a possibility of spreading TB to the peritoneal cavity during ventriculoperitoneal shunt; however, in practice, this is not common [6]. Endoscopic third ventriculostomy (ETV) is a well-known and increasingly utilized alternative way for CSF diversion for hydrocephalus that does not involve hardware susceptible to obstruction or the risk of infection secondary to being a retained foreign body. However, ETV is not ideal for acute cases of TBMH given the difficulty of the surgical technique and the risk for failure, both secondary to the murky character of the CSF that makes visualization very difficult, along with the presence of an inflamed thin vascular ventricular floor creates a worrisome combination. Poor visualization during ETV leads to case abortion and increases the risks for complications. Current recommendations are for at least four weeks of ventricular drainage with ATT prior to attempting ETV [6]. HIV co-infected patients may potentially be more suited to ETV as the HIV-TBMH patients have lower ICP and benefit significantly from a reduced shunt infection risk [4].

6 Other CNS Tuberculosis Manifestations

TBM, TBH, and tuberculomas are by far the most common clinical manifestations of a TB infection reaching the CNS. Several other less common CNS-TB-associated conditions will be touched on briefly.

TB abscess is similar to tuberculoma that does not achieve the same granulomatous reaction as in tuberculoma. This condition resembles a pyogenic abscess much more closely and is quite rare compared to tuberculomas. There is also some evidence that TB abscess is over-diagnosed in situations where a tuberculoma has significant caseous necrosis. Management is similar to that of a tuberculoma, and definitive diagnosis requires excision of the abscess with the pathological examination of the abscess wall, which will lack a granulomatous reaction [6].

TB arachnoiditis is often a consequence of TBM and can form in various locations in the CNS. Opticochiasmatic arachnoiditis from TB can result in profound vision loss. TB basal arachnoiditis can form a thick gelatinous exudate around the base of the brain and the cranial nerves leading to significant cranial

neuropathies. This arachnoiditis can also affect the spinal cord. Spinal TB arachnoiditis consists of the encasement of the spinal cord over several weeks forming a similar gelatinous exudate. The mass effect on the spinal cord and nerve roots leads to conditions ranging from radiculopathy to myelopathy and may even mimic cauda equina syndrome [1]. Diagnosis typically requires MRI demonstrating arachnoiditis and CSF with a markedly increased protein count along with evidence of systemic TB or spinal tissue samples suggestive of TB.

7 Conclusion

CNS-TB takes many forms and can have various clinical consequences. It affects developed nations and endemic areas in different ways. It may be an incidentally found tuberculoma on cranial imaging or devastating meningitis, leaving the patient comatose with a high likelihood of mortality. The mainstay of treatment is medical therapy with certain key surgical interventions. The choice of medical therapy depends on the clinical presentation, and there is a significant need for further data to identify the ideal medical treatment regimens for the various manifestations of CNS-TB. Identifying TBMH patients with altered sensorium who will benefit from shunt placement will lead to improved outcomes in this devastating population without performing unnecessary procedures on those who will not improve. Improved imaging modalities and *M. tb* identification on less-invasive biopsy strategies will reduce the need for surgical intervention.

Core Messages

- CNS-TB is a potentially devastating infection with high rates of morbidity and mortality.
- Diagnostic accuracy is critical to the outcome of CNS-TB.
- Medical management is the mainstay for most types of CNS-TB.
- CNS-TB pathologies such as TBH or large tuberculoma may be responsive to surgical intervention.

References

1. Leonard JM (2017) Central nervous system tuberculosis. Microbiol Spectr 5. https://doi.org/10.1128/microbiolspec.TNMI7-0044-2017
2. Schaller MA, Wicke F, Foerch C, Weidauer S (2019) Central nervous system tuberculosis: etiology, clinical manifestations and neuroradiological features. Clin Neuroradiol 29:3–18
3. Be NA, Kim KS, Bishai WR, Jain SK (2009) Pathogenesis of central nervous system tuberculosis

4. Thwaites GE, Schoeman JF (2009) Update on tuberculosis of the central nervous system: pathogenesis, diagnosis, and treatment. Clin Chest Med 30:745–754
5. Rich A, McCordick H (1933) The pathogenesis of tuberculous meningitis. Bull Johns Hopkins Hosp 52:5–37
6. Rajshekhar V (2015) Surgery for brain tuberculosis: a review. Acta Neurochir 157:1665–1678
7. Alorheidi K, Dodin J, Berg J, Hoffman W (2017) Brain tuberculoma: a case report and literature review. S D Med 70:298–301
8. Ramachandran R, Muniyandi M, Iyer V et al (2017) Dilemmas in the diagnosis and treatment of intracranial tuberculomas. J Neurol Sci 381:256–264
9. Davis A, Meintjes G, Wilkinson RJ (2018) Treatment of tuberculous meningitis and its complications in adults. Curr Treat Opt Neurol 20

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Pleural Tuberculosis

35

José M. Porcel and Laura Porcel

Grandes médicos son el sol, el aire, el silencio y el arte.
Great doctors are the sun, the air, the silence and the art.

Santiago Ramón y Cajal *(He fell ill with tuberculosis in 1878 and was admitted to the sanatorium of Panticosa (Huesca, España).)*

Summary

Tuberculosis (TB) is the most common etiology behind pleural effusion in regions where this infection is endemic. The rupture of a subpleural parenchymatous focus into the pleural space is assumed to be the primary cause of TB effusions. A subacute febrile sickness with pleuritic chest discomfort, cough, and variable degrees of dyspnea are common in patients with this condition. Patients present with unilateral, small to moderate in size, and often loculated effusions in association with lung disease on chest radiographs in nearly 30% of the cases. Exudates are always seen in pleural fluid studies, with lymphocytic predominance in 90% of cases, increased adenosine deaminase (ADA) levels in 92%, positive cultures in 30%, and a positive Xpert test in 50%. Hospitalized patients should be

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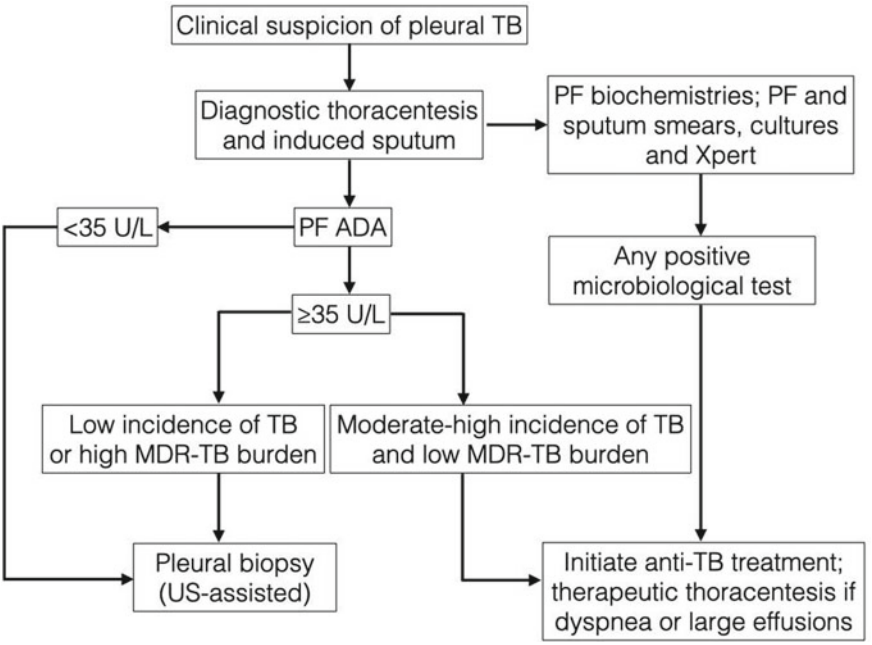
isolated since around 40% of sputum specimens yield bacilli. Other than pleural fluid ADA, unstimulated interferon-γ and interleukin-27 are accurate markers of TB effusions. Pleural biopsy, preferably under ultrasound assistance, also has a high diagnostic sensitivity when pleural tissue is processed for histology, culture, and polymerase chain reaction-based techniques. In regions where TB is widespread to moderate to high degrees and multidrug-resistant TB is of low prevalence, a presumptive diagnosis can be made and empirical anti-TB treatment initiated based on clinical-epidemiologic and pleural fluid ADA data. TB effusions are treated the same way as pulmonary TB. In cases of moderate to large effusions or having trouble breathing, a therapeutic thoracentesis may be warranted. Rarely do TB effusions complicate with TB empyema, which may need a prolonged course of anti-TB drugs and surgical decortication.

Graphical Abstract



A suggested algorithmic approach for the management of suspected TB effusions. Low incidence of TB refers to <10 cases/100,000 population per year, whereas rates of 40/100,000 or greater are considered a high incidence of TB. The high burden of MDR-TB implies a local prevalence of >4% of new cases of TB. ADA, adenosine deaminase; MDR-TB, multidrug-resistant tuberculosis; PF, pleural fluid; TB, tuberculosis; US, ultrasound. Adapted with permission from [1]

Keywords

Adenosine deaminase · Pleural biopsy · Pleural effusion · Tuberculosis · *Tuberculous empyema*

1 Introduction

Tuberculosis (TB) is a public health issue in underdeveloped nations. About 9.9 million people fell in with TB in 2020, and 1.5 million people died from it [2]. This corresponded to a global incidence of 127 cases/100,000 population, ranging from less than ten cases/100,000 in many European countries to more than 500 cases/100,000 in Southern Africa and the Philippines. Geographically, more than 85% of the cases occurred in South-East Asia, Africa, and the Western Pacific. Human immunodeficiency virus (HIV) co-occurred with TB in about 8% of all TB cases [2], but this figure exceeded 50% in some countries [3]. Moreover, in 2020, there were about 158,000 cases of multidrug-resistant TB (MDR-TB) [2].

Extrapulmonary TB (EPTB) commonly involves the pleura. In Catalonia (Spain), there were 1079 notified cases of TB in 2019 (incidence 14.1/100,000), of which pulmonary TB (PTB) accounted for 64.4%, and EPTB for 35.3% [4]. The two most common extrapulmonary sites of involvement were lymph nodes (46%) and pleura (24%, or 11.5% of all TB cases). Yet pleural TB was the predominant cause of extrapulmonary disease in two large series from Zimbabwe (40.7% of 2,024 cases [3]) and Pakistan (29.6% of 15,790 cases [5]), followed by TB lymphadenitis (16.7% and 22.7%, respectively).

Between 1994 and 2013, TB was the fourth major cause of pleural effusions after heart failure, cancer, and pneumonia at our institution in Lleida (Catalonia, Spain) [6]. However, the incidence of TB pleuritis in this area has consistently reduced since then, with just 13 (< 4%) of 356 tapped effusions being related to TB in 2018.[1] TB, on the other hand, is the leading cause of pleural effusion in locations where it is endemic [1].

2 Pathogenesis

In locations where TB is widespread, TB effusions are thought to be caused by a primary infection, while they are thought to be caused by a reactivation of latent TB infection (LTBI) in areas where TB is not common. When *Mycobacterium tuberculous* (*M. tb*) bacilli are released into the pleural space by a ruptured subpleural parenchymal lesion, an immune reaction is triggered in the surrounding area, which leads to pleural TB effusion. Initiated by neutrophils, monocyte migration and a T-helper (Th) type 1 lymphocyte response with the production of interferon-gamma

[1] Unpublished data.

(IFN-γ), other Th-1 type cytokines, and chemokines are all part of the process. Pleural capillaries become more permeable as a result of the inflammatory process. It paves the way for the intense lymphocytic infiltration and granulomatous pleuritis that occlude the lymphatic stomata, resulting in pleural fluid (PF) formation and accumulation [1].

3 Clinical Manifestations

Pleural TB patients are mostly younger than 45 years (with a slight predominance of males), though they tend to be older in developed regions with low TB prevalence where disease reactivation is more common. The disease presents as an acute or, mostly, a subacute syndrome (median duration of symptoms of two to three weeks) associated with fever (80%), pleuritic chest pain (70%), cough (60%), dyspnea (40%), and other potential manifestations (malaise, diaphoresis, weakness, weight loss) [7–10]. HIV co-infected patients may present with a more protected illness, constitutional symptoms, and evidence of disseminated disease [11]. In contrast to bacterial pneumonia, the peripheral leukocyte count in patients with TB effusions is usually normal.

TB effusions are mostly unilateral, modest to moderate in size, and loculated in around half of the instances [10]. In one series, these effusions occupied half or more of the hemithorax in about 40% of TB patients ($N = 320$) [10]. Another study found that pleural TB was the third most common cause of large or massive pleural effusions (12%) after malignancy and pneumonia [12]. Co-existing pulmonary changes are seen in 30% of chest radiographs and 50–85% of computed tomography (CT) scans, mostly in the ipsilateral lungs (70%); however, these percentages vary depending on the kind of TB pleuritis, whether a primary infection or reactivation [9, 10, 13]. Depending on the duration of the effusion, ultrasound findings might range from free-flowing fluids to complicated echogenic and/or septated fluids. At least one-fifth of tuberculin skin test (TST) results are negative [10]. It is imperative that all patients with TB effusions be tested for HIV infection because, as mentioned in the preceding text, around 8% have HIV-TB co-infection.

All suspected TB effusions should be taken for testing if there is enough fluid. According to Light's criteria, the fluid is always an exudate [14]. Lymphocytes predominate (>50% of the total leukocyte count) in 90% of instances, with polymorphonuclear cells predominating in 10%. The percentage of lymphocytes (mostly T lymphocytes) surpasses 90% in more than half of the lymphocyte-rich fluids. Moreover, some neutrophilic TB effusions become lymphocytic predominant on re-aspiration. A PF protein concentration >5 g/dL, glucose level <60 mg/dL, and pH < 7.20 occur in 70%, 25% and 9% of patients, respectively [10].

A distinct entity from TB effusion is TB empyema. It is currently a rare entity, although it may account for up to 9% of all TB effusions in certain areas with a high incidence of TB [15]. TB empyema is caused by an active, persistent pleural

infection, resulting in purulent PF that is loaded with *M. tb* [16]. Presumably, it arises from various potential mechanisms, including progression of an untreated primary TB effusion, direct pleural infection from a burst lymph node, transmission via the bloodstream, post-pneumonectomy, or following a number of outdated pleural procedures (artificial pneumothorax, thoracoplasty, ball plombage, oleothorax). Patients often present with the symptoms of a long-term disease such as fatigue, low-grade fever, weight loss, or even an empyema necessitatis–a burst of thick pus through the chest wall. A thick, calcified pleural rind and rib thickening surround loculated PF on the radiograph. Diagnostic thoracentesis yields a purulent fluid with neutrophilic predominance, low pH and glucose, and a smear markedly positive for *M. tb*. Finally, TB may seldom be complicated by a cholesterol effusion or pseudo-chylothorax–effusion with calcified pleura, which is common in patients with long-term pleural effusions (trapped lung). The PF has a turbid or milky appearance in half the cases and typically contains cholesterol crystals and a high concentration of cholesterol (>200 mg/dL) [16].

4 Diagnosis

The detection of *M. tb* (by microscopy, culture, or nucleic acid amplification tests [NAATs]) in sputum, PF, or pleural biopsy specimens is required for the conclusive diagnosis of TB effusions. In the adequate clinical context, a pleural biopsy showing granuloma or an increased PF ADA concentration may also be used to make the diagnosis [1].

4.1 Smear Microscopy and Cultures

Pleural TB is thought to be a paucibacillary infection. Data from more than 4800 patients with TB pleuritis were used to establish the following estimated mean sensitivities for microbiological tests [17]:

- acid-fast smears (Ziehl–Neelsen or auramine) of sputum have a sensitivity of 11%;
- cultures of sputum on solid (Lowenstein-Jensen or Ogawa) or liquid media have a sensitivity of 42%;
- acid-fast smears of PF have a sensitivity of 4%; and
- cultures of PF on solid or liquid media have a sensitivity of 31%.

In three studies comprising patients with TB pleuritis ($N = 544$), there were 59% of positive cultures on PF and 47% on sputum samples using liquid media [18–20], which contrast with two other studies ($N = 536$) where liquid cultures were positives in only 17% of PF specimens [21, 22]. At the moment, the valid practice is to culture PF on both solid and liquid media; however, the latter provides a faster time

to positivity than the former (two vs. four to six weeks). For inoculation into a liquid medium, just five mL of PF seems to be sufficient [19].

M. tb is more likely to be isolated from PF and sputum in HIV-positive patients and those with neutrophil-rich fluids. For example, one study [23] reported that positive PF Lowenstein-Jensen cultures were more than doubled in HIV-positive ($N = 33$) versus HIV-negative ($N = 78$) TB patients (63.6% vs. 29.5%). These discrepancies might be explained by poor bacterial clearance from the pleural area in the setting of immunosuppression. In another study, the yield of mycobacterial cultures of sputum and PF were higher in 24 neutrophilic-predominant TB effusions (50% for each) than in 190 lymphocytic-rich ones (25% for sputum and 10% for PF) [24]. It may be claimed that in the early stages of infection, when neutrophils are the most prevalent cell type, the immune system has not yet developed an adequate defense and the intrapleural mycobacterial burden is greater. Finally, a study suggested that a second PF culture performed within two days from the first one may increase the microbiological yield by approximately 13% [25].

Studying patients with TB pleuritis ($N = 517$), 24% and 53% of participants were positive for Ziehl–Neelsen staining and Lowenstein-Jensen cultures, respectively, in the pleural biopsy specimens [26]. Likewise, the sensitivity of pleural tissue cultures was 42% in a thoracoscopic series of 473 TB pleuritis [27].

It is difficult to make fast clinical decisions and provide timely anti-TB therapy (ATT) based on the low yield of cultures and the time it takes them to turn positive. This has stimulated the search for rapid and reliable tests, including NAAT and PF biomarkers.

4.2 Nucleic Acid Amplification Assays

NAATs, which use a polymerase chain reaction (PCR) to amplify *M. tb*-specific nucleic acid sequences, can identify *M. tb* in clinical samples such as sputum, PF, and tissue biopsies. Because of their near-perfect specificity, these tests are considered as confirmation tests for TB, much like cultures. Nevertheless, PCR techniques have a limited sensitivity related to the low burden of bacilli which results in an absolute quantity of genetic material that is below the threshold of detection by these means. A number of molecular techniques have been approved by the World Health Organization (WHO) for rapid (2 h) diagnosis of TB and rifampicin resistance, which is considered a surrogate marker of MDR-TB (Xpert® MTB/RIF and Xpert® MTB/RIF Ultra, Cepheid, Sunnyvale, USA; Truenat® MTB and MTB Plus system, Molbio Diagnostics, Goa, India) [28].

In two meta-analyses of 24 and 27 studies, comprising 2486 and 4006 PF specimens, respectively, the Xpert® MTB/RIF had an overall sensitivity and specificity of around 50% and 100%, respectively, using mycobacterial culture as the reference standard [29, 30]. However, the test only detected 23% [29] to 30% [31] of culture-negative TB effusions. Xpert® MTB/RIF yields in sputum and pleural biopsy specimens are also greater than those of traditional cultures. In a series including 102 patients with TB pleuritis without parenchymal changes on

chest radiographs, the examination of just one sample of induced sputum resulted in sensitivity for smears, cultures, and Xpert® MTB/RIF assay of 7.8%, 21.6%, and 34.3%, respectively [32]. In another study, 198 patients with pleural effusions underwent pleural biopsies by medical thoracoscopy [33]. Pleural TB was identified in 134 individuals using pleural tissue histology and responsiveness to ATT as the diagnostic reference standard. Pleural biopsy cultures had a sensitivity of 41% and a specificity of 100%, compared to the Xpert® MTB/RIF test in the pleural tissue, which had a sensitivity of 52% and a specificity of 100%.

Xpert® MTB/RIF Ultra, an upgraded version of Xpert, offers a better sensitivity for *M. tb* detection, although its performance data for pleural TB is more limited. In a prospective head-to-head study of 208 individuals with pleural TB and 84 with non-TB effusions, Xpert Ultra on PF samples demonstrated a specificity of 98.8% and an overall higher sensitivity (44.2%) than smears (1.4%), cultures (26.4%), and Xpert (19.2%) [34]. When it came to culture-positive samples, Xpert Ultra was more sensitive than Xpert (83.6% vs. 50.9%) but identified only 19.8% of those microbiological-negative ones. In another series of 108 TB pleuritis, the overall sensitivity of Xpert Ultra again doubled that of Xpert (66.1% vs. 34.3%); a proportion which was maintained for both culture-positive (84.2% vs. 49.1%) and culture-negative (35.3% vs. 17.6%) TB cases [35]. Lastly, a recent meta-analysis of four studies that examined 678 PF samples reported the following operating characteristics of Xpert Ultra and Xpert tests: sensitivity, 47% and 25%; specificity, 97% and 99%; positive likelihood ratio (LR), 18.3 and 18.5; and negative LR, 0.54 and 0.76, respectively, to diagnose TB pleurisy [36].

Other commercially available NAATs, such as Genotype MTBDR*plus* (Hain Lifescience, Germany) and Fluorotype® MTB (Hain Lifescience, Germany), have shown a sensitivity of only 13% in clinically diagnosed pleural TB patients [37, 38] and, therefore, cannot be recommended in this context.

4.3 Pleural Fluid Biomarkers

Although many PF biomarkers of TB have been evaluated over the last decades, only three are qualified for use in clinical practice: adenosine deaminase (ADA), unstimulated IFN-γ, and interleukin-27 (IL-27). High levels in any of the three are valuable indicators for ruling in TB effusions in high prevalence settings, whereas low levels in any of the three are valuable indicators for ruling out TB effusions in all prevalence settings.

4.3.1 Adenosine Deaminase

Adenosine and deoxyadenosine are converted to inosine and deoxyinosine by ADA, a T-lymphocyte enzyme. Since first reported in 1978 [39] and further established in 1983 [40], diagnosing TB using PF ADA measurement has shown to be very accurate, as exemplified in the eight meta-analyses [41–48] published to date (Table 1). According to them, PF ADA has an approximate sensitivity of 92%, a specificity of 90%, a positive LR of 8.9, a negative LR of 0.09, and an area under

Table 1 Meta-analyses on the diagnostic accuracy of pleural fluid adenosine deaminase for tuberculous pleural effusions

References	No. of studies	Total No. of patients/ No. of TB effusions	Sensitivity (%)	Specificity (%)	AUC
Goto et al. [41]	40	5485/1857	92.2	92.2	0.92
Greco et al. [42]	31	4738/1621	92.0	89.0	0.93
Morisson and Neves [43][a]	9	1674/857	91.8	88.4	0.97
Liang et al. [44]	63	8093/2796	92.0	90.0	0.96
Gui and Xiao [45]	12	2244/865	86.0	88.0	0.93
Aggarwal et al. [46][b]	40	3524/2058	94.0	89.0	0.96
Palma et al. [47][c]	16	4147/1172	93.0	92.0	0.97
Aggarwal et al. [48]	174	27,009/10,696	92.0	90.0	0.91

AUC the area under the curve; *TB* tuberculosis
Performed exclusively in [a]Brazilian, [b]Indian, and [c]Spanish populations

the curve (AUC) of 0.94 for labeling TB. Regardless of the measuring approach used (colorimetric Giusti or manual or automated kinetic), ADA's outstanding performance is unaffected [47]. The diagnostic limit for the ADA is 35–40 U/L. Unlike other biomarkers such as unstimulated IFN-γ and IL-27, ADA concentrations decrease with age, presumably as a result of the immune dysfunction of macrophages and lymphocytes [49, 50]. Therefore, perhaps lower limits are better to be applied to elderly patients to prevent false-negatives results (e.g., a cutoff decrease of about 10 U/L for patients above the age of 55 has been suggested) [49]. It should be noted, however, that sometimes an initial low concentration of PF ADA in TB patients increases above the diagnostic value in a second thoracentesis. Co-infection with HIV has no effect on ADA's diagnostic accuracy [51].

Additionally, complicated parapneumonic effusions, empyema, and lymphomas are also related to elevated PF ADA levels [52]. More than 30% of lymphomatous effusions test positive for TB exceeding the diagnostic threshold value of the ADA [53]. The clinical presentation and the prevalence of neutrophils in the PF help distinguish parapneumonic effusion and empyema from TB. It is extremely indicative of empyema or lymphoma, rather than TB when PF ADA activity is more than 250 U/L [52].

ADA1 and ADA2 are the two isoenzymes of ADA. ADA1, a ubiquitous enzyme produced by a range of cell types, including neutrophils, accounts for the bulk of false-positive non-TB effusions. ADA2 is only secreted by monocytes and macrophages (85% of TB effusions) [24]. However, since ADA2 assays are not now standard, and the test contributes little to total ADA evaluation, it is not frequently employed in TB diagnosis.

The use of ADA to replace blind pleural biopsies has facilitated TB diagnosis. In regions with a low disease burden, TB almost never is the cause of effusions with an ADA content less than 35 U/L [52, 54]. Despite substantial evidence to the contrary, ADA is still not often considered an aid to speeding up clinical decision-making. The test does not offer information on treatment sensitivity or

definitive verification of infection in places where MDR-TB is prevalent. However, the easy and rapid information provided by PF ADA should never preclude requesting timely microbiological tests.

4.3.2 Interferon-Gamma

IFN-γ is an inflammatory cytokine. Th-1 cells, cytotoxic T cells, and natural killer cells all can generate this cytokine, which, in turn, stimulates macrophages to boost their mycobactericidal activity. When evaluated by enzyme-linked immunosorbent assay (ELISA), free, unstimulated IFN-γ levels in PF are useful in identifying TB effusions with accuracy comparable to or slightly better than ADA [42]. A meta-analysis of 22 studies, encompassing 782 patients with TB effusions and 1319 patients with non-TB effusions, found that PF IFN-γ in the diagnosis of TB pleurisy had an AUC of 0.99, a sensitivity of 89%, a specificity of 89%, and positive and negative likelihood ratios (LR) of 23.4 and 11.1, respectively [55]. A high price and a lack of acknowledged threshold values make this test a less attractive option than the easy and affordable ADA.

IFN-γ release assays (IGRA), which detect IFN-γ produced by T cells in response to stimulation by particular mycobacterial antigens, are usually believed to be of limited diagnostic utility compared to unstimulated IFN-γ. There are two major IGRA: the QuantiFERON-TB Gold In-Tube Plus (QFT-GIT Plus) assay and the T-SPOT.TB assay. The former is an ELISA-based whole blood test, while the latter is an enzyme-linked immunospot that is done on isolated peripheral blood mononuclear cells (PBMCs). However, like TST, they are designed for diagnosing LTBI and so are unable to distinguish LTBI from active TB disease. Blood and PF samples can be processed for IGRA and have been investigated for the diagnosis of TB effusions. In this sense, while the performance of blood IGRA is disappointing, that of PF IGRA is conflicting. A meta-analysis of 21 studies assessed the performance of blood (N = 1085) and PF (N = 727) IGRA in detecting pleural TB. It was found that the pooled sensitivity was 77% and 72%, respectively, while the respective specificities were 71% and 78% [56]. Conversely, another meta-analysis that only included studies that evaluated T-SPOT on PF (13 studies, with 997 TB and 656 non-TB effusions) yielded a pool test sensitivity of 91% and specificity of 88% [57]. Therefore, whereas the former meta-analysis argues against the diagnostic use of IGRA, the latter suggests that, at least, PF-based T-SPOT test might have some role in the identification of pleural TB. The problem is that there are neither standard protocols for PF T-SPOT performance nor uniform criteria for the interpretation of results.

4.3.3 Interleukin-27

IL-27 belongs to the IL-12 family that participates in IFN-γ responses and Th-1 type immunity. PF IL-27 was revealed to help diagnose TB effusions, as reported in meta-analyses [58–61]. In one of them, before proceeding with the meta-analysis, the authors performed a head-to-head comparison study of PF ADA, IFN-γ, and IL-27 in two prospective independent cohorts [59]. In terms of diagnostic accuracy, IL-27 levels more than 591.4 ng/L were comparable to those of IFN-γ levels greater

than 116.1 ng/L and somewhat better than those of ADA levels greater than 21.4 U/L (respective AUC of 0.983, 0.973, and 0.900). The largest meta-analysis included 11 studies with 502 TB effusions and 952 non-TB effusions [61]. The pooled sensitivity, specificity, positive LR, and negative LR of PF IL-27 assays were 95%, 91%, 13.9, and 0.07, respectively, while the AUC was 0.983 [61]. It is necessary to find acceptable cutoff values for IL-27 levels while the assay technology and experience continue to favor the simple and affordable ADA test. However, a few studies suggest a combination of the two markers may contribute to a more efficient diagnosis of TB effusions [62–64].

4.4 Pleural Biopsy

A pleural biopsy for histology and cultures (which provide information on drug resistance [DR]) may be needed to confirm a diagnosis of TB. Biopsies can be performed blindly (blinded or closed pleural biopsy), with radiological guidance (ultrasound or CT), or with medical thoracoscopy or pleuroscopy. Given the diffuse involvement of the pleural surface in TB, closed pleural biopsy has long been the modality of choice. However, the approach to get pleural tissue depends on local competence and resource availability. One or more of the following findings in pleural biopsy samples is considered to be indicative of TB:

i. presence of *M. tb* on stains, culture, or NAAT;
ii. caseating granulomas; and
iii. non-caseating granulomas with no other explanation than TB.

The yield of closed pleural biopsy for TB effusions averages 70% [65] but depends on factors such as operator expertise, the number of samples collected, the diameter of the needle, or the assistance of the procedure by imaging. Closed pleural biopsies may obtain inadequate specimens because of a lack of pleural tissue or satisfactory pleural tissue (i.e., fat and/or intercostal muscle predominate). This occurred in 23% of 1,013 samples in one study [66]. It was shown that a closed pleural biopsy was most sensitive when more than six specimens were extracted, which on average included more than two specimens of the parietal pleura [67]. However, a subsequent small study found that a single biopsy was diagnostic in 81% of TB cases ($N = 16$) [68]. In addition, among 66 pleural TB patients, ultrasound-assisted pleural biopsies using an Abrams needle were more likely to include pleura and had much greater diagnostic sensitivity for pleural TB than Tru-cut samples (81.8% vs. 65.2%) [69].

Because the pathologist is given many samples picked under visual control, medical thoracoscopy improves diagnostic sensitivity for TB by more than 90% [27, 70–72]. Gross macroscopic findings on thoracoscopy include miliary sago-like nodules, fibrinous adhesions, hyperemia, and pleural thickening [71]. From the histological standpoint, granulomas are seen in about 75% of the cases [26, 72], especially in younger people [27]. A randomized trial compared cutting-needle

(18G) biopsy with ultrasound guidance versus thoracoscopic pleural biopsy in diagnosing TB [73]. Patients allocated to the ultrasound-guided procedure ($N = 98$) were allowed to be subjected to a second biopsy if the first one had been inconclusive. Overall, the two modalities were comparable in terms of sensitivity (82% for the ultrasound group and 90% for the thoracoscopic group). However, when it came to a pleural thickness less than one cm, the ultrasound group showed lower sensitivity than the thoracoscopic group (66% vs. 86%).

Since pleural TB can be diagnosed on thoracentesis alone in almost 80% of cases [26, 74], a pleural biopsy is indicated when any of the following conditions are met [75]:

i. measurement of ADA is not available;
ii. PF ADA is less than 35 U/L and TB suspected, in areas with moderate to high rates of TB;
iii. PF ADA is greater than 35 U/L in areas with low rates of TB; and
iv. MDR-TB is a concern, provided microbiological tests in PF or sputum are non-informative.

Risk factors for DR-TB include recent immigrating from or residing in locations with a 4% or more MDR-TB prevalence, previous TB treatment without rifampicin, and exposure to known DR-TB cases [76]. Table 2 summarizes measures of diagnostic accuracy for tests that identify a TB effusion.

Table 2 Sensitivity and specificity of different tests for identifying tuberculous effusions

Test	Sensitivity (%)	Specificity (%)
Pleural fluid microbiological tests		
Smear microscopy (Ziehl–Neelsen or auramine)	4	~100
Cultures (solid and liquid media)	31	100
Xpert® MTB/RIF	51	99
Pleural fluid biochemical tests		
ADA > 35–40 U/L	92	90
Unstimulated IFN-γ	89	97
IGRA	72	78
IL-27	94	92
Pleural biopsy		
Closed or blinded	70	100
Ultrasound-guided	80	100
Thoracoscopy	90–100	100
Smear microscopy	24	~100
Cultures	47	100
Xpert® MTB/RIF	52	~100
Granulomas (histology)	75	95
Sputum		
Smear microscopy	11	~100
Cultures	42	100

ADA adenosine deaminase; *IFN-γ* interferon-gamma; *IGRA* IFN-γ release assays; *IL* interleukin

5 Treatment

TB pleurisy is often self-limiting. However, since the disease reactivates in more than half the untreated patients within five years, when there is reasonable clinical suspicion, ATT should be started awaiting culture findings [1]. The treatment for TB effusions is the same as that for PTB, including isolation in single rooms using airborne precautions for hospitalized patients. The standardized regimen of four first-line drugs (isoniazid, rifampicin, pyrazinamide, and ethambutol) for two months, followed by isoniazid and rifampicin for four months, is the most commonly prescribed. Alternative treatment courses have been tested. For example, in 200 HIV-negative participants, six months of isoniazid and rifampicin was found to be equally effective as six months of isoniazid and rifampicin along with pyrazinamide for the first two months, with significantly fewer adverse effects in the former group [77].

The treatment duration of TB pleurisy is identical in HIV-positive and HIV-negative patients. All HIV co-infected patients with TB pleurisy should be started on antiretroviral therapy if they were not already receiving it. Antiretrovirals should be started after two weeks of commencing TB medication for cases with CD4 < 50 cells/mm^3, but antiretrovirals should be started within eight weeks of starting ATT for CD4 > 50 cells/mm^3 [78]. The rationale behind these time frames between ATT and antiretroviral drugs is to minimize the risk of immune reconstitution inflammatory syndrome (IRIS), which often emerges shortly after antiretrovirals and is characterized by a transient but sometimes severe worsening of symptoms and/or radiological features of TB. IRIS occurs in about one-quarter of HIV-TB co-infected patients, though it does not influence subsequent TB treatment outcomes [79].

Isoniazid and rifampicin-resistant *M. tb* strains are designated as MDR-TB. Mono-rifampicin resistance is rare, and thus resistance to rifampicin is indicative of MDR-TB, which should be assumed pending documentation of susceptibility to isoniazid. If the incidence of MDR-TB is high, persons should be treated as if they had MDR-TB, and rapid NAAT is critical for clinical decision-making. The prevalence of MDR-TB varies widely among geographical areas. In 2018, MDR-TB was found in 3.4% of new TB infections and 18% of previously treated patients worldwide [2]. In 2019, the authors' geographical area (Catalonia) registered an MDR-TB incidence of about 1%, with 13.8% of isolates being resistant to a first-line drug and 7.4% to isoniazid [4]. On the opposite end of the scale, MDR-TB was found in more than 30% of newly diagnosed TB patients in 2018 in the Russian Federation [2]. Having resistance to one of the fluoroquinolones and an injectable medication used in MDR-TB treatment is required for a diagnosis of XDR-TB (extended drug resistance in TB). Different regimens have been recommended for MDR-TB [80–82], among which the administration of a mixture of oral bedaquiline, pretomanid, and linezolid for six months represents a viable option [76]. Management of XDR-TB requires expert consultation.

TB pleurisy usually responds well to treatment, with fever going away in two weeks and PF resorbing in six weeks or more, depending on the amount of effusion [83]. An initial therapeutic thoracentesis in patients with dyspnea or those with a moderate to a large amount of PF may shorten the symptomatic period, speed up the removal of the pleural effusion, and possibly (controversial) lessen the amount of residual pleural thickening (RPT) [84–87]. In loculated effusions or those with a complex-septated echogenic sonographic pattern [88], complete drainage of the pleural space may require the instillation of intrapleural fibrinolytics [85, 87, 89, 90], a situation in which there is somewhat more consensus on the beneficial effects of fluid drainage on RPT. RPT more than ten millimeters has been documented in around 25% of the patients after therapy [91]. However, it diminishes with time and has little effect on daily life. Two meta-analyses of 590 [92] and 957 [93] patients with TB effusions concluded, with a low level of evidence, that the adjunctive treatment with corticosteroids may be used to speed up the remission of symptoms and pleural effusions, and also the risk of RPT, but at the expense of more adverse events. They may be used in a limited percentage of patients who still have significant systemic symptoms following two weeks of ATT and therapeutic thoracentesis [1]. Finally, a theoretical advantage of draining TB effusions relies on the fact that the penetration of anti-TB drugs into the PF is inconsistent, except perhaps for isoniazid. This may potentially favor the selection of resistance populations, whereas drainage would reduce the bacterial load of the pleural space [94].

Effusions may paradoxically grow, and symptoms may reappear or remain in a variable number of patients during the first three to 12 weeks of ATT, but this does not imply that the treatment has failed. This is called paradoxical reaction, a phenomenon partly equivalent in HIV-negative patients to the above-mentioned IRIS. In two studies, worsening radiological findings were described in 16% of 458 [95] and 23% of 129 [96] HIV-negative patients with pleural TB, after a mean follow-up of 61 and 51 days after starting ATT, respectively. Among the patients with paradoxical response, 44% and 56%, respectively, required effusion drainage and/or steroids for symptom control [95, 96].

Tuberculous empyema may need longer TB regimens in patients with ongoing active infection after six months of therapy. Decortication through video-assisted thoracoscopic surgery or open surgery is suggested to control symptomatic trapped lung or overt fibrothorax, multiloculated empyema not responding to chest tubes, and persistent bronchopleural fistula [97, 98].

6 Conclusion

Pleural TB is, along with lymphadenitis, the most prevalent kind of EPTB. It is important to take TB into account of the differential diagnoses of any pleural effusion in locations where the disease is endemic. Pleural TB can be the result of either primary or reactivation TB. It is considered a paucibacillary disease, and, therefore, microbiological tests are often non-informative. The diagnosis mainly

relies on the combination of clinical-epidemiological and PF (i.e., lymphocytic exudate with high levels of ADA) and/or pleural biopsy data (demonstration of caseating granulomas). The treatment is the same as for pulmonary TB, and complications are very uncommon. Graphical Abstract illustrates an algorithmic approach to pleural TB management.

Core Messages

- The typical TB PF profile is that of a lymphocytic-predominant exudate with ADA levels >35 U/L.
- When PF ADA is not available or informative, pleural biopsy under ultrasound guidance is recommended.
- Pleural TB should receive the same standard ATT as pulmonary TB.
- Therapeutic thoracentesis is advised for moderate to large effusions.

References

1. Porcel JM (2009) Tuberculous pleural effusion. Lung 187(5):263–270
2. World Health Organization. Global tuberculosis report 2021. Available at: https://www.who.int/publications/i/item/9789240037021
3. Martino RJ, Chirenda J, Mujuru HA, Ye W, Yang Z (2020) characteristics indicative of tuberculosis/HIV coinfection in a high-burden setting: lessons from 13,802 incident tuberculosis cases in Harare, Zimbabwe. Am J Trop Med Hyg 103(1):214–220. https://doi.org/10.4269/ajtmh.19-0856
4. La tuberculosi a Catalunya l'any 2019. Available at: https://canalsalut.gencat.cat/ca/salut-a-z/t/tuberculosi/recursos-per-a-professionals/epidemiologia/
5. Tahseen S, Khanzada FM, Baloch AQ, Abbas Q, Bhutto MM, Alizai AW, Zaman S, Qasim Z, Durrani MN, Farough MK, Ambreen A, Safdar N, Mustafa T (2020) Extrapulmonary tuberculosis in Pakistan—a nation-wide multicenter retrospective study. PLoS ONE 15(4):e0232134
6. Porcel JM, Esquerda A, Vives M, Bielsa S (2014) Etiología del derrame pleural: análisis de más de 3000 toracocentesis consecutivas. Arch Bronconeumol 50:161–165
7. Qiu L, Teeter LD, Liu Z, Ma X, Musser JM, Graviss EA (2006) Diagnostic associations between pleural and pulmonary tuberculosis. J Infect 53(6):377–386
8. Valdés L, San José ME, Pose A, Gude F, González-Barcala FJ, Alvarez-Dobaño JM, Sahn SA (2010) Diagnosing tuberculous pleural effusion using clinical data and pleural fluid analysis A study of patients less than 40 years-old in an area with a high incidence of tuberculosis. Respir Med 104(8):1211–1217
9. Macías A, Sánchez-Montalvá A, Salvador F, Villar A, Tórtola T, Saborit N, Molina I (2019) Epidemiology and diagnosis of pleural tuberculosis in a low incidence country with high rate of immigrant population: a retrospective study. Int J Infect Dis 78:34–38
10. Bielsa S, Acosta C, Pardina M, Civit C, Porcel JM (2019) Derrame pleural tuberculoso: características clínicas de 320 pacientes. Arch Bronconeumol 55(1):17–22
11. Shaw JA, Diacon AH, Koegelenberg CFN (2019) Tuberculous pleural effusion. Respirology 24(10):962–971

12. Porcel JM, Vives M (2003) Etiology and pleural fluid characteristics of large and massive effusions. Chest 124(3):978–983
13. Ko JM, Park HJ, Kim CH (2014) Pulmonary changes of pleural TB: up-to-date CT imaging. Chest 146(6):1604–1611
14. Porcel JM, Light RW (2013) Pleural effusions. Dis Mon 59(2):29–57
15. Wen P, Wei M, Han C, He Y, Wang MS (2019) Risk factors for *Tuberculous empyema* in pleural tuberculosis patients. Sci Rep 9(1):19569
16. Porcel JM (2017) Derrames pleurales benignos persistentes. Rev Clin Esp 217(6):336–341
17. Porcel JM (2018) Biomarkers in the diagnosis of pleural diseases: a 2018 update. Ther Adv Respir Dis 12:1753466618808660
18. Ruan SY, Chuang YC, Wang JY, Lin JW, Chien JY, Huang CT, Kuo YW, Lee LN, Yu CJ (2012) Revisiting tuberculous pleurisy: pleural fluid characteristics and diagnostic yield of mycobacterial culture in an endemic area. Thorax 67(9):822–827
19. von Groote-Bidlingmaier F, Koegelenberg CF, Bolliger CT, Chung PK, Rautenbach C, Wasserman E, Bernasconi M, Friedrich SO, Diacon AH (2013) The yield of different pleural fluid volumes for *Mycobacterium tuberculosis* culture. Thorax 68(3):290–291
20. Lee BH, Yoon SH, Yeo HJ, Kim DW, Lee SE, Cho WH, Lee SJ, Kim YS, Jeon D (2015) Impact of implementation of an automated liquid culture system on diagnosis of tuberculous pleurisy. J Korean Med Sci 30(7):871–875
21. Kim CH, Cha SI, Lee J (2015) Letter to the editor: respective contribution of liquid and solid media to mycobacterial yields from pleural fluid in tuberculous pleural effusion. J Korean Med Sci 30(12):1922–1923
22. Zhang Q, Zhou C (2017) Comparison of laboratory testing methods for the diagnosis of tuberculous pleurisy in China. Sci Rep 7(1):4549
23. Marjani M, Yousefzadeh A, Baghaei P, Tabarsi P, Moniri A, Masjedi MR, Velayati AA (2016) Impact of HIV infection on tuberculous pleural effusion. Int J STD AIDS 27(5):363–369
24. Bielsa S, Palma R, Pardina M, Esquerda A, Light RW, Porcel JM (2013) Comparison of polymorphonuclear- and lymphocyte-rich tuberculous pleural effusions. Int J Tuberc Lung Dis 17(1):85–89
25. Ko Y, Song J, Lee SY, Moon JW, Mo EK, Park JY, Kim JH, Park S, Hwang YI, Jang SH, Jhun BW, Sim YS, Shin TR, Kim DG, Hong JY, Lee CY, Lee MG, Kim CH, Hyun IG, Park YB (2017) Does repeated pleural culture increase the diagnostic yield of *Mycobacterium tuberculosis* from tuberculous pleural effusion in HIV-negative individuals? PLoS ONE 12 (7):e0181798
26. Sahn SA, Huggins JT, San José ME, Álvarez-Dobaño JM, Valdés L (2013) Can tuberculous pleural effusions be diagnosed by pleural fluid analysis alone? Int J Tuberc Lung Dis 17 (6):787–793
27. Zhao T, Xu Y, Song Q, Wang X, Jin M, Lin D (2019) Medical thoracoscopy for tuberculous pleurisy: a retrospective analysis of 575 cases. Ann Thorac Med 14(2):134–140
28. WHO (2020) Rapid communication: Molecular assays as initial tests for the diagnosis of tuberculosis and rifampicin resistance. Available at: https://www.who.int/tb/publications/2020/rapid-communications-molecular-assays/en/
29. Sehgal IS, Dhooria S, Aggarwal AN, Behera D, Agarwal R (2016) Diagnostic performance of Xpert MTB/RIF in tuberculous pleural effusion: systematic review and meta-analysis. J Clin Microbiol 54(4):1133–1136
30. Kohli M, Schiller I, Dendukuri N, Dheda K, Denkinger CM, Schumacher SG, Steingart KR (2018) Xpert® MTB/RIF assay for extrapulmonary tuberculosis and rifampicin resistance. Cochrane Database Syst Rev 8(8):CD012768.
31. Tadesse M, Abebe G, Bekele A, Bezabih M, Yilma D, Apers L, de Jong BC, Rigouts L (2019) Xpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis: a diagnostic evaluation study. Clin Microbiol Infect 25(8):1000–1005

32. Sumalani KK, Akhter N, Chawla D, Rizvi NA (2019) Diagnostic yield of sputum induction in patients with pleural tuberculosis at a tertiary care hospital in Karachi. Int J Tuberc Lung Dis 23(11):1213–1216
33. Akhter N, Sumalani KK, Chawla D, Ahmed Rizvi N (2019) Comparison between the diagnostic accuracy of Xpert MTB/Rif assay and culture for pleural tuberculosis using tissue biopsy. ERJ Open Res 5(3):00065–02019
34. Wang G, Wang S, Yang X, Sun Q, Jiang G, Huang M, Huo F, Ma Y, Chen X, Huang H (2020) Accuracy of Xpert MTB/RIF ultra for the diagnosis of pleural TB in a multicenter cohort study. Chest 157(2):268–275
35. Wang G, Wang S, Jiang G, Yang X, Huang M, Huo F, Ma Y, Dai G, Li W, Chen X, Huang H (2019) Xpert MTB/RIF ultra improved the diagnosis of paucibacillary tuberculosis: a prospective cohort study. J Infect 78(4):311–316
36. Jiang J, Yang J, Shi Y, Jin Y, Tang S, Zhang N, Lu Y, Sun G (2020) Head-to-head comparison of the diagnostic accuracy of Xpert MTB/RIF and Xpert MTB/RIF ultra for tuberculosis: a meta-analysis. Infect Dis (Lond):1–13. https://doi.org/10.1080/23744235.2020.1788222
37. Irfan M, Idrees F, Jabeen K, Zubairi ABS, Butt S, Hasan R (2020) Accuracy of genotype MTBDRplus line probe assay in patients with tuberculous pleural effusion: comparison with clinical and culture-based diagnosis. Infect Dis (Lond) 52(4):235–241
38. Bielsa S, Bernet A, Civit C, Acosta C, Manonelles A, Porcel JM (2020) FluoroType® MTB in pleural fluid for diagnosing tuberculosis (2021). FluoroType® MTB en líquido pleural para el diagnóstico de tuberculosis]. Rev Clin Esp 221(3):139–144
39. Piras MA, Gakis C, Budroni M, Andreoni G (1978) Adenosine deaminase activity in pleural effusions: an aid to differential diagnosis. Br Med J 2(6154):1751–1752
40. Ocaña I, Martinez-Vazquez JM, Segura RM, Fernández-De-Sevilla T, Capdevila JA (1983) Adenosine deaminase in pleural fluids. Test for diagnosis of tuberculous pleural effusion. Chest 84(1):51–53
41. Goto M, Noguchi Y, Koyama H, Hira K, Shimbo T, Fukui T (2003) Diagnostic value of adenosine deaminase in tuberculous pleural effusion: a meta-analysis. Ann Clin Biochem 40 (Pt 4):374–381
42. Greco S, Girardi E, Masciangelo R, Capoccetta GB, Saltini C (2003) Adenosine deaminase and interferon gamma measurements for the diagnosis of tuberculous pleurisy: a meta-analysis. Int J Tuberc Lung Dis 7(8):777–786
43. Morisson P, Neves DD (2008) Evaluation of adenosine deaminase in the diagnosis of pleural tuberculosis: a Brazilian meta-analysis. J Bras Pneumol 34(4):217–224
44. Liang QL, Shi HZ, Wang K, Qin SM, Qin XJ (2008) Diagnostic accuracy of adenosine deaminase in tuberculous pleurisy: a meta-analysis. Respir Med 102(5):744–754
45. Gui X, Xiao H (2014) Diagnosis of tuberculosis pleurisy with adenosine deaminase (ADA): a systematic review and meta-analysis. Int J Clin Exp Med 7(10):3126–3135
46. Aggarwal AN, Agarwal R, Sehgal IS, Dhooria S, Behera D (2016) Meta-analysis of Indian studies evaluating adenosine deaminase for diagnosing tuberculous pleural effusion. Int J Tuberc Lung Dis 20(10):1386–1391
47. Palma RM, Bielsa S, Esquerda A, Martínez-Alonso M, Porcel JM (2019) Eficacia diagnóstica de la adenosina desaminasa en líquido pleural para diagnosticar tuberculosis. Metaanálisis de estudios españoles. Arch Bronconeumol 55(1):23–30
48. Aggarwal AN, Agarwal R, Sehgal IS, Dhooria S (2019) Adenosine deaminase for diagnosis of tuberculous pleural effusion: a systematic review and meta-analysis. PLoS ONE 14(3): e0213728
49. Korczynski P, Klimiuk J, Safianowska A, Krenke R (2019) Impact of age on the diagnostic yield of four different biomarkers of tuberculous pleural effusion. Tuberculosis (Edinb) 114:24–29

50. Jiang CG, Wang W, Zhou Q, Wu XZ, Wang XJ, Wang Z, Zhai K, Shi HZ (2020) Influence of age on the diagnostic accuracy of soluble biomarkers for tuberculous pleural effusion: a post hoc analysis. BMC Pulm Med 20(1):178
51. Baba K, Hoosen AA, Langeland N, Dyrhol-Riise AM (2008) Adenosine deaminase activity is a sensitive marker for the diagnosis of tuberculous pleuritis in patients with very low CD4 counts. PLoS ONE 3(7):e2788
52. Porcel JM, Esquerda A, Bielsa S (2010) Diagnostic performance of adenosine deaminase activity in pleural fluid: a single-center experience with over 2100 consecutive patients. Eur J Intern Med 21(5):419–423
53. Porcel JM, Cuadrat I, García-Cerecedo T, Pardina M, Bielsa S (2019) Pleural effusions in diffuse large B-cell lymphoma: clinical and prognostic significance. Lung 197(1):47–51
54. Arnold DT, Bhatnagar R, Fairbanks LD, Zahan-Evans N, Clive AO, Morley AJ, Medford AR, Maskell NA (2015) Pleural fluid adenosine deaminase (pfADA) in the diagnosis of tuberculous effusions in a low incidence population. PLoS ONE 10(2):e0113047
55. Jiang J, Shi HZ, Liang QL, Qin SM, Qin XJ (2007) Diagnostic value of interferon-gamma in tuberculous pleurisy: a metaanalysis. Chest 131(4):1133–1141
56. Aggarwal AN, Agarwal R, Gupta D, Dhooria S, Behera D (2015) Interferon gamma release assays for diagnosis of pleural tuberculosis: a systematic review and meta-analysis [published correction appears in J Clin Microbiol. 2016 Feb;54(2):508]. J Clin Microbiol 53(8):2451–2459
57. Luo Y, Xue Y, Guo X, Lin Q, Tang G, Yu J, Mao L, Wang F, Sun Z (2020) Diagnostic value of pleural fluid T-SPOT for tuberculous pleurisy: an updated meta-analysis. Tuberculosis (Edinb) 122:101941
58. Li M, Zhu W, Khan RSU, Saeed U, Wang R, Shi S, Luo Z (2017) Accuracy of interleukin-27 assay for the diagnosis of tuberculous pleurisy: a PRISMA-compliant meta-analysis. Medicine (Baltimore) 96(50):e9205
59. Wang W, Zhou Q, Zhai K, Wang Y, Liu JY, Wang XJ, Wang Z, Zhang JC, Tong ZH, Shi HZ (2018) Diagnostic accuracy of interleukin 27 for tuberculous pleural effusion: two prospective studies and one meta-analysis. Thorax 73(3):240–247
60. Liu Q, Yu YX, Wang XJ, Wang Z, Wang Z (2018) Diagnostic accuracy of interleukin-27 between tuberculous pleural effusion and malignant pleural effusion: a meta-analysis. Respiration 95(6):469–477
61. Zhang Q, Ma Y, Zhang M, Wang Y, Wu W (2020) Diagnostic value of interleukin-27 in tuberculous pleurisy: a meta-analysis. QJM 114(8):568–576. https://doi.org/10.1093/qjmed/hcaa215
62. Wu YB, Ye ZJ, Qin SM, Wu C, Chen YQ, Shi HZ (2013) Combined detections of interleukin 27, interferon-γ, and adenosine deaminase in pleural effusion for diagnosis of tuberculous pleurisy. Chin Med J (Engl) 126(17):3215–3221
63. Valdés L, San José E, Ferreiro L, Golpe A, Gude F, Álvarez-Dobaño JM, Pereyra MF, Toubes ME, González-Barcala FJ (2014) Interleukin 27 could be useful in the diagnosis of tuberculous pleural effusions. Respir Care 59(3):399–405
64. Skouras VS, Magkouta SF, Psallidas I, Tsilioni I, Maragozidis P, Gourgoulianis KI, Kalomenidis I (2015) Interleukin-27 improves the ability of adenosine deaminase to rule out tuberculous pleural effusion regardless of pleural tuberculosis prevalence. Infect Dis (Lond) 47(7):477–483
65. Zhang T, Wan B, Wang L, Li C, Xu Y, Wang X, Liu H, Song Y, Lin D, Zhan P, Lv T (2020) The diagnostic yield of closed needle pleural biopsy in exudative pleural effusion: a retrospective 10-year study. Ann Transl Med 8(7):491
66. Edgar JR, Wong ML, Hale M, Menezes CN (2018) Histopathological diagnoses on pleural biopsy specimens over a 15-year period at Chris Hani Baragwanath Academic Hospital, Johannesburg, South Africa: a retrospective review. S Afr Med J 109(1):53–57

67. Kirsch CM, Kroe DM, Azzi RL, Jensen WA, Kagawa FT, Wehner JH (1997) The optimal number of pleural biopsy specimens for a diagnosis of tuberculous pleurisy. Chest 112 (3):702–706
68. Jiménez D, Pérez-Rodriguez E, Diaz G, Fogue L, Light RW (2002) Determining the optimal number of specimens to obtain with needle biopsy of the pleura. Respir Med 96(1):14–17
69. Koegelenberg CF, Bolliger CT, Theron J, Walzl G, Wright CA, Louw M, Diacon AH (2010) Direct comparison of the diagnostic yield of ultrasound-assisted Abrams and Tru-Cut needle biopsies for pleural tuberculosis. Thorax 65(10):857–862
70. Diacon AH, Van de Wal BW, Wyser C, Smedema JP, Bezuidenhout J, Bolliger CT, Walzl G (2003) Diagnostic tools in tuberculous pleurisy: a direct comparative study. Eur Respir J 22 (4):589–591
71. Wang Z, Xu LL, Wu YB, Wang XJ, Yang Y, Zhang J, Tong ZH, Shi HZ (2015) Diagnostic value and safety of medical thoracoscopy in tuberculous pleural effusion. Respir Med 109 (9):1188–1192
72. Thomas M, Ibrahim WH, Raza T, Mushtaq K, Arshad A, Ahmed M, Taha S, Al Sarafandi S, Karim H, Abdul-Sattar HA (2017) Medical thoracoscopy for exudative pleural effusion: an eight-year experience from a country with a young population. BMC Pulm Med 17(1):151
73. Zhou X, Jiang P, Huan X, Li W, Chen Y, Gao H, Qi X, Wu J, Wang X, Ou Y, Jia X (2018) Ultrasound-guided versus thoracoscopic pleural biopsy for diagnosing tuberculous pleurisy following inconclusive thoracentesis: a randomized, controlled trial. Med Sci Monit 24:7238–7248
74. Koegelenberg CF, Irusen EM, von Groote-Bidlingmaier F, Bruwer JW, Batubara EM, Diacon AH (2015) The utility of ultrasound-guided thoracentesis and pleural biopsy in undiagnosed pleural exudates. Thorax 70(10):995–997
75. Porcel JM (2017) The case against performing pleural biopsies for the etiological diagnosis of exudates. ¿Se debe realizar una biopsia pleural para el diagnóstico etiológico de los exudados? No. Rev Clin Esp 217(7):423–426
76. Gilbert DN, Chambers HF, Saag MS, Pavia AT, Boucher HW (eds) (2021) The Sandord guide to antimicrobial therapy 2020, 51st edn. Antimicrobial Therapy Inc., Sperryville
77. García-Rodríguez JF, Valcarce-Pardeiro N, Álvarez-Díaz H, Mariño-Callejo A (2019) Long-term efficacy of 6-month therapy with isoniazid and rifampin compared with isoniazid, rifampin, and pyrazinamide treatment for pleural tuberculosis. Eur J Clin Microbiol Infect Dis 38(11):2121–2126
78. Panel on antiretroviral guidelines for adults and adolescents (2019) Guidelines for the use of antiretroviral agents in adults and adolescents with HIV. Department of Health and Human Services. Available at https://aidsinfo.nih.gov/guidelines/html/1/adult-and-adolescent-arv/37/whats-new-in-the-guidelines-
79. Narendran G, Jyotheeswaran K, Senguttuvan T, Vinhaes CL, Santhanakrishnan RK, Manoharan T, Selvaraj A, Chandrasekaran P, Menon PA, Bhavani KP, Reddy D, Narayanan R, Subramanyam B, Sathyavelu S, Krishnaraja R, Kalirajan P, Angamuthu D, Susaimuthu SM, Ganesan RRK, Tripathy SP, Swaminathan S, Andrade BB (2020) Characteristics of paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome and its influence on tuberculosis treatment outcomes in persons living with HIV. Int J Infect Dis 98:261–267
80. Caminero JA, García-García JM, Caylà JA, García-Pérez FJ, Palacios JJ, Ruiz-Manzano J (2020) Update of SEPAR guideline «Diagnosis and treatment of drug-resistant tuberculosis». Actualización de la normativa SEPAR «Diagnóstico y tratamiento de la tuberculosis con resistencia a fármacos». Arch Bronconeumol 56(8):514–521
81. Nahid P, Mase SR, Migliori GB, Sotgiu G, Bothamley GH, Brozek JL, Cattamanchi A, Cegielski JP, Chen L, Daley CL, Dalton TL, Duarte R, Fregonese F, Horsburgh CR Jr, Ahmad Khan F, Kheir F, Lan Z, Lardizabal A, Lauzardo M, Mangan JM, Marks SM, McKenna L, Menzies D, Mitnick CD, Nilsen DM, Parvez F, Peloquin CA, Raftery A, Schaaf HS, Shah NS, Starke JR, Wilson JW, Wortham JM, Chorba T, Seaworth B (2019)

Treatment of drug-resistant tuberculosis. An official ATS/CDC/ERS/IDSA clinical practice guideline. Am J Respir Crit Care Med 200(10):e93–e142. Erratum in: Am J Respir Crit Care Med. 15 Feb 2020; 201(4):500–501
82. WHO consolidated guidelines on tuberculosis: module 4: treatment—drug-resistant tuberculosis treatment (2020). World Health Organization, Geneva
83. Cohen M, Sahn SA (2001) Resolution of pleural effusions. Chest 119(5):1547–1562
84. Lai YF, Chao TY, Wang YH, Lin AS (2003) Pigtail drainage in the treatment of tuberculous pleural effusions: a randomised study. Thorax 58(2):149–151
85. Chung CL, Chen CH, Yeh CY, Sheu JR, Chang SC (2008) Early effective drainage in the treatment of loculated tuberculous pleurisy. Eur Respir J 31(6):1261–1267
86. Bhuniya S, Arunabha DC, Choudhury S, Saha I, Roy TS, Saha M (2012) Role of therapeutic thoracentesis in tuberculous pleural effusion. Ann Thorac Med 7(4):215–219
87. Cao GQ, Li L, Wang YB, Shi ZZ, Fan DY, Chen HY (2015) Treatment of free-flowing tuberculous pleurisy with intrapleural urokinase. Int J Tuberc Lung Dis 19(11):1395–1400
88. Lai YF, Su MC, Weng HH, Wu JT, Chiu CT (2009) Sonographic septation: a predictor of sequelae of tuberculous pleurisy after treatment. Thorax 64(9):806–809
89. Kwak SM, Park CS, Cho JH, Ryu JS, Kim SK, Chang J, Kim SK (2004) The effects of urokinase instillation therapy via percutaneous transthoracic catheter in loculated tuberculous pleural effusion: a randomized prospective study. Yonsei Med J 45(5):822–828
90. Cases Viedma E, Lorenzo Dus MJ, González-Molina A, Sanchis Aldás JL (2006) A study of loculated tuberculous pleural effusions treated with intrapleural urokinase. Respir Med 100 (11):2037–2042
91. Porcel JM, Rubio-Caballero M (2005) Secuelas del derrame pleural tuberculoso. Med Clin (Barc) 124(13):494.6
92. Ryan H, Yoo J, Darsini P (2017) Corticosteroids for tuberculous pleurisy. Cochrane Database Syst Rev 3(3):CD001876
93. Xie S, Lu L, Li M, Xiong M, Zhou S, Zhang G, Peng A, Wang C (2017) The efficacy and safety of adjunctive corticosteroids in the treatment of tuberculous pleurisy: a systematic review and meta-analysis. Oncotarget 8(47):83315–83322
94. Jutte PC, Rutgers SR, Van Altena R, Uges DR, Van Horn JR (2004) Penetration of isoniazid, rifampicin and pyrazinamide in tuberculous pleural effusion and psoas abscess. Int J Tuberc Lung Dis 8(11):1368–1372
95. Jeon K, Choi WI, An JS, Lim SY, Kim WJ, Park GM, Park SS, Choi HS, Lee BH, Choi JC, Na MJ, Park J, Kim JY (2012) Paradoxical response in HIV-negative patients with pleural tuberculosis: a retrospective multicentre study. Int J Tuberc Lung Dis 16(6):846–851
96. Jung JW, Shin JW, Kim JY, Park IW, Choi BW, Seo JS, Choi JC (2011) Risk factors for development of paradoxical response during anti-tuberculosis treatment in HIV-negative patients with pleural tuberculosis. Tohoku J Exp Med 223(3):199–204
97. Chen B, Zhang J, Ye Z, Ye M, Ma D, Wang C, Zhu C (2015) Outcomes of video-assisted thoracic surgical decortication in 274 patients with *Tuberculous empyema*. Ann Thorac Cardiovasc Surg 21(3):223–228
98. Kumar A, Asaf BB, Lingaraju VC, Yendamuri S, Pulle MV, Sood J (2017) Thoracoscopic decortication of stage III *Tuberculous empyema* is effective and safe in selected cases. Ann Thorac Surg 104(5):1688–1694

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Ocular Tuberculosis

36

Samir S. Shoughy and Khalid F. Tabbara

The biggest disease today is not leprosy or tuberculosis, but rather the feeling of being unwanted.

Mother Teresa

Summary

Tuberculosis (TB) is a major infectious disease that primarily affects the lungs. Extrapulmonary organs, including the eyes, might be involved. Ocular TB represents an extrapulmonary spread of TB. The clinical spectrum of ocular TB is variable. It may affect the ocular adnexa with or without intraocular involvement. A high index of suspicion is needed to establish the diagnosis of ocular TB. The definitive diagnosis of TB of the eye can be established through isolation of the *Mycobacterium tuberculosis* bacilli in ocular tissue samples by microscopic examination or culture on specific media. Recently, evidence and experience-based guidelines for treating tubercular uveitis were proposed. This

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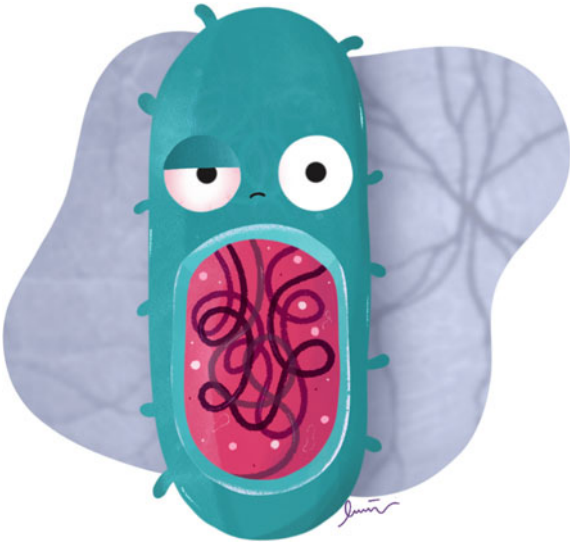
chapter reviews the ocular manifestations of TB and the recent guidelines for managing TB uveitis established by The Collaborative Ocular Tuberculosis Study group.

Graphical Abstract



Ocular tuberculosis

Keywords

Extrapulmonary · Inflammation · *Mycobacterium tuberculosis* · Ocular tuberculosis · Tuberculosis

1 Introduction

Tuberculosis (TB) is a major infectious disease that primarily affects the lungs. Extrapulmonary organs, including the eyes, might be involved [1]. Extrapulmonary TB (EPTB) accounts for up to 20% of the health burden of TB and increases to 50% in patients with concomitant HIV infection [2, 3]. Ocular TB represents extrapulmonary dissemination of *Mycobacterium tuberculosis* (*M. tb*). Ocular involvement may present clinically with the pulmonary disease; however, it was found that about 92% of patients with tubercular ocular involvement show no associated pulmonary TB (PTB) [3].

The clinical spectrum of the lesions seen in the eye in patients with TB is variable. It may be unilateral or bilateral. Involvement of the eye may result from active mycobacterial infection or immunologic response.

Tubercular infection may reach the eye through different routes, including [4]:

- hematogenous spread involving mainly the uveal tract due to its high vascularity;
- primary exogenous ocular infection may occur in the lids, conjunctiva, cornea, sclera, lacrimal gland, and lacrimal sac; and
- secondary ocular infection may occur via direct extension from adjacent tissues.

2 Tubercular External Eye Disease

Table 1 provides a summary of these diseases.

Table 1 Tubercular external eye disease

Structure	Clinical findings
Eyelid	Chronic blepharitis Localized nodule Recurrent chalazia Ulcerated skin nodules Cicatricial eyelid changes Lupus vulgaris Cold abscess
Conjunctival involvement	Conjunctivitis Conjunctival nodular lesions Tuberculomas Conjunctival ulceration Phlyctenulosis
Orbit	Tuberculous periostitis Bony destruction Orbital granuloma Cold abscess Orbital apex syndrome Lacrimal gland granuloma Lacrimal gland abscess Tuberculous dacryoadenitis Lacrimal sac granuloma
Cornea	Interstitial keratitis Disciform keratitis Corneal erosions
Sclera and episcleral	Anterior scleritis Posterior scleritis Nodular episcleritis

2.1 Eyelid

Tubercular involvement of the eyelid is rare to present an isolated ocular finding. It may display a clinical picture of chronic blepharitis or a localized nodule that mimics a chalazion [4]. The skin of the lids may also show cutaneous TB, which may present in different ways, including recurrent chalazia, subepithelial nodules, ulcerated skin nodules, plaques, and cicatricial eyelid disease [5, 6]. Lupus vulgaris is a common form of cutaneous TB, which may affect the skin of the lids [4]. TB of the lids may also present in the form of an acute abscess or in the form of soft fluctuant mass without acute inflammation [4].

2.2 Conjunctiva

TB of the conjunctiva may present as conjunctivitis, conjunctival nodular lesions, polyps, tuberculomas, conjunctival granuloma with ulceration or phlyctenulosis [1, 5]. Although the conjunctiva is more commonly involved in association with systemic disease, isolated involvement of the conjunctiva may occur [5]. Patients may complain of redness, ocular pain, mucopurulent discharge, and/or lid swelling [4].

2.3 Orbit

Tubercular involvement of the orbit is a rare form of EPTB [1]. It may present as proptosis secondary to mass effect or in the form of diplopia resulting from involvement of the extraocular muscles or the cranial nerves [7]. Other presenting features may include pain, headache, lid swelling, decreased vision, visual field abnormalities, chemosis, and epiphora [4]. The mycobacteria may reach the orbit via the hematogenous route or by direct spread from the surrounding paranasal sinuses.

Orbital TB may present as tuberculous periostitis, bony destruction, orbital granuloma, cold abscess, and orbital apex syndrome [4, 5]. The affection of the lacrimal system can be in the form of lacrimal gland granuloma, lacrimal gland abscess, tuberculous dacryoadenitis, nasolacrimal duct involvement, and/or lacrimal sac granuloma [4, 5].

2.4 Cornea

Patients with tubercular corneal involvement may complain of photophobia, ocular pain, tearing, and blepharospasm [8]. Tubercular corneal involvement may be in the form of corneal erosions, interstitial keratitis, disciform keratitis, or phlyctenular keratoconjunctivitis [9, 10]. The corneal findings are manifestations of mycobacterial infection or represent an immunologic response to the mycobacterial antigen

[11]. The keratitis may be associated with scleritis and uveitis [12]. Sclerokeratitis is more common than isolated keratitis.

2.5 Sclera and Episclera

TB of the sclera may occur in the form of anterior scleritis and, less commonly, posterior scleritis [13]. Tuberculous anterior scleritis may be nodular or diffuse. The severely inflamed sclera may undergo necrosis and may perforate [14]. Tuberculoma or infective posterior scleritis may rarely occur in patients with ocular tuberculosis [13, 15]. TB can also cause nodular episcleritis [16].

3 Tubercular Uveitis

Ocular involvement in patients with TB most often manifests as uveitis [17, 18]. Tubercular uveitis may be the initial presentation of TB infection [19] (Table 2).

3.1 Tubercular Anterior Uveitis

The term tubercular anterior uveitis (TAU) was used by the COTS group to describe the inflammation limited to the anterior segment (inflammation mainly involves the iris and the ciliary body) [20, 21]. Tubercular anterior uveitis may be unilateral or bilateral, acute or chronic, granulomatous or non-granulomatous (less frequently) [8]. The granulomatous form of tuberculous anterior uveitis manifests by features such as mutton-fat keratic precipitates and nodular iris granulomas (Koeppe and Busacca nodules), and anterior chamber angle nodules [8]. Broad-based posterior synechiae may lead to iris bombe [22]. Non-granulomatous uveitis may also occur in the form of iritis or iridocyclitis in patients with TB, usually presenting with fine white keratic precipitates and inflammatory cells with no accompanying iris nodules [1, 8]. Cyclitis is frequently seen and may be

Table 2 Tubercular uveitis

Structure	Clinical findings
Iris and ciliary body	Anterior uveitis
Pars plana and vitreous	Intermediate uveitis
Retina and/or the choroid	Tubercular serpiginous-like choroiditis Tubercular multifocal choroiditis Orbital granuloma Tubercular focal choroiditis Tuberculoma
Anterior chamber, vitreous, and retina/choroid	Panuveitis

associated with necrosis and calcification, leading to caseating granuloma [8]. Pigmented hypopyon, anterior pupillary membrane, and cataracts may also develop in patients with tubercular anterior uveitis [1, 7, 8].

3.2 Tubercular Intermediate Uveitis

Tubercular intermediate uveitis (TIU) was defined by the COTS group as the inflammation which mainly involves the vitreous [20, 21]. TIU has non-specific features. It may present with features of simulating pars planitis and other forms of intermediate uveitis. Patients usually present with a blurring of vision and floaters. The ocular signs include mild chronic uveitis with vitritis, snowballs, snow banking, and peripheral vascular sheathing [23–25].

3.3 Tubercular Posterior Uveitis

Tubercular posterior uveitis (TPU) was defined by the COTS group as the inflammation which involves the retina with or without the involvement of the choroid [20, 21]. Posterior segment involvement is frequent in patients with ocular TB. The most commonly encountered sign of tubercular involvement of the posterior segment is multifocal choroiditis [26]. There are four distinct clinical forms of tubercular choroiditis (TBC) as defined by the COTS study group [20]:

i. tubercular serpiginous-like choroiditis (TB SLC): The lesions involving the choroid may be single or multiple. The lesions are discreet fuzzy, and the edges are slightly raised. The lesions progress by a wave-like pattern. It is characterized by an active serpiginous-like edge and central healing;
ii. tubercular multifocal choroiditis (TMC): This pattern may simulate other types of choroiditis such as acute posterior multifocal placoid pigment epitheliopathy and idiopathic multifocal choroiditis. Choroidal tubercles were included under tubercular multifocal choroiditis;
iii. tubercular focal choroiditis (TFC): This pattern occurs in the form of unifocal choroiditis lesions which do not show features of tubercular serpiginous-like choroiditis;
iv. tuberculoma: The lesions may be single or multiple in the form of yellowish subretinal lesions. The lesions possess indistinct borders and surrounding exudates. Tubercular subretinal abscesses are included under this category.

3.4 Tubercular Panuveitis

Tubercular panuveitis (TBP) uveitis was defined by the COTS group as the inflammation involving the whole uveal tissues, including the anterior chamber, vitreous, and retina/choroid [20, 21].

3.5 Tubercular Retinal Vasculitis (TRV)

Patients with ocular TB may present with isolated retinal vasculitis. The vasculitis may affect either arteries or veins and might be occlusive [20]. The veins are more commonly affected than the arteries in patients with tubercular retinal vasculitis [27]. Eales disease is not the same as tubercular retinal vasculitis and should not be used to describe such cases [20, 21]. The characteristic features include vitritis, retinal hemorrhages, neovascularization, perivascular cuffing with dense exudates, and neuroretinitis [7]. Tubercular retinal vasculitis is most commonly occlusive. This may predispose to variable sequelae, including branch retinal vein occlusion, branch and/or central retinal artery occlusion with subsequent extensive peripheral capillary nonperfusion, retinal neovascularization, optic disc neovascularization, bleeding into the vitreous, and subsequent retinal traction detachment [8]. Iris neovascularization may develop with subsequent neovascular glaucoma [8].

4 Optic Nerve Involvement

Infection may reach the optic nerve by either contiguous spread of infection from the choroid or by hematogenous spread from PTB [1, 7]. Clinical presentation may be in the form of papillitis, retrobulbar neuritis, compressive optic neuropathy, ischemic optic neuropathy, optic nerve tubercle, optic atrophy, papilledema, and optochiasmatic arachnoiditis [28].

5 Endophthalmitis and Panophthalmitis

Endophthalmitis and panophthalmitis have been rarely reported in patients with ocular TB [29–31]. It may result from hematogenous spread, untreated choroidal or subretinal abscesses, and less commonly following cataract surgery.

6 Diagnosis and Management

The clinician should keep a high index of suspicion to establish the diagnosis of ocular TB because of the smoldering chronic course and variable clinical manifestations. The correct diagnosis of ocular TB requires isolation of the *mycobacterial* bacilli in ocular tissue samples by microscopic examination or culture on specific media [4]. However, demonstration of acid-fast bacilli with Ziehl–Neelsen stain or detection of the organism by culture usually has a low yield. This low yield could be explained in part by the low volume of the obtained sample, the paucibacillary nature of ocular TB, and the proposed role of immune-mediated

mechanisms in the development of ocular inflammation [32]. Demonstration of necrotizing granulomatous inflammation in an ocular biopsy from orbital and eyelid lesions may be helpful in the diagnosis; however, large sample size is needed to help in the diagnosis of tubercular ocular involvement [4, 32].

Detection of mycobacterial DNA by polymerase chain reaction (PCR) has been employed in the diagnosis of tubercular eye disease [33]. However, PCR cannot be used as a gold standard for the diagnosis of ocular TB because of the wide variations of the test [33]. Furthermore, PCR is unable to distinguish active from latent infection [32].

Interferon-γ-release assay (IGRA) and the tuberculin skin test are the two immunological tests most commonly used in the diagnosis of ocular TB. Both tests cannot distinguish between active disease and latent infection [33].

Radiological tests, including chest X-ray and computed tomography (CT) of the chest, are used to image the most common primary TB infection site. Radiological findings include hilar lymphadenopathy, parenchymal scarring, and pleural disease [32].

Tabbara proposed guidelines for the diagnosis of ocular TB. They include a combination of clinical ocular findings suggestive of TB, including chorioretinitis and anterior granulomatous uveitis, positive purified protein derivative, positive therapeutic response to antitubercular therapy within four weeks, and exclusion of other causes of uveitis [34].

The COTS group developed experience and evidence-based therapeutic guidelines for the treatment of tubercular uveitis (TBU) [35]. Experts agreed to start ATT in the following conditions in the presence of positive results for either of the immunologic tests together with radiological evidence suggestive of past tubercular infection [35]:

- recurrent episodes of tubercular anterior uveitis
- cases of tubercular intermediate uveitis
- cases of tubercular posterior uveitis
- cases of active tubercular retinal vasculitis.

In cases with the first episode of tubercular anterior uveitis and patients with inactive tubercular retinal vasculitis, it was agreed to start antitubercular medications only when both immunological tests and radiological tests were positive [35].

In cases of tubercular choroiditis, it was agreed to start antitubercular medications in the presence of positive results for any one of the immunologic tests together with radiologic features suggestive of TB [36].

In cases of tuberculoma and tubercular serpiginous-like choroiditis, positive results from even one positive immunologic test were considered sufficient to recommend ATT, even in the absence of features suggestive of TB by radiology [36].

Anti-TB therapy was defined by the COTS group as multidrug therapy, which includes four drugs, namely rifampicin, isoniazid, ethambutol, and pyrazinamide. The administration of these drugs should comply with the health strategy of each

country [35, 36]. The use of systemic steroids in patients with ocular TB is controversial. It is advised to delay initiation of systemic steroids until after initiation of ATT in patients with tubercular uveitis unless there is a high risk of severe inflammation with subsequent damage to the ocular tissues [37]. Hamade and colleagues found that early administration of steroids without ATT in patients with tubercular ocular involvement may lead to poor visual outcomes compared to patients who were not given steroids prior to presentation [38].

7 Conclusion

In conclusion, the clinical diagnosis of ocular TB is a challenging issue as the clinical manifestations are not specific. Further studies are needed to establish accurate criteria for the diagnosis and management of ocular TB.

Core Messages

- The clinical spectrum of ocular TB is variable.
- The definitive diagnosis of ocular TB requires isolation of the *M. tb* bacilli.
- Treatment of ocular TB depends on a combination of clinical findings, immunological tests, and chest imaging.
- Future studies are needed to determine the specific indications, doses, and duration of anti-TB medications.

References

1. Gupta V, Shoughy SS, Mahajan S et al (2015) Clinics of ocular tuberculosis. Ocul Immunol Inflamm 23(1):14–24
2. Dheda K, Barry CE, Maartens G (2016) Tuberculosis. Lancet 387:1211–1226
3. Agrawal R, Gunasekeran DV, Grant R et al (2017) Clinical features and outcomes of patients with tubercular uveitis treated with antitubercular therapy in the collaborative ocular tuberculosis study (COTS)–1. JAMA Ophthalmol 135(12):1318–1327
4. Albert DM, Raven ML (2016) Ocular tuberculosis. Microbiol Spectr 4(6):10.1128
5. Dalvin LA, Smith WM (2015) Orbital and external ocular manifestations of *Mycobacterium tuberculosis*: a review of the literature. J Clin Tuberc Other Mycobact Dis 4:50–57
6. Salam T, Uddin JM, Collin JR, Verity DH, Beaconsfield M, Rose GE (2015) Periocular tuberculous disease: experience from a UK eye hospital. Br J Ophthalmol 99(5):582–585
7. Sharma A, Thapa B, Lavaju P (2011) Ocular tuberculosis: an update. Nepal J Ophthalmol 3 (1):52–67
8. Tabbara KF (2005) Ocular tuberculosis: anterior segment. Int Ophthalmol Clin 45(2):57–69
9. Sheu SJ, Shyu JS, Chen LM, Chen YY, Chirn SC, Wang JS (2001) Ocular manifestations of tuberculosis. Ophthalmology 108(9):1580–1585
10. Aclimandos WA, Kerr-Muir M (1992) Tuberculous keratoconjunctivitis. Br J Ophthalmol 76 (3):175–176

11. Arora R, Mehta S, Gupta D, Goyal J (2010) Bilateral disciform keratitis as the presenting feature of extrapulmonary tuberculosis. Br J Ophthalmol 94(6):809–810
12. Shoughy SS, Jaroudi MO, Tabbara KF (2016) Clinical manifestations and outcome of tuberculous sclerokeratitis. Br J Ophthalmol 100(9):1301–1303
13. Gupta A, Gupta V, Pandav SS, Gupta A (2003) Posterior scleritis associated with systemic tuberculosis. Indian J Ophthalmol 51(4):347–349
14. Bloomfield SE, Mondino B, Gray GF (1976) Scleral tuberculosis. Arch Ophthalmol 94 (6):954–956
15. Velasco e Cruz AA, Chahud F, Feldman R, Akaishi PM (2011) Posterior scleral tuberculoma: case report. Arq Bras Oftalmol 74(1):53–54
16. Bathula BP, Pappu S, Epari SR, Palaparti JB, Jose J, Ponnamalla PK (2012) Tubercular nodular episcleritis. Indian J Chest Dis Allied Sci 54(2):135–136
17. Bouza E, Merino P, Muñoz P, Sanchez-Carrillo C, Yáñez J, Cortés C (1997) Ocular tuberculosis. A prospective study in a general hospital. Medicine (Baltimore) 76:53–61
18. Biswas J, Badrinath SS (1995) Ocular morbidity in patients with active systemic tuberculosis. Int Ophthalmol 19:293–298
19. Shah JS, Shetty N, Shah SK, Shah NK (2016) Tubercular uveitis with ocular manifestation as the first presentation of tuberculosis: a case series. J Clin Diagn Res10:NR01–NR03
20. Agrawal R, Agarwal A, Jabs DA et al (2019) Standardization of nomenclature for ocular tuberculosis—results of collaborative ocular tuberculosis study (COTS) workshop. Ocul Immunol Inflamm 1–11. Published online ahead of print, 10 Dec 2019
21. Jabs DA, Nussenblatt RB, Rosenbaum JT (2005) Standardization of uveitis nomenclature (SUN) working group. Standardization of uveitis nomenclature for reporting clinical data. Results of the first international workshop. Am J Ophthalmol 140(3):509–516
22. Gupta A, Bansal R, Gupta V et al (2010) Ocular signs predictive of tubercular uveitis. Am J Ophthalmol 4:562–570
23. Ness T, Virchow JC (2001) Posteriore uveitis: sarkoidose oder tuberkulose [posterior uveitis: sarcoidosis or tuberculosis]. Ophthalmologe 98(2):207–211
24. Gupta V, Gupta A, Rao NA (2007) Intraocular tuberculosis—an update. Surv Ophthalmol 52:561–587
25. Babu K, Bhat SS (2014) Unilateral snow banking in tuberculosisrelated intermediate uveitis. J Ophthalmic Inflamm Infect 10:4
26. Islam SM, Tabbara KF (2002) Causes of uveitis at the eye center in Saudi Arabia: a retrospective review. Ophthalmic Epidemiol 9(4):239–249
27. Gupta A, Gupta V, Arora S et al (2001) PCR-positive tubercular retinal vasculitis: clinical characteristics and management. Retina 21:435–444
28. Davis EJ, Rathinam SR, Okada AA et al (2012) Clinical spectrum of tuberculous optic neuropathy. J Ophthalmic Inflamm Infect 2:183–189
29. Raina UK, Tuli D, Arora R et al (2000) Tubercular endophthalmitis simulating retinoblastoma. Am J Ophthalmol 130:843–845
30. Dvorak-Theobald G (1958) Acute tuberculous endophthalmitis; report of a case. Am J Ophthalmol 45:403–407
31. Mehta SA, Vaidya AR (2011) Post cataract endophthalmitis due to *Mycobacterium tuberculosis*. Ocul Immunol Inflamm 19:232–233
32. Ang M, Chee SP (2017) Controversies in ocular tuberculosis. Br J Ophthalmol 101(1):6–9
33. Agarwal A, Agrawal R, Gunasekaran DV, Raje D, Gupta B, Aggarwal K, Murthy SL, Westcott M, Chee SP, McCluskey P, Ling HS, Teoh S, Cimino L, Biswas J, Narain S, Agarwal M, Mahendradas P, Khairallah M, Jones N, Tugal-Tutkun I, Babu K, Basu S, Carreño E, Lee R, Al-Dhibi H, Bodaghi B, Invernizzi A, Goldstein DA, Herbort CP, Barisani-Asenbauer T, González-López JJ, Androudi S, Bansal R, Moharana B, Mahajan S, Esposti S, Tasiopoulou A, Nadarajah S, Agarwal M, Abraham S, Vala R, Singh R, Sharma A, Sharma K, Zierhut M, Kon OM, Cunningham E, Nguyen QD, Pavesio C, Gupta V (2019) The collaborative ocular tuberculosis study (COTS)-1 report 3: polymerase chain reaction in the

diagnosis and management of tubercular uveitis: global trends. Ocul Immunol Inflamm 27 (3):465–473
34. Tabbara KF (2007) Tuberculosis. Curr Opin Ophthalmol 18(6):493–501
35. Agrawal R, Testi I, Bodaghi B, Barisani-Asenbauer T, McCluskey P, Agarwal A, Kempen JH, Gupta A, Smith JR, de Smet MD, Yuen YS, Mahajan S, Kon OM, Nguyen QD, Pavesio C, Gupta V, Collaborative Ocular Tuberculosis Study Consensus Group (2020) Collaborative ocular tuberculosis study consensus guidelines on the management of tubercular uveitis-report 2: guidelines for initiating antitubercular therapy in anterior uveitis, intermediate uveitis, panuveitis, and retinal vasculitis. Ophthalmology 27:S0161–6420(20)30598-4
36. Agrawal R, Testi I, Mahajan S, Yuen YS, Agarwal A, Kon OM, Barisani-Asenbauer T, Kempen JH, Gupta A, Jabs DA, Smith JR, Nguyen QD, Pavesio C, Gupta V, Collaborative Ocular Tuberculosis Study Consensus Group (2020) Collaborative ocular tuberculosis study consensus guidelines on the management of tubercular uveitis-report 1: guidelines for initiating antitubercular therapy in tubercular choroiditis. Ophthalmology11:S0161–6420(20) 30013-0
37. Agrawal R, Gunasekeran DV, Grant R et al (2017) Clinical features and outcomes of patients with tubercular uveitis treated with antitubercular therapy in the collaborative ocular tuberculosis study (COTS)-1. JAMA Ophthalmol 135(12):1318–1327
38. Hamade IH, Tabbara KF (2010) Complications of presumed ocular tuberculosis. Acta Ophthalmol 88(8):905–909

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Ocular Tuberculosis: Biomarkers for Risk Stratification

37

Rina La Distia Nora, Wandya Hikmahwati, and Ikhwanuliman Putera

The knowledge of anything, since all things have causes, is not acquired or complete unless it is known by its causes.

Ibn Sina

Summary

Mycobacterium tuberculosis (*M. tb*) can occur in organs other than the lungs, including the eye, called ocular tuberculosis (TB). This infection causes abnormalities of some eye parts, primarily involving the uvea. About 30–50% of uveitis cases in developing countries are caused by infection, including TB. The clinical manifestation of uveitis associated with *M. tb* can mimic other causes. Since uveitis is a vision-threatening inflammatory disease, an accurate diagnosis is crucial. Ocular fluids collected for acid-fast smear, culture, or *M. tb* polymerase, often do not provide evidence of infection because of its low sensitivity and morbidity associated with invasive sample collection. It is essential to know the status of latent or active TB to guide in giving proper treatment for the right patient. Biomarkers, the parameters that can be used as an objective measure of a normal or

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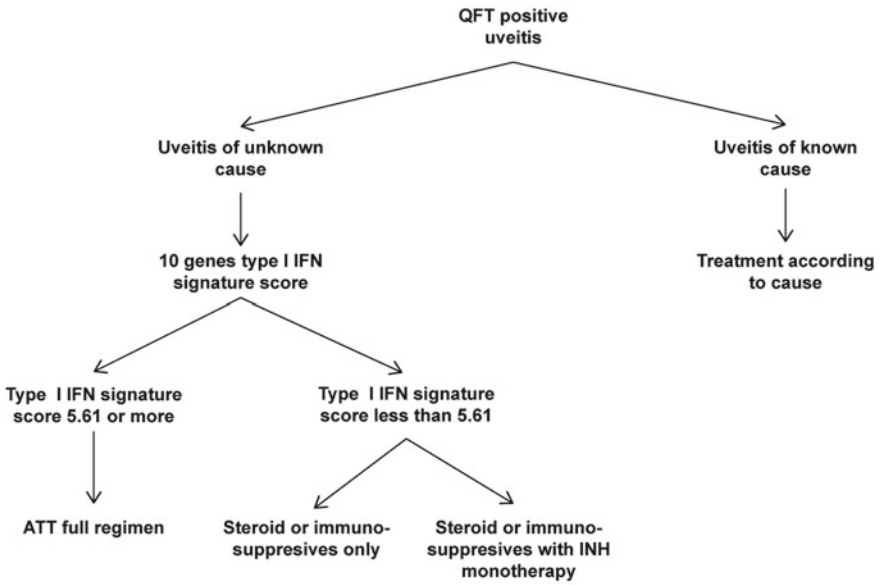
pathogenic biological process, can be helpful to overcome the problems. Several candidate biomarkers being studied, especially host-derived biomarkers, have shown promising results in ocular TB diagnosis. These include assessment of gene expression, microRNAs, protein analysis, and cellular immunophenotyping. These biomarker candidates can be developed further to stratify ocular TB and overcome the limitation of finding *M. tb* in ocular tissues.

Graphical Abstract



An algorithm proposed to use type 1 IFN-inducible genes to stratify patients with QFT positive uveitis who are at high risk of having TB uveitis. Reproduced from [1]; Copyright, © 2018 La Distia Nora et al. under the terms of the Creative Commons Attribution License

Keywords

Biomarker · Cellular immunophenotyping · Diagnosis · Gene expression · microRNA · Ocular tuberculosis · Protein · Risk stratification · Uveitis

1 Introduction

Tuberculosis (TB) remains a major burden of infection worldwide. Infection with *Mycobacterium tuberculosis* (*M. tb*) may not manifest symptoms and is considered inactive in approximately 90% of individuals, the so-called latent TB infection (LTBI).

The clinical disease might occur at any moment later as the infection can progress further depending on the immunological status of individuals [2]. In 2018, World Health Organization (WHO) stated that the South-East Asian area had the most significant number of new TB cases (44%), followed by the African (24%) and Western Pacific regions (18%). India, Indonesia, China, Pakistan, Philippines, Bangladesh, Nigeria, and South Africa accounted for around two-thirds of new TB cases [3].

M. tb is often found in oxygen-rich tissues [2]. About 20% of *M. tb* infections occur in organs other than the lungs, including the eyes, called ocular TB. In primary ocular TB cases, the port of entry for *M. tb* is through the eyes. This primary infection causes conjunctiva, cornea, and/or sclera abnormalities. On the other hand, the organism spreads to the ocular tissue by circulation in secondary infection as occurred in ocular TB [4]. Uveitis is a vision-threatening inflammatory disease that might significantly impact the uveal tract (iris, ciliary body, and choroid) as it serves as the blood-supplying layer in the eyes) and nonetheless influences surrounding tissues [5]. Since its varying clinical signs, uveitis can be classified based on its main anatomical inflammation site, onset, chronicity, and disease course using the Standardization of Uveitis Nomenclature (SUN) criteria [6]. The etiology of uveitis can be infections, autoimmune diseases, side effects of drugs, or idiopathic. It was estimated that around 30–50% of uveitis cases in developing countries were related to infection, including TB [5, 7].

Intraocular TB may present with dispersed clinical spectra, depending on its primary inflammation site. Granulomatous anterior uveitis presents with either one or a combination of large-greasy (mutton-fat) keratic precipitates, iris nodules, or ciliary body granulomas. Iris nodules may appear at the pupillary margin (Koeppe nodule) or central anterior surface of the iris (Busacca nodule). Exudates might appear in pars plana or peripheral uvea, resembling granulomatous or, in some instances, non-granulomatous inflammation. Several characteristics of posterior segment inflammation could also emerge as retinitis, retinal vasculitis (usually occlusive retinal vasculitis), subretinal neovascularization, cystoid macular edema, neuroretinitis (with macular star), and/or even optic neuropathy. Choroidal involvement can also present as choroid tubercle, choroidal tuberculoma, subretinal abscess, serpiginous-like, or serpiginous choroiditis. Panuveitis, endophthalmitis, or panophthalmitis were also possible [8–10]. Interestingly, TB is one of the most common etiologies of uveitis with regard to systemic infections. On the other hand, uveitis was considered the most common form of ocular TB [11]. Since uveitis could lead to blindness if improperly treated, accurate diagnosis is a fundamental yet uneasy task for ophthalmologists.

2 Challenge in Tuberculosis-Associated Uveitis Diagnosis

Clinically, the manifestation of TB uveitis can mimic uveitis from other causes. Most suspected ocular TB cases are treated with anti-TB treatment (ATT), potentially resulting in overtreatment and/or undertreatment [12]. Since there is no

evidence-based guideline in establishing ocular TB diagnosis and timing of ATT initiation, treatment strategies may vary and contribute to different outcomes worldwide [13].

Ocular fluids collected for acid-fast smear, culture, or polymerase chain reaction (PCR) often do not prove confirmed ocular TB because of the low sensitivity and morbidity associated with invasive procedures [14]. Paucibacillary infection of *M. tb* could result in a low bacillus reading of the ocular fluid [11]. In many areas, patients from an endemic TB region have a history of contact with TB patients and tested positive for either tuberculin skin test (TST) or interferon-gamma release assays (IGRA). Moreover, ocular signs could lead to suspicion of ocular TB with other potential causes that had already been excluded. Worth noting that a positive response to the ATT with or without systemic steroids may also lead to suspected ocular TB diagnosis [15].

A concern has been raised as TST was considered to have lower sensitivity and specificity than expected when diagnosing TB, despite its widespread use. In areas where most people received the Bacillus Calmette-Guerin (BCG) vaccine or who have been exposed to mycobacterium other than *M. tb*, false-positive TST results might obscure its utility as it detects an indirect sign of systemic TB infection. Moreover, in patients with severe disease, such as in clinically active TB and immunocompromised patients, the diagnostic value of TST poses limitations to serve as a reliable diagnostic marker [16]. Nonetheless, TST remains a valuable screening tool for TB-associated uveitis [17].

The increased utility of IGRA in recent years has helped overcome TST shortcomings in latent TB diagnosis, especially in regions with routine BCG vaccination. IGRA is based on interferon-gamma secreted by sensitive T-cells in response to certain antigens from *M. tb* (i.e., early secretory antigen target-6 [ESAT-6], culture filtrate protein-10 [CFP-10]). QuantiFERON-TB Gold (QFT) (Cellestis, Australia) and ELISpot assay (T-SPOT.TB) (Oxford Immunotec, Oxford, UK) are two available IGRA types. In many clinical settings, QFT is preferred due to its lower cost and less sensitivity to temperature than T-SPOT.TB [16]. In addition, QuantiFERON-TB GOLD In-Tube (QFT-GIT) offers the third antigen, TB7.7, besides the former two even though all three IGRA types are considered to have better specificity to diagnose latent TB [16]. Meanwhile, the patient's immunosuppressed condition could affect the sensitivity of QFT-GIT, unlike T-SPOT.TB, which is only slightly affected by immunosuppressed status [18].

Differentiating active TB and LTBI by solely using IGRA or TST is difficult as both are indirect immunological tests utilizing T-cells response to prior *M. tb* exposure [19]. New techniques such as transcript microarrays, flow cytometry of intracellular cytokines, and multiplex micro-based immunoassay of cytokines were developed to overcome the shortcoming [20]. By knowing the status of TB, latent or active, a clinician can judiciously give ATT to the right patient. Appropriate ATT administration influence the treatment outcome, with special consideration to the length of ATT.

3 Potential Biomarkers to Improve Diagnosis Accuracy

Biomarkers are parameters that can be used as an objective measure of a normal or pathogenic biological process and serve as indicators of pharmacological response to a therapeutic intervention. In daily practice, biomarkers might help stratify high- and low-risk patients based on the suspected disease and potentially be utilized to monitor disease progression or targeted interventions being administered [21]. Biomarkers can be either pathogen-specific or host-specific. They may represent the pathogenesis of certain diseases being investigated. Biomarkers may also reflect an individual's current condition and the likelihood of developing a disease based on comparative assessment with the general healthy population [22].

3.1 *M. tb*-Derived Biomarker

A variety of samples, including sputum, blood, or urine, can be tested for the presence of *M. tb*. *M. tb* DNA can be found in the blood and urine of pulmonary TB (PTB) patients [23]. The molecules of pathogenic derivates should pass detectable amounts in the sample matrix before they can be considered targets for antigen detection tests. These antigens must be specific and ubiquitous in clinical samples [21].

A significant portion of the *M. tb* cell wall is composed of lipoarabinomannan (LAM), accounting for approximately 15% of *M. tb* mass [24]. It has four main structures:

i. Glycophospholipid attachment that is non-covalently bound to the *M. tb* cell membrane;
ii. The extension of phosphatidylinositol mannoside that serves as a mannan core and is inheritably present across species;
iii. Variable branching arabinan side chain; and
iv. Variable capping motive that provides species diversity.

LAM can be classified into three family structures based on its cap:

i. The LAM derived from non-pathogenic mycobacterial species (i.e., fast-growing *M. smegmatis*);
ii. LAM is covered in phosphatidyl-myoinositol (PILAM), which has an inositol phosphate cap; and
iii. ManLAM, it is the LAM with an additional cap element attached to its mannopyranose structure. This LAM is also generally detected in pathogenic mycobacterial species (i.e., *M. tb*, *M. kansasii*, *M. avium*, and *M. leprae*) and associated with fast-growing capacity. The presence of 5-deoxy-5-methylthio-xylofuranose as an element of *M. tb* ManLAM might help differentiate it from other species [25].

LAM is considered a virulence factor associated with the pathogenesis of *M. tb* infection. During infection, its presence in body fluids can be a potential biomarker for detecting infected individuals [24]. The replicating *M. tb* found in systemic circulation may pass through the glomerular basement membrane and release into the urine. Its detection with an uncomplicated immunoassay kit may help TB diagnosis using a urine sample [25]. However, studies concerning the utility of LAM as a biomarker for ocular TB have not been carried out. This is because LAM that uses urine for the specimen cannot reflect the presence of bacteria in the eye, considering the fact that even PCR examination from ocular fluid alone has low sensitivity [26].

Epitope as part of an antigen is also considered helpful for diagnosing ocular TB. To discover immunodominant (ID) peptides, seven *M. tb* antigens (Rv1965, Rv1971, Rv2351c, Rv2675c, Rv3121, Rv1837c, and Rv3874) were screened using intraocular fluid in patients confirmed with intraocular TB and non-intraocular TB patients as controls. The additional antigens potentially expressed in the intraocular environment were also identified by screening the *M. tb* genome in the artificial intraocular fluid. ID epitopes in seven antigens recognized by antibodies in intraocular fluid from intraocular TB patients are identified. Moreover, the transcriptome of replicating *M. tb* in artificial intraocular fluid provided insight into the adaptation of *M. tb* in the intraocular environment. This can lead to finding potential biomarkers for ocular TB diagnosis [12].

In general, the present body of knowledge proves it is challenging to detect *M. tb* in ocular tissues. The inflammation could be due to paucibacillary infection or delayed-type hypersensitivity reaction without apparent *M. tb* found in ocular tissue [11].

3.2 Host-Derived Biomarker of *M. tb* Infection

Although finding the *M. tb* remains the gold standard of a confirmed diagnosis of ocular TB [27], various challenges described above make researchers try to find alternative host-derived biomarkers to assist the diagnosis of ocular TB. Fortunately, this limitation can be overcome by leveraging the host's response to infection [28]. Host-derived biomarkers have a variety of non-sputum-based tests for diagnosing active TB [23]. The concept of identifying the host immune response due to an infection to determine the diagnosis emerges in this context. Gene expression, proteins, metabolites/wastes, cytokines, and other potential host indicators are useful candidates to assist clinical diagnosis, with no exception to be implemented in ocular TB [28].

Host-based gene expression changes rapidly and provides valuable information in response to exposure (including infection). A previous study showed that it could differentiate active infection from colonization. In addition, it also could help determine many types of pathogens (for example, viral from a bacterial pathogen), provides prognostic information, and predicts disease severity [28].

Varying results from asymptomatic clearance, latent infection, and active disease are determined by host and pathogen factors given host-agent-environment interactions. Secreted cytokines in response to specific antigens of *M. tb* reflect the immunological process involved in TB pathogenesis [29].

Polychromatic flow cytometry may visualize *M. tb*-specific T cells' subsets contributing to LTBI and active TB. This technique employs a laser beam through thousands of cells per second from samples. It permits phenotypic discrimination between antigen-specific lymphocyte subsets in various stages of TB infection by determining the number of cells expressing a certain cytokine [30]. Therefore, gene expression, cytokines, and cellular immunophenotype analysis can be adopted as promising biomarkers to guide the diagnosis of active TB infection, including ocular TB.

3.2.1 Host-Derived Biomarker to Distinguish Active and Latent Pulmonary Tuberculosis

Gene Expression

A study to identify candidates for the expressed genes to discriminate active and latent *M. tb* infection was done by isolating RNA from peripheral blood mononuclear cells (PMBC). Patients with active and latent TB were included in the study, along with healthy controls with negative results on all tests related to TB. In active TB and LTBI, 169 genes were expressed differently. In LTBI, 103 genes were upregulated and 66 genes were downregulated compared to active TB. Subsequently, elevated gene transcripts not associated with common respiratory tract infections and inflammatory responses were identified: NEMF (nuclear export mediator factor), ASUN (asunder spermatogenesis regulator), and DHX29 (DEAH (Asp-Glu-Ala-His) box polypeptide 29). Transcripts regarded as a reference for TB disease, PTPRC (protein tyrosine phosphatase, receptor type, C) and CD45, were used as a comparison. It is observed that the ASUN level differed significantly between active or latent TB and controls. Meanwhile, DHX29 potentially distinguished active TB and healthy individuals. NEMF, on the other hand, had not proven to be less useful as a surrogate biomarker [31].

The PTPRC, ASUN, and DXH29 are expressed in TB infection. Parallel with another study, Ordway et al. found the escalation of PTPRC in the Guinea pig after *M. tb* exposure. The PTPRC decreased after the infection had lasted a long time [32]. PTPRC or CD45 is related to the pathogenesis of TB because it is essential in antigen receptor signal transduction and lymphocyte development [33]. ASUN is also involved in regulating the mitotic cell cycle [34]. Moreover, ASUN may play a role in T lymphocyte differentiation or proliferation and serve as a basis in differentiating TB infection spectra [35]. On the other hand, DHX29 serves as a helicase protein that initiates translation and may be altered in TB [31, 35].

MicroRNAs

The recent development of biomarkers arises with microRNA (miRNA). This small fragment of non-coding RNAs may alter gene expression through a

post-transcriptional modification that regulates protein being translated [36]. Thus, the miRNA pathway implicated in TB pathomechanism and its exploration enriched our understanding in distinguishing active and latent TB [37].

A study using microarray analysis to study potential miRNA implicated in TB infection spectra found that whole blood-derived miRNA signature consisting of the-miR-150, hsa-miR-21, hsa-miR-29c, and hsa-miR-194 could differentiate active pulmonary TB from latent TB and controls [38]. Wang et al. found another miRNAs signature. Four miRNAs implicated in hematopoietic response: hsa-miR-223, hsa-miR-424, hsa-miR-451, and hsa-miR-144, were highly elevated in TB disease compared to LTBI. Moreover, a study in the Taiwanese population found that hsa-miR-146a-5p and hsa-miR-150-5p were helpful in differentiating active and latent TB [39, 40]. By using the same approach, Xu et al. also demonstrated that active TB, LTBI, and healthy controls gave different miRNA signatures. From 26 differentially regulated miRNA, 23 were considered helpful for active TB diagnosis. Further in silico analysis with three different miRNA target gene predictors resulted in different mRNA targets, yet they postulated that many miRNAs contribute to the same mRNA target. Besides, miR-21* was a signature for active disease, while mir-15b* was highly expressed in healthy individuals [41].

Lipomannan, an *M. tb* cell wall element, may inhibit macrophages from secreting TNF. This was associated with deregulated miRNAs: upregulated miR-125b and downregulated miR-155 expressions. Different mycobacteria may potentially influence the balance of these two miRNAs' expression. For instance, non-pathogenic *M. smegmatis* might increase miR-155 but decrease miR-125b with elevated TNF production [42].

Analysis of co-regulatory network performed by Lin and coworkers found genes contributing to the immunological response of *M. tb*. Their pathway analysis found transcription factors (TF) in *M. tb* response: SP11, CEBPB, STAT1, STAT2, STAT3, STAT4, and STAT5 that constitute transcription factors-miRNA network. Six genes named *EBPB, FCGR1A, FCGR3A, ITGAX, ITGAM*, and *IL1B* were highly deregulated and involved in the TB signaling pathway. The TF-miRNA gene co-regulatory networks may become therapeutic targets and biomarkers in TB [43].

Protein

A cross-sectional study by Wang et al. in BCG-vaccinated individuals living in TB endemic areas was carried out to assess the potential of IP-10, IL-2, and TNF-α in differentiating active and latent TB by doing QFT and ELISA examination. With ESAT-6, CFP-10, and TB7.7 proteins as TB antigen stimulation, and without antigen stimulation, the release of IFN-γ, IP-10, IL-2, and TNF-α was measured. A combination of QFT with IL-2 and IP-10 increased the detection rate for active TB. Active TB patients and household contacts had higher baseline IP-10 levels than healthy controls [44]. A high IP-10 level correlates with individual response to *M. tb* as it serves as a chemoatractor for T lymphocytes and monocytes upon *M. tb*-induced inflammation [45, 46]. The high IP-10 level in LTBI was argued to be associated with a subclinical immune response to control *M. tb* infection, whereas low IP-10 in active TB was due to the immunosuppressive state of cellular immunological

response. Those who tested positive for QFT and had a high baseline level of IP-10 tended to have a higher risk of developing the active disease [44]. Moreover, the ratio of IL-2/IFN-γ, which reflects Th1-type $CD4^+$ T cells response, was closely related to *M. tb* infection. The antigen-stimulated IL-2/IFN-γ ratio was elevated in LTBI, potentially distinguishing patients with LTBI from those with active TB [44].

Frahm et al. found 25 promising biomarkers, namely IL-1β, IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 p40/70, IL-13, IL-15, IL-17, TNF-α, IFN-α, IFN-γ, GM-CSF, MIP-1α, MIP-1β, IP-10, MIG, Eotaxin, RANTES, and MCP-1, which corresponded to specific TB antigen responses [46]. When active and latent TB patients were compared to healthy control, the IP-10 response was much higher. Even though a different cut-off for IP-10 was implemented, this was consistent with another study [47]. The combination of IL-15, which stimulates cathelicidin, and MCP-1 can also distinguish between those with active TB and those with LTBI. The sensitivity and specificity of both measurements were 83 and 88% in differentiating active versus latent TB [46].

Cellular Immunophenotype

Th1 cells contribute significantly to the immune response to *M. tb* infection. Estévez et al. demonstrated a lower systemic Th1/Th2 ratio in active TB primarily due to fewer Th1 detected in circulation. However, this ratio has never been firmly used as a sign to describe active TB in clinical practice [48]. A study by Batoni et al. found that NK-Bright cells (CD3-CD56++) were a source of immunological regulatory cytokines and produced IFN-γ after BCG stimulation, suggesting that they could help control *M. tb* during latent infection [49]. In another study that evaluated $CD4^+$ T-cells activation in *M. tb* response, CD154 expression was superior to secreted IFN-γ [48]. Compared to other cytokines or activation molecules, CD154 was more sensitive and specific to reflect *M. tb*-specific Th cells, as demonstrated in Li et al.'s study. This molecule was transiently expressed in T cells upon specific antigen stimulation and demonstrated co-stimulatory signals to the antigen-presenting cells [50]. Furthermore, monocyte-to-lymphocyte (MLR) and neutrophil-to-lymphocyte (NLR) ratios are considered more affordable and accessible to discriminate active TB from either latent TB or healthy state. Higher MLR and NLR ratios were observed among patients with active TB but not in those with latent TB or controls [48].

3.2.2 Current Situation of the Host-Derived Biomarker to Assist Ocular Tuberculosis Diagnosis

Gene Expression

In our previous study, type 1 interferon (IFN)-inducible gene expressions from peripheral blood samples showed the ability to discriminate active pulmonary TB. We applied its utility to patients with undifferentiated uveitis or uveitis with an unknown cause who had positive QFT. We found a system to classify those with high and low risk of having ocular TB based on type 1 IFN-inducible gene expressions score [1]. Our analysis found ten out of 35 type 1 IFN-inducible genes

significantly dysregulated in active pulmonary TB: *UBE2L6*, *FCGR1B*, *GBP1*, *IL1B, MYD88*, *TLR8*, *IRF7*, *STAT1*, *SERPING1*, and *IFIT2*. Subsequently, a type 1 IFN signature score was developed, and 5.61 was determined as the optimal cut-off level. Interestingly, we found that QFT-positive undifferentiated uveitis cases with type 1 IFN signature score <5.61 seemed unlikely to have TB-related uveitis [1].

Protein

An intraocular fluid study examining aqueous cytokines and chemokines in TB-related uveitis was performed by Ang et al., who recruited patients with strong clinical presentation associated with TB uveitis who tested positive for TST/IGRA and gave positive responses to ATT. According to their findings, TB uveitis demonstrated a significantly higher level of IL-6, CXCL8, CXCL9, CXCL 10, and IP-10 compared to control groups. This study suggested that patients with uveitis who respond to ATT do not have an active ocular TB infection but rather autoimmune-related ocular inflammation provoked by TB [51].

Cellular Immunophenotype

To identify the various phenotypes of ocular TB and evaluate the effect of treatment, Hutchinson et al. measured the proportions and activation marker phenotypes of *M. tb* responding $CD4^+$ T cells [52]. As previously reported, TNF-α+ CD154 +IFNg +CD27- and TNF-α+CD154+GM-CSF+CD27-$CD4^+$ T cells were specific for *M. tb* response and could separate those with active TB from those with LTBI [53]. Even though T cells profiling among ocular TB patients demonstrated a promising ability to classify treatment responders and nonresponders, there were no remarkable phenotype-specific T cells found. Of note, the small sample size could limit its generalization. In addition, they postulated that bacterial load in pulmonary versus ocular TB might result in different peripheral T cells profiling. However, their finding serves as a basis for further study to evaluate peripheral *M. tb*-specific $CD4^+$ T-cell utility to guide ATT administration. Moreover, they found that $CD4^+$ T-cell markers were negatively correlated with vitritis [52]. This conflicting result needs to be addressed in a prospective study with a larger sample size.

Phenotypic and T cells profiling using intraocular fluid samples was performed by Tagirasa et al. Given limited T cells obtained in intraocular fluids, the breach of blood-retinal-barrier by *M. tb* invasion potentially allows influx of autoreactive T cells and get activated by local self-antigenic stimuli. The retinal antigen-specific response was observed in 40% of vitreous samples challenged with the crude retinal extract. The presence of autoreactive T cells in vitreous humor perhaps contributes to the intraocular inflammatory state in ocular TB with potential involvement of self immune responses. These autoreactive T cells demonstrated deviant activation-induced cell death with a significant decrease in apoptotic markers [54]. Interestingly, retinal antigen-specific T lymphocytes were also observed in other uveitis entities like birdshot chorioretinopathy [55].

Table 1 Summary of current biomarker candidates to improve ocular TB diagnosis

Level of detection	Samples	Method of detection	Biomarker candidate	Potential biological significance	References
Gene expression	PBMC	Microarray and validated by qPCR	ASUN	Regulation of mitotic cell cycle	[31]
			DXH29	Translation initiation	[31]
	PPD stimulated PBMC	Microarray	ATP10A	Transmembrane movement of small molecules	[58]
			TLR6	Trigger MyD88-dependent and independent pathways involving initiation of T-cell mediated immunity	[58]
	PBMC	RT-PCR	UBE2L6 FCGR1B GBP1 IL1B MYD88 TLR8 IRF7 STAT1 SERPING1 IFIT2	Induced by Type 1 IFN	[1]
miRNAs	PBMC	Microarray and validated by qPCR	hsa-miR-150	Negative regulator of NK cell maturation	[38]
			hsa-miR-21	Reduction of Th1 response	[38, 39]
			hsa-miR-29c	Supress immune response to intracellular pathogens by targeting IFN-γ mRNA	[38]
		Microarray	hsa-miR-146a-5p	Require NF-kB pathways to supress inflammatory response from *M. tb*	[40]
			hsa-miR-150-5p	Chemokine signalling pathway	[40]
			hsa-miR-16-5p	Inducer of apoptosis	[40]
			hsa-miR-221-3p	MAPK, B-cell receptor, T-cell receptor signalling pathway	[40]
			miR-21	Increased by activation of naïve $CD4^+$ T cells	[41, 59]

(continued)

Table 1 (continued)

Level of detection	Samples	Method of detection	Biomarker candidate	Potential biological significance	References
		Microarray and validated by RT-PCR			
		Microarray and qPCR	hsa-miR-223	Regulate chemotaxis via CXCL2, CCL3, IL-6	[39, 59]
			hsa-miR-424	Induce monocyte-macrophage differentiation	[39]
			hsa-miR-451	Erythroid differentiation and homeostasis	[39]
			hsa-miR-144	Erythroid differentiation and homeostasis	[39]
Protein	PPD stimulated PBMC	Microarray	CXCL10/IP-10	Chemokine produced by several cell types, monocytes and T cells	[58]
	M. tb antigen stimulated PBMC	ELISA	IP-10	Chemokine produced by several cell types, monocytes and T cells	[44]
			IL-2/IFN- γ ratio	IL-2 promotes T cell replication and essential for cellular immunity and granuloma formation	[44]
		ELISA and multiplexed bead arrays	IP-10	Chemokine produced by several cell types, monocytes and T cells	[46]
			IL-15	Stimulate $CD8^+/CD4^+$ T cells and NK cells	[46, 60]
			MCP-1	Regulate migration and infiltration of monocytes/ macrophages	[46, 61]
		ELISA	IL-15	Stimulate $CD8^+/CD4^+$ T cells and NK cells	[60]
			IL-32	Caspase-mediated apoptosis or autophagy, increasing antimycobacterial peptides	[60]
		Multiple cytokines (bioplex) assay	IFN-γ (stimulated)	Activation of phagocytes, stimulation of antigen precenting cells, and modulation of other cytokines involved in *M. tb* killing	[62]
			IP-10 (stimulated)	Chemokine produced by several cell types, monocytes and T cells	[62]
			IL-1Ra (stimulated)	Secreted by monocytes, neutrophils and such structural cells as epithelial cells. Competitive inhibition of IL-1α and IL-1β	[62]

(continued)

Table 1 (continued)

Level of detection	Samples	Method of detection	Biomarker candidate	Potential biological significance	References
			IP-10 (unstimulated)	Chemokine produced by several cell types, monocytes and T cells	[62]
			VEGF (stimulated)	Can be produced by macrophages, epithelioid cells, and inflammatory cells around active TB lesions to supplement blood supply	[62]
			IL-12 (stimulated)	Regulate IFN-γ production and cytotoxic effector function of *M. tb* antigen-specific T cells	[62]
		Multiple cytokines assay	IL-10	Supress macrophage and dendritic cell functions, inhibit formation of mature fibrotic granuloma during *M. tb* infection	[63]
			MCP-1	Induce chemotaxis of monocytes and granulocytes	[63]
			IL-1RA	Secreted by monocytes, neutrophils and such structural cells as epithelial cells. Competitive inhibition of IL-1α and IL-1β	[63]
		ELISA	IL-10	Supress macrophage and dendritic cell functions, inhibit formation of mature fibrotic granuloma during *M. tb* infection	[64]
			IL-17	Enhance Th-1 memory response to reduce mycobacterial burden	[64]
	Aqueous humour	Multiple cytokines assay	IL-6	Induce autophagy formation in virulent *M. tb* infection in THP-1 cells	[51, 65]
			CXCL8/IL-8	Bind to tubercle bacilli and increase the ability neutrophils and macrophages to phagocyte *M. tb*	[51, 66]
			CXCL9	Induced by ESAT6. A chemotactic ligand produced by macrophages and other APC at the site of inflammation	[51, 67]
			CXC10 / IP-10	Chemokine produced by several cell types, monocytes and T cells	[51]

(continued)

Table 1 (continued)

Level of detection	Samples	Method of detection	Biomarker candidate	Potential biological significance	References
Cellular immunophenotype	PBMC	Flow cytometry	CD45/PTPRC	Modulation of T and B cell receptor signal transduction	[31]
		Flow cytometry	CD154	Activated T cell or macrophage marker	[48]
			CD3-CD56++	NKT-like cells marker	[48]
		Flow cytometry	TNF-α+ CD154 +IFNg+CD27-	T cells marker	[53]
			TNF-α+CD154 +GM-CSF +CD27-	T cells marker	[53]
Miscellaneous	PBMC	Flow cytometry	Th1/Th2 ratio	–	[48]
			MLR	–	[48]
			NLR	–	[48]

PBMC peripheral blood mononuclear cell; *qPCR* quantitative polymerase chain reaction; *PPD* purified protein derivative; *RT-PCR* reverse transcription-polymerase chain reaction, *NK* natural killer; *mRNA* messenger RNA; *MAPK* mitogen-activated protein kinase; *QFT* QuantiFERON-TB Gold; ELISA, enzyme-linked immunosorbent assay; *M. tb Mycobacterium tuberculosis*; APC, antigen-presenting cell; *MLR* monocyte-to-lymphocyte ratio; *NLR* neutrophil-to-lymphocyte ratio

4 Ocular Tuberculosis in HIV Patients: A Condition that Can Affect Potential Biomarkers

Several notable studies about ocular TB infection in HIV patients have been performed [56, 57]. The immunodeficiency state in HIV patients could influence biomarkers evaluation. The CD4 decline observed in HIV-infected individuals may implicate TB risk, including those who have already been treated with antiretrovirals. Using intracellular staining on IFNγ+CD4$^+$ T cells obtained from the HIV-infected and non-HIV-infected group, it was found that HLA-DR expression was highly upregulated. HLA-DR expressed in ESAT-6/CFP-10 stimulated CD4$^+$ T cells in HIV-infected individuals with active TB. This discovery opens the door to further research into the use of immunophenotyping to distinguish between active and latent tuberculosis in HIV-infected individuals [56].

In an experimental study utilizing *M. tb* peptides, malate synthase and MPT51 were reported to be useful in detecting early active TB among HIV-infected individuals. Anti-MPT51 antibody response was highly elevated in active TB patients and potentially served as a sensitive marker to diagnose TB in HIV patients before any clinical symptoms were present. MPT51 is an immunodominant protein with alpha/beta hydrolases. This molecule contributes to *M. tb* pathogenicity, particularly by facilitating *M. tb* adhesion by its fibronectin-binding capacity [57].

Table 1 provides a summary of current biomarker candidates in ocular TB diagnosis.

5 Conclusion

Ocular TB is a form of active extrapulmonary TB that can be caused by either direct infection due to the presence of *M. tb* in ocular tissue or indirect delayed-type hypersensitivity reaction in the eyes. Currently, establishing a definitive or confirmed diagnosis requires detection of *M. tb* in intraocular samples/tissues. However, detecting *M. tb* in the eyes is challenging in clinical practice. Both TST and IGRA tests have limitations in distinguishing active or latent TB infection. Several candidate biomarkers, especially host-derived biomarkers, can help confirm ocular TB diagnosis, including assessment of gene expression, miRNAs, protein analysis, and cellular immunophenotyping. These biomarker candidates can be developed further to stratify ocular TB and overcome the limitation of finding *M. tb* in ocular tissues. It should be emphasized that our current understanding of the pathogenesis of ocular TB is lacking. Determination of an applicable biomarker needs to elaborate on the mechanism of the disease. There is no single reliable biomarker to be implemented in clinical practice yet. Thus, prospective studies on biomarkers are needed in ocular TB diagnosis and prognosis risk stratification.

We learn and research medicine mainly to name all things, just like Adam PBUH was taught the names — all of them.

Rina La Distia Nora, Wandya Hikmahwati, Ikhwanuliman Putera

Core Messages

- Ocular TB is a paucibacillary eye infection representing immunological responses without apparent *M. tb* invasion.
- Indirect immunological responses serve as host-derived biomarker candidates to establish ocular TB diagnosis.
- Direct extrapolation of biomarkers in pulmonary TB may not truly reflect intraocular inflammatory response in ocular TB.
- Validation studies are needed to evaluate biomarkers' applicability in guiding diagnosis and prognosis of ocular TB.

References

1. La Distia Nora R, Sitompul R, Bakker M, Versnel MA, Swagemakers SMA, van der Spek PJ et al (2018) Type 1 interferon-inducible gene expression in QuantiFERON Gold TB-positive uveitis: a tool to stratify a high versus low risk of active tuberculosis? PLoS ONE 13(10): e0206073. https://doi.org/10.1371/journal.pone.0206073
2. Shakarchi FI (2015) Ocular tuberculosis: current perspectives. Clin Ophthalmol 9:2223–2227. https://doi.org/10.2147/opth.S65254
3. World Health Organization (2020) Tuberculosis. https://www.who.int/news-room/fact-sheets/detail/tuberculosis
4. Foster CS, Vitale AT (2013) Diagnosis & treatment of uveitis. Jaypee Brothers,Medical Publishers Pvt. Limited
5. Barisani-Asenbauer T, Maca SM, Mejdoubi L, Emminger W, Machold K, Auer H (2012) Uveitis—a rare disease often associated with systemic diseases and infections—a systematic review of 2619 patients. Orphanet J Rare Dis 7:57. https://doi.org/10.1186/1750-1172-7-57
6. Jabs DA, Nussenblatt RB, Rosenbaum JT (2005) Standardization of uveitis nomenclature for reporting clinical data. Results of the first international workshop. Am J Ophthalmol 140 (3):509–516. https://doi.org/10.1016/j.ajo.2005.03.057
7. London NJ, Rathinam SR, Cunningham ET Jr (2010) The epidemiology of uveitis in developing countries. Int Ophthalmol Clin 50(2):1–17. https://doi.org/10.1097/IIO.0b013e3181d2cc6b
8. Dalvin LA, Smith WM (2017) Intraocular manifestations of *Mycobacterium tuberculosis*: a review of the literature. J Clin Tuberc Other Mycobact Dis 7:13–21. https://doi.org/10.1016/j.jctube.2017.01.003
9. Gupta V, Gupta A, Rao NA (2007) Intraocular tuberculosis—an update. Surv Ophthalmol 52 (6):561–587. https://doi.org/10.1016/j.survophthal.2007.08.015
10. Gupta V, Shoughy SS, Mahajan S, Khairallah M, Rosenbaum JT, Curi A et al (2015) Clinics of ocular tuberculosis. Ocul Immunol Inflamm 23(1):14–24. https://doi.org/10.3109/09273948.2014.986582
11. Kashyap B, Goyal N, Das GK, Singh NP, Kaur IR (2018) Ophthalmic presentation of disseminated tuberculosis with relapse-immunological profile. Indian J Clin Biochem 33 (4):483–486. https://doi.org/10.1007/s12291-018-0741-2
12. Kaur K, Ryndak MB, Agarwal A, Verma I, Gupta V, Laal S (2019) *Mycobacterium tuberculosis* (*M. tb*) antibody and antigen biomarkers for rapid diagnosis of intra-ocular tuberculosis. Investig Ophthalmol Visual Sci 60(9):827–827

13. Agrawal R, Gunasekeran DV, Grant R, Agarwal A, Kon OM, Nguyen QD et al (2017) Clinical features and outcomes of patients with tubercular uveitis treated with antitubercular therapy in the collaborative ocular tuberculosis study (COTS)–1. JAMA Ophthalmol 135 (12):1318–1327. https://doi.org/10.1001/jamaophthalmol.2017.4485
14. Bajema KL, Pakzad-Vaezi K, Hawn T, Pepple KL (2017) Tuberculous uveitis: association between anti-tuberculous therapy and clinical response in a non-endemic country. J Ophthal Inflamm Infect 7(1):19. https://doi.org/10.1186/s12348-017-0137-0
15. Al-Shakarchi FI (2014) Pattern of uveitis at a referral center in Iraq. Middle East Afr J Ophthalmol 21(4):291–295. https://doi.org/10.4103/0974-9233.142263
16. Lee C, Agrawal R, Pavesio C (2016) Ocular tuberculosis—a clinical conundrum. Ocul Immunol Inflamm 24(2):237–242. https://doi.org/10.3109/09273948.2014.985387
17. Hong BK, Khanamiri HN, Bababeygy SR, Rao NA (2014) The utility of routine tuberculosis screening in county hospital patients with uveitis. Br J Ophthalmol 98(8):1091–1095. https://doi.org/10.1136/bjophthalmol-2013-303937
18. Du F, Xie L, Zhang Y, Gao F, Zhang H, Chen W et al (2018) Prospective comparison of QFT-GIT and T-SPOT.TB assays for diagnosis of active tuberculosis. Sci Rep 8(1):5882. https://doi.org/10.1038/s41598-018-24285-3
19. Sester M, Sotgiu G, Lange C, Giehl C, Girardi E, Migliori GB et al (2011) Interferon-γ release assays for the diagnosis of active tuberculosis: a systematic review and meta-analysis. Eur Respir J 37(1):100–111. https://doi.org/10.1183/09031936.00114810
20. Won EJ, Choi J-H, Cho Y-N, Jin H-M, Kee H, Park Y et al (2016) Biomarkers for discrimination between latent tuberculosis infection and active tuberculosis disease. J Infect 74. https://doi.org/10.1016/j.jinf.2016.11.010
21. Tucci P, Gonzalez-Sapienza G, Marín M (2014) Pathogen-derived biomarkers for active tuberculosis diagnosis. Front Microbiol 5:549. https://doi.org/10.3389/fmicb.2014.00549
22. McNerney R, Maeurer M, Abubakar I, Marais B, McHugh TD, Ford N et al (2012) Tuberculosis diagnostics and biomarkers: needs, challenges, recent advances, and opportunities. J Infect Dis 205 Suppl 2:S147–S158. https://doi.org/10.1093/infdis/jir860
23. Goletti D, Petruccioli E, Joosten SA, Ottenhoff TH (2016) Tuberculosis biomarkers: from diagnosis to protection. Infect Dis Rep 8(2):6568. https://doi.org/10.4081/idr.2016.6568
24. Correia-Neves M, Fröberg G, Korshun L, Viegas S, Vaz P, Ramanlal N et al (2019) Biomarkers for tuberculosis: the case for lipoarabinomannan. ERJ Open Res 5(1). https://doi.org/10.1183/23120541.00115-2018
25. Bulterys MA, Wagner B, Redard-Jacot M, Suresh A, Pollock NR, Moreau E et al (2019) Point-of-care urine LAM tests for tuberculosis diagnosis: a status update. J Clin Med 9(1). https://doi.org/10.3390/jcm9010111
26. Ang M, Vasconcelos-Santos DV, Sharma K, Accorinti M, Sharma A, Gupta A et al (2018) Diagnosis of ocular tuberculosis. Ocul Immunol Inflamm 26(2):208–216. https://doi.org/10.1080/09273948.2016.1178304
27. Gupta A, Sharma A, Bansal R, Sharma K (2015) Classification of intraocular tuberculosis. Ocul Immunol Inflamm 23(1):7–13. https://doi.org/10.3109/09273948.2014.967358
28. Holcomb ZE, Tsalik EL, Woods CW, McClain MT (2017) Host-based peripheral blood gene expression analysis for diagnosis of infectious diseases. J Clin Microbiol 55(2):360–368. https://doi.org/10.1128/jcm.01057-16
29. Torrado E, Cooper AM (2013) Cytokines in the balance of protection and pathology during mycobacterial infections. Adv Exp Med Biol 783:121–140. https://doi.org/10.1007/978-1-4614-6111-1_7
30. Rovina N, Panagiotou M, Pontikis K, Kyriakopoulou M, Koulouris NG, Koutsoukou A (2013) Immune response to mycobacterial infection: lessons from flow cytometry. Clin Dev Immunol 2013:464039. https://doi.org/10.1155/2013/464039
31. Lee SW, Wu LS, Huang GM, Huang KY, Lee TY, Weng JT (2016) Gene expression profiling identifies candidate biomarkers for active and latent tuberculosis. BMC Bioinform 17(Suppl 1):3. https://doi.org/10.1186/s12859-015-0848-x

32. Ordway D, Palanisamy G, Henao-Tamayo M, Smith EE, Shanley C, Orme IM et al (2007) The cellular immune response to *Mycobacterium tuberculosis* infection in the guinea pig. J Immunol 179(4):2532–2541. https://doi.org/10.4049/jimmunol.179.4.2532
33. Hermiston ML, Xu Z, Weiss A (2003) CD45: a critical regulator of signaling thresholds in immune cells. Annu Rev Immunol 21:107–137. https://doi.org/10.1146/annurev.immunol.21.120601.140946
34. Anderson MA, Jodoin JN, Lee E, Hales KG, Hays TS, Lee LA (2009) Asunder is a critical regulator of dynein-dynactin localization during *Drosophila* spermatogenesis. Mol Biol Cell 20(11):2709–2721. https://doi.org/10.1091/mbc.e08-12-1165
35. Parsyan A, Shahbazian D, Martineau Y, Petroulakis E, Alain T, Larsson O et al (2009) The helicase protein DHX29 promotes translation initiation, cell proliferation, and tumorigenesis. Proc Natl Acad Sci U S A 106(52):22217–22222. https://doi.org/10.1073/pnas.0909773106
36. Sohel MM (2016) Extracellular/circulating MicroRNAs: release mechanisms, functions and challenges. Achiev Life Sci 10. https://doi.org/10.1016/j.als.2016.11.007
37. Sabir N, Hussain T, Shah SZA, Peramo A, Zhao D, Zhou X (2018) miRNAs in tuberculosis: new avenues for diagnosis and host-directed therapy. Front Microbiol 9:602. https://doi.org/10.3389/fmicb.2018.00602
38. Latorre I, Leidinger P, Backes C, Domínguez J, de Souza-Galvão ML, Maldonado J et al (2015) A novel whole-blood miRNA signature for a rapid diagnosis of pulmonary tuberculosis. Eur Respir J 45(4):1173–1176. https://doi.org/10.1183/09031936.00221514
39. Wang C, Yang S, Sun G, Tang X, Lu S, Neyrolles O et al (2011) Comparative miRNA expression profiles in individuals with latent and active tuberculosis. PLoS ONE 6(10):e25832. https://doi.org/10.1371/journal.pone.0025832
40. Wu LS, Lee SW, Huang KY, Lee TY, Hsu PW, Weng JT (2014) Systematic expression profiling analysis identifies specific microRNA-gene interactions that may differentiate between active and latent tuberculosis infection. Biomed Res Int 2014:895179. https://doi.org/10.1155/2014/895179
41. Xu Y, Ren W, Liu Y, Zhang X, Li C, Sun Z (2013) Tuberculosis-related miRNAs have potential as disease biomarkers. J Tuberc Res 01(02):11. https://doi.org/10.4236/jtr.2013.12005
42. Rajaram MV, Ni B, Morris JD, Brooks MN, Carlson TK, Bakthavachalu B et al (2011) *Mycobacterium tuberculosis* lipomannan blocks TNF biosynthesis by regulating macrophage MAPK-activated protein kinase 2 (MK2) and microRNA miR-125b. Proc Natl Acad Sci U S A 108(42):17408–17413. https://doi.org/10.1073/pnas.1112660108
43. Lin Y, Duan Z, Xu F, Zhang J, Shulgina MV, Li F (2017) Construction and analysis of the transcription factor-microRNA co-regulatory network response to *Mycobacterium tuberculosis*: a view from the blood. Am J Transl Res 9(4):1962–1976
44. Wang S, Diao N, Lu C, Wu J, Gao Y, Chen J et al (2012) Evaluation of the diagnostic potential of IP-10 and IL-2 as biomarkers for the diagnosis of active and latent tuberculosis in a BCG-vaccinated population. PLoS ONE 7(12):e51338. https://doi.org/10.1371/journal.pone.0051338
45. Azzurri A, Sow OY, Amedei A, Bah B, Diallo S, Peri G et al (2005) IFN-gamma-inducible protein 10 and pentraxin 3 plasma levels are tools for monitoring inflammation and disease activity in *Mycobacterium tuberculosis* infection. Microbes Infect 7(1):1–8. https://doi.org/10.1016/j.micinf.2004.09.004
46. Frahm M, Goswami ND, Owzar K, Hecker E, Mosher A, Cadogan E et al (2011) Discriminating between latent and active tuberculosis with multiple biomarker responses. Tuberculosis (Edinb) 91(3):250–256. https://doi.org/10.1016/j.tube.2011.02.006
47. Ruhwald M, Bjerregaard-Andersen M, Rabna P, Kofoed K, Eugen-Olsen J, Ravn P (2007) CXCL10/IP-10 release is induced by incubation of whole blood from tuberculosis patients with ESAT-6, CFP10 and TB7.7. Microbes Infect 9(7):806–812. https://doi.org/10.1016/j.micinf.2007.02.021

48. Estevez O, Anibarro L, Garet Fernández ME, Martínez A, Peña A, Barcia L et al (2020) Multi-parameter flow cytometry immunophenotyping distinguishes different stages of tuberculosis infection. J Infect 81. https://doi.org/10.1016/j.jinf.2020.03.064
49. Batoni G, Esin S, Favilli F, Pardini M, Bottai D, Maisetta G et al (2005) Human CD56bright and CD56dim natural killer cell subsets respond differentially to direct stimulation with *Mycobacterium bovis* bacillus Calmette-Guérin. Scand J Immunol 62(6):498–506. https://doi.org/10.1111/j.1365-3083.2005.01692.x
50. Li L, Qiao D, Fu X, Lao S, Zhang X, Wu C (2011) Identification of *Mycobacterium tuberculosis*-specific Th1, Th17 and Th22 cells using the expression of CD40L in tuberculous pleurisy. PLoS ONE 6(5):e20165. https://doi.org/10.1371/journal.pone.0020165
51. Ang M, Cheung G, Vania M, Chen J, Yang H, Li J et al (2012) Aqueous cytokine and chemokine analysis in uveitis associated with tuberculosis. Mol Vis 18:565–573
52. Hutchinson PE, Kee AR, Agrawal R, Yawata N, Tumulak MJ, Connolly JE et al (2020) Singapore ocular tuberculosis immunity study (SPOTIS): role of T-lymphocyte profiling in patients with presumed ocular tuberculosis. Ocular Immunol Inflamm 1–7. https://doi.org/10.1080/09273948.2020.1767791
53. Hutchinson P, Barkham TM, Tang W, Kemeny DM, Chee CB, Wang YT (2015) Measurement of phenotype and absolute number of circulating heparin-binding hemagglutinin, ESAT-6 and CFP-10, and purified protein derivative antigen-specific CD4 T cells can discriminate active from latent tuberculosis infection. Clin Vaccine Immunol 22(2):200–212. https://doi.org/10.1128/cvi.00607-14
54. Tagirasa R, Parmar S, Barik MR, Devadas S, Basu S (2017) Autoreactive T cells in immunopathogenesis of TB-associated uveitis. Invest Ophthalmol Vis Sci 58(13):5682–5691. https://doi.org/10.1167/iovs.17-22462
55. Kuiper JJ, Rothova A, Schellekens PA, Ossewaarde-van Norel A, Bloem AC, Mutis T (2014) Detection of choroid- and retina-antigen reactive CD8(+) and CD4(+) T lymphocytes in the vitreous fluid of patients with birdshot chorioretinopathy. Hum Immunol 75(6):570–577. https://doi.org/10.1016/j.humimm.2014.02.012
56. Riou C, Berkowitz N, Goliath R, Burgers WA, Wilkinson RJ (2017) Analysis of the phenotype of *Mycobacterium tuberculosis*-Specific $CD4^+$ T cells to discriminate latent from active tuberculosis in HIV-Uninfected and HIV-infected individuals. Front Immunol 8:968. https://doi.org/10.3389/fimmu.2017.00968
57. Wilson RA, Maughan WN, Kremer L, Besra GS, Fütterer K (2004) The structure of *Mycobacterium tuberculosis* MPT51 (FbpC1) defines a new family of non-catalytic alpha-beta hydrolases. J Mol Biol 2:519–530. https://doi.org/10.1016/j.jmb.2003.11.001
58. Lu C, Wu J, Wang H, Wang S, Diao N, Wang F, Gao Y, Chen J, Shao L, Weng X, Zhang Y, Zhang W, Ahmed N (2011) Novel biomarkers distinguishing active tuberculosis from latent infection identified by gene expression profile of peripheral blood mononuclear cells. PLoS ONE 6(8):e24290. https://doi.org/10.1371/journal.pone.0024290
59. Kleinsteuber K, Heesch K, Schattling S, Kohns M, Sander-Jülch C, Walzl G, Hesseling A, Mayatepek E, Fleischer B, Marx FM, Jacobsen M, Torrelles JB (2013) Decreased expression of miR-21 miR-26a miR-29a and miR-142-3p in $CD4^+$ T cells and peripheral blood from tuberculosis patients. PLoS ONE 8(4):e61609. https://doi.org/10.1371/journal.pone.0061609
60. Hong Y, Kim Y, Lee JJ, Lee MG, Lee CY, Kim Y, Heo J, Han S-S, Lee S-J, Kim WJ, Hong JY (2019) Levels of vitamin D-associated cytokines distinguish between active and latent tuberculosis following a tuberculosis outbreak. BMC Infect Dis 19(1):151. https://doi.org/10.1186/s12879-019-3798-5
61. Deshmane SL, Kremlev S, Amini S, Sawaya BE (2009) Monocyte Chemoattractant Protein-1 (MCP-1): an overview. J Interferon Cytokine Res 29(6):313–326. https://doi.org/10.1089/jir.2008.0027
62. Wang S, Li Y, Shen Y, Wu J, Gao Y, Zhang S, Shao L, Jin J, Zhang Y, Zhang W (2018) Screening and identification of a six-cytokine biosignature for detecting TB infection and discriminating active from latent TB. J Transl Med 16(1):206. https://doi.org/10.1186/s12967-018-1572-x

63. Suzukawa M, Akashi S, Nagai H, Nagase H, Nakamura H, Matsui H, Hebisawa A, Ohta K, Wilkinson K (2016) Combined analysis of IFN-γ IL-2 IL-5 IL-10 IL-1RA and MCP-1 in QFT supernatant is useful for distinguishing active tuberculosis from latent infection. PLOS ONE 11(4):e0152483. https://doi.org/10.1371/journal.pone.0152483
64. Hur Y-G, Gorak-Stolinska P, Ben-Smith A, Lalor MK, Chaguluka S, Dacombe R, Doherty TM, Ottenhoff TH, Dockrell HM, Crampin AC, Kumar A (2013) Combination of cytokine responses indicative of latent TB and active TB in Malawian adults. PLoS ONE 8 (11):e79742. https://doi.org/10.1371/journal.pone.0079742
65. Dutta RK, Kathania M, Raje M, Majumdar S (2012) IL-6 inhibits IFN-γ induced autophagy in Mycobacterium tuberculosis H37Rv infected macrophages. Int J Biochem Cell Biol 44 (6):942–954. S1357272512000775. https://doi.org/10.1016/j.biocel.2012.02.021
66. Krupa A, Fol M, Dziadek BR, Kepka E, Wojciechowska D, Brzostek A, Torzewska A, Dziadek J, Baughman RP, Griffith D, Kurdowska AK (2015) Binding of CXCL8/IL-8 to Mycobacterium tuberculosis modulates the innate immune response. Mediators of Inflamm 2015:1–11. https://doi.org/10.1155/2015/124762
67. Hasan Z, Jamil B, Ashraf M, Islam M, Yusuf MS, Khan JA, Hussain R, Ojcius DM (2009) ESAT6-Induced IFNγ and CXCL9 can differentiate severity of tuberculosis. PLoS ONE 4(4): e5158. https://doi.org/10.1371/journal.pone.0005158

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Bone and Joint Tuberculosis

38

Amer Hayat Khan

It's almost unbelievable that an illness that can be diagnosed and treated can be overlooked as a serious health concern. We must do everything possible to help mitigate, treat, and in the end, eradicate this crippling and limiting illness.

Baroness Julia Cumberlege (member House of Lords, former UK health minister, prominent osteoporosis advocate. Message on the occasion of the 2nd IOF Women Leaders Roundtable, 2006)

Summary

Despite the availability of effective medication, tuberculosis (TB) is on the rise worldwide. The rising number of immunocompromised patients because of the use of chemotherapeutic agents for the treatment of other diseases like cancer, diabetes, and HIV/AIDS has much to do with this resurrection. Multiple drug-resistant (DR) strains of TB and the aging population are also linked. Additional forms of mycobacteria besides *Mycobacterium tuberculosis* or *Mycobacterium bovis* are known as the cause of bone and joint infections. The potential causative conditions are environmental problems, malnutrition, and abject poverty. TB of the musculoskeletal system begins with hematogenous seeding of the bacteria soon after the infection has occurred (pulmonary, in most cases). Medical symptoms differ in terms of onset, discomfort, swelling of the joint, and mobility.

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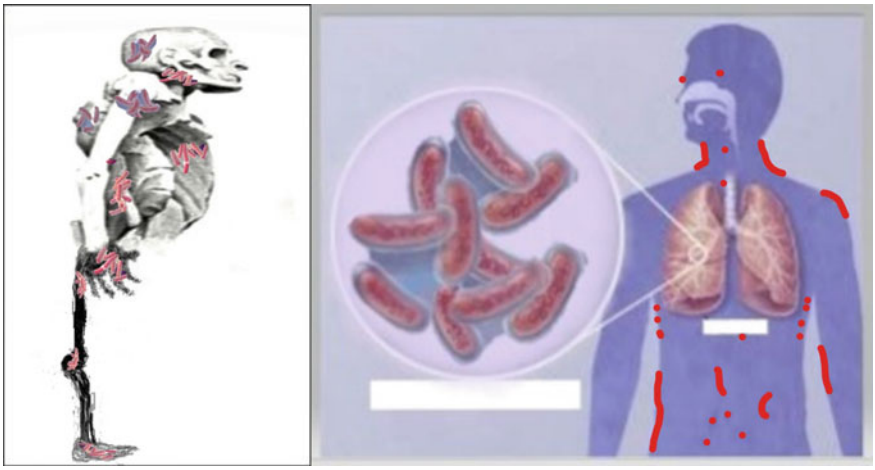
Histopathological analysis, Mantoux test, radiological imaging, surgical biopsy, fine-needle aspiration biopsy, bacteriological examination, and polymerase chain reaction are among the procedures used in cases suspected of having the disease. Multidrug anti-TB chemotherapy is the critical enabler to treatment. Surgery is recommended for those who develop abscesses, have intractable pain, cannot tolerate spinal weakness, have elevated kyphosis, and do not respond to chemotherapy. The key factors contributing to the below-average results include delayed diagnosis, comorbidities, DR, and poor adherence.

Graphical Abstract



Mycobacterium tuberculosis: from human lungs to bone

Keywords

Bone and joint tuberculosis · Mycobacterial bone infections · Osteomyelitis tuberculosis · Spinal tuberculosis · Tuberculosis

1 Introduction

Skeletal tuberculosis (TB) involves the joints and bones [1]. The disease has been found in Egyptian mummies dating back over 9000 years. Post-Columbian skeletons in Chile demonstrated 2% of the time that the people had lesions consistent with bone TB. A further molecular study has confirmed the existence of *Mycobacterium tuberculosis* (*M. tb*) complex DNA in ancient bone specimens [2].

Musculoskeletal TB is seen in around 10–25% of extrapulmonary TB (EPTB) cases. The majority of infection sites are located in the spine (with 50–69% prevalence), then the hip, knee, and ankle/foot (with 10–13% prevalence each) [3, 4]. There is only limited data on childhood musculoskeletal TB [4]. Every year, 20 children under the age of 12 are treated for musculoskeletal TB at a tertiary care hospital in Africa's Sub-Saharan region.

Though there remains an issue of osteoporosis among TB patients, there is little in the way of research. This problem has previously been documented in Taiwan and Korea. There were 53 new TB cases per 100,000 Taiwanese people in 2012. Osteoporosis/fragility fracture was found in Taiwan at 9.61 per 1000 people per year among TB patients, and another result discovered was 4.31 per 1000 people per year among TB patients [5, 6]. The estimated TB incidence in Korea in 2015 was 80 per 100,000 populations, placing Korea in the center of the world when it comes to TB burden. The findings of a Korean study indicate that 19.6% of the population had TB disease, 36.6% had TB scars on their chest X-ray, and 45% had both TB disease and scars [7].

Osteoporosis is an ailment featured by a decrease in bone mass and microstructure stagnation that causes bones to become more fragile and increases fracture risk. Both of these features contribute to a very high mortality rate. BMD, or bone mineral density, is used to calculate bone strength and is affected by calcium content [8]. It is challenging to pin down the exact diagnosis early in the present scenario because the physician dealt with TB but overlooked the osteopenia because of the same circumstance. Population-based research was recently completed in Taiwan and published in the European Respiratory Journal; the results indicated that 4.31 and 1.80 per 1000 person-years in 3725 TB patients and 14,900 control subjects ($p < 0.001$) were osteoporotic, respectively [9]. Additionally, TB is considered a risk factor for osteoporosis (HR 1.82, 95% CI 1.38–2.40). TB patients are not regularly screened for osteoporosis, thus leaving it in doubt for their future.

The results can be interpreted as T- or Z-scores using quantitative ultrasound (QUS) and dual-energy X-ray absorptiometry (DXA). A significant number of fractures (approximately 1.5 million per year) are caused by osteoporosis, which results in osteoporosis-related fractures. In osteoporosis, both traumatic and non-traumatic fractures occur. The WHO and NIH classify BMD as normal, osteopenia, and osteoporosis depending on T-score or Z-score. The Z-score is commonly used to predict BMD in a group of matching people of a similar age, gender, and ethnicity. For a Z-score of ≤ -2 SD (standard deviations), BMD was perceived as being poor [10–12].

2 Bacteriology of Bone and Joint Tuberculosis

Mycobacteria other than *Mycobacterium tuberculosis* (*M. tb*) or *M. bovis* may cause bone or joint infections [13]. Bacillemia can result in seeding organisms in bone and synovial tissue during *M. tb* primary infection. Tissue and pus samples are

obtained, and different culturing methods are used. The growth of *M. tb* is slow, taking several weeks to identify a colony, or neither is possible on a conventional culture medium. Streamlined culturing techniques include the standard Löwenstein-Jensen (L.J.) and BACTEC MGIT 960 methods to validate bone and joint TB cases. To offer a brief description, the specimen is decontaminated first, then diluted for 15 min with 2% sodium hydroxide and 0.5% NALC (N-acetyl-l-cysteine-sodium hydroxide). After diluting the tube with 0.1 M phosphate buffer (pH 6.5), it was centrifuged at 4000 × *g* for 20 min. The re-suspended pellets were centrifuged again, and the final pellet was re-suspended in phosphate buffer to make enough space for the liquid MGIT 960 and the L.J. growth medium. A 0.5 ml deposit was inoculated into and out of each culture media [14]. Colony numbers for joint tuberculosis are typically a thousand times lower than those for pulmonary TB. Because of the accessibility of infection sites, histopathological diagnosis is frequently a worry, and patients are less reluctant to undertake procedures that may be uncomfortable. Other variables in the spread of atypical mycobacteria include the use of local steroid shots and other anticancer drugs, traumatic surgery, and diabetes mellitus [13, 15, 16].

3 Pathophysiology

Through the hematogenous route of spread, TB of the musculoskeletal system will shortly follow the initial lung infection. Bone and joint involvement observed with TB is of two types: caseous exudative and granular. It causes bone breakdown, localized inflammation, abscess, sinus, and various constitutional symptoms in young children. A granular type appears to be more subtle and less disruptive, and abscess formation is uncommon. Adults are more likely to be affected. TB that originates in bone growth plates appears to spread to the joint spaces. However, it can be transmitted by the lymphatic system, as well. One of the most prevalent causes of joint infection is a lack of activation of the lymphatic or vascular supply [17–21]. TB is thought to have formed in the epiphyseal plates of long bones, resulting in tubercle growth in the marrow. As a result, this would possibly result in a serious infection. The synovitis can deal with *M. tb*, which causes the growth of granulation tissue, followed by marginal erosions, and, finally, bone degradation, which ultimately results in periarticular demineralization. To minimize the risk of cartilage destruction, TB inhibits proteolytic enzyme development, decreasing the chance of proteolysis and thereby lessening cartilage destruction. This rise in abscess formation around the abscess can be because this condition has not been treated. Bone sequestration is uncommon. Bone breakdown and limited new bone growth are both evident in the active phases of tuberculous osteomyelitis [22, 23].

4 Clinical Features

Bone and joint TB may occur at any age. TB can infiltrate almost all of the bones in the body, and the majority of people with arthritis have monoarticular arthritis. Around 70% of most childhood diseases arise in the spine. Many of the joints involved in everyday activities are weight-bearing joints, including the hips, knees, and elbows. Usually, people would experience discomfort and swelling in the affected region, accompanied by limitation of movement. A significant weakening of the regional muscle, as well as an increase in deformity, can occur. Additionally, the cold abscess (painless) condition was the only noticeable presentation in a limited number of cases. Tubercular arthritis might involve more than one site in 5–30% of cases [24]. The risk of a recurrence of TB arthritis is as high as 17–34%. Most often, it happens in the hip joint [15].

Skeletal TB accounts for about 50% of all cases of this form of TB. TB infection spreads through the subligamentous tissue, traveling through the spine through paravertebral spaces and into the soft tissues that surround the spine. As a result, the vertebrae develop osteonecrosis, cease to function, and are destroyed [25–28]. Acid-fast bacilli are a key element in bone loss. It is likely that compression of the spine, either by bone or the development of an abscess and granulation tissue directly covering the cord and leptomeninges, is responsible for spinal cord involvement [29]. The beginnings of neurological deficiencies are incremental relative to other conditions, for example, diabetic foot [30]. "Skipped lesions" are when spinal TB affects vertebral bodies at two or three separate locations [31]. Monoarticular TB usually occurs in the spine and weight-bearing joints, such as the knee, hip, and ankle. Furthermore, synovial-type TB arthritis is usually found in the cited joints [32].

The onset can be as subtle as subtle joint swelling or as obvious as acute joint swelling, with discomfort and joint mobility limitation being the most common symptoms [33]. Sinuses can present as a single symptom, misinterpreted as a diabetic foot or a pyogenic infection [34]. A joint deformity can occur, as can granulomatous processes that result in a lumpy or doughy feeling. Besides, localized pain could occur earlier than other inflammatory or radiographic changes [21]. Ultimately, the health-related quality of life and reduced functional abilities are more likely to occur in a patient with bone and articular cartilage destroyed, particularly if the diagnosis is made later [35]. Spine TB, in the majority of cases, presents insidiously. Tightness, joint pain in all directions, and extreme muscle spasms are common in the surrounding area. In nearly all situations, an abscess in the soft tissues results from a pre-existing lesion that has spread into the intermuscular spaces over time. Kyphosis in the spine can be followed by local tenderness [13]. The most common appearance of psoas abscess is a strong, hard swelling in the upper thigh. Serious spinal deformity, such as angular kyphosis, is very common in low-income neighborhoods and is particularly prevalent in children. Delayed diagnosis of spinal TB is common among these people.

5 Diagnosis

The most difficult component of detecting skeletal TB is the lack of documentation of critical chest illness in more than half of the patients [36]. Also, the delay in making a confirmed diagnosis for bone and skeletal TB makes the treatment more complicated. A patient's history is essential to make a confirmed diagnosis, and the medical staff should take a detailed history to identify the source of the infection. In HIV patients and patients with relatively high numbers of CD4 cells, the detection of bone and musculoskeletal TB is commonly missed, especially among asymptomatic TB patients [37].

After taking a microscopic tissue sample, a verified diagnosis can be made. Samples can be taken using a simple needle aspiration or tissue biopsy using the assistance of computed tomography (CT) whenever it is available [38]. Clinical examination, Mantoux testing, imaging, fine needle aspiration, the suction of purulent material or bacteriological synovial fluids, and histopathological biopsy are some other popular diagnostic methods [39, 40]. The polymerase chain reaction (PCR) has been introduced to tissue biopsy for the initial detection of tuberculous arthritis. However, in the case of osseous TB diagnosis, mycobacterium cell culture of bone or synovial fluids remains the standard practice [41]. At an early stage of TB treatment, the skin test produces negative results, so the tuberculin skin test (TST) should be done after six weeks of the initiation of arthritis [41]. Positive TST results for children should be taken seriously in any child who has monoarticular arthritis.

In communities where TB prevalence is high, the Mantoux skin is helpful for skeletal diseases among children [42, 43]. Approximately 14% of Mantoux test results were reported as a false negative, indicating that, despite its advantages, the test has a considerable margin of error [44, 45].

Non-specific anomalies can occur in early-stage radiological studies, but they are not readily apparent; instead, they may be asymptomatic, and as the disease progresses, soft tissue swelling and a small amount of periosteal reaction, osteopenia, joint space narrowing, and subchondral erosion are observed [45].

MRI is preferred as the most effective tool for recognizing granulation tissue and abscess and detecting soft tissue masses and bone degradation [46]. While in CT scan, bone abnormalities like calcifications and sequestra and bone anatomy are more clear and easier to spot [47]. The radiographic imaging of the chest can identify around 50% of osteoarticular TB. However, active chest disease is present in one in every 536 [41]. It has been reported that around 73% of cases were associated with granulomatous lesions with or without caseation necrosis [48].

PCR is more sensitive in detecting mycobacteria and can distinguish non-tuberculous mycobacteria connected to soft tissue diseases that are often misdiagnosed with TB. Direct smearing detected mycobacteria in 27% of operational specimens of purulent synovial fluid and 63% on culture tests [49]. The culture of the sinus-track specimen can sometimes identify the organism when pathological and clinical examination cannot do so [50]. For that, the sinus-track

specimen has been considered a great source for the isolation of mycobacteria. It is also advisable to consider TB bone infection when *Staphylococcus epidermidis* is observed during the usual aerobic and anaerobic sinus culture [45]. A bone biopsy is recommended to clear up the confusion and make a confirmed diagnosis. It is the most sensitive test for TB arthritis [51, 52]. Histological investigations should be performed whenever the microbiological tests come negative.

DNA-Based PCR is a sensitive test; however, it might fail to identify non-variable bacilli [53]. Studies found the PCR test sensitive in 57.7% of synovial fluid samples, 81% of the sputum samples, and 64.2% of the pleural fluid samples [54–56]. The widespread prevalence of latent TB causes an increase in false-positive PCR findings [57, 58].

There are some serious limitations for the most recent diagnostic techniques, such as the high cost and specific expensive equipment required, in addition to the expertise and trained staff required to conduct tests such as the rapid automated nucleic acid probe assays, which drastically reduce the application of these tests in developing countries [59].

The novel tests based on the automated nucleic acid amplification method, the Xpert MTB/RIF assay, detected *M. tb* and rifampin resistance, and its preliminary findings suggested that it could help diagnose musculoskeletal TB [60]. In most cases, an acute or chronic pathogen infection like *Staphylococcus aureus*, osteomyelitis, brucellosis, melioidosis, actinomycosis, candidiasis, or histoplasmosis would be required for the diagnosis of skeletal TB [41]. Sometimes multifocal bone appearance can lead to misdiagnosis of musculoskeletal TB as metastatic cancer [61]. Pott's disease, spondyloarthropathy, vertebral body collapse due to osteopenia, pyogenic spinal infection, and malignancies share common clinical symptoms, thus raising the complexity of a conclusive diagnosis [41, 62].

6 Treatment

Most patients can usually recover from osteoarticular TB when diagnosed and treated early. Multidrug anti-TB chemotherapy plays a major role in recovery besides active training and non-weight-bearing exercises [63, 64]. The therapy should begin with enough rest, followed by progressively increasing mobilization based on professional advice and constant monitoring. Structural spinal supports are available in many forms, including collars, corsets, and braces that can be helpful during treatment [64]. Proper initial support is crucial for a successful treatment.

The prime target of the treatment is firstly to contain the infection and then eradicate it, as well as to relieve the pain and preserve and restore the bone and joint functions [65, 66]. Late diagnosis is considered the major reason for poor treatment outcomes [67]. The CDC and the American Thoracic Society (ATS) in the USA currently recommend osseous TB treatment in two phases:

i. the first one is a two-month treatment course with isoniazid, rifampicin, pyrazinamide, and ethambutol; and
ii. the second phase is a six to 12 months treatment with isoniazid and rifampicin [68].

Thoracic and spinal TB should be treated with ambulatory chemotherapy conferring to The Joint Tuberculosis Committee of the British Thoracic Society, which comprises six months of combined therapy with ethambutol, rifampicin, pyrazinamide, and isoniazid. Afterward, this treatment is extended for further four months with the addition of isoniazid and rifampicin [69]. Surgery is also recommended for some cases, along with chemotherapy in case of spinal cord compression or instability [69]. Indian studies, on the other hand, suggest treatment that does not wait until there is radiographic evidence of bone healing for patients with bone and muscle TB, which may take up to six months to emerge [70].

Multiple studies have observed the advantages of surgical treatments for spinal TB. The surgical intervention group had fewer neurological impairments, less angel for kyphosis, and shorter inpatient time than the non-surgical therapy group [70]. A meta-analysis, however, indicated no significant difference between surgical intervention groups and simply chemotherapy groups [71].

The optimal treatment duration for skeletal TB remains controversial. Some studies favored a prolonged duration of treatment to optimize post-treatment recovery, while others concluded that six months of bacilli treatment in the lesion is optimal [72]. For pediatric TB, some studies show that prolonged treatment of a 15-month course of therapy might effectively eliminate bacilli [73]. However, medical treatment duration has been recorded in several cases ranging from six months to 18 months for sacral TB and other spinal sites [74, 75]. Drug resistance (DR) should be considered after four to six months of continuous treatment with poor or slow improvement. If resistance has been observed, the second-line intensive treatment should be started [76].

7 Prevention

According to the British National Health Service, TB vaccination can be done for babies, children, and adults, starting from one year old. However, only healthcare professionals at a greater risk of contracting TB get the Bacillus Calmette-Guerin (BCG) vaccination since it has been demonstrated to have declining efficiency and does not provide enough protection over time [77].

Several vaccinations have been created to defend against tuberculosis; several are now in clinical testing since the existing BCG vaccine offers insufficient protection and varies considerably across people. Also, there are some ongoing investigations to improve the BCG vaccine effectiveness [78]. Until then, the BCG vaccine remains the best option, especially for children and babies, besides using more sophisticated bioinformatics analysis to identify the biosignature of BCG protection against TB [78].

8 Patient's Adherence

Poor TB medication adherence has been connected to chemotherapy treatment failure and, eventually, DR, which is a significant issue [79]. Many factors or barriers can lead to patients' non-compliance. Incomprehension, loss of income, stigma, a lack of social support, drug side effects, and a long treatment time are the most common barriers to treatment adherence [80, 81]. Many factors, such as accessibility to healthcare facilities, direct and clear communication, and acceptance and understanding of attitudes from healthcare professionals, help improve patients' quality of life [81]. Furthermore, offering extensive teaching campaigns for healthcare workers, patients' family members, and the community will simplify developing a social support framework for the patient, which will aid in acceptance and adherence and make resolving any emergent concerns much easier.

9 Conclusion

Bone and joint TB constitute a more challenging situation in developing and undeveloped countries. In endemic regions, if there is evidence of bone degradation and spinal vertebrae preservation and epidural masses, it is reasonable to suspect TB, specifically a spinal infection. TB-related osteoarticular symptoms linked to the prosthetic limb, the trochanteric region, and non-tuberculous mycobacterial infections should be investigated in-depth. In such a condition, surgery combined with extended but recommended anti-TB chemotherapy would be an ultimate option for the ideal treatment outcomes. Controversies on whether surgery should be performed and how long therapy should last remain. Patient adherence and concordance are also advised for ideal care outcomes.

Core Messages

- Diagnosis of TB of bones and joints might be missed at an early stage, which makes things delayed and complicated.
- TB of bones and joints will be challenging in children's scenarios.
- Patients with TB of bones and joints need long-term follow-up.

References

1. Pigrau SC, Rodríguez PD (2013) Bone and joint tuberculosis. Eur Spine J 22(4):556–566
2. Taylor G, Murphy E, Hopkins R, Rutland P, Chistov Y (2007) First report of *Mycobacterium bovis* DNA in human remains from the Iron Age. Microbiology 153(Pt 4):1243–1249

3. Johansen IS, Nielsen SL, Hove M et al (2015) Characteristics and clinical outcome of bone and joint tuberculosis from 1994 to 2011: a retrospective register-based study in Denmark. Clin Infect Dis 61:554–562
4. Maqungo S, Oleksak M, Dix PS, Hoffman EB (2012) Tuberculosis of the foot and ankle in children. SA Orthopaedic J 11:23–28
5. Chen Y, Feng J, Ting W, Yen Y, Chuang P, Pan S et al (2017) Increased risk of incident osteoporosis and osteoporotic fracture in tuberculosis patients: a population-based study in a tuberculosis-endemic area. Osteoporosis Int 28(5):1711–1721
6. Yeh J, Wang Y, Lin C, Lin C, Hsu W (2016) Association of respiratory tuberculosis with incident bone fracture: bridging the tuberculosis airway infection and the osteoporotic bone. PLoS ONE 11(12)
7. Choi C, Choi W, Kim C, Lee S, Kim K (2017) Risk of sarcopenia and osteoporosis in male tuberculosis survivors: Korea National Health and Nutrition Examination Survey. Sci Rep 7 (13127):1–10
8. Carnevale V, Romagnoli E, D'Erasmo E (2004) Skeletal involvement in patients with diabetes mellitus. Diabetes Metab Res Rev 20(3):196–204
9. Jia YF, Ying YC, Yung FY, Sheng WP, Wei JS (2016) Active tuberculosis increases the risk of incident osteoporosis—a nationwide population-based cohort study. Eur Respir J 48. https://doi.org/10.1183/13993003.congress-2016.OA4823
10. Cournil A, Eymard DS, Diouf A, Moquet C, Coutherut J, Gueye et al (2012) Reduced quantitative ultrasound bone mineral density in HIV-infected patients on antiretroviral therapy in Senegal. PLoS ONE 7(2)
11. Handa K, Maalouf DB, Chandra P, Thimmaraju K, Samanta S, Raju S (2012) Study of serum magnesium values in pulmonary tuberculosis patients. J Adv Health Med Sci 4(1):54–57
12. Cosman F, De Beur S, LeBoff M, Lewiecki E, Tanner B, Randal S et al (2014) Clinician's guide to prevention and treatment of osteoporosis. Osteoporos Int 25(8)
13. Sankaran B (1993) Tuberculosis of bones and joints. Ind J Tub 40:109–118
14. Chen S, Zhao L, Dong W, Gu Y, Li Y, Dong L et al (2015) The clinical features and bacteriological characterizations of bone and joint tuberculosis in China. Sci Rep 5(1):1–9
15. Abdulaziz S, Almoallim H, Ibrahim A, Samannodi M, Shabrawishi M, Meeralam Y et al (2012) Poncet's disease (reactive arthritis associated with tuberculosis): retrospective case series and review of the literature. Clin Rheumatol 31(10):1521–1528
16. Denis DN, Merrien D, Billaud E, Besnier J, Duhamel E, Hutin P et al (1998) Extrapulmonary tuberculosis in the central-western region. A retrospective study of 217 cases (Gericco 1991–1993). Presse Med (Paris, France: 1983) 27(8):341–346
17. Tuli SM (2016) Tuberculosis of the skeletal system. JP Medical Ltd.
18. Lenaerts A, Barry CE III, Dartois V (2015) Heterogeneity in tuberculosis pathology, microenvironments and therapeutic responses. Immunol Rev 264(1):288–307
19. Iseman MD (2000) A clinician's guide to tuberculosis. Lippincott Williams and Wilkins. Philadelphia, pp 162–170
20. Wright T, Sundaram M, McDonald D (1996) Radiologic case study: tuberculous osteomyelitis and arthritis. Orthopedics 19:699–702
21. Isselbacher KJ, Braunwald E, Petersdorf RG, Wilson JD, Martin JB, Fauci AS (1987) Infectious arthritis. Harrison's principles of internal medicine, 12th edn. McGraw-Hill, New York, pp 544–548
22. Davidson PT, Horowitz I (1970) Skeletal tuberculosis: a review with patient presentations and discussion. Am J Med 48:77–84
23. Kahn DS, Pritzker KPH (1973) The pathophysiology of bone infection. Clin Orthop 7 (96):12–19
24. Parasca I, Damian L, Albu A (2006) Infectious muscle disease. Rom J Intern Med 44(2):131–141
25. Rajasekaran S, Shanmugasundaram TK, Parabhakar R et al (1998) Tuberculous lesions of the lumbosacral region. A 15-year follow-up patients treated by ambulant chemotherapy. Spine 23:1163–1167

26. Hodgson AR, Stock FE (1994) Anterior spinal fusion: a preliminary communication on the medical treatment of Pott's and Pott's paraplegia. Clin Orthop Rel Res 300:16–23
27. Meghji S, White PA, Nair SP et al (1997) *Mycobacterium tuberculosis* chaperanin 10 stimulates bone resorption: a potential contributory factor in Pott's disease. J Exp Med 186:1241–1246
28. Korkusuz Z, Islam C (1997) Prevention of post-operative late kyphosis in Pott's disease by anterior decompression and intervertebral grafting. World J Surg 21:524–528
29. Shanley DJ (1995) Tuberculosis of the spine: imaging features. Am J Res 164:659–664
30. Hsu LCS, Leong JCY (1984) Tuberculosis of the lower cervical spine (C2 to C7). J Bone Joint Surg (Br) 66:1–5
31. Turgut M (2001) Multifocal extensive spinal tuberculosis (Pott's disease) involving the cervical, thoracic and lumbar vertebrae. Br J Neurosurg 15:142–147
32. Sequeira W, Co H, Block JA (2000) Osteoarticular tuberculosis: current diagnosis and treatment. Am J Ther 7(6):393–398
33. Hunfield KP, Rittmeister M, Wichelhaus TA et al (1998) Two cases of chronic arthritis of the forearm due to *Mycobacterium tuberculosis*. Eur J Clin Microbiol Infect Dis 17:344–348
34. Mousa HA (1998) Tuberculosis of bones and joints: diagnostic approaches. Int Orthop 22:245–246; Chen WS, Wang CJ, Eng HL (1997) Tuberculous arthritis of the elbow. Int Orthop 21:367–370
35. Titov AG, Vyshnevskaya EB, Mazurenko SI et al (2004) Use of polymerase chain reaction to diagnose tuberculous arthritis from joint tissues and synovial fluid. Arch Pathol Lab Med 128:205–209
36. Moule MG, Cirillo JD (2020) *Mycobacterium tuberculosis* dissemination plays a critical role in pathogenesis. Front Cell Infect Microbiol 10:65
37. Jilani TN, Avula A, Zafar Gondal A, Siddiqui AH (2020) Active tuberculosis. In: StatPearls. StatPearls Publishing, Treasure Island (FL). Available at: http://www.ncbi.nlm.nih.gov/books/NBK513246/. Accessed 3 Sept 2020
38. Wang P, Liao W, Cao G, Jiang Y, Rao J, Yang Y (2020) Characteristics and management of spinal tuberculosis in tuberculosis endemic area of Guizhou Province: a retrospective study of 597 patients in a teaching hospital. Biomed Res Int
39. Jami SA, Jiandang S, Mobarak SA, Hao LC (2020) Recent diagnosis and treatment progress of spinal tuberculosis. Int J Spine Surg 2(1):1–6
40. Jacquier H, Fihman V, Amarsy R, Vicaut E, Bousson V, Cambau E et al (2019) Benefits of polymerase chain reaction combined with culture for the diagnosis of bone and joint infections: a prospective test performance study. Open Forum Infect Dis 6(12)
41. Procopie I, Popescu EL, Huplea V, Plesea RM, Ghelase ȘM, Stoica GA et al (2017) Osteoraticular tuberculosis—brief review of clinical morphological and therapeutic profiles. Curr Health Sci J 43(3):171–901
42. Gualano G, Mencarini P, Lauria FN, Palmieri F, Mfinanga S, Mwaba P et al (2019) Tuberculin skin test—outdated or still useful for Latent TB infection screening? Int J Infect Dis 80:S20–S22
43. Nayak S, Acharjya B (2012) Mantoux test and its interpretation. Indian Dermatol Online J 3 (1):2–6
44. Pahal P, Sharma S (2020) PPD skin test (tuberculosis skin test). In: StatPearls [Internet]. StatPearls Publishing, Treasure Island (FL)
45. Qian Y, Han Q, Liu W, Yuan W-E, Fan C (2018) Characteristics and management of bone and joint tuberculosis in native and migrant population in Shanghai during 2011 to 2015. BMC Infect Dis 18(1):543
46. Kukreja R, Mital M, Gupta PK (2018) Evaluation of spinal tuberculosis by plain X-rays and magnetic resonance imaging in a tertiary care hospital in Northern India—a prospective study. Int J Contemp Med Res 5(2):6
47. Moulopoulos LA, Koutoulidis V, Hillengass J, Zamagni E, Aquerreta JD, Roche CL et al (2018) Recommendations for acquisition, interpretation, and reporting of whole-body low

dose CT in patients with multiple myeloma and other plasma cell disorders: a report of the IMWG Bone Working Group. Blood Cancer J 8(10):1–9
48. Masood S (1992) Diagnosis of tuberculosis of bone and soft tissue by fine-needle aspiration biopsy. Diagn Cytopathol 8(5):451–455
49. Ahmed NA, Huda N (2013) Osteoarticular tuberculosis—a three years' retrospective study. J Clin Diagn Res 7(10):2189–2192
50. Haworth CS, Banks J, Capstick T, Fisher AJ, Gorsuch T, Laurenson IF et al (2017) British Thoracic Society guidelines for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD). Thorax 72(2):ii1–ii64
51. Hughes P, Miranda R, Doyle AJ (2019) MRI imaging of soft tissue tumours of the foot and ankle. Insights Imaging 10(1):60
52. Al-Sayyad MJ, Abumunaser LA (2011) Tuberculous arthritis revisited as a forgotten cause of monoarticular arthritis. Ann Saudi Med 31(4):398–401
53. Kralik P, Ricchi M (2017) A basic guide to real-time PCR in microbial diagnostics: definitions, parameters, and everything. Front Microbiol 8:108
54. Asnaashari AMH, Towhidi M, Farid R, Abbaszadegan MR, Attaran D, Fatemi SS et al (2011) Evaluation of polymerase chain reaction for diagnosis of "tuberculous pleurisy." Tanaffos 10 (1):12–18
55. Vorster MJ, Allwood BW, Diacon AH, Koegelenberg CFN (2015) Tuberculous pleural effusions: advances and controversies. J Thorac Dis 7(6):981–991
56. Li Q, Pan YX, Zhang CY (1994) Specific detection of *Mycobacterium tuberculosis* in clinical material by PCR and Southern blot. Zhonghua Jie He He Hu Xi Za Zhi 17(4):238–240, 256
57. Titov AG, Vyshnevskaya EB, Mazurenko SI, Santavirta S, Konttinen YT (2004) Use of polymerase chain reaction to diagnose tuberculous arthritis from joint tissues and synovial fluid. Arch Pathol Lab Med 128(2):205–209
58. Wu M, Su J, Jan F, Cai L, Deng Z (2018) Skipped multifocal extensive spinal tuberculosis involving the whole spine. Medicine 97(3)
59. Yiyan L, Xing Y, Weian Z (2017) Emerging microtechnologies and automated systems for rapid bacterial identification and antibiotic susceptibility testing. Slas Technol 22(6):585–608
60. Wen H, Li P, Ma H, Lv G (2017) Diagnostic accuracy of Xpert MTB/RIF assay for musculoskeletal tuberculosis: a meta-analysis. Infect Drug Resist 10:299–305
61. Ye M, Huang J, Wang J, Ren J, Tu J, You W et al (2015) Multifocal musculoskeletal tuberculosis mimicking multiple bone metastases: a case report. BMC Infect Dis 16(1):34
62. Amidon RF, Ordookhanian C, Vartanian T, Kaloostian P (2020) A rare form of Pott's disease with multifaceted pathological complications. Cureus 12(6)
63. Hazra A, Laha B (2005) Chemotherapy of osteoarticular tuberculosis. Indian J Pharmacol 37 (1):5
64. Bhat ZS, Rather MA, Maqbool M, Ahmad Z (2018) Drug targets exploited in *Mycobacterium tuberculosis*: Pitfalls and promises on the horizon. Biomed Pharmacother 103:1733–1747
65. Hawkinson NV, DNP, RN, RNFA (2019) Corsets: a type of spinal brace. SpineUniverse. Available at: https://www.spineuniverse.com/treatments/bracing/corsets-type-spinal-brace. Accessed 4 Sept 2020
66. Issar S (2003) *Mycobacterium tuberculosis* pathogenesis and molecular determinants of virulence. Clin Microbiol Rev 16(3):463–496
67. Stephen K (2020) Osteomyelitis treatment & management: approach considerations, medical therapy, surgical therapy. Medscape. Available at: https://emedicine.medscape.com/article/1348767-treatment, Accessed 4 Sept 2020
68. Ali MK, Karanja S, Karama M (2017) Factors associated with tuberculosis treatment outcomes among tuberculosis patients attending tuberculosis treatment centres in 2016–2017 in Mogadishu, Somalia. Pan Afr Med J 28(1)
69. Nahid P, Dorman SE, Alipanah N, Barry PM, Brozek JL, Cattamanchi A et al (2016) Executive Summary: Official American Thoracic Society/Centers for Disease Control and

Prevention/Infectious Diseases Society of America Clinical Practice Guidelines: treatment of drug-susceptible tuberculosis. Clin Infect Dis 63(7):853–867
70. Joint Tuberculosis Committee of the British Thoracic Society (1998) Chemotherapy and management of tuberculosis in the United Kingdom: recommendations 1998. Thorax 53 (7):536–548
71. Garg RK, Somvanshi DS (2011) Spinal tuberculosis: a review. J Spinal Cord Med 34(5):440–454
72. Zhang X, Ji J, Liu B (2013) Management of spinal tuberculosis: a systematic review and meta-analysis. J Int Med Res 41(5):1395–1407
73. Rasouli MR, Mirkoohi M, Vaccaro AR, Yarandi KK, Rahimi-Movaghar V (2012) Spinal tuberculosis: diagnosis and management. Asian Spine J 6(4):294–308
74. Hosalkar HS, Agrawal N, Reddy S, Sehgal K, Fox EJ, Hill RA (2009) Skeletal tuberculosis in children in the Western world: 18 new cases with a review of the literature. J Child Orthop 3 (4):319–324
75. Wellons JC, Zomorodi AR, Villaviciencio AT, Woods CW, Lawson WT, Eastwood JD (2004) Sacral tuberculosis: a case report and review of the literature. Surg Neurol 61(2):136–139; discussion 139–141
76. Subasi M, Bukte Y, Kapukaya A, Gurkan F (2004) Tuberculosis of the metacarpals and phalanges of the hand. Ann Plast Surg 53(5):469–472
77. Nahid P, Mase SR, Migliori GB, Sotgiu G, Bothamley GH, Brozek JL et al (2019) Treatment of drug-resistant tuberculosis. An official ATS/CDC/ERS/IDSA Clinical Practice Guideline. Am J Respir Crit Care Med 200(10):e93–142
78. BCG tuberculosis (TB) vaccine overview (2019) NHS.UK. Available at: https://www.nhs.uk/conditions/vaccinations/bcg-tuberculosis-tb-vaccine/. Accessed 4 Sept 2020
79. Hazel MD, Steven GS (2017) What have we learnt about BCG vaccination in the last 20 years? Front Immunol 8:1134
80. Rajasekaran S, Khandelwal G (2013) Drug therapy in spinal tuberculosis. Eur Spine J 22 (4):587–593
81. Gebreweld FH, Kifle MM, Gebremicheal FE, Simel LL, Gezae MM, Ghebreyesus SS et al (2018) Factors influencing adherence to tuberculosis treatment in Asmara, Eritrea: a qualitative study. J Health Popul Nutr 37(1):1

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Abdominal Tuberculosis: Pathogenesis, Clinical Features, and Diagnosis

39

Ashish Gupta

The biggest disease today is not leprosy or tuberculosis, but rather the feeling of being unwanted.

Mother Teresa

Summary

Tuberculosis (TB) is a morbid infectious disorder that can involve multiple organ systems in the body. Pulmonary infection is the commonest site, followed by the abdomen. Abdominal TB can have a varied presentation and generally mimics malignancy in endemic areas. The diagnosis is established after a definitive biopsy. Recent advances in serology and biochemical parameters may aid in non-invasive diagnosis. However, significant overlap in the signs and symptoms of TB and malignancy makes invasive biopsy mandatory.

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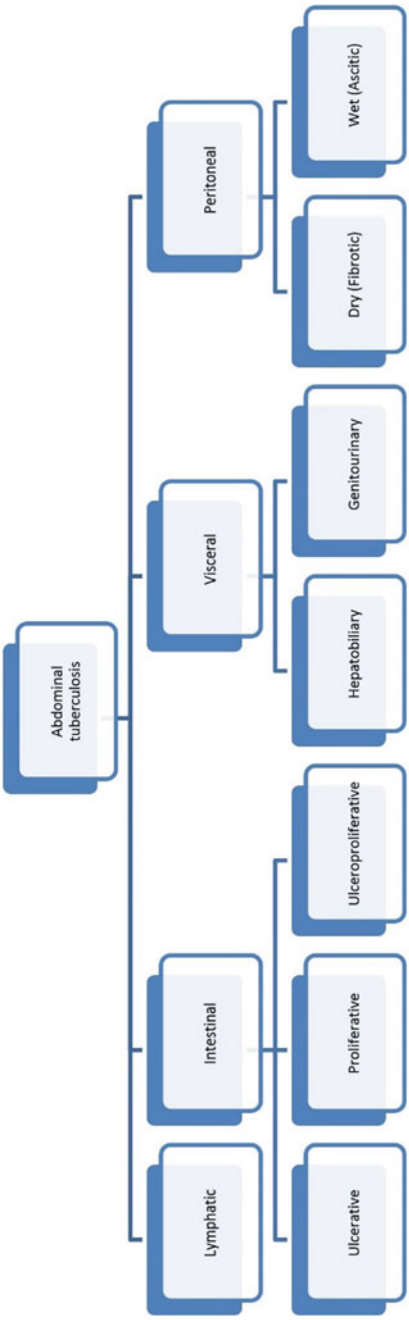
Graphical Abstract



Abdominal tuberculosis classification

Keywords

Abdomen tuberculosis · Gastrointestinal tuberculosis · Miliary tuberculosis · Peritoneal tuberculosis

1 Introduction

Tuberculosis (TB) is a common infection encountered in third-world countries [1]. With increasing immunodeficiency disorders, it is becoming a major health problem in the western world [2]. The pulmonary system is the commonest system infected by *Mycobacterium tuberculosis* (*M. tb*). Concomitant respiratory and abdomen is involved in 15–25% of the cases [3]. There is a significant overlap of symptoms between malignancies as well as TB of the abdomen [4]. Various reports have already been published highlighting diagnostic as well as therapeutic misadventure. A cheap, reliable, and reproducible biomarker can help differentiate these ailments.

TB of the abdomen, being a complex host of multiple viscera, peritoneum, and lymphatics, manifests with a wide range of signs and symptoms [5]. These can range from isolated abdominal pain to a cocoon abdomen in widespread involvement. Radiological tests like ultrasonogram and contrast-enhanced computerized tomogram (CECT) can aid in the diagnosis of TB [6]. Biochemical ascitic fluid analysis can help diagnose the ascitic form of peritoneal TB. Histopathological examination of the peritoneal or the lymph node (LN) biopsy may aid the investigation. The gastrointestinal (GI) system can be approached directly using endoscopes, and a biopsy can be obtained simultaneously. Effective chemotherapeutic agents are available for the non-surgical management of the ailment. However, multidrug-resistant forms of bacilli are widely prevalent in immunocompromised hosts [7].

2 Pathogenesis

M. tb gains its entry into the host through the respiratory route [8]. Further dissemination of the infection in the body depends upon the immunity and nourishment status of the infected individual. Immunosuppression in any form leads to dissemination through lymphatics and blood. The gastrointestinal system is involved after ingestion of non-boiled and infected milk and sputum [9]. The bacteria involve the mucosa and cause ulceration and necrosis. The healing of these ulcers leads to stricture formation [10], whereas transmural necrosis of the bowel wall causes perforation and secondary peritonitis. Visceral involvement, though uncommon, can occur as a result of the miliary process, either through direct involvement through the surrounding peritoneum and LNs or hematogenous dissemination through the portal and hepatic supply in the case of liver and spleen

[11]. The peritoneum is affected directly from the viscera like fallopian tubes, GI system, and rupture of the involved LNs [12].

The ileocecal region is the commonest site involved in the GI tract. The alkaline nature of the fluid, extensive lymphatic tissue, large absorptive surface area, and stasis of the infected fluid due to the presence of the ileocecal valve are hypotheses behind this occurrence [10]. The immunity of the affected patient then decides the clinical form of the infection. It can be either ulcerative, hyperplastic, or ulcero-hyperplastic. The ulcerative form is a sign of underlying immunosuppression and malnutrition. The hyperplastic forms present with a mass typically in the right iliac fossa, which is formed by the omentum, mesentery, and the involved bowel loops [13].

Peritoneal TB can be of the ascitic (wet) type or fibrotic (dry) type [14]. The wet type of peritoneal involvement presents with exudative ascites, and fibrotic form causes omental thickening with nodules and adhesions between the bowel loops. This classification, however, does not help in clinical practice as a significant overlap of both forms is encountered.

Viscera are rarely involved in the tubercular process. These organs are inherently resistant to TB infections as the alkaline nature of the biliary and pancreatic fluid is hostile for *M. tb* [11]. Spleen is involved in the hematogenous spread, and primary splenic TB is rarely encountered [2]. The genitourinary system is the commonest viscera involved and is discussed elsewhere [15]. The bacteria become lodged in the visceral parenchyma, resulting in granulomatous caseation and abscess formation. These features challenge the clinical acumen of the treating physician as they mimic more common benign as well as malignant visceral lesions. These organs are involved as a part of direct involvement, miliary process, or through hematogenous spread. TB in these organs presents as tubercular mass or abscess. The classification of abdominal TB is depicted in Graphical Abstract.

3 Clinical Features

3.1 Generalized Symptoms

The patients with TB present with varied symptoms. It commonly affects individuals between 20 and 40 years of age with equal sex preponderance [10]. Abdominal pain is the most common symptom [16]. It may be colicky in nature due to luminal narrowing. It gets relieved after the passage of flatus. A dull aching character may be noticed in mesenteric or omental lymphadenitis. These patients may also experience loss of weight which is unintentional. Fever may be observed in 25–40% of the cases. Patients with ascites may notice abdominal distension and associated malnutrition. These patients give a history of chronic anemia with other micronutrient deficiencies [17]. Constipation and diarrhea may be observed in a few cases depending on the extent of the luminal compromise and associated mucosal damage.

3.2 Site-Specific Symptoms

3.2.1 Visceral Tuberculosis

Patients with visceral TB may present as pyrexia of unknown origin. These are evaluated, and one may find a focus of abscess in the hepatobiliary system or spleen. Primary visceral involvement without any other focus of TB is rare. Gallbladder (GB) TB may present as malignancy with clinical features like abdominal pain, loss of appetite, and obstructive jaundice with portal lymphadenopathy [4, 11]. Many cases of GB-TB mimicking a malignant GB mass have been reported [18]. The pancreas, when involved by TB, presents as a pancreatic mass with or without obstructive jaundice. Peripancreatic lymphadenopathy, ascites, and peritoneal nodules are common in both malignant as well as tubercular processes [19]. The presence of multiple visceral metastases is a subtle finding of malignancy, whereas mesenteric lymphadenopathy and omental caking and thickening are commonly seen in the tubercular abdomen [11]. These findings are not specific for either of the disease and ultimately land the patient for a morbid surgical procedure.

3.2.2 Gastroduodenal Tuberculosis

The gastric mucosa is inherently resistant to TB infection because of the presence of an acidic milieu [10]. When infected (0.5–2%), these patients may experience epigastric discomfort, perforation, and pyloric stenosis in late cases. Patients with pyloric stenosis may have non-bilious vomiting with weight loss and appetite. Duodenum can be involved either intrinsically or extrinsically by the infected preduodenal LNs [20]. These lesions cause luminal compromise, and patients present with bilious non-projectile vomiting, loss of weight, and abdominal pain [21]. The patients may also present with hematemesis requiring significant blood transfusions. These patients may also suffer choleretic diarrhea secondary to the fistulization of the duodenal mass into the large bowel.

3.2.3 Ileo-Jejunal Tuberculosis

The small bowel is the commonest site of gastrointestinal involvement. As discussed earlier, the presence of a large absorptive area along with the highest concentration of lymphatic tissue predisposes this segment of the bowel to TB [10]. The patients typically present with abdominal pain, which is colicky in nature, and it gets relieved after the passage of flatus. The ulcer might perforate, causing secondary peritonitis. Visible intestinal peristalsis can be clinically noted in case of absolute obstruction. These patients are dehydrated and require surgical correction.

3.2.4 Colonic Tuberculosis

The colon is rarely involved in TB. Human immunodeficiency virus (HIV) infection has led to an increased incidence of colonic TB. The right-sided disease is common, and it is involved as a continuous infection with terminal ileum [10]. The transverse colon, sigmoid, and rectum segmental involvement causes abdominal pain, anemia, rectal bleeding, and obstruction in severe cases. These cases require biopsy for the definitive diagnosis, and surgical correction is warranted in rare cases [22].

3.3 Tubercular Complications

Many patients in endemic areas come to emergency with secondary peritonitis due to bowel perforation or absolute constipation [23]. These patients are dehydrated and hemodynamically unstable. They require aggressive fluid resuscitation, broad-spectrum antibiotics, organ support, and surgery for damage control [24]. The extent of operative procedure and type of surgery depends upon the condition of the involved bowel segment and hemodynamic stability of the patients [25]. The outcome of the aggressive management in these cases is usually dismal, as the mortality varies between 8 and 30% even after recent advances in perioperative care [26]. Many surviving patients have multiple ostomies and experience ostomy-related complications like electrolyte imbalance, peristomal excoriation, stomal prolapsed or retraction, and nutrient deficiencies [27, 28]. These complications depend on the surgical expertise and the segment of the bowel that has been exteriorized.

4 Diagnosis

Diagnosis of abdominal TB is challenging, given the varied clinical and radiological presentation. None of the biochemical and radiological tests is the gold standard for it. Histopathology of the affected tissue may aid in diagnosis to a certain extent. Recent advances like polymerase chain reaction (PCR) can help detect bacterial DNA and diagnose it in many cases [29].

5 Hematological Tests

Blood investigations may show elevated erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and total leucocyte counts (TLC) [11]. Hypoalbuminemia may occur as a result of loss of appetite and malabsorption. While receiving anti-TB therapy (ATT), liver enzymes may be elevated and must be monitored regularly.

6 Radiological Investigations

6.1 Plain Chest X-Ray

A plain X-ray of the chest may reveal the active focus of pulmonary TB (PTB) in 25% of the cases. Many patients (75%) show a normal chest roentgenogram depicting primary abdominal involvement. Active PTB may show multiple parenchymal infiltrates with pleural effusion and mediastinal widening due to mediastinal lymphadenopathy. In healed cases, multiple fibrotic bands with pleural thickening may be noticed [30].

6.2 Plain Abdomen X-Ray

A plain X-ray of the abdomen may reveal multiple air-fluid levels with dilated bowel loops in case of obstruction. These patients may also show enterolith, which denotes the presence of anon passable stricture in the small bowel. The bowel may be centralized or clumped with a relative scarcity of rectal air. The free air under the diaphragm denotes the perforation of the hollow viscus, and it may result in secondary peritonitis [31].

6.3 Barium Meal

Barium meal (follow-through) is done after ingestion of radio-opaque contrast medium and subsequently taking the radiographic images of the abdominal cavity. It detects any intraluminal strictures and partial obstruction. The barium meal shows multiple strictures with dilated bowel loops in between [9]. These studies should be done with utmost precautions as barium impaction may cause complete obstruction, and free spillage of barium into the peritoneal cavity leads to chemical peritonitis requiring urgent surgical correction [32]. With the advent of computerized cross-sectional imaging, the utility of these historical investigations, even in underdeveloped countries, is reduced to academic purposes.

6.4 Ultrasonogram

Ultrasonogram is widely available nowadays. Operator dependency and low diagnostic yield are the limitations of this modality. It is non-invasive and provides the gross representation of the abdominal viscera. The ultrasonogram shows free fluid and omental caking in the peritoneal form of TB. The lymphatic form of TB might cause enlargement of the mesenteric, celiac, periportal, and retroperitoneal LNs. Matting the lymph nodes leads to mass formation, and an ultrasonogram will reveal a hyperechoic peripheral area with central liquefaction. Calcific speculations may be observed in healed calcified nodes [33].

A visceral form of TB may show a large mass involving the affected organ associated with lymphadenopathy. The mass may have central liquefaction as noticed in large malignant tumors. The lymph node enlargement of the draining area adds to the clinical challenge [4]. Patients with pyrexia of unknown origin may show multiple visceral abscesses, which are also difficult to differentiate from the bacterial abscess [2]. Multiple liver abscesses may also be confused with hepatic plate dysgenesis like polycystic liver and von Meyenburg complexes [34].

Bowel thickening may be noticed in the enteric form of TB. The thickening involves the ileocecal junction primarily. The bowel thickening is also associated with cecal involvement, appendicular thickening, and omental adhesions. Pseudo kidney sign may be seen in pulled up caecal mass when it is seen in subhepatic

space [9]. These signs have significant overlap with other benign conditions and malignancies. Biopsy of the affected segment provides the only chance of differentiation between these conditions.

6.5 Abdomen Contrast-Enhanced Computed Tomography

Cross-sectional imaging like CECT can aid in clinical diagnosis [35]. These investigations provide the image of the viscera along with the associated structures. The imaging assists the clinical judgment and justifies the empirical diagnosis. The cross-sectional imaging shows enlarged mesenteric and celiac LNs [4]. These LNs may be discrete or matted due to TB periadenitis. The matted LN mass might have areas of central caseation necrosis, which on CECT will appear as a hypointense area with peripheral hyperintense rim associated with surrounding stranding [9]. The healed or dead nodes may demonstrate calcifications. Lymphadenopathy is relatively sparse in retroperitoneum, unlike lymphoma, where retroperitoneal lymphadenopathy is a common phenomenon. The biopsy of the affected LN provides the definitive diagnosis.

As described above ileocecal junction and terminal ileum are the commonest site of gastrointestinal TB. CECT of the abdomen in these cases shows a mass in the right iliac fossa, which is formed by the thickened terminal ileum and caecum, matted omentum, small bowel mesentery, and appendix. The mass is inflammatory in nature. The terminal ileum and caecum are pulled up to lie in the subhepatic space [36]. There may be a visualization of dilated small bowel loops along with the intervening strictures. These strictures may be long or small, multiple or single, and passable or non-passable depending on the length of the involved bowel. The free air in the peritoneal cavity signifies the free perforation of the small bowel. CECT enteroclysis may show the direct transit of the oral contrast from the affected segment to the large bowel or distant bowel, signifying the fistulization of the segment into the distal large intestine. These patients present with the passage of loose stools with loss of weight.

Viscera, when involved in the tubercular process, mimic the intraabdominal visceral malignancy or the infective process. The hepatobiliary involvement will be seen as a large mass with heterointense areas [11]. There may be calcifications seen in the periphery of the mass, and it may be associated with portal, periportal, and celiac lymphadenopathy. The presence of ascites and peritoneal nodules may also confuse the clinical diagnosis. Pancreatic TB presents as a large necrotic mass in the head and tail of the pancreas [37]. There may be peripancreatic stranding as well as LN involvement in the peripancreatic areas. The presence of mass with obstructive jaundice or pain favors malignancy, and TB as a primary disease is considered rarely. Spleen is involved in the miliary process, and hematogenous dissemination is commonly seen. Splenic TB may present as multiple splenic hypointense areas signifying splenic abscesses with enlargement [2]. These visceral findings camouflage as malignancy, and all patients are subjected to the intense treatment protocol, only to be a histological surprise.

6.6 Diagnostic Laparoscopy

Minimally invasive surgery has emerged as a useful alternative to the conventional open laparotomy [38]. A minimally invasive approach has led to early recovery, reduced post-operative pain, and scarring. These advantages of laparoscopy have increased its utility in diagnosing abdominal infections as well as disseminated malignancy. Diagnostic laparoscopy is the initial investigation before contemplating the definitive surgery. The presence of whitish nodules over the peritoneum and bowel surface can be seen in TB and metastatic disease as well [39]. Subjecting these deposits for intraoperative frozen section and histopathology has diagnosed TB in many patients.

Diagnostic laparoscopy offers the advantage of viewing the whole intraabdominal cavity, including the pelvis and reproductive organs. The presence of thickened fallopian tubes along with active fimbrial discharge is a sign of active TB pelvic inflammatory disease.

6.7 Ascitic Fluid Tap

Intraperitoneal free fluid can be appreciated in many patients with abdominal TB. The free fluid is exudative in nature. Biochemical analysis of the fluid shows increased proteins with low glucose levels. There may be lymphocytosis, and its yield for acid-fast bacilli (AFB) is very low. Peritoneal biopsy has the highest yield of AFB among peritoneal fluid and biopsy. Raised adenosine deaminase (ADA) (> 37) has typically been associated with abdominal TB [10].

6.8 Nuclear Scans

Nuclear scans have evolved as a diagnostic modality for malignancy. The tumor cells possess a high metabolic rate, and these selectively take up the glucose tracer well [40]. The inflammatory cells lack this capability, and hence inflammatory lesions are less avid on nuclear scans. The peritoneal involvement of TB is seen as diffuse uptake, whereas it is isolated nodular uptake in malignancy. Multiple reports have been published in literature where the abdominal TB has mimicked malignancy, and even in PET-CT presence, TB was diagnosed only after definitive histopathology. Gallium 67 scintigraphy has traditionally been used to initiate and monitor chemotherapy response in abdominal TB [41].

6.9 Histopathology

Histology is the standard gold test for diagnosis. Histology of the tissue shows caseating granuloma with epithelioid and Langhan's cells with AFB [11]. The central caseation is typical of TB in endemic areas. The diagnosis of TB is established only if the following clinical and diagnostic criteria are met:

- demonstration of a caseating granuloma on histology;
- demonstration of AFB;
- isolation of *M. tb* when the tissue is subjected to culture; and
- clinical resolution of disease after the initiation of chemotherapy.

The yield of fine-needle aspiration (FNA) is low compared to the biopsy. The false-negative yield of FNA is 10–15%. A negative test in a patient with typical signs and symptoms should be reassessed, especially in the endemic areas.

7 Treatment

The treatment of abdominal TB primarily comprises ATT. The treatment regimens and complications of ATT have been described elsewhere.

8 Conclusion

TB is an infective disease with high morbidity and mortality, especially in co-infection with HIV. Abdominal TB poses a challenge to the treating physician as it is a great mimicker of abdominal malignancy. Modern-day molecular diagnostic modalities help differentiate these pathologies and initiate the appropriate treatment of the affected patients. Though healed after appropriate chemotherapy, the patients still suffer from the sequel of infections and present with subacute intestinal obstruction, abdominal pain, etc. These patients also prove to be a nightmare for the surgeons who operate them for some other surgical ailment as there are dense adhesions between the bowel and mesentery, patients are malnourished, and the prognosis with these comorbidities is poor.

Core Messages

- TB still affects people, especially in the developing world.
- TB is treatable.
- Surgery is required in rare cases with obstruction or perforation.

References

1. Pai M, Kalantri S, Aggarwal AN, Menzies D, Blumberg HM (2006) Nosocomial tuberculosis in India. Emerg Infect Dis 12(9):1311
2. Gupta A (2018) Splenic tuberculosis: a comprehensive review of literature. Pol Przegl Chir 90:49–51

3. Horvath KD, Whelan RL (1998) Intestinal tuberculosis: return of an old disease. Am J Gastroenterol 93(5):692–696
4. Durgapal P, Joshi PP, Gupta A, Gupta A, Kishore S, Singh A (2019) Granulomatous inflammation still fooling surgeons. Trop Doct 49(3):252–253
5. Lazarus AA, Thilagar B (2007) Abdominal tuberculosis. Dis Mon 53(1):32–38
6. Epstein BM, Mann JH (1982) CT of abdominal tuberculosis. Am J Roentgenol 139(5):861–866
7. Millard J, Ugarte-Gil C, Moore DA (2015) Multidrug resistant tuberculosis. BMJ 350
8. Cudahy P, Shenoi SV (2016) Diagnostics for pulmonary tuberculosis. Postgrad Med J 92 (1086):187–193
9. Debi U, Ravisankar V, Prasad KK, Sinha SK, Sharma AK (2014) Abdominal tuberculosis of the gastrointestinal tract: revisited. World J Gastroenterol: WJG 20(40):14831
10. Sharma MP, Bhatia V (2004) Abdominal tuberculosis. Indian J Med Res 120:305–315
11. Gupta A, Gupta A, Anjum R, Agrawal S, Mallik D (2018) A comprehensive review on primary gallbladder tuberculosis. Pol Przegl Chir 90(2):10–12
12. McGuinness FE, Hamilton D, Al Nabulsi J (2000) Clinical imaging in non-pulmonary tuberculosis. Springer, New York, NY
13. Rasheed S, Zinicola R, Watson D, Bajwa A, McDonald PJ (2007) Intra-abdominal and gastrointestinal tuberculosis. Colorectal Dis 9(9):773–783
14. Ahamed ZR, Shah J, Agarwala R, Kumar-M P, Mandavdhare HS, Gupta P, Singh H, Sharma A, Dutta U, Sharma V (2019) Controversies in classification of peritoneal tuberculosis and a proposal for clinico-radiological classification. Expert Rev Anti-Infect Ther 17(8):547–555
15. Cek M, Lenk S, Naber KG, Bishop MC, Johansen TEB, Botto H, Grabe M, Lobel B, Redorta JP, Tenke P (2005) EAU guidelines for the management of genitourinary tuberculosis. Eur Urol 48(3):353–362
16. Chow KM, Chow VCY, Hung LCT, Wong SM, Szeto CC (2002) Tuberculous peritonitis–associated mortality is high among patients waiting for the results of mycobacterial cultures of ascitic fluid samples. Clin Infect Dis 35(4):409–413
17. Uzunkoy A, Harma M, Harma M (2004) Diagnosis of abdominal tuberculosis: experience from 11 cases and review of the literature. World J Gastroenterol: WJG 10(24):3647
18. Deo KB, Sharma V, Mandavdhare H, Kumar Basher R, Rohilla M, Singh H (2019) An uncommon case of gall bladder mass: gall bladder tuberculosis. Trop Doct 49(2):136–138
19. Chaudhary P, Bhadana U, Arora MP (2015) Pancreatic tuberculosis. Indian J Surg 77(6):517–524
20. Dahiya D, Garg M, Kaman L, Rana S, Rao C, Behera A (2013) Duodenal tuberculosis—a rare case report and review of literature. Pol Przegl Chir 85(8):464–466
21. Sharma SK, Mohan A (2019) Extrapulmonary tuberculosis. In: Hasnain S, Ehtesham N, Grover S (eds) *Mycobacterium tuberculosis*: molecular infection biology, pathogenesis, diagnostics and new interventions. Springer, Singapore. https://doi.org/10.1007/978-981-32-9413-4_4
22. Misra SP, Misra V, Dwivedi M, Gupta S (1999) Colonic tuberculosis: clinical features, endoscopic appearance and management. J Gastroenterol Hepatol 14(7):723–729
23. Pattanayak S, Behera S (2015) Is abdominal tuberculosis a surgical problem? Ann R Coll Surg Engl 97(6):414–419
24. Kumar-M P, Shafiq N, Kumar P, Gupta A, Malhotra S, Gautam V, Ray P, Gupta R, Gupta V, Deen Yadav T, Verma GR (2019) Antimicrobial susceptibility patterns of organisms causing secondary abdominal infections in patients with perforated abdominal viscus. Ther Adv Infect Dis 6:2049936119865796
25. Gupta A, Chakaravarthi K, Pattnaik B, Kaman L (2016) Duplication cyst of ileum presenting as acute intestinal obstruction in an adult. Case Rep 2016:bcr2016214775
26. Jhobta RS, Attri AK, Kaushik R, Sharma R, Jhobta A (2006) Spectrum of perforation peritonitis in India-review of 504 consecutive cases. World J Emerg Surg 1(1):1–4

27. Jayarajah U, Samarasekara AM, Samarasekera DN (2016) A study of long-term complications associated with enteral ostomy and their contributory factors. BMC Res Notes 9(1):1–6
28. Butler DL (2009) Early post-operative complications following ostomy surgery: a review. J Wound Ostomy Contin Nurs 36(5):513–519
29. Nikam C, Kazi M, Nair C, Jaggannath M, Manoj M, Vinaya R, Shetty A, Rodrigues C (2014) Evaluation of the Indian TrueNAT micro RT-PCR device with GeneXpert for case detection of pulmonary tuberculosis. Int J Mycobacteriol 3(3):205–210
30. World Health Organization (2016) Chest radiography in tuberculosis detection: summary of current WHO recommendations and guidance on programmatic approaches (No. WHO/HTM/TB/2016.20). World Health Organization
31. Urabinahatti KA, Singh AK, Nayak A, Gupta R, Jain M, Dubey C, Garg RK (2016) Abdominal tuberculosis: an epidemiological profile and management of 40 cases in a tertiary set up. Int Surg J 3(3):1502–1508
32. Pandit N, Singh H, Jaiswal LS (2018) Barium peritonitis: a disastrous complication of an unnecessary diagnostic study. Trop Doct 48(2):171–173
33. von Hahn T, Bange FC, Westhaus S, Rifai K, Attia D, Manns M, Potthoff A, Gebel M (2014) Ultrasound presentation of abdominal tuberculosis in a German tertiary care center. Scand J Gastroenterol 49(2):184–190
34. Gupta A, Pattnaik B, Das A, Kaman L (2016) Von Meyenburg complex and complete ductal plate malformation along with Klatskin tumour: a rare association. Case Rep 2016: bcr2016215220
35. Deshpande SS, Joshi AR, Deshpande SS, Phajlani SA (2019) Computed tomographic features of abdominal tuberculosis: unmask the impersonator! Abdom Radiol 44(1):11–21
36. Harisinghani MG, McLoud TC, Shepard JAO, Ko JP, Shroff MM, Mueller PR (2000) Tuberculosis from Head to Toe 1: (CME available in print version and on RSNA Link). Radiographics 20(2):449–470
37. Miri MB, Safari MT, Alizadeh AHM (2015) Pancreatic tuberculosis: an overview. JOP J Pancreas 16(3):232–238
38. Gupta A, Gupta A, Gupta S, Chauhan U, Joshua LM (2018) Are incidentally detected gall bladder cancers really incidental? A report of two cases from a developing nation. Trop Doct 48(4):355–358
39. Krishnamurthy G, Rajendran J, Sharma V, Kumar H, Singh H (2018) Incidental peritoneal tuberculosis: surgeon's dilemma in endemic regions. Ther Adv Infect Dis 5(5):97–102
40. Ramia JM, Muffak K, Fernández A, Villar J, Garrote D, Ferron JA (2006) Gallbladder tuberculosis: false-positive PET diagnosis of gallbladder cancer. World J Gastroenterol: WJG 12(40):6559
41. Goldfarb CR, Colp C, Ongseng F, Finestone H, Havas J (1997) Gallium scanning in the 'new' tuberculosis. Clin Nucl Med 22(7):470–474

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Bovine Tuberculosis at the Interface of Cattle, Wildlife, and Humans

40

Mitchell V. Palmer, Carly Kanipe, Jason E. Lombard, and Paola M. Boggiatto

An attempt to eradicate the disease [tuberculosis] from humans without also eliminating it from domestic animals could only be futile.

Myers and Steele

Summary

General awareness of zoonotic diseases has been heightened by the SARS-CoV-2 pandemic, but in truth, emerging zoonotic diseases have been and remain burdensome to public health. Many zoonotic diseases involve domestic animals and/or wildlife, and transmission occurs at the domestic animal-wildlife-human interface. Bovine tuberculosis (TB) due to *Mycobac-*

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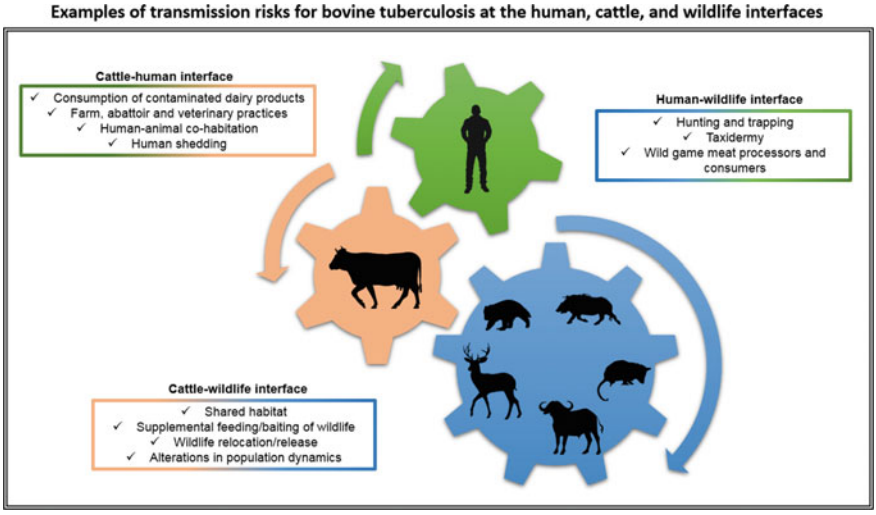
terium bovis (*M. bovis*) exemplifies a zoonotic disease at the interface of cattle, wildlife, and humans. An interface represents not only a physical location but also interspecies interactions occurring at that location. There are various wildlife reservoirs of *M. bovis*, and when disease transmits to cattle, it does so by various means ranging from sharing of feed to licking possums. Human-animal interfaces may be in the forest, in the barn, or in the kitchen and the interactions range from eating cheese to field dressing wild game. Disease on a global scale like bovine TB is difficult to eradicate, and few countries have been successful. Wildlife reservoirs of disease and wildlife-to-cattle transmission further complicate the eradication of *M. bovis*, making it difficult, if not impossible, to eliminate the disease from cattle. Disease eradication requires thorough knowledge and understanding of these interfaces.

Graphical Abstract



Risks and causes of *M. bovis* transmission at the cattle-wildlife-human interface

Keywords

Cattle · Interface · *Mycobacterium bovis* · Tuberculosis · Wildlife · Zoonoses

1 Introduction

As a result of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, the general public is more aware of the potential for disease transmission between humans and animals and the respective health and economic consequences. In truth, numerous emerging zoonotic events represent a burden on global public health [1]. An analysis of 335 emerging disease events occurring between 1940 and 2004 showed that more than 60% were zoonotic, and the majority (over 70%) had an epidemiologically important wildlife host [1]. For disease transmission to occur among humans, wildlife, and domestic animals, some level of interspecies interaction is required. This interaction is often described as an interface (e.g., wildlife-domestic animal, wildlife-human, domestic animal-human) [2].

An interface can be defined as a space or location where unrelated populations meet and interact. An interface, therefore, is a combination of both a physical space or location and the interspecies interactions that occur there. In the context of infectious disease, not all pathogens can successfully transmit at the animal-human interface or the domestic animal-wildlife interface. One pathogen, *Mycobacterium bovis* (*M. bovis*), the cause of tuberculosis (TB) in cattle and wildlife, and the cause of zoonotic TB in humans, represents an excellent model, as it successfully transmits at the cattle-wildlife, cattle-human, and wildlife-human interfaces [3].

As for bovine TB, the physical location of a cattle-wildlife interface may be feed storage yards or pastures located in the middle of heavily forested areas of Northern Michigan where infection with *M. bovis* in wild white-tailed deer (*Odocoileus virginianus*) is endemic (Fig. 1). The interaction may be the consumption of livestock feed by deer. In doing so, infected deer leave *M. bovis* behind in their saliva and nasal secretions, which is then indirectly transmitted to cattle as they consume contaminated feed [4]. This interface further demonstrates that interspecies interactions need not be direct nor require physical contact.

In other cases, the physical location and interaction that define a particular interface may not be known. For example, in the United Kingdom (UK) and the Republic of Ireland (ROI), the role of the European badger (*Meles meles*) as a source of *M. bovis* infection in cattle has long been suspected, if not generally accepted [5–7]. However, the exact means by which direct or indirect badger-to-cattle transmission occurs remain unclear [8]. In the following sections, we will use *M. bovis* as a model to describe cattle-wildlife, cattle-human, and wildlife-human interfaces.

2 Cattle-Wildlife Interface

Motivations for controlling bovine TB were related to both public health and animal health. As such, early eradication efforts focused exclusively on controlling disease in cattle, thereby decreasing the cattle-to-human transmission of *M. bovis* through decreasing contaminated milk and meat [9]. Unfortunately, in some regions, while

Fig. 1 Farmstead in Michigan, the USA, surrounded by dense forest, which is ideal white-tailed deer habitat, placing deer in proximity to cattle and cattle feed storage areas. Photo courtesy of Graham Hickling, Manaaki Whenua-Landcare Research, Lincoln, NZ

attention was centered on the relatively high disease prevalence in cattle, *M. bovis* was *spilling over* from cattle into susceptible wildlife hosts. As eradication efforts successfully decreased disease in cattle generally, eradication foundered in some areas, and disease prevalence in cattle remained elevated due to the sustained presence of *M. bovis* in local wildlife populations. Disease, having spilled over from cattle to wildlife, was now *spilling back* from wildlife to cattle [10]. The insidious and chronic nature of TB makes it especially difficult to identify in wildlife, resulting in years of intra- and interspecies transmission before it is recognized [11–13].

Today, wildlife populations where *M. bovis* infection is maintained represent wildlife reservoirs of infection and obstacles to bovine TB eradication. Well-acknowledged wildlife reservoirs of *M. bovis* include the European badger in the UK and the ROI, the brushtail possum (*Trichosurus vulpecula*) in New Zealand, the European wild boar (*Sus scrofa*) in the Iberian Peninsula, the African Cape buffalo (*Syncerus caffe*r) and Greater kudu (*Tragelaphus strepsiceros*) in South Africa, and the white-tailed deer in the United States (US) [14]. The common and most disconcerting characteristic of a wildlife reservoir is that *M. bovis* infection can be maintained in the population without an external source of infection [14]. Thus, once established in wildlife reservoir hosts, elimination of TB from the

source population (e.g., cattle) will not eliminate the disease in the wildlife population.

The cattle-wildlife interface may be as straightforward as comingling or sharing pasture by cattle and wildlife. In South Africa and Uganda, *M. bovis* is maintained in Cape buffalo herds in the Kruger National Park and Queen Elizabeth National Park, respectively. In these regions, both cattle and buffalo numbers are increasing, thus limiting resource availability. In search of suitable food and water, cattle wander inside park boundaries and buffalo wander outside of park boundaries, increasing the chance of interaction between these two species [15]. Transmission between cattle and buffalo has been confirmed using molecular methodology [16–18]. In these areas, the lack of effective bovine TB control programs exacerbates the problem [17].

In other cases, the interface is not so straightforward. Fourteen domestic and wild species in New Zealand have been shown to be infected with *M. bovis*, among which the brushtail possum is of greatest importance. Possums were originally imported to New Zealand from Australia in the mid-nineteenth century; however, the population has increased to a current estimate of 60–70 million possums that cover more than 90% of New Zealand's land area [19]. This represents a possum density 20 times higher than that seen in their native Australia [19]. The interaction between cattle and *M. bovis*-infected brushtail possums in New Zealand is intriguing. Generally speaking, healthy possums tend to avoid contact with cattle [20]. TB in possums is often progressive and severe, with most succumbing within six months of infection. In contrast to healthy possums, those with advanced disease manifest increased daytime activity and exhibit abnormal behaviors such as rolling, falling, and stumbling, all of which attract inquisitive cattle. Researchers used moderately-sedated possums to mimic terminally-ill possums, showing that cattle express great interest in unusually behaving possums [21]. More precisely, cattle could be drawn in from a distance of 50 m and would spend significant time within 1.5 m, a distance at which *M. bovis* can be transmitted through aerosols. Cattle often sniffed, licked, rolled, lifted, chewed, and kicked the possums providing ample opportunity for *M. bovis* transmission [20]. Furthermore, disease transmission is facilitated by the pathogenesis of the disease in possums, where highly infectious material is expelled from both the respiratory tract and discharging sinuses from superficial lymph node lesions.

The lack of a complete understanding of the interface location or interaction activities may lead to an increase in wildlife-cattle disease transmission even when mitigation strategies are implemented. European badgers are a recognized source of *M. bovis* in the UK and the ROI [22, 23] and are believed to be partially responsible for many cattle herd TB breakdowns. A great amount of research has explored the means by which badgers transmit *M. bovis* to cattle. It has been suggested that infected badgers climb into livestock feed bunks or water tanks and leave behind *M. bovis*, which is then indirectly transmitted to cattle through feeding or watering. It has also been proposed that inquisitive cattle investigate badger latrines where they are exposed to urine or feces containing *M. bovis*. To make matters worse, *M. bovis*-infected badgers may survive for three to four years, during which time they may be

shedding *M. bovis* [24]. In spite of various reasonable hypotheses, the details of the badger-to-cattle transmission of *M. bovis* remain unclear. Mounting rates of bovine TB led to an extensive study of the epidemiological significance of badgers in relation to bovine TB and possible badger management measures [25]. The study showed that badgers serve as a reservoir of infection for cattle [26]. However, the study also demonstrated that selective removal of badgers in response to new bovine TB diagnoses within a study area (reactive badger culling) actually increased the incidence of tuberculin reactors in cattle herds in the study area [27, 28]. In contrast, after five years of removing all badgers from a study area (proactive badger culling), the incidence of tuberculin reactors in cattle in the study area was down 23%. The beneficial effect was ongoing as one to two years after the last proactive cull; the tuberculin reactor rate was down 54% [25]. It is believed that the removal of some but not all of the badgers within a study area resulted in a restructuring of social groups and increased home ranges for remaining badgers [29]. Expanded ranging behavior likely increased contact (and disease transmission) with cattle, as well as other badgers [30]. In the UK, badger culling remains highly controversial and hotly contested [31]. In this case, sociopolitical forces also influence the cattle-wildlife interface. Public attitudes do not favor badger culling in the UK, and surveys demonstrate that people value conservation and animal welfare over disease prevention [3].

Spillover transmission from infected cattle is believed to have introduced *M. bovis* into North American wildlife. In North America, reports of TB in free-ranging wildlife, specifically deer, date back to the 1920s [10, 11, 32]. At that time, efforts to eradicate TB from cattle were progressing, and it was assumed that once TB was eradicated from cattle, the cattle-to-deer transmission would cease, and TB in deer would disappear. Any deer-to-deer transmission was considered insignificant, thereby assuming that wild deer were an improbable reservoir host population. In Michigan, currently, as well as in Minnesota, historically, the presence of TB in cattle can be linked to white-tailed deer through whole-genome sequencing of isolates [11, 32–34]. Similarly, by *M. bovis* strain comparison, the presence of TB in cattle near Riding Mountain National Park in Manitoba, Canada, can be linked to *M. bovis*-infected elk (*Cervus elaphus manitobensis*) within the park [35].

In the case of Michigan, the physical locations and interactions which characterize the cattle-wildlife interface are often heavily influenced by human activities. The proximity of ideal deer habitat to livestock feeding areas allows deer and cattle to share feed. Direct contact is not required, as deer leave behind feed contaminated by saliva and nasal secretions containing *M. bovis*, which is subsequently consumed by cattle. Thus, deer-to-cattle transmission can be indirect [4]. Epidemiological studies linking these cattle TB outbreaks to deer are supported not only by the proximity of cattle herds to areas where TB in wild deer is endemic but also by DNA typing of *M. bovis* isolates.

Supplemental feeding of deer by humans, especially during winter months, is a major factor in the generation and persistence of TB in Michigan white-tailed deer [36–39]. Enticing wild deer to congregate around feed piles increases the deer-to-deer transmission of TB, thus contributing to the maintenance of TB (and other diseases)

in this population. Moreover, when feed piles intended for deer are placed in areas accessible to cattle, deer-to-cattle transmission is increased. Encroachment of humans and cattle on wildlife habitat and the actions of humans, well-meaning or not, are increasing cattle-wildlife, as well as human-wildlife contact [40, 41].

The wildlife ranching or deer farming industries have also created new cattle-wildlife interfaces. In recent decades, the development of recreational, commercial hunting has resulted in marked changes to the number and distribution of wildlife populations in southern Spain, namely wild boar and red deer (*Cervus elaphus*), both of which can serve as reservoirs of *M. bovis* [42–44]. In these scenarios, large areas of suitable habitat have been fenced in, and artificial feeding and watering locations have been provided, rendering wildlife populations essentially captive [45]. Within these confines, cattle are also often raised, creating cattle-wildlife interactions. Land management that forces the coexistence of wildlife and cattle increases interspecies interactions and disease transmission.

Game ranching and deer farming have gained popularity in North America [46]. In addition to increasing animal density, thus facilitating disease transmission, other common practices include the release of exotic and/or native deer species into semi-free ranging environments for hunting [10]. There, comingling with cattle and other susceptible species such as feral swine is enhanced, thereby fostering interspecies disease transmission.

Management limitations at some interfaces may influence the efficiency of disease transmission. In most countries, surveillance testing is focused on cattle due to the lack of surveillance methods for wildlife, impeding detection of TB in wildlife populations. Diagnostic tests are generally developed for cattle and are not suitable for most wildlife species. Moreover, it is difficult and costly to trap, test, and track wildlife, severely restricting the ability to recognize the emergence of wildlife reservoirs of TB.

3 Human-Animal Interface

Complicating the sylvatic cycle between cattle and wildlife is the human-animal interface. This can be further broken down into human-wildlife and human-cattle interfaces, though the delineation is frequently muddled and sometimes nonexistent. Human-cattle interactions can represent a public health risk as well as an impediment to disease eradication efforts. As with cattle-wildlife interactions, wildlife habitat is continuously encroached upon by humans for agricultural purposes as the global demand for food increases. As such, we can expect human-animal (wildlife and livestock) interactions to increase in the coming decades. One study examining the conversion of natural habitats to agricultural or urban ecosystems found such changes in land use expanded hazardous interfaces between humans, livestock, and wildlife. One ominous finding was that mammalian species, which harbor more zoonotic pathogens were more likely to thrive in these areas of converted ecosystems [47].

The public health implications of *M. bovis* infection in humans (zoonotic TB) were suspected as early as 1865 by Chauveau, who confirmed disease transmission between cattle through ingestion of diseased material [9, 48]. Similarly, experiments by Villemin in 1868 showed that diseased material could transmit TB to various animal species [49]. This led both scientists to posit that TB transmission was achievable in both man and animals by consuming meat or milk from diseased animals. Later, in 1888, Professor Thomas Walley, Principal of the Royal Dick Veterinary College at Edinburgh, taught that TB in cattle could be transmitted to humans through milk, meat, or merely by contact with infected cattle inside dairy barns [50]. In 1877, in the US, Cornell's James Law taught that zoonotic TB was the most important zoonotic disease of the time [51]. Despite admonitions from veterinary professionals, acceptance was slow, both by government officials and physicians. Even as late as the early 1900s, *M. bovis* accounted for 25–30% of all human TB in the US and Europe [52, 53]. More distressing was that most of these cases were in children, the primary consumers of unpasteurized milk [54]. A survey in 1890 of 18,000 US physicians showed that most doctors did not consider unpasteurized milk to be a source of TB for humans [51, 55]. Today, in underdeveloped countries with little or no bovine TB control programs, ingestion of unpasteurized milk containing *M. bovis* remains a public health problem. In contrast, in developed countries, human cases linked to ingestion of *M. bovis* are mostly limited to artisanal cheeses produced with raw unpasteurized milk [56–58]. As such, the human-cattle interface involves both cattle and cattle products, making the kitchen an interface location and our ingestion of unpasteurized dairy products an interface interaction.

The human-cattle interface has been substantially influenced by livestock importation and exportation. In the 1800s, Britain exported large numbers of Hereford cattle to their many colonies, including the Americas. With cattle, they also exported their specific strain of *M. bovis*. This is reflected in the similar *M. bovis* spoligotype patterns (clonal complexes) in Britain and in former British colonies and trading partners [59]. As international trade increased, so did the spread of this particular clonal complex, making it one of the most prominent worldwide. Today, it is possible to delineate different strains within clonal complexes, allowing for more specific traceback, something critical in a time of global trade. For example, in the US, the importation of cattle from Mexico has led to disease in areas that previously had none. In some cases, these animals are untested or falsely negative to the tuberculin skin test upon importation; however, later spoligotyping and whole-genome sequencing can show the strains originated in Mexico [60].

Human-to-human transmission of *Mycobacterium tuberculosis* (*M. tb*) is primarily through aerosolization of droplet nuclei from infected individuals. Coughing, talking, and singing can produce droplet nuclei, which are small (< 5 μm) remnants of evaporated droplets that can float in the air for hours. Infectious bacilli can be associated with these tiny droplets. In contrast, larger droplets, which may or may not contain infectious bacilli, tend to fall to the ground quickly and are less likely to result in disease transmission. Due to their small size, infectious droplet

nuclei can travel deep in the lung to pulmonary alveoli once inhaled. Larger droplets, if inhaled, are generally cleared from the nasal passages and upper respiratory tract [61–63]. Aerosol transmission from cattle to humans is believed to occur through a similar mechanism involving aerosolization of droplet nuclei [64, 65]. In addition to direct aerosol transmission from cattle, acid-fast bacilli have been found in dried cattle sputum on the walls of their enclosures, posing an additional route by which humans may come into contact with the bacteria, especially when enclosures are being cleaned [65]. Livestock owners, caretakers, and veterinarians in close contact with infected cattle are at higher risk of airborne or fomite-driven infection [66–68]. Livestock owners are more likely to be infected via the aerosol route, while veterinarians and abattoir workers are more likely infected through accidental cutaneous inoculation or contact with fomites [67]. Cutaneous TB wounds were once so common in abattoir workers as to be called "butcher's warts" [65]. A recent survey of four slaughterhouses in Italy demonstrated that workers involved with the slaughter of tuberculin-positive cattle are at high risk of exposure to *M. bovis*. Carcasses, workers' hands, and rinse water were all found to contain *M. bovis* DNA. Moreover, the carcasses of healthy cattle slaughtered on the day after processing tuberculin-positive cattle were also found to be contaminated [69]. In these cases, procedures used to decontaminate slaughterhouses after processing tuberculin-positive cattle were ineffective and insufficient. Furthermore, the study reported that none of the personnel wore mandatory personal protective equipment, other than disposable latex gloves and waterproof aprons [69].

Although the human-wildlife interface could involve wildlife interactions with any human, it is more likely that hunters, trappers, taxidermists, wild game meat processors, and consumers of wild game meat would have interactions that could lead to disease transmission [70]. However, even in regions of the US where TB is endemic among wild white-tailed deer, a popular game species from which venison is widely consumed, human *M. bovis* infections linked to deer are rare [71]. Between 1995 and 2007, 13 cases of human *M. bovis* infections were identified in Michigan. No genetic or epidemiological link to deer could be identified in 11 of those cases [71]. In one of the two remaining cases, pulmonary lesions were present, while the other case was limited to cutaneous TB on the hand, thought to be the result of superficial cuts sustained during field dressing a deer. Similarly, in New Zealand, a veterinary surgeon surveying possums with *M. bovis* was infected [72], developing tenosynovitis of the forearm and secondary carpal tunnel syndrome.

The human-cattle interface is generally thought to be unidirectional, with humans being infected by cattle. This is not always the case. Human-to-cattle transmission (reverse zoonosis) has been reported in multiple countries, including Africa, India, Europe, China, and the US [73–91]. The source of infection for cattle is most often traced to active TB patients expelling bacilli through sputum, urine, and feces [74]. Grange cites examples of numerous people (most of which had pulmonary TB) infecting over 100 herds, resulting in the slaughter of over 1000 animals [92].

M. tb is the most commonly reported *M. tb* complex (MTBC) species transmitted from humans to cattle, which is predictable given that more than 90% of human TB cases are caused by *M. tb* [93]. Although not reported, there is also the possibility of cattle transmitting *M. tb* back to humans. Studies report that cattle are either generally resistant to infection by *M. tb* or have low numbers of *M. tb* detected in lymph nodes with no gross pathology [94, 95]. Young cattle, usually heifers, are reported as being infected with *M. tb* more commonly than adult cattle suggesting there might be differences in the immune systems' ability to prevent infection in younger cattle. In cattle, experimental infection studies with *M. tb* and *M. bovis* showed varying responses [94]. *M. bovis* infection was associated with colonization and lesion formation, while *M. tb* colonized tissues without inducing lesions. Antibody responses persisted in the *M. bovis*-infected cattle, while antibody responses waned in *M. tb*-infected cattle, lasting only six to 16 weeks after infection.

Airborne human-to-cattle transmission is possible with people suffering from pulmonary TB. Other interactions that promote human-to-cattle transmission of TB may include practices, such as bringing cattle into a farmer's home at night or expectorating chewed tobacco juice directly into the oral cavity of cattle as an anti-parasite treatment [96]. In some countries, humans urinate on the hay to provide salt to the cows. As summarized by Grange and Collins in 1987 [97], of fifty herds infected from human sources, 24 herds were infected from humans with renal TB [98]. Urinary tract infection is one of the manifestations of human *M. bovis* infection and is not usually diagnosed until transmission to animals has already occurred. One patient with genitourinary *M. bovis* infected 48 cattle in four different herds by urinating in the cow barn [92, 99].

In many countries with active bovine TB eradication programs, the human-to-cattle transmission of *M. bovis* is not generally recognized as a significant risk. Admittedly, published reports of human-to-cattle transmission are infrequent in developed countries; however, evidence suggests the human component of disease exposure to cattle should be considered and addressed within a "One Health" approach. As long ago as 1969, long before the "One Health" approach became popular, it was recognized that eradicating the disease from humans without eliminating it from cattle would be ineffective [100]. Similarly, efforts to eradicate TB from cattle without addressing the TB status of people working with cattle may prevent or delay eradication.

4 Conclusion

According to the 2017 world health organization (WHO) roadmap for zoonotic TB, "One Health" is defined in terms of "the interdependence of the health of people, animals, and the environment" [101]. The burden of disease in people cannot be

reduced without managing the disease in animals, whether domestic or wild. Eliminating a disease from a domestic population is difficult; one need only look at the US bovine TB eradication campaign that began in 1917 and is still in effect today. Eliminating a disease from a wild population is vastly more difficult. Even gathering data to understand the extent of a potential wildlife disease problem poses great challenges [102]. Except for Australia, no country with a wildlife reservoir of *M. bovis* has successfully eradicated TB from cattle or wildlife. Moreover, as seen in Africa, numerous wildlife species may serve as reservoirs, each interacting with cattle and humans in its own fashion. Differing social attitudes create further obstacles. In New Zealand, the brushtail possum is an invasive pest, destroying natural flora and fauna. Wholesale poisoning of possums with 1080 (sodium fluoroacetate) is considered acceptable [103, 104]. In stark contrast, the European badger is beloved by many in the UK. Almost any contact with badgers may be met with resistance; hence the passage of the Protection of Badger Act of 1992, which makes it illegal to

- "wilfully kill, injure, take or attempt to kill, injure or take a badger;
- possess a dead badger or any part of a badger;
- cruelly ill-treat a badger;
- use badger tongs in the course of killing, taking or attempting to kill a badger;
- dig for a badger;
- sell or offer for sale or control any live badger;
- mark, tag or ring a badger;
- interfere with a badger sett by:
  - damaging a sett or any part there of;
  - destroying a sett;
  - obstructing access to a sett;
  - causing a dog to enter a sett;
  - disturbing a badger while occupying a sett" [105].

Dealing with persistent *M. bovis* infection in European badgers in the UK may look very different from the management of infected brushtail possums in New Zealand. Each wildlife reservoir of *M. bovis* and its interactions at the cattle-wildlife-human interfaces will vary. Each case will need to be closely examined and social, environmental, and scientific factors well understood if bovine TB is to be eradicated.

Efforts to eradicate tuberculosis from cattle without addressing the tuberculosis status of people working with cattle may prevent or delay bovine tuberculosis eradication.

Mitchell V. Palmer, Carly Kanipe, Jason E. Lombard, Paola M. Boggiatto

Core Messages

- Emerging zoonotic diseases remain a public health concern.
- Tuberculosis due to *Mycobacterium bovis* represents a zoonosis at the interface of cattle, wildlife, and humans.
- Cattle-wildlife, human-cattle, and human-wildlife interfaces exist.
- The physical space or location and the interspecies interactions at each location may differ.
- To eradicate bovine tuberculosis, each interface requires individual attention and interface-specific management.

References

1. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P (2008) Global trends in emerging infectious diseases. Nature 451(7181):990–993. https://doi.org/10.1038/nature06536
2. Wiethoelter AK, Beltran-Alcrudo D, Kock R, Mor SM (2015) Global trends in infectious diseases at the wildlife-livestock interface. Proc Natl Acad Sci U S A 112(31):9662–9667. https://doi.org/10.1073/pnas.1422741112
3. Palmer MV, Thacker TC, Waters WR, Gortazar C, Corner LA (2012) *Mycobacterium bovis*: a model pathogen at the interface of livestock, wildlife, and humans. Vet Med Int 2012:236205. https://doi.org/10.1155/2012/236205
4. Palmer MV, Waters WR, Whipple DL (2004) Investigation of the transmission of *Mycobacterium bovis* from deer to cattle through indirect contact. Am J Vet Res 65 (11):1483–1489. https://doi.org/10.2460/ajvr.2004.65.1483
5. Muirhead RH, Gallagher J, Burn KJ (1974) Tuberculosis in wild badgers in Gloucestershire: epidemiology. Vet Rec 95:552–555
6. Wilesmith JW (1983) Epidemiological features of bovine tuberculosis in cattle herds in Great Britain. J Hyg (Lond) 90(2):159–176. https://doi.org/10.1017/s0022172400028837
7. Ni Bhuachalla D, Corner LA, More SJ, Gormley E (2015) The role of badgers in the epidemiology of *Mycobacterium bovis* infection (tuberculosis) in cattle in the United Kingdom and the Republic of Ireland: current perspectives on control strategies. Vet Med (Auckl) 6:27–38. https://doi.org/10.2147/VMRR.S53643
8. Campbell EL, Byrne AW, Menzies FD, McBride KR, McCormick CM, Scantlebury M, Reid N (2019) Interspecific visitation of cattle and badgers to fomites: a transmission risk for bovine tuberculosis? Ecol Evol 9(15):8479–8489. https://doi.org/10.1002/ece3.5282
9. Palmer MV, Waters WR (2011) Bovine tuberculosis and the establishment of an eradication program in the United States: role of veterinarians. Vet Med Int 2011:816345. https://doi.org/10.4061/2011/816345
10. Miller RS, Sweeney SJ (2013) *Mycobacterium bovis* (bovine tuberculosis) infection in North American wildlife: current status and opportunities for mitigation of risks of further infection in wildlife populations. Epidemiol Infect 141(7):1357–1370. https://doi.org/10.1017/S0950268813000976
11. Schmitt SM, Fitzgerald SD, Cooley TM, Bruning-Fann CS, Sullivan L, Berry D, Carlson T, Minnis RB, Payeur JB, Sikarskie J (1997) Bovine tuberculosis in free-ranging white-tailed deer from Michigan. J Wildl Dis 33(4):749–758. https://doi.org/10.7589/0090-3558-33.749

12. Ekdahl MO, Smith BL, Money DFL (1970) Tuberculosis in some wild and feral animals in New Zealand. NZ Vet J 18:44–45. https://doi.org/10.1053/rvsc.2000.0422
13. Woodford MH (1982) Tuberculosis in wildlife in the Ruwenzori National Park, Uganda (Part II). Trop Anim Health Prod 14(3):155–160
14. Palmer MV (2013) *Mycobacterium bovis*: characteristics of wildlife reservoir hosts. Transbound Emerg Dis 60(s1):1–13. https://doi.org/10.1111/tbed.12115
15. Musoke J, Hlokwe T, Marcotty T, du Plessis BJ, Michel AL (2015) Spillover of *Mycobacterium bovis* from wildlife to livestock, South Africa. Emerg Infect Dis 21(3):448–451. https://doi.org/10.3201/eid2103.131690
16. Hlokwe TM, van Heiden P, Michel AL (2014) Evidence of increasing intra and inter-species transmission of *Mycobacterium bovis* in South Africa: are we losing the battle? Prev Vet Med 115(1–2):10–17. https://doi.org/10.1016/j.prevetmed.2014.03.011
17. Sichewo PR, Hlokwe TM, Etter EMC, Michel AL (2020) Tracing cross species transmission of *Mycobacterium bovis* at the wildlife/livestock interface in South Africa. BMC Microbiol 20(1):49. https://doi.org/10.1186/s12866-020-01736-4
18. Plumptre A, Kukirakwinga D, Moyer D, Driciru M, Rwetsiga A (2010) Greater Virunga landscape large mammal surveys. Uganda Wildlife Authority, Kampala, Uganda
19. O'Neil BD, Pharo HJ (1995) The control of bovine tuberculosis in New Zealand. NZ Vet J 43(7):249–255
20. Paterson BM, Morris RS (1995) Interactions between beef cattle and simulated tuberculous possums on pasture. NZ Vet J 43(7):289–293
21. Sauter CM, Morris RS (1995) Behavioural studies on the potential for direct transmission of tuberculosis from feral ferrets (*Mustela furo*) and possums (*Trichosurus vulpecula*) to farmed livestock. NZ Vet J 43(7):294–300. https://doi.org/10.1080/00480169./1995.35909
22. Little TW, Naylor PF, Wilesmith JW (1982) Laboratory study of *Mycobacterium bovis* infection in badgers and calves. Vet Rec 111(24):550–557
23. Cheeseman CL, Wilesmith JW, Stuart FA (1989) Tuberculosis: the disease and its epidemiology in the badger, a review. Epidemiol Infect 103(1):113–125
24. Hutchings MR, Harris S (1997) Effects of farm management practices on cattle grazing behaviour and the potential for transmission of bovine tuberculosis from badgers to cattle. Vet J 153(2):149–162. https://doi.org/10.1016/s1090-0233(97)80035-4
25. Wilson GJ, Carter SP, Delahay RJ (2011) Advances and prospects for management of TB transmission between badgers and cattle. Vet Microbiol 151(21450417):43–50. https://doi.org/10.1016/j.vetmic.2011.02.024
26. Bourne FJ, Donnelly CA, Cox DR, Gettinby G, McInerney JP, Morrison WI, Woodroffe R (2006) TB policy and the badger culling trials. Vet Rec 158(19):671–672. https://doi.org/10.1136/vr.158.19.671
27. Donnelly CA, Woodroffe R, Cox DR, Bourne J, Gettinby G, Le Fevre AM, McInerney JP, Morrison WI (2003) Impact of localized badger culling on tuberculosis incidence in British cattle. Nature 426(6968):834. https://doi.org/10.1038/nature02192
28. Woodroffe R, Donnelly CA, Jenkins HE, Johnston WT, Cox DR, Bourne FJ, Cheeseman CL, Delahay RJ, Clifton-Hadley RS, Gettinby G, Gilks P, Hewinson RG, McInerney JP, Morrison WI (2006) Culling and cattle controls influence tuberculosis risk for badgers. Proc Natl Acad Sci USA 103(40):14713–14717. https://doi.org/10.1073/pnas.0606251103
29. Woodroffe R, Donnelly CA, Cox DR, Gilks P, Jenkins HE, Johnston WT, Le Fevre AM, Bourne FJ, Cheeseman CL, Clifton-Hadley RS, Gettinby G, Hewinson RG, McInerney JP, Mitchell AP, Morrison WI, Watkins GH (2009) Bovine tuberculosis in cattle and badgers in localized culling areas. J Wildl Dis 45(1):128–143. https://doi.org/10.7589/0090-3558-45.1.128
30. Vial F, Donnelly CA (2011) Localized reactive badger culling increases risk of bovine tuberculosis in nearby cattle herds. Biol Lett 8(1):50–53. https://doi.org/10.1098/rsbl.2011.0554

31. Bennett RM (2017) The political economy of bovine tuberculosis in Great Britain. Rev Sci Tech 36(1):105–114. https://doi.org/10.20506/rst.36.1.2614
32. Schmitt SM, O'Brien DJ, Bruning-Fann CS, Fitzgerald SD (2002) Bovine tuberculosis in Michigan wildlife and livestock. Ann NY Acad Sci 969(1):262–268
33. Portacci K (2008) Assessment of risk associated with the Minnesota proposed plan to split-state status for *Mycobacterium bovis* (bovine tuberculosis). United States Department of Agriculture, Animal and Plant Health Inspection Service
34. Carstensen M, Doncarlos MW (2011) Preventing the establishment of a wildlife disease reservoir: a case study of bovine tuberculosis in wild deer in Minnesota, USA. Vet Med Int 2011:1–10. https://doi.org/10.4061/2011/413240
35. Lees VW, Copeland S, Rousseau P (2003) Bovine tuberculosis in elk (*Cervus elaphus manitobensis*) near Riding Mountain National Park, Manitoba, from 1992 to 2002. Can Vet J 44(10):830–831
36. Palmer MV, Waters WR, Whipple DL (2004) Shared feed as a means of deer-to-deer transmission of *Mycobacterium bovis*. J Wildl Dis 40(1):87–91. https://doi.org/10.7589/0090-3558-40.1.87
37. Miller R, Kaneene JB (2006) Evaluation of historical factors influencing the occurrence and distribution of *Mycobacterium bovis* infection among wildlife in Michigan. Am J Vet Res 67 (4):604–615. https://doi.org/10.2460/ajvr.67.4.604
38. O'Brien DJ, Schmitt SM, Fierke JS, Hogle SA, Winterstein SR, Cooley TM, Moritz WE, Diegel KL, Fitzgerald SD, Berry DE, Kaneene JB (2002) Epidemiology of *Mycobacterium bovis* in free-ranging white-tailed deer, Michigan, USA, 1995–2000. Prev Vet Med 54 (1):47–63. https://doi.org/10.1016/s0167-5877(02)00010-7
39. O'Brien DJ, Schmitt SM, Fitzgerald SD, Berry DE, Hickling GJ (2006) Managing the wildlife reservoir of *Mycobacterium bovis*: the Michigan, USA, experience. Vet Microbiol 112(2–4):313–323. https://doi.org/10.1016/j.vetmic.2005.11.014
40. Daszak P, Cunningham AA, Hyatt AD (2001) Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Trop 78(2):103–116. https://doi.org/10.1016/s0001-706x(00)00179-0
41. Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife—threats to biodiversity and human health. Science 287(5452):443–449. https://doi.org/10.1126/science.287.5452.443
42. Naranjo V, Gortazar C, Vicente J, de la Fuente J (2008) Evidence of the role of European wild boar as a reservoir of *Mycobacterium tuberculosis* complex. Vet Microbiol 127 (18023299):1–9. https://doi.org/10.1016/j.vetmic.2007.10.002
43. Vicente J, Hofle U, Garrido JM, Fernandez-De-Mera IG, Juste R, Barral M, Gortazar C (2006) Wild boar and red deer display high prevalences of tuberculosis-like lesions in Spain. Vet Res 37(1):107–119. https://doi.org/10.1051/vetres:2005044
44. Gortazar C, Delahay RJ, McDonald RA, Boadella M, Wilson GJ, Gavier-Widen D, Acevedo P (2012) The status of tuberculosis in European wild mammals. Mammal Rev 42 (3):193–206. https://doi.org/10.1111/j.1365-2907.2011.00191.x
45. Vicente J, Hofle U, Garrido JM, Fernandez-de-Mera IG, Acevedo P, Juste R, Barral M, Gortazar C (2007) Risk factors associated with the prevalence of tuberculosis-like lesions in fenced wild boar and red deer in south central Spain. Vet Res 38(3):451–464. https://doi.org/10.1051/vetres:2007002
46. Elbein A (2020) The Texotics. The Texas Observer. https://www.texasobserver.org/the-texotics/. Accessed 13 Aug 2020
47. Gibb R, Redding DW, Chin KQ, Donnelly CA, Blackburn TM, Newbold T, Jones KE (2020) Zoonotic host diversity increases in human-dominated ecosystems. Nature. https://doi.org/10.1038/s41586-020-2562-8
48. Pearson L, Ravenel MP (1901) Tuberculosis of cattle and the Pennsylvania plan for its repression. Wm. Stanely Ray, State Printer of Pennsylvania, Harrisburg, PA

49. Daniel TM (2006) The history of tuberculosis. Respir Med 100(11):1862–1870. https://doi.org/10.1016/j.rmed.2006.08.006
50. Pritchard DG (1988) A century of bovine tuberculosis 1888–1988: conquest and controversy. J Comp Pathol 99(4):357–399
51. Rosenkrantz BG (1985) The trouble with bovine tuberculosis. Bull Hist Med 59(2):155–175
52. Rodwell TC, Kapasi AJ, Moore M, Milian-Suazo F, Harris B, Guerrero LP, Moser K, Strathdee SA, Garfein RS (2010) Tracing the origins of *Mycobacterium bovis* tuberculosis in humans in the USA to cattle in Mexico using spoligotyping. Int J Infect Dis. https://doi.org/10.1016/j.ijid.2009.11.037
53. Olmstead A, Rhode P (2004) An impossible undertaking the eradication of bovine TB from the US. J Econ Hist 64(3):1–39. https://doi.org/10.1017/S0022050704002955
54. Crimmins EM, Condran GA (1983) Mortality variation in U.S. cities in 1900: a two-level explanation by cause of death and underlying factors. Soc Sci Hist 7(1):31–60
55. Miller EB (1989) Tuberculous cattle problem in the United States to 1917. Hist Med Vet 14 (1–2):1–64
56. Cezar RD, Lucena-Silva N, Borges JM, Santana VL, Pinheiro Junior JW (2016) Detection of *Mycobacterium bovis* in artisanal cheese in the state of Pernambuco, Brazil. Int J Mycobacteriol 5(3):269–272. https://doi.org/10.1016/j.ijmyco.2016.04.007
57. Harris NB, Payeur J, Bravo D, Osorio R, Stuber T, Farrell D, Paulson D, Treviso S, Mikolon A, Rodriguez-Lainz A, Cernek-Hoskins S, Rast R, Ginsberg M, Kinde H (2007) Recovery of *Mycobacterium bovis* from soft fresh cheese originating in Mexico. Appl Environ Microbiol 73(3):1025–1028. https://doi.org/10.1128/AEM.01956-06
58. Pereira-Suarez AL, Estrada-Chavez Y, Zuniga-Estrada A, Lopez-Rincon G, Hernandez DU, Padilla-Ramirez FJ, Estrada-Chavez C (2014) Detection of *Mycobacterium tuberculosis* complex by PCR in fresh cheese from local markets in Hidalgo, Mexico. J Food Prot 77 (5):849–852. https://doi.org/10.4315/0362-028X.JFP-13-389
59. Smith NH (2012) The global distribution and phylogeography of *Mycobacterium bovis* clonal complexes. Infect Genet Evol 12(4):857–865. https://doi.org/10.1016/j.meegid.2011.09.007
60. Milian-Suazo F, Harris B, Arriaga Diaz C, Romero Torres C, Stuber T, Alvarez Ojeda G, Morales Loredo A, Perez Soria M, Payeur JB (2008) Molecular epidemiology of *Mycobacterium bovis*: usefulness in international trade. Prev Vet Med 87(3–4):261–271. https://doi.org/10.1016/j.prevetmed.2008.04.004
61. Wells WF, Ratcliffe HL, Grumb C (1948) On the mechanics of droplet nuclei infection; quantitative experimental airborne tuberculosis in rabbits. Am J Hyg 47(1):11–28
62. Loudon RG, Roberts RM (1967) Droplet expulsion from the respiratory tract. Am Rev Respir Dis 95(3):435–442
63. Loudon RG, Roberts RM (1968) Singing and the dissemination of tuberculosis. Am Rev Respir Dis 98(2):297–300
64. O'Reilly LM, Daborn CJ (1995) The epidemiology of *Mycobacterium bovis* infections in animals and man: a review. Tubercle Lung Dis 76(Suppl 1):1–46
65. Grange JM (2001) *Mycobacterium bovis* infection in human beings. Tuberculosis (Edinb) 81 (1–2):71–77. https://doi.org/10.1054/tube.2000.0263
66. Fanning A, Edwards S (1991) *Mycobacterium bovis* infection in human beings in contact with elk (*Cervus elaphus*) in Alberta, Canada. Lancet 338(8777):1253–1255. https://doi.org/10.1016/0140-6736(91)92113-G
67. Vayr F, Martin-Blondel G, Savall F, Soulat JM, Deffontaines G, Herin F (2018) Occupational exposure to human *Mycobacterium bovis* infection: a systematic review. PLoS Negl Trop Dis 12(1):e0006208. https://doi.org/10.1371/journal.pntd.0006208
68. Fanning A, Edwards S, Hauer G (1991) *Mycobacterium bovis* infection in humans exposed to elk in Alberta. Can Dis Wkly Rep 17(44):239–240
69. Ciambrone L, Gioffre A, Musarella R, Samele P, Visaggio D, Pirolo M, Clausi MT, Di Natale R, Gherardi M, Spatari G, Visca P, Casalinuovo F (2020) Presence of *Mycobacterium*

bovis in slaughterhouses and risks for workers. Prev Vet Med 181:105072. https://doi.org/10.1016/j.prevetmed.2020.105072
70. Wilkins MJ, Bartlett PC, Frawley B, O'Brien DJ, Miller CE (2001) Boulton ML (2003) *Mycobacterium bovis* (bovine TB) exposure as a recreational risk for hunters: results of a Michigan Hunter Survey. Int J Tuberc Lung Dis 7(10):1001–1009
71. Wilkins MJ, Meyerson J, Bartlett PC, Spieldenner SL, Berry DE, Mosher LB, Kaneene JB, Robinson-Dunn B, Stobierski MG, Boulton ML (2008) Human *Mycobacterium bovis* infection and bovine tuberculosis outbreak, Michigan, 1994–2007. Emerg Infect Dis 14 (4):657–660
72. Cooke MM, Gear AJ, Naidoo A, Collins DM (2002) Accidental *Mycobacterium bovis* infection in a veterinarian. NZ Vet J 50(1):36–38. https://doi.org/10.1080/00480169.2002.36248
73. Adesokan HK, Akinseye VO, Streicher EM, VanHelden P, Warren RM, Cadmus SI (2019) Reverse zoonotic tuberculosis transmission from an emerging Uganda I strain between pastoralists and cattle in South-Eastern Nigeria. BMC Vet Res 15:437. https://doi.org/10.1186/s12917-019-2185-1
74. Ameni G, Tadesse K, Hailu E, Deresse Y, Medhin G, Aseffa A, Hewinson G, Vordermeier M, Berg S (2013) Transmission of *Mycobacterium tuberculosis* between farmers and cattle in central Ethiopia. PLoS ONE 8(10):e76891. https://doi.org/10.1371/journal.pone.0076891
75. Ibrahim S (2016) Molecular identification of *Mycobacterium tuberculosis* transmission between cattle and man: a case report. J Microbiol Exp 3(3). https://doi.org/10.15406/jmen.2016.03.00091
76. Hlokwe TM, Said H, Gcebe N (2017) *Mycobacterium tuberculosis* infection in cattle from the Eastern Cape Province of South Africa. BMC Vet Res 13(1):299. https://doi.org/10.1186/s12917-017-1220-3
77. Mittal M, Chakravarti S, Sharma V, Sanjeeth BS, Churamani CP, Kanwar NS (2014) Evidence of presence of *Mycobacterium tuberculosis* in bovine tissue samples by multiplex PCR: possible relevance to reverse zoonosis. Transbound Emerg Dis 61(2):97–104. https://doi.org/10.1111/tbed.12203
78. Prasad HK, Singhal A, Mishra A, Shah NP, Katoch VM, Thakral SS, Singh DV, Chumber S, Bal S, Aggarwal S, Padma MV, Kumar S, Singh MK, Acharya SK (2005) Bovine tuberculosis in India: potential basis for zoonosis. Tuberculosis (Edinb) 85(5–6):421–428. https://doi.org/10.1016/j.tube.2005.08.005
79. Srivastava K, Chauhan DS, Gupta P, Singh HB, Sharma VD, Yadav VS, Sreekumaran, Thakral SS, Dharamdheeran JS, Nigam P, Prasad HK, Katoch VM (2008) Isolation of *Mycobacterium bovis* & *M. tuberculosis* from cattle of some farms in north India—possible relevance in human health. Indian J Med Res 128(18820355):26–31
80. Sweetline Anne N, Ronald BSM, Kumar TMAS, Kannan P, Thangavelu A (2017) Molecular identification of *Mycobacterium tuberculosis* in cattle. Vet Microbiol 198:81–87. https://doi.org/10.1016/j.vetmic.2016.12.013
81. Foddai A, Nielsen LR, Krogh K, Alban L (2015) Assessment of the probability of introducing *Mycobacterium tuberculosis* into Danish cattle herds. Prev Vet Med 122(1–2):92–98. https://doi.org/10.1016/j.prevetmed.2015.08.005
82. Lesslie IW (1960) Tuberculosis in attested herds caused by the human type tubercle bacillus. Vet Rec 72:S218–S224
83. Krajewska M, Kozinska M, Zwolska Z, Lipiec M, Augustynowicz-Kopec E, Szulowski K (2012) Human as a source of tuberculosis for cattle. First evidence of transmission in Poland. Vet Microbiol 159(1–2):269–271. https://doi.org/10.1016/j.vetmic.2012.04.001
84. Ocepek M, Pate M, Zolnir-Dovc M, Poljak M (2005) Transmission of *Mycobacterium tuberculosis* from human to cattle. J Clin Microbiol 43(7):3555–3557. https://doi.org/10.1128/JCM.43.7.3555-3557.2005

85. Guta S, Casal J, Napp S, Saez JL, Garcia-Saenz A, Perez de Val B, Romero B, Alvarez J, Allepuz A (2014) Epidemiological investigation of bovine tuberculosis herd breakdowns in Spain 2009/2011. PLoS ONE 9(8):e104383. https://doi.org/10.1371/journal.pone.0104383
86. Romero B, Rodriguez S, Brezos J, Diaz R, Copano MF, Meredez I, Minguez O, Marques S, Palacios JJ, Garcia de Viedma D, Saez JL, Mateos A, Aranaz A, Dominguez L, de Juan L (2011) Humans as a source of *Mycobacterium tuberculosis* infection in cattle, Spain. Emerg Infect Dis 17(12):2393–2395
87. Fritsche A, Engel R, Buhl D, Zellweger JP (2004) *Mycobacterium bovis* tuberculosis: from animal to man and back. Int J Tuberc Lung Dis 8(7):903–904
88. Chen Y, Chao Y, Deng Q, Liu T, Xiang J, Chen J, Zhou J, Zhan Z, Kuang Y, Cai H, Chen H, Guo A (2009) Potential challenges to the Stop TB Plan for humans in China; cattle maintain *M. bovis* and *M. tuberculosis*. Tuberculosis 89:95–100. https://doi.org/10.1016/j.tube.20
89. Du Y, Qi Y, Yu L, Lin J, Liu S, Ni H, Pang H, Liu H, Si W, Zhao H, Wang C (2011) Molecular characterization of *Mycobacterium tuberculosis* complex (MTBC) isolated from cattle in northeast and northwest China. Res Vet Sci 90(3):385–391. https://doi.org/10.1016/j.rvsc.2010.07.020
90. Esfandbod M (2011) Images in clinical medicine. Tuberculoid leprosy. N Engl J Med 364 (21524216):1657. https://doi.org/10.1056/NEJMicm1011992
91. Baldwin JH (1968) Pulmonary bovine tuberculosis in an owner and his dairy herd. Cornell Veterinarian 58(1):81–87
92. Grange JM, Yates MD (1994) Zoonotic aspects of *Mycobacterium bovis* infection. Vet Microbiol 40(8073621):137–151
93. WHO (2019) WHO Global Tuberculosis Report. https://www.who.int/tb/publications/global_report/en/. Accessed 3 Aug 2020
94. Waters WR, Whelan AO, Lyashchenko KP, Greenwald R, Palmer MV, Harris BN, Hewinson RG, Vordermeier HM (2010) Immune responses in cattle inoculated with *Mycobacterium bovis*, *Mycobacterium tuberculosis*, or *Mycobacterium kansasii*. Clin Vaccine Immunol 17(2):247–252. https://doi.org/10.1128/CVI.00442-09
95. Firdessa R, Berg S, Hailu E, Schelling E, Gumi B, Erenso G, Gadisa E, Kiros T, Habtamu M, Hussein J, Zinsstag J, Robertson BD, Ameni G, Lohan AJ, Loftus B, Comas I, Gagneux S, Tschopp R, Yamuah L, Hewinson G, Gordon SV, Young DB, Aseffa A (2013) Mycobacterial lineages causing pulmonary and extrapulmonary tuberculosis, Ethiopia. Emerg Infect Dis 19(3):460–463. https://doi.org/10.3201/eid1903.120256
96. Ameni G, Vordermeier M, Firdessa R, Aseffa A, Hewinson G, Gordon SV, Berg S (2011) *Mycobacterium tuberculosis* infection in grazing cattle in central Ethiopia. Vet J 188 (3):359–361. https://doi.org/10.1016/j.tvjl.2010.05.005
97. Grange JM, Collins CH (1987) Bovine tubercle bacilli and disease in animals and man. Epidmiol Infect 99(2):221–234
98. Huitema H (1969) The eradication of bovine tuberculosis in cattle in the Netherlands and the significance of man as a source of infection for cattle. Sel Pap R Neth Tuberc Ass 12:62–67
99. Schliesser T (1974) "Tierversuch" der Vergangenheit [Control of bovine tuberculosis —"animal experiment" of the past]. Prax Pneumol 28(Suppl):87–874
100. Meyers JA, Steele JH (1969) Bovine tuberculosis control in man and animals. Warren E Green, Inc
101. WHO (2017) Roadmap for zoonotic tuberculosis. https://www.who.int/tb/publications/2017/zoonotic_TB/en/. Accessed 1 Jan 2020
102. Stallknecht D (2007) Impediments to wildlife disease surveillance, research and diagnostics. In: Childs JE, Mackenzie JS, Richt JA (eds) Wildlife and emerging zoonotic diseases: the biology, circumstances and consequences of cross-species transmission, vol 315. Springer, Berlin, Heidelberg, pp 445–461
103. Ryan TJ, Livingstone PG, Ramsey DS, de Lisle GW, Nugent G, Collins DM, Buddle BM (2006) Advances in understanding disease epidemiology and implications for control and

eradication of tuberculosis in livestock: the experience from New Zealand. Vet Microbiol 112(2–4):211–219. https://doi.org/10.1016/j.vetmic.2005.11.025
104. Tweddle NE, Livingstone P (1994) Bovine tuberculosis control and eradication programs in Australia and New Zealand. Vet Microbiol 40(1–2):23–39. https://doi.org/10.1016/0378-1135(94)90044-2
105. Protection of Badgers Act 1992 (1992) The Stationary Office Limited, London

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Evolution and Molecular Characteristics of *Mycobacterium tuberculosis* and *Mycobacterium bovis*

41

Teresa Rito, Osvaldo Inlamea, Olena Oliveira, Raquel Duarte, Pedro Soares, and Margarida Correia-Neves

Millions of people die from tuberculosis every year - and it's totally treatable. This is a disease we can eradicate in our lifetime.

Jennifer Wright

Summary

In the last decade, a major genomics revolution took place in life and health sciences that shed light on the biology and evolution of all living organisms. The pathogenic *Mycobacterium tuberculosis* and *Mycobacterium bovis,* members of the *Mycobacterium tuberculosis* complex, are perfect examples of important genomic studies on their interspecific evolutionary relationships as well as intraspecific evolution and their spread across the world. Mechanisms

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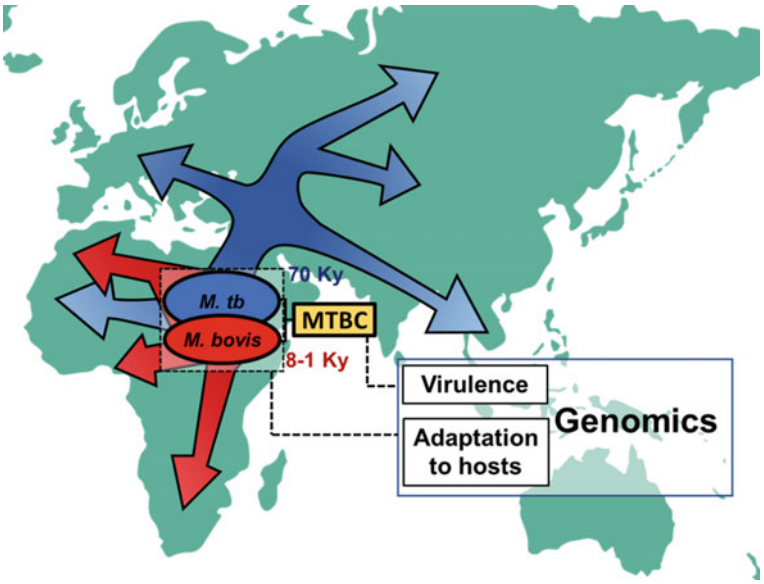
underlying pathogenicity and virulence are being identified in the annotated genomes, crucial for understanding clinical outcomes. Genomic diversity within these mycobacteria is being exploited to pinpoint transmission events and transmission chains, providing a valuable tool for public health agents. In conclusion, genomics and evolution provide valuable tools, data, and inferences within an interdisciplinary approach that is crucial for tuberculosis eradication.

Graphical Abstract



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Keywords

Co-evolution · Genomics · Genotyping · Molecular epidemiology · Phylogenetics · Phylogeography

1 Introduction

Mycobacterium tuberculosis and *Mycobacterium bovis* are two of the most commonly found bacteria associated with tuberculosis (TB) lesions related to some degree of pathogenicity. Despite a continuous decrease of TB worldwide, this disease continues to be one of the most worrisome diseases worldwide, causing a high number of fatalities. *M. tb* and *M. bovis* are the two most studied mycobacteria. They are respectively the pathogenic agents responsible for TB in humans and bovine TB (bTB), a form of TB that affects cattle, being of high economic importance in livestock production.

The genus Mycobacterium is highly complex, with about 200 species currently described. Mycobacteria are typically classified into rapidly-growing bacteria within the genus, including environmental mycobacteria and slow-growing bacteria that include known pathogens. Beyond the mentioned *M. tuberculosis* and *M. bovis* that cause TB in a wide range of mammals and are part of the named *M. tuberculosis* complex (MTBC), the genus includes other pathogens, namely the causal agent of leprosy and a second complex, the *M. avium* complex (MAC).

In the last couple of decades, we witnessed a revolution in genetic and genomic analyses in the various fields of health and life sciences. The drastic increase in genomic resolution is allowing the relevant information about these species to accumulate fast, including their evolutionary relationships, functional aspects of the pathogens, adaptations for virulence and drug-resistance against therapies, or to unravel the deep history of the pathogens and trace recent possible transmissions in a public health scenario. This chapter aims to review the basics of how genomics led to breakthroughs in various aspects of our understanding of the evolution of pathogenic *M. tuberculosis* and *M. bovis*.

2 The *M. tuberculosis* Complex

M. tuberculosis and *M. bovis* belong to Mycobacteriaceae, a family integrated into the order Actinomycetales, class Actinobacteria, phylum Actinobacteria, and Bacteria domain [1]. The genus Mycobacterium contains species typically grouped into complexes, such as the MTBC. Figure 1 displays a maximum-likelihood phylogenetic tree establishing relationships between MTBC species and other

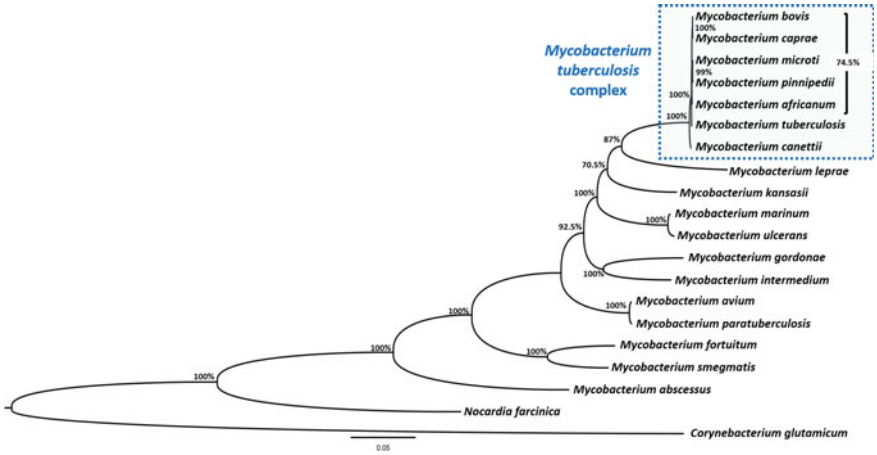

Fig. 1 Maximum-likelihood phylogenetic tree establishing relationships between mycobacterial species. "*Mycobacterium tuberculosis* complex" species are highlighted. For the phylogenetic reconstruction, two hundred concatenated conserved genes in the different species were used. *Corynebacterium glutamicum* and *Nocardia farcinica* were used as outgroups. Percentages within nodes correspond to the bootstrap values for 500 samples of the tree

mycobacteria, either pathogenic or environmental, established by comparing nearly 200 concatenated conserved genes in the different species. Genetic studies suggested previously that slow-growing pathogenic bacteria, including the MTBC, evolved from a fast-growing phenotype [2].

The MTBC consists of several highly relevant species for animal health, including *M. canetti*, *M. africanum*, *M. pinnipedii*, *M microti*, *M. caprae*, *M. bovis,* and *M. tuberculosis*. MTBC members present tropism to a given host or group of hosts. For example, *M. tuberculosis*, *M. africanum,* and *M. canettii* are associated with infection in humans, while *M. bovis* infects mostly cattle, *M. caprae* is a causal agent of TB in goats, and *M. pinnipedii* in seals. However, *M. bovis* can also cause TB in humans, mainly in the case of immunocompromised individuals, making it a zoonotic public health threat [3]. On the general structure of the MBTC, some diversity might still be hidden: a recent study showed the existence of a sister clade to the whole MTBC causing TB in patients in the region of the Great Lakes in Africa [4].

The genome of the different species of the MTBC has above 99% similarity. The *M. bovis* and *M. tuberculosis* genomes have above 99.9% identity, which is reflected in the low level of discrimination in Fig. 1 when considering only housekeeping genes. The reference genome of *M. tuberculosis* (strain H37Rv, NC_000962.3) is 4.41 Mb-long with over 4,000 genes identified, where 3,906 are protein-coding genes as reported in NCBI (genome[1] section). The genome of *M. bovis* typically used as reference (strain AF2122/97) is 4.35 Mb long with 4,105

[1] National Center for Biotechnology Information, see https://www.ncbi.nlm.nih.gov/.

genes and 3,989 protein-coding genes described. In both genomes, only a single copy of each ribosomal RNA exists, e.g., 5S, 16S, and 23S, and over 40 transfer ribonucleic acids (tRNAs). As typical in Actinobacteria, the GC content is high, at around 65%. Another feature of the MTBC genome is the high number of repetitive elements, namely the widely studied proline-glutamate (PE) or proline-proline-glutamate (PPE) family genes [5] that represent up to 10% of the genome. These characteristics make the assembly of the genomes difficult when dealing with short reads in the raw data.

Despite the high homology between the genomes of *M. bovis* and *M. tuberculosis*, the adaptation to different hosts specifies genomic differences between both, most of these still to be unraveled [6]. Successive deletions/insertions of large deoxyribonucleic acid (DNA) segments have been pointed out as major sources of speciation and differences in pathogenesis between species within the MTBC. Fourteen regions of differences (RD) were previously determined that were lost in several lineages of the MTBC [6, 7]. These regions are present in the laboratory reference strain of *M. tuberculosis* H37Rv but absent in the attenuated strain *M. bovis* BCG used in TB vaccines, highlighting their potential roles in virulence. It is, however, clear now that hundreds of nucleotide substitutions are present when comparing coding regions of the genomes of both species, possibly with functional implications. The transcriptome also displays altered features in terms of gene expression profiles caused by differences in regulation [8], perhaps leading to some of the major differences observed between MTBC species. As a zoonotic species, infection with *M. bovis* in humans also leads to different proteomic regulations in humans compared with infection with *M. tuberculosis*, again pointing to differences in the pathogenic profile between the two species [9].

Horizontal gene transfer has been pointed out previously as playing a major role in the acquisition and evolution of pathogenicity in mycobacteria [10, 11], including gene transfer from eukaryotic organisms [12]. It is often uncertain what would be the organisms that represented the source of those transferred genes. If horizontal transfer played a major role in the early evolution of mycobacteria, current data suggests that these episodes are extremely rare or absent in mycobacteria nowadays. Nevertheless, horizontal gene transfer was also proposed to be responsible for specific host adaptations in pathogenic bacteria [13].

3 Functional Genomics

The annotated genome of the several Mycobacterium species allows us to understand general variation patterns that underlined the adaptation of the specimens either to a pathogenic profile in general or to specific hosts. However, while the genome of MTBC species is generally well-annotated, several hypothetical genes still exist with functions unknown. Also, as mentioned above, on the finer-scale (namely within the MTBC), substitutions on specific genes between species might explain adaptations that are not related to differential gene content between MTBC species.

The success of mycobacteria as intracellular pathogens relates to the capacity to circumvent the immune response and to use macrophages as the main host cell. Virulent mycobacteria can modulate the activation and functions of these cells. Another major aspect of virulence is the capacity to use fats and cholesterol as the major carbon source, even during chronic infection when nutrients are lacking. Identifying genes related to these aspects is essential for understanding the virulence of *M. tuberculosis* and *M. bovis*. The genomic revolution in life science enabled major advances through comparative genomics that increased our understanding of the functional mechanisms that explain pathogenicity, virulence, or how pathogenic mycobacteria can persist within the host.

One obvious common feature of the genus Mycobacteria is an extension of the lipid metabolism genes. Figure 2 displays an annotation using Eggnog-mapper [14] of *M. tuberculosis*, *M. bovis,* and *Corynebacterium glutamicum* to compare the different classes of proteins. Using the COG (clusters of orthologous groups) classification, it is evident that lipid metabolism plays a major role in mycobacteria compared with an actinobacterium, more precisely a Corynebacteria. As mentioned above, fatty-acid metabolism plays an essential role in infection and dormancy in *M. tuberculosis*, where fatty acids become the major energy source.

Another previously observed trend in pathogenic mycobacteria is the increment of alternative pathways for "replication and repair" [15]. Figure 2 also displays a set of COG categories extended in relation to *Corynebacterium glutamicum*, but a detailed analysis would be required to distinguish what general trends are from the specific comparisons between the species involved only. A major adaptation within the genus Mycobacterium is a shift from a fast-growing phenotype in the ancestry of all mycobacteria [2] to an intra-cellular slow-growing existence. Such shift includes the loss of cellular amino acid transportation capacity (including the loss of livFGMH and ABC transporter operons) that might limit fast growth [13].

It is a known fact that the outcome of TB infection is derived from multiple factors, from the host's immune system to environmental variables, but it is clear that the genetics of the bacteria plays a major factor. There exists a vast number of parameters to define virulence without a complete consensus on its definition. At its core, virulence is defined as the capacity for mycobacteria to cause disease while being able to survive within the host cell against the immune system of the host. In terms of molecular assays, a possible definition relates with genes whose inactivation with the pathogen results in a specific decrease in virulence considering different validated TB models. Nevertheless, screening approaches and comparative genomics identified several genes as important for pathogenicity and virulence, even though the overlap between approaches is often limited, highlighting that much information still needs to be discovered.

Much of the genes identified as virulence genes relate to the host's capacity to survive and persist, coding for several lipid pathways, cell surface proteins, regulators, and signal transduction systems. Mycobacteria lack toxin-producing genes, and virulence genes are mostly conserved in non-pathogenic mycobacteria, showing that adaptation from environmental to pathogenic occurred with very little gene content shift but instead evolution of existing genes. Considering that

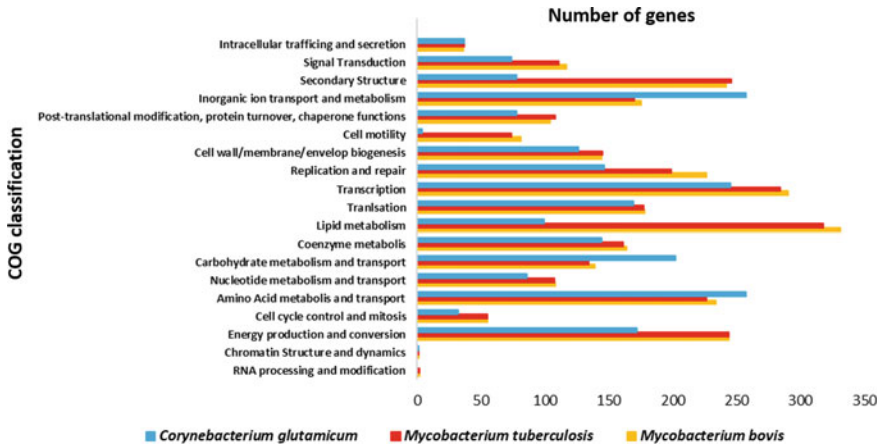

Fig. 2 Number of genes in the different classes of proteins of *M. tuberculosis*, *M. bovis,* and *Corynebacterium glutamicum*, categorized using the COG (clusters of orthologous groups) classification. The genomes of *M. tuberculosis*, *M. bovis,* and *Corynebacterium glutamicum* also contained 877, 880, and 566 genes classified as "function unknown" not included in the figure

mycobacteria take advantage of the host inflammatory signal to spread within the host, it is likely that the bases of pathogenicity are related to adaptations to the hosts.

One important virulence factor in MTBC is the presence of mycolic acids in the cell wall, a feature that characterizes mycobacteria. These cell wall components play a crucial role in invading the host cells allowing the evasion of the immune system. The cell wall also maintains antigens in the cell surface that affects the host's immune response. The enzymatic pathways involved in the biosynthesis and transport of mycolates [16] are relatively known and represent major drug targets. Lipoproteins in the cell surface have also been linked to virulence. There are nearly 100 putative lipoproteins in the *M. tuberculosis* genome-related immune modulation and induction of protective responses [17]. These include lipoproteins LpqH, LppX, Mpt83, LprG, RpfB, LpqS, LprN, Lprl, and PstS (as reviewed in [18]). ESAT-6 is a widely studied protein that is abundantly secreted by *M. tuberculosis*, and it is an important virulence factor whose inactivation substantially reduces the pathogen's virulence. Its role relates to interaction with some host cellular factors. ESAT-6 modulates inflammatory response by the host through the alteration of several intracellular signaling pathways affecting macrophages and T cells, as well as epithelial cells [19, 20].

Mce genes (mammalian cell entry) are members of an assembly within the extracellular membrane of MTBC species that are likely lipid transporters that allow the nutrient uptake in the host environment. However, recent research also suggests a broader role through interaction with the host signaling proteins [21].

Transporters across the cell wall might represent important virulent factors performed by specialized proteins [18]. The twin-arginine transporter in the cytoplasmic membrane transports folded proteins that include elements related to wall biosynthesis, resistance to antibiotics, and transport of virulence-related proteins. A widely studied group of proteins in MTBC are the PE-PPE proteins that are either secreted or are located on the cell surface. They are thought to represent a potential source of antigenic variation [5] and modulate the innate immune system, representing an important mechanism for evading the host immune response. It is thought that PE/PPE evolution greatly impacted the evolution of pathogenic mycobacteria [22]. Another transport system that might play a pivotal role in virulence is the ESX transporter system encoded by a set of ESX genes. For example, the ESX-1 system is deleted in *M. bovis* strain BCG (in the lost RD1), an attenuated form of the pathogen [23]. The ESX systems are related to cell death, PE/PPE secretion, and drug resistance.

Furthermore, enzymes such as PtpA, SapM, and Ndk are responsible for evading the immune system through phagosome arresting and/or survival within the phagosome [24–26]. A great part of the success of MTBC species as a pathogen agent is its capacity to circumvent the oxidative stress conditions established by the host as a response to the infection. Analyses of the genome also revealed redox sensors that can detect various types of redox stress [27] and activate mechanisms to regulate the redox level within the pathogen, including catalase peroxidases, peroxiredoxins, and glutathione peroxidase [28]. Citrate lyase activity was also relevant for the pathogen to survive oxidative stress [29].

As mentioned above, several studies exist that aim to characterize virulence genes with slight overlapping. These represent a far from an exhaustive list of hypothetical virulence genes/proteins, but other studies exist that point out the use of a nitrogen source provider or control of apoptosis as important virulence factors.

4 Genotyping and Genetic Epidemiology of *Mycobacterium tuberculosis* and *Mycobacterium bovis*

In recent years, genomics coupled with advanced statistical and phylogenetic methods allowed an astonishing comprehension of the evolution of a given pathogen and the expansion of the diseases caused by these bacteria, with various examples from recent cases, as the rapid worldwide spread of COVID-19 [30] to diseases with thousands of years of co-evolution with the host namely TB [31] and bTB [32] discussed here. Beyond the global picture, genetic epidemiology allows a fine-scale analysis and evaluation of hypothetical transmission episodes and chains of transmission within a molecular epidemiology approach. We will briefly describe the four main genotyping methodologies used in molecular epidemiology in TB and bTB, the implications of each genotyping technique, and their level of discrimination in interpreting public health scenarios.

In the last few years, the genetic epidemiology of *M. tuberculosis* entered a genomic era, where whole genomes are being used to establish and test hypothetical transmission events and transmissions chains. However, other genotyping methods were used to access lineage information on the studied clinical and epidemiological cases in the recent past. One previous reference method was the use of restriction enzymes under a restriction fragment length polymorphism (RFLP) approach and a probe for an inserted sequence called IS6110 within the fragments [33]. With lower discrimination and more dubious interpretation of the profiles compared with the following genotyping methods, this method was not applicable for *M. bovis,* where polymorphic inserts are mostly absent.

A reference genotyping method that followed was based on the characterization of direct repeats (DR) using probes [34], however, with similar issues of interpretation and standardization. However, the application of PCR technology allowed an assay to analyze a set of spacers (sequences between DRs where primers would hybridize) that would provide a barcode of 43 spacers, labeled 1–43, that are stable between labs, a genotyping assay called spoligotyping [35]. It identifies spoligotypes genotyped on different labs, allowing the comparison between studies, periods, and geographic locations. It also allowed the identification of members of the MTBC. In the case of *M. bovis*, this methodology permitted discrimination of genotypes, including the so-called European 1 and 2 clonal complexes, missing spacers 11 and 21 respectively, and African 1 and 2 complexes, missing spacer 30 in the first and spacers 3 and 7 in the latter [36, 37].

For *M. tuberculosis*, a greater level of discrimination was achieved by introducing the MIRU (mycobacterial interspersed repetitive units) system that employs a variable number of tandem repeat *loci* [38]. MIRU-VNTR was then proposed as a standardized method for analyzing variation in clinical isolates [38]. The last version, still widely used nowadays, contains a total of 24 VNTR *loci* (*loci* 154, 424, 2401, 2461, 577, 2531, 580, 2687, 802, 2996, 960, 3007, 1644, 3171, 1955, 3192, 2059, 3690, 2163b, 4052, 2165, 4156, 2347 and 4348). Advantages concerning previous genotyping methods in *M. tuberculosis* are various, including a much higher discriminatory capacity. VNTR *loci* have higher mutation rates and greater alleles than previously used markers that were mostly biallelic. As with spoligotyping data, MIRU-VNTR profiles can also be easily recorded. In this case, the genotype corresponds to the number of repetitions in each of the 24 *loci*, again allowing comparison between studies, years, and regions. Using platforms like MIRU-VNTRplus [39], it is possible to classify the isolates of *M. tuberculosis* into specific branches of the *M. tuberculosis* tree. Clustering methodologies, like spanning trees, help establish hypothetical transmission events or clusters of transmission in the population during an outbreak [40]. Using appropriate phylogenetic methodologies like parsimony, median vectors, and a weighting scheme for the different *loci*, a broad evolution of the genotypes can be established, and monophyletic clades corresponding to these major *M. tuberculosis* branches can be obtained [41, 42], allowing a rough evolutionary perspective to be obtained. While MIRU is a great improvement concerning previous methods, its value falls short compared with whole-genome sequencing, whose prices have been drastically

decreasing, leading to the continuous abandonment of other methodologies like spoligotyping and MIRU-VNTR.

In terms of *M. bovis*, the discriminatory power of MIRU-VNTR is far from the one obtained for *M. tuberculosis*. From the 24 loci present in the system [38], only five have shown to be relevant in terms of polymorphic status in *M. bovis* (2165, 2461, 577, 580, and 3192) [36, 37]. Given that fact, MIRU-VNTR does not offer a higher discrimination power than spoligotyping [37]. Although these two systems are not as powerful tools as in *M. tuberculosis*, the use of whole genomes of *M. bovis* specifically for molecular epidemiology is much rarer than in *M. tuberculosis*. Nevertheless, a better level of discrimination can be obtained by combining MIRU-VNTR loci with spoligotyping profiles that will differentiate geographic clusters across Africa [32, 36] and gene flow between neighboring regions.

In terms of genomic epidemiology, geneticists and public health researchers have access to the maximum possible discrimination for *M. tuberculosis* and *M. bovis* using whole-genome sequencing. A genomic sequence not only offers a detailed relationship between strains in a public health scenario but also in a global scenario, offering a fine-scale placing of the lineages into the *M. tuberculosis* worldwide tree [40, 43, 44]. The lineages are classified into known groups with differential virulence and other features, offering a direct tool for public health. It also allows the search for mutations promoting drug resistance to first and second-line drugs [45–47]. The high resolution afforded can also detect mixed infections [48]. What are the possible drawbacks of using genomic data? The major caveat until just a handful of years ago was the price, but the continued decrease in sequencing prices makes the price of a whole genome somehow similar to that of MIRU in several reference laboratories. Some laboratories might find the bioinformatics pipelines and the size of the datasets a challenge to less informatics-prone researchers. One minor difficulty in assembling genomes of pathogenic mycobacteria is the relatively high number of repetitive elements, but these can be excluded for nearly the same resolution.

One single point that can generate some interpretational issues relates to the instantaneous mutation rates of the pathogens. Non-matching genotypes in IS6110, spoligotyping, or MIRU-VNTR profiles between isolates can directly exclude an epidemiological connection between cases. In these same systems matching genetic results between two clinical cases does not necessarily prove transmission but shows proximity between cases within the transmission chain that can be analyzed epidemiologically. Whole genomes do not have such a direct interpretation. Several mutations can occur within a whole genome between direct transmissions. Given the random fluctuation of the mutational process, the possible heterogeneous nature of mutation rate between lineages [49], and the emergence of variants associated with drug resistance is difficult to define that value. Nevertheless, that is a minor issue that can be addressed by an integrative, multidisciplinary approach with epidemiologists and public health agents to study the non-genetic links between cases simultaneously.

5 Genetic History of *Mycobacterium tuberculosis* and *Mycobacterium bovis*

M. tuberculosis and *M. bovis* are members of the MTBC. There is no doubt that MTBC originated from fast-growing environmental mycobacteria. However, there are doubts regarding the point of origin and the time of the emergence of this pathogenic clade. Previous studies hypothesized that *M. bovis* and *M. tuberculosis* represented the same branch or lineage within the MTBC. In this scenario, *M. tuberculosis* likely originated from *M. bovis,* and the bacteria were transmitted to man from domesticated animals in a zoonotic scenario [50]. This theory is directly related to the emergence of the Neolithic in the Near East, and more specifically claimed that *M. bovis* was an existing pathogen for bovines, and following their domestication during the development of agricultural societies, the proximity between human beings and the animals prompted a zoonotic transmission of *M. bovis* to humans, where further adaptations to the host led to the existing *M. tuberculosis*. However, the first whole genomes of both species revealed that, while being members of the MTBC, *M. tuberculosis* and *M. bovis* represented independent branches of the complex. If a direction were implicit in their evolution, it would be the opposite as the most divergent branch of the MTBC, *M. canetti*, infects humans (Fig. 1), and a sister clade to the MTBC was also detected in human TB patients in Africa [4]. While *M. bovis* is mostly present in Africa (possibly resulting from transmission control elsewhere), *M. tuberculosis* is spread worldwide. However, genetic or genomic studies suggested that both have an African source.

The most accepted theory nowadays indicates that TB might have followed human evolution for over 70,000 years. Modern humans originated in Africa at least a few hundreds of thousands of years ago, where most of the species' evolution took place. While modern humans probably left Africa multiple times during their evolution, it is unlikely that any exit before 70,000 years ago left any descendants until the present day. After 70,000 years, prompted by ideal climate conditions and a probable rise in cultural and technical innovations [51, 52], modern humans left Africa and colonized the world, successfully settling most of the globe, a model named Out-of-Africa. The similarity of the *M. tuberculosis* tree and the human mitochondrial DNA (mtDNA) branch labeled L3, which represents all mtDNA lineages outside Africa and dates to about 70,000 years [51], was the motivation to propose a similar genetic history. A theory was put forward that *M. tuberculosis* accompanied modern humans in their expansion outside Africa (Fig. 3) [31]. While there are some controversies regarding molecular clocks in *M. tuberculosis* [53] and the fact that the study of Comas and collaborators offered a great degree of circularity in their calibration (as the time-frame was partially calibrated assuming the age of mtDNA haplogroup L3), several studies suggested the same time frame using different calibration methodologies [53].

The major branches of *M. tuberculosis* outside Africa would follow a similar pattern of modern human evolution that, after reaching Southwestern Asia, migrated North and West into Europe where a major branch exists, and through the

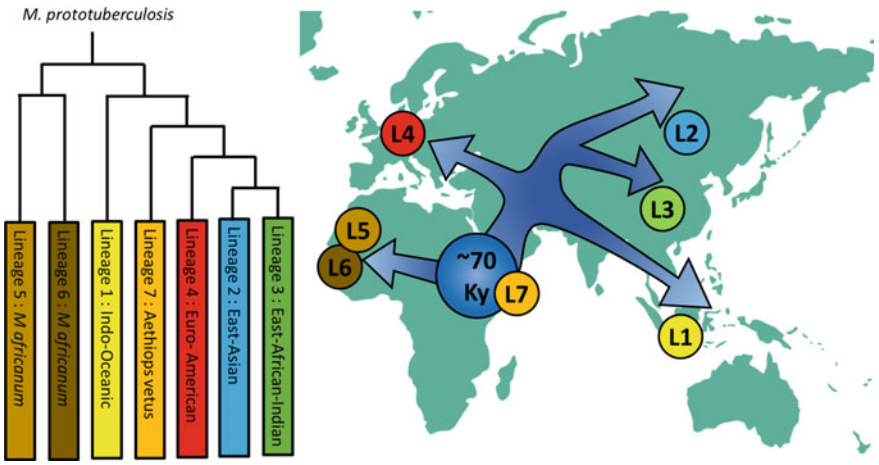

Fig. 3 Hypothetical spread of *M. tuberculosis* across the world, starting in Africa about 70,000 years ago. Phylogenetic relationship between major branches of the *M. tuberculosis* tree is displayed, as well as the geographic distribution of the branches

Asian southern coastal area through South Asia into Southeast Asia and Oceania and into East Asia [54] where major branches of *M. tuberculosis* also exist (Fig. 3). The geographic clustering of *M. tuberculosis* diversity also corresponds roughly to the same geographic patterns of modern human diversity. One possible implication of such a model is that *M. tuberculosis* migrated with modern humans and evolved and adapted to the human populations in different geographic regions [55]. Currently, seven main lineages or branches of *M. tuberculosis* are described, including three in Africa, the lineages 5 and 6 of *M. africanum* in West Africa and lineage 7 Aethiops vetus in East Africa in the Horn of Africa [7], lineage 1 Indo-Oceanic in the Indian Ocean, lineage 2 East-Asian, lineage 3 East-African-Indian in the Asian continent, and lineage L4 Euro-American in Europe (Fig. 3). Using somewhat misrepresentative terminology, lineages 1, 5, and 6 within Africa are called "ancient," while lineages 2–4 are called "modern" lineages. Evolutionarily wise, lineages have the same level of evolution and co-evolution in the populations they co-evolved in (with the term "ancient" suggesting some level of non-evolution and an ancient state). A growing body of evidence suggests that the variation of *M. tuberculosis* strains has functional implications: different variations in *M. tuberculosis* lineages are associated with specific phenotypes of the disease. Although genetic diversity can influence the clinical severity of TB in humans, the specific factors for these heterogeneous presentations of the disease in different scenarios are mainly unknown. One lineage – part of lineage 2 – commonly associated with major outbreaks and virulence, is the Beijing/W strain that can model some aspects of intensified virulence [56].

While *M. tuberculosis* might have co-evolved with modern humans across 70,000 years, the pathogen was not persisting in a demographic scenario similar to the one observed today. Early modern human populations of hunter-gatherers were present in drastically lower density minimizing large episodes of transmission, which might have caused positive selection for variants that maximize efficiency in latency [55]. With the Neolithic and sedentary behavior and lifestyle of the populations that developed stable agricultural practices in the last 10,000 years, population densities increased in specific areas, leading to another co-expansion of *M. tuberculosis* with modern humans [31]. At this point, more virulent strains, more efficient in transmission might have been selected. It is difficult to know the burden of TB in ancient human populations, but a recent ancient DNA study suggested that it was high enough to cause major shifts in human allele frequencies [57]. Recent studies also suggested that different populations have different backgrounds of hypothetical alleles of genetic susceptibility to TB [58–61].

In *M. bovis*, studies indicate a much shallower level of evolution with a much more recent ancestry of the pathogen. *M. bovis* mostly present in Africa, and as *M. tuberculosis*, it likely had an African origin or at least a nearby origin (as in Southwestern Asia). Two studies published in 2020 suggested that the spread of *M. bovis* in Africa started in Eastern Africa [32, 62], although with different time-frames. One study is based on whole genomes, presenting an extremely higher resolution [62]. The authors estimated the age for the emergence of *M. bovis* between the third to the twelfth century AD, indicating that the pathogen emerged less than 1800 years ago. Nevertheless, despite the very high resolution, the authors mention that the age estimates for older clades (including the emergence of *M. bovis*) might be underestimated, which means that the chronology might need revision in the future. The other study, starting from a previous estimate of 6,000 years for *M. bovis* [63], and using a combination of MIRU-VNTR and spoligotyping data allowed to estimate a higher diversity of *M. bovis* in Eastern Africa [32], followed by Northern Africa diversity, and a high degree of geographic clustering of genotypes across Africa. The hypothesis raised by the authors was that *M. bovis* emerged with the introduction of pastoralist societies around 5,000 to 4,000 years in Eastern Africa, without excluding an origin in Southwestern Asia where animal domestication took place, and it was the source for the introduction of cattle species and dairy practices to Africa (as no African animals were domesticated). The maintenance of a higher concentration of animals in the same area than before should characterize this period as animals, beyond a source of meat and hides, provided a novel source of protein, milk, in the so-called Secondary Product Revolution. According to archaeology, the levels of diversity of *M. bovis* across different regions also correlated well with the early introduction of cattle across Africa [32]. The two studies [32, 62], even considering the different levels of resolution and chronology of the origin of *M. bovis,* reached some similar conclusions: a higher diversity in Eastern Africa and probable source of expansion into the remaining continent; cases outside Africa, namely in Europe, represent later gene flow from the African continent; and the evolution of *M. bovis* is much more shallow than for *M. tuberculosis*, with age estimates at least ten times lower.

6 Conclusion

Whole-genome sequencing is a powerful tool that has been drastically increasing in resolution and importance in the last decade. Genomics is a field of science that is an ideal interdisciplinary player. It brings to the fight against TB a set of researchers, including geneticists, evolutionists, and bioinformaticians. Data generated on annotation and comparative genomics of the genomes provide the basis for biochemists, cell biologists, and immunologists to study the biology of the pathogens and understand virulence and survival mechanisms of the mycobacteria—a knowledge that can be eventually transformed into therapies' development. Genomic characterization of the strain allows identifying more virulent strains and variants associated with drug resistance prompting a more focused treatment by the clinicians. The evolution of the strains permits to understand the current distribution of the pathogens worldwide and how local adaptations might have taken place, and on the short-term evolution, it allows to test with maximum discriminatory power hypothetical transmission events and transmission chains during outbreaks which allows public health agents to take actions to break transmission chains. The fight against TB can also be won through an integrative, interdisciplinary approach, and in this approach, genomics plays an invaluable role as a binder of multiple fields of research.

Core Messages

- Pathogenic mycobacteria evolved from slow-growing bacteria.
- Comparative genomics shows the evolution of the *M. tuberculosis* complex into pathogenicity and specific adaptations to the host.
- Genetics and genomics are essential tools for understanding transmission events in public health.
- *M. tuberculosis* probably evolved with modern humans for at least 70,000 years following an origin in Africa.
- *M. bovis* has a much more recent origin, with an early evolution in Eastern Africa.

References

1. Saviola B, Bishai W (2006) The genus Mycobacterium—medical. The Prokaryotes. Springer, New York, pp 919–933. https://doi.org/10.1007/0-387-30743-5_34
2. Wee WY, Dutta A, Choo SW (2017) Comparative genome analyses of mycobacteria give better insights into their evolution. PLoS ONE 12:e0172831. https://doi.org/10.1371/journal.pone.0172831
3. Michel AL, Müller B, van Helden PD (2010) *Mycobacterium bovis* at the animal-human interface: a problem, or not? Vet Microbiol Elsevier 371–381. https://doi.org/10.1016/j.vetmic.2009.08.029

4. Ngabonziza JCS, Loiseau C, Marceau M, Jouet A, Menardo F, Tzfadia O, Antoine R, Niyigena EB, Mulders W, Fissette K, Diels M, Gaudin C, Duthoy S, Ssengooba W, André E, Michel K, Kaswa MK, Habimana YM, Brites D, Affolabi D, Mazarati JB, Jong BC, Rigouts L, Gagneux S, Meehan CJ, Supply P (2020) A sister lineage of the *Mycobacterium tuberculosis* complex discovered in the African Great Lakes region. Nat Commun 11:1–11. https://doi.org/10.1038/s41467-020-16626-6
5. Akhter Y, Ehebauer MT, Mukhopadhyay S, Hasnain SE (2012) The PE/PPE multigene family codes for virulence factors and is a possible source of mycobacterial antigenic variation: perhaps more? Biochimie Elsevier 110–116. https://doi.org/10.1016/j.biochi.2011.09.026
6. Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, Eiglmeier K, Garnier T, Gutierrez C, Hewinson G, Kremer K, Parsons LM, Pym AS, Samper S, van Soolingen D, Cole ST (2002) A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. Proc Natl Acad Sci U S A 99:3684–3689. https://doi.org/10.1073/pnas.052548299
7. Nebenzahl-Guimaraes H, Yimer SA, Holm-Hansen C, De Beer J, Brosch R, Van Soolingen D (2016) Genomic characterization of *Mycobacterium tuberculosis* lineage 7 and a proposed name: 'Aethiops vetus.' Microb Genomics 2. https://doi.org/10.1099/mgen.0.000063
8. Golby P, Nunez J, Witney A, Hinds J, Quail MA, Bentley S, Simon Harris S, Smith N, Hewinson RG, Gordon SV (2013) Genome-level analyses of *Mycobacterium bovis* lineages reveal the role of SNPs and antisense transcription in differential gene expression. BMC Genomics 14:710. https://doi.org/10.1186/1471-2164-14-710
9. Li P, Wang R, Dong W, Hu L, Zong B, Zhang Y, Wang X, Guo A, Zhang A, Xiang Y, Chen H, Tan C (2017) Comparative proteomics analysis of human macrophages infected with virulent *Mycobacterium bovis*. Front Cell Infect Microbiol 7:65. https://doi.org/10.3389/fcimb.2017.00065
10. Reva O, Korotetskiy I, Ilin A (2015) Role of the horizontal gene exchange in evolution of pathogenic Mycobacteria. BMC Evol Biol 15:1–8. https://doi.org/10.1186/1471-2148-15-S1-S2
11. Jang J, Becq J, Gicquel B, Deschavanne P, Neyrolles O (2008) Horizontally acquired genomic islands in the tubercle bacilli. Trends Microbiol 16:303–308. https://doi.org/10.1016/j.tim.2008.04.005
12. Gamieldien J, Ptitsyn A, Hide W (2002) Eukaryotic genes in *Mycobacterium tuberculosis* could have a role in pathogenesis and immunomodulation. Trends Genet Elsevier Ltd. 5–8. https://doi.org/10.1016/S0168-9525(01)02529-X
13. Bachmann NL, Salamzade R, Manson AL, Whittington R, Sintchenko V, Earl AM, Marais BJ (2020) Key transitions in the evolution of rapid and slow growing mycobacteria identified by comparative genomics. Front Microbiol 10:3019. https://doi.org/10.3389/fmicb.2019.03019
14. Huerta-Cepas J, Forslund K, Coelho LP, Szklarczyk D, Jensen LJ, Von Mering C, Bork P (2017) Fast genome-wide functional annotation through orthology assignment by eggNOG-mapper. Mol Biol Evol 34:2115–2122. https://doi.org/10.1093/molbev/msx148
15. McGuire AM, Weiner B, Park ST, Wapinski I, Raman S, Dolganov G, Peterson M, Riley R, Zucker J, Abeel T, White J, Sisk P, Stolte C, Koehrsen M, Yamamoto RT, Iacobelli-Martinez M, Kidd MJ, Maer AM, Schoolnik GK, Regev A, Galagan J (2012) Comparative analysis of mycobacterium and related actinomycetes yields insight into the evolution of *Mycobacterium tuberculosis* pathogenesis. BMC Genomics 13:1–27. https://doi.org/10.1186/1471-2164-13-120
16. Jamet S, Quentin Y, Coudray C, Texier P, Laval F, Daffé M, Fichant G, Cam K (2015) Evolution of mycolic acid biosynthesis genes and their regulation during starvation in *Mycobacterium tuberculosis*. J Bacteriol 197:3797–3811. https://doi.org/10.1128/JB.00433-15
17. Becker K, Sander P (2016) *Mycobacterium tuberculosis* lipoproteins in virulence and immunity—fighting with a double-edged sword. FEBS Lett Wiley Blackwell 3800–3819. https://doi.org/10.1002/1873-3468.12273

18. Echeverria-Valencia G, Flores-Villalva S, Espitia CI (2018) Virulence factors and pathogenicity of Mycobacterium. In: Mycobacterium—research and development. InTech. https://doi.org/10.5772/intechopen.72027
19. Sreejit G, Ahmed A, Parveen N, Jha V, Valluri VL, Ghosh S, Mukhopadhyay S (2014) The ESAT-6 protein of *Mycobacterium tuberculosis* interacts with beta-2-microglobulin (β2M) affecting antigen presentation function of macrophage. PLoS Pathog 10:e1004446. https://doi.org/10.1371/journal.ppat.1004446
20. Mishra BB, Moura-Alves P, Sonawane A, Hacohen N, Griffiths G, Moita LF, Anes E (2010) *Mycobacterium tuberculosis* protein ESAT-6 is a potent activator of the NLRP3/ASC inflammasome. Cell Microbiol 12:1046–1063. https://doi.org/10.1111/j.1462-5822.2010.01450.x
21. Fenn K, Wong CT, Darbari VC (2020) *Mycobacterium tuberculosis* uses Mce proteins to interfere with host cell signaling. Front Mol Biosci Frontiers Media S.A. 149. https://doi.org/10.3389/fmolb.2019.00149
22. Fishbein S, van Wyk N, Warren RM, Sampson SL (2015) Phylogeny to function: PE/PPE protein evolution and impact on *Mycobacterium tuberculosis* pathogenicity. Mol Microbiol 96:901–916. https://doi.org/10.1111/mmi.12981
23. Tiwari S, Casey R, Goulding CW, Hingley-Wilson S, Jacobs WR (2019) Infect and inject: how *Mycobacterium tuberculosis* exploits its major virulence-associated type VII secretion system, ESX-1. In: Bacteria and intracellularity. American Society of Microbiology, pp 113–126. https://doi.org/10.1128/microbiolspec.bai-0024-2019
24. Sun J, Singh V, Lau A, Stokes RW, Obregón-Henao A, Orme IM, Wong D, Av-Gay Y, Hmama Z (2013) *Mycobacterium tuberculosis* nucleoside diphosphate kinase inactivates small GTPases leading to evasion of innate immunity. PLoS Pathog 9. https://doi.org/10.1371/journal.ppat.1003499
25. Wang J, Ge P, Qiang L, Tian F, Zhao D, Chai Q, Zhu M, Zhou R, Meng G, Iwakura Y, Gao GF, Liu CH (2017) The mycobacterial phosphatase PtpA regulates the expression of host genes and promotes cell proliferation. Nat Commun 8:1–16. https://doi.org/10.1038/s41467-017-00279-z
26. Puri RV, Reddy PV, Tyagi AK (2013) Secreted acid phosphatase (SapM) of *Mycobacterium tuberculosis* is indispensable for arresting phagosomal maturation and growth of the pathogen in Guinea Pig tissues. PLoS One 8. https://doi.org/10.1371/journal.pone.0070514
27. Bhat SA, Singh N, Trivedi A, Kansal P, Gupta P, Kumar A (2012) The mechanism of redox sensing in *Mycobacterium tuberculosis*. Free Radic Biol Med 53:1625–1641. https://doi.org/10.1016/j.freeradbiomed.2012.08.008
28. Rhee SG, Yang KS, Kang SW, Woo HA, Chang TS (2005) Controlled elimination of intracellular H2O2: regulation of peroxiredoxin, catalase, and glutathione peroxidase via post-translational modification. Antioxid Redox Signal 619–626. https://doi.org/10.1089/ars.2005.7.619
29. Arora G, Chaudhary D, Kidwai S, Sharma D, Singh R (2018) CitE Enzymes are essential for *Mycobacterium tuberculosis* to establish infection in macrophages and Guinea Pigs. Front Cell Infect Microbiol 8:385. https://doi.org/10.3389/fcimb.2018.00385
30. Rito T, Richards MB, Pala M, Correia-Neves M, Soares PA (2020) Phylogeography of 27,000 SARS-CoV-2 genomes: Europe as the major source of the COVID-19 pandemic. Microorganisms 8:1678. https://doi.org/10.3390/microorganisms8111678
31. Comas I, Coscolla M, Luo T, Borrell S, Holt KE, Kato-Maeda M, Parkhill J, Malla B, Berg S, Thwaites G, Yeboah-Manu D, Bothamley G, Mei J, Wei L, Bentley S, Harris SR, Niemann S, Diel R, Aseffa A, Gao Q, Young D, Gagneux S (2013) Out-of-Africa migration and neolithic coexpansion of *Mycobacterium tuberculosis* with modern humans. Nat Genet 45:1176–1182. https://doi.org/10.1038/ng.2744
32. Inlamea OF, Soares P, Ikuta CY, Heinemann MB, Achá SJ, Machado A, Ferreira Neto JS, Correia-Neves M, Rito T (2020) Evolutionary analysis of *Mycobacterium bovis* genotypes

across Africa suggests co-evolution with livestock and humans. Yang R (ed) PLoS Negl Trop Dis 14:e0008081. https://doi.org/10.1371/journal.pntd.0008081
33. McEvoy CRE, Falmer AA, van Pittius NCG, Victor TC, van Helden PD, Warren RM (2007) The role of IS6110 in the evolution of *Mycobacterium tuberculosis*. Tuberculosis (Edinb) pp 393–404. https://doi.org/10.1016/j.tube.2007.05.010
34. Beggs ML, Cave MD, Marlowe C, Cloney L, Duck P, Eisenach KD (1996) Characterization of *Mycobacterium tuberculosis* complex direct repeat sequence for use in cycling probe reaction. J Clin Microbiol 34:2985–2989. https://doi.org/10.1128/jcm.34.12.2985-2989.1996
35. Streicher EM, Victor TC, Van Der Spuy G, Sola C, Rastogi N, Van Helden PD, Warren RM (2007) Spoligotype signatures in the *Mycobacterium tuberculosis* complex. J Clin Microbiol 45:237–240. https://doi.org/10.1128/JCM.01429-06
36. Machado A, Rito T, Ghebremichael S, Muhate N, Maxhuza G, Macuamule C, Moiane I, Macucule B, Marranangumbe AS, Baptista J, Manguele J, Koivula T, Streicher EM, Warren RM, Kallenius G, van Helden P, Correia-Neves M (2018) Genetic diversity and potential routes of transmission of *Mycobacterium bovis* in Mozambique. Vinetz JM (ed) PLoS Negl Trop Dis 12:e0006147. https://doi.org/10.1371/journal.pntd.0006147
37. Guimaraes AMS, Zimpel CK (2020) *Mycobacterium bovis*: from genotyping to genome sequencing. Microorganisms. MDPI AG. https://doi.org/10.3390/microorganisms8050667
38. Supply P, Allix C, Lesjean S, Cardoso-Oelemann M, Rüsch-Gerdes S, Willery E, Savine E, de Haas P, van Deutekom H, Roring S, Bifani P, Kurepina N, Kreiswirth B, Sola C, Rastogi N, Vatin V, Gutierrez MC, Fauville M, Niemann S, Skuce R, Kremer K, Locht C, van Soolingen D (2006) Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of *Mycobacterium tuberculosis*. J Clin Microbiol 44:4498–4510. https://doi.org/10.1128/JCM.01392-06
39. Weniger T, Krawczyk J, Supply P, Niemann S, Harmsen D (2010) MIRU-VNTRplus: a web tool for polyphasic genotyping of *Mycobacterium tuberculosis* complex bacteria. Nucleic Acids Res 38. https://doi.org/10.1093/nar/gkq351
40. Roetzer A, Diel R, Kohl TA, Rückert C, Nübel U, Blom J, Wirth T, Jaenicke S, Schuback S, Rüsch-Gerdes S, Supply P, Kalinowski J, Niemann S (2013) Whole genome sequencing versus traditional genotyping for investigation of a *Mycobacterium tuberculosis* outbreak: a longitudinal molecular epidemiological study. PLoS Med 10:e1001387. https://doi.org/10.1371/journal.pmed.1001387
41. Rito T, Matos C, Carvalho C, Machado H, Rodrigues G, Oliveira O, Ferreira E, Gonçalves J, Maio L, Morais C, Ramos H, Guimarães JT, Santos CL, Duarte R, Correia-Neves M (2018) A complex scenario of tuberculosis transmission is revealed through genetic and epidemiological surveys in Porto. BMC Infect Dis 18:1–12. https://doi.org/10.1186/s12879-018-2968-1
42. Oliveira O, Gaio R, Carvalho C, Correia-Neves M, Duarte R, Rito T (2019) A nationwide study of multidrug-resistant tuberculosis in Portugal 2014–2017 using epidemiological and molecular clustering analyses. BMC Infect Dis 19:567. https://doi.org/10.1186/s12879-019-4189-7
43. Kohl TA, Diel R, Harmsen D, Rothgänger J, Meywald Walter K, Merker M, Weniger T, Niemann S (2014) Whole-genome-based *Mycobacterium tuberculosis* surveillance: a standardized, portable, and expandable approach. J Clin Microbiol 52:2479–2486. https://doi.org/10.1128/JCM.00567-14
44. Meehan CJ, Moris P, Kohl TA, Pečerska J, Akter S, Merker M, Utpatel C, Beckert P, Gehre F, Lempens P, Stadler T, Kaswa MK, Kühnert D, Niemann S, de Jong BC (2018) The relationship between transmission time and clustering methods in *Mycobacterium tuberculosis* epidemiology. EBioMedicine 37:410–416. https://doi.org/10.1016/j.ebiom.2018.10.013
45. Cohen KA, Manson AL, Desjardins CA, Abeel T, Earl AM (2019) Deciphering drug resistance in *Mycobacterium tuberculosis* using whole-genome sequencing: progress, promise, and challenges. Genome Med BioMed Central Ltd. 1–18. https://doi.org/10.1186/s13073-019-0660-8

46. Coll F, McNerney R, Preston MD, Guerra-Assunção JA, Warry A, Hill-Cawthorne G, Mallard K, Nair M, Miranda A, Alves A, Perdigão J, Viveiros M, Portugal I, Hasan Z, Hasan R, Glynn JR, Martin N, Pain A, Clark TG (2015) Rapid determination of anti-tuberculosis drug resistance from whole-genome sequences. Genome Med 7:1–10. https://doi.org/10.1186/s13073-015-0164-0
47. Walker TM, Kohl TA, Omar SV, Hedge J, Del Ojo Elias C, Bradley P, Iqbal Z, Feuerriegel S, Niehaus KE, Wilson DJ, Clifton DA, Kapatai G, Ip CLC, Bowden R, Drobniewski FA, Allix-Béguec C, Gaudin C, Parkhill J, Diel R, Supply P, Crook DW, Smith EG, Walker AS, Ismail N, Niemann S, Peto TEA (2015) Modernizing medical microbiology (MMM) informatics group. Whole-genome sequencing for prediction of *Mycobacterium tuberculosis* drug susceptibility and resistance: a retrospective cohort study. Lancet Infect Dis 15:1193–1202. https://doi.org/10.1016/S1473-3099(15)00062-6
48. Sobkowiak B, Glynn JR, Houben RMGJ, Mallard K, Phelan JE, Guerra-Assunção JA, Banda L, Mzembe T, Viveiros M, McNerney R, Parkhill J, Crampin AC, Clark TG (2018) Identifying mixed *Mycobacterium tuberculosis* infections from whole genome sequence data. BMC Genomics 19:1–15. https://doi.org/10.1186/s12864-018-4988-z
49. Ford CB, Shah RR, Maeda MK, Gagneux S, Murray MB, Cohen T, Johnston JC, Gardy J, Lipsitch M, Fortune SM (2013) *Mycobacterium tuberculosis* mutation rate estimates from different lineages predict substantial differences in the emergence of drug-resistant tuberculosis. Nat Genet 45:784–790. https://doi.org/10.1038/ng.2656
50. O'Reilly LM, Daborn CJ (1995) The epidemiology of *Mycobacterium bovis* infections in animals and man: a review. Tuber Lung Dis 76:1–46. https://doi.org/10.1016/0962-8479(95)90591-X
51. Soares P, Rito T, Pereira L, Richards MB (2016) A genetic perspective on African prehistory. In: Vertebrate paleobiology and paleoanthropology. Springer, pp 383–405. https://doi.org/10.1007/978-94-017-7520-5_18
52. Rito T, Vieira D, Silva M, Conde-Sousa E, Pereira L, Mellars P, Richards MB, Soares P (2019) A dispersal of Homo sapiens from southern to eastern Africa immediately preceded the out-of-Africa migration. Sci Rep 9:1–10. https://doi.org/10.1038/s41598-019-41176-3
53. Menardo F, Duchêne S, Brites D, Gagneux S (2019) The molecular clock of *Mycobacterium tuberculosis*. Biek R (ed) PLOS Pathog 15:e1008067. https://doi.org/10.1371/journal.ppat.1008067
54. Richards MB, Soares P, Torroni A (2016) Palaeogenomics: mitogenomes and migrations in Europe's past. Curr Biol Cell Press R243–R246. https://doi.org/10.1016/j.cub.2016.01.044
55. Gagneux S (2012) Host-pathogen co-evolution in human tuberculosis. Philos Trans Royal Soc B: Biol Sci Royal Society 850–859. https://doi.org/10.1098/rstb.2011.0316
56. Mikheecheva NE, Zaychikova MV, Melerzanov AV, Danilenko VN (2017) A nonsynonymous SNP catalog of *Mycobacterium tuberculosis* virulence genes and its use for detecting new potentially virulent sublineages. Genome Biol Evol 9:887–899. https://doi.org/10.1093/gbe/evx053
57. Kerner G, Laval G, Patin E, Boisson-Dupuis S, Abel L, Casanova JL, Quintana-Murci L (2021) Human ancient DNA analyses reveal the high burden of tuberculosis in Europeans over the last 2000 years. Am J Hum Genet 108:517–524. https://doi.org/10.1016/j.ajhg.2021.02.009
58. Rito T, Ferreira J, Cavadas B, Soares P, Oliveira O, Richards MB, Duarte R, Pereira L, Correia-Neves M (2019) Association of leukotriene A4 hydrolase with tuberculosis susceptibility using genomic data in Portugal. Microorganisms 7:650. https://doi.org/10.3390/microorganisms7120650
59. Möller M, Kinnear CJ (2020) Human global and population-specific genetic susceptibility to *Mycobacterium tuberculosis* infection and disease. Curr Opin Pulm Med Lippincott Williams and Wilkins 302–310. https://doi.org/10.1097/MCP.0000000000000672
60. Miao R, Ge H, Xu L, Sun Z, Li C, Wang R, Ding S, Yang C, Xu F (2016) Genetic variants at 18q11.2 and 8q24 identified by genome-wide association studies were not associated with

pulmonary tuberculosis risk in Chinese population. Infect Genet Evol 40:214–218. https://doi.org/10.1016/j.meegid.2016.03.005
61. Rolandelli A, Pellegrini JM, Hernández Del Pino RE, Tateosian NL, Amiano NO, Morelli MP, Castello FA, Casco N, Levi A, Palmero DJ, García VE (2019) The non-synonymous rs763780 single-nucleotide polymorphism in IL17F gene is associated with susceptibility to tuberculosis and advanced disease severity in Argentina. Front Immunol 10:2248. https://doi.org/10.3389/fimmu.2019.02248
62. Loiseau C, Menardo F, Aseffa A, Hailu E, Gumi B, Ameni G, Berg S, Rigouts L, Robbe-Austerman S, Zinsstag J, Gagneux S, Brites D (2020) An African origin for *Mycobacterium bovis*. Evol Med Public Heal 2020:49–59. https://doi.org/10.1093/emph/eoaa005
63. Wirth T, Hildebrand F, Allix-Béguec C, Wölbeling F, Kubica T, Kremer K, van Soolingen D, Rüsch-Gerdes S, Locht C, Brisse S, Meyer A, Supply P, Niemann S (2008) Origin, spread and demography of the *Mycobacterium tuberculosis* complex. Achtman M (ed) PLoS Pathog 4: e1000160. https://doi.org/10.1371/journal.ppat.1000160

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Animal Tuberculosis: Gross Lesions and Anatomopathological Diagnosis

42

Hélder Quintas, Justina Prada, Maria da Conceição Fontes, Ana Cláudia Coelho, and Isabel Pires

Restart… If you can. Without anguish. And without haste. And the steps you take, On this hard path. Of the future. Give them with freedom. Until you get there. Don't rest. Of no fruit be pleased with only half …

Miguel Torga

Summary

Animal tuberculosis (TB) is a zoonotic disease with worldwide distribution that can cause serious animal infections with economic and public health concerns. In its turn, human TB is currently one of the leading causes of death in the world

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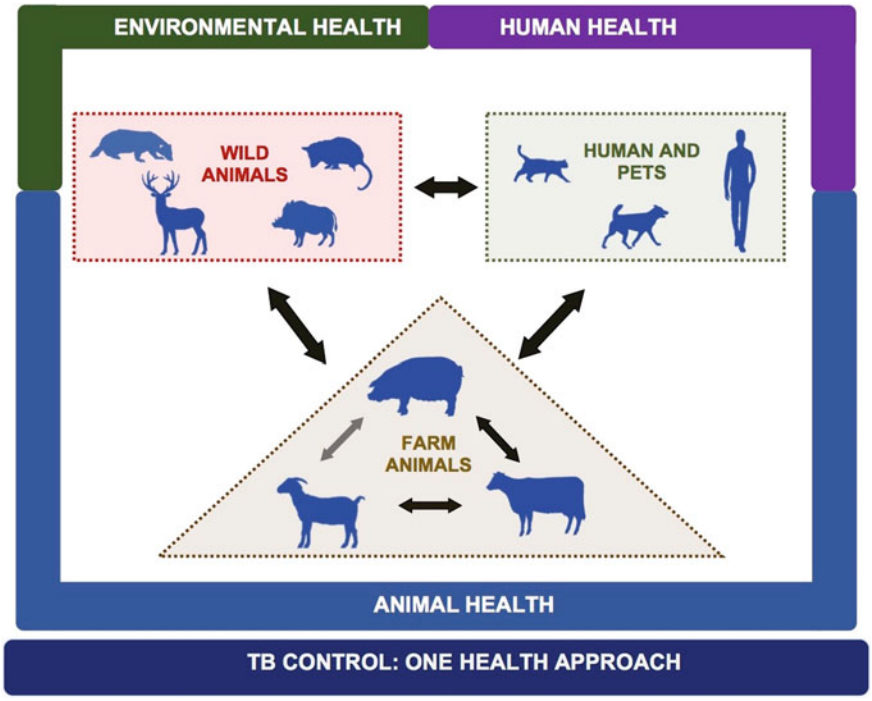
due to a curable disease. Although the diagnosis demands microbiological culture confirmation, anatomopathological diagnosis of suggestive lesions often provides a presumptive diagnosis of TB and is used by veterinary professionals as a central tool, either through monitoring at slaughter or when and where applicable, in clinical practice. Throughout this chapter, the anatomopathological diagnosis of animal TB will be reviewed, underling the peculiarities and similarities of TB lesions in ruminants, dogs, cats, pigs, and horses. Additionally, the importance of TB anatomopathological diagnosis in meat inspection is discussed. The last aim of this chapter is to emphasize that veterinarians and their teams, whether clinicians, pathologists, microbiologists, epidemiologists, anatomopathologists, and meat inspectors, have a central role in TB control and eradication. Only with the involvement of multidisciplinary teams of veterinary and human health professionals will it be possible to effectively combat animal and human TB in an integrated "One Health" perspective.

Graphical Abstract



A "One Health" approach, with a close link between human, veterinary, and environmental health teams: A key to effective tuberculosis (TB) control

Keywords

Anatomopathological diagnosis · Meat inspection · *Mycobacterium bovis* · *Mycobacterium caprae* · One Health · Tuberculosis

1 Introduction

Zoonotic diseases are responsible for 60.3% of emerging infectious diseases, and 71.8% of these are of wildlife origin. Diseases that spread between humans and animals, including wildlife, are extremely important and deserve special attention from the public and veterinary health systems [1, 2].

Tuberculosis (TB) is a serious re-emerging disease and undoubtedly a relevant health problem in animals and humans caused by several members of the *Mycobacterium tuberculosis (M. tb)* complex [3]. A particularly important zoonosis is human or zoonotic TB due to *Mycobacterium bovis (M. bovis)* [4]. This disease is becoming increasingly important in developing countries, where humans and animals share the same environment [5]. *M. bovis* infection has been reported from a multiplicity of domestic and wildlife species. The closeness between infected animals and humans and the practice of consuming unpasteurized milk, raw meat, and other undercooked meat products are important predisposing factors [3, 6]. Human-to-human transmission of *M. bovis* is infrequent but has been observed, mainly associated with immunodeficiency disorders [7]. *Mycobacterium caprae (M. caprae)* has also been identified as the etiological agent of TB in pets, wildlife, and humans [6].

2 The Anatomopathological Diagnosis of Animal Tuberculosis

The identification of TB-compatible lesions is essential for the diagnosis of the disease. Based on a presumptive diagnosis, it is possible to collect material for exams to confirm or exclude TB diagnosis and, if applicable, to take measures to prevent its spread [8]. This disease must always be seen from a "One Health" perspective from the first suspicion. The One Health concept can be understood as a transdisciplinary and collaborative attempt, carried out by teams working in public health, food safety, human health, animal health, social science, health economics, and ecosystem health, working both locally and globally, with the aim of achieving most favorable health for our environment, animals, and people [9–11]. It is noted that One Health owns up that domestic animal, wildlife, and human health are interrelated within the context of ecosystem health and produces a useful theoretical framework for the deployment of effective solutions to universal health challenges and safeguarding natural resources [10, 12, 13].

The anatomopathological diagnosis of animal TB could be challenging to the veterinary, both in clinical practice and in meat inspection activity. Morphological features are variable, with different types of lesions in different species. The spectrum of TB lesions is also linked to the method of disease transmission (route of infection), the etiological agent, infection mechanisms, the animal state of health (that modulate the immune system's intensity and adequacy of reaction), the influence of the type of tissue or organ affected, and the existence of reinfection [14–17].

The major transmission routes are aerogenous, digestive, transplacental, and cutaneous. In general, the most common route in animals is via the aerogenous one. The digestive transmission commonly implies more bacilli to cause disease, with lesions mainly located in the intestine and mesenteric lymph nodes. Transplacental transmission (usually associated with TB lesions of the progenitor endometrium) leads to hepatic and portal lymph nodes lesions in the fetus. Cases of iatrogenic, cutaneous, and genital transmission are also reported [14, 18–20].

When the animal first comes into contact with the TB agent, primary TB appears. The primary complex is defined as the affected organ and its draining lymph node. Typically, the lesions are proliferative. Post-primary TB follows when there is reactivation or reinfection, and lesions are predominantly exudative. Gross lesions in primary or post-primary lesions can be quite heterogeneous, even in the same species, since different organs may be affected. Moreover, the post-primary lesions show different aspects, depending on the evolutionary phase of the disease. Generally, standard TB lesions vary between small pale foci, sometimes encapsulated, with central calcification, to extensive foci of caseous necrosis, which can undergo liquefaction [8, 14, 21–23]. Microscopically, the typical lesion is the tubercle granuloma with caseous necrosis in the central area, surrounded by multinucleated Langhans-type giant cells, epithelioid cells, and lymphocytes at the lesion periphery. A fibrous capsule could be noted [8, 24, 25].

Although the definitive diagnosis is by microbiological methods, histopathology can be a valuable aid in identifying acid-resistant bacilli with Zielh Nielsen stain [26, 27]. TB lesions have similarities in the different species (Figs. 1 and 2); nevertheless, there are peculiarities [17, 28].

2.1 Tuberculosis in Ruminants

Bovine, caprine, and ovine TB are mainly caused by *M. bovis* and *M. caprae*. However, cases of infection by *M. tb* have been described. Lesions are characteristic, as described above [28, 29].

In adult cattle, primary lesions are observed in the lung and mediastinal lymph nodes. The infection occurs mainly through the digestive tract in calves, and lesions are observed in bowel mucosa [30]. In small ruminants, injuries are more frequent in the respiratory tract. However, the generalization of the process can occur, and lesions develop in different organs [21, 31]. Table 1 summarizes the lesional features of ruminant TB.

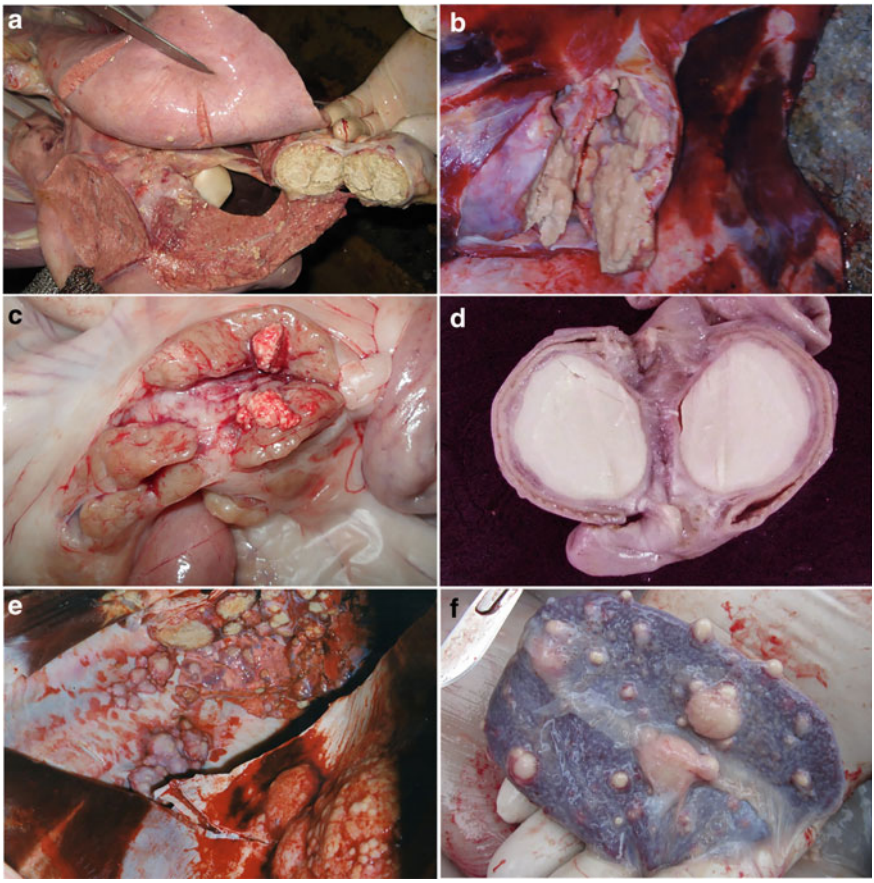


Fig. 1 Tuberculosis compatible lesions in different species: **a** goat, pulmonary primary complex; **b** cow, prescapular lymph node; **c** pig; mesenteric lymph node; **d** red deer, mesenteric lymph node; **e** cow, serosal lesions; **f** goat, spleen. (Fig. 1c *adapted with permission from the courtesy of Carla Miranda*)

The pulmonary tissue is the most frequently affected. Usually, in primary complex lesions, the lungs present pale nodules with caseous necrosis. In contrast, post-primary lesions, in general, show miliary nodules, extensive granulomas, and/or areas of coalescent caseification with mollification of the caseous leading to collapse of the bronchial walls and cave formation. Lymph nodes often present caseification lesions with necrosis, calcification, and fibrosis phenomena. Primary complex lesions in hepatic tissue are frequently incomplete. In generalized processes, other organs may present several granulomatous lesions due to bacillus dissemination. In the liver, lesions range from miliary to large nodules lesions. Serous membranes can present granulomatous serositis, with umpteen granulomas of different sizes spread by serosa, also known as "pearl tuberculosis." Peyer's

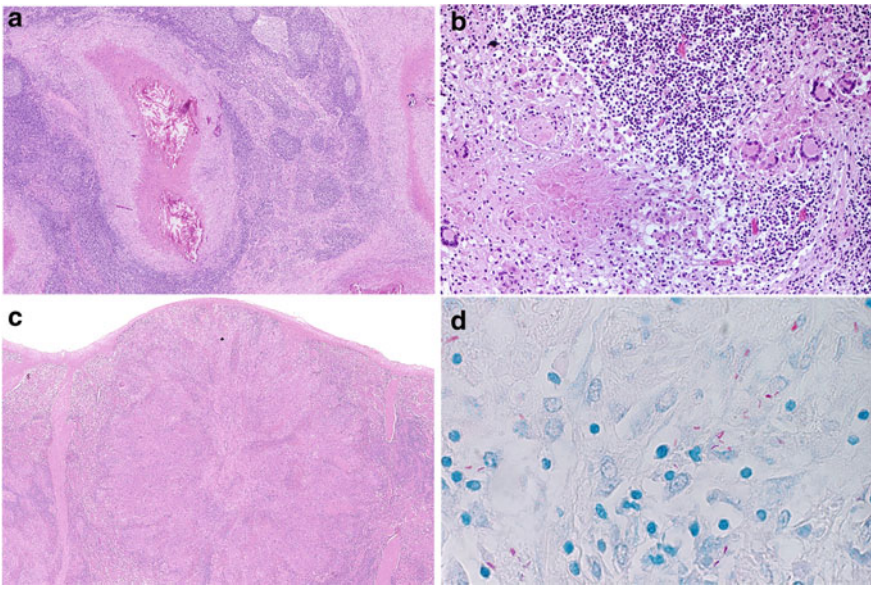


Fig. 2 Microscopic tuberculosis compatible lesions: **a** cow, lymph node granulomas (H&E, 40x); **b** goat; lymph node (note the abundant Langerhans cells, H&E, 200x); **c** pig, spleen (H&E, 40x); **d** goat, liver; alcool acid-resistant bacilli (Zielh-Nielsen, 1000x)

Table 1 Lesion features of caprine and bovine tuberculosis(*Prepared with data from* [21, 24])

Tuberculosis				
Primary	Complex	Complete	Proliferative lesions	Predominant cellular immune response
		Incomplete		
	Early generalization	Miliary		
		Slow-early		
		Large nodule		Predominant type IV hypersensitivity reactions (delayed hypersensitivity)
		Serosas		
Post Primary	Organic–chronic	Acinous–nodular	Proliferative–exsudative lesions	
		Cavernae		
	Late generalization	Caseous lobular pneumonia	Exsudative lesions	

plaques and lymphoid tissue may show solid and dense plaques, miliary lesions, or caseous ulcers. The forestomachs and abomasum only occasionally present lesions [21, 25, 32].

Microscopically, TB-compatible lesions have different aspects. According to lesion morphology and prominent components, some authors have classified lesions into proliferative, exudative, or both [21, 33] (Table 2). In other ruminant species

Table 2 Microscopic lesions in ruminant tuberculosis

Proliferative lesions	Proliferative-exsudatives lesions	Exsudatives lesions
✓ Small diameter granulomas with a reduced amount of central necrosis due to secondary calcification ✓ Calcified granulomas surrounded by epithelioid cells and some giant Langham cells, lymphocytes, and a conjunctive capsule, whose degree of development depends on the disease stage ✓ Reduced number of bacilli	✓ Extensive calcification necrotic nodules sometimes calcified ✓ These masses are increased by the inclusion of small peripheral granulomas, constituted by a diffuse infiltrate of epithelioid cells, giant Langham cells and lymphocytes, surrounded by a proteinaceous matrix (coagulated plasma)	✓ Primary caseification necrosis ✓ Large areas of the pulmonary parenchyma with necrosis, without calcification, surrounded by a specific or nonspecific cellular infiltrate, and large areas with coagulated plasma ✓ Pattern of fibrinous pneumonia ✓ A large number of bacilli in intracellular and extracellular locations

(*Prepared with data from* [21, 24])

such as red deer (*Cervus elaphus elaphus*), fallow deer (*Dama dama*), and elk (*Cervus elaphus nelsoni*), the gross features of the lesions are different from cattle. Suppurative inflammation may be evident; calcification is variable, while giant cells and fibrosis are discrete compared to cattle lesions. Sika deer (*C. nippon*) lesions are non-encapsulated with aggregates of epithelioid macrophages and giant cells in high number, with an irregular shape, scarce necrosis and calcification, and minimal neutrophils [14, 34, 35].

2.2 Tuberculosis in Dogs and Cats

TB in naturally infected pets is more often a subclinical disease. The etiological agents implied are not only *M. tb* but also *M. bovis* and *M. avium* [36–38]. In cats, *M. tb is rarely found. M. microti* and *M. bovis* are more frequent etiological agents in this species [39].

The most frequently affected sites in dogs with clinical signs are lungs and regional lymph nodes [40]. However, there are rare cases of disseminated TB reported [36, 41]. In cats, the skin is usually affected, mostly in the head. One or several lesions may have been found, occasionally ulcerated or discharged. Lymph nodes, mostly submandibular, are commonly involved. Pulmonary, abdominal, and systemic involvement are uncommon [42–44].

Typical TB granulomas are rare in dogs and cats. When present, granulomas are discrete, with epithelioid cells, some agglomerations of plasma cells and lymphocytes, and fibroblasts at the periphery. Larger granulomas exhibit necrosis at the center of the lesion. A granulomatous lesion with randomly scattered macrophages and rare large cells is the most common kind of lesion [14, 36].

2.3 Tuberculosis in Pigs

Pigs are susceptible to *M. bovis* [45], *M. avium* [46, 47], and *M. tb* [48]. TB infection occurs in domestic pigs, wild boars, and feral pigs. Pigs are considered spill-over hosts rather than sources of infection [39, 49]. The oral route is the most frequent route of infection. Macroscopic lesions are variable, and their incidence increases with age, usually occurring in mandibular lymph nodes and extending to retropharyngeal and thoracic lymph nodes with generalization. The lesions include caseification, caseification and calcification, and miliary spots [50, 51]. Fibrous encapsulated granulomas with central necrosis with different degrees of calcification are the most frequent lesions observed. Granulomas without peripheral fibroplasia are also common [52].

2.4 Tuberculosis in Horses

Horses are considered resistant to TB. However, there are occasional cases of *M. bovis* [53], *M. avium* [54], and *M. tb* infection [55]. Although respiratory transmission is to be considered, the main transmission route in horses is digestive. Thus, the primary lesions occur within the mesenteric lymph nodes. The intestine, other lymph nodes, lung, liver, spleen, and kidney can then be affected with miliary or nodular lesions [54, 56–58]. Although less frequent, lesions on the skin and eyes can also occur [59, 60]. The lesions exhibit necrosis in advanced cases with a lardaceous appearance. The microscopic lesion presents a granulomatous cell infiltration comprising epithelioid macrophages, plasma cells, multinucleated giant cells, and fibroblasts. Caseous necrosis does not occur or is minimal [39, 61].

3 The Importance of Tuberculosis Anatomopathological Diagnosis in Meat Inspection

Meat inspection's ultimate objective is to safeguard consumers' health, detecting and preventing hazards (physical, biological) in meat and allowing only meat that meets the proper hygienic and nutritional characteristics to be declared proper for human consumption, thereby contributing to food safety [12, 62]. Furthermore, meat inspection contributes to animal disease surveillance, namely of emerging infectious and zoonotic diseases, providing useful information and data. Meat inspection allows the early detection of potential conditions that can influence public health in its aspects of human and animal health [63–65].

According to the European Union, the meat inspection system consists of three key components: inspection of all animals destined for human consumption, analysis of food chain information (FCI), and slaughterhouse monitoring (antemortem and post-mortem inspection). The antemortem and post-mortem meat inspection can improve from the adequate utilization of FCI since it can help them focus on animal and public health concerns [65].

Traditional meat inspection procedures include visualization, palpation, incision of organs and lymph nodes, and, when required, laboratory examination. However, Some limitations are associated with traditional health inspection, either in the occurrence of cross-contamination of carcasses or even in terms of detection of macroscopic lesions [65, 66].

Post-mortem inspection of carcasses and offal has a long history in detecting pathological findings in TB. However, a visual-only inspection reduces detection effectiveness in TB and would have an overall negative impact on surveillance programs, especially in countries officially free of bovine TB [65].

The primary method of TB control is test-and-slaughter; however, it has faced increasing resistance and disapproval from public opinion. Besides that, in developing countries, for social and economic reasons, this method is often untenable and, therefore, alternative control methods are needed [67]. Some major weaknesses found in these nations include a lack of sustainable disease prevention and eradication initiatives, such as periodic herd testing through tuberculin tests, an efficient test and slaughter policy, corpse condemnation at abattoirs, and a thorough compensation plan. In some of these countries, where the disease is endemic, bovine TB detection occurs only by observing macroscopic lesions of slaughter animals instead of surveillance focused on the One Health paradigm [2]. In the developing world, a recognized problem is the lack of appropriate facilities and trained veterinarians to offer qualified meat inspection services and standards [69].

Inspection of animals in slaughter is more critical in preventing zoonotic TB in developing countries than in developed ones. The antemortem inspection helps in identifying animals that can be infected, and post-mortem inspection detects gross lesions which indicate meat is not desirable for human consumption [68], removing contaminated meat from the food chain and the traceback of animals to potentially infected herds of origin [69]. It is unquestionable that the sanitary inspection of cattle with TB provides important information about the occurrence of TB in a given region and, as such, the likelihood of public health being compromised through the consumption of contaminated meat [69].

The approach of One Health in anatomopathological diagnosis in meat inspection means that TB is not solely a matter of end-product testing or inspection at slaughterhouse but a system that includes the entire chain from farm-to-fork. This paradigm applied to food safety needs interaction among all participants. Any findings during the inspection procedures performed at the slaughterhouse are reported back to the farm and to the veterinarian field and thus can be addressed at the farm level (Regulation (EC) 178/2002; [70]). In the One Health concept, collecting epidemiological data on the state of bovine TB from slaughterhouses and using mathematical models may help and enhance understanding the epidemiology of bovine TB and the risk and preventative factors in animals and people [71, 72].

4 Conclusion

The anatomopathological diagnosis is essential for the diagnosis of animal TB. In this context, the work of clinical veterinarians, meat inspectors, and pathologists play a central role in the quick identification of lesions compatible with TB that microbiology laboratories can later confirm. Without underestimating the importance of TB in small animals, veterinarians of farm animals and meat inspectors should have special training in identifying and collecting lesions, especially in regions where animal TB has a worrying prevalence.

Once the disease is identified, the work of other professionals (i.e., veterinary epidemiologists, vet staff from official agencies, among others) will allow stipulating suitable measures to fight against TB. Regardless of their professional specialty and as a member of multidisciplinary teams, veterinary professionals must be an asset in this area. From a One Health perspective, a close link between human health and veterinary teams can be the key to effectively controlling this zoonotic disease.

Core Messages

- The anatomopathological diagnosis is essential for the diagnosis of animal TB.
- TB lesions have similarities in the different species; however, some peculiarities must be considered.
- Veterinarians (clinicians, anatomopathologists, meat inspectors, and microbiologists) must be trained in TB diagnosis.
- Only with multidisciplinary teams in a "One Health" approach, effective TB control plans can be developed.

References

1. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL et al (2008) Global trends in emerging infectious diseases. Nature 451(7181):990–993. https://doi.org/10.1038/nature06536
2. Mohamed A (2020) Bovine tuberculosis at the human-livestock-wildlife interface and its control through one health approach in the Ethiopian Somali Pastoralists: a review. One Health 9:100113. https://doi.org/10.1016/j.onehlt.2019.100113
3. Thoen CO, Kaplan B, Thoen TC, Gilsdorf MJ, Shere JA (2016) Zoonotic tuberculosis. A comprehensive ONE HEALTH approach. Medicina 76(3):159–165
4. Macedo Couto R, Ranzani OT, Waldman EA (2019) Zoonotic tuberculosis in humans: control, surveillance, and the one health approach. Epidemiol Rev 41(1):130–144. https://doi.org/10.1093/epirev/mxz002

5. Biru AAG, Sori T, Desissa F, Teklu A, Tafess K (2014) Epidemiology and public health significance of bovine tuberculosis in and around Sululta District. Central Ethiopia. Afr J Microbiol Res 8:2352–2358. https://doi.org/10.5897/AJMR2013.6325
6. Sternberg Lewerin S (2015) Tuberculosis and one health—what is in a name? Front Vet Sci 2:1–4. https://doi.org/10.3389/fvets.2015.00054
7. Perez-Lago L, Navarro Y, Garcia-de-Viedma D (2014) Current knowledge and pending challenges in zoonosis caused by *Mycobacterium bovis*: a review. Res Vet Sci 97(Suppl): S94–S100. https://doi.org/10.1016/j.rvsc.2013.11.008
8. Cunha MV, Monteiro M, Carvalho P, Mendonca P, Albuquerque T, Botelho A (2011) Multihost tuberculosis: insights from the Portuguese control program. Vet Med Int 2011:795165. https://doi.org/10.4061/2011/795165
9. Gibbs EP (2014) The evolution of one health: a decade of progress and challenges for the future. Vet Rec 174(4):85–91. https://doi.org/10.1136/vr.g143
10. Sleeman JM, DeLiberto T, Nguyen N (2017) Optimization of human, animal, and environmental health by using the one health approach. J Vet Sci 18(S1):263–268. https://doi.org/10.4142/jvs.2017.18.S1.263
11. Boqvist S, Soderqvist K, Vagsholm I (2018) Food safety challenges and one health within Europe. Acta Vet Scand 60(1):1. https://doi.org/10.1186/s13028-017-0355-3
12. Garcia SNOB, Jay-Russell MT (2020) One health for food safety, food security, and sustainable food production. Front Sustain Food Syst 4:1–9. https://doi.org/10.3389/fsufs.2020.00001
13. BM G (1991) Higiene y Inspeccion de Carnes, vol I. Graficas Celaryan S. A, Leon Spain
14. Caswell JL, Williams KJ (2016) Chapter 5 respiratory system. In: GRANT MAXIE M, Jubb KV, Kennedy PC, Palmer N (ed) Pathology of domestic animals, vol 2. Elsevier, Inc, pp 547–561
15. Quintas H Estudo de um foco de tuberculose em caprinos de Trás-os-Montes UTAD
16. Cassidy JP, Bryson DG, Pollock JM, Evans RT, Forster F, Neill SD (1998) Early lesion formation in cattle experimentally infected with *Mycobacterium bovis*. J Comp Pathol 119 (1):27–44. https://doi.org/10.1016/s0021-9975(98)80069-8
17. Pereira AC, Reis AC, Ramos B, Cunha MV (2020) Animal tuberculosis: Impact of disease heterogeneity in transmission, diagnosis and control. Transbound Emerg Dis. https://doi.org/10.1111/tbed.13539
18. Peart LK, Schneider JW, Jordaan HF, Wright CA (2005) Fine needle aspiration biopsy of postvaccination disseminated *Mycobacterium bovis* infection presenting as a solitary cutaneous papule. Acta Cytol 49(2):230–231
19. Mateos Colino A, Sousa Escandon MA, Golpe Gomez R, Garcia Figueras R, Perez Valcarcel J, Fernandez MA (2003) Tuberculous epididymitis caused by *Mycobacterium bovis*. Archivos espanoles de urologia 56(2):175–178
20. Rayner EL, Pearson GR, Hall GA, Gleeson F, McIntyre A, Smyth D et al (2015) Early lesions following aerosol challenge of rhesus macaques (Macaca mulatta) with *Mycobacterium tuberculosis* (Erdman strain). J Comp Pathol 152(2–3):217–226. https://doi.org/10.1016/j.jcpa.2014.10.002
21. Bernabé A, Gómez MA, Navarro JA, Gómez S, Sánchez J, Sidrach J, Menchen V, Vera A, Sierra MA (1990) Morphopathology of caprine tuberculosis. I. Pulmonary tuberculosis. Anales de Veterinaria de Murcia 9–20
22. Hunter RL (2011) Pathology of post primary tuberculosis of the lung: an illustrated critical review. Tuberculosis 91(6):497–509. https://doi.org/10.1016/j.tube.2011.03.007
23. Cassidy JP (2006) The pathogenesis and pathology of bovine tuberculosis with insights from studies of tuberculosis in humans and laboratory animal models. Vet Microbiol 112(2–4):151–161. https://doi.org/10.1016/j.vetmic.2005.11.031
24. Bernabe AG, Gomez MA, Navarro JA, Gomez S, Sánchez J, Sidrach J, Menchénc V (1991) Pathological changes of spontaneous dual infection of tuberculosis and paratuberculosis in goats. Small Ruminant Res 5(4):337–390. https://doi.org/10.1016/0921-4488(91)90075-2

25. Buendia AJ, Navarro JA, Salinas J, McNair J, de Juan L, Ortega N et al (2013) Ante-mortem diagnosis of caprine tuberculosis in persistently infected herds: influence of lesion type on the sensitivity of diagnostic tests. Res Vet Sci 95(3):1107–1113. https://doi.org/10.1016/j.rvsc.2013.10.003
26. Ulrichs T, Lefmann M, Reich M, Morawietz L, Roth A, Brinkmann V et al (2005) Modified immunohistological staining allows detection of Ziehl-Neelsen-negative *Mycobacterium tuberculosis* organisms and their precise localization in human tissue. J Pathol 205(5):633–640. https://doi.org/10.1002/path.1728
27. Gutierrez Cancela MM, Garcia Marin JF (1993) Comparison of Ziehl-Neelsen staining and immunohistochemistry for the detection of *Mycobacterium bovis* in bovine and caprine tuberculous lesions. J Comp Pathol 109(4):361–370. https://doi.org/10.1016/s0021-9975(08)80299-x
28. Gutierrez M, Samper S, Jimenez MS, van Embden JD, Marin JF, Martin C (1997) Identification by spoligotyping of a caprine genotype in *Mycobacterium bovis* strains causing human tuberculosis. J Clin Microbiol 35(12):3328–3330. https://doi.org/10.1128/JCM.35.12.3328-3330.1997
29. Sanchez J, Tomas L, Ortega N, Buendia AJ, del Rio L, Salinas J et al (2011) Microscopical and immunological features of tuberculoid granulomata and cavitary pulmonary tuberculosis in naturally infected goats. J Comp Pathol 145(2–3):107–117. https://doi.org/10.1016/j.jcpa.2010.12.006
30. Domingo M, Vidal E, Marco A (2014) Pathology of bovine tuberculosis. Res Vet Sci 97(Suppl):S20–29. https://doi.org/10.1016/j.rvsc.2014.03.017
31. Luboya LW, Malangu M, Kaleka M, Ngulu N, Nkokele B, Maryabo K et al (2017) An assessment of caprine tuberculosis prevalence in Lubumbashi slaughterhouse, Democratic Republic of Congo. Trop Anim Health Prod 49(4):875–878. https://doi.org/10.1007/s11250-017-1252-5
32. Momotani E, Yoshino T (1984) Pathological changes of spontaneous dual infection of tuberculosis and paratuberculosis in beef cattle. Nihon juigaku zasshi Japan J Vet Sci 46(5):625–631. https://doi.org/10.1292/jvms1939.46.625
33. Liebana E, Johnson L, Gough J, Durr P, Jahans K, Clifton-Hadley R et al (2008) Pathology of naturally occurring bovine tuberculosis in England and Wales. Vet J 176(3):354–360. https://doi.org/10.1016/j.tvjl.2007.07.001
34. Rhyan JC, Saari DA (1995) A comparative study of the histopathologic features of bovine tuberculosis in cattle, fallow deer (Dama dama), sika deer (Cervus nippon), and red deer and elk (Cervus elaphus). Vet Pathol 32(3):215–220. https://doi.org/10.1177/030098589503200301
35. Rhyan JC, Saari DA, Williams ES, Miller MW, Davis AJ, Wilson AJ (1992) Gross and microscopic lesions of naturally occurring tuberculosis in a captive herd of wapiti (Cervus elaphus nelsoni) in Colorado. J Vet Diagn Invest: Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc 4(4):428–433. https://doi.org/10.1177/104063879200400411
36. Martinho AP, Franco MM, Ribeiro MG, Perrotti IB, Mangia SH, Megid J et al (2013) Disseminated *Mycobacterium tuberculosis* infection in a dog. Am J Trop Med Hyg 88(3):596–600. https://doi.org/10.4269/ajtmh.12-0332
37. Horn B, Forshaw D, Cousins D, Irwin PJ (2000) Disseminated Mycobacterium avium infection in a dog with chronic diarrhoea. Aust Vet J 78(5):320–325. https://doi.org/10.1111/j.1751-0813.2000.tb11781.x
38. Megid J, Bracarense AP, dos Reis AC, Sturion DJ, Martin LM, Pinheiro SR (1994) Canine tuberculosis and its importance in public health. Rev Saude Publica 28(4):309–310. https://doi.org/10.1590/s0034-89101994000400011
39. Pesciaroli M, Alvarez J, Boniotti MB, Cagiola M, Di Marco V, Marianelli C et al (2014) Tuberculosis in domestic animal species. Res Vet Sci 97(Suppl):S78-85. https://doi.org/10.1016/j.rvsc.2014.05.015

40. Parsons SD, Gous TA, Warren RM, van Helden PD (2008) Pulmonary *Mycobacterium tuberculosis* (Beijing strain) infection in a stray dog. J S Afr Vet Assoc 79(2):95–98. https://doi.org/10.4102/jsava.v79i2.252
41. Szalus-Jordanow O, Augustynowicz-Kopec E, Czopowicz M, Olkowski A, Lobaczewski A, Rzewuska M et al (2016) Intracardiac tuberculomas caused by *Mycobacterium tuberculosis* in a dog. BMC Vet Res 12(1):109. https://doi.org/10.1186/s12917-016-0731-7
42. Broughan JM, Downs SH, Crawshaw TR, Upton PA, Brewer J, Clifton-Hadley RS (2013) *Mycobacterium bovis* infections in domesticated non-bovine mammalian species. Part 1: review of epidemiology and laboratory submissions in Great Britain 2004–2010. Vet J 198 (2):339–345. https://doi.org/10.1016/j.tvjl.2013.09.006
43. Fitzgerald SD, Hollinger C, Mullaney TP, Bruning-Fann CS, Tilden J, Smith R et al (2016) Herd outbreak of bovine tuberculosis illustrates that route of infection correlates with anatomic distribution of lesions in cattle and cats. J Vet Diagnostic Invest: Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc 28(2):129–132. https://doi.org/10.1177/1040638715626484
44. Eroksuz Y, Baydar E, Otlu B, Dabak M, Eroksuz H, Karabulut B et al (2019) Case report: systemic tuberculosis caused by *Mycobacterium bovis* in a cat. BMC Vet Res 15(1):9. https://doi.org/10.1186/s12917-018-1759-7
45. Nugent G, Gortazar C, Knowles G (2015) The epidemiology of *Mycobacterium bovis* in wild deer and feral pigs and their roles in the establishment and spread of bovine tuberculosis in New Zealand wildlife. NZ Vet J 63(Suppl 1):54–67. https://doi.org/10.1080/00480169.2014.963792
46. Domingos M, Amado A, Botelho A (2009) IS1245 RFLP analysis of strains of Mycobacterium avium subspecies hominissuis isolated from pigs with tuberculosis lymphadenitis in Portugal. Vet Rec 164(4):116–120. https://doi.org/10.1136/vr.164.4.116
47. Miranda C, Matos M, Pires I, Correia-Neves M, Ribeiro P, Alvares S et al (2012) Diagnosis of Mycobacterium avium complex in granulomatous lymphadenitis in slaughtered domestic pigs. J Comp Pathol 147(4):401–405. https://doi.org/10.1016/j.jcpa.2012.05.005
48. Arega SM, Conraths FJ, Ameni G (2013) Prevalence of tuberculosis in pigs slaughtered at two abattoirs in Ethiopia and molecular characterization of *Mycobacterium tuberculosis* isolated from tuberculous-like lesions in pigs. BMC Vet Res 9:97. https://doi.org/10.1186/1746-6148-9-97
49. Vicente J, Hofle U, Garrido JM, Fernandez-De-Mera IG, Juste R, Barral M et al (2006) Wild boar and red deer display high prevalences of tuberculosis-like lesions in Spain. Vet Res 37 (1):107–119. https://doi.org/10.1051/vetres:2005044
50. Corner LA, Barrett RH, Lepper AW, Lewis V, Pearson CW (1981) A survey of mycobacteriosis of feral pigs in the Northern Territory. Aust Vet J 57(12):537–542. https://doi.org/10.1111/j.1751-0813.1981.tb00428.x
51. Di Marco V, Mazzone P, Capucchio MT, Boniotti MB, Aronica V, Russo M et al (2012) Epidemiological significance of the domestic black pig (Sus scrofa) in maintenance of bovine tuberculosis in Sicily. J Clin Microbiol 50(4):1209–1218. https://doi.org/10.1128/JCM.06544-11
52. Santos N, Correia-Neves M, Ghebremichael S, Kallenius G, Svenson SB, Almeida V (2009) Epidemiology of *Mycobacterium bovis* infection in wild boar (Sus scrofa) from Portugal. J Wildl Dis 45(4):1048–1061. https://doi.org/10.7589/0090-3558-45.4.1048
53. Keck N, Dutruel H, Smyej F, Nodet M, Boschiroli ML (2010) Tuberculosis due to *Mycobacterium bovis* in a Camargue horse. Vet Rec 166(16):499–500. https://doi.org/10.1136/vr.b4785
54. Lofstedt J, Jakowski RM (1989) Diagnosis of avian tuberculosis in a horse by use of liver biopsy. J Am Vet Med Assoc 194(2):260–262
55. Lyashchenko KP, Greenwald R, Esfandiari J, Lecu A, Waters WR, Posthaus H et al (2012) Pulmonary disease due to *Mycobacterium tuberculosis* in a horse: zoonotic concerns and limitations of antemortem testing. Vet Med Int 2012:642145. https://doi.org/10.1155/2012/642145

56. Milligan ET (1929) Two cases of tuberculosis of horse-shoe kidney. Proc R Soc Med 22 (10):1379–1380
57. Hlokwe TM, Sutton D, Page P, Michel AL (2016) Isolation and molecular characterization of *Mycobacterium bovis* causing pulmonary tuberculosis and epistaxis in a Thoroughbred horse. BMC Vet Res 12(1):179. https://doi.org/10.1186/s12917-016-0813-6
58. Mair TS, Taylor FG, Gibbs C, Lucke VM (1986) Generalized avian tuberculosis in a horse. Equine Vet J 18(3):226–230. https://doi.org/10.1111/j.2042-3306.1986.tb03607.x
59. Leifsson PS, Olsen SN, Larsen S (1997) Ocular tuberculosis in a horse. Vet Rec 141(25):651–654
60. Flores JM, Sanchez J, Castano M (1991) Avian tuberculosis dermatitis in a young horse. Vet Rec 128(17):407–408. https://doi.org/10.1136/vr.128.17.407
61. Monreal L, Segura D, Segales J, Garrido JM, Prades M (2001) Diagnosis of *Mycobacterium bovis* infection in a mare. Vet Rec 149(23):712–714
62. Vidal E, Tolosa E, Espinar S, de Val BP, Nofrarias M, Alba A et al (2016) Six-Year follow-up of slaughterhouse surveillance (2008–2013): the Catalan slaughterhouse support network (SESC). Vet Pathol 53(3):532–544. https://doi.org/10.1177/0300985815593125
63. Alton GD, Pearl DL, Bateman KG, McNab WB, Berke O (2010) Factors associated with whole carcass condemnation rates in provincially-inspected abattoirs in Ontario 2001–2007: implications for food animal syndromic surveillance. BMC Vet Res 6:42. https://doi.org/10.1186/1746-6148-6-42
64. Alton GD, Pearl DL, Bateman KG, McNab WB, Berke O (2012) Suitability of bovine portion condemnations at provincially-inspected abattoirs in Ontario Canada for food animal syndromic surveillance. BMC Vet Res 8:88. https://doi.org/10.1186/1746-6148-8-88
65. EFSA EPoBHB, on Contaminants in the Food Chain (CONTAM), and on Animal Health and Welfare (AHAW) (2013) Scientific opinion on the public health hazards to be covered by inspection of meat (bovine animals). EFSA J 11(6):3266. https://doi.org/10.2903/j.efsa.2013.3266
66. Collins DSHR (2015) Zoonotic tuberculosis in humans: control, surveillance, and the one health approach. Epidemiol Rev 41:130–144. https://doi.org/10.1093/epirev/mxz002
67. OIE (2019) Controlling bovine tuberculosis: a one health challenge. Bull Panorama 1:1–185
68. Musoke D, Ndejjo R, Atusingwize E, Halage AA (2016) The role of environmental health in one health: a Uganda perspective. One Health 2:157–160. https://doi.org/10.1016/j.onehlt.2016.10.003
69. WHO (2019) Key political commitment documents for tuberculosis prevention and care and their intended influence in the WHO European region analytical report. Technical report. Accessed 14 Aug 2020
70. Regulation (EC) No 178/2002 (consolidated text) of the European parliament and of the council laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJEC L31
71. Bach M, Jordan S, Hartung S, Santos-Hovener C, Wright MT (2017) Participatory epidemiology: the contribution of participatory research to epidemiology. Emerg Themes Epidemiol 14:2. https://doi.org/10.1186/s12982-017-0056-4
72. Nega MMH, Mekonen G (2012) Prevalence and zoonotic implications of bovine tuberculosis in Northwest Ethiopia. Int J Med Sci 2:188–192

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Estimation of Microbial Mutation Rates in Tuberculosis Research

43

Qi Zheng

Anybody can do it – after he has been shown how.

Christopher Columbus, as retold by James Baldwin

Summary

Antibiotic resistance is a dominant theme in tuberculosis research. Quantitative studies on microbial mutation rates play a key role in drug resistance research. Despite recent rapid advances in whole-genome sequencing, the classic Luria-Delbrük fluctuation test continues to be the choice of method for measuring microbial mutation rates. To help researchers new to this field, the author of this chapter provides detailed descriptions and practical guidelines pertaining to the proper use of this classic protocol. The discussion focuses on practical issues that are still bewildering to many tuberculosis researchers. Future developments in the field are discussed from a personal perspective.

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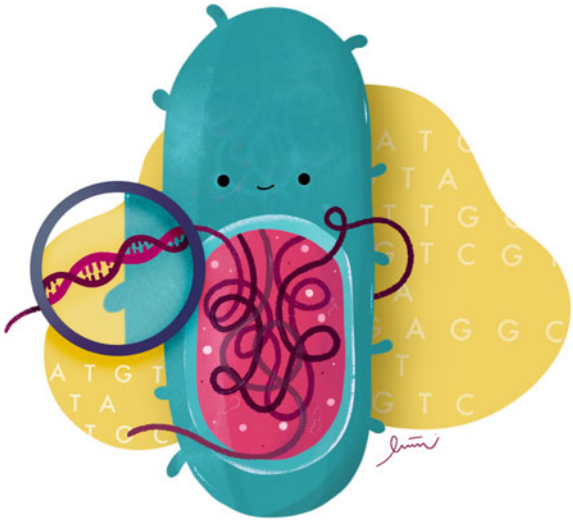
Graphical Abstract



Keywords

Antibiotic resistance · Likelihood-ratio test · Luria-Delbrük experiment · Mutant distribution · Mutation rate

1 Introduction

Microbial drug resistance is a dominant theme in today's tuberculosis research, and mutation is the underlying cause of microbial drug resistance. Experimental determination of microbial mutation rates therefore plays an integral role in tuberculosis research. This chapter aims to provide a historical overview of research on the determination of microbial mutation rates, focusing on theoretical developments pertaining to antibiotic resistance of *Mycobacterium tuberculosis*, the etiological organism of tuberculosis. The ultimate goal of this chapter is to help tuberculosis researchers better appreciate and utilize available data analytic tools that are vital in determining microbial mutation rates in their research. The information in this chapter will also help the reader see more clearly future directions in furthering quantitative understanding of drug resistance of *M. tuberculosis*.

It would seem impossible to pinpoint a year or an event that marks the beginning of experimental research conducted to measure mutation rates of *M. tuberculosis* to antibiotic resistance. However, it appears certain that such research began after 1943 when Luria and Delbrück published their pathbreaking paper [1] introducing a novel experimental protocol called the fluctuation test. The work of Luria and Delbrück was driven by a strong intellectual curiosity about whether beneficial mutations could

occur spontaneously, a topic hotly debated at the time. Luria discovered the fluctuation test serendipitously at a country club in Indiana, inspired by a mundane slot machine—but after months of intense thinking [2, p. 75]. However, it was Delbrück's work to put Luria's idea on a firm mathematical basis that gave birth to the modern concept of the mutation rate. Delbrück defined the mutation rate as the probability of mutation per cell division, or equivalently, the probability of mutation per bacterium per generation. Without this precise definition, the determination of microbial mutation rates would be an impossible or meaningless task. Ref. [3] recounts Delbrück's contribution in this regard.

The work of Luria and Delbrück focused on *Escherichia coli* cells, and the mutation of interest was that which rendered the bacteria resistant to certain bacteriophages. In the ensuing four year or so, a multitude of fluctuation tests were conducted by other microbiologists and by Luria himself. *E. coli* continued to be the favorite organism for fluctuation tests, partly due to its short life span and easy availability. But antibiotics quickly replaced bacteriophages, possibly because antibiotics were more effective or more manageable as selective agents. Antitubercular drugs were often chosen, but most investigators who chose those drugs did not seem to be directly interested in tuberculosis research.

The work by David [4] in 1970 is the first milestone in the study of mutation rates of *M. tuberculosis* to antibiotic resistance. David was the first to employ the fluctuation test to show that *M. tuberculosis* can acquire resistance to antitubercular drugs via spontaneous mutation, although he declared that this was not the impetus for his investigation. David was also the first to use the fluctuation test to determine in vitro mutation rates of *M. tuberculosis* to drug resistance and to express mutation rates explicitly in units of "mutations per bacterium per generation." David's work encompassed four antitubercular drugs, namely, isoniazid, streptomycin, rifampin, and ethambutol, but it involved only the H37Rv strain.

The investigation by Werngren and Hoffner [5] some 30 years later is the second milestone in the study of *M. tuberculosis* mutation rates. These two investigators took the fluctuation test not merely as a tool for measuring mutation rates, but used it as a novel tool to address a fundamental question: Do strains of the Beijing genotype acquire resistance to rifampin more rapidly than their non-Beijing cousins? They included 6 Beijing strains and 7 non-Beijing strains in their study. Their experiments did not yield evidence supporting the hypothesis that Beijing strains enjoyed elevated mutability. In 2013, Ford et al. [6] launched a similar investigation, but they reached a different conclusion. Their investigation involved 4 Beijing strains and 5 non-Beijing strains. They concluded that Beijing strains tend to exhibit higher mutation rates to resistance to rifampin than non-Beijing strains. In addition, they made a refreshing observation that *M. tuberculosis* mutates at a constant rate per unit time. (For conceptual aid, the reader may consult Ref. [3].) Efforts have been made to reconcile these two conclusions, but reconciliation between the two conclusions remains a stubborn open problem [7].

To understand and extend these and other related investigations, researchers new to this filed need to understand the Luria-Delbrück protocol and major theoretical developments associated with the fluctuation test.

2 Basics of the Fluctuation Experiment

The fluctuation experiment [1] was proposed in 1943 to resolve a theoretical controversy in evolutionary biology. Soon it was recognized as an effective experimental protocol for measuring microbial mutation rates in the laboratory. After almost eight decades of research and application, the fluctuation experiment is now widely regarded as the preferred method for estimating microbial mutation rates. The protocol is customarily called the fluctuation test due to its historical origin, but it is also commonly known as the Luria-Delbrück experiment, the fluctuation experiment or the fluctuation assay.

Figure 1 is a diagram of a fluctuation experiment. A fluctuation experiment comprises a number of similar liquid cultures, often contained in glass tubes and referred to as parallel cultures or sister cultures. The experimenter inoculates a small number (denoted by N_0) of wile-type bacterial cells (nonmutants) into each of the sister cultures and incubates the cultures for an appropriate period of time, e.g., an 8-hour period for *Escherichia coli* or a $3 \sim 4$ week period for *M. tuberculosis*. At the end of the incubation period, the total number of cells in each tube increases to N_t. The incubation period ideally should coincide with the logarithmic phase of the nonmutant cell population, which ensures that the nonmutants grow and divide unimpeded throughout the whole incubation period. With a small probability, each nonmutant cell division triggers a mutation of interest (see Fig. 2), and the resulting mutant would proliferate like its nonmutant companions. Because backward mutation is assumed to be negligible, all offspring of a mutant are mutants. At the end of the incubation period, the experimenter transfers either the entirety or a portion of the contents of each tube onto a solid culture (an agar plate) coated with a selective agent (e.g., an antibiotic) that kills wild-type cells but that allows drug-resistant mutant cells to grow and divide. This process is called plating. If the experimenter plates only a fraction of the liquid culture, the process is called partial plating, and the proportion plated (denoted by the symbol ϵ), is called the plating efficiency. Ideally, the selective agent should instantly kill the wild-type cells, and each mutant cell that has been transferred to the solid culture should form a visible colony on the surface of the solid culture.

What connection did Luria see between the above laboratory procedure and a slot machine? If no mutations occur in any of the tubes, one may or may not see mutant colonies on the plates. However, if mutant colonies do appear on any of the plates, each colony would indicate a mutation occurring after plating. The reason is that mutants descended from the same ancestral mutant would clump together to form a single colony, as these mutants cannot separate on the surface of a solid culture. On the other hand, when mutations occur in the tubes, all resultant mutants can move about freely in the liquid culture and hence they are likely to separate. As a result, mutants derived from the same mutation would form distinct colonies when transferred to an agar plate. That is, the number of colonies on a plate in the latter case indicates the number of mutants existing in the tube, provided that the selective agent is so lethal that mutations occurring after plating are negligible. Luria saw an exciting analogy between slot machine returns and the number of

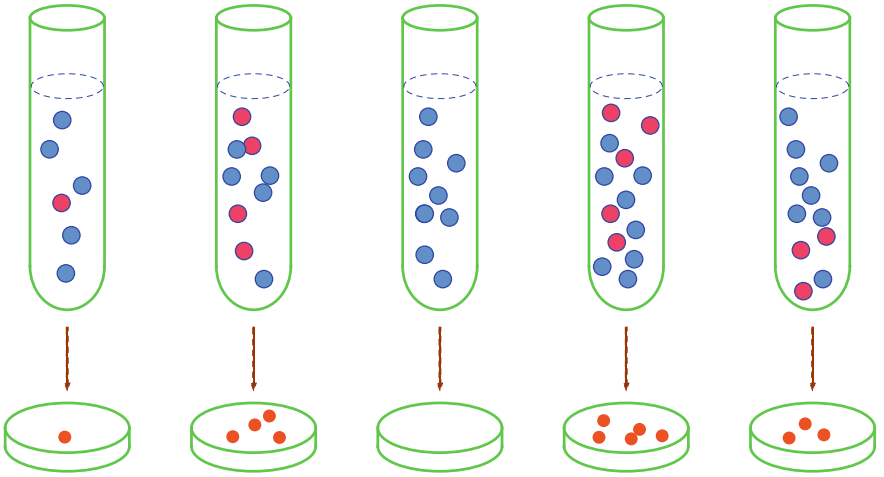


Fig. 1 Diagram illustrating the Luria-Delbrück protocol. A blue circle represents a nonmutant cell in each tube, while a red circle represents a mutant cell. Cells of either type in the tubes are invisible to the naked eye, but they are here enlarged to highlight the fundamental assumption that each mutant cell in a tube will form a visible colony on the corresponding plate after plating (given the plating efficiency is 100%)

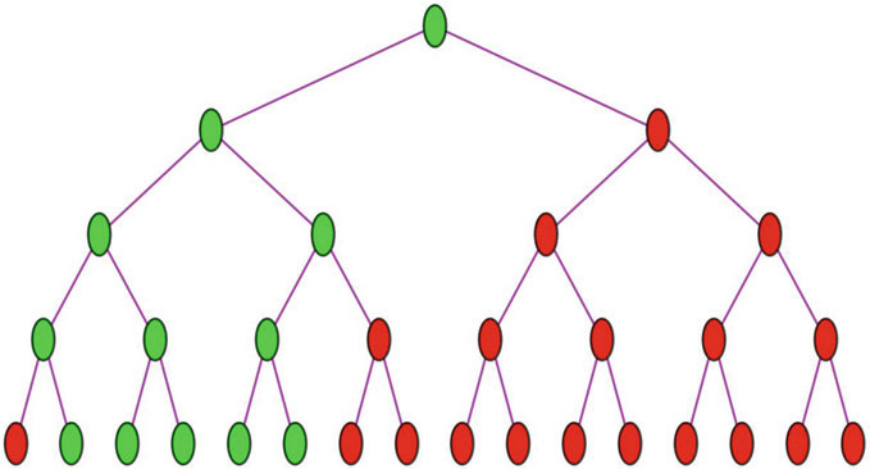


Fig. 2 Diagram showing a possible four-generation pedigree of a nonmutant cell (green) in a fluctuation experiment. Cells generally grow asynchronously, but synchronous growth is portrayed here to highlight the distinction between a mutation and a mutant. Three mutations occur, but they result in eleven mutants (red). Adapted from [70],

mutant colonies on each plate, because an early-occurring mutation would give a jackpot, not in terms of dollar values, but in terms of mutant colonies. This distinction was so fundamental that Zheng [8] dubbed it the mutation-mutant principle. The numbers of mutant colonies on the plates in Luria's experiments showed slot machine payout patterns, providing evidence supporting the random mutation hypothesis. The following question concretizes the random mutation hypothesis: Did *M. tuberculosis* cells ever acquire mutations that convey resistance to isoniazid before 1812, a century before the drug was first synthesized? [9, p. 2]. The random mutation hypothesis would give an affirmative answer to that question, while the alternative hypothesis would deny that possibility.

For nearly three decades the tuberculosis research community remained impervious to Luria's idea, but it was surprisingly receptive to the view of random mutation just a few years after the publication of the Luria-Delbrück paper. Pyle [10] in 1947 reported that streptomycin-resistant *M. tuberculosis* cells were found in sputum cultures of tuberculosis patients prior to streptomycin treatment, and she concluded that "any large population of tubercle bacilli may be expected to contain organisms which are relatively resistant to streptomycin without having been exposed to the drug." Vennesland et al. [11] in 1947, and Yegian and Venderlinde [12] in 1948, took slightly different approaches, but reached similar conclusions. Science historians may puzzle over whether these investigators were somehow influenced by findings of Luria and Delbrück. Historians may also wonder why it took some 27 years for the fluctuation protocol to penetrate indefinable barriers to greet such an important organism as *M. tuberculosis* [4].

This chapter focuses on the estimation of rates at which random mutations occur in the tubes. (Some investigators also apply the fluctuation protocol to study antibiotic-induced mutations; see e.g., Ref. [13].) Note that the occurrence of mutation is not observable. Information available to the experimenter is the number of mutant colonies on each plate: $y_1, y_2, \ldots, y_n$, where n is the number of tubes in the experiment. The number of colonies appearing on a plate is either the same as the number of mutant cells prior to plating in the tube, or an ϵ-fraction random sample thereof. But it is not the same as the number of mutations (see Fig. 2). Fortunately, the mean number of mutations per culture, often denoted by the symbol m, can be inferred from the mutant count data $y_1, y_2, \ldots, y_n$, by means of an appropriately chosen mutant distribution. Once an estimate of m is available, the mutation rate is estimated by the relation

$$\mu = \frac{m}{N_t - N_0} \approx \frac{m}{N_t}. \tag{1}$$

3 Mutation Rate or Mutant Frequency?

David [4] introduced the concept of the mutation rate to tuberculosis research some half a century ago, but the tuberculosis research community as a whole is still slow in embracing the mutation rate as a fundamental concept in routine research. The

mutant frequency, mainly due to its computational simplicity, is still favored by some investigators [14,15]. Delbrück [16, p. 226] was the first to caution against indiscriminate use of the mutant frequency: "It is obvious that the fraction of mutants is a poor measure of the mutation rate, since the number of mutants depends on two factors, namely, the number of mutations occurring in the culture and when they occurred." Mutant frequencies in routine work are often referred to as mutation frequencies, a long-running practice that has helped blur the important distinction between the two concepts. To better appreciate this distinction, one should pay attention to how David [4] used these two concepts in reporting his pioneering work. David calculated mutant frequencies for all tubes, and he cited the wide fluctuation in the mutant frequencies as evidence to support the claim that drug resistant bacteria in his experiments occurred randomly. But David used the same mutant count data to calculate mutation rates in order to examine the organism's tendency to mutate.

Occasionally, some investigators used the two concepts interchangeably. For example, in a study comparing Beijing and non-Beijing genotype strains of *M. tuberculosis* [17], the authors mentioned "mutation rate" in the abstract, but used "mutation frequency" throughout the main text. That study, taking many peers by surprise, led to a debate [18,19], but none of the participants in the debate saw the unintentional mix of the two concepts as a source for confusion. The unexpectedly large fold changes in mutant frequencies would probably decrease considerably if fold changes in mutation rates were considered instead. In that investigation it would seem more appropriate to use mutation rates in place of mutant frequencies. It is helpful to keep in mind that the mutant frequency increases with the number of elapsed cell generations, as shown graphically in Ref. [20]. This should be another reason for caution in adopting the mutant frequency in an investigation.

4 Relevant Developments and Associated Formulas

Luria regarded mathematical humbug with aversion [2, p. 74]; thus an understanding of Luria's ideas underlying the fluctuation test requires no mathematics. However, to appreciate Delbrück's work on the estimation of microbial mutation rates demands considerable mathematics. Parkinson [21] passionately believed that Delbrück's mathematical work was unnecessary. In fact, Parkinson unintentionally blurred the lines between two kinds of contributions made by Luria and Delbruck. Mathematical models are indispensable in extracting mutation rates from fluctuation assay data. More mathematics is needed to better estimate mutation rates. The vast literature on various mathematical models associated with the Luria-Delbrück protocol, produced by researchers working in a multitude of disciplines in some 79 years, is understandably bewildering to any quantitative researcher, let alone to a microbiologist specializing in tuberculosis. Foster [22,23] and Rosche and Foster [24] methodically distilled a large portion of this vast literature and cataloged all major mathematical formulas that are important to microbiologists wishing to calculate mutation rates by themselves. Here I offer a similar, selective list of relevant mathematical formu-

las, aiming at helping tuberculosis researchers understand key concepts and sidestep common pitfalls.

The earliest methods are the P_0 method and the method of the mean, proposed by Luria and Delbrück [1]. In light of the claim made in the footnote at the bottom of the first page of the classic paper [1, p. 491], Delbrück, a physicist by training, was likely the sole architect of both methods. In fact, as Luria [2, p. 39] recounted the exciting days leading to the discovery of the fluctuation test some 40 years later, Delbrück alone worked out the underlying mathematics within a week after Luria wrote Delbrück about the novel experiments he conducted. The P_0 method is defined by

$$m = -\log(p_0), \tag{2}$$

where p_0 is the proportion of null cultures, that is, cultures that do not contain mutant cells at the end of the first incubation period (prior to plating). This method is conceptually straightforward, but it was the origin of the persistent log 2 confusion. In converting a value of m obtained from Eq. (2) to a mutation rate, Luria and Delbrück [1, p. 507] used the relation $\mu = (\log 2)m/N_t$ in place of the correct relation $\mu = m/N_t$ given by Eq. (1). The unwarranted log 2 factor became entrenched partly because several authors constructed various arguments to justify it [25–28]. Kondo appeared to be the first to argue against the erroneous log 2 factor. Before submitting for publication a theoretical paper criticizing the log 2 factor, Kondo communicated with Delbrück about his manuscript [29, p. 374] and received comments from Delbrück. Hence, it seems likely that Delbrück was aware of Kondo's critiques and somehow sanctioned Kondo's views. Further warnings and arguments against the log 2 factor were given by Zheng [3,30].

The second method of Luria and Delbrück was based on Delbrück's concept of a likely average. The method in its original form is expressed by

$$r = aN_t \log(N_t Ca), \tag{3}$$

where r is the average number of observed mutant colonies per culture and C is the number of cultures. An estimate of the mutation rate is obtained by numerically solving Eq. (3) for the unknown a. This method is often called the method of the mean due to its reliance on the sample mean r. Performance can be somewhat improved by replacing the mean r with the median number of mutants. As elaborated in Ref. [31], Delbrück's idea can spawn a large family of estimators, and the most enduring ones are the Lea-Coulson method [32]

$$m \log(m) + 1.24\, m = r \tag{4}$$

and the Drake formula [27]

$$m \log(m) = r. \tag{5}$$

In contrast to Eq. (3), these two equations are in terms of m, not directly in terms of the mutation rate. These three methods, inspired by the idea of the likely average,

are all suboptimal [31]. In drug resistance research, Eq. (5) was recommended for experiments in which $m \geq 30$, possibly to eschew the seemingly strenuous process of applying maximum likelihood (ML) estimators [33–35]. This outworn rule of thumb can lead to unwieldy mutant counts. As first noticed by Sarkar [36], JBS Haldane in about 1946 suggested $m \approx 2$ for pragmatic reasons. The probability of a culture containing above 200 mutant cells is 0.01 when $m = 2$; that probability increases to about 0.31 when $m = 30$. Excessive numbers of mutants may force the experimenter to resort to partial plating, a different kind of complexity. On the other hand, Eq. (4) is the most reliable among the three sister estimators, and it was recently recommended for a different scenario [37, p. 98]:

> For experiments with zeros or a large spread between low and high numbers of mutants (happens with lower rate assays), use the Method of the Median to calculate the rate.

Still, this view is counterproductive. Investigators should always apply maximum likelihood estimators. In particular, investigators should discard the method of the mean given in Eq. (3), despite its recent appearance in a popular textbook [38, p. 72].

Stewart et al. [39] were the first to tackle the problem of imperfect plating, proposing a two-step estimation process. First, assuming a perfect plating efficiency, compute an estimate of m called m_{obs}. Next, calculate the desired estimate of m called m_{act} by applying the formula

$$m_{\text{act}} = \frac{\epsilon - 1}{\epsilon \log(\epsilon)} m_{\text{obs}}. \tag{6}$$

Jones et al. [40] later tackled the problem directly, offering a median-based estimator

$$\hat{m} = \frac{\hat{\xi}_{0.5}/\epsilon - \log 2}{\log(\hat{\xi}_{0.5}/\epsilon) - \log(\log 2)}, \tag{7}$$

where $\hat{\xi}_{0.5}$ is the median mutant count. Based on work of Stewart [41], Zheng [42] devised an algorithm for computing ML estimates of m that accounted for the effect of plating efficiency.

In 1949, Lea and Coulson [32] proposed a variant of the Luria-Delbrück model. In the original Luria-Delbrück model, mutations occur randomly, but mutant cells grow deterministically. The Lea-Coulson model allows mutant cells to grow stochastically. The crown jewel of their work is the probability generating function of the mutant distribution:

$$g(z) = e^{-m} \exp\left\{ m \left(\frac{z}{1 \times 2} + \frac{z^2}{2 \times 3} + \frac{z^3}{3 \times 4} + \dots \right) \right\}. \tag{8}$$

Theoretically, the probability of a tube containing k mutants is simply the coefficient of z^k of the above generating function. But the method proposed by Lea and Coulson to calculate these coefficients was too cumbersome to be widely used in practice. Some 43 year later, Ma et al. [43] published a far simpler, recursive approach to

calculate the mutant probabilities:

$$p_0 = e^{-m};\ p_k = \frac{m}{k}\sum_{i=0}^{k-1}\frac{p_i}{k-i+1}\ \ (k \geq 1). \tag{9}$$

This was a stunning discovery. Stewart [41] implemented this algorithm in his program "mutants c" before the paper of Ma et al. went to press. Years later Foster [22] wondered about the origin of this technique and found that it was a technique known to those who were familiar with the contagious distribution [44]. In fact, the basic form of this technique appeared in a popular calculus textbook by a Russian mathematician in the 1950s; see Refs. [45, p. 411] and [46, p. 448]. Stewart [47] called Eq. (9) the MSS algorithm and was the first to use it to compute ML estimates of m. Steward [47] also laid a theoretical foundation for constructing approximate confidence intervals (CIs) for m. Rosche and Foster [24] slightly modified Stewart's formulas and gave the following equations for the lower and upper 95% confidence limits for $\log(m)$:

$$\begin{aligned} CL_{+0.95} &= \log(\hat{m}) + 1.96\sigma\exp(-0.315 \times 1.96\sigma) \\ CL_{-0.95} &= \log(\hat{m}) - 1.96\sigma\exp(+0.315 \times 1.96\sigma) \end{aligned} \tag{10}$$

with σ given by

$$\sigma = \frac{1.225\hat{m}^{-0.315}}{\sqrt{n}}. \tag{11}$$

Here n is the number of cultures. Exponentiating the above confidence limits would yield an approximate 95% CI for m.

A diligent reader may notice the odd absence of N_0 and N_t in the generating function in Eq. (8). As Coulson learned later, Eq. (8) was approximate. The exact generating function, given first by D.G. Kendall [49, p.3], depends also on a secondary parameter ϕ:

$$g(z) = \exp\left[\frac{m}{\phi}\left(\frac{1}{z} - 1\right)\log(1 - \phi z)\right]. \tag{12}$$

Here, the oft-ignored parameter ϕ is given by

$$\phi = 1 - \frac{N_0}{N_t}. \tag{13}$$

In all applications, $0 < \phi < 1$. But setting $\phi = 1$ gives the Lea-Coulson generating function (8). Nádas et al. [48] called attention to possibly grave errors in applications when ϕ is much smaller than unity. However, when $N_0/N_t < 0.001$, which occurs in most applications, the Lea-Coulson generating function is a satisfactory approximation to the exact generating function. The exact generating function first appeared in print in a paper of Armitage [25] and readers interested in its intriguing provenance are referred to Ref. [49]. rSalvador [50] is so far the only software

Table 1 Mutant count data of Demerec [9] is analyzed under the assumption that mutants were counted in the gth generation for selected values of g

g	5	8	10	15	20	∞
ϕ	0.969	0.996	0.999	> 0.999	> 0.999	1.0
$\hat{m}$	13.222	11.056	10.884	10.845	10.844	10.844

Maximum likelihood estimates of m are computed using rSalvador that adopts the exact generating function given by Eq. (12). The values of ϕ are assumed to be $1 - 2^{-g}$

package that allows the user to choose the exact generating function by specifying a value of ϕ. To help see the effect of ϕ on the estimation of m, I here apply the exact model (12) to the well-known Demerec data [51]. The values of ϕ are chosen by $\phi = 1 - 1/2^g$ for some integer values of g, and corresponding ML estimates of m obtained by rSalvador are given in Table 1. If cells were allowed to divide for just 5 generations, $\hat{m}$ would be markedly different from the estimate obtained under the approximate model (8). However, when $g \geq 15$, the difference is negligible. In practice, g should not be too large either, as cells must be plated while they are still undergoing exponential growth.

In 2005, Zheng [52] devised algorithms for computing likelihood-ratio (LR) confidence intervals for m, and hence for mutation rates. It is a well-known fact in statistical theory that the likelihood interval is preferable to the Wald interval when sample size is not large. As Pawitan put it [53, p. 48], "the applicability of the likelihood-based CI is much wider and, consequently, it is much safer to use than the Wald interval." In practice, most fluctuation experiments consist of a small number of cultures, but Wald intervals are more commonly used due to their relative computational simplicity. For example, the predecessor of rSalvador originally gave only Wald intervals [54]. In 2016, Zheng [55] devised algorithms for computing LR test statistics that allow mutation rates to be compared. The superiority of the LR test over other approximate tests is an established fact in modern statistics [56, p. 220]. Agresti's advice [57, p. 107] remains relevant in the context of fluctuation assay data: "Although the Wald test is adequate for large samples, the likelihood-ratio test is more powerful and more reliable for sample sizes often used in practice." At present only rSalvador [50] offers LR-based CIs and LR tests for mutation rates. Investigators may sometimes prefer to use fold change to compare mutation rates. Recently Zheng [58] devised algorithms allowing investigators to report mutation rate fold changes along with corresponding LR-based CIs.

The problem of variation in N_t also received considerable theoretical consideration. As N_t is determined by serial dilutions, variation in N_t has a distinct human dimension. Earlier investigators such as Wierdl et al. [59] and Schmidt et al. [60] devised statistical inference methods that took account of variation in N_t. However, Foster [23, p. 682] expressed a concern regarding how severely it might affect the estimation of m. In 2009, Wu et al. [61] launched a relatively thorough investigation

into the effects of variation in N_t, and Zheng [62] in 2011 proposed a gamma-mixture model to account for variation in N_t. The two most recent studies, of Ycart and Veziris [63] and of Zheng [64], seemed to aim at opposite directions. While the former emphasizes possible grave consequences of ignoring variation in N_t, the latter holds a relatively optimistic view, suggesting avoiding unnecessary corrections as long as the coefficient of variation (CV) for N_t is small. And Zheng [64] found that in most applications CVs were often too small to be cause for concern, and he proposed a theoretical basis for this observation. If an exceedingly large CV for N_t is encountered, say $CV > 0.5$, the investigator should probably first ask whether the serial dilution process was properly conducted. If measurement errors are too large, the N_t measurements may not represent the actual size of cultures in which cells divide and mutate. Corrections based on inflated CVs would not be helpful.

In 2012, Hamon and Ycart [65] proposed a novel estimation method based on the empirical probability generating function (GF), aiming at tackling real and imagined difficulties haunting the likelihood paradigm. The GF method kept improving for several years, showing at once impressive performance and disheartening underperformance [7]. Research into the GF method helped crystallize a simple fact that parameter identifiability problem is a common anathema to the GF method, as well as to the likelihood method. Attempts to conquer the notorious identifiability problem led to a software package named flan [66]. An interesting feature of flan version 0.8 is that comparison of mutation rates seems to be based on the following formula:

$$t = \left(\frac{\hat{m}_1}{N_1} - \frac{\hat{m}_2}{N_2}\right) \times \left(\frac{var(\hat{m}_1)}{N_1^2} + \frac{var(\hat{m}_2)}{N_2^2}\right)^{-0.5}. \tag{14}$$

Here, $\hat{m}_1$ and $\hat{m}_2$ are estimates of m of the two groups, and N_1 and N_2 are the N_t measurements of the two groups. Fisher information might be used to estimate the variances of $\hat{m}_1$ and $\hat{m}_2$. The test statistic is reported as a T value, but it is compared with percentile points of the standard normal distribution in calculating p values.

5 An Example

Most investigators understandably prefer tools that require minimal programming skills. Since its inception, the web tool FALCOR [67] has been the favorite tool for fluctuation assay, as it relieves the user of the burden of learning the basics of an unfamiliar computer language. However, FALCOR has two important drawbacks. The first drawback is that it constructs CIs using an ad hoc procedure inspired by Schmidt et al. [60]. (Wierdl et al. [59] and Wu et al. [61] used similar methods in their investigations.) Specifically, FALCOR applies Eq. (4) to each individual culture to obtain a sequence of culture-specific mutation rates. It then uses the ranks of these culture-specific mutation rates to form a CI for the mutation rate. In other words, although FALCOR computes mutation rates using the likelihood principle, it constructs CIs

without relying on the likelihood function. The performance of this ad hoc approach is largely unknown. This hybrid strategy can confuse an unwary user. For example, most of the CIs reported in the study of Nyinoh and McFadden [68] do not contain the point estimates of the targeted mutation rates. The second drawback of FALCOR is that it does not have the capability to account for plating efficiency. The web tool FluCalc [69] was designed to overcome these two drawbacks of FALCOR. FluCalc employs Eq. (10) to construct CIs and Eq. (6) to adjust for plating efficiency. The recent appearance of webSalvador [70] offers more flexibility to those investigators who prefer web tools to traditional software packages. (webSalvador is a web user interface to rSalvador.)

This section aims to help the reader determine when to use FluCalc fruitfully and when it would pay off to make an effort to use rSalvador or its new cousin webSalvador. I will use data provided by the authors of FluCalc [69, p. 425]. This possibly fictitious experiment comprises 12 cultures. One half of each 200-μL culture is plated to determine the number of mutants, and the other half is used to determine the total cell count N_t. Hence, the plating efficiency is $\epsilon = 0.5$. The mutant colony counts are as follows.

```
27 53 47 73 21 40 22 30 45 19 32 38
```

At a dilution of 1/10,000, the recorded numbers of viable cells are

```
34 60 55 43 34 59 42 32 44 56 31 58
```

Now, to use rSalvador, we create a vector of mutant counts by

```
> y=c(27,53,47,73,21,40,22,30,45,19,32,38)
```

Clearly, we need another vector for the calculation of N_t.

```
> wild=c(34,60,55,43,34,59,42,32,44,56,31,58)
> Nt=mean(wild)*20,000
> Nt
[1] 913333.3
```

Because N_t denotes the number of cells in the whole culture, in the above code we multiply the numbers of cells in the diluted cultures by a factor of 20,000, not 10,000. Assuming a perfect plating efficiency, we obtain an estimate of an imagined m, and hence also an estimate of an imagined mutation rate.

```
> newton.LD(y)
[1] 11.07257
> newton.LD(y)/Nt
[1] 1.212325e-05
```

As expected, both results agree with those by FluCalc. Now we try to obtain a LR-based 95% CI for the imagined mutation rate.

```
> confint.LD(y)/Nt
[1] 8.624179e-06 1.592181e-05
```

Both confidence limits differ only slight from those generated by FluCalc, indicating that formulas (10) perform satisfactorily. However, all these results are of little interest to the investigators, because the fact $\epsilon = 0.5$ must now be accounted for. Using Eq. (6), we get an estimate of the actual m

```
> (0.5-1)/0.5/log(0.5)*11.07257
[1] 15.97434
```

This is what FluCalc accomplishes. But we can also find an ML estimate of the actual m directly, as follows.

```
> newton.LD.plating(y,e=0.5)
[1] 18.63416
```

Now the difference is noticeable—the two estimates differ by about 14%. Not surprisingly, an LR-based CI will not in general coincide with that generated by FluCalc. An LR-based 95% CI for the mutation rate is

```
> confint.LD.plating(y,e=0.5)/Nt
[1] 1.492171e-05 2.623816e-05
```

As noted in Ref. [31], the bias would be larger when the plating efficiency is farther away from unity. Consider the case $\epsilon = 0.1$, which is a common plating efficiency encountered in practice. Using Eq. (6), we get

$$m_{act} = \frac{-0.9 \times 11.0726}{0.1 \times \log(0.1)} = 43.28$$

which coincides with the FluCalc output when the V_{sel} parameter defined in FluCalc is set to 20 (μL). But we can obtain an authentic ML estimate of m as follows.

```
> newton.LD.plating(y,e=0.1)
[1] 66.19399
```

and we see that the difference is now about 34.6%. Therefore, an ML estimate of the mutation rate is

```
> newton.LD.plating(y,e=0.1)/Nt
```

```
[1] 7.247517e-05
```

and an approximate 95% CI for the mutation rate is

```
> confint.LD.plating(y,e=0.1)/Nt
[1] 5.561605e-05 8.989704e-05
```

On the other hand, FluCalc would yield an estimated mutation rate of 4.74×10^{-5} with a 95% CI $[3.31, 6.35] \times 10^{-5}$. In summary, FluCalc has overcome an important drawback of FALCOR, and users can use it with ease to apply the Lea-Coulson model (8) to their data. However, when it comes to imperfect plating, caution is strongly recommended.

Lack of algorithmic methods for comparing mutation rates using fluctuation assay data has been an important roadblock to many recent investigations. To circumvent this obstacle, Ford et al. [6] chose to compare mutant frequencies between strains by the Mann-Whitney U test. A more appropriate approach available in rSalvador is the LR test tailored for fluctuation assay data [55]. As an illustration, I pretend here that the vector `wild` contains mutant counts for another strain. Suppose that the average N_t measurement for this strain is twice that for the first strain, whose mutant counts are in the vector `y`. Assume further that $\epsilon = 1.0$ in both experiments. A comparison of the two mutation rates can proceed as follows, yielding a p-value of 0.03:

```
> LRT.LD(y,wild,R=2)
[1] 4.61645915 0.03166654
```

Investigators may also find it desirable to compare mutation rates using FALCOR. For instance, Ramiro et al. [71] identified significant differences in mutation rates between clones by detecting nonoverlapping 95% CIs. This could be a viable approach if the investigators had chosen to use 84% CIs in place of 95% CIs [72]. FluCalc can offer this option by substituting the normal percent point 1.96 in Eq. (10) with $z_{0.08} = 1.405$. A better option is to perform LR tests offered by rSalvador [50].

6 Likelihood Function or Generating Function?

The introduction of the GF method into fluctuation data analysis by Hamon and Ycart [65] was motivated by their disheartening failures to compute ML estimates using samples containing mutant counts exceeding 1,000. The initial excitement greeting the discovery of the GF method led some to believe that the likelihood method might be outworn—at least for fluctuation assay data. This sentiment led to a GF-only web tool called bz-rates, crafted by Gillet-Markowska et al. [73] under the impression that

> ML estimators can become unstable for fluctuation assays involving cultures with large numbers of mutants. In such cases, the empirical probability generating function (GF) remains robust and is preferred over ML ...

It is now clear that a major cause of the imagined deficiency of the ML method was an overlooked implementation detail. SALVADOR 2.0 [52,74], written in the high-level Mathematica language [75], employs the C language to execute certain highly repetitive, computation intensive procedures that are characteristic of mutant distributions. In their initial implementation, Hamon and Ycart [65] relied solely on the R language. When the R package flan [66] adopted a similar strategy to incorporate the C language to improve computational efficiency, the strategy greatly ameliorated the computational difficulties supposedly inherent to the likelihood method. Meanwhile, inherent flaws of the GF method began to surface.

The unpredictable performance of the GF method was first illustrated in Ref. [50], and it was later found to be partly responsible for a misleading conclusion about the Beijing strains of *M. tuberculosis* [7]. Here we consider a simpler testing case, which consists of 7 cultures.

```
> testing=c(299,33,5,32,78,9,490)
```

Now consider the Lea-Coulson model. The ML estimate of m by flan version 0.8 is

```
> mutestim(testing,fitness=1,method='ML')
$mutations
[1] 7.499931
$sd.mutations
[1] 1.493889
```

which agrees with both FluCalc and rSalvador:

```
> newton.LD(testing)
[1] 7.499931
```

However, flan gives a strikingly different GF estimate of m:

```
>  mutestim(testing,fitness=1,method='GF')
$mutations
[1] 9.975086
$sd.mutations
[1] 2.469583
```

The difference is about 33%. Moreover, bz-rates disagrees with flan, as it gives an estimate of 11.6. This GF estimate of m by bz-rates differs from the ML estimate by about 54.6%. The standard errors by the two versions of the GF method also differ noticeably. Such problematic examples can be found with relative ease. At present,

the GF method lacks a firm theoretical foundation comparable to that undergirding the likelihood paradigm. But the GF method seems an efficient way of supplying initial guesses to an ML estimator [66].

7 Conclusion

The fluctuation experiments of David [4] mark the beginning of earnest efforts to study mutation rates as part of tuberculosis research. In the past 52 years the study of mutation rates of *M. tuberculosis* to antibiotic resistance has made great strides. This chapter has offered an overview of major developments that are of interest to tuberculosis researchers studying mutation rates and antibiotic resistance. At this point, it is natural to be curious about possible further developments in the future. I deem the following areas highly relevant, challenging and exciting.

First, it has been a long-running trend in microbial mutation research that researchers increasingly favor the bacterium *Escherichia coli*. In the meantime, antituberculosis drugs, e.g., rifampin, are becoming increasingly synonymous with antibiotics in the mutation research community. These two trends present a challenge to tuberculosis researchers and computational biologists. Current knowledge of in vitro mutation rates of *E. coli* to resistance to antitubercular drugs is relatively rich. But it remains a daunting task to translate mutation rates of *E. coli* to clinically useful knowledge about mutation rates of *M. tuberculosis*. To accelerate research, some investigators [68] considered *M. smegmatis* as a safer, faster-growing alternative organism that may exhibit mutation rates more relevant to *M. tuberculosis*. However, little is know about how mutation rates between these two organisms are quantitatively related.

Second, understanding in vivo mutation rates from knowledge of in vitro mutation rates is the ultimate goal of conducting fluctuation experiments in tuberculosis research [76]. The pioneering investigation by Ford et al. [77] is the closest to a human in vivo study of mutation rates of *M. tuberculosis*. These investigators enlisted 9 macaque monkeys and employed both the fluctuation test and whole genome sequencing (WGS) techniques to measure mutation rates of *M. tuberculosis* to resistance to rifampin. But such a herculean task may not be undertaken routinely. Mathematical modeling may be helpful in bridging the gap between in vitro and in vivo mutation rates.

Third, further mathematical and statistical work may help the fluctuation protocol and sequencing techniques to work synergistically. Contrary to conventional wisdom, the fluctuation protocol remains more popular than sequencing-based methods, and some believe that the old protocol is more precise [78, p. 2]. A litany of simplifying assumptions underlying the fluctuation protocol has been repeatedly scrutinized in the past, but sequencing-based methods are so far exempt from such harsh scrutiny. As a result, newcomers tend to embrace sequencing-based methods without reservation, regarding such methods as de facto gold standard. Thus, when an estimate by the fluctuation assay disagrees with what a sequencing-based method suggests, they

tend to believe that the old protocol either underestimated or overestimated the true mutation rate. In addition to the inherent errors in SNP (single-nucleotide polymorphism) calling [79,80], WGS-based methods for mutation rate estimation require their own simplifying assumptions. Ford et al. [77] articulated one such assumption as follows: "SNPs observed multiple times within the same lesion were assumed to have arisen once and then replicated; as such they were each only counted once." The fluctuation protocol does not have this drawback, as a mutant distribution takes into account the possibility that de novo mutations associated with the same phenotype can occur multiple times.

Core Messages

- The investigation by David in 1970 marks the beginning of earnest efforts to determine mutation rates of *M. tuberculosis* to antibiotic resistance.
- Mutation rate should supplant mutant frequency in most quantitative studies of microbial mutation to drug resistance.
- The maximum likelihood method is still the standard tool for estimating microbial mutation rates.
- The likelihood-ratio test is the most appropriate for the comparison of mutation rates, considering sample sizes commonly used in fluctuation assays.

References

1. Luria SE, Delbrück M (1943) Mutations of bacteria from virus sensitivity to virus resistance. Genetics 28:491–511
2. Luria SE (1984) A slot machine, a broken test tube: an autobiography. Harper & Row, New York
3. Zheng Q (2017) Toward a unique definition of the mutation rate. Bull Math Biol 79:683–692
4. David HL (1970) Probability distribution of drug-resistant mutants in unselected populations of *Mycobacterium tuberculosis*. Appl Microbiol 20:810–814
5. Werngren J, Hoffner SE (2003) Drug-susceptible *Mycobacterium tuberculosis* Beijing genotype does not develop mutation-conferred resistance to rifampin at an elevated rate. J Clin Microbiol 41:1520–1524
6. Ford CB, Shah RR, Meeda MK et al (2013) *Mycobacterium tuberculosis* mutation rate estimates from different lineages predict substantial differences in the emergence of drug-resistant tuberculosis. Nat Genet 45:784–790

7. Zheng Q, Werngren J (2018) An unbiased attitude is vital to exploring the Beijing genotype of Mycobacterium tuberculosis. Tuberculosis 111:193–197
8. Zheng Q (2003) Mathematical issues arising from the directed mutation controversy. Genetics 164:373–379
9. Chakraborty S, Rhee KY (2015) Tuberculosis drug development: history and evolution of the mechanism-based paradigm. Cold Spring Harb Perspect Med 5:a021147
10. Pyle M (1947) Relative numbers of resistant tubercle bacilli in sputa of patients before and during treatment with streptomycin. Proc Staff Meet Mayo Clin 22:465–473
11. Vennesland K, Ebert RH, Bloch RG (1947) The demonstration of naturally-occurring streptomycin-resistant variants in the human strain of tubercle bacillus H-37RV. Science 106:476–477
12. Yegian D, Vanderlinde RJ (1948) A quantitative analysis of the resistance of Mycobacteria to streptomycin. J Bacteriol 56:177–186
13. Kohanski MA, DePristo MA, Collins JJ (2010) Sublethal antibiotic treatment leads to multidrug resistance via radical-induced mutagenesis. Mol Cell 37:311–320
14. Henderson-Begg SK, Sheppard CL, George RC, Livermore DM, Hall LMC (2010) Mutant frequency in antibiotic-resistant and -susceptible isolates of Streptococcus pneumoniae. Int J Antimicrob Agents 35:342–346
15. Kayigire XA, Friedrich SO, van der Merwe L, Diacon AH (2017) Acquisition of rifampin resistance in pulmonary tuberculosis. Antimicrob Agents Chemother 61:e02220-16
16. Delbrück M (1945) Spontaneous mutations of bacteria. Ann Mo Bot Garden 32:223–233
17. de Steenwinkel JEM, ten Kate MT, de Knegt GJ, Kremer K, Aarnoutse RE, Boeree MJ, Verbrugh HA, van Soolingen D, Bakker-Woudenberg IAJM (2012) Drug susceptibility of Mycobacterium tuberculosis Beijing genotype and association with MDR TB. Emerg Infect Dis 18:660–663
18. den Hertog AL, Menting S, van Soolingen D, Anthony RM (2014) Letter to the editor: Mycobacterium tuberculosis Beijing genotype resistance to transient rifampin exposure. Emerg Infect Dis 20:1932–1933
19. Werngren J (2013) Letter to the editor: *Mycobacterium tuberculosis* Beijing type mutation frequency. Emerg Infect Dis 19:522
20. Zheng Q (2018) A cautionary note on the mutation frequency in microbial research. Mutat Res 809:51–55
21. Parkinson JS (2016) Look, Max—no math required! J Bacteriol 17:2281–2282
22. Foster PL (2006) Methods for determining spontaneous mutation rates. Methods Enzymol 409:195–213
23. Foster PL (2007) Measuring spontaneous mutation rates. In: Reddy C, Beveridge T, Breznak J, Marzluf G, Schmidt T, Snyder L (eds) Methods for general and molecular microbiology, 3rd edn. ASM Press, Washington, DC, pp 676–683
24. Rosche WA, Foster PL (2000) Determining mutation rates in bacterial populations. Methods 20:4–17
25. Armitage P (1952) The statistical theory of bacterial population subject to mutation. J R Stat Soc Ser B 14:1–44
26. Armitage P (1953) Statistical concepts in the theory of bacterial mutation. J Hygiene 51:162–184
27. Drake JW (1970) The molecular basis of mutation. Holden-Day, San Francisco
28. Hayes W (1968) The genetics of bacteria and their viruses: studies in basic genetics and molecular biology, 2nd edn. Wiley, New York
29. Kondo S (1972) A theoretical study on spontaneous mutation rate. Mutat Res 14:365–374
30. Zheng Q (2005) Update on estimation of mutation rates using data from fluctuation experiments. Genetics 171:861–864
31. Zheng Q (2015) A new practical guide to the Luria-Delbrück protocol. Mutat Res 781:7–13

32. Lea EA, Coulson CA (1949) The distribution of the numbers of mutants in bacterial populations. J Genet 49:264–285
33. Gillespie SH, Voelker LL, Ambler JE et al (2003) Fluoroquinolone resistance in Streptococcus pneumoniae: evidence that gyrA mutations arise at a lower rate and that mutation in gyrA or parC predisposes to further mutation. Microb Drug Resist 9:17–24
34. Gillespie SH, Basu A, Dickens AL et al (2005) Effect of subinhibitory concentration of ciprofloxacin on Mycobacterium fortuitum mutation rates. J Antimicrob Chemother 56:344–348
35. Pope CF, Gillespie SH, Moore JE, McHugh TD (2010) Approaches to measure the fitness of Burkholderia cepacia complex isolates. J Med Microbiol 59:679–689
36. Sarkar A (1991) Haldane's solution of the Luria-Delbrück distribution. Genetics 127:257–261
37. Polleys EJ, Freudenreich CH (2020) Genetic assays to study repeat fragility in Saccharomyces cerevisiae. In: Guy-Frank Richard (ed) Trinucleotide repeats: methods and protocols, methods in molecular biology, vol 2056. Springer, pp 83–101 (Chapter 5)
38. Stephenson FH (2016) Calculations for molecular biology and biotechnology: a guide to mathematics in the laboratory, 3rd edn. Academic, San Diego, CA
39. Stewart FM, Gordon DM, Levin BR (1990) Fluctuation analysis: the probability distribution of the number of mutants under different conditions. Genetics 124:175–185
40. Jones ME, Thomas SM, Rogers A (1994) Luria-Delbrück fluctuation experiments: design and analysis. Genetics 136:1209–1216
41. Stewart FM (1991) Fluctuation analysis: the effect of plating efficiency. Genetica 84:51–55
42. Zheng Q (2008) A note on plating efficiency in fluctuation experiments. Math Biosci 216:150–153
43. Ma WT, Vh Sandri G, Sarkar S (1992) Analysis of the Luria and Delbrück distribution using discrete convolution powers. J Appl Prob 29:255–267
44. Gurland J (1958) A generalized class of contagious distribution. Biometrics 14:229–249
45. Fichtenholtz GM (1954) Differential and integral calculus, Chinese language edition. Higher Education Press, Beijing (8th edition 2006)
46. Fichtenholtz GM (1954) Differential- und Integralrechnung. Band 2. VEB Deutscher Verlag der Wissenschaften, Berlin (10th edition 1964, 1990)
47. Stewart FM (1994) Fluctuation tests: how reliable are the estimates of mutation rates? Genetics 137:1139–1146
48. Nádas A, Goncharova EI, Rossman TG (1996) Mutations and infinity: improved statistical methods for estimating spontaneous rates. Environ Mol Mutagen 28:90–99
49. Zheng Q (1999) Progress of a half century in the study of the Luria-Delbrück distribution. Math Biosci 162:1–32
50. Zheng Q (2017) rSalvador: an R package for the fluctuation experiment. G3 (Bethesda) 7:3849–3856
51. Demerec M (1945) Production of Staphylococcus strains resistant to various concentrations of penicillin. Proc Natl Acad Sci USA 31:16–24
52. Zheng Q (2005) New algorithms for Luria-Delbrück fluctuation analysis. Math Biosci 196:198–214
53. Pawitan Y (2001) In all likelihood: statistical modeling and inference using likelihood. Clarendon Press, Oxford
54. Zheng Q (2002) Statistical and algorithmic methods for fluctuation analysis with SALVADOR as an implementation. Math Biosci 176:237–252
55. Zheng Q (2016) Comparing mutation rates under the Luria-Delbrück protocol. Genetica 144:351–359
56. Lindsey JK (1996) Parametric statistical inference. Clarendon Press, Oxford
57. Agresti A (2007) An introduction to categorical data analysis, 2nd edn. Wiley, Hoboken, NJ
58. Zheng Q (2021) New approaches to mutation rate fold change in Luria-Delbrück fluctuation experiments. Math Biosci 335:108572

59. Wierdl M, Greens CN, Datta A et al (1996) Destabilization of simple repetitive DNA sequences by transcription in yeast. Genetics 143:713–721
60. Schmidt KH, Pennaneach V, Putamn CD, Kolodner RD (2006) Analysis of gross-chromosomal rearrangements in Saccharomyces cerevisiae. Methods Enzymol 409:462–476
61. Wu X, Strome ED, Meng Q et al (2009) A robust estimator of mutation rates. Mutat Res 661:101–109
62. Zheng Q (2011) A Bayesian two-level model for fluctuation assay. Genetica 139:1409–1416
63. Ycart B, Veziris N (2014) Unbiased estimation of mutation rates under fluctuating final counts. PLoS ONE 9(7):e101434
64. Zheng Q (2016) A second look at the final number of cells in a fluctuation experiment. J Theor Biol 401:54–63
65. Hamon A, Ycart B (2012) Statistics for the Luria-Delbrück distribution. Electron J Stat 6:1251–1272
66. Mazoyer A, Drouilhet R, Despréaux S, Ycart B (2017) flan: an R package for inference on mutation models. R J 9:334–351
67. Hall BM, Ma C-X, Liang P, Singh KK (2009) Fluctuation AnaLysis CalculatOR: a web tool for the determination of mutation rate using Luria-Delbrück fluctuation analysis. Bioinformatics 25:1564–1565
68. Nyinoh IW, McFadden J (2019) Spontaneous mutations conferring antibiotic resistance to antitubercular drugs at a range of concentrations in Mycobacterium smegmatis. Drug Dev Res 80:147–154
69. Radchenko EA, McCinty RJ, Aksenova AY, Neil AJ, Mirkin SM (2018) Quantitative analysis of the rates of repeat-mediated genome instability in a yeast experimental system. In: Muzi-Falconi M, Brown GW (eds) Genome instability: methods and protocols, methods in molecular biology, vol 1672. Springer, pp 421–438 (Chapter 29)
70. Zheng Q (2021) webSalvador: a web tool for the Luria-Delbrück experiment. Microbiol Resource Announc 10:e00314-21
71. Ramiro RS, Durão P, Bank C, Gordo I (2020) Low mutational load and high mutation rate variation in gut commensal bacterial. PLoS Biol 18(3):e000617
72. Zheng Q (2015) Methods for comparing mutation rates using fluctuation assay data. Mutat Res 777:20–22
73. Gillet-Markowska A, Louvel G, Fischer G (2015) bz-rates: a web-tool to estimate mutation rates from fluctuation analysis. G3 (Bethesda) 5:2323–2327
74. Zheng Q (2005) SALVADOR 2.0: a tool for fluctuation analysis. https://library.wolfram.com/infocenter/MathSource/5556. Accessed 22 Feb 2022
75. Wolfram Research, Inc. (2020) Mathematica, version 12.1. Champaign, IL
76. Bergval IL, Schuiteme ARJ, Klatser PR, Anthony RM (2009) Resistant mutants of Mycobacterium tuberculosis selected in vitro do not reflect the in vivo mechanism of isoniazid resistance. J Antimicrob Chemother 64:515–523
77. Ford CB, Lin PL, Chase MR et al (2011) Use of whole genome sequencing to estimate the mutation rate of Mycobacterium tuberculosis during latent infection. Nat Genet 43:482–486
78. Pauly MD, Procario MC, Lauring AS (2017) A novel twelve class fluctuation test reveals higher than expected mutation rates for influenza A viruses. eLife 6:e26437
79. Altmann A, Weber P, Bader D et al (2012) A beginners guide to SNP calling from high-throughput DNA-sequencing data. Hum Genet 131:1541–1554
80. Hwang S, Kim E, Lee I, Marcotte EM (2015) Systematic comparison of variant calling pipelines using gold standard personal exome variants. Sci Rep 5:17875

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The Role of Epigenetics in the Development of Anti-Tuberculosis Drug Resistance

44

Musa Marimani, Aijaz Ahmad, and Adriano Duse

It always seems impossible until it's done.

Nelson Mandela, former president of the Republic of South Africa

Summary

Mycobacterium tuberculosis (*M. tb*) is an airborne infectious agent that causes tuberculosis (TB). The Bacillus Calmette-Guérin (BCG) vaccine is utilized to provide anti-TB immunity. However, this vaccine generates variable immune responses in different individuals depending on their geographic location. Current TB treatment necessitates combinatorial use of different anti-TB drugs for six months to about two years, depending on the drug susceptibility or resistance profile of the *M. tb* strain. Unfortunately, long-term combination therapy is generally associated with the manifestation of unintended clinical complications. Epigenetic processes induce a change in gene expression

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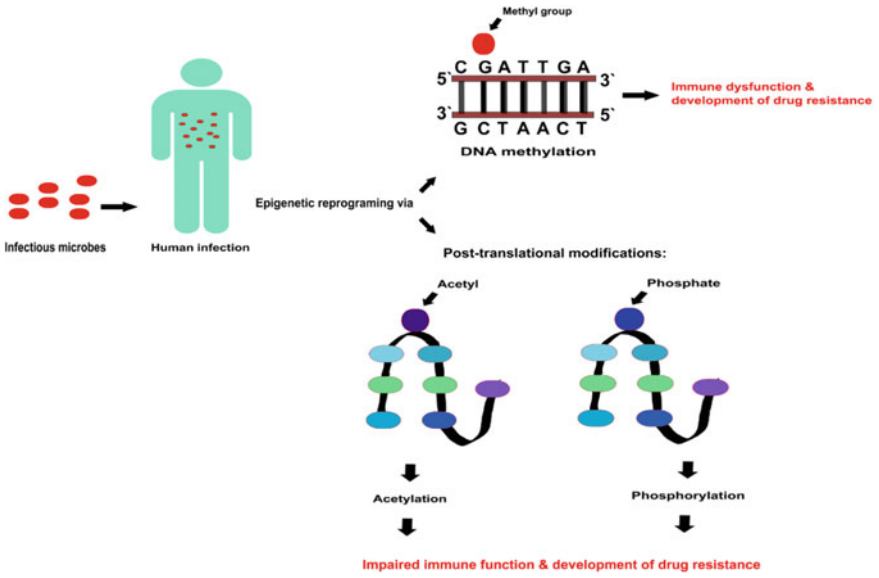
patterns. However, these changes do not modify the nucleotide sequence in DNA. Epigenetic mechanisms are used by *M. tb* and other pathogens to hijack and reprogram important host cellular processes to advance microbial proliferation. Therefore, the current book chapter will examine the devastating impact of acetylation, DNA methylation, and phosphorylation on target gene expression, protein function, and host immunity. Importantly, the role of these epigenetic marks on the emergence of drug-resistant *M. tb* strains is also interrogated.

Graphical Abstract



Diagrammatic representation of epigenetic processes introduced by *Mycobacterium tuberculosis (M. tb)* after the cellular invasion

Keywords

Drug resistance · Epigenetics · Mycobacterium tuberculosis · Tuberculosis

1 Introduction

1.1 Epidemiology of Tuberculosis

Tuberculosis (TB) is a lung disease due to the infection with *Mycobacterium tuberculosis* (*M. tb*). This microbial pathogen is airborne, and transmission is mediated by the inhalation of contaminated droplets. Following the invasion of human cells, *M. tb* initiates replication leading to active TB [1, 2] or may become dormant, resulting in latent TB infection (LTBI) [3–5]. Individuals with active TB disease exhibit symptoms such as chest pains, fever, nausea, and anemia, while patients with LTBI do not display the clinical symptoms associated with TB disease. These asymptomatic individuals cannot transmit the disease to other people but may later develop active TB disease. The transition from LTBI to an active disease state is known as TB reactivation. The propensity for TB reactivation is about 5–10%, and many individuals progress to active disease within five years after infection.

Bacterial entry into host cells is facilitated by interactions between *M. tb* surface ligands and host receptors such as CD14 [6], complement [7], macrophage scavenger receptors [8], mannose [9], surfactant protein A [10], and the protein receptor known as the dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin (DC-SIGN) [11]. The rate of bacterial invasion [12] depends on strain virulence and the immune status of the infected individual [13, 14]. Approximately ten million new TB cases and nearly 1.2 million mortalities were documented in HIV-negative patients in 2018 [15]. Besides, TB disease was responsible for about 251,000 fatalities in HIV-positive individuals [15]. Globally, nations with high TB incidences include South Africa, Nigeria, Bangladesh, the Philippines, China, India, and Pakistan [15]. These nations contributed 66% of new TB incidences.

1.2 Diagnosis of Tuberculosis

Traditionally, TB was diagnosed from patient sputum by employing the sputum smear microscopy method. Unfortunately, this technique had many disadvantages, including decreased sensitivity and specificity as well as the inability to identify paucibacillary TB among HIV-positive individuals. Moreover, this diagnostic method is unable to identify drug-resistant (DR) *M. tb* strains. Serological tests that detect the presence of anti-*M. tb* antibodies are also applied to identify TB infection. These tests are divided into in vitro and in vivo assays. The tuberculin skin test (TST) is an in vivo diagnostic method, while the interferon-gamma release assay (IGRA) is an example of an in vitro assay used for TB detection. The major limitation with these serological assays is their incapability to discriminate between LTBI and the active form of the disease [16] and failure to determine the probability of TB reactivation [17]. Other TB diagnostic approaches include the culture method and chest radiography.

Recently, highly specific molecular-based diagnostic methods have been developed. The GeneXpert MTB/RIF® [18–20] method is a routine technique used to detect active TB infection and rifampicin-resistant *M. tb* strains with a turnaround time of about two hours to seven days depending on the hospital setup. Additionally, this method may also be employed for diagnosing pediatric TB as well as suspected TB cases. Impressively, line probe assays (LPAs) have versatile diagnostic capabilities as they can be applied to identify a wide variety of DR-*M. tb* strains. Indeed, these tests may be applied to detect genetic mutations that promote bacterial survival in the presence of isoniazid and rifampicin, resulting in multi-drug-resistant (MDR) [21, 22] and extensively drug-resistant (XDR) microorganisms. Unfortunately, these mutant strains are not responsive to second-line drugs employed for TB treatment [23–25]. Owing to their useful clinical attributes, LPAs and the GeneXpert MTB/RIF® techniques are highly recommended for TB diagnosis as they have enhanced sensitivity and specificity relative to other diagnostic methods.

1.3 Prevention of Tuberculosis

The spread of TB can be prevented by adhering to the prescribed treatment plan, covering the mouth during coughing or sneezing, frequent hand washing after coughing or sneezing, avoiding contact with infected individuals as well as provision of efficient ventilation. Bacillus Calmette-Guérin (BCG) is the only vaccine applied for TB immunization. This prophylactic agent consists of an attenuated *Mycobacterium bovis* strain [26]. This attenuated strain is employed as a proxy to induce immune response directed against *M. tb* and other mycobacterial pathogens. In addition to providing anti-mycobacterial immunity, BCG is also utilized to prevent the development of tuberculous meningitis [27, 28], Buruli ulcer [29, 30], leprosy [31, 32], and bladder cancer [33]. Generally, the BCG vaccine generates immune protection ranging between 60 and 80% in infants [27, 34], while its activity against pulmonary TB is mainly determined by the geographical location of treated individuals [35, 36]. Disappointingly, limitations of administering BCG vaccine include poor elicitation of host immune response [35], inefficient efficacy in elderly patients, and failure to enhance immunity after vaccine re-administration [37]. These limitations are further compounded by high disparity levels in the generation and sustainability of anti-TB immunity depending on patient age, environmental conditions, and socioeconomic status. Therefore, the development of effective and novel vaccines capable of eliciting consistent and durable host immune responses needs to be urgently pursued.

1.4 Treatment of Tuberculosis

A wide range of anti-TB drugs has been synthesized to alleviate clinical symptoms and eradicate TB disease. These antibiotics have been divided into first-line, second-line, and third-line antibiotics. Pyrazinamide, rifabutin or rifapentine,

ethambutol, isoniazid, and rifampicin are first-line anti-TB agents [38], while second-line drugs include amikacin, capreomycin, and pyrazinamide. The third-line category of anti-TB drugs is comprised of antibiotics such as amoxicillin, clofazimine, imipenem, linezolid, and clarithromycin [38]. The drug bedaquiline is used in combination with other antibiotics to treat pulmonary disease in elderly patients infected with MDR-TB [39]. In general, the therapeutic efficacy of anti-TB molecules ranges from 60 to 90%, with effects lasting for about 19 years [40]. Satisfactory patient recovery and therapeutic efficacy of anti-TB agents necessitate the administration of drugs for about six months-two years and proper compliance with the treatment plan. Compliance with the treatment program leads to the eradication of TB disease, while non-compliance practices such as early cessation and drug misuse exert extensive selection pressure on the therapeutic molecule [41–43]. This generates genetic mutations that support microbial persistence and often lead to the development of various DR-*M. tb* variants [41–43] (Fig. 1). The presence of DR strains has necessitated the application of combination therapy to abolish TB disease. However, this practice frequently results in side effects such as coughing, nausea, hemoptysis, hepatic cytolysis, and hepatotoxicity [44–46]. This then creates a research platform for testing new molecular markers and the potential generation of new therapeutic compounds. Moreover, it also provides an opportunity to unravel and augment the bacterial and host cellular mechanisms that are critical for disease progression, susceptibility to genetic mutations, and development of DR.

2 Epigenetics

Epigenetics is the process whereby chemical modification of biological molecules such as DNA and proteins induces heritable changes in gene expression and protein activity without altering their respective nucleotide or amino acid sequence. Chemical modifications are acquired during processes such as DNA methylation, acetylation, ADP-ribosylation, and ubiquitination [47]. The presence of these and other chemical groups in biological molecules changes DNA accessibility, DNA–protein interaction, and chromatin structure. Subsequently, this leads to a change in gene expression profiling and is often accompanied by dysregulation of critical intracellular signaling pathways and disease development. Various studies have illustrated a pronounced change in the host epigenetic and cellular machinery after infection with microorganisms.

One of the key strategies employed by these infectious agents is to modify gene expression after introducing epigenetic modifications in target genes responsible for microbial recognition, clearance, and apoptosis [48, 49]. Thus, manipulation of host gene expression has been utilized by HIV [50, 51], *M. tb* [52, 53], and other pathogens to successfully colonize host cells and disseminate them into other tissues. Notably, epigenetic mechanisms mediated by microbial invasion induce gene

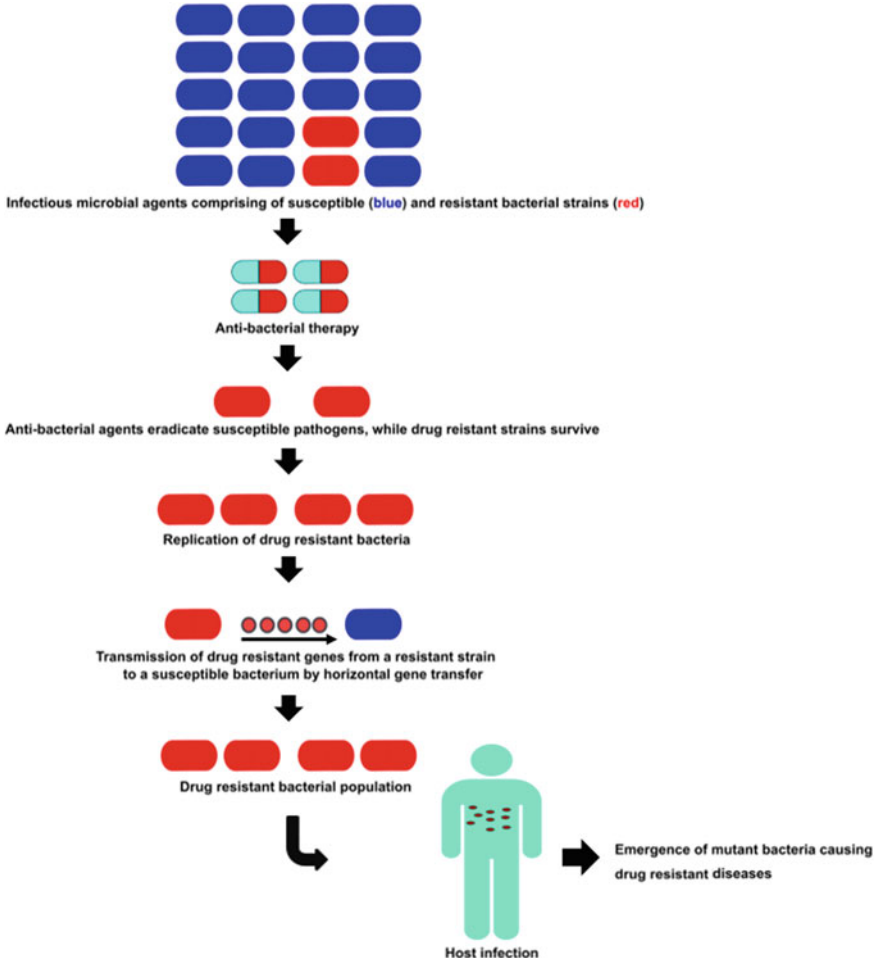


Fig. 1 Emergence and transmission of drug-resistant bacteria. The microbial population is composed of susceptible and resistant strains. Treatment with antibacterial agents eradicates susceptible bacteria, but the resistant strains persist and replicate. Transmission of resistant genes from a drug-resistant bacterial pathogen to a susceptible strain occurs via horizontal gene transfer. Infection of host cells and tissues by drug-resistant strains leads to the manifestation of diseases that are not responsive to conventional antibacterial agents

dysregulation and improper protein function and may also confer resistance to antimicrobial agents [53, 54]. The current book chapter aims to discuss the molecular and clinical impact of epigenetic mechanisms such as DNA methylation, acetylation, and phosphorylation on *M. tb* pathogenesis, survival, stress response, and in regulating the bacterial drug susceptibility or resistant profile.

2.1 The DNA Methylation Epigenetic Mark

DNA methylation occurs when the protein DNA methyltransferase adds a methyl moiety to DNA. Unlike acetylation and phosphorylation, which are post-translational mechanisms (PTMs) that modify proteins, epigenetic reprogramming by DNA methylation occurs at a DNA level. Consequently, the epigenetic changes in DNA may potentially be transferred to mRNA during gene expression and to proteins during translation (Fig. 2). The presence of these epigenetic markers in the host genome is of critical importance in modulating gene expression, gene function, protein synthesis, protein function, and catalytic activity (Fig. 2). DNA methylation controls several important cellular signaling pathways in humans. These include both innate and adaptive immune signaling and cell differentiation [55]. Structurally, the presence of a methyl group on the gene promoter prevents interactions with transcriptional proteins and results in the silencing of candidate gene transcription [56–58]. This alters the host gene expression patterns, protein production, and function (Fig. 2).

Therefore, following infection with microorganisms such as *M. tb*, the altered gene expression profile incapacitates the host from mounting a potent innate and adaptive immune response. Ultimately, this promotes bacterial proliferation and dissemination and has a devastating clinical impact, particularly in young children, pregnant women, and older individuals. Devastatingly, DNA methylation has also been associated with cancer development [59–61] and microbial virulence. Infection with *M. tb* leads to the development of a broad range of TB clinical conditions, including LTBI and active TB infection. Furthermore, an increasing number of mutant *M. tb* variants that are not responsive to conventional antibiotics has been noted. These include strains responsible for the manifestation of DR-, MDR-, and XDR-TB diseases. The intrinsic DNA methylation profile coupled with altered methylation of vitamin D receptors in various racial groups and ethnicities has been associated with TB predisposition [62]. In infected host cells, innate immunity directed against TB infection is mainly mediated by toll-like receptor 2 (TLR2). As such, research was undertaken to investigate the impact of DNA methylation on the function of TRL2 after TB infection [63]. In this study, DNA methylation patterns were evaluated in 20 5'—C—phosphate—G—3' (CpG) regions within the *TLR2* gene and promoter regions in 77 healthy participants and 99 patients with pulmonary TB. Relative to healthy individuals, TB patients displayed a remarkable elevation in DNA methylation levels in 5 CpG regions, *TLR2* expression in natural killer cells, tumor necrosis factor-α (TNF-α), and interferon-gamma (IFN-γ) [63]. Conversely, a marked decline in *TLR2* expression in monocytes was observed in infected individuals. Encouragingly, the administration of anti-TB therapeutics reversed the effects of TLR2 methylation within six months [63]. Data originating from this investigation indicated that methylation of *TLR2* significantly decreased its expression levels, impaired its immunological function, and was associated with increased bacterial infection and proliferation.

Application of TB therapy restored the normal expression levels and function of *TLR2,* leading to symptom alleviation in treated patients, thus, highlighting the

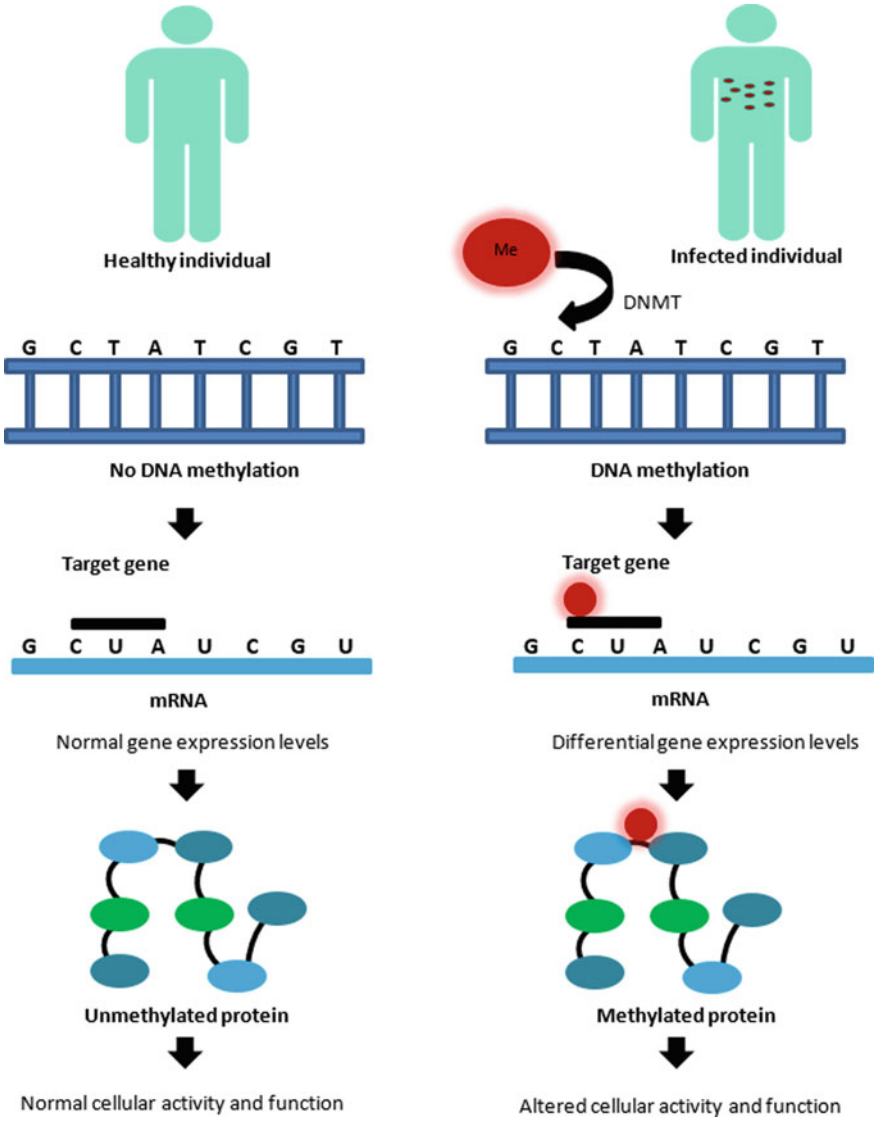


Fig. 2 Change in target gene expression mediated by DNA methylation. Methylation is accomplished when the enzyme DNA methyltransferase transfers the methyl group to the DNA molecule after microbial infection. Subsequently, genetic traits contained in the modified DNA are transferred to RNA during transcription and to proteins following translation. The methylated molecule displays distinct gene expression and protein synthesis profile as well as altered functional characteristics relative to the unmodified counterpart. Properties attained by epigenetic reprogramming of the host cellular systems via DNA methylation promote pathogenicity survival and regulate the drug susceptibility or resistant profile of pathogens Me, methylation; DNMT, DNA methyltransferase enzyme

important role of anti-TB treatment in reversing the negative effects induced by TLR2 methylation [63]. Following infection, *M. tb* reprograms cellular functions by using epigenetic mechanisms such as DNA methylation to alter the cytokine expression levels in the macrophages of infected host cells [55]. Differential DNA methylation patterns were studied in patients with active and latent TB as well as in T helper-1 cells obtained from macrophages infected with several *M. tb* clinical strains. Microarray analysis showed that DNA methylation of cytokines and corresponding receptors was associated with TB infection. The effect of the DNA methylation profile on cytokine expression and function was shown to be strain- and host-dependent [55]. Following infection with Beijing/W strains, a marked increase in DNA methylation was reported in interleukin-4 receptor (IL-4R), IL-6R, and IL-17R in host macrophages. In TB patients, DNA methylation had a negative effect on the normal expression and function of *IL17* and associated genes [55]. This study provided valuable information pertaining to the impact of DNA methylation in modifying cellular and immunological functions in infected host macrophages [55]. Chromatin modifications modulate host transcription levels during *M. tb* infection. The H3K4me is an epigenetic modification that indicates the trimethylation of the lysine residue located in position 4 of the DNA packaging protein histone H3. The virulent H37Rv and non-virulent H37Ra bacterial strains have recently been employed to assess the effect of epigenetic modifications on the chromatin using immunoprecipitation and DNA sequencing [64].

The H3K4me modification induced substantial variations in the dual-specificity MAP kinase phosphatase 4 (DUSP4) and sequence binding protein 1 (SATB1) in macrophages infected with the virulent or non-virulent strains. Notably, SATB1 suppressed gp91phox, which prompted the production of reactive oxygen species (ROS) upon microbial infection. The production of HOX transcript antisense RNA (HOTAIR) was markedly enhanced in macrophage cells following infection with the H37Ra non-virulent strain [64]. In contrast to this, a significant decline in HOTAIR production was observed in host macrophages treated with the H37Rv virulent strain. The overexpression of HOTAIR was associated with the removal of the suppressive effects of the H3K4me modification in the transcriptional start sites of *SATB1* and *DUSP4* genes and indicated that reversal of this epigenetic mark promotes expression of *SATB1*, *DUSP4,* and other related genes [64]. Therefore, this investigation elucidated the importance of the H3K4me trimethylation modification in regulating host immunity and determining the level of TB infection in host macrophages infected with either virulent or non-virulent bacterial pathogens [64]. In a separate current study, the ability of the 6 kDa early secretory antigenic target-6 (ESAT-6) to trigger the development of bone marrow-derived macrophages into epithelioid cells and elucidation of the precise molecular pathway was investigated [65]. The ESAT-6 recombinant protein was expressed in *Escherichia coli* (*E. coli*) and harvested following induction using isopropyl-β-D-thiogalactopyranoside. The bone marrow-derived macrophages were extracted from mice bone marrow collected from hind legs. Data emanating from this study illustrated that ESAT-6 caused

a marked increase in gene expression and protein synthesis levels [65]. However, these changes were absent in mutant mice lacking the TLR2.

Furthermore, ESAT-6 also induced a remarkable increase in the generation of nitric oxide and inducible nitric oxide synthase, which are associated with the suppression of H3K27 trimethylation in *M. tb*-infected cells. These promising outcomes suggest that ESAT-6 and TLR2 elicit pronounced nitric oxide synthase and nitric oxide levels that inhibit the trimethylation of H3K27 cells. Subsequently, this promotes the transition of bone marrow-derived macrophages into epithelioid macrophages [65] and plays a crucial function in combating TB progression in infected host cells [65]. Methylation of the cytosine nucleotide in the DNA sequence suppresses gene expression in prokaryotes [66] and higher eukaryotes [67]. In addition to this, ribosomal RNA methylation is commonly induced during *M. tb* invasion and has been thought to advance bacterial persistence in host cells [68, 69]. Specific correlations have been established between DNA methylation patterns and the development of DR. For example, the methylation profile generated by Erm(38) in *Mycobacterium smegmatis* (*M. smegmatis*) induces resistance to erythromycin and lincosamide antibiotics [70]. Unfortunately, Erm(37) is also present in all bacterial species that form the *Mycobacterium tuberculosis* complex (MTBC) [70–72] and confers resistance to macrolide-lincosamide-streptogramin (MLS) via methylation of 23S ribosomal RNA [73]. On the other hand, the enzyme adenine dimethyltransferase is encoded by the *KsgA* gene and catalyzes methylation of A1518 and A1519 regions of 16S ribosomal RNA, which confers *M. tb* resistance to clarithromycin [72]. Moreover, global DNA methylation and transcriptome changes in *M. tb* have been demonstrated to be associated with conferring resistance to isoniazid and rifampicin resistance [74].

2.2 Acetylation

Acetylation describes a process whereby an acetyl group is transferred to a molecule. The reaction is catalyzed by the acetyltransferase enzyme and is one of the key epigenetic mechanisms leading to PTMs of proteins. Acetylation plays a major role in determining protein synthesis, properties, and subsequent cellular function. Lysine acetylation is also known as N^{ε}-lysine acetylation, and O-acetylation are PTMs that occur in protein molecules. N^{ε}-lysine acetylation involves the transfer of the acetyl residue to the amino group of lysine, while O-acetylation is the process whereby the hydroxyl group of threonine and serine are acetylated by acetyltransferase enzymes. Both N^{ε}-lysine acetylation and O-acetylation regulate specific cellular mechanisms in *M. tb*. However, many investigations have highlighted the importance of O-acetylation in kinase substrates, which modulates various *M. tb* signaling pathways [75, 76]. This reversible epigenetic modification necessitates the addition of acetyl to the ε-amino groups present in lysine moieties in protein molecules [77, 78] (Fig. 3). The presence of this modification has significant functional implications as it affects protein stability and normal catalytic activity (Fig. 3). Lysine acetylation also alters downstream cellular processes that include

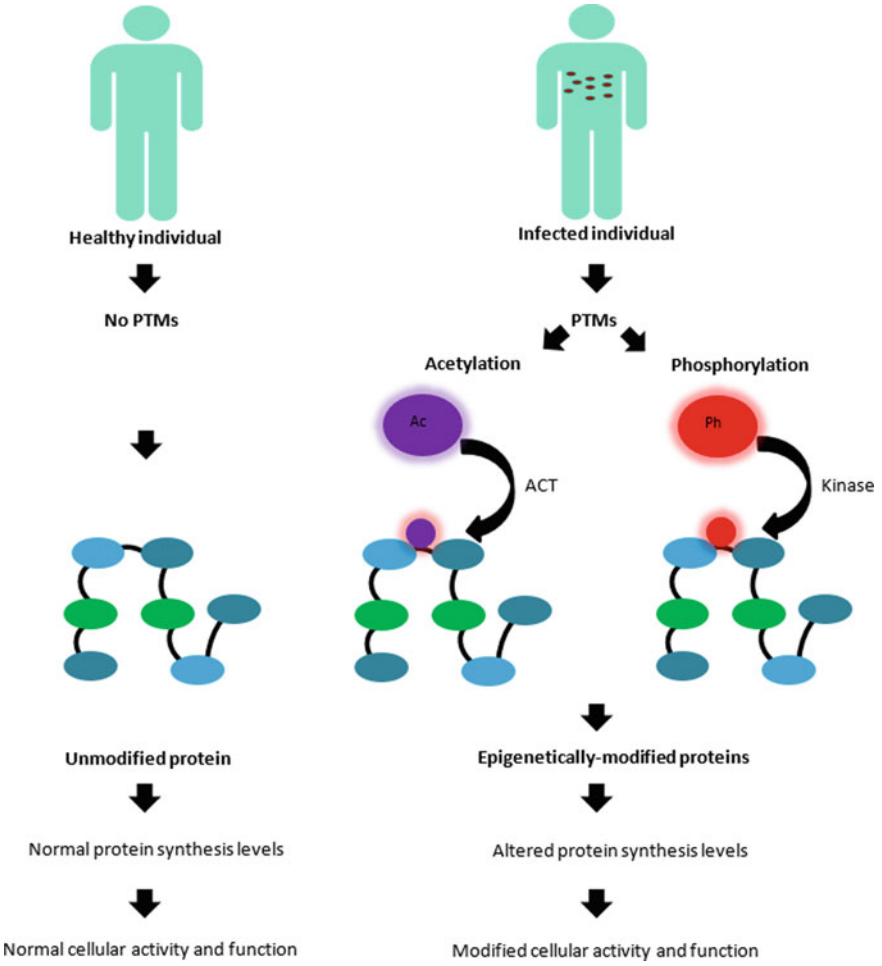


Fig. 3 Altered protein synthesis and catalytic activity induced by PTMs. Protein acetylation and phosphorylation are PTMs catalyzed by acetyltransferase and kinase enzymes, respectively, following microbial infection. The presence of acetyl or phosphate groups on protein molecules changes the chromatin structure and the DNA–protein interactions. This enables the modified protein to exhibit different catalytic and functional properties as compared to the unmodified protein. These acquired properties induce epigenetic reprogramming of the host signaling pathways to advance microbial virulence persistence and also contribute to modulating microbial susceptibility or resistance to therapeutic agents. PTMs represent post-translational modifications, Ac indicates acetylation, ACT denotes acetyltransferase enzyme, Ph illustrates phosphorylation

protein synthesis, protein catalytic activity, interactions between protein species, carbon utilization, and metabolic function [77, 78]. A number of studies focusing on lysine acetylation have been conducted in histone proteins, transcriptionally associated protein molecules [79, 80], and non-histone proteins found in various eukaryotic species. These acetylated non-histone proteins were found to be essential

in a number of biological processes that include protein production, metabolic activity, and cell cycle, which demonstrated that the impact of lysine acetylation is not only limited to transcription [81–85]. Lysine acetylation patterns have also been elucidated in other clinically relevant microbial species such as *Vibrio parahaemolyticus* (*V. parahaemolyticus*), *Streptomyces roseosporus* (*S. roseosporus*), *Salmonella enterica* (*S. enterica*), *Bacillus subtilis* (*B. subtilis*), *Erwinia amylovora* (*E. amylovora*), and *E. coli*. In these microorganisms, lysine acetylation was shown to be involved in regulating protein synthesis, central metabolism, and microbial virulence [86–92].

The universal stress protein (USP) was classified as an acetylated molecule that can undergo lysine acetylation in *M. tb*. This process is catalyzed by the AMP-regulated protein lysine acetyltransferase enzyme Rv0998 [93]. Additionally, the ortholog of this protein in *M. smegmatis* called MSMEG 5458 has also been implicated to be associated with lysine acetylation [93]. Specifically, *M. tb* lysine acetylation determines the degree of protein stability and compartmentalization, which in turn regulates a wide range of cellular processes [94, 95]. For example, the incorporation of acetyl residues into the histone-like nucleoid protein of *M. tb* has been shown to control genome organization and DNA binding capacity [96]. Additionally, in *M. tb*, lysine acetylation modulates fatty acid metabolism and immunogenicity of HspX protein [97] and has also been demonstrated to regulate propionate metabolism in *M. smegmatis* [98, 99]. Interestingly, lysine acetylation is controlled by enzyme-mediated and non-enzyme-mediated processes [100]. The enzymatic reactions are catalyzed by acetyltransferases which transfer the acetyl moiety to lysine, and deacetylases which remove the acetyl residue from lysine. Acetyltransferase is responsible for catalyzing acetylation of *M. tb* Rv0998, Rv2170 [101], and Rv3423 proteins and results in a marked decrease in their catalytic properties. In bacterial cells and mitochondria, lysine acetylation also occurs in a non-enzymatic fashion. In this case, acetyl-coenzyme A and the secondary metabolite acetyl phosphate act as an acetyl donor [102–104]. This non-enzymatic reaction is initiated following the binding of acetyl-coenzyme A to the target protein at a high pH such as that found in the mitochondria [105, 106]. Proteins that participate in deacetylation in *M. tb* include histone deacetylase, which regulates anti-mycobacterial activity in host macrophages [107], and Rv1151c, which controls the catalytic activity of acetyl-CoA synthase [97, 108, 109]. Generally, inhibition of deacetylase activity in *M. tb* results in a significant change in stress response, biofilm formation, and morphological characteristics [97]. The extent of lysine acetylation has been studied in *M. tb* to evaluate the biological impact of this epigenetic mechanism [110].

Xie et al. undertook an investigation whereby 1128 regions were noted on acetylated protein molecules in *M. tb* [110]. Analysis of generated acetylome data indicated that acetylated protein molecules in *M. tb* were involved in modulating protein production and a wide range of cellular metabolic processes. Twenty proteins harboring acetyl residues displayed a high degree of sequence similarity with acetylated proteins in *B. subtilis, E. coli*, *S. enterica,* and *S. roseosporus* and suggested that acetylated proteins are highly conserved. Importantly, some

acetylated protein molecules such as isocitrate lyase were shown to contribute towards bacterial virulence, persistence, and antibiotic resistance. Furthermore, the incorporation of mutations into the isocitrate lyase acetylation site led to a significant decline in enzyme activity, thus, highlighting the critical role of lysine acetylation in regulating cellular functions and *M. tb* virulence [110]. The DosR regulatory system is exploited by *M. tb* to survive under harsh environmental conditions such as hypoxia. It has been established that in an oxygen-deprived environment, *M. tb* transitions to a non-replicating state which allows the pathogen to be resistant to antibacterial agents. For this course, a study was performed to comprehensively elucidate cellular pathways that promote bacterial persistence in the non-replicating bacterial state. In this study, it was shown that the enzyme acetyltransferase (Mt-pat/Rv0998) was implicated in catalyzing cellular processes that promoted *M. tb* survival and also changed the carbon flux from oxidative to reductive tricarboxylic acid (TCA) [111].

The newly generated reductive TCA requires the enzyme malate dehydrogenase, which regulates the redox reaction of NAD^+/NADH. As a result, chemical inhibition or genetic alteration of this enzyme caused a substantial decrease in the microbial load in both the cell culture and mouse models [111]. These results illustrated the importance of protein acetylation performed by acetyltransferase in driving *M. tb* survival and antibacterial resistance under unfavorable hypoxic conditions [111]. Therefore, this protein may potentially serve as an attractive drug target to eradicate non-replicating bacterial cells and combat DR strains. In a closely related study, it was highlighted that the conserved DNA-binding lysine moiety 182 (K182) of DosR regulates DNA binding [112]. Transfer of acetyl groups to K182 inhibited the binding of DosR to DNA in cultured cells. DosR acetylation was accompanied by a substantial decline in the level of acetylated K182 proteins and resulted in altered *M. tb* survival under hypoxic conditions in vitro and in vivo [112]. Acetylation also played a major role in modulating DosR regulon genes that are essential for bacterial infection, survival, and proliferation in host cells and may act as a key domain for epigenetic-based therapeutic strategy [112]. The N-acetyltransferase protein triggers enhanced intracellular survival in *M. tb* and confers resistance to many aminoglycoside antibiotics. Previously, this enzyme has been demonstrated to confer kanamycin resistance in some clinical cases of XDR TB. Consequently, the activity of this protein in *M. tb* and its homolog in *M. smegmatis* was tested against a range of lysine and anti-TB agents [113]. In both bacterial species, N-acetyltransferase induced acetylation of capreomycin and some lysine agents. However, this enzyme did not acetylate non-aminoglycoside drugs. Analysis of modeling data predicted that the site of acetylation on capreomycin was located on one of the primary amines in the drug β-lysine chain. Nuclear magnetic resonance illustrated that in *M. tb*, capreomycin acetylation occurred on the first amine on the β-lysine chain [113]. Similar to *M. tb*, capreomycin acetylation in *M. smegmatis* was also reported to trigger DR. This study showed that N-acetyltransferase promotes enhanced intracellular survival of both *M. tb* and *M. smegmatis* by triggering acetylation of aminoglycoside drugs and some lysine compounds leading to bacterial replication and DR [113].

Birhanu et al. investigated global acetylation profiles in different *M. tb* strains. Acetylome data unraveled 2490 acetylation regions [114]. These sites were comprised of 2349 regions with O-acetylation and 141 with N^{ε}-acetylation. These epigenetically-altered proteins were involved in various cellular mechanisms, including protein synthesis, stress response, metabolic activity, and antibiotic resistance. Specifically, 261 acetylation regions found on 165 protein molecules were distinctively modulated in lineage 4 and 7 bacterial strains [114]. Moreover, hypoacetylation was reported in 257 acetylation sites identified in 161 protein species in lineage 7 strains. Notably, these modified proteins were shown to be important for bacterial infection, virulence, stress responses, and bioenergetics. This investigation demonstrated the presence of two acetylation mechanisms in *M. tb*: N^{ε} and O-acetylation, which are essential in regulating bacterial infection, virulence, and persistence [114]. Therefore, therapeutic molecules directed against these acetylation mechanisms may combat critical pathways necessary for bacterial virulence, dissemination, and DR. In an investigation conducted with the primary aim of identifying several acetyltransferase enzymes involved in epigenetic modification, 47 proteins were found in *M. tb* with varying amino acid sequences, antibiotic substrates, and other chemical compounds [115].

Acetylome analysis revealed one acetyltransferase enzyme, which had remarkable alterations and deletions within the *N*-terminal region. These variations suggested that this protein may possess different physiochemical characteristics relative to other enzyme molecules [115]. Another enzyme that catalyzed three post-translational epigenetic mechanisms was also identified. These processes include glutarylation, succinylation, and lysine acetylation. Evidence gathered from genomic data demonstrated that a large proportion of enzymes and their corresponding genes were part of the same operon [115]. Since several acetylation enzymes investigated in this study were involved in establishing *M. tb* infection, the development of novel therapeutic agents may deactivate the various epigenetic mechanisms performed by these proteins and may potentially lead to the synthesis of effective TB treatment and prevention of antibiotic resistance.

2.3 Phosphorylation

Phosphorylation is a process whereby the phosphate group is transferred to a compound. This process is performed by a specific group of enzymes called protein kinases (Fig. 3). Phosphorylation is one of the most documented PTMs epigenetic mechanisms that have a significant effect on cellular and physiological functions (Fig. 3). In the case of TB infection, the presence or absence of phosphorylation influences the extent of bacterial infection, dissemination, persistence, and the host's immune competence. Therefore, the application of molecules that target and disrupt the activity of protein kinases may reverse the devastating immunological and subsequent clinical effects associated with protein phosphorylation. The success of *M. tb* in establishing infection and the capacity to survive in diverse host environmental conditions is attributed to its ability to adapt, persist and develop

mechanisms that allow bacterial proliferation. Indeed, these strategies allow the pathogen to escape immune surveillance, thus, promoting bacterial replication and transmission in infected host cells. Amongst others, *M. tb* has developed mechanisms that modulate bacterial virulence, such as the 11 Serine/Threonine protein kinases (STPKs), an intracellular network that is comprised of 12 regulatory systems, three phosphatase enzymes, and one tyrosine protein [75, 116, 117]. In particular, the STPKs, 12 regulatory systems and tyrosine kinase induce phosphorylation of substrates on the aspartate amino acid leading to the generation of highly stable phosphorylated products.

Usually, the hydrolysis of phosphoryl-aspartate only occurs for a few hours, while the Serine/Threonine/Tyrosine phosphor-esters generate signals that may persist for weeks and necessitate the activity of the phosphatase enzyme to be reversed [118]. Similarly, the addition of the phosphate group to amino acids (i.e., *O*-phosphorylation) on the protein molecule produces comparable molecular signals and events [118]. As a result, *M. tb* conveniently employs Serine/Threonine/Tyrosine phosphorylation to trigger long-term global responses, while phosphoryl-aspartate is utilized to produce localized short-term responses. This versatility is advantageous as it enables the pathogen to adapt and flourish in various cellular conditions. Except for PknC, the sequences of all other STPKs possess a transmembrane that is useful for connecting the *N*-terminal kinase region located intracellularly to the extracellular *C*-terminal sensory domain. Previous structural investigations have disclosed that dimerization activates these transmembrane receptor kinase enzymes. This subsequently leads to kinase activation, and this process is mediated by phosphorylation of the activation loop [119]. Moreover, data emanating from other studies have indicated that the STPKs in *M. tb* are subjected to tyrosine phosphorylation in their respective activation segments and illustrated the dual-functionality of the STPKs [120]. The protein tyrosine kinase (PtkA) has previously been shown to undergo phosphorylation on the Serine/Threonine amino acid residues resulting in specific interaction with various STPKs [121]. This observation consolidated the existence of a cross-phosphorylation system between tyrosine and STPKs in *M. tb*. Compared to tyrosine phosphorylation, the degree of Serine/Threonine phosphorylation is remarkably higher in *M. tb* and has been reported in more than 500 regions [122].

In STPKs, phosphorylation is a complex epigenetic mechanism that requires three stages, including master regulators (PknB and PknH), signal transducers (PknJ and PknE) as well as substrate kinase proteins (PknD, PknA, PknL, PknF, and PknK). The master regulator kinase enzymes are activated by auto-phosphorylation and allow cross-phosphorylation of downstream kinase proteins [123]. In the same way, kinases involved in signal transduction can also undergo auto-phosphorylation resulting in cross-phosphorylation of downstream kinases, which are critical for transmitting signals to intracellular molecules. However, substrate kinase enzymes were found to be incapable of phosphorylating other STKPs and suggested that they were exclusively involved in targeting other protein substrates. The intracellular mechanisms regulated by STPKs induce a change in gene expression, protein synthesis, and activity, as well as binding

capacity between proteins. Collectively, these phosphorylation-mediated epigenetic alterations enhance bacterial adaptation, metabolic activity, and survival while minimizing or deactivating the host immune response [123].

The presence of several *M. tb* strains, including DR, MDR, and XDR pathogens, necessitates lengthy treatment programs and generally leads to poor compliance to anti-TB therapeutic schedules by TB patients. To circumvent this, Napier et al. evaluated the advantage of applying the anti-kinase drug imatinib to target and inhibit the activity of the host Ab1 kinases and associated tyrosine kinase enzymes [124]. A marked reduction in markers of TB replication in *Mycobacterium marinum* (*M. marinum*) and *M. tb* that includes bacterial load and inflammation were observed in mice following imatinib administration. Importantly, this drug was effective when applied as a therapeutic or prophylactic agent. Moreover, co-administration of imatinib with licensed TB drugs rifabutin or rifampicin led to a pronounced suppression in markers of bacterial infection and replication [124]. Preliminary results reported in this investigation highlighted that imatinib might be conveniently applied as a prophylactic or therapeutic drug to inhibit bacterial proliferation. This drug also provides additional versatility as it targets the host kinase enzymes and may be co-administered with first-line anti-TB agents to induce enhanced antibacterial activity [124]. These drug properties are crucial, particularly in combating diseases caused by various DR-*M. tb* strains. The Src tyrosine kinase protein is a distinguished type of enzyme that is involved in catalyzing the phosphorylation of tyrosine moieties in other tyrosine kinases. This host protein is essential for mycobacterial pathology, particularly bacterial persistence in infected host cells [125]. For this reason, potent anti-Src compounds may be applied to inhibit bacterial survival and dissemination, and the utility of this therapeutic approach has previously been tested [125]. Application of the anti-Src molecule (AZD0530) triggered a substantial decrease in the replication and persistence of the virulent H37Rv, MDR, and XDR strains in cultured mammalian cells. Impressively, markers of bacterial replication and persistence were also markedly suppressed in guinea pigs infected with H37Rv, MDR, and XDR pathogens following treatment of these animals with AZD0530 [125]. Valuable therapeutic evidence recovered from this undertaking indicates that AZD0530 may be utilized to eradicate TB infection in vitro and in vivo by suppressing Src activity in both susceptible and DR-*M. tb* strains.

Protein tyrosine phosphatase (PtpA) is involved in dephosphorylation and is secreted by *M. tb* during infection. This protein binds to the host H^+-ATPase complex in the vacuole to inhibit acidification of the phagosome and prevents phagosome-lysosome interaction [126, 127]. By contrast, PtkA catalyzes the phosphorylation of PtpA and results in enhanced dephosphorylation activity [128], and the same operon encodes these two protein molecules [129]. Previously, the bacterial wild-type *PtkA* and mutant Δ*PtkA* strains were explored for their ability to infect and survive in host macrophages [130]. The outcome of this investigation was that the wild-type strain efficiently infected and displayed effective intracellular persistence in the T helper-1 macrophage model. However, the Δ*PtkA* mutant produced a significantly poor infection profile, displayed poor intracellular survival

in host cells, and was incapable of inhibiting phagocytosis and phagosome acidification [130]. Compared to the wild-type *PtkA*, upregulation of *PtpA* gene expression and enhanced production of TrxB2 (i.e., a substrate for PtkA phosphorylation) were reported in the mutant Δ*PtkA* strain. These results inevitably provided evidence that PtkA is important for establishing bacterial infection, intracellular survival, preventing phagocytosis, and phagosome acidification [130], and may, as such, serve as an ideal molecular target to combat TB infection.

Pulmonary TB infection remains one of the critical public health concerns. The emergence and spread of DR mutants enable the pathogen to continue the infection cycle, and it has been noted that chromosomal mutations that alter drug targets are responsible for inducing DR in *M. tb*. The benefit of phosphorylation on the multifunctional RecA serine 207, which modulates DNA damage response in mycobacterial species, has been established [131]. It was demonstrated that after DNA damage, RecA serine 207 was phosphorylated, leading to the deactivation of pathways responsible for triggering DR. This was achieved by specific suppression of the LexA coprotease catalytic activity of RecA without altering its essential strand exchange or ATPase enzymatic function. This protein component was found to interact with the cytoplasmic membrane during DNA damage response in bacterial cells. Cardiolipin subsequently exerts its function by solely suppressing the coprotease activity of the unmodified, but not that of the phosphorylated RecA serine 207 [131]. This confirmed that bolstering pathways susceptible to epigenetic manipulation may decrease microbial infections and the emergence of DR mycobacterial pathogens.

3 Discussion

Since the advent of antibiotic production, several microbial diseases have been treated and eradicated following the timeous application of these antimicrobial molecules. Thus, different antibiotic drugs have been synthesized to treat a broad range of bacterial infections. However, improper clinical use, over-prescription, and non-compliance to treatment schedules resulted in the development of DR microbial strains. These microorganisms can tolerate, persist, and replicate even after being exposed to conventional therapeutics intended to abolish or diminish their virulence. The survival of these microbial pathogens is mainly driven by the presence of DR genes that have been intrinsically developed by the pathogen after inducing selection pressure on the therapeutic agent or acquired from other microorganisms via horizontal gene transfer. Taken together, all these factors necessitated the development of novel antibiotics, combination therapy [132–134], and the utilization of powerful antibiotics [135–137]. Unfortunately, various pathogens have massively evolved advanced survival strategies that enable them to persist even after being exposed to powerful antibiotic groups such as carbapenems [138–140].

Epigenetic processes induce a change in gene expression without modifying the genomic DNA sequence. The pathogen *M. tb* is one of the microorganisms that exploit epigenetic mechanisms to invade host cells, persist, proliferate, and induce resistance to different antibiotic agents. Indeed epigenetic markers such as acetylation [110, 112–114], DNA methylation [55, 63–65], and phosphorylation [124, 125, 130, 131] have been widely exploited by *M. tb* to advance pathogenicity by triggering DR. Following infection, this bacterial pathogen utilizes epigenetic mechanisms to alter the chromatin structure and accessibility, DNA–protein interactions, protein-protein communication, and binding affinity. This results in a remarkable change in the gene expression and protein synthesis profiles. These drastic cellular alterations lead to successful reprogramming of the host cellular systems and signaling pathways. As a consequence, epigenetic processes allow *M. tb* to manipulate critical pathways that regulate both innate and adaptive immune responses. This results in improved bacterial virulence, persistence, and resistance to various antibiotic classes [74, 131, 141–144].

Epigenetic modifications such as DNA methylation occurs at a DNA level. As a result, genetic traits induced by this epigenetic mechanism may be replicated during the DNA replication cycle and transmitted to RNA during transcription. Ultimately, these genetic alterations embedded in host genes may potentially be transferred to proteins during protein synthesis. This results in the production of protein molecules with distinct catalytic and functional characteristics [145–147]. On the other hand, epigenetic processes such as acetylation and phosphorylation are PTMs that manifest after protein synthesis and have a marked effect on protein function and catalytic capabilities [96, 97, 112, 120, 148, 149]. Encouragingly, previous and recent investigations have demonstrated that the utilization of antimicrobial agents that target and disrupt microbial-induced epigenetic processes are capable of suppressing, inhibiting, and reversing the devastating clinical manifestations caused by DNA methylation [55, 63–65], acetylation [110, 112–114], and phosphorylation [124, 125, 130, 131]. Therefore, epigenetic therapy has been developed to target and suppress microbial [125] or host molecular targets [124] that play a key role in advancing epigenetic-mediated change in host gene expression. Clinically, the versatility of epigenetic drugs in targeting the host or microbial therapeutic targets is essential for disease treatment and minimizing the development of DR strains. Identification and comprehensive characterization of target genes that are amenable to several epigenetic modifications are critical for the synthesis of new and potent antimicrobial drugs. Moreover, detection and disruption of microbial genes that initiate a cascade of epigenetic reprogramming events in the host cells may serve as valuable molecular targets for both disease treatment and diagnostic applications.

Alternative treatment approaches that include the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (cas9) and RNA interference (RNAi) may be employed to inhibit the expression of virulent genes and eradicate the emergence of DR microorganisms. The RNAi process occurs naturally in eukaryotes and is responsible for modulating gene expression. The molecules that partake in various RNAi steps include microRNAs (miRNAs), precursor miRNAs (pre-miRNAs), and primary-microRNAs (pri-miRNAs). Partial

base-pairing between the RNAi substrate molecules and the target mRNA results in suppression of protein synthesis. Oppositely, accurate base-pairing causes mRNA degradation. The use of molecules that closely resemble miRNAs, pre-miRNAs, or pri-miRNAs triggers the RNAi mechanism. Consecutively, this represses target gene expression. Accordingly, artificial short hairpin RNAs (shRNAs) designed to replace pre-miRNAs are applied to silence viral [150] and *M. tb* gene expression [151, 152].

Correspondingly, synthetic small interfering RNAs (siRNAs) synthesized to resemble miRNAs have previously been used to knockdown gene expression in *M. tb* [153–155], DR *B. subtilis* [156], and DR cancer diseases [157–159]. Conversely, CRISPR-cas9 is a gene-editing mechanism employed to create mutations in the DNA after DNA repair has been established. The newly-formed mutations greatly compromise the stability and functionality of target DNA and result in the generation of defective RNA and protein molecules. For this purpose, the CRISPR-cas9 strategy has been used to induce transcriptional repression in *M. tb* [160, 161] and DR bacterial pathogens [162, 163]. Both RNAi and CRISPR-cas9 approaches may be specifically tailored to disrupt the expression of virulence genes required for host invasion and replication. Remarkably, various delivery systems have been developed to facilitate the efficient transportation of effector molecules responsible for RNAi or CRISPR-cas9-mediated target gene knockdown. Indeed, viral and non-viral delivery methods have been utilized to protect effector molecules from enzymatic degradation, thus increasing their half-life in the circulation and causing prolonged therapeutic activity in target host cells and tissues. The non-viral delivery mechanism is generally preferred; it is relatively safe as opposed to viral vectors that tend to persist in the host cells.

Ingestion of foods containing nutrients that inhibit or suppress epigenetic mechanisms is another feasible method of combating bacterial infection, survival, dissemination, and emergence of DR pathogens. Consumption of food with bioactive nutrients can reverse epigenetic modifications introduced by microorganisms [164]. In particular, foods rich in betaine, folate, choline, and vitamin B12 can alter the degree of methylation in DNA and histone proteins. Certain molecules produced following 1-carbon metabolism may regulate DNA and histone methylation profiles. These molecules include *S*-adenosylhomocysteine, which suppresses the activities of DNA methyltransferases and *S*-adenosylmethionine. Consequently, foods containing bioactive nutrients and nutritious supplements capable of modulating the amount of *S*-adenosylmethionine and *S*-adenosylhomocysteine may be applied to control the level of histone and DNA methylation. Vitamin H (biotin) plays a crucial role in the biotinylation of histones, while vitamin B3 (niacin) is involved in the acetylation and ribosylation of histone proteins [164]. Furthermore, consumption of foods containing high levels of vitamin D has been demonstrated to repress markers of *M. tb* replication by regulating the DNA methylation profile in cultured mammalian cells and mice [165] as well as in TB patients [166]. Accordingly, bioactive nutrients contained in certain foods such as genistein and catechin block the catalytic function of proteins involved in epigenetic mechanisms [164]. The application of efficient and novel immunostimulatory agents is another

strategy to counter bacterial invasion. This will trigger and equip the host immunity to provide protection against pathogenic *M. tb* strains irrespective of their drug susceptibility or resistant profiles.

4 Conclusion

In conclusion, a combinatorial approach involving the application of novel antimicrobial compounds, epigenetic molecules in conjunction with immunostimulatory agents, and ingestion of foods containing nutrients capable of targeting and eradicating epigenetic effects on the host cellular machinery may be the most effective therapeutic strategy to counter *M. tb* invasion, pathogenicity, persistence, and emergence of DR mutant strains. Specifically, ingestion of bioactive nutrients naturally present in most vegetables and fruits effectively arms the host immunity, neutralizes microbial invasion, and eradicates the pathogen from circulation. This natural and cheap source of vitalizing immune agents is particularly important for individuals residing in rural and other underdeveloped regions.

Core Messages

- Infection by *M. tb* and other microbial agents induce epigenetic changes in infected individuals.
- Epigenetic processes regulated by *M. tb* include DNA methylation, acetylation, and phosphorylation.
- Chemical groups added during epigenetic modification change the DNA accessibility and chromatin structure.
- Epigenetic marks reprogram the host cellular machinery leading to altered gene expression and protein synthesis levels.
- Altered cellular function impairs the host's immunity and promotes the development of DR microorganisms.

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References

1. Collaborators GBDT (2018) The global burden of tuberculosis: results from the Global Burden of Disease Study 2015. Lancet Infect Dis 18:261–284. https://doi.org/10.1016/S1473-3099(17)30703-X
2. Peto HM, Pratt RH, Harrington TA, LoBue PA, Armstrong LR (2009) Epidemiology of extrapulmonary tuberculosis in the United States, 1993–2006. Clin Infect Dis 49:1350–1357. https://doi.org/10.1086/605559

3. Houben RMGJ, Dodd PJ (2016) The global burden of latent tuberculosis Infection: a re-estimation using mathematical modelling. PLoS Med 13:e1002152–e1002152. https://doi.org/10.1371/journal.pmed.1002152
4. Chen B, Gu H, Wang X, Wang F, Peng Y, Ge E et al. (2019) Prevalence and determinants of latent tuberculosis infection among frontline tuberculosis healthcare workers in southeastern China: a multilevel analysis by individuals and health facilities. Int J Infect Dis 79:26–33. https://doi.org/10.1016/j.ijid.2018.11.010
5. Pontarelli A, Marchese V, Scolari C, Capone S, El-Hamad I, Donato F et al. (2019) Screening for active and latent tuberculosis among asylum seekers in Italy: a retrospective cohort analysis. Travel Med Infect Dis 27:39–45. https://doi.org/10.1016/j.tmaid.2018.10.015
6. Pugin J, Heumann D, Tomasz A, Kravchenko VV, Akamatsu Y, Nishijima M et al (1994) CD14 Is a pattern recognition receptor. Immunity 1:509–516. https://doi.org/10.1016/1074-7613(94)90093-0
7. Schlesinger LS, Bellinger-Kawahara CG, Payne NR, Horwitz MA (1990) Phagocytosis of Mycobacterium tuberculosis is mediated by human monocyte complement receptors and complement component C3. J Immunol 144:2771 LP–2780. Available: http://www.jimmunol.org/content/144/7/2771.abstract
8. Zimmerli S, Edwards S, Ernst JD (1996) Selective receptor blockade during phagocytosis does not alter the survival and growth of Mycobacterium tuberculosis in human macrophages. Am J Respir Cell Mol Biol 15:760–770. https://doi.org/10.1165/ajrcmb.15.6.8969271
9. Schlesinger LS (1993) Macrophage phagocytosis of virulent but not attenuated strains of Mycobacterium tuberculosis is mediated by mannose receptors in addition to complement receptors. J Immunol 150:2920–2930
10. Downing JF, Pasula R, Wright JR, Twigg HL 3rd, Martin WJ 2nd (1995) Surfactant protein a promotes attachment of Mycobacterium tuberculosis to alveolar macrophages during infection with human immunodeficiency virus. Proc Natl Acad Sci USA 92:4848–4852. https://doi.org/10.1073/pnas.92.11.4848
11. Tailleux L, Schwartz O, Herrmann J-L, Pivert E, Jackson M, Amara A et al (2003) DC-SIGN is the major Mycobacterium tuberculosis receptor on human dendritic cells. J Exp Med 197:121–127. https://doi.org/10.1084/jem.20021468
12. Menozzi FD, Reddy VM, Cayet D, Raze D, Debrie A-S, Dehouck M-P et al (2006) Mycobacterium tuberculosis heparin-binding haemagglutinin adhesin (HBHA) triggers receptor-mediated transcytosis without altering the integrity of tight junctions. Microbes Infect 8:1–9. https://doi.org/10.1016/j.micinf.2005.03.023
13. Menozzi FD, Bischoff R, Fort E, Brennan MJ, Locht C (1998) Molecular characterization of the mycobacterial heparin-binding hemagglutinin, a mycobacterial adhesin. Proc Natl Acad Sci USA 95:12625–12630. https://doi.org/10.1073/pnas.95.21.12625
14. De Lima CS, Marques MAM, Debrie A-S, Almeida ECC, Silva CAM, Brennan PJ et al (2009) Heparin-binding hemagglutinin (HBHA) of Mycobacterium leprae is expressed during infection and enhances bacterial adherence to epithelial cells. FEMS Microbiol Lett 292:162–169. https://doi.org/10.1111/j.1574-6968.2009.01488.x
15. Organization WH. Global tuberculosis report 2019. Geneva PP-Geneva: World Health Organization. Available: https://apps.who.int/iris/handle/10665/329368
16. Mack U, Migliori GB, Sester M, Rieder HL, Ehlers S, Goletti D et al. (2009) LTBI: latent tuberculosis infection or lasting immune responses to M. tuberculosis? A TBNET consensus statement. Eur Respir J 33:956–973. https://doi.org/10.1183/09031936.00120908
17. Zellweger J-P, Sotgiu G, Block M, Dore S, Altet N, Blunschi R et al. (2015) Risk Assessment of tuberculosis in contacts by IFN-γ release assays. A tuberculosis network european trials group study. Am J Respir Crit Care Med 191:1176–1184. https://doi.org/10.1164/rccm.201502-0232OC

18. Kolia-Diafouka P, Carrère-Kremer S, Lounnas M, Bourdin A, Kremer L, Van de Perre P et al. (2019) Detection of Mycobacterium tuberculosis in paucibacillary sputum: performances of the Xpert MTB/RIF ultra compared to the Xpert MTB/RIF, and IS6110 PCR. Diagn Microbiol Infect Dis 94:365–370. https://doi.org/10.1016/j.diagmicrobio.2019.02.008
19. Joon D, Nimesh M, Gupta S, Kumar C, Varma-Basil M, Saluja D (2019) Development and evaluation of rapid and specific sdaA LAMP-LFD assay with Xpert MTB/RIF assay for diagnosis of tuberculosis. J Microbiol Methods 159:161–166. https://doi.org/10.1016/j.mimet.2019.03.002
20. Sun Q, Wang S, Dong W, Jiang G, Huo F, Ma Y et al. (2019) Diagnostic value of Xpert MTB/RIF Ultra for osteoarticular tuberculosis. J Infect 79:153–158. https://doi.org/10.1016/j.jinf.2019.06.006
21. Makhado NA, Matabane E, Faccin M, Pinçon C, Jouet A, Boutachkourt F et al. (2018) Outbreak of multidrug-resistant tuberculosis in South Africa undetected by WHO-endorsed commercial tests: an observational study. Lancet Infect Dis 18:1350–1359. https://doi.org/10.1016/S1473-3099(18)30496-1
22. Gupta R, Thakur R, Kushwaha S, Jalan N, Rawat P, Gupta P et al. (2018) Isoniazid and rifampicin heteroresistant Mycobacterium tuberculosis isolated from tuberculous meningitis patients in India. Indian J Tuberc 65:52–56. https://doi.org/10.1016/j.ijtb.2017.08.005
23. Ennassiri W, Jaouhari S, Cherki W, Charof R, Filali-Maltouf A, Lahlou O (2017) Extensively drug-resistant tuberculosis (XDR-TB) in Morocco. J Glob Antimicrob Resist 11:75–80. https://doi.org/10.1016/j.jgar.2017.07.002
24. Zeng X, Jing W, Zhang Y, Duan H, Huang H, Chu N (2017) Performance of the MTBDRsl Line probe assay for rapid detection of resistance to second-line anti-tuberculosis drugs and ethambutol in China. Diagn Microbiol Infect Dis 89:112–117. https://doi.org/10.1016/j.diagmicrobio.2016.06.011
25. Timire C, Sandy C, Kumar AMV, Ngwenya M, Murwira B, Takarinda KC et al (2019) Access to second-line drug susceptibility testing results among patients with Rifampicin resistant tuberculosis after introduction of the Hain® Line Probe Assay in Southern provinces. Zimbabwe Int J Infect Dis 81:236–243. https://doi.org/10.1016/j.ijid.2019.02.007
26. Karlson AG, Lessen EF (1970) *Mycobacterium bovis* nom. nov. Int J Syst Evol Microbio 20 (3):105–112
27. Trunz BB, Fine PEM, Dye C (2006) Effect of BCG vaccination on childhood tuberculous meningitis and miliary tuberculosis worldwide: a meta-analysis and assessment of cost-effectiveness. Lancet 367:1173–1180. https://doi.org/10.1016/S0140-6736(06)68507-3
28. Van Bui T, Lévy-Bruhl D, Che D, Antoine D, Jarlier V, Robert J (2015) Impact of the BCG vaccination policy on tuberculous meningitis in children under 6 years in metropolitan France between 2000 and 2011. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull 20. https://doi.org/10.2807/1560-7917.es2015.20.11.21064
29. Portaels F, Aguiar J, Debacker M, Guédénon A, Steunou C, Zinsou C et al (2004) Mycobacterium bovis BCG vaccination as prophylaxis against Mycobacterium ulcerans osteomyelitis in Buruli ulcer disease. Infect Immun 72:62–65. https://doi.org/10.1128/iai.72.1.62-65.2004
30. Phillips RO, Phanzu DM, Beissner M, Badziklou K, Luzolo EK, Sarfo FS et al (2015) Effectiveness of routine BCG vaccination on buruli ulcer disease: a case-control study in the Democratic Republic of Congo, Ghana and Togo. PLoS Negl Trop Dis 9:e3457. https://doi.org/10.1371/journal.pntd.0003457
31. Setia MS, Steinmaus C, Ho CS, Rutherford GW (2006) The role of BCG in prevention of leprosy: a meta-analysis. Lancet Infect Dis 6:162–170. https://doi.org/10.1016/S1473-3099(06)70412-1
32. Barreto ML, Pilger D, Pereira SM, Genser B, Cruz AA, Cunha SS et al (2014) Causes of variation in BCG vaccine efficacy: examining evidence from the BCG REVAC cluster

randomized trial to explore the masking and the blocking hypotheses. Vaccine 32:3759–3764. https://doi.org/10.1016/j.vaccine.2014.05.042
33. Kandeel W, Abdelal A, Elmohamady BN, Sebaey A, Elshaaer W, Elbarky E et al. (2015) A comparative study between full-dose and half-dose intravesical immune bacille Calmette-Guérin injection in the management of superficial bladder cancer. Arab J Urol 13:233–237. https://doi.org/10.1016/j.aju.2015.07.002
34. Rodrigues LC, Mangtani P, Abubakar I (2011) How does the level of BCG vaccine protection against tuberculosis fall over time? BMJ 343:d5974. https://doi.org/10.1136/bmj.d5974
35. Colditz GA, Brewer TF, Berkey CS, Wilson ME, Burdick E, Fineberg HV et al (1994) Efficacy of BCG vaccine in the prevention of tuberculosis. Meta Anal Published Lit JAMA 271:698–702
36. Zodpey SP, Shrikhande SN (2007) The geographic location (latitude) of studies evaluating protective effect of BCG vaccine and it's efficacy/effectiveness against tuberculosis. Indian J Public Health 51:205–210
37. Rodrigues LC, Pereira SM, Cunha SS, Genser B, Ichihara MY, de Brito SC et al (2005) Effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: the BCG-REVAC cluster-randomised trial. Lancet (London, England) 366:1290–1295. https://doi.org/10.1016/S0140-6736(05)67145-0
38. Zumla A, Nahid P, Cole ST (2013) Advances in the development of new tuberculosis drugs and treatment regimens. Nat Rev Drug Discov 12:388–404. https://doi.org/10.1038/nrd4001
39. Andries K, Villellas C, Coeck N, Thys K, Gevers T, Vranckx L et al. (2014) Acquired resistance of Mycobacterium tuberculosis to Bedaquiline. van Veen HW ed. PLoS One 9:e102135. https://doi.org/10.1371/journal.pone.0102135
40. Getahun H, Matteelli A, Abubakar I, Aziz MA, Baddeley A, Barreira D et al. (2015) Management of latent Mycobacterium tuberculosis infection: WHO guidelines for low tuberculosis burden countries. Eur Respir J 46:1563–1576. https://doi.org/10.1183/13993003.01245-2015
41. Wáng YXJ, Chung MJ, Skrahin A, Rosenthal A, Gabrielian A, Tartakovsky M (2018) Radiological signs associated with pulmonary multi-drug resistant tuberculosis: an analysis of published evidences. Quant Imaging Med Surg 8:161–173. https://doi.org/10.21037/qims.2018.03.06
42. Dixit A, Freschi L, Vargas R, Calderon R, Sacchettini J, Drobniewski F et al (2019) Whole genome sequencing identifies bacterial factors affecting transmission of multidrug-resistant tuberculosis in a high-prevalence setting. Sci Rep 9:5602. https://doi.org/10.1038/s41598-019-41967-8
43. DeNegre AA, Ndeffo Mbah ML, Myers K, Fefferman NH (2019) Emergence of antibiotic resistance in immunocompromised host populations: a case study of emerging antibiotic resistant tuberculosis in AIDS patients. PLoS ONE 14:e0212969–e0212969. https://doi.org/10.1371/journal.pone.0212969
44. Mejri I, Ourari B, Cherif H, Amar JB, Zaibi H, Azzabi S et al. (2016) Pulmonary tuberculosis and lung cancer: a complex interaction. Eur Respir J 48:PA3721. https://doi.org/10.1183/13993003.congress-2016.PA3721
45. Lima GC, Silva EV, Magalhães P de O, Naves JS (2017) Efficacy and safety of a four-drug fixed-dose combination regimen versus separate drugs for treatment of pulmonary tuberculosis: a systematic review and meta-analysis. Braz J Microbiol 48:198–207. https://doi.org/10.1016/j.bjm.2016.12.003
46. Al-Shaer MH, Mansour H, Elewa H, Salameh P, Iqbal F (2017) Treatment outcomes of fixed-dose combination versus separate tablet regimens in pulmonary tuberculosis patients with or without diabetes in Qatar. BMC Infect Dis 17:118. https://doi.org/10.1186/s12879-017-2231-1
47. Kouzarides T (2007) Chromatin modifications and their function. Cell 128:693–705. https://doi.org/10.1016/j.cell.2007.02.005

48. Bi J, Wang Y, Yu H, Qian X, Wang H, Liu J et al (2017) Modulation of Central Carbon Metabolism by Acetylation of Isocitrate Lyase in Mycobacterium tuberculosis. Sci Rep 7:44826. https://doi.org/10.1038/srep44826
49. Wang X, Ao H, Song M, Bai L, He W, Wang C et al (2019) Identification of DNA methylation regulated novel host genes relevant to inhibition of virus replication in porcine PK15 cell using double stranded RNA mimics and DNA methyltransferase inhibitor. Genomics 111:1464–1473. https://doi.org/10.1016/j.ygeno.2018.09.020
50. Mbonye U, Wang B, Gokulrangan G, Shi W, Yang S, Karn J (2018) Cyclin-dependent kinase 7 (CDK7)-mediated phosphorylation of the CDK9 activation loop promotes P-TEFb assembly with Tat and proviral HIV reactivation. J Biol Chem 293:10009–10025. https://doi.org/10.1074/jbc.RA117.001347
51. Espíndola MS, Soares LS, Galvão-Lima LJ, Zambuzi FA, Cacemiro MC, Brauer VS et al (2018) Epigenetic alterations are associated with monocyte immune dysfunctions in HIV-1 infection. Sci Rep 8:5505. https://doi.org/10.1038/s41598-018-23841-1
52. Yaseen I, Kaur P, Nandicoori VK, Khosla S (2015) Mycobacteria modulate host epigenetic machinery by Rv1988 methylation of a non-tail arginine of histone H3. Nat Commun 6:8922. https://doi.org/10.1038/ncomms9922
53. Sakatos A, Babunovic GH, Chase MR, Dills A, Leszyk J, Rosebrock T et al. (2018) Post-translational modification of a histone-like protein regulates phenotypic resistance to isoniazid in mycobacteria. Sci Adv 4:eaao1478. https://doi.org/10.1126/sciadv.aao1478
54. Gomez-Gonzalez PJ, Andreu N, Phelan JE, de Sessions PF, Glynn JR, Crampin AC et al (2019) An integrated whole genome analysis of Mycobacterium tuberculosis reveals insights into relationship between its genome, transcriptome and methylome. Sci Rep 9:5204. https://doi.org/10.1038/s41598-019-41692-2
55. Zheng L, Leung ETY, Wong HK, Lui G, Lee N, To K-F et al. (2016) Unraveling methylation changes of host macrophages in Mycobacterium tuberculosis infection. Tuberculosis (Edinb). 98:139–148. https://doi.org/10.1016/j.tube.2016.03.003
56. Bobetsis YA, Barros SP, Lin DM, Weidman JR, Dolinoy DC, Jirtle RL et al (2007) Bacterial infection promotes DNA Hypermethylation. J Dent Res 86:169–174. https://doi.org/10.1177/154405910708600212
57. Franco R, Schoneveld O, Georgakilas AG, Panayiotidis MI (2008) Oxidative stress, DNA methylation and carcinogenesis. Cancer Lett 266:6–11. https://doi.org/10.1016/j.canlet.2008.02.026
58. Minárovits J (2009) Microbe-induced epigenetic alterations in host cells: the coming era of patho-epigenetics of microbial infections. a review. Acta Microbiol Immunol Hung 56:1–19. https://doi.org/10.1556/AMicr.56.2009.1.1
59. Yasmin R, Siraj S, Hassan A, Khan AR, Abbasi R, Ahmad N (2015) Epigenetic Regulation of inflammatory Cytokines and associated genes in human Malignancies. Pouliot M (ed) Mediators Inflamm 2015:201703. https://doi.org/10.1155/2015/201703
60. Tang W, Wan S, Yang Z, Teschendorff AE, Zou Q (2017) Tumor origin detection with tissue-specific miRNA and DNA methylation markers. Bioinformatics 34:398–406. https://doi.org/10.1093/bioinformatics/btx622
61. Capper D, Jones DTW, Sill M, Hovestadt V, Schrimpf D, Sturm D et al (2018) DNA methylation-based classification of central nervous system tumours. Nature 555:469–474. https://doi.org/10.1038/nature26000
62. Andraos C, Koorsen G, Knight JC, Bornman L (2011) Vitamin D receptor gene methylation is associated with ethnicity, tuberculosis, and TaqI polymorphism. Hum Immunol 72:262–268. https://doi.org/10.1016/j.humimm.2010.12.010
63. Chen Y-C, Hsiao C-C, Chen C-J, Chao T-Y, Leung S-Y, Liu S-F et al (2014) Aberrant Toll-like receptor 2 promoter methylation in blood cells from patients with pulmonary tuberculosis. J Infect 69:546–557. https://doi.org/10.1016/j.jinf.2014.08.014

64. Subuddhi A, Kumar M, Majumder D, Sarkar A, Ghosh Z, Vasudevan M et al (2020) Unraveling the role of H3K4 trimethylation and lncRNA HOTAIR in SATB1 and DUSP4-dependent survival of virulent Mycobacterium tuberculosis in macrophages. Tuberculosis 120:101897. https://doi.org/10.1016/j.tube.2019.101897
65. Lin J, Jiang Y, Liu D, Dai X, Wang M, Dai Y (2020) Early secreted antigenic target of 6-kDa of Mycobacterium tuberculosis induces transition of macrophages into epithelioid macrophages by downregulating iNOS / NO-mediated H3K27 trimethylation in macrophages. Mol Immunol 117:189–200. https://doi.org/10.1016/j.molimm.2019.11.013
66. Blow MJ, Clark TA, Daum CG, Deutschbauer AM, Fomenkov A, Fries R et al (2016) The Epigenomic landscape of Prokaryotes. PLoS Genet 12:e1005854–e1005854. https://doi.org/10.1371/journal.pgen.1005854
67. Wu H, Zhang Y (2014) Reversing DNA Methylation: mechanisms, genomics, and biological functions. Cell 156:45–68. https://doi.org/10.1016/j.cell.2013.12.019
68. Shell SS, Prestwich EG, Baek S-H, Shah RR, Sassetti CM, Dedon PC et al. (2013) DNA methylation impacts gene expression and ensures hypoxic survival of Mycobacterium tuberculosis. PLoS Pathog 9:e1003419–e1003419. https://doi.org/10.1371/journal.ppat.1003419
69. Wong SY, Javid B, Addepalli B, Piszczek G, Strader MB, Limbach PA et al. (2013) Functional Role of Methylation of G518 of the 16S rRNA 530 Loop by GidB in <span class="named-content genus-species" id=" named-content-1">Mycobacterium tuberculosis</span&gt. Antimicrob Agents Chemother 57:6311 LP–6318. https://doi.org/10.1128/AAC.00905-13
70. Madsen CT, Jakobsen L, Douthwaite S (2005) Mycobacterium smegmatis Erm(38) is a reluctant dimethyltransferase. Antimicrob Agents Chemother 49:3803–3809. https://doi.org/10.1128/AAC.49.9.3803-3809.2005
71. Madsen CT, Jakobsen L, Buriánková K, Doucet-Populaire F, Pernodet J-L, Douthwaite S (2005) Methyltransferase Erm(37) slips on rRNA to confer atypical resistance in Mycobacterium tuberculosis. J Biol Chem 280:38942–38947. https://doi.org/10.1074/jbc.M505727200
72. Phunpruch S, Warit S, Suksamran R, Billamas P, Jaitrong S, Palittapongarnpim P et al (2013) A role for 16S rRNA dimethyltransferase (ksgA) in intrinsic clarithromycin resistance in Mycobacterium tuberculosis. Int J Antimicrob Agents 41:548–551. https://doi.org/10.1016/j.ijantimicag.2013.02.011
73. Buriánková K, Doucet-Populaire F, Dorson O, Gondran A, Ghnassia J-C, Weiser J et al. (2004) Molecular basis of Intrinsic Macrolide Resistance in the Mycobacterium tuberculosis Complex. Antimicrob Agents Chemother 48:143 LP–150. https://doi.org/10.1128/AAC.48.1.143-150.2004
74. Chen L, Li H, Chen T, Yu L, Guo H, Chen Y et al. (2018) Genome-wide DNA methylation and transcriptome changes in Mycobacterium tuberculosis with rifampicin and isoniazid resistance. Int J Clin Exp Pathol 11:3036–3045. Available: https://pubmed.ncbi.nlm.nih.gov/31938429
75. Chao J, Wong D, Zheng X, Poirier V, Bach H, Hmama Z et al (2010) Protein kinase and phosphatase signaling in Mycobacterium tuberculosis physiology and pathogenesis. Biochim Biophys Acta Proteins Proteomics 1804:620–627. https://doi.org/10.1016/j.bbapap.2009.09.008
76. Cousin C, Derouiche A, Shi L, Pagot Y, Poncet S, Mijakovic I (2013) Protein-serine/threonine/tyrosine kinases in bacterial signaling and regulation. FEMS Microbiol Lett 346:11–19. https://doi.org/10.1111/1574-6968.12189
77. Xu H, Hegde SS, Blanchard JS (2011) Reversible acetylation and inactivation of Mycobacterium tuberculosis acetyl-CoA synthetase is dependent on cAMP. Biochemistry 50:5883–5892. https://doi.org/10.1021/bi200156t

78. Xu J-Y, Zhao L, Liu X, Hu H, Liu P, Tan M et al (2018) Characterization of the Lysine Acylomes and the substrates regulated by protein Acyltransferase in Mycobacterium smegmatis. ACS Chem Biol 13:1588–1597. https://doi.org/10.1021/acschembio.8b00213
79. Yang H, Sha W, Liu Z, Tang T, Liu H, Qin L et al (2018) Lysine acetylation of DosR regulates the hypoxia response of Mycobacterium tuberculosis. Emerg Microbes Infect 7:1–14. https://doi.org/10.1038/s41426-018-0032-2
80. Zhang F, Zhou Q, Yang G, An L, Li F, Wang J (2018) A genetically encoded 19F NMR probe for lysine acetylation. Chem Commun 54:3879–3882. https://doi.org/10.1039/C7CC09825A
81. Kim SC, Sprung R, Chen Y, Xu Y, Ball H, Pei J et al (2006) Substrate and functional diversity of lysine acetylation revealed by a proteomics survey. Mol Cell 23:607–618. https://doi.org/10.1016/j.molcel.2006.06.026
82. Choudhary C, Kumar C, Gnad F, Nielsen ML, Rehman M, Walther TC et al. (2009) Lysine Acetylation Targets Protein Complexes and Co-Regulates Major Cellular Functions. Science (80-) 325:834 LP–840. https://doi.org/10.1126/science.1175371
83. Weinert BT, Wagner SA, Horn H, Henriksen P, Liu WR, Olsen JV et al. (2011) Proteome-wide mapping of the Drosophila acetylome demonstrates a high degree of conservation of lysine acetylation. Sci Signal 4:ra48. https://doi.org/10.1126/scisignal.2001902
84. Finkemeier I, Laxa M, Miguet L, Howden AJM, Sweetlove LJ (2011) Proteins of Diverse function and subcellular location Are Lysine Acetylated in Arabidopsis. Plant Physiol 155:1779 LP–1790. https://doi.org/10.1104/pp.110.171595
85. Jeffers V, Sullivan Jr WJ (2012) Lysine acetylation is widespread on proteins of diverse function and localization in the protozoan parasite Toxoplasma gondii. Eukaryot Cell 11:735–742. https://doi.org/10.1128/EC.00088-12
86. Wang Q, Zhang Y, Yang C, Xiong H, Lin Y, Yao J et al. (2010) Acetylation of Metabolic Enzymes Coordinates Carbon Source Utilization and Metabolic Flux. Science (80-) 327:1004 LP–1007. https://doi.org/10.1126/science.1179687
87. Wu X, Vellaichamy A, Wang D, Zamdborg L, Kelleher NL, Huber SC et al (2013) Differential lysine acetylation profiles of Erwinia amylovora strains revealed by proteomics. J Proteomics 79:60–71. https://doi.org/10.1016/j.jprot.2012.12.001
88. Kim D, Yu BJ, Kim JA, Lee Y-J, Choi S-G, Kang S et al (2013) The acetylproteome of Gram-positive model bacterium Bacillus subtilis. Proteomics 13:1726–1736. https://doi.org/10.1002/pmic.201200001
89. Lee D-W, Kim D, Lee Y-J, Kim J-A, Choi JY, Kang S et al (2013) Proteomic analysis of acetylation in thermophilic Geobacillus kaustophilus. Proteomics 13:2278–2282. https://doi.org/10.1002/pmic.201200072
90. Zhang K, Zheng S, Yang JS, Chen Y, Cheng Z (2013) Comprehensive profiling of Protein Lysine Acetylation in Escherichia coli. J Proteome Res 12:844–851. https://doi.org/10.1021/pr300912q
91. Pan J, Ye Z, Cheng Z, Peng X, Wen L, Zhao F (2014) Systematic analysis of the lysine acetylome in vibrio parahemolyticus. J Proteome Res 13:3294–3302. https://doi.org/10.1021/pr500133t
92. Liao G, Xie L, Li X, Cheng Z, Xie J (2014) Unexpected extensive lysine acetylation in the trump-card antibiotic producer Streptomyces roseosporus revealed by proteome-wide profiling. J Proteomics 106:260–269. https://doi.org/10.1016/j.jprot.2014.04.017
93. Nambi S, Basu N, Visweswariah SS (2010) cAMP-regulated protein lysine acetylases in mycobacteria. J Biol Chem 285:24313–24323. https://doi.org/10.1074/jbc.M110.118398
94. Okkels LM, Müller E-C, Schmid M, Rosenkrands I, Kaufmann SHE, Andersen P et al (2004) CFP10 discriminates between nonacetylated and acetylated ESAT-6 of Mycobacterium tuberculosis by differential interaction. Proteomics 4:2954–2960. https://doi.org/10.1002/pmic.200400906

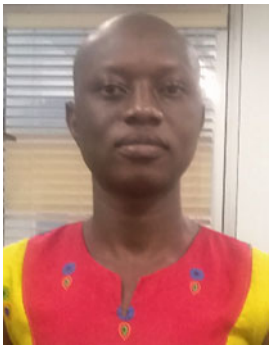
95. van Els CACM, Corbière V, Smits K, van Gaans-van den Brink JAM, Poelen MCM, Mascart F et al. (2014) Toward understanding the essence of post-translational modifications for the Mycobacterium tuberculosis Immunoproteome. Front Immunol 5:361. https://doi.org/10.3389/fimmu.2014.00361
96. Ghosh S, Padmanabhan B, Anand C, Nagaraja V (2016) Lysine acetylation of the Mycobacterium tuberculosis HU protein modulates its DNA binding and genome organization. Mol Microbiol 100:577–588. https://doi.org/10.1111/mmi.13339
97. Liu F, Yang M, Wang X, Yang S, Gu J, Zhou J et al. (2014) Acetylome analysis reveals diverse functions of lysine acetylation in Mycobacterium tuberculosis Mol & Cell Proteomics 13:3352 LP–3366. https://doi.org/10.1074/mcp.M114.041962
98. Hayden JD, Brown LR, Gunawardena HP, Perkowski EF, Chen X, Braunstein M (2013) Reversible acetylation regulates acetate and propionate metabolism in Mycobacterium smegmatis. Microbiology 159:1986–1999. https://doi.org/10.1099/mic.0.068585-0
99. Nambi S, Gupta K, Bhattacharyya M, Ramakrishnan P, Ravikumar V, Siddiqui N et al. (2013) Cyclic AMP-dependent protein lysine acylation in mycobacteria regulates fatty acid and propionate metabolism. J Biol Chem 288:14114–14124. https://doi.org/10.1074/jbc.M113.463992
100. Carabetta VJ, Cristea IM (2017) Regulation, function, and detection of protein acetylation in bacteria. J Bacteriol 199:e00107-e117. https://doi.org/10.1128/JB.00107-17
101. Lee W, VanderVen BC, Walker S, Russell DG (2017) Novel protein acetyltransferase, Rv2170, modulates carbon and energy metabolism in Mycobacterium tuberculosis. Sci Rep 7:72. https://doi.org/10.1038/s41598-017-00067-1
102. Weinert BT, Iesmantavicius V, Wagner SA, Schölz C, Gummesson B, Beli P et al. (2013) Acetyl-Phosphate is a critical determinant of Lysine Acetylation in E. coli. Mol Cell 51:265–272. https://doi.org/10.1016/j.molcel.2013.06.003
103. Kuhn ML, Zemaitaitis B, Hu LI, Sahu A, Sorensen D, Minasov G et al (2014) Structural, kinetic and proteomic characterization of acetyl phosphate-dependent bacterial protein acetylation. PLoS ONE 9:e94816–e94816. https://doi.org/10.1371/journal.pone.0094816
104. Kosono S, Tamura M, Suzuki S, Kawamura Y, Yoshida A, Nishiyama M et al (2015) Changes in the Acetylome and Succinylome of Bacillus subtilis in response to carbon source. PLoS ONE 10:e0131169–e0131169. https://doi.org/10.1371/journal.pone.0131169
105. Wagner GR, Payne RM (2013) Widespread and enzyme-independent Nε-acetylation and Nε-succinylation of proteins in the chemical conditions of the mitochondrial matrix. J Biol Chem 288:29036–29045. https://doi.org/10.1074/jbc.M113.486753
106. Baeza J, Smallegan MJ, Denu JM (2015) Site-specific reactivity of non-enzymatic lysine acetylation. ACS Chem Biol 10:122–128. https://doi.org/10.1021/cb500848p
107. Moreira JD, Koch BEV, van Veen S, Walburg KV, Vrieling F, Mara Pinto Dabés Guimarães T et al. (2020) Functional Inhibition of Host Histone Deacetylases (HDACs) Enhances in vitro and in vivo Anti-mycobacterial Activity in Human Macrophages and in Zebrafish. Front Immunol 11:36. https://doi.org/10.3389/fimmu.2020.00036
108. Gu J, Deng J-Y, Li R, Wei H, Zhang Z, Zhou Y et al (2009) Cloning and characterization of NAD-dependent protein deacetylase (Rv1151c) from Mycobacterium tuberculosis. Biochemistry (Mosc) 74:743–748. https://doi.org/10.1134/s0006297909070062
109. Cain JA, Solis N, Cordwell SJ (2014) Beyond gene expression: The impact of protein post-translational modifications in bacteria. J Proteomics 97:265–286. https://doi.org/10.1016/j.jprot.2013.08.012
110. Xie L, Wang X, Zeng J, Zhou M, Duan X, Li Q et al (2015) Proteome-wide lysine acetylation profiling of the human pathogen Mycobacterium tuberculosis. Int J Biochem Cell Biol 59:193–202. https://doi.org/10.1016/j.biocel.2014.11.010
111. Rittershaus ESC, Baek S-H, Krieger I V, Nelson SJ, Cheng Y-S, Nambi S, et al. (2018) A Lysine Acetyltransferase contributes to the metabolic adaptation to hypoxia in mycobacterium tuberculosis. Cell Chem Biol 25:1495–1505.e3. https://doi.org/10.1016/j.chembiol.2018.09.009

112. Bi J, Gou Z, Zhou F, Chen Y, Gan J, Liu J et al (2018) Acetylation of lysine 182 inhibits the ability of Mycobacterium tuberculosis DosR to bind DNA and regulate gene expression during hypoxia. Emerg Microbes Infect. 7:108. https://doi.org/10.1038/s41426-018-0112-3
113. Houghton JL, Green KD, Pricer RE, Mayhoub AS, Garneau-Tsodikova S (2013) Unexpected N-acetylation of capreomycin by mycobacterial Eis enzymes. J Antimicrob Chemother 68:800–805. https://doi.org/10.1093/jac/dks497
114. Birhanu AG, Yimer SA, Holm-Hansen C, Norheim G, Aseffa A, Abebe M et al. (2017) N (ε)- and O-Acetylation in Mycobacterium tuberculosis Lineage 7 and Lineage 4 Strains: Proteins Involved in Bioenergetics, Virulence, and Antimicrobial Resistance Are Acetylated. J Proteome Res 16:4045–4059. https://doi.org/10.1021/acs.jproteome.7b00429
115. Xie L, Yang W, Fan X, Xie J (2019) Comprehensive analysis of protein acetyltransferases of human pathogen Mycobacterium tuberculosis. Biosci Rep 39:BSR20191661. https://doi.org/10.1042/BSR20191661
116. Wong D, Chao JD, Av-Gay Y (2013) Mycobacterium tuberculosis-secreted phosphatases: from pathogenesis to targets for TB drug development. Trends Microbiol 21:100–109. https://doi.org/10.1016/j.tim.2012.09.002
117. Prisic S, Husson RN (2014) Mycobacterium tuberculosis Serine/Threonine Protein Kinases. Microbiol Spectr 2. https://doi.org/10.1128/microbiolspec.MGM2-0006-2013
118. Sickmann A, Meyer HE (2001) Phosphoamino acid analysis. Proteomics 1:200–206. https://doi.org/10.1002/1615-9861(200102)1:2%3c200::AID-PROT200%3e3.0.CO;2-V
119. Alber T (2009) Signaling mechanisms of the Mycobacterium tuberculosis receptor Ser/Thr protein kinases. Curr Opin Struct Biol 19:650–657. https://doi.org/10.1016/j.sbi.2009.10.017
120. Kusebauch U, Ortega C, Ollodart A, Rogers RS, Sherman DR, Moritz RL, et al. (2014) Mycobacterium tuberculosis supports protein tyrosine phosphorylation. Proc Natl Acad Sci 111:9265 LP–9270. https://doi.org/10.1073/pnas.1323894111
121. Zhou P, Wong D, Li W, Xie J, Av-Gay Y (2015) Phosphorylation of Mycobacterium tuberculosis protein tyrosine kinase A PtkA by Ser/Thr protein kinases. Biochem Biophys Res Commun 467:421–426. https://doi.org/10.1016/j.bbrc.2015.09.124
122. Prisic S, Dankwa S, Schwartz D, Chou MF, Locasale JW, Kang C-M et al. (2010) Extensive phosphorylation with overlapping specificity by Mycobacterium tuberculosis serine/threonine protein kinases. Proc Natl Acad Sci USA. 107:7521–7526. https://doi.org/10.1073/pnas.0913482107
123. Richard-Greenblatt M, Av-Gay Y (2017) Epigenetic phosphorylation control of mycobacterium tuberculosis infection and persistence. Microbiol Spectr 5. https://doi.org/10.1128/microbiolspec.TBTB2-0005-2015
124. Napier RJ, Rafi W, Cheruvu M, Powell KR, Zaunbrecher MA, Bornmann W et al (2011) Imatinib-sensitive tyrosine kinases regulate mycobacterial pathogenesis and represent therapeutic targets against tuberculosis. Cell Host Microbe 10:475–485. https://doi.org/10.1016/j.chom.2011.09.010
125. Chandra P, Rajmani RS, Verma G, Bhavesh NS, Kumar D. Targeting drug-sensitive and -resistant strains of Mycobacterium tuberculosis by Inhibition of Src Family Kinases Lowers Disease Burden and Pathology. Stallings CL, editor. mSphere. 2016;1: e00043–15. doi:https://doi.org/10.1128/mSphere.00043-15
126. Bach H, Papavinasasundaram KG, Wong D, Hmama Z, Av-Gay Y (2008) Mycobacterium tuberculosis virulence is mediated by PtpA dephosphorylation of human vacuolar protein sorting 33B. Cell Host Microbe 3:316–322. https://doi.org/10.1016/j.chom.2008.03.008
127. Wong D, Bach H, Sun J, Hmama Z, Av-Gay Y (2011) Mycobacterium tuberculosis protein tyrosine phosphatase (PtpA) excludes host vacuolar-H+-ATPase to inhibit phagosome acidification. Proc Natl Acad Sci USA 108:19371–19376. https://doi.org/10.1073/pnas.1109201108

128. Zhou P, Li W, Wong D, Xie J, Av-Gay Y (2015) Phosphorylation control of protein tyrosine phosphatase a activity in Mycobacterium tuberculosis. FEBS Lett. 589:326–331. https://doi.org/10.1016/j.febslet.2014.12.015
129. Bach H, Wong D, Av-Gay Y (2009) Mycobacterium tuberculosis PtkA is a novel protein tyrosine kinase whose substrate is PtpA. Biochem J 420:155–162. https://doi.org/10.1042/BJ20090478
130. Wong D, Li W, Chao JD, Zhou P, Narula G, Tsui C et al (2018) Protein tyrosine kinase, PtkA, is required for Mycobacterium tuberculosis growth in macrophages. Sci Rep 8:155. https://doi.org/10.1038/s41598-017-18547-9
131. Wipperman MF, Heaton BE, Nautiyal A, Adefisayo O, Evans H, Gupta R et al. (2018) Mycobacterial Mutagenesis and drug resistance are controlled by phosphorylation- and Cardiolipin-mediated inhibition of the RecA Coprotease. Mol Cell 72:152–161.e7. https://doi.org/10.1016/j.molcel.2018.07.037
132. Maryandyshev A, Pontali E, Tiberi S, Akkerman O, Ganatra S, Sadutshang TD et al. (2017) Bedaquiline and Delamanid combination treatment of 5 patients with pulmonary extensively drug-resistant tuberculosis. Emerg Infect Dis 23:1718–1721. https://doi.org/10.3201/eid2310.170834
133. Ferlazzo G, Mohr E, Laxmeshwar C, Hewison C, Hughes J, Jonckheere S et al (2018) Early safety and efficacy of the combination of bedaquiline and delamanid for the treatment of patients with drug-resistant tuberculosis in Armenia, India, and South Africa: a retrospective cohort study. Lancet Infect Dis 18:536–544. https://doi.org/10.1016/S1473-3099(18)30100-2
134. Kim CT, Kim T-O, Shin H-J, Ko YC, Hun Choe Y, Kim H-R et al (2018) Bedaquiline and delamanid for the treatment of multidrug-resistant tuberculosis: a multicentre cohort study in Korea. Eur Respir J 51:1702467. https://doi.org/10.1183/13993003.02467-2017
135. Hazra S, Xu H, Blanchard JS (2014) Tebipenem, a new carbapenem antibiotic, is a slow substrate that inhibits the β-lactamase from Mycobacterium tuberculosis. Biochemistry 53:3671–3678. https://doi.org/10.1021/bi500339j
136. Tiberi S, D'Ambrosio L, De Lorenzo S, Viggiani P, Centis R, Sotgiu G et al. (2016) Ertapenem in the treatment of multidrug-resistant tuberculosis: first clinical experience. Eur Respir J 47:333 LP–336. https://doi.org/10.1183/13993003.01278-2015
137. Kaushik A, Gupta C, Fisher S, Story-Roller E, Galanis C, Parrish N et al. (2017) Combinations of avibactam and carbapenems exhibit enhanced potencies against drug-resistant Mycobacterium abscessus. Future Microbiol 12:473–480. https://doi.org/10.2217/fmb-2016-0234
138. Wang W, Liu F, Peng Z, Li F, Ma A (2016) Complete Genome Sequence of Salmonella enterica subsp. enterica Serovar Indiana C629, a Carbapenem-Resistant Bacterium Isolated from Chicken Carcass in China. Genome Announc 4:e00662–16. https://doi.org/10.1128/genomeA.00662-16
139. Kumar P, Kaushik A, Bell DT, Chauhan V, Xia F, Stevens RL et al (2017) Mutation in an unannotated protein confers Carbapenem resistance in Mycobacterium tuberculosis. Antimicrob Agents Chemother 61:e02234-e2316. https://doi.org/10.1128/AAC.02234-16
140. Rojas LJ, Salim M, Cober E, Richter SS, Perez F, Salata RA et al (2017) Colistin resistance in Carbapenem-Resistant Klebsiella pneumoniae: laboratory detection and impact on mortality. Clin Infect Dis 64:711–718. https://doi.org/10.1093/cid/ciw805
141. Kaspy I, Rotem E, Weiss N, Ronin I, Balaban NQ, Glaser G (2013) HipA-mediated antibiotic persistence via phosphorylation of the glutamyl-tRNA-synthetase. Nat Commun 4:3001. https://doi.org/10.1038/ncomms4001
142. Esterhuyse MM, Weiner J 3rd, Caron E, Loxton AG, Iannaccone M, Wagman C et al (2015) Epigenetics and proteomics join transcriptomics in the quest for tuberculosis biomarkers. MBio 6:e01187. https://doi.org/10.1128/mBio.01187-15

143. Unissa AN, Sukumar S, Hanna LE (2017) The role of N-Acetyl transferases on isoniazid resistance from mycobacterium tuberculosis and human: an in silico approach. Tuberc Respir Dis (Seoul) 80:255–264. https://doi.org/10.4046/trd.2017.80.3.255
144. Li H, Guo H, Chen T, Wang W, Wu Z, Chen L et al (2020) Potential genes related to levofloxacin resistance in mycobacterium tuberculosis based on Transcriptome and Methylome overlap analysis. J Mol Evol 88:202–209. https://doi.org/10.1007/s00239-019-09926-z
145. Wei M, Wang L, Wu T, Xi J, Han Y, Yang X, et al. (2016) NLRP3 activation was regulated by DNA Methylation modification during Mycobacterium tuberculosis Infection. Biomed Res Int 2016:4323281. https://doi.org/10.1155/2016/4323281
146. Phelan J, de Sessions PF, Tientcheu L, Perdigao J, Machado D, Hasan R et al (2018) Methylation in Mycobacterium tuberculosis is lineage specific with associated mutations present globally. Sci Rep 8:160. https://doi.org/10.1038/s41598-017-18188-y
147. Koh H-J, Kim Y-R, Kim J-S, Yun J-S, Kim S, Kim SY et al (2018) CD82 hypomethylation is essential for tuberculosis pathogenesis via regulation of RUNX1-Rab5/22. Exp Mol Med 50:62. https://doi.org/10.1038/s12276-018-0091-4
148. Vilchèze C, Molle V, Carrère-Kremer S, Leiba J, Mourey L, Shenai S et al. (2014) Phosphorylation of KasB regulates virulence and acid-fastness in Mycobacterium tuberculosis. PLOS Pathog 10:e1004115. Available: https://doi.org/10.1371/journal.ppat.1004115
149. Baronian G, Ginda K, Berry L, Cohen-Gonsaud M, Zakrzewska-Czerwińska J, Jakimowicz D et al. (2015) Phosphorylation of Mycobacterium tuberculosis ParB participates in regulating the ParABS chromosome segregation system. PLoS One 10:e0119907. Available: https://doi.org/10.1371/journal.pone.0119907
150. Ahmed OB, Lage H (2019) Bacteria-mediated delivery of RNAi effector molecules against viral HPV16-E7 eradicates oral squamous carcinoma cells (OSCC) via apoptosis. Cancer Gene Ther 26:166–173. https://doi.org/10.1038/s41417-018-0054-x
151. Li Q, Karim AF, Ding X, Das B, Dobrowolski C, Gibson RM et al (2016) Novel high throughput pooled shRNA screening identifies NQO1 as a potential drug target for host directed therapy for tuberculosis. Sci Rep 6:27566. https://doi.org/10.1038/srep27566
152. Wang F, Zhang Y, Wang X, Wang C, Wang X, Wu J et al (2016) A small hairpin RNA targeting myeloid cell leukemia-1 enhances apoptosis in host macrophages infected with Mycobacterium tuberculosis. J Microbiol 54:330–337. https://doi.org/10.1007/s12275-016-5627-5
153. Pires D, Marques J, Pombo JP, Carmo N, Bettencourt P, Neyrolles O et al (2016) Role of Cathepsins in Mycobacterium tuberculosis Survival in Human Macrophages. Sci Rep 6:32247. https://doi.org/10.1038/srep32247
154. Khan A, Mann L, Papanna R, Lyu M-A, Singh CR, Olson S et al (2017) Mesenchymal stem cells internalize Mycobacterium tuberculosis through scavenger receptors and restrict bacterial growth through autophagy. Sci Rep 7:15010. https://doi.org/10.1038/s41598-017-15290-z
155. Koli U, Nilgiriwala K, Sriraman K, Jain R, Dandekar P (2019) Targeting tuberculosis infection in macrophages using chitosan oligosaccharide nanoplexes. J Nanoparticle Res 21:200. https://doi.org/10.1007/s11051-019-4623-1
156. Sun D, Zhang W, Li N, Zhao Z, Mou Z, Yang E et al (2016) Silver nanoparticles-quercetin conjugation to siRNA against drug-resistant Bacillus subtilis for effective gene silencing: in vitro and in vivo. Mater Sci Eng C Mater Biol Appl 63:522–534. https://doi.org/10.1016/j.msec.2016.03.024
157. Zhang L, Yang X, Li Y, Zheng W, Jiang X (2017) Hollow carbon nanospheres as a versatile platform for co-delivery of siRNA and chemotherapeutics. Carbon N Y 121:79–89. https://doi.org/10.1016/j.carbon.2017.05.084
158. Ding J, Liang T, Min Q, Jiang L, Zhu J-J (2018) Stealth and fully-laden drug carriers: self-assembled Nanogels Encapsulated with Epigallocatechin Gallate and siRNA for drug-resistant Breast Cancer Therapy. ACS Appl Mater Interfaces 10:9938–9948. https://doi.org/10.1021/acsami.7b19577

159. Li P-C, Tu M-J, Ho PY, Jilek JL, Duan Z, Zhang Q-Y et al. (2018) Bioengineered NRF2-siRNA is effective to interfere with NRF2 pathways and improve chemosensitivity of human cancer cells. Drug Metab Dispos 46:2–10. https://doi.org/10.1124/dmd.117.078741
160. Rock JM, Hopkins FF, Chavez A, Diallo M, Chase MR, Gerrick ER et al (2017) Programmable transcriptional repression in mycobacteria using an orthogonal CRISPR interference platform. Nat Microbiol 2:16274. https://doi.org/10.1038/nmicrobiol.2016.274
161. Rawat K, Das S, Vivek Vinod BS, Vekariya U, Garg T, Dasgupta A et al (2019) Targeted depletion of BTF3a in macrophages activates autophagic pathway to eliminate Mycobacterium tuberculosis. Life Sci 220:21–31. https://doi.org/10.1016/j.lfs.2019.01.035
162. Kang YK, Kwon K, Ryu JS, Lee HN, Park C, Chung HJ (2018) Correction to nonviral genome editing based on a polymer-derivatized crispr nanocomplex for targeting bacterial pathogens and antibiotic resistance. Bioconjug Chem 29:3936. https://doi.org/10.1021/acs.bioconjchem.8b00771
163. Rodrigues M, McBride SW, Hullahalli K, Palmer KL, Duerkop BA (2019) Conjugative delivery of CRISPR-Cas9 for the selective depletion of antibiotic-resistant enterococci. Antimicrob Agents Chemother 63. https://doi.org/10.1128/AAC.01454-19
164. Choi S-W, Friso S (2010) Epigenetics: a new bridge between nutrition and health. Adv Nutr 1:8–16. https://doi.org/10.3945/an.110.1004
165. Yang Y, Liu X, Yin W, Xie D, He W, Jiang G et al (2016) 5-Aza-2′-deoxycytidine enhances the antimicrobial response of vitamin D receptor against Mycobacterium tuberculosis. RSC Adv 6:61740–61746. https://doi.org/10.1039/C6RA10647A
166. Wang M, Kong W, He B, Li Z, Song H, Shi P et al (2018) Vitamin D and the promoter methylation of its metabolic pathway genes in association with the risk and prognosis of tuberculosis. Clin Epigenetics 10:118. https://doi.org/10.1186/s13148-018-0552-6



Musa Marimani is a South African researcher currently serving as a lecturer in the Department of Anatomical Pathology at the University of the Witwatersrand, Johannesburg, South Africa. He is interested in investigating the role of epigenetic mechanisms in advancing TB and HIV infection. Some of the epigenetic modifications are introduced in HIV and *Mycobacterium tuberculosis* to subvert the host immunity leading to high microbial infection and transmission. Our research projects are aimed at exploring epigenetic modifications, including acetylation, DNA methylation, and phosphorylation introduced by HIV and TB in human hosts following infection. Therefore, these mechanisms may potentially be utilized as useful indicators or markers for diagnostic procedures and the development of effective therapeutic drugs and vaccines. In addition to conducting research, Dr. Marimani also provides Molecular Biology teaching and research training for Honours students as well as Molecular Medicine practical training for medical registrars.

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Adriano G. Duse is the Head of Clinical Microbiology and Infectious Diseases in the School of Pathology in the Faculty of Health Sciences at Wits University, South Africa. He started his career as a medical technologist and completed his MBBCh at Wits, specializing as a Microbiologist. Professor Duse has held positions of Chairman of the Infection Control Society of South Africa, Sub-Saharan Ambassador for the American Society for Microbiology (ASM), and Council Member of the International Society for Infectious Diseases (ISID). He currently holds the post of Southern African Chair for the Global Antibiotic Resistance Partnership (GARP). He also serves on the Advisory Committee of the International Federation of Home Hygiene and as External Infection Prevention and Control Expert Consultant for Viral Hemorrhagic Fevers (VHF) to the WHO and organizations such as International SOS. In 2014 he was deployed to Liberia, Sierra Leone, and Nigeria to assist with the containment of the Ebola virus outbreak in his capacity as a VHF infection prevention and control expert. Professor Duse is the Case Management and Infection Prevention and Control Lead at the newly created Emergency Operations Centre, National Institute for Communicable Diseases, South Africa.

Multiomics Integration of Tuberculosis Pathogenesis

45

Jae Jin Lee, Philip Sell, and Hyungjin Eoh

Unity, not uniformity, must be our aim. We attain unity only through variety. Differences must be integrated, not annihilated, not absorbed.

Mary Parker Follett

Summary

Advances in high-throughput technology have made it possible to quantitatively monitor changes in multiple sets of biological molecules under different environmental stresses. Microbial adaptation to stresses can be monitored by genomics, transcriptomics, proteomics, and metabolomics. When combined, the resulting multiomics approach provides a much more comprehensive perspective of biological systems than using any single omics alone. Integrated multiomics has improved our understanding of the complex adaptive mechanisms of pathogens and allows for more accurate predictions of pathogenic outcomes. A vast amount of research has been carried out on tuberculosis (TB)

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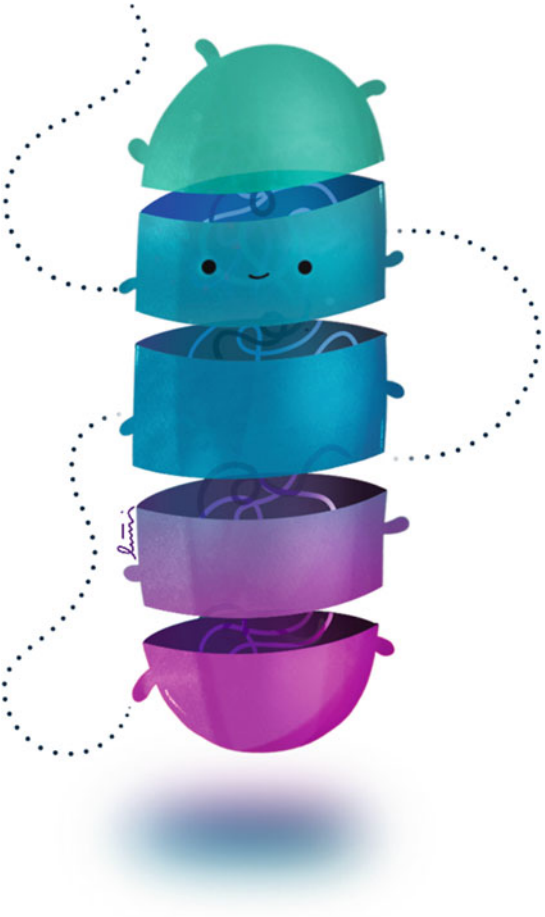
pathogenesis. These studies decipher the biological molecules, pathways, and components of *Mycobacterium tuberculosis (M. tb)*, the etiological agent of TB, involved in the adaptive strategies required for virulence. This chapter summarizes our current knowledge drawn from studies investigating the metabolic adaptation of *M. tb* and its survival in different phenotypic states. The collective interpretation of diverse but essential metabolic networks in *M. tb* will provide new insights for more effective TB interventions.

Graphical Abstract



Multiomics integration of tuberculosis pathogenesis

Keywords

Metabolic essentiality · Metabolic remodelling · Multiomics · Pathogenesis · Phenotypic heterogeneity

1 Introduction

Tuberculosis (TB) research continues to persevere towards developing efficient treatment. Despite continuous advances in research technology to reveal TB pathogenesis, *Mycobacterium tuberculosis (M. tb)* remains a formidable foe against the countless therapies that have been invented to date [1–3].

The hallmark of *M. tb* pathogenesis is its ability to adapt to various antimicrobial environments identified within hosts. *M. tb* can replicate even under an intact immune system; soon after recruitment, functional immune cells release reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI), then in response, *M. tb* slows its replication rate, which enhances the phenotypic heterogeneity within the population [4–12]. Adequate immune responses recruit various immune cells, which convene into a multicellular structure called a granuloma which functions as a trap to keep uncleared *M. tb* bacilli within a restricted area [13, 14]. The interior of the granuloma is full of biochemical stresses, including hypoxia, nutrient starvation, low pH, and accumulation of ROS and RNI, wherein the majority of *M. tb* bacilli resides in the non- or slowly- replicating state by limiting its replication [15–20]. Thus, granuloma formation is a critical innate immune strategy [21, 22]. As a countermeasure, inside the granuloma, *M. tb* survives by shifting to a non-replicating persistent (NRP) state. Infected individuals with properly functioning immune systems may present clinically asymptomatic where *M. tb* mostly lives in an NRP but a metabolically active state. The NRP state of *M. tb* is phenotypically tolerant to nearly all TB antibiotics because they target cellular processes active only during replication [23–27]. This phenotypic quiescence and drug tolerance explain long TB treatment durations, which provides favorable conditions for the emergence of drug-resistant (DR) mutations [28–36]. Moreover, this NRP population serves as a reservoir for disease reactivation [37, 38]. A study at Cornell University using an NRP-TB mouse model showed that *M. tb* bacterial burden was undetectable after bactericidal antibiotic treatment, but reactivation occurred in response to immunosuppressive agents, such as glucocorticoids, in the absence of new infections [39–41].

Although immunological and biochemical stresses slow its replication, a significant portion of *M. tb* continues to replicate throughout infection in mouse models [9, 42]. This implies that the bacillary number in the NRP state is largely maintained by balancing the number of replicating, non-replicating, and dying bacilli at the site of infection under the active immune system in a state of phenotypic heterogeneity. Within granulomas, *M. tb* is trapped inside macrophages or

in extracellular niches at necrotic centers of granulomas. *M. tb* can also be found in the upper lobe of the lung on the inner cavity epithelium, where they can exit the NRP state [43–46]. Thus, the physiological understanding of *M. tb* bacilli in various phenotypic states is the unmet clinical and scientific need to develop more powerful therapeutic interventions.

As a unicellular organism, diversity is key for *M. tb* to survive harsh conditions [47, 48]. Diversity in a microbial population has been considered an important strategy to maximize adaptation in rapidly changing environments through metabolic flexibility and mutual interaction with neighboring siblings. *M. tb* always retains a level of metabolic diversity; subpopulations that encode identical genetic information can exhibit phenotypic heterogeneity [9–11, 49, 50]. This strategy allows *M. tb* to secure its species as a bet-hedging tactic [51–53]. This heterogenous population is typically composed of a random mixture of phenotypic states from fully replicating to completely NRP [53, 54]. Consequently, *M. tb* relies on nongenetic mechanistic strategies, including optimization of cellular fitness and metabolic shifts, which are difficult to elucidate by analyzing a single set of biological molecules.

Whole-genome sequencing was first conducted to uncover the *Haemophilus influenzae* genome in the mid-1990s [55, 56]. This served as a milestone in systems biology, but the volume of data went beyond a complete interpretation at the time. Bioinformatic tools have been co-developed to understand relevant genetic information among copious data. Details on transcription of genetic information to mRNA, subsequent translation into proteins, and finally, the substrates and products of protein complexes all must be included to reach a more comprehensive representation of the data. The nuanced intermediates involved in metabolic networks are all crucial to holistically understanding microbial pathogenesis [57–59].

This chapter provides the characteristics associated with phenotypic heterogeneity of *M. tb* that have been validated by the multiomics approach. We focus on the metabolic activities essential for survival in each major phenotypic state: replicating, NRP, and reactivation. All cellular processes stem from the foundation of metabolism, thereby making metabolism an explication of pathogenicity. This perspective will direct efforts in a more sophisticated way to better develop effective treatments and strategies to address the *M. tb* pandemic and quell its widespread transmission.

2 Multiomics Integration to Study *M. Tuberculosis* Pathogenesis

2.1 Multiomics Technology as a Window to Visualize *M. Tuberculosis* Physiology

The whole-genome sequence of *M. tb* H_{37}Rv was first published in 1998 [60]. This genomics study identified over 3900 open reading frames, of which less than 50% were annotated with known functions. Manipulating gene function using gene

knockouts aided in defining the essential pathways for *M. tb* growth and adaption to adverse environments [61, 62]. Single-gene knockout methods have been used alongside global methods, such as transposon (Tn) mutagenesis, to inactivate genes. Tn libraries were generated by random insertion within the *M. tb* genome and monitored for essentiality in the presence of selective conditions. DNA microarrays were then used to map Tn abundance, termed Tn site hybridization (TraSH). Sassetti et al. applied the TraSH method using a bacteriophage vehicle and successfully identified 614 genes essential for in vitro growth [63, 64]. These genes are involved in biosynthesizing amino acids, cofactors, and nucleic acids, including many unknown functions.

Conditional expression methods use an inducible promotor (TetON or TetOFF) to investigate genes' essentiality and functions. This strategy was used by Schnappinger et al. to control 474 genes by Tet regulation which identified greater than 8.5 million chemical-genetic interactions [65–67].

More recently, Clustered Regularly Interspaced Short Palindromic Repeats Interference (CRISPR*i*) was applied to essential gene screening in mycobacterial genomics [68–70]. This system works by targeting dCas9 nuclease, with nonfunctional nuclease activity, to *M. tb* genes with a sgRNA (single guide RNA). When the dCas9-sgRNA complex is formed, transcription of targeted genes is stalled by sterically blocking access of RNA polymerase to the promotor. The level of gene silencing is determined by sgRNA length and sequence, which makes it possible to study the impact of essential gene depletion where traditional approaches to completely knock out genes would be lethal to *M. tb*. The CRISPR*i*-dCas9 system was also used to generate an approximately 90,000 sgRNA library, enabling high-throughput platforms to screen for essential genes [68]. Rock et al. used sgRNA to make *M. smegmatis* folate metabolism hypomorphs to show the essentiality of this pathway for replication [69]. CRISPR*i* is a useful tool for genetic studies, but a potential caveat is the off-target effects from nonspecific interactions of dCas9.

Studying the *M. tb* transcriptome adds another avenue to connect genomic information [71]. Transcriptomics can take snapshots of gene expression levels in *M. tb* during adaptation to various environments, making it an excellent method to explore *M. tb* pathogenesis. Beyond understanding *M. tb* physiology, transcriptomics has been useful in assigning gene function and essentiality, discovering drug targets, and exploring an antimicrobial's mode of action [72–75]. Transcriptomics has identified the intermediary pathways required by intracellular *M. tb* to utilize host lipids: the β-oxidation pathway, glyoxylate shunt, methylcitrate cycle (MCC), and cholesterol metabolism [65, 76–81]. Another transcriptomics study found induction of glutamate synthase (GltB) and glutamate decarboxylase (GadB) as an adaptive strategy to counteract intracellular acidification arising from MCC activation [82]. Transcriptomics profiling of in vitro NRP models revealed significant remodeling in electron transport chain (ETC) activities [83, 84]. Combining transcriptomics with gene essentiality datasets offers a beginning to the multiomics understanding of TB pathogenesis.

Metabolomics is the holistic evaluation of metabolite networks in an organism or system of biological study. It is crucial for understanding the metabolic processes underlying *M. tb* adaptation to adverse environments [57, 85–90]. Carvalho et al. aerobically grew *M. tb* in the presence of fully ^{13}C labeled glycerol, acetate, or glucose and analyzed the ^{13}C enrichment of each metabolite in central carbon metabolism (CCM) pathways [91]. They demonstrated that *M. tb* could co-catabolize multiple carbon substrates simultaneously through glycolysis and the tricarboxylic acid (TCA) cycle. This co-catabolism is mediated by the compartmentalization of each carbon component to a distinct metabolic direction [92]. A study led by Agapova et al. observed that *M. tb* could also utilize multiple amino acids as nitrogen sources [93]. Serafini et al. found a new role of MCC as a source of propionyl-CoA and cell wall lipids through the reverse direction by assimilation of pyruvate and lactate [94]. Additionally, Dutta et al. used metabolomics to compare wild-type and Rel-deficient *M. tb* to verify that the Rel stringent response regulator functions in *M. tb's* transition to the non-replicating, quiescent NRP state [95]. As such, metabolomics defines metabolic networks and helps pinpoint the essential pathways used in the adaptive strategies of *M. tb*.

2.2 Metabolic Networks of *M. Tuberculosis* in Diverse Phenotypic States

2.2.1 Actively Replicating State

Permissive Carbon Sources

Intracellular pathogens acquire nutrients needed to generate energy and biomass from the host [96]. It is technically challenging to study intracellular *M. tb* physiology due to the indirect nature of all experimental trials; earlier methods to probe main carbon sources largely relied on infection with auxotrophs [97]. Zimmerman et al. integrated information from metabolomics and transcriptomics data to reveal that intracellular *M. tb* utilizes up to 33 different nutrients from the host, of which three are solely used for biomass and the remaining 30 for ATP biosynthesis [98]. These included various subclasses of lipids such as monoacylglycerols and phosphatidylinositol phosphate, suggesting that *M. tb* is exposed to various nutrients within macrophages.

The foregoing multiomics integration of intracellular *M. tb* datasets pinpointed cholesterol as one of the most prevalent permissive carbon sources by detecting 4,5-9,10-diseco-3-hydroxy-5,9,17-tri-oxoandrosta-1(10),2-diene-4-oic acid (DSHA) accumulation. Since DSHA is a non-mammalian metabolite, it is degraded from cholesterol by *M. tb*. Supporting this, during infection, they observed host cholesterol levels decrease by $\sim$ 80% and depletion of other cholesterol derivatives [99].

Simultaneous consumption of multiple carbon and nitrogen sources illustrates that *M. tb* has evolved highly modular metabolic networks that adapt to nutritionally adverse environments. Genomics studies have demonstrated that *M. tb* lacks the classical phosphotransferase (PTS) and carbon catabolite repression

(CCR) system–canonical adaptive strategies used to consume external carbon nutrients, especially by gram-negative and gram-positive bacteria [60, 91]. Hence, the metabolic network topology of *M. tb* is evolutionarily designed for optimal and unique pathogenesis.

Cholesterols

Although *M. tb* can simultaneously co-catabolize multiple carbon and nitrogen sources, accumulating genomics studies designate cholesterol as the most probable carbon source for *M. tb* growth and even survival in the NRP state from various in vitro and in vivo models [100–102].

Mce (mammalian cell entry) membrane proteins (Mce1-4) import lipid substances into *M. tb* (Fig. 1). Pandey et al. identified Mce4 as the major import route of cholesterol [103]. Mce4 is thought to confer *M. tb* pathogenesis by promoting its intracellular growth, whereby *mce4* deficient *M. tb* replicates poorly in media with cholesterol as a sole carbon substrate and in mice [104]. Multiple transcriptomics and proteomics analyses showed significantly higher expression of *mce1–mce4* transcripts and their protein levels in later stages of growth than in the early exponential stage, indicating growth phase-dependent functionality of Mce proteins. The functional essentiality of Mce4 was supported by the enhanced expression level of *mce4* in *M. tb* while residing within macrophages [105].

Once imported, *M. tb* degrades cholesterol to exclusively biosynthesize pyruvate, acetyl-CoA, propionyl-CoA, and succinyl-CoA, all precursor substrates of CCM pathways (Fig. 1) [96]. Combining metabolomics and transcriptome analysis by RNA-Seq from *M. tb* during infection determined overlapping metabolic networks, enzymes, and metabolites, several of which belonged to cholesterol degradation pathways [98, 106]. The final products of cholesterol degradation are now acknowledged as key metabolic intermediates required to fuel *M. tb* CCM, including the TCA cycle, glyoxylate shunt, MCC, methylmalonyl-CoA pathway, and gluconeogenic pathway (Fig. 1) [107, 108]. Propionyl-CoA is an initial substrate for the methylmalonyl-CoA pathway and MCC [109]. Biosynthesis of methyl-branched polyketide lipids and long-chain fatty acids occurs in the methylmalonyl-CoA pathway. The methylmalonyl-CoA and MCC pathways fuel CCM intermediates, thereby balancing toxic effects associated with cholesterol consumption, propionyl-CoA accumulation, and biosynthesis of essential intermediary metabolites of the TCA cycle [81, 109, 110]. Indeed, *M. tb* bacilli deficient in isocitrate lyase (ICL), the last enzyme in MCC, stopped replication and died from an accumulation of toxic intermediates and starvation of essential TCA cycle intermediates [80, 81]. Isotope metabolomics of *M. tb* deficient in ICL (Δ ICL) proved this vulnerability by showing that metabolic defects in Δ ICL stemmed from the conversion of the MCC into a dead-end pathway. Subsequent accumulation of MCC intermediates, such as 2-methylcitrate and 2-methylisocitrate, led to altered NADH/NAD + ratio, membrane potential, and intracellular acidification [81].

Vitamin B12-dependent shunting of propionyl-CoA through the methylmalonyl-CoA pathway minimizes the MCC intermediate-mediated toxicity while biosynthesizing lipids including sulfolipid-1 (SL-1) and phthiocerol

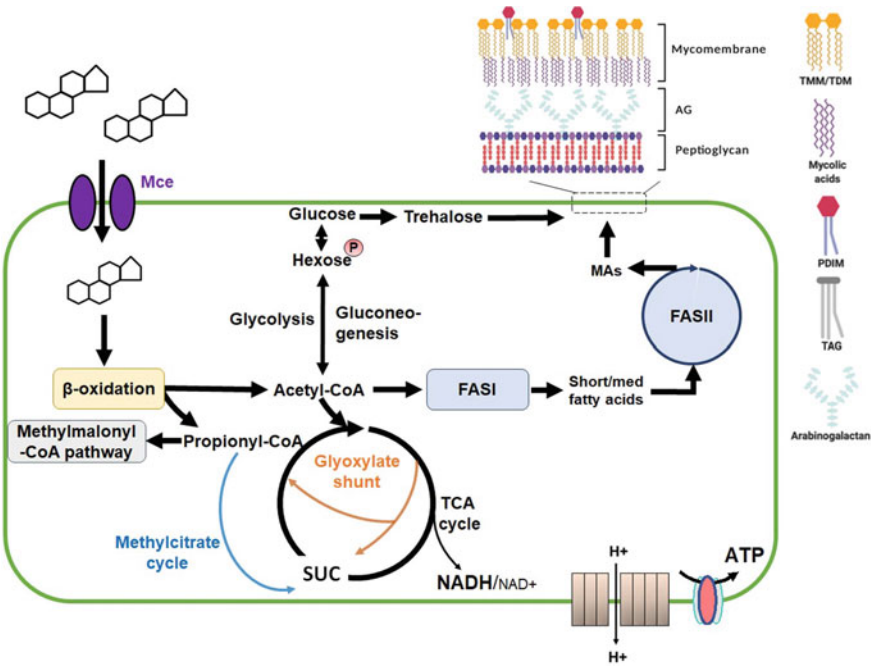


Fig. 1 Metabolic networks of replicating *M. tb*. *M. tb* membrane protein, Mce 4 (Mammalian Cell Entry), is used to import cholesterol. Cholesterol serves as a substrate of β-oxidation that leads to the biosynthesis of acetyl-CoA, propionyl-CoA, and pyruvate. These metabolites are substrates of the TCA cycle, glyoxylate shunt, methylcitrate cycle, methylmalonyl-CoA pathway, and gluconeogenesis. Acetyl-CoA is a substrate of the TCA cycle and subsequent production of NADH, a reduced electron carrier required for the production of ATP via electron transport chain (ETC). The set of FASI and FASII activities is required for the biosynthesis of mycolic acids (MA). Mycolic acids are condensed with trehalose to produce trehalose dimycolate (TDM), one of the most prevalent PAMPs (pathogen-associated molecular pattern). Hexose P, glucose phosphate and its isomers; MAs, mycolic acids; TMM, trehalose monomycolate; AG, arabinogalactan; SUC, succinate; TAG, triacylglycerol; PDIM, phthiocerol dimycocerosate. (*Created with BioRender.com*)

dimycocerosate (PDIM) [81, 82, 109]. *M. tb* enhances the biosynthesis of PDIM and SL-1 when residing inside the host by coupling to catabolic cholesterol metabolism. This metabolic channeling is associated with *M. tb* pathogenesis because PDIM is known to aid in the recruitment and invasion of macrophages at the site of infection and help evade immune responses by masking cell surface PAMP (pathogen-associated molecular pattern) molecules [111, 112].

Lee et al. performed metabolomics analysis with BCG (Bacillus Calmette–Guérin), a live TB vaccine strain, and *M. tb* H_{37}Rv after culturing in media containing propionate or inside the macrophages [82]. The results supported an essential role of MCC in optimal intracellular replication and *M. tb* pathogenesis. They also observed an increase in glutamate synthase (GltB/D) activity, converting

glutamine to glutamate and GABA (γ-aminobutyric acid), as a metabolic effort to neutralize MCC intermediates and propionyl-CoA mediated toxicity. Indeed, increased GltB/D activity improved the survival of Δ ICL cultured in media containing propionate. Metabolic networks in *M. tb* are interconnected such that MCC intermediates or propionyl-CoA mediated toxicity on ETC and bioenergetics can be corrected by GltB/D activity in nitrogen metabolism, an example of the process termed metabolic adaptation. Collectively, the metabolic plasticity of *M. tb* required for permissive carbon uptake and consumption is thus accepted as a hallmark of its pathogenesis.

Antibiotic Targets of Actively Replicating *M. Tuberculosis*

Mycolic Acid Synthesis

Isonicotinic acid hydrazide, or isoniazid (INH), has been studied extensively and is a main component of the conventional first-line TB drug regimen. INH is significantly more potent in killing replicating *M. tb* than all other TB antibiotics [113–116]. The antimicrobial kinetics of INH was delayed by around four days, during which accumulated INH could not be washed from INH-susceptible *M. tb* while it could be washed away from resistant bacilli [116]. The accumulation of INH within INH-susceptible strains was accompanied by a wrinkled cell surface phenotype and a loss of its acid-fastness, a bactericidal phenotype specifically shown after INH treatment. Jacobs et al. characterized *M. smegmatis* mutants resistant to INH to find the drug target to be InhA, an NADH-dependent enoyl-ACP reductase [117–119]. InhA is part of the type II fatty acid synthesis pathway (FAS II) for mycolic acid biosynthesis (Fig. 1). The missense mutations found in these mutants at inhA and *katG* (catalase-peroxidase) conferred an order of magnitude increase of INH minimal inhibitory concentration (MIC). They could be rescued by replacing wild-type *inhA* through allelic exchange [120]. Inversely, the degree of INH resistance can be predicted by identifying the mutations of *inhA*, although missense mutations at other genes, including *kasA* or *katG,* must also be considered. A study confirmed that INH specifically inhibits the InhA step in the FAS II pathway by two-dimensional clustering of whole protein expression profiles of *M. tb* after INH treatment. Wilson et al. used a microarray hybridization to confirm that the mode of action of INH is associated with FAS II by showing operonic clustering of the genes encoding FAS II (Rv2243–Rv2247) and FbpC (Ag85C) as interacting partners.

INH effects on *M. tb* mycolic acid biosynthesis and its specificity to actively replicating *M. tb* suggest that the metabolic activities linked to mycolic acid biosynthesis are essential for *M. tb* replication and survival.

Membrane Energetics

Energy production from the ETC occurs via oxidative phosphorylation (OXPHOS), a ubiquitous and essential metabolic pathway for *M. tb* replication [121, 122]. Nutrients are used to fuel OXPHOS and subsequently generate an electrochemical gradient, called the proton motive force (PMF), that serves as a major source of

intracellular protons and ATP by driving ATP synthases (Fig. 1). In *M. tb*, the generation of the PMF is mediated by the proton-pumping components of ETC, including cytochrome bc1-aa3 complex and, albeit a less exergonic reaction, cytochrome bd oxidase [84, 123, 124]. Recently, the discovery of small molecules targeting proton-pumping components in *M. tb* ETC has targeted the cytochrome bc1 subunit [125–127]. The lead compounds to date are a series of imidazopyridine amides. The most promising drug candidate of the series, Q203, is currently in phase II clinical trials under the FDA Investigational New Drug Application [128].

The discovery of bedaquiline (BDQ), a mycobacterial F1F0-ATP synthase inhibitor, validated OXPHOS as an essential component for survival in the replicating state [129–131]. BDQ became an important TB drug to treat multidrug-drug-resistant (MDR) or extensively drug-resistant (XDR) TB patients. BDQ showed an unusually slowed time-dependent killing termed as weak-early bactericidal activity, where the four $\log_{10}$ reductions in bactericidal phase just began four days after treatment following a period of bacteriostatic activity only [129, 132–135]. Transcriptomics and proteomics analysis of *M. tb* treated with BDQ observed induction of around 39 genes that belong to the dormancy regulon [135]; these genes may function to counteract damaged F1F0-ATP synthase, which would explain the temporal maintenance of bacteriostatic viability during initial exposure. Recent genetics studies using *Staphylococcus aureus* and *Escherichia coli* showed that ATP depletion is a signal that triggers bacterial dormancy and persistence [136–140]. The early bactericidal activity of BDQ differs depending upon the carbon substrates available in the media. Fermentable energy sources can be consumed for ATP biosynthesis through glycolysis or OXPHOS. However, *M. tb* cannot break down fatty acids by glycolysis, so in this case, ATP production solely relies on OXPHOS. *M. tb* displayed increased susceptibility to BDQ when cultured in media containing fatty acids as the only source of carbon [135]. A recent metabolomics study led by Wang et al. validated BDQ's effect on *M. tb* in a replicating state with the combination of direct ATP biosynthesis inhibition via interfering with F1F0-ATP synthase activity and indirect metabolic consequences arising from hundreds of annotated ATP-dependent reactions including glutamine synthase activity [141].

2.2.2 Non-Replicating Persistent State

Alternate Carbon Sources

NRP *M. tb* downregulates activities of key components in the ETC, which decreases OXPHOS activity and ATP biosynthesis to approximately 10% to that of its replicating counterparts [83, 142, 143]. The notable characteristic of NRP *M. tb* is its phenotypic drug tolerance or nonheritable resistance, which allows persistence under effective chemotherapies [31, 144–147]. This is largely because antibiotics that effectively kill replicating *M. tb* are no longer effective in killing NRP *M. tb* (Tables 1 and 2). For this reason, the physiology of NRP *M. tb* has emerged as a central feature of its pathogenesis [26]. Sarathy et al. used an established in vitro nutrient starvation NRP model to measure the intracellular level of diverse anti-TB

Table 1 Antibiotics targeting actively replicating *M. tb*

Sites of action	Antibiotics	Mechanisms of action
Cell wall	Isoniazid	Inhibits nicotinamide adenine dinucleotide (NADH)-specific enoyl-acyl carrier protein (ACP) reductase involved in the fatty acid synthesis
	Ethambutol	Inhibits arabinosyl transferases involved in cell wall biosynthesis
	D-Cycloserine	Inhibits synthesis of peptidoglycan and cell wall maintenance
	Ethionamide	Inhibits the *inhA* gene product enoyl-ACP reductase
	Prothionamide	
	SQ109	Inhibits the MmpL3 TMM exporter
DNA replication	Moxifloxacin	Inhibits the ATP-dependent enzymes topoisomerase II (DNA gyrase) and topoisomerase IV
	Gatifloxacin	
	Levofloxacin	Inhibits the DNA-gyrase, which in turn inhibits the relaxation of supercoiled DNA
Transcription	Rifampin	Inhibits the activity of the essential *rpoB* gene product β-subunit of DNA-dependent RNA polymerase
	Rifabutin	
	Rifalazil	
	Rifapentine	
Translation	Amikacin	Inhibits protein synthesis by binding to the conserved A site of 16S rRNA in the 30S ribosomal subunit
	Kanamycin	
	Streptomycin	
	Capreomycin	Inhibits protein synthesis by interacting with the ribosome
	Clarithromycin	Inhibits protein synthesis by binding to the 50S ribosomal subunit
	Linezolid	Inhibits protein synthesis by binding to 23S rRNA in the early phase to prevent proper binding of formyl-methionine tRNA
Cytoplasmic process	Para-aminosalicylic acid (PAS)	Acts as a metabolic precursor (prodrug) that generates a toxic dihydrofolate analog that subsequently inhibits DHFR (dihydrofolate reductase) activity
ATP synthesis	Bedaquiline	Inhibits mycobacterial F1F0-ATP synthase by binding to proton pump subunit

antibiotics such as fluoroquinolones, rifampin, and linezolid; they found that penetration in NRP state bacilli was significantly lower than in replicating bacilli [148–150]. Efflux pump inhibitors were used to confirm that reduced accumulation of antibiotics within the bacilli was independent of efflux processes [151]. Recent ^{13}C isotope metabolomics studies using the in vitro biofilm culture system also showed that the relationship between the enrichment of NRP populations and the ATP biosynthesis and consumption rate of external carbon sources is inversely

Table 2 Antibiotics targeting non-replicating persistent (NRP) *M. tb*

Antibiotics	Mechanisms of action
Bedaquiline	Inhibits mycobacterial F1F0-ATP synthase by binding to proton pump subunit
Pretomanid (PA-824)	Generates the ROS
AM-0016	Causes cell envelope damage and rapidly collapses membrane potential (A novel xanthone-based antibacterial)
Pyrazinamide	Pyrazinamide enters bacteria and is converted into POA. Under acidic pH, POA accumulates inside the cells and causes a deenergized membrane and acidification of the cytoplasm
Rhodanine (D155931)	Targets dihydrolipoamide acyltransferase (DlaT) activity
TCA1	TCA1 is activated by decaprenyl-phosphoryl-β-D-ribofuranose oxidoreductase DprE1 and MoeW, enzymes involved in the cell wall and molybdenum cofactor biosynthesis

proportional [34]. Similar findings were observed in otherwise permissive *M. tb* adapting to hypoxic conditions [142, 143], suggesting that NRP *M. tb* can withstand adverse environmental stresses by depleting its ATP level to restrict the uptake of external antibiotics and carbon sources.

Existing knowledge of *M. tb* in an NRP state derives from experimental models of varying physiologic relevance and, despite controversial debates, almost all evidence includes alteration in ETC activity. Even NRP *M. tb* needs a basal metabolic activity level sufficient to maintain an energized membrane potential, PMF, and core cellular processes [83, 142, 152]. For example, when biosynthesizing low levels of ATP, NRP *M. tb* must continuously recycle the reducing equivalent, NAD^+, to maintain the PMF. A study using isotopologue analysis and metabolomics examined the change of CCM pathway intermediates during transitioning into NRP state [142, 152], confirmed by a significant change in the NADH/NAD^+ ratio. As a compensatory mechanism, NRP *M. tb* initiates a fermentative respiratory strategy by net incorporation of CO_2 and active secretion of succinate by activating a reversed TCA cycle (Fig. 2). Another metabolomics work measured TCA cycle intermediates during the NRP state metabolism shift to show the critical role of succinate. However, fatty acids were provided to mimic conditions relevant to the host. Eoh et al. discovered that the glyoxylate shunt mediates hypoxia adaptation by contributing to the biosynthesis of succinate and glycine as an end product of the glyoxylate shunt, which is divergent from its traditional role in fatty acid metabolism required for active replication (Fig. 2) [142].

Transcriptomics studies using nutrient starvation identified the general depletion of genes associated with aerobic ETC components, suggesting metabolic changes that are similar to those during hypoxic adaptation [153–155]. These include upregulation of ICL and fumarate reductase with downregulation of F1F0-ATP synthase and type I NADH dehydrogenase (Ndh1). Separate transcriptomics and

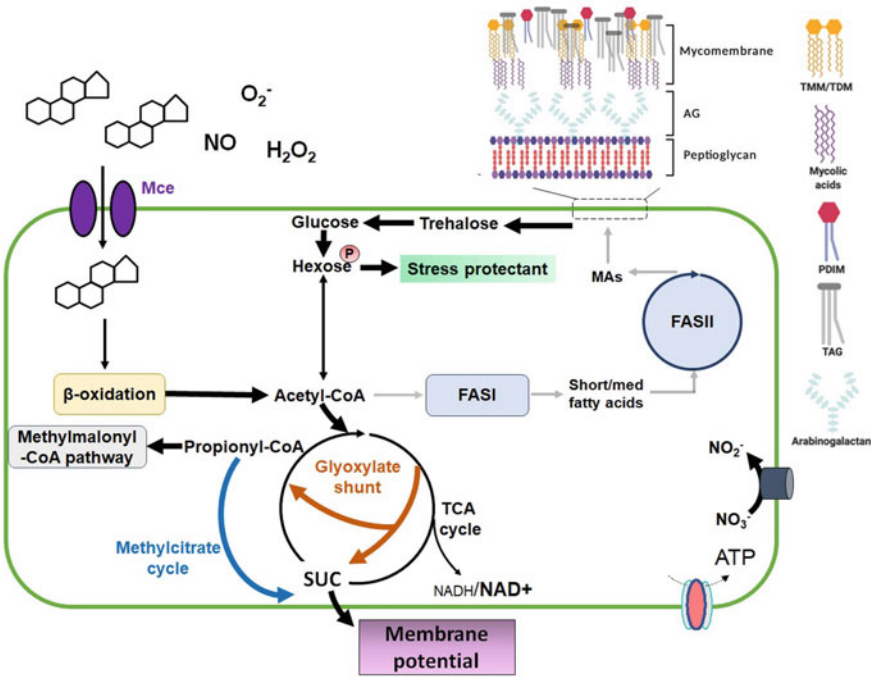


Fig. 2 Metabolic remodeling occurring within NRP *M. tb*. When adapting to biochemical stresses, *M. tb* restricts the uptake of external carbon sources, including cholesterol, while depleting its ATP level. Host fatty acids are still used as the primary source of the glyoxylate shunt, methylcitrate cycle, and methylmalonyl-CoA pathway. Methylmalonyl-CoA pathway contributes to host immune evasion through biosynthesis of phthiocerol dimycocerosate (PDIM). Meanwhile, NRP *M. tb* utilizes preexisting trehalose to fuel glycolysis and the pentose phosphate pathway (PPP) as an alternate source for ATP and antioxidants. Shunting trehalose towards the biosynthesis of glycolysis and PPP intermediates results in depletion of TDM content, which allows for the stealth invasion under an intact immune system. Without O_2, nitrate (NO_3) or fumarate can be an alternate electron acceptor for NRP *M. tb*, but none of them could support *M. tb* growth. Extra-biosynthesis of succinate (SUC) is secreted as an end product, which serves as an electrogenic process used to maintain membrane potential and ATP biosynthesis(*Created with BioRender.com*)

proteomics studies of *M. tb* following treatment with INH and linezolid displayed dysregulated pathways, including lipid metabolism, cell wall processes, intermediary metabolism, and ETC activity [156–158]. The expression profile of genes in CCM and ETC suggests a significant overlap in the adaptive strategies used by NRP *M. tb* in response to nutrient starvation, hypoxic environment, or even antibiotic treatment [156, 159]. Multiple studies of chronic phase *M. tb* infection in mice and humans showed altered gene expression in *aceA*, *narK2*, *nuo*, *nadC*, *menA*, *lld2*, and *ppdK*, involved in respiration, and *aceA* and *echA15*, involved in lipid metabolism, that paralleled results found from in vitro hypoxia, nutrient starvation, or antibiotic treatment models. The significant overlap of genes

identified from all of these studies is largely restricted to the ETC, indicating that the ETC is a general sensor of stresses. We speculate that NRP *M. tb* uses ETC activity to sense environmental stresses and then restrict the penetration of toxic metabolites for securing survival through adaptive metabolism. Accordingly, NRP *M. tb* can endure significantly limited support from external nutrients.

Two independent metabolomics studies, using NRP *M. tb* recapitulated under the in vitro biofilm system and hypoxia, validated the contribution of preexisting, intrinsic *M. tb* metabolites as an alternate carbon source [34, 143]. Metabolic profiling specific to NRP *M. tb* and multivariate pathway mapping ranked the trehalose metabolism and glycolysis as the two top pathways. Targeted metabolomics showed the depletion of trehalose with a reciprocal accumulation of glucose phosphate, an initial substrate for intermediates of the pentose phosphate pathway (PPP) and glycolysis. This suggests that preexisting trehalose is an internal source of carbon substrates to fuel PPP and glycolysis while transitioning into NRP *M. tb*. The PPP and glycolysis are replenished by preexisting trehalose that serves as an alternative source of ATP, NADPH, and antioxidants to compensate for ATP depletion and limited external carbon sources (Fig. 2). Genetic experiments to interfere with trehalose carbon consumption confirmed that the lack of catabolic trehalose activity invoked *M. tb* hypersensitivity to INH and BDQ.

The Center for Disease Control and Prevention (CDC) predicted that lengthy TB treatment could be shortened significantly by the addition of antimicrobials that target the NRP subgroup within phenotypically heterogeneous populations of *M. tb* [160]. Known stringencies include nutritional deficiency, ROS, RNI, acidic, hypoxic, and membrane perturbing stresses. Understanding the essential cellular processes during NRP transition in response to the stringent environments will provide insights into the desperately needed improvements to existing TB chemotherapies.

Trehalose

Transcriptomics and proteomics studies commonly indicate trehalose metabolism as one of the essential activities required for survival of NRP *M. tb* [161–163]. Trehalose is a natural, nonreducing glucose disaccharide with an α, α-1,1-glycosidic linkage [α-D-glucopyranosyl-(1 → 1)-α-D-glucopyranoside] [164]. Soon after its discovery, trehalose has been appreciated as a bioprotectant of stress in many biological systems.

Trehalose in *M. tb* is most widely recognized as a structural component of cell wall glycolipids, like trehalose dimycolate (TDM; cord factor) that also performs essential immunomodulatory functions [34, 165–167]. Another example is SL-1 which has major implications for the pathogenesis and transmission of TB [168, 169]. It was found that trehalose auxotrophs of *M. smegmatis* lose their viability in media lacking free trehalose, suggesting the important role of free trehalose in *M. tb*. Free trehalose has been suspected of having two main functions: stress protection and intracellular carbon storage. *M. tb* likely uses trehalose in both ways. In a replicating state, *M. tb* utilizes trehalose as a carbon source and as a substrate for the biosynthesis of cell envelope glycolipids such as TDM and SL-1. A recent

metabolomics study confirmed that trehalose could also serve as an intracellular storage compound that is internally mobilized during the transition to the NRP state (Fig. 2) [34, 143]. Furthermore, shunting trehalose towards the biosynthesis of CCM intermediates for energy production resulted in depletion of TDM and, presumably, SL-1 content. Indeed, the incubation of NRP *M. tb* cells fixed with paraformaldehyde and mouse bone-marrow-derived macrophages (BMDM) showed the diminished ability of induced pro-inflammatory cytokine secretion by BMDM [143]. This result correlates immunoreactivity with the shift in *M. tb* trehalose metabolism during NRP state survival.

Antibiotic Targets of Non-Replicating *M. Tuberculosis*

Altered Membrane Bioenergetics

The essentialities of bioenergetics and the ETC have been proven in studies with NRP *M. tb* adapting to hypoxia. *M. tb* is an obligate aerobic bacterium, but mounting experimental evidence demonstrates that within granulomas, where O_2 content is below 1% and nitric oxide (NO) content is dramatically elevated, both intracellular and extracellular spaces serve as niches that are colonized by *M. tb* [146, 170–172]. Microbiological and biochemical studies suggest that *M. tb* cannot replicate under hypoxic conditions; however, by shifting its metabolism, it can survive as non- or slowly replicating for decades when oxygen content is at even below 0.06% (NRP phase 2 in Wayne in vitro hypoxia model) [26, 173]. Genetic studies showed upregulation of proline dehydrogenase in NRP *M. tb* adapted to nutrient starvation [154, 174, 175]. Proline dehydrogenase and pyrroline-5-carboxylate dehydrogenase are involved in proline degradation to biosynthesize glutamate and transfer four electrons to the ETC. In these steps, FAD is reduced to $FADH_2$, which is coupled to the reduction of NAD^+ to NADH, an important electron donor for NRP *M. tb*.

M. tb modulates oxidase expression to adapt its ETC according to varying oxygen availability [176, 177]. Under low oxygen conditions and high NO content, while transitioning into NRP state, cytochrome bd oxidase expression is upregulated [174]. Transcriptomics of intracellular *M. tb* showed downregulation of Ndh1, ATP synthase, and menaquinol-cytochrome c oxidase compared to those of replicating counterparts to affirm less demand for generation of energy in the NRP state. On the other hand, nitrate oxidase, Ndh2, and fumarate reductase were shown to be upregulated in the NRP state, suggesting that NRP *M. tb* maintains the ETC and OXPHOS by shifting the functional activity of its components. Sustaining ETC activity is critical for energy generation and recycling of NAD^+ in the NRP state.

Alternate electron acceptors are required for NRP *M. tb* metabolism when O_2 is not present. Nitrate (NO_3) and fumarate have been studied as potential alternatives for NRP *M. tb* electron acceptors, but none of them could support the *M. tb* growth (Fig. 2) [83, 152, 178, 179]. This finding suggests that in the absence of O_2, the ETC in NRP *M. tb* plays a more critical part in redox homeostasis and disposal of reducing equivalents. Biosynthesis of NO_3 by oxidation of NO and NarK mediates the import of NO_3. Intriguingly, the expression of *narK2* and *narU* is upregulated in anaerobic conditions to secure an alternative electron acceptor. Transcriptional

control of *narK2* is regulated by the DosR/DevR dormancy regulon, which is influenced by environmental changes in O_2, NO, and CO levels. The *M. tb* genome contains the *narGHJI* (Rv1161-Rv1164) operon, which encodes a putative membrane-bound nitrate reductase complex. Expression of this operon is not upregulated under hypoxic or nutrient starvation conditions in *M. tb*. An alternate role of NarGHJI is to maintain the redox balance in NRP *M. tb* adapted to hypoxia. Sohaskey et al. have shown that NO_3 helped *M. tb* rapidly adapt to oxygen depletion [43, 180, 181].

Recent metabolomics studies using ^{13}C tracing experiments showed a TCA cycle shift in NRP *M. tb* induced by hypoxia, provoking the biosynthesis of extra succinate as a product of active secretion [142, 152]. The overproduction and secretion of succinate are essential to maintain the NRP viability by acting as an anaplerotic precursor to downstream TCA cycle intermediates and serving as an electrogenic process to maintain membrane potential and ATP biosynthesis (Fig. 2). The essentiality of succinate for membrane bioenergetic function in NRP *M. tb* was proved by in vitro and mouse model studies of succinate dehydrogenase (Sdh1), the membrane anchor for the second ETC component [179, 182–185]. The essentiality of ETC, membrane bioenergetics, and accompanied redox homeostasis is supported by the clinical outcomes of BDQ, delamanid (OPC-67683), and pretomanid (PA-824), which cause ETC poisoning by the accumulation of NO from deazaflavin dependent nitroreductase (Ddn) activity, thereby effectively killing NRP *M. tb* [146, 186–189]. Similar antimicrobial activity targeting NRP *M. tb* with the aforementioned compounds has also been observed in both animals and humans [190] (Table 2).

2.2.3 Exiting from the Non-Replicating Quiescent State

Carbon Sources

Integration of molecular typing with epidemiological studies proved that in most cases, the same strain was responsible for both initial infection and disease relapse found in low-TB burdened areas, suggestive of reactivation. On the other hand, reinfection was implicated in higher-TB burdened countries where various strains were associated with disease relapse [191–193]. Latently infected hosts will produce a positive TB skin test, although they have no clinical symptoms. These latently infected individuals that are immune-competent have a 10% risk of disease reactivation during their lifetime. This chance increases in individuals with a compromised immune system. Common examples of immune systems impairments include co-infection with HIV and/or treatment with immunosuppressive agents, such as TNF-neutralizing drugs. An estimated one-fourth of the world population is latently infected, a huge reservoir for possible reactivation [194, 195].

Some useful in vivo reactivation models of *M. tb* include rabbits and guinea pigs. Cost-effective in vitro experimental strategies have been attempted; nonetheless, knowledge about NRP reactivation and underlying mechanisms for NRP transition to replicating state is limited. Most multiomics studies of reactivation have employed the NRP *M. tb* adapted to hypoxia [196, 197]. Transcriptomic analysis

using this process showed that reactivation altered several pathways, such as restoration of DosR regulon-mediated transcriptional remodeling, DNA repair, ETC activity, and cell wall biosynthesis. Interestingly, a recent biochemical study provoking NRP *M. tb* reactivation by K^+-limiting conditions showed a transcriptional activation in two stages:

i. initial de novo mRNA synthesis, activating cell defense mechanisms and lipid metabolism;
ii. secondary active cell proliferation through inducing central metabolism reactions [198].

Although we intuitively assume the transition from NRP state to active replication is reflected by resuming bacterial growth, we cannot assume the metabolic activities required to exit from the NRP state should be identical to those required in the replicating state. Two recent metabolomics studies showed accumulation of glycolysis, aminosugar biosynthesis, and PPP intermediates in NRP *M. tb* adapted to hypoxia with a decrease in the upstream disaccharide, trehalose [34, 143]. These accumulations were later proven to promote de novo peptidoglycan synthesis for opportunistic reactivation (Fig. 3). This study demonstrated that the accumulation of these metabolites acts as an anticipatory metabolic action to provide a sufficient carbon source required for successful reentry into the normal cell cycle.

A separate metabolomics study of NRP *M. tb* under hypoxia also validated the role of succinate as a potential carbon source used to exit the NRP state (Fig. 3) [142, 152]. Some of the unused succinates are stored, which may later be used to quickly resume carbon flow downstream of the TCA cycle accompanied with NADH and ATP synthesis upon reaeration. Since the succinate/fumarate redox couple midpoint potential is almost neutral ($\varepsilon^{0`} = +0.03$ V), it can help redox balance by fermentation product accumulation through fumarate reductase. Succinate serves as the bridge between fermentative and oxidative metabolic states by its wide range of ability to maintain ATP synthesis and membrane potential and feed the TCA cycle.

Potential Antibiotic Targets Preventing Reactivation of Non-Replicating *M. Tuberculosis*

Succinate Dehydrogenase

Succinate oxidation is coupled to quinone reduction by succinate dehydrogenases (Sdh). Sdh assists in the biosynthesis of TCA cycle intermediates and the generation of the PMF. There are two Sdh complexes found in the *M. tb* genome: Rv0249c-Rv0247c (Sdh1) and Rv3316-Rv3319 (Sdh2) [179, 182]. Sdh complexes have been an attractive target for antifungal agents, including oxadiazole carbohydrazide and thiazole carboxamide [199, 200]. Recent metabolomics and biochemical studies showed that 3-nitropropionate (3NP) is an inhibitor of *M. tb* Sdh by targeting the dicarboxylate-binding site of the subunit A [122, 142]. Treatment of 3NP did not cause a reduction but rather an accumulation of succinate in the TCA cycle, confirming that 3NP is a specific Sdh inhibitor and not an ICL inhibitor.

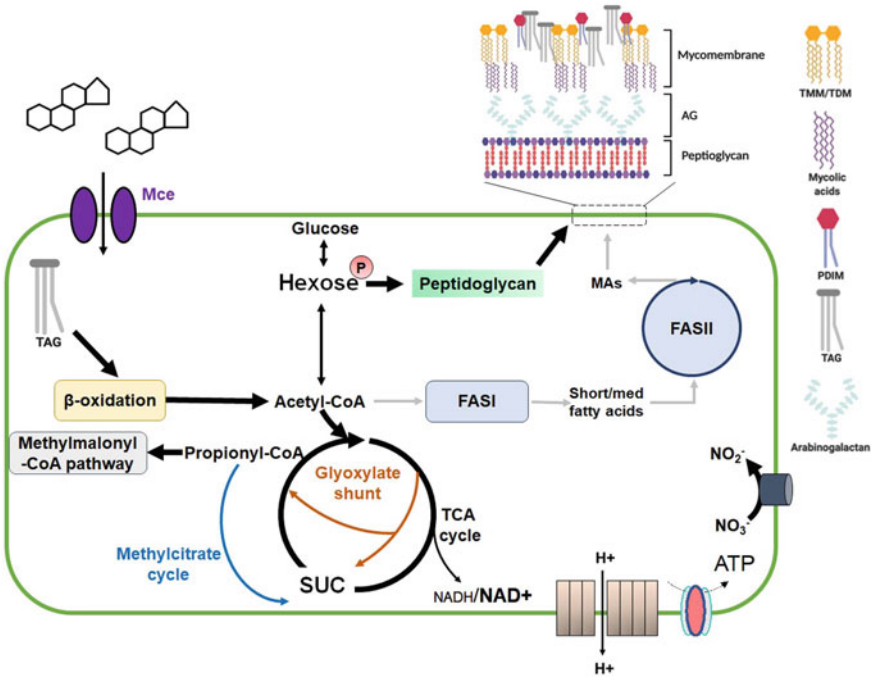


Fig. 3 Initial carbon source candidates required for *M. tb* reactivation. Some of the unused succinates facilitate the resumption of carbon flow through the TCA cycle and accompany biosynthesis of NADH, leading to the generation of proton motive force and ATP. *M. tb* also uses intermediates from the aminosugar biosynthesis pathway as substrates of MurA, the rate-limiting step of peptidoglycan biosynthesis. Other than succinate and aminosugars, triacylglycerol (TAG) is also a potential carbon source used by *M. tb* to exit the NRP state. (*Created with BioRender.com*)

Treatment of 3NP in NRP *M. tb* adapted to hypoxia resulted in a time-dependent delayed regrowth upon reaeration, suggesting the functional essentiality of accumulated succinate as an initial carbon source required for exit from the NRP state. Sdh is an appealing potential drug target to prevent *M. tb* reactivation.

UDP-N-Acetylglucosamine 1-carboxylvinyltransferase (MurA)

A metabolomics study using hypoxic *M. tb* identified accumulation of a discrete set of intermediates in the early portion of glycolysis and aminosugar biosynthesis pathway, including glucose phosphates and UDP-N-Acetyl Glucosamine (UDP-GlcNAc) [143]. During reaeration, following metabolic labeling with ^{13}C-acetate during hypoxia, only the unlabeled fraction of foregoing intermediates exhibited time-dependent depletion with reciprocal induction of UDP-N-Acetyl Muramic acid (UDP-MurNAc) by condensing UDP-GlcNAc with newly synthesized PEP. This is the first committed step of de novo peptidoglycan biosynthesis that is catalyzed by MurA [201]. These findings link the hypoxia-induced

accumulation of glycolysis and aminosugar biosynthesis pathway intermediates to carbon substrates used to reinitiate peptidoglycan biosynthesis of *M. tb* upon reaeration. MurA serves as a potential drug target to block the reactivation of NRP *M. tb* because it catalyzes a critical step in the anticipatory metabolic response.

3 Conclusion

Metabolic remodeling promotes *M. tb* pathogenesis through multiple and complicated operations that go far beyond the energy generation and macromolecule biosynthesis required for replication. These include biochemical regulatory functions to maintain viability such as nutritional homeostasis, membrane bioenergetics, allosteric regulation, antioxidant activity, and extrinsic factors required for survival, such as antibiotic tolerance and host immune interaction. Accumulating evidence supports that metabolic remodeling leads to cellular adaptation in a phenotype-specific process. It is important to recognize that metabolic remodeling is responsible for the diverse phenotypic roles in *M. tb* pathogenesis. The advent of integrated multiomics technologies allowed direct measurements, replacing the indirect inferences made from sequence homology methods. Not only is the knowledge of *M. tb* pathogenesis greatly expanded by these technologies, but more complex questions can be addressed through the interpretation of vast datasets using computational bioinformatics-based modeling. For example, the pharmacological objective is to target metabolically and phenotypically heterogenous *M. tb* populations containing bacilli from replicating to NRP states and subsequent reactivation. Working towards the answers, it is imperative to recognize conceptually novel approaches to identify previously unprecedented new TB interventions.

Core Messages

- Population diversity is an adaptive strategy to environmental changes by metabolic flexibility and mutual interaction.
- Enhancing phenotypic heterogeneity is a bet-hedging tactic against intact immune systems and antibiotic stresses.
- The phenotypic diversity of *M. tb* can be monitored by genomics, transcriptomics, proteomics, and metabolomics.
- Multiomics integration can provide a much more powerful comprehensive knowledge than using single omics alone.
- Multiomics analysis of *M. tb* metabolism aids in novel TB therapeutics discovery to cure drug-resistant TB patients.

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References

1. Nathan C (2012) Fresh approaches to anti-infective therapies. Sci Transl Med 4 (140):140sr–142
2. Nathan C, Barry CE 3rd (2015) TB drug development: immunology at the table. Immunol Rev 264(1):308–318
3. Allen TW (1989) Tuberculosis remains a formidable adversary. J Am Osteopath Assoc 89 (5):617–618
4. Nathan CF, Hibbs JB Jr (1991) Role of nitric oxide synthesis in macrophage antimicrobial activity. Curr Opin Immunol 3(1):65–70
5. MacMicking JD, North RJ, LaCourse R, Mudgett JS, Shah SK, Nathan CF (1997) Identification of nitric oxide synthase as a protective locus against tuberculosis. Proc Natl Acad Sci USA 94(10):5243–5248
6. Chan J, Tanaka K, Carroll D, Flynn J, Bloom BR (1995) Effects of nitric oxide synthase inhibitors on murine infection with *Mycobacterium tuberculosis*. Infect Immun 63(2):736–740
7. Chan J, Xing Y, Magliozzo RS, Bloom BR (1992) Killing of virulent *Mycobacterium tuberculosis* by reactive nitrogen intermediates produced by activated murine macrophages. J Exp Med 175(4):1111–1122
8. Voskuil MI, Bartek IL, Visconti K, Schoolnik GK (2011) The response of *mycobacterium tuberculosis* to reactive oxygen and nitrogen species. Front Microbiol 2:105
9. Manina G, Dhar N, McKinney JD (2015) Stress and host immunity amplify *Mycobacterium tuberculosis* phenotypic heterogeneity and induce nongrowing metabolically active forms. Cell Host Microbe 17(1):32–46
10. Dhar N, McKinney JD (2007) Microbial phenotypic heterogeneity and antibiotic tolerance. Curr Opin Microbiol 10(1):30–38
11. Ackermann M (2015) A functional perspective on phenotypic heterogeneity in microorganisms. Nat Rev Microbiol 13(8):497–508
12. Ernst JD (2012) The immunological life cycle of tuberculosis. Nat Rev Immunol 12(8):581–591
13. Davis JM, Ramakrishnan L (2009) The role of the granuloma in expansion and dissemination of early tuberculous infection. Cell 136(1):37–49
14. Ramakrishnan L (2012) Revisiting the role of the granuloma in tuberculosis. Nat Rev Immunol 12(5):352–366
15. Ehlers S, Schaible UE (2012) The granuloma in tuberculosis: dynamics of a host-pathogen collusion. Front Immunol 3:411
16. Flynn JL, Chan J (2001) Tuberculosis: latency and reactivation. Infect Immun 69(7):4195–4201
17. Voskuil MI, Schnappinger D, Visconti KC, Harrell MI, Dolganov GM, Sherman DR, Schoolnik GK (2003) Inhibition of respiration by nitric oxide induces a *Mycobacterium tuberculosis* dormancy program. J Exp Med 198(5):705–713
18. Gomez JE, McKinney JD (2004) *M. tuberculosis* persistence, latency, and drug tolerance. Tuberculosis (Edinb) 84(1–2):29–44
19. Cosma CL, Sherman DR, Ramakrishnan L (2003) The secret lives of the pathogenic mycobacteria. Annu Rev Microbiol 57:641–676

20. Chan J, Flynn J (2004) The immunological aspects of latency in tuberculosis. Clin Immunol 110(1):2–12
21. Rubin EJ (2009) The granuloma in tuberculosis–friend or foe? N Engl J Med 360(23):2471–2473
22. Paige C, Bishai WR (2010) Penitentiary or penthouse condo: the tuberculous granuloma from the microbe's point of view. Cell Microbiol 12(3):301–309
23. Kohanski MA, Dwyer DJ, Collins JJ (2010) How antibiotics kill bacteria: from targets to networks. Nat Rev Microbiol 8(6):423–435
24. Dutta NK, Karakousis PC (2014) Latent tuberculosis infection: myths, models, and molecular mechanisms. Microbiol Mol Biol Rev 78(3):343–371
25. Parrish NM, Dick JD, Bishai WR (1998) Mechanisms of latency in *Mycobacterium tuberculosis*. Trends Microbiol 6(3):107–112
26. Wayne LG, Sohaskey CD (2001) Non-replicating persistence of *mycobacterium tuberculosis*. Annu Rev Microbiol 55:139–163
27. Sacchettini JC, Rubin EJ, Freundlich JS (2008) Drugs versus bugs: in pursuit of the persistent predator *Mycobacterium tuberculosis*. Nat Rev Microbiol 6(1):41–52
28. Brauner A, Fridman O, Gefen O, Balaban NQ (2016) Distinguishing between resistance, tolerance and persistence to antibiotic treatment. Nat Rev Microbiol 14(5):320–330
29. Cohen NR, Lobritz MA, Collins JJ (2013) Microbial persistence and the road to drug resistance. Cell Host Microbe 13(6):632–642
30. Van den Bergh B, Michiels JE, Wenseleers T, Windels EM, Boer PV, Kestemont D, De Meester L, Verstrepen KJ, Verstraeten N, Fauvart M, Michiels J (2016) Frequency of antibiotic application drives rapid evolutionary adaptation of *Escherichia coli* persistence. Nat Microbiol 1:16020
31. Windels EM, Michiels JE, Fauvart M, Wenseleers T, Van den Bergh B, Michiels J (2019) Bacterial persistence promotes the evolution of antibiotic resistance by increasing survival and mutation rates. ISME J 13(5):1239–1251
32. Davies J, Davies D (2010) Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 74(3):417–433
33. Kohanski MA, DePristo MA, Collins JJ (2010) Sublethal antibiotic treatment leads to multidrug resistance via radical-induced mutagenesis. Mol Cell 37(3):311–320
34. Lee J, Lee S, Song N, Nathan T, Swarts B, Eum S, Ehrt S, Cho S, Eoh H (2019) Transient drug-tolerance and permanent drug-resistance rely on the trehalose-catalytic shift in *Mycobacterium tuberculosis*. Nat Commun 2, 10(1):2928
35. Levin-Reisman I, Ronin I, Gefen O, Braniss I, Shoresh N, Balaban NQ (2017) Antibiotic tolerance facilitates the evolution of resistance. Science 355(6327):826–830
36. Lewis K, Shan Y (2017) Why tolerance invites resistance. Science 355(6327):796
37. Gengenbacher M, Kaufmann SH (2012) *Mycobacterium tuberculosis*: success through dormancy. FEMS Microbiol Rev 36(3):514–532
38. Esmail H, Barry CE 3rd, Young DB, Wilkinson RJ (2014) The ongoing challenge of latent tuberculosis. Philos Trans R Soc Lond B Biol Sci 369(1645):20130437
39. Cha SB, Jeon BY, Kim WS, Kim JS, Kim HM, Kwon KW, Cho SN, Shin SJ, Koh WJ (2015) Experimental reactivation of pulmonary *Mycobacterium avium* complex infection in a modified cornell-like murine model. PLoS ONE 10(9):e0139251
40. McCune RM, Jr., McDermott W, Tompsett R (1956) The fate of *Mycobacterium tuberculosis* in mouse tissues as determined by the microbial enumeration technique. II. The conversion of tuberculous infection to the latent state by the administration of pyrazinamide and a companion drug. J Exp Med 104(5):763–802
41. McCune RM, Jr, Tompsett R (1956) Fate of Mycobacterium tuberculosis in mouse tissues as determined by the microbial enumeration technique. I. The persistence of drug-susceptible tubercle bacilli in the tissues despite prolonged antimicrobial therapy. J Exp Med 104 (5):737–762

42. Munoz-Elias EJ, Timm J, Botha T, Chan WT, Gomez JE, McKinney JD (2005) Replication dynamics of *Mycobacterium tuberculosis* in chronically infected mice. Infect Immun 73 (1):546–551
43. Boshoff HI, Barry CE 3rd (2005) Tuberculosis-metabolism and respiration in the absence of growth. Nat Rev Microbiol 3(1):70–80
44. Russel WF, Dressler SH, Middlebrook G, Denst J (1955) Implications of the phenomenon of open cavity healing for the chemotherapy of pulmonary tuberculosis. Am Rev Tuberc 71(3, Part 1):441–446
45. Gadkowski LB, Stout JE (2008) Cavitary pulmonary disease. Clin Microbiol Rev 21 (2):305–333, table of contents
46. Kaplan G, Post FA, Moreira AL, Wainwright H, Kreiswirth BN, Tanverdi M, Mathema B, Ramaswamy SV, Walther G, Steyn LM, Barry CE 3rd, Bekker LG (2003) *Mycobacterium tuberculosis* growth at the cavity surface: a microenvironment with failed immunity. Infect Immun 71(12):7099–7108
47. Forrellad MA, Klepp LI, Gioffre A, Sabio y Garcia J, Morbidoni HR, de la Paz Santangelo M, Cataldi AA, Bigi F (2013) Virulence factors of the *Mycobacterium tuberculosis* complex. Virulence 4(1):3–66
48. Pieters J (2008) *Mycobacterium tuberculosis* and the macrophage: maintaining a balance. Cell Host Microbe 3(6):399–407
49. Dhar N, McKinney J, Manina G (2016) Phenotypic Heterogeneity in *Mycobacterium tuberculosis*. Microbiol Spectr 4(6)
50. Rego EH, Audette RE, Rubin EJ (2017) Deletion of a mycobacterial divisome factor collapses single-cell phenotypic heterogeneity. Nature 546(7656):153–157
51. Villa Martin P, Munoz MA, Pigolotti S (2019) Bet-hedging strategies in expanding populations. PLoS Comput Biol 15(4):e1006529
52. Venturelli OS, Zuleta I, Murray RM, El-Samad H (2015) Population diversification in a yeast metabolic program promotes anticipation of environmental shifts. PLoS Biol 13(1): e1002042
53. Lenaerts A, Barry CE 3rd, Dartois V (2015) Heterogeneity in tuberculosis pathology, microenvironments and therapeutic responses. Immunol Rev 264(1):288–307
54. Barry CE 3rd, Boshoff HI, Dartois V, Dick T, Ehrt S, Flynn J, Schnappinger D, Wilkinson RJ, Young D (2009) The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. Nat Rev Microbiol 7(12):845–855
55. Fraser CM, Gocayne JD, White O, Adams MD, Clayton RA, Fleischmann RD, Bult CJ, Kerlavage AR, Sutton G, Kelley JM, Fritchman RD, Weidman JF, Small KV, Sandusky M, Fuhrmann J, Nguyen D, Utterback TR, Saudek DM, Phillips CA, Merrick JM, Tomb JF, Dougherty BA, Bott KF, Hu PC, Lucier TS, Peterson SN, Smith HO, Hutchison CA 3rd, Venter JC (1995) The minimal gene complement of *Mycoplasma genitalium*. Science 270 (5235):397–403
56. Fleischmann RD, Adams MD, White O, Clayton RA, Kirkness EF, Kerlavage AR, Bult CJ, Tomb JF, Dougherty BA, Merrick JM et al (1995) Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. Science 269(5223):496–512
57. Rhee K (2013) Minding the gaps: metabolomics mends functional genomics. EMBO Rep 14 (11):949–950
58. Rhee KY, de Carvalho LP, Bryk R, Ehrt S, Marrero J, Park SW, Schnappinger D, Venugopal A, Nathan C (2011) Central carbon metabolism in *Mycobacterium tuberculosis*: an unexpected frontier. Trends Microbiol 19(7):307–314
59. Hasin Y, Seldin M, Lusis A (2017) Multi-omics approaches to disease. Genome Biol 18 (1):83
60. Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry CE 3rd, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Krogh A, McLean J, Moule S, Murphy L, Oliver K, Osborne J, Quail MA,

Rajandream MA, Rogers J, Rutter S, Seeger K, Skelton J, Squares R, Squares S, Sulston JE, Taylor K, Whitehead S, Barrell BG (1998) Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature 393(6685):537–544

61. Parish T, Stoker NG (2000) Use of a flexible cassette method to generate a double unmarked *Mycobacterium tuberculosis* tlyA plcABC mutant by gene replacement. Microbiology 146 (Pt 8):1969–1975. https://doi.org/10.1099/00221287-146-8-1969
62. Eoh H, Brown AC, Buetow L, Hunter WN, Parish T, Kaur D, Brennan PJ, Crick DC (2007) Characterization of the *Mycobacterium tuberculosis* 4-diphosphocytidyl-2-C-methyl-D-erythritol synthase: potential for drug development. J Bacteriol 189(24):8922–8927
63. Sassetti CM, Boyd DH, Rubin EJ (2003) Genes required for mycobacterial growth defined by high density mutagenesis. Mol Microbiol 48(1):77–84
64. Sassetti CM, Rubin EJ (2003) Genetic requirements for mycobacterial survival during infection. Proc Natl Acad Sci U S A 100(22):12989–12994
65. Schnappinger D, Ehrt S, Voskuil MI, Liu Y, Mangan JA, Monahan IM, Dolganov G, Efron B, Butcher PD, Nathan C, Schoolnik GK (2003) Transcriptional adaptation of *Mycobacterium tuberculosis* within macrophages: insights into the phagosomal environment. J Exp Med 198(5):693–704
66. Ehrt S, Guo XV, Hickey CM, Ryou M, Monteleone M, Riley LW, Schnappinger D (2005) Controlling gene expression in mycobacteria with anhydrotetracycline and Tet repressor. Nucleic Acids Res 33(2):e21
67. Schnappinger D, Ehrt S (2014) Regulated expression systems for mycobacteria and their applications. Microbiol Spectr 2(1)
68. Rock J (2019) Tuberculosis drug discovery in the CRISPR era. PLoS Pathog 15(9): e1007975
69. Rock JM, Hopkins FF, Chavez A, Diallo M, Chase MR, Gerrick ER, Pritchard JR, Church GM, Rubin EJ, Sassetti CM, Schnappinger D, Fortune SM (2017) Programmable transcriptional repression in mycobacteria using an orthogonal CRISPR interference platform. Nat Microbiol 2:16274
70. Choudhary E, Thakur P, Pareek M, Agarwal N (2015) Gene silencing by CRISPR interference in mycobacteria. Nat Commun 6:6267
71. Mashabela GT, de Wet TJ, Warner DF (2019) *Mycobacterium tuberculosis* metabolism. Microbiol Spectr 7(4)
72. Boshoff HI, Myers TG, Copp BR, McNeil MR, Wilson MA, Barry CE 3rd (2004) The transcriptional responses of *Mycobacterium tuberculosis* to inhibitors of metabolism: novel insights into drug mechanisms of action. J Biol Chem 279(38):40174–40184
73. Paananen J, Fortino V (2019) An omics perspective on drug target discovery platforms. Brief Bioinform 27:bbz122
74. Gomez-Gonzalez PJ, Andreu N, Phelan JE, de Sessions PF, Glynn JR, Crampin AC, Campino S, Butcher PD, Hibberd ML, Clark TG (2019) An integrated whole genome analysis of *Mycobacterium tuberculosis* reveals insights into relationship between its genome, transcriptome and methylome. Sci Rep 9(1):5204
75. Benjak A, Uplekar S, Zhang M, Piton J, Cole ST, Sala C (2016) Genomic and transcriptomic analysis of the streptomycin-dependent *Mycobacterium tuberculosis* strain 18b. BMC Genomics 17:190
76. Rohde KH, Veiga DF, Caldwell S, Balazsi G, Russell DG (2012) Linking the transcriptional profiles and the physiological states of *Mycobacterium tuberculosis* during an extended intracellular infection. PLoS Pathog 8(6):e1002769
77. Rachman H, Strong M, Ulrichs T, Grode L, Schuchhardt J, Mollenkopf H, Kosmiadi GA, Eisenberg D, Kaufmann SH (2006) Unique transcriptome signature of *Mycobacterium tuberculosis* in pulmonary tuberculosis. Infect Immun 74(2):1233–1242

78. Lavalett L, Ortega H, Barrera LF (2020) Human alveolar and splenic macrophage populations display a distinct transcriptomic response to infection with *Mycobacterium tuberculosis*. Front Immunol 11:630
79. Munoz-Elias EJ, McKinney JD (2006) Carbon metabolism of intracellular bacteria. Cell Microbiol 8(1):10–22
80. Munoz-Elias EJ, McKinney JD (2005) *Mycobacterium tuberculosis* isocitrate lyases 1 and 2 are jointly required for *in vivo* growth and virulence. Nat Med 11(6):638–644
81. Eoh H, Rhee KY (2014) Methylcitrate cycle defines the bactericidal essentiality of isocitrate lyase for survival of *Mycobacterium tuberculosis* on fatty acids. Proc Natl Acad Sci USA 111(13):4976–4981
82. Lee JJ, Lim J, Gao S, Lawson CP, Odell M, Raheem S, Woo J, Kang SH, Kang SS, Jeon BY, Eoh H (2018) Glutamate mediated metabolic neutralization mitigates propionate toxicity in intracellular *Mycobacterium tuberculosis*. Sci Rep 8(1):8506
83. Rao SP, Alonso S, Rand L, Dick T, Pethe K (2008) The protonmotive force is required for maintaining ATP homeostasis and viability of hypoxic, non-replicating *Mycobacterium tuberculosis*. Proc Natl Acad Sci USA 105(33):11945–11950
84. Lamprecht DA, Finin PM, Rahman MA, Cumming BM, Russell SL, Jonnala SR, Adamson JH, Steyn AJ (2016) Turning the respiratory flexibility of *Mycobacterium tuberculosis* against itself. Nat Commun 10(7):12393
85. Baughn AD, Rhee KY (2014) Metabolomics of central carbon metabolism in *Mycobacterium tuberculosis*. Microbiol Spectr 2(3)
86. Eoh H (2014) Metabolomics: a window into the adaptive physiology of *Mycobacterium tuberculosis*. Tuberculosis (Edinb) 94(6):538–543
87. Reaves ML, Rabinowitz JD (2011) Metabolomics in systems microbiology. Curr Opin Biotechnol 22(1):17–25
88. Trivedi DK, Hollywood KA, Goodacre R (2017) Metabolomics for the masses: the future of metabolomics in a personalized world. New Horiz Transl Med 3(6):294–305
89. Xu EY, Schaefer WH, Xu Q (2009) Metabolomics in pharmaceutical research and development: metabolites, mechanisms and pathways. Curr Opin Drug Discov Devel 12 (1):40–52
90. Johnson CH, Ivanisevic J, Siuzdak G (2016) Metabolomics: beyond biomarkers and towards mechanisms. Nat Rev Mol Cell Biol 17(7):451–459
91. de Carvalho LP, Fischer SM, Marrero J, Nathan C, Ehrt S, Rhee KY (2010) Metabolomics of *Mycobacterium tuberculosis* reveals compartmentalized co-catabolism of carbon substrates. Chem Biol 17(10):1122–1131
92. Noy T, Vergnolle O, Hartman TE, Rhee KY, Jacobs WR Jr, Berney M, Blanchard JS (2016) Central role of pyruvate kinase in carbon co-catabolism of *Mycobacterium tuberculosis*. J Biol Chem 291(13):7060–7069
93. Agapova A, Serafini A, Petridis M, Hunt DM, Garza-Garcia A, Sohaskey CD, de Carvalho LPS (2019) Flexible nitrogen utilisation by the metabolic generalist pathogen *Mycobacterium tuberculosis*. Elife 8:e41129
94. Serafini A, Tan L, Horswell S, Howell S, Greenwood DJ, Hunt DM, Phan MD, Schembri M, Monteleone M, Montague CR, Britton W, Garza-Garcia A, Snijders AP, VanderVen B, Gutierrez MG, West NP, de Carvalho LPS (2019) *Mycobacterium tuberculosis* requires glyoxylate shunt and reverse methylcitrate cycle for lactate and pyruvate metabolism. Mol Microbiol 112(4):1284–1307
95. Dutta NK, Klinkenberg LG, Vazquez MJ, Segura-Carro D, Colmenarejo G, Ramon F, Rodriguez-Miquel B, Mata-Cantero L, Porras-De Francisco E, Chuang YM, Rubin H, Lee JJ, Eoh H, Bader JS, Perez-Herran E, Mendoza-Losana A, Karakousis PC (2019) Inhibiting the stringent response blocks *Mycobacterium tuberculosis* entry into quiescence and reduces persistence. Sci Adv 5(3):eaav2104
96. Wilburn KM, Fieweger RA, VanderVen BC (2018) Cholesterol and fatty acids grease the wheels of *Mycobacterium tuberculosis* pathogenesis. Pathog Dis 76(2):fty021

97. Ehrt S, Rhee K, Schnappinger D (2015) Mycobacterial genes essential for the pathogen's survival in the host. Immunol Rev 264(1):319–326
98. Zimmermann M, Kogadeeva M, Gengenbacher M, McEwen G, Mollenkopf HJ, Zamboni N, Kaufmann SHE, Sauer U (2017) Integration of metabolomics and transcriptomics reveals a complex diet of *Mycobacterium tuberculosis* during early macrophage infection. mSystems 2(4):e00057–17
99. Yam KC, D'Angelo I, Kalscheuer R, Zhu H, Wang JX, Snieckus V, Ly LH, Converse PJ, Jacobs WR Jr, Strynadka N, Eltis LD (2009) Studies of a ring-cleaving dioxygenase illuminate the role of cholesterol metabolism in the pathogenesis of *Mycobacterium tuberculosis*. PLoS Pathog 5(3):e1000344
100. Kovarova-Kovar K, Egli T (1998) Growth kinetics of suspended microbial cells: from single-substrate-controlled growth to mixed-substrate kinetics. Microbiol Mol Biol Rev 62 (3):646–666
101. Nazarova EV, Montague CR, La T, Wilburn KM, Sukumar N, Lee W, Caldwell S, Russell DG, VanderVen BC (2017) Rv3723/LucA coordinates fatty acid and cholesterol uptake in *Mycobacterium tuberculosis*. Elife 6:e26969
102. VanderVen BC, Fahey RJ, Lee W, Liu Y, Abramovitch RB, Memmott C, Crowe AM, Eltis LD, Perola E, Deininger DD, Wang T, Locher CP, Russell DG (2015) Novel inhibitors of cholesterol degradation in *Mycobacterium tuberculosis* reveal how the bacterium's metabolism is constrained by the intracellular environment. PLoS Pathog 11(2):e1004679
103. Pandey AK, Sassetti CM (2008) Mycobacterial persistence requires the utilization of host cholesterol. Proc Natl Acad Sci USA 105(11):4376–4380
104. Griffin JE, Gawronski JD, Dejesus MA, Ioerger TR, Akerley BJ, Sassetti CM (2011) High-resolution phenotypic profiling defines genes essential for mycobacterial growth and cholesterol catabolism. PLoS Pathog 7(9):e1002251
105. Casali N, Riley LW (2007) A phylogenomic analysis of the Actinomycetales mce operons. BMC Genomics 8:60
106. Pisu D, Huang L, Grenier JK, Russell DG (2020) Dual RNA-Seq of Mtb-infected macrophages *In Vivo* reveals ontologically distinct host-pathogen interactions. Cell Rep 30 (2):335–350 e334
107. Griffin JE, Pandey AK, Gilmore SA, Mizrahi V, McKinney JD, Bertozzi CR, Sassetti CM (2012) Cholesterol catabolism by *Mycobacterium tuberculosis* requires transcriptional and metabolic adaptations. Chem Biol 19(2):218–227
108. Venugopal A, Bryk R, Shi S, Rhee K, Rath P, Schnappinger D, Ehrt S, Nathan C (2011) Virulence of *Mycobacterium tuberculosis* depends on lipoamide dehydrogenase, a member of three multienzyme complexes. Cell Host Microbe 9(1):21–31
109. Savvi S, Warner DF, Kana BD, McKinney JD, Mizrahi V, Dawes SS (2008) Functional characterization of a vitamin B12-dependent methylmalonyl pathway in *Mycobacterium tuberculosis*: implications for propionate metabolism during growth on fatty acids. J Bacteriol 190(11):3886–3895
110. Munoz-Elias EJ, Upton AM, Cherian J, McKinney JD (2006) Role of the methylcitrate cycle in *Mycobacterium tuberculosis* metabolism, intracellular growth, and virulence. Mol Microbiol 60(5):1109–1122
111. Stanley SA, Cox JS (2013) Host-pathogen interactions during *Mycobacterium tuberculosis* infections. Curr Top Microbiol Immunol 374:211–241
112. Arbues A, Lugo-Villarino G, Neyrolles O, Guilhot C, Astarie-Dequeker C (2014) Playing hide-and-seek with host macrophages through the use of mycobacterial cell envelope phthiocerol dimycocerosates and phenolic glycolipids. Front Cell Infect Microbiol 4:173
113. Robitzek EH, Selikoff IJ (1952) Hydrazine derivatives of isonicotinic acid (rimifon marsilid) in the treatment of active progressive caseous-pneumonic tuberculosis; a preliminary report. Am Rev Tuberc 65(4):402–428

114. Robitzek EH, Selikoff IJ, Ornstein GG (1952) Chemotherapy of human tuberculosis with hydrazine derivatives of isonicotinic acid; preliminary report of representative cases. Q Bull Sea View Hosp 13(1):27–51
115. Winder FG, Collins PB (1970) Inhibition by isoniazid of synthesis of mycolic acids in *Mycobacterium tuberculosis*. J Gen Microbiol 63(1):41–48
116. Chakraborty S, Rhee KY (2015) Tuberculosis drug development: history and evolution of the mechanism-based paradigm. Cold Spring Harb Perspect Med 5(8):a021147
117. Vilcheze C, Jacobs WR Jr (2014) Resistance to isoniazid and ethionamide in *Mycobacterium tuberculosis*: genes, mutations, and causalities. Microbiol Spectr 2(4):MGM2–0014–2013
118. Banerjee A, Dubnau E, Quemard A, Balasubramanian V, Um KS, Wilson T, Collins D, de Lisle G, Jacobs WR Jr (1994) *inhA*, a gene encoding a target for isoniazid and ethionamide in *Mycobacterium tuberculosis*. Science 263(5144):227–230
119. Vilcheze C, Wang F, Arai M, Hazbon MH, Colangeli R, Kremer L, Weisbrod TR, Alland D, Sacchettini JC, Jacobs WR Jr (2006) Transfer of a point mutation in *Mycobacterium tuberculosis* inhA resolves the target of isoniazid. Nat Med 12(9):1027–1029
120. Heym B, Alzari PM, Honore N, Cole ST (1995) Missense mutations in the catalase-peroxidase gene, katG, are associated with isoniazid resistance in *Mycobacterium tuberculosis*. Mol Microbiol 15(2):235–245
121. Bald D, Villellas C, Lu P, Koul A (2017) Targeting energy metabolism in *Mycobacterium tuberculosis*, a new paradigm in Antimycobacterial drug discovery. MBio 8(2):e00272-e317
122. Cook GM, Hards K, Dunn E, Heikal A, Nakatani Y, Greening C, Crick DC, Fontes FL, Pethe K, Hasenoehrl E, Berney M (2017) Oxidative phosphorylation as a target space for tuberculosis: success, caution, and future directions. Microbiol Spectr 5(3)
123. Cumming BM, Addicott KW, Adamson JH, Steyn AJ (2018) *Mycobacterium tuberculosis* induces decelerated bioenergetic metabolism in human macrophages. Elife 7:e39169
124. Iqbal IK, Bajeli S, Akela AK, Kumar A (2018) Bioenergetics of mycobacterium: an emerging landscape for drug discovery. Pathogens 7(1)
125. von Jagow G, Ljungdahl PO, Graf P, Ohnishi T, Trumpower BL (1984) An inhibitor of mitochondrial respiration which binds to cytochrome b and displaces quinone from the iron-sulfur protein of the cytochrome bc1 complex. J Biol Chem 259(10):6318–6326
126. Rybniker J, Vocat A, Sala C, Busso P, Pojer F, Benjak A, Cole ST (2015) Lansoprazole is an antituberculous prodrug targeting cytochrome bc1. Nat Commun 6:7659
127. Lu P, Heineke MH, Koul A, Andries K, Cook GM, Lill H, van Spanning R, Bald D (2015) The cytochrome bd-type quinol oxidase is important for survival of *Mycobacterium smegmatis* under peroxide and antibiotic-induced stress. Sci Rep 5:10333
128. Pethe K, Bifani P, Jang J, Kang S, Park S, Ahn S, Jiricek J, Jung J, Jeon HK, Cechetto J, Christophe T, Lee H, Kempf M, Jackson M, Lenaerts AJ, Pham H, Jones V, Seo MJ, Kim YM, Seo M, Seo JJ, Park D, Ko Y, Choi I, Kim R, Kim SY, Lim S, Yim SA, Nam J, Kang H, Kwon H, Oh CT, Cho Y, Jang Y, Kim J, Chua A, Tan BH, Nanjundappa MB, Rao SP, Barnes WS, Wintjens R, Walker JR, Alonso S, Lee S, Oh S, Oh T, Nehrbass U, Han SJ, No Z, Lee J, Brodin P, Cho SN, Nam K (2013) Discovery of Q203, a potent clinical candidate for the treatment of tuberculosis. Nat Med 19(9):1157–1160
129. Andries K, Verhasselt P, Guillemont J, Gohlmann HW, Neefs JM, Winkler H, Van Gestel J, Timmerman P, Zhu M, Lee E, Williams P, de Chaffoy D, Huitric E, Hoffner S, Cambau E, Truffot-Pernot C, Lounis N, Jarlier V (2005) A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. Science 307(5707):223–227
130. Huitric E, Verhasselt P, Andries K, Hoffner SE (2007) In vitro antimycobacterial spectrum of a diarylquinoline ATP synthase inhibitor. Antimicrob Agents Chemother 51(11):4202–4204
131. Huitric E, Verhasselt P, Koul A, Andries K, Hoffner S, Andersson DI (2010) Rates and mechanisms of resistance development in *Mycobacterium tuberculosis* to a novel diarylquinoline ATP synthase inhibitor. Antimicrob Agents Chemother 54(3):1022–1028

132. Cohen J (2013) Infectious disease. Approval of novel TB drug celebrated—with restraint. Science 339(6116):130
133. Cholo MC, Mothiba MT, Fourie B, Anderson R (2017) Mechanisms of action and therapeutic efficacies of the lipophilic antimycobacterial agents clofazimine and bedaquiline. J Antimicrob Chemother 72(2):338–353
134. Diacon AH, Dawson R, von Groote-Bidlingmaier F, Symons G, Venter A, Donald PR, van Niekerk C, Everitt D, Winter H, Becker P, Mendel CM, Spigelman MK (2012) 14-day bactericidal activity of PA-824, bedaquiline, pyrazinamide, and moxifloxacin combinations: a randomised trial. Lancet 380(9846):986–993
135. Koul A, Vranckx L, Dhar N, Gohlmann HW, Ozdemir E, Neefs JM, Schulz M, Lu P, Mortz E, McKinney JD, Andries K, Bald D (2014) Delayed bactericidal response of *Mycobacterium tuberculosis* to bedaquiline involves remodelling of bacterial metabolism. Nat Commun 5:3369
136. Conlon BP, Rowe SE, Gandt AB, Nuxoll AS, Donegan NP, Zalis EA, Clair G, Adkins JN, Cheung AL, Lewis K (2016) Persister formation in *Staphylococcus aureus* is associated with ATP depletion. Nat Microbiol 1:16051
137. Fisher RA, Gollan B, Helaine S (2017) Persistent bacterial infections and persister cells. Nat Rev Microbiol 15(8):453–464
138. Lewis K (2010) Persister cells. Annu Rev Microbiol 64:357–372
139. Shan Y, Brown Gandt A, Rowe SE, Deisinger JP, Conlon BP, Lewis K (2017) ATP-Dependent persister formation in *Escherichia coli*. MBio 8(1):e02267-e2316
140. Wang Y, Bojer MS, George SE, Wang Z, Jensen PR, Wolz C, Ingmer H (2018) Inactivation of TCA cycle enhances Staphylococcus aureus persister cell formation in stationary phase. Sci Rep 8(1):10849
141. Wang Z, Soni V, Marriner G, Kaneko T, Boshoff HIM, Barry CE III, Rhee K (2019) Mode-of-action profiling reveals glutamine synthetase as a collateral metabolic vulnerability of *M. tuberculosis* to bedaquiline. Proc Natl Acad Sci USA 116(39):19646–19651
142. Eoh H, Rhee KY (2013) Multifunctional essentiality of succinate metabolism in adaptation to hypoxia in *Mycobacterium tuberculosis*. Proc Natl Acad Sci USA 110(16):6554–6559
143. Eoh H, Wang Z, Layre E, Rath P, Morris R, Branch Moody D, Rhee KY (2017) Metabolic anticipation in *Mycobacterium tuberculosis*. Nat Microbiol 2:17084
144. Balaban NQ, Helaine S, Lewis K, Ackermann M, Aldridge B, Andersson DI, Brynildsen MP, Bumann D, Camilli A, Collins JJ, Dehio C, Fortune S, Ghigo JM, Hardt WD, Harms A, Heinemann M, Hung DT, Jenal U, Levin BR, Michiels J, Storz G, Tan MW, Tenson T, Van Melderen L, Zinkernagel A (2019) Definitions and guidelines for research on antibiotic persistence. Nat Rev Microbiol 17(7):441–448
145. Balaban NQ, Merrin J, Chait R, Kowalik L, Leibler S (2004) Bacterial persistence as a phenotypic switch. Science 305(5690):1622–1625
146. Ehrt S, Schnappinger D, Rhee KY (2018) Metabolic principles of persistence and pathogenicity in *Mycobacterium tuberculosis*. Nat Rev Microbiol 16(8):496–507
147. Jayaraman R (2008) Bacterial persistence: some new insights into an old phenomenon. J Biosci 33(5):795–805
148. Sarathy JP, Via LE, Weiner D, Blanc L, Boshoff H, Eugenin EA, Barry CE 3rd, Dartois VA (2018) Extreme drug tolerance of *Mycobacterium tuberculosis* in caseum. Antimicrob Agents Chemother 62(2):e02266-e2317
149. Sarathy J, Blanc L, Alvarez-Cabrera N, O'Brien P, Dias-Freedman I, Mina M, Zimmerman M, Kaya F, Ho Liang HP, Prideaux B, Dietzold J, Salgame P, Savic RM, Linderman J, Kirschner D, Pienaar E, Dartois V (2019) Fluoroquinolone Efficacy against tuberculosis is driven by penetration into lesions and activity against resident bacterial populations. Antimicrob Agents Chemother 63(5):e02516-e2518
150. Sarathy J, Dartois V, Dick T, Gengenbacher M (2013) Reduced drug uptake in phenotypically resistant nutrient-starved non-replicating *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 57(4):1648–1653

151. Lopez-Agudelo VA, Baena A, Ramirez-Malule H, Ochoa S, Barrera LF, Rios-Estepa R (2017) Metabolic adaptation of two in silico mutants of *Mycobacterium tuberculosis* during infection. BMC Syst Biol 11(1):107
152. Watanabe S, Zimmermann M, Goodwin MB, Sauer U, Barry CE 3rd, Boshoff HI (2011) Fumarate reductase activity maintains an energized membrane in anaerobic *Mycobacterium tuberculosis*. PLoS Pathog 7(10):e1002287
153. Betts JC, Lukey PT, Robb LC, McAdam RA, Duncan K (2002) Evaluation of a nutrient starvation model of *Mycobacterium tuberculosis* persistence by gene and protein expression profiling. Mol Microbiol 43(3):717–731
154. Lin W, de Sessions PF, Teoh GH, Mohamed AN, Zhu YO, Koh VH, Ang ML, Dedon PC, Hibberd ML, Alonso S (2016) Transcriptional profiling of *Mycobacterium tuberculosis* exposed to *In Vitro* Lysosomal stress. Infect Immun 84(9):2505–2523
155. Voskuil MI, Visconti KC, Schoolnik GK (2004) *Mycobacterium tuberculosis* gene expression during adaptation to stationary phase and low-oxygen dormancy. Tuberculosis (Edinb) 84(3–4):218–227
156. Briffotaux J, Liu S, Gicquel B (2019) Genome-wide transcriptional responses of Mycobacterium to antibiotics. Front Microbiol 10:249
157. Betts JC (2002) Transcriptomics and proteomics: tools for the identification of novel drug targets and vaccine candidates for tuberculosis. IUBMB Life 53(4–5):239–242
158. Argyrou A, Jin L, Siconilfi-Baez L, Angeletti RH, Blanchard JS (2006) Proteome-wide profiling of isoniazid targets in *Mycobacterium tuberculosis*. Biochemistry 45(47):13947–13953
159. Keren I, Minami S, Rubin E, Lewis K (2011) Characterization and transcriptome analysis of *Mycobacterium tuberculosis* persisters. MBio 2(3):e00100-00111
160. Cadena AM, Fortune SM, Flynn JL (2017) Heterogeneity in tuberculosis. Nat Rev Immunol 17(11):691–702
161. Rao PK, Li Q (2009) Protein turnover in mycobacterial proteomics. Molecules 14(9):3237–3258
162. Marrero J, Trujillo C, Rhee KY, Ehrt S (2013) Glucose phosphorylation is required for *Mycobacterium tuberculosis* persistence in mice. PLoS Pathog 9(1):e1003116
163. Korte J, Alber M, Trujillo CM, Syson K, Koliwer-Brandl H, Deenen R, Kohrer K, DeJesus MA, Hartman T, Jacobs WR Jr, Bornemann S, Ioerger TR, Ehrt S, Kalscheuer R (2016) Trehalose-6-Phosphate-Mediated Toxicity determines essentiality of OtsB2 in *Mycobacterium tuberculosis In Vitro* and in Mice. PLoS Pathog 12(12):e1006043
164. Kalscheuer R, Koliwer-Brandl H (2014) Genetics of Mycobacterial Trehalose Metabolism. Microbiol Spectr 2(3)
165. Indrigo J, Hunter RL Jr, Actor JK (2002) Influence of trehalose 6,6'-dimycolate (TDM) during mycobacterial infection of bone marrow macrophages. Microbiology 148 (Pt 7):1991–1998
166. Kalscheuer R, Weinrick B, Veeraraghavan U, Besra GS, Jacobs WR Jr (2010) Trehalose-recycling ABC transporter LpqY-SugA-SugB-SugC is essential for virulence of *Mycobacterium tuberculosis*. Proc Natl Acad Sci USA 107(50):21761–21766
167. Patin EC, Geffken AC, Willcocks S, Leschczyk C, Haas A, Nimmerjahn F, Lang R, Ward TH, Schaible UE (2017) Trehalose dimycolate interferes with FcgammaR-mediated phagosome maturation through Mincle, SHP-1 and FcgammaRIIB signalling. PLoS ONE 12 (4):e0174973
168. Ishikawa E, Ishikawa T, Morita YS, Toyonaga K, Yamada H, Takeuchi O, Kinoshita T, Akira S, Yoshikai Y, Yamasaki S (2009) Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. J Exp Med 206(13):2879–2888
169. Ruhl CR, Pasko BL, Khan HS, Kindt LM, Stamm CE, Franco LH, Hsia CC, Zhou M, Davis CR, Qin T, Gautron L, Burton MD, Mejia GL, Naik DK, Dussor G, Price TJ, Shiloh MU (2020) *Mycobacterium tuberculosis* sulfolipid-1 activates nociceptive neurons and induces cough. Cell 181(2):293–305 e211

170. Aly S, Wagner K, Keller C, Malm S, Malzan A, Brandau S, Bange FC, Ehlers S (2006) Oxygen status of lung granulomas in *Mycobacterium tuberculosis*-infected mice. J Pathol 210(3):298–305
171. Via LE, Lin PL, Ray SM, Carrillo J, Allen SS, Eum SY, Taylor K, Klein E, Manjunatha U, Gonzales J, Lee EG, Park SK, Raleigh JA, Cho SN, McMurray DN, Flynn JL, Barry CE 3rd (2008) Tuberculous granulomas are hypoxic in guinea pigs, rabbits, and nonhuman primates. Infect Immun 76(6):2333–2340
172. Heng Y, Seah PG, Siew JY, Tay HC, Singhal A, Mathys V, Kiass M, Bifani P, Dartois V, Herve M (2011) *Mycobacterium tuberculosis* infection induces hypoxic lung lesions in the rat. Tuberculosis (Edinb) 91(4):339–341
173. Wayne LG, Hayes LG (1996) An *in vitro* model for sequential study of shiftdown of *Mycobacterium tuberculosis* through two stages of non-replicating persistence. Infect Immun 64(6):2062–2069
174. Cook GM, Hards K, Vilcheze C, Hartman T, Berney M (2014) Energetics of Respiration and oxidative phosphorylation in mycobacteria. Microbiol Spectr 2(3)
175. Berney M, Cook GM (2010) Unique flexibility in energy metabolism allows mycobacteria to combat starvation and hypoxia. PLoS ONE 5(1):e8614
176. May EE, Sershen CL (2016) Oxygen availability and metabolic dynamics during *Mycobacterium tuberculosis* latency. IEEE Trans Biomed Eng 63(10):2036–2046
177. Cook GM, Berney M, Gebhard S, Heinemann M, Cox RA, Danilchanka O, Niederweis M (2009) Physiology of mycobacteria. Adv Microb Physiol 55(81–182):318–189
178. Shi L, Sohaskey CD, Kana BD, Dawes S, North RJ, Mizrahi V, Gennaro ML (2005) Changes in energy metabolism of *Mycobacterium tuberculosis* in mouse lung and under *in vitro* conditions affecting aerobic respiration. Proc Natl Acad Sci USA 102(43):15629–15634
179. Hartman T, Weinrick B, Vilcheze C, Berney M, Tufariello J, Cook GM, Jacobs WR Jr (2014) Succinate dehydrogenase is the regulator of respiration in *Mycobacterium tuberculosis*. PLoS Pathog 10(11):e1004510
180. Sohaskey CD (2008) Nitrate enhances the survival of *Mycobacterium tuberculosis* during inhibition of respiration. J Bacteriol 190(8):2981–2986
181. Sohaskey CD, Wayne LG (2003) Role of narK2X and narGHJI in hypoxic upregulation of nitrate reduction by *Mycobacterium tuberculosis*. J Bacteriol 185(24):7247–7256
182. Cook GM, Greening C, Hards K, Berney M (2014) Energetics of pathogenic bacteria and opportunities for drug development. Adv Microb Physiol 65:1–62
183. Kim JS, Cho DH, Heo P, Jung SC, Park M, Oh EJ, Sung J, Kim PJ, Lee SC, Lee DH, Lee S, Lee CH, Shin D, Jin YS, Kweon DH (2016) Fumarate-Mediated Persistence of *Escherichia coli* against Antibiotics. Antimicrob Agents Chemother 60(4):2232–2240
184. Pecsi I, Hards K, Ekanayaka N, Berney M, Hartman T, Jacobs WR Jr, Cook GM (2014) Essentiality of succinate dehydrogenase in *Mycobacterium smegmatis* and its role in the generation of the membrane potential under hypoxia. MBio 5(4)
185. Tian J, Bryk R, Itoh M, Suematsu M, Nathan C (2005) Variant tricarboxylic acid cycle in *Mycobacterium tuberculosis*: identification of alpha-ketoglutarate decarboxylase. Proc Natl Acad Sci U S A 102(30):10670–10675
186. Dawson R, Diacon AH, Everitt D, van Niekerk C, Donald PR, Burger DA, Schall R, Spigelman M, Conradie A, Eisenach K, Venter A, Ive P, Page-Shipp L, Variava E, Reither K, Ntinginya NE, Pym A, von Groote-Bidlingmaier F, Mendel CM (2015) Efficiency and safety of the combination of moxifloxacin, pretomanid (PA-824), and pyrazinamide during the first 8 weeks of antituberculosis treatment: a phase 2b, open-label, partly randomised trial in patients with drug-susceptible or drug-resistant pulmonary tuberculosis. Lancet 385(9979):1738–1747
187. Diacon AH, Pym A, Grobusch M, Patientia R, Rustomjee R, Page-Shipp L, Pistorius C, Krause R, Bogoshi M, Churchyard G, Venter A, Allen J, Palomino JC, De Marez T, van Heeswijk RP, Lounis N, Meyvisch P, Verbeeck J, Parys W, de Beule K, Andries K, Mc

Neeley DF (2009) The diarylquinoline TMC207 for multidrug-resistant tuberculosis. N Engl J Med 360(23):2397–2405
188. Gler MT, Skripconoka V, Sanchez-Garavito E, Xiao H, Cabrera-Rivero JL, Vargas-Vasquez DE, Gao M, Awad M, Park SK, Shim TS, Suh GY, Danilovits M, Ogata H, Kurve A, Chang J, Suzuki K, Tupasi T, Koh WJ, Seaworth B, Geiter LJ, Wells CD (2012) Delamanid for multidrug-resistant pulmonary tuberculosis. N Engl J Med 366(23):2151–2160
189. Cellitti SE, Shaffer J, Jones DH, Mukherjee T, Gurumurthy M, Bursulaya B, Boshoff HI, Choi I, Nayyar A, Lee YS, Cherian J, Niyomrattanakit P, Dick T, Manjunatha UH, Barry CE 3rd, Spraggon G, Geierstanger BH (2012) Structure of Ddn, the deazaflavin-dependent nitroreductase from *Mycobacterium tuberculosis* involved in bioreductive activation of PA-824. Structure 20(1):101–112
190. Singh R, Manjunatha U, Boshoff HI, Ha YH, Niyomrattanakit P, Ledwidge R, Dowd CS, Lee IY, Kim P, Zhang L, Kang S, Keller TH, Jiricek J, Barry CE 3rd (2008) PA-824 kills non-replicating *Mycobacterium tuberculosis* by intracellular NO release. Science 322 (5906):1392–1395
191. Zong Z, Huo F, Shi J, Jing W, Ma Y, Liang Q, Jiang G, Dai G, Huang H, Pang Y (2018) Relapse versus reinfection of recurrent tuberculosis patients in a national tuberculosis specialized hospital in Beijing. China Front Microbiol 9:1858
192. McIvor A, Koornhof H, Kana BD (2017) Relapse, reinfection and mixed infections in tuberculosis disease. Pathog Dis 75(3)
193. Trinh QM, Nguyen HL, Nguyen VN, Nguyen TV, Sintchenko V, Marais BJ (2015) Tuberculosis and HIV co-infection-focus on the Asia-Pacific region. Int J Infect Dis 32:170–178
194. Nathan C (2009) Taming tuberculosis: a challenge for science and society. Cell Host Microbe 5(3):220–224
195. Nathan C (2014) Drug-resistant tuberculosis: a new shot on goal. Nat Med 20(2):121–123
196. Rustad TR, Sherrid AM, Minch KJ, Sherman DR (2009) Hypoxia: a window into *Mycobacterium tuberculosis* latency. Cell Microbiol 11(8):1151–1159
197. Du P, Sohaskey CD, Shi L (2016) Transcriptional and physiological changes during *Mycobacterium tuberculosis* reactivation from non-replicating persistence. Front Microbiol 7:1346
198. Salina EG, Grigorov AS, Bychenko OS, Skvortsova YV, Mamedov IZ, Azhikina TL, Kaprelyants AS (2019) Resuscitation of Dormant "Non-culturable" Mycobacterium tuberculosis Is characterized by immediate transcriptional Burst. Front Cell Infect Microbiol 9:272. https://doi.org/10.3389/fcimb.2019.00272
199. Wu YY, Shao WB, Zhu JJ, Long ZQ, Liu LW, Wang PY, Li Z, Yang S (2019) Novel 1,3,4-Oxadiazole-2-carbohydrazides as prospective agricultural antifungal agents potentially targeting succinate Dehydrogenase. J Agric Food Chem 67(50):13892–13903
200. Li Y, Geng J, Liu Y, Yu S, Zhao G (2013) Thiadiazole-a promising structure in medicinal chemistry. ChemMedChem 8(1):27–41
201. Brown ED, Vivas EI, Walsh CT, Kolter R (1995) MurA (MurZ), the enzyme that catalyzes the first committed step in peptidoglycan biosynthesis, is essential in *Escherichia coli*. J Bacteriol 177(14):4194–4197

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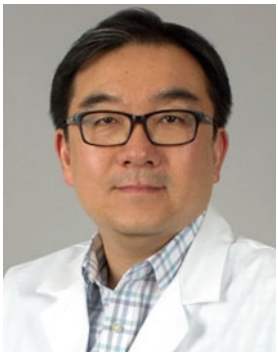

Hyungjin Eoh earned his DVM degree in Seoul National University, Republic of Korea, and a Ph.D. degree in Colorado State University, USA. Prof. Eoh studied in Weill Cornell Medicine, Cornell University as a postdoctoral research associate. He applied LC-MS mediated metabolomics to elucidate M. tuberculosis metabolic topology while adapting to adverse environmental stresses and redefining functional essentialities of new antibiotic targets. Prof. Eoh established his independent laboratory at the Dept. of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California. In his laboratory, he has expanded his multiomics expertise to study various infectious diseases such as viral infection-induced cancers, Candidiasis, Leprosy, and recently COVID-19, as well as tuberculosis. For these researches, Prof. Eoh has collaborated with various national and international research groups and over 30 research articles in top-tier journals.

Lung Microbiome in Tuberculosis

46

Jorge Cervantes

Messieurs, c'est les microbes qui auront le dernier mot
Gentlemen, it is the microbes who will have the last word.

Louis Pasteur

Summary

The lung microbiota serves as a barrier providing resistance to colonization by respiratory pathogens, while maturing and maintaining the respiratory immune system's balance. Studies on changes occurring in lung microbial communities associated with tuberculosis (TB) continue to emerge. As anti-TB treatment is a prolonged antibiotic treatment that requires multiple drugs, it may bring not only long-term detrimental effects on the microbiome, but these, in turn, may affect the treatment outcome. The study of how the lung's microbiota interacts with *M. tb* infection could open the door for a potential role of probiotics in TB treatment and prevention.

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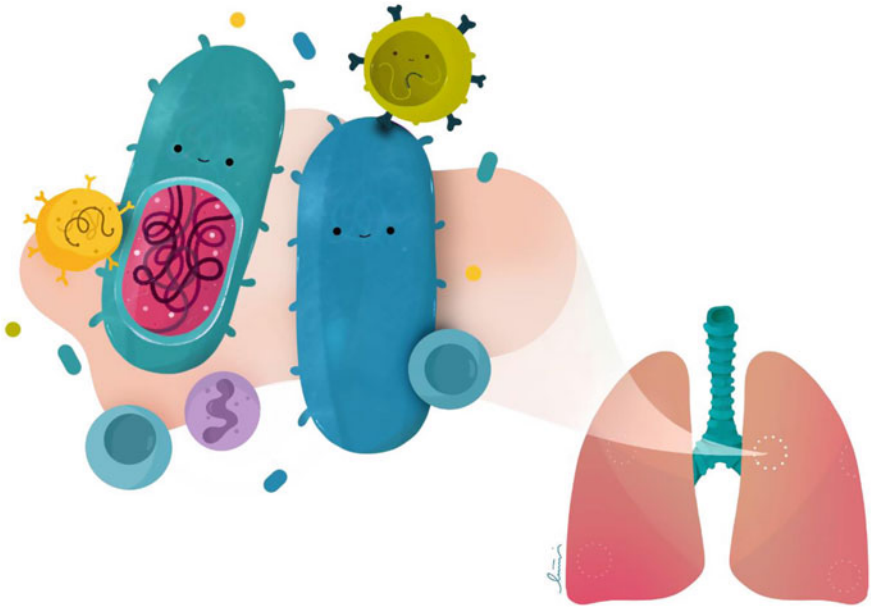
Graphical Abstract



Lung microbiome in tuberculosis. Adapted with permission from the Association of Science and Art (ASA), Universal Scientific Education and Research Network (USERN); Made by Nastaran-Sadat Hosseini

Keywords

Antituberculous therapy · Lung microbiota · *Mycobacterium tuberculosis*

1 Introduction

Tuberculosis (TB) remains a public health threat, despite the global strategies in place aiming to eliminate it [1]. Global efforts to reduce and eradicate TB have to face many obstacles, including the rapid emergence of drug-resistant (DR) strains, as well as the limited efficacy of BCG as a vaccine for pulmonary TB [2]. The immunological response in the lung after exposure to *Mycobacterium tuberculosis (M. tb)* with the formation of a stable granuloma [3] is of utmost importance in

determining whether this exposure results in a successful infection or a protective immunity. Several studies have demonstrated the important role that the host microbiota has in the immune system, its development, maintenance, and in continuously directing an effective immune response against infections. Advancing our understanding of the interactions occurring between the bacterial communities present in the lung might help develop improved ways for the diagnosis, treatment, and prevention of pulmonary TB.

2 The Lung Microbiota in Tuberculosis

The lung microbiota refers to the diverse microbial communities present in the lower respiratory tract. In health, the lung microbiota resembles that one of the oropharynx and probably originates from microaspirations occurring at night [4]. Several studies on the lung microbiota in humans have shown that its diversity and composition can be affected by multiple factors, including antibiotic therapy, environmental factors, and socio-demographic factors. The lung microbiota not only provides resistance to colonization by respiratory pathogens but also plays a role in the maturation and maintenance of homeostasis of the immune system in the lung [5]. The alteration or dysbiosis of the airway microbiota, observed in patients with pulmonary TB, suggests that it could play a significant role in the different pathophysiological processes occurring in TB [6].

Studies describing changes in lung microbial communities associated with TB continue to emerge [7, 8]. These are, however, still limited in number, and the results they provide are inconsistent and sometimes conflicting. Sampling methodologies, and differences associated with the geographic origin of the studies, along with inherent immunogenetic factors, may be partially responsible for the contrasting results [6, 8]. Nevertheless, a diminished diversity in the lung microbiota in TB patients appears to be a common denominator [7, 9–11].

As summarized in Table 1, some reports describe a significant reduction in the abundance of the genus *Streptococcus* along with an increased abundance of *Mycobacterium* in TB patients [9, 10, 12]. *Streptococcus*, *Neisseria*, *Prevotella*, and *Veillonella* are in co-abundance in TB patients. *Actinomyces, Fusobacterium, Leptotrichia, Prevotella, Streptococcus*, and *Veillonella* were previously proposed to represent major genera in the TB sputum microbiota [13]. *Prevotella, Gammaproteobacteria*, *Streptococcus, Neisseria, Selenomonas, Bifidobacterium*, and *Haemophilus* have been largely seen in healthy controls [9, 12, 14]. A recent report showed overrepresentation of *Anoxybacillus* in TB patients, while *Prevotella, Alloprevotella, Veillonella,* and *Gemella* were enriched in *M. tb*-negative patients [11].

Two meta-analyses have been conducted to compare the composition of the lung microbiota of TB patients with that of healthy controls. The first one concentrated on lower respiratory tract microbiota composition (i.e., sputum or broncho-alveolar

Table 1 Genera associated with active tuberculosis (TB) and healthy lower respiratory tract

Possible core genera in TB	Active TB	Healthy controls
Actinomyces, Fusobacterium, Leptotrichia, Prevotella, Streptococcus, and Veillonella [13]	*Anoxybacillus, Streptococcus, Neisseria, Prevotella, Veillonella, Caulobacter, Actinomyces, Rothia, Leuconostoc* [7, 11–13]	*Prevotella, Lactobacillus, Gammaproteobacteria, Actinobacillus, Streptococcus, Neisseria, Selenomonas, Bifidobacterium, Tumebacillus, Propionibacterium, Haemophilus, Alloprevotella, Veillonella, and Gemella* [7, 9, 11, 12, 14]

lavage). TB patients had an increased abundance of *Caulobacter henricus, Actinomyces graevenitzii, Rothia mucilaginosa,* and *M. tb*, whereas healthy controls had *Tumebacillus ginsengisoli, Propionibacterium acnes,* and *Haemophilus parahaemolyticus* as distinct species signatures [7]. Additionally, *R. mucilaginosa* served as the connecting link amongst many other bacterial species in close proximity to *M. tb* infections, as shown using network analysis of co-abundance [7]. A second, more recent meta-analysis found that *Veillonella, Rothia,* and *Leuconostoc* were exclusively found in TB patients, while *Lactobacillus, Gammaproteobacteria, Haemophilus,* and *Actinobacillus* were only found in healthy controls [12].

Even the microbiome present at distal sites from the lung can exert an important function in respiratory health. Pathogens like *Helicobacter hepaticus* can affect microbiota integrity and decrease resistance to *M. tb* [15]. The gut–lung axis is bidirectional, allowing for the flow of microbial products, endotoxins, metabolites, and immune mediators–cytokines–into the circulation through the intestinal niche. The implications of the disturbances to the lung-gut microbiota caused by *M. tb* are crucial, as lung inflammation can impact the microbiota in the lung and the gut and vice versa [15]. The host microbiota plays a crucial role in the prevention of *M. tb* lung colonization in the early stages of infection [16]. A reduced number of *Streptococcus, Haemophilus*, and *Neisseria* appear to be involved in developing a Th1-response in TB patients [17]. Modulation of the Th1-response seems important in maintaining the integral structure of the granuloma [3].

3 The Lung Microbiota in Antituberculous Treatment

Anti-TB treatment (ATT) for four to nine months that comprise several medications is now used to manage TB. Although ATT can effectively kill *M. tb*, they have important side effects, such as liver toxicity. Any short course of antibiotics can cause long-term gut microbiota perturbations [18]. While effectively targeting

M. tb, prolonged use of antibiotics causes considerable damage to the commensal bacteria. ATT may have long-term detrimental effects on the host microbiome of patients under treatment [8, 19]. Furthermore, changes in the lung microbiota may even be associated with treatment outcomes [14].

The most extensively used anti-TB medications, e.g., isoniazid, rifampicin, and pyrazinamide, have been demonstrated to impact the gut microbiota composition in animals [20]. This poses the concern that overuse of antibiotics could potentially become a risk factor for reactivating latent or treated TB [21].

As multidrug-resistant tuberculosis (MDR-TB) continues to grow as a major global concern [22], so has the search for new antibiotics and alternative approaches to fight TB [16, 23].

4 Future Prospects

Our understanding of the mechanisms by which *M. tb* infection (and the antibiotic treatment to eradicate it) alters the lung's microbiota could open the possibilities for a potential role for the use of probiotics as adjuvant TB treatment and prevention [16, 24]. New information on the interactions between the lung microbiota with alveolar epithelial cells, innate immune cells, and subsequent adaptive immune responses [25] could provide clues on the pathogenesis of *M. tb* infection and prompt new therapies for protection.

5 Conclusion

The lung microbiome exhibits changes in microbial communities associated with TB. Lung microbiota alterations linked to prolonged antibiotic treatment required to cure TB have long-term detrimental effects and may affect treatment outcomes.

Core Messages

- The lung microbiome exhibits changes in microbial communities associated with TB.
- Lung microbiota alterations linked to prolonged antibiotic treatment required to cure TB have long-term detrimental effects and may affect treatment outcomes.
- Advancing our knowledge of the lung microbiome in TB can help to develop better approaches to diagnose, treat, and prevent this disease.

References

1. Sakamoto H, Lee S, Ishizuka A, Hinoshita E, Hori H, Ishibashi N et al (2019) Challenges and opportunities for eliminating tuberculosis—leveraging political momentum of the UN high-level meeting on tuberculosis. BMC Public Health 19(1):76. https://doi.org/10.1186/s12889-019-6399-8
2. Daley CL (2019) The global fight against tuberculosis. Thorac Surg Clin 29(1):19–25. https://doi.org/10.1016/j.thorsurg.2018.09.010
3. Balcells ME, Yokobori N, Hong BY, Corbett J, Cervantes J (2019) The lung microbiome, vitamin D, and the tuberculous granuloma: a balance triangle. Microb Pathog 131:158–163. https://doi.org/10.1016/j.micpath.2019.03.041
4. Bassis CM, Erb-Downward JR, Dickson RP, Freeman CM, Schmidt TM, Young VB et al (2015) Analysis of the upper respiratory tract microbiotas as the source of the lung and gastric microbiotas in healthy individuals. mBio 6(2):e00037. https://doi.org/10.1128/mBio.00037-15
5. Man WH, de Steenhuijsen Piters WA, Bogaert D (2017) The microbiota of the respiratory tract: gatekeeper to respiratory health. Nat Rev Microbiol 15(5):259–270. https://doi.org/10.1038/nrmicro.2017.14
6. Adami AJ, Cervantes JL (2015) The microbiome at the pulmonary alveolar niche and its role in *Mycobacterium tuberculosis* infection. Tuberculosis (Edinb) 95(6):651–658. https://doi.org/10.1016/j.tube.2015.07.004
7. Hong BY, Paulson JN, Stine OC, Weinstock GM, Cervantes JL (2018) Meta-analysis of the lung microbiota in pulmonary tuberculosis. Tuberculosis (Edinb) 109:102–108. https://doi.org/10.1016/j.tube.2018.02.006
8. Hong BY, Maulen NP, Adami AJ, Granados H, Balcells ME, Cervantes J (2016) Microbiome changes during tuberculosis and antituberculous therapy. Clin Microbiol Rev 29(4):915–926. https://doi.org/10.1128/CMR.00096-15
9. Hu Y, Cheng M, Liu B, Dong J, Sun L, Yang J et al (2020) Metagenomic analysis of the lung microbiome in pulmonary tuberculosis - a pilot study. Emerg Microbes Infect 9(1):1444–1452. https://doi.org/10.1080/22221751.2020.1783188
10. Vazquez-Perez JA, Carrillo CO, Iniguez-Garcia MA, Romero-Espinoza I, Marquez-Garcia JE, Falcon LI et al (2020) Alveolar microbiota profile in patients with human pulmonary tuberculosis and interstitial pneumonia. Microb Pathog 139:103851. https://doi.org/10.1016/j.micpath.2019.103851
11. Hu Y, Kang Y, Liu X, Cheng M, Dong J, Sun L et al (2020) Distinct lung microbial community states in patients with pulmonary tuberculosis. Sci China Life Sci. https://doi.org/10.1007/s11427-019-1614-0
12. Eshetie S, van Soolingen D (2019) The respiratory microbiota: new insights into pulmonary tuberculosis. BMC Infect Dis 19(1):92. https://doi.org/10.1186/s12879-019-3712-1
13. Cheung MK, Lam WY, Fung WY, Law PT, Au CH, Nong W et al (2013) Sputum microbiota in tuberculosis as revealed by 16S rRNA pyrosequencing. PLoS ONE 8(1):e54574. https://doi.org/10.1371/journal.pone.0054574
14. Wu J, Liu W, He L, Huang F, Chen J, Cui P et al (2013) Sputum microbiota associated with new, recurrent and treatment failure tuberculosis. PLoS ONE 8(12):e83445. https://doi.org/10.1371/journal.pone.0083445
15. Cervantes J, Hong BY (2017) The gut-lung axis in tuberculosis. Pathog Dis 75(8)
16. Dumas A, Corral D, Colom A, Levillain F, Peixoto A, Hudrisier D et al (2018) The host microbiota contributes to early protection against lung colonization by Mycobacterium tuberculosis. Front Immunol 9:2656. https://doi.org/10.3389/fimmu.2018.02656
17. Nakhaee M, Rezaee A, Basiri R, Soleimanpour S, Ghazvini K (2018) Relation between lower respiratory tract microbiota and type of immune response against tuberculosis. Microb Pathog 120:161–165. https://doi.org/10.1016/j.micpath.2018.04.054

18. Cervantes J (2016) Use your antibiotics wisely. Consequences to the intestinal microbiome. FEMS Microbiol Lett 363(10). https://doi.org/10.1093/femsle/fnw081
19. Namasivayam S, Maiga M, Yuan W, Thovarai V, Costa DL, Mittereder LR et al (2017) Longitudinal profiling reveals a persistent intestinal dysbiosis triggered by conventional anti-tuberculosis therapy. Microbiome 5(1):71. https://doi.org/10.1186/s40168-017-0286-2
20. Khan N, Mendonca L, Dhariwal A, Fontes G, Menzies D, Xia J et al (2019) Intestinal dysbiosis compromises alveolar macrophage immunity to Mycobacterium tuberculosis. Mucosal Immunol 12(3):772–783. https://doi.org/10.1038/s41385-019-0147-3
21. Osei Sekyere J, Maningi NE, Fourie PB (2020) Mycobacterium tuberculosis, antimicrobials, immunity, and lung-gut microbiota crosstalk: current updates and emerging advances. Ann N Y Acad Sci 1467(1):21–47. https://doi.org/10.1111/nyas.14300
22. Rao M, Ippolito G, Mfinanga S, Ntoumi F, Yeboah-Manu D, Vilaplana C et al (2019) Improving treatment outcomes for MDR-TB—Novel host-directed therapies and personalised medicine of the future. Int J Infect Dis 80S:S62–S67. https://doi.org/10.1016/j.ijid.2019.01.039
23. Nguta JM, Appiah-Opong R, Nyarko AK, Yeboah-Manu D, Addo PG (2015) Current perspectives in drug discovery against tuberculosis from natural products. Int J Mycobacteriol 4(3):165–183. https://doi.org/10.1016/j.ijmyco.2015.05.004
24. Mortaz E, Adcock IM, Folkerts G, Barnes PJ, Paul Vos A, Garssen J (2013) Probiotics in the management of lung diseases. Mediators Inflamm 2013:751068. https://doi.org/10.1155/2013/751068
25. Gupta N, Kumar R, Agrawal B (2018) New players in immunity to tuberculosis: the host microbiome, lung epithelium, and innate immune cells. Front Immunol 9:709. https://doi.org/10.3389/fimmu.2018.00709

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The Correlation of Microbiota and Host Epigenome in Tuberculosis

47

Samira Tarashi, Mir Davood Omrani, Arfa Moshiri, Abolfazl Fateh, Seyed Davar Siadat, and Andrea Fuso

As you start to walk on the way, the way appears.

Rumi

Summary

Tuberculosis (TB) infection still represents a relevant global health issue affecting millions of patients worldwide, although some may remain undiagnosed and untreated. The diagnosis, treatment, and control strategies of TB may not be as effective as they should be. In this regard, the role of the microbiota community and epigenetic mechanisms in TB infection can be fundamental but not widely

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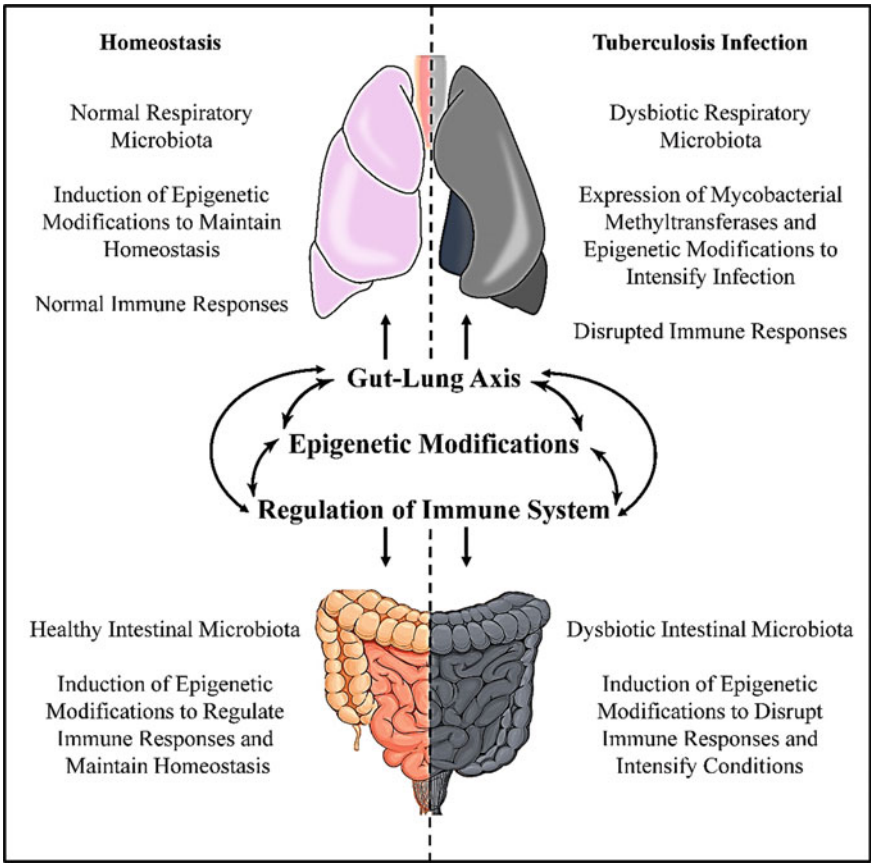
deciphered. It appears that the development of microbiota and epigenetics analysis can be operative for innovative and more effective control strategies and targeted therapeutics of TB infection. Hence, an overview of the correlation between microbiota community and epigenetic mechanisms occurring upon TB infection can represent an opportunity to improve control of TB infection while stressing that additional evaluations are necessary to uncover this correlation.

Graphical Abstract



The gut-lung axis, epigenetics, and immunity in tuberculosis

Keywords

Epigenetics · Microbiota · Mycobacterial methyltransferase · *Mycobacterium tuberculosis*

1 Introduction

Mycobacterium tuberculosis (*M. tb*) mostly affects the lungs and induces tuberculosis (TB), an infectious disease. Historically, TB is defined as one of the devastating epidemiological challenges in the health system. The latest World Health Organization (WHO) report has indicated 9.96 million new TB cases in 2020 [1]. Among all the newly infected cases by *M. tb*, about 15% could not receive efficient treatment. Each case of active TB can infect ten to 15 people annually [2]. Besides, an epidemiological report predicted that without effective control and treatment of *M. tb*, this bacterial agent could infect almost 225 million individuals during 1998–2030 [3]. Hence, rapid detection and impressible treatment are two major objects for TB infection control [4]. Nevertheless, despite the intense research on TB, the control of this infection is still largely suboptimal, and the mechanisms of its progression cannot be completely explained. Therefore, deciphering novel strategies to amend TB control is of great relevance. WHO has recently introduced the top priority target product profiles (TPPs) to rapidly recognize TB infection [5]. These contain non-sputum-based tests to quickly diagnose TB infection to start the most efficient treatment on the same day. Thus, introducing distinctive and specific biomarkers for rapid and correct detection may be a crucial need. It has been recently shown that the specific microbiota pattern and epigenetic mechanisms at each stage of TB infection should be more evaluated as a novel and potential biomarker [6, 7]. Nevertheless, a few biomarkers based on microbiota and epigenetic marks are currently included in clinical inquiries. Besides, in order to limit the global TB burden, the WHO announced the End TB Strategy [8]. It is typically highlighted that a combined interface of microbiota and epigenetic mechanisms rather than just the effect of a single infectious agent may cause TB infection. Taken together, microbiota and epigenetic analyses may be useful to achieve this goal. The human microbiota and the epigenetic mechanisms, as interesting new fields, have important implications for more successful and effective diagnosis, treatment, and control of TB infection.

The human microbiota is involved in the immune system's maturation and development, including immunomodulation, triggering *M. tb*reactivation from latent form, transmission, antibiotic resistance, and disease severity. Subsequently, the dysbiotic microbiota community is a significant issue in the *M. tb*pathogenesis, and its evaluation may upgrade control strategies of TB infection in the future. Hence, consideration of the microbiota community in different aspects of TB infection can be important for better detection of this disease. In recent years, increasing studies have been focused on different parts of the correlation between microbiota and TB infection. On the other hand, several bacterial by-products have been reported as central regulatory factors with the potential to active epigenetic alterations and, consequently, the host transcriptional profile [9]. It is well established that these by-products are important messengers in the interactions between microbiota and host cells [10]. In addition to the critical involvement of microbiota in different epigenetic alterations to maintain homeostasis, several pathogens also

induce these alterations in the host cells or even their genome modulating the severity of the disease and escaping the host defense mechanisms [11]. *M. tb* is one of the classic examples of those pathogens that may epigenetically modify the host genome for its survival. Overall, considering the possible association between epigenetic mechanisms and TB progression represents an interesting area. A study of the correlation of microbiota community and epigenetic mechanisms may assist in revealing important evidence about TB severity and host susceptibility [12, 13]. However, various features of the correlation inside the microbiota community, epigenetic alterations, and TB infection are not fully uncovered. An overview of the interaction between the microbiota communities and the epigenetic alterations occurring in TB infection will be outlined in the next section. This object sheds new light on how the association between the microbiota communities and epigenetic mechanisms is associated with TB infection.

1.1 Human Microbiota and Tuberculosis

Initially, Lederberg and McCray introduced the concept of human microbiota [14], which referred to the microbial community in habitats of different human body sites such as the gut, oral cavity, vagina, skin, and lower respiratory tract [15]. The entire genome of this bacterial community is introduced as the microbiome [16]. The healthy microbiota community and related metabolites play a beneficial and crucial role in maintaining a healthy state by imprinting metabolism and immunity to the host [17]. An imbalance in the normal microbiota community, named dysbiosis, is associated with various pathogens' impaired immune system function and colonization [18]. Although numerous studies have investigated the potential association between TB infection and variations in the respiratory and gut microbiota, the mechanisms and causative factors associated with the development of TB infection have not been widely addressed [19], and recent evidence highlighted epigenetic alterations might be important in this correlation. Figure 1 presents a schematic view of the gut-lung axis and the importance of induced epigenetic mechanisms by microbiota and *M. tb* in homeostasis and TB infection.

Some evaluations used animal models to confirm the feasible role of gut microbiota in TB infection. For example, it is shown that a special strain of *Helicobacter* may affect the outcomes of TB infection [20]. Winglee et al. studied mice infected by *M. tb* until death and stated a significant decrease in the Bacteroidetes and Clostridiales in the gut microbiota during infection [21]. Besides, the microbiota community has evaluated several human studies in different stages of TB infection. The main bacterial genera specified in recurrent TB infection include *Collinsella*, *Streptococcus, Prevotella*, *Bacteroides*, and *Escherichia*, which are not meaningfully different from the new TB cases [22]. Recent findings show that the abundance of mycobacteria was significantly reduced one month after treatment, and some of the associated pathways to a healthy gut microbiota were improved [23]. It is shown that treatment changed the taxonomic microbiota composition without changing total diversity. Depleting *Bacteroides* and increasing

Faecalibacterium, *Ruminococcus,* and *Eubacterium* species are confirmed in cured TB patients [24]. Also, a better assessment of the immune status is suggested in HIV patients, which may activate immunity against *M. tb*, anti-TB treatment, and infection control [25]. Specifically, the respiratory microbiota suppresses immune responses against *M. tb* and enhances its risk, particularly in HIV patients, by producing short-chain fatty acids (SCFAs), such as propionate and butyrate. These studies provide a paradigm example to show why respiratory and gut microbiota changes are associated with the severity of the inflammatory response in the lungs. In general, dysbiotic respiratory microbiota causes an impaired immune response and creates a favorable condition for the proliferation and growth of various pathogens that can cause further inflammation and dysbiosis (Fig. 1) [26]. *M. tb* is identified as one of the most complex pathogens that may quickly colonize a subject after the respiratory microbiota dysbiosis; its chronicity and progression may contribute to the greater development of dysbiosis [26]. Like the respiratory microbiota, dysbiosis of the gut microbiota is also correlated with the development of multiple diseases [27]. Growing evidence demonstrates the close immunological link between the respiratory and gut microbiota, described as the gut-lung axis. The concept of the gut-lung axis represents the regulatory impact of respiratory microbiota on gut immunity and vice versa. In this regard, disruption of the gut and/or respiratory microbiota communities is linked with the altered immune responses and the TB infection severity (Fig. 1) [28]. The microbiota's by-products (endotoxins, metabolites, and extracellular vesicles (EVs), etc.) are released into the bloodstream, thus connecting the respiratory and gut niches. In this way, it is possible that they pass through the liver and in consequence, activate some immune cells such as macrophages and neutrophils, i.e., the most important cells activated in TB infection [29]. Moreover, the lymphatic system obstruction in the gut-lung axis decreases the release of multiple cytokines (interleukin (IL)-10, IL-1β, and vascular endothelial growth factor (VEGF), etc.) in the lungs [30]. This evidence suggests that the study of both respiratory and gut microbiota can be fundamental in the pathogenesis, prevention, and treatment of TB infection by epigenetic mechanisms. Nevertheless, little is known about the effect of epigenetic mechanisms in the gut-lung axis on TB infection. Altogether, the protective effect of the gut microbiota against lung infections and the related epigenetic alterations could become a promising field of research to improve control strategies of TB infection.

2 Human Microbiota and Epigenetics

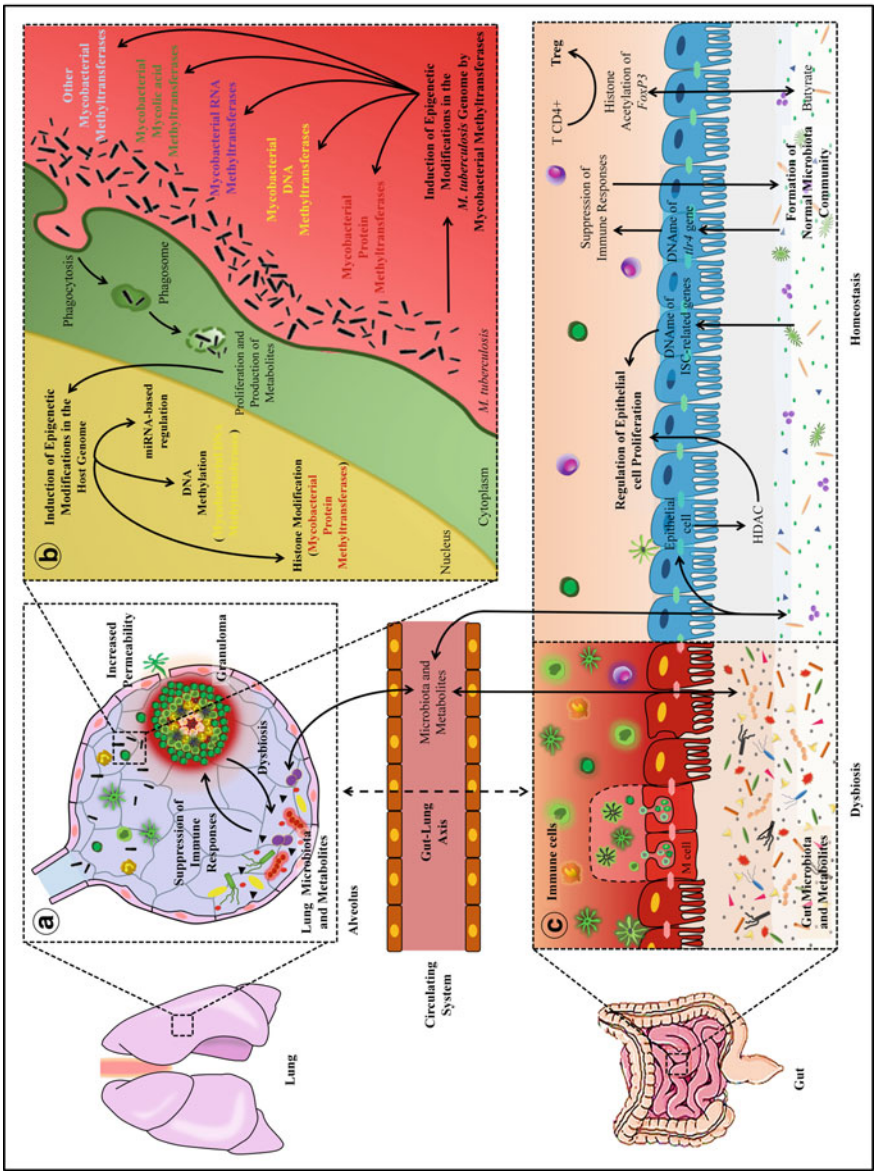
The total number of microorganisms that normally inhabit our mucosal surfaces is greater than the number of eukaryotic cells (about 10^{13} cells) in the human body. The microbiota community impacts homeostasis state and disease progression and changes over the lifetime. The gut habitat of each individual includes on average 300 to 500 different bacterial species. Various interactions between host cells and microorganisms are likely evaluated in no other places as significant as the

gastrointestinal tract. The normal gut microbiota by these pathways has homeostatic roles beyond the traditional concepts such as defense against potential pathogens and fermentation of indigestible nutrients [31]. It is proposed that such pathways directly impact the well-being of the gastrointestinal tract. It is now mostly defined that exposure to bacteria induces epigenetic alterations and may explain to some extent homeostatic maintenance or disease progression.

The term "epigenetic" derives from the Greek word "epigenesis," which means "above genetics/genesis." Waddington first coined the term in 1942 as a biological phenomenon with no direct relationship between the genotype of a gene and its phenotype [32]. Epigenetic mechanisms modify gene function remarkably without any direct change in the DNA sequence [33]. Several epigenetic mechanisms, including chromatin remodeling, histone alterations, DNA methylation, and RNA-based regulation, are today well characterized. Generally, evaluation of the epigenetic mechanisms helped to solve complex relationships and molecular mechanisms related to different cellular processes in complex and multifactorial diseases by deciphering the functioning of genes. In the frameshift of infectious diseases, epigenetic studies allowed us to understand better how microorganisms earn protection from host immunity [34]. Epigenetic mechanisms govern most health indicators and a wide variety of infections and disorders. However, the extent of various epigenetic mechanisms for the development of human diseases is underestimated compared to genetic variation. Despite that, epigenetic alterations may be proved as better biomarkers than genetic factors [35]. Therefore, the assessment of epigenetic mechanisms in the homeostatic states and different pathologies represents an opportunity to introduce useful biomarkers with great potential for prevention, diagnosis, and even identification of new targets for medical therapy [36].

The microbiota community may impact epigenetic mechanisms and makeup even before birth [37]. Formerly, the fetus was presumed to remain in a sterile condition, and the microbiota community forms only during and after birth. This dogma is in stark contrast to numerous studies [38, 39]. Early exposure to microbiota appears to play a major role during fetal life and may effectively maintain hemostasis or risk of subsequent disease, possibly with inherited epigenetic alterations [40]. The microbiota is considered an important part of normal human physiology because its microbial genome allows for multiple metabolic processes

Fig. 1 A schematic figure of the correlation between microbiota, epigenetics, and tuberculosis. ▶ **a** The effect of *M. tuberculosis (M. tb)*inhalation on the lung microbiota and epigenetics. The immune cells surround *M. tuberculosis* and form a granuloma, while *M. tb* induces dysbiosis, suppressing immune responses in the lung. The microbiota and *M. tb* can increase epigenetic alterations to reach their suitable conditions; **b** The induced epigenetic alterations upon TB infection. *M. tb* may epigenetically affect its genome by producing different mycobacterial methyltransferases (presented in Fig. 2 in detail). In addition, *M. tb* epigenetically changes the host genome to increase its susceptibility; **c** The immunologically relationship between the gut and respiratory microbiota by the gut-lung axis. The gut microbiota in both dysbiotic and homeostatic status can affect the respiratory microbiota by the gut-lung axis. This axis is bidirectional. The induced epigenetic alterations by gut microbiota to maintain homeostasis are presented on the right side


Lung
Gut
Circulating System
Alveolus
Gut-Lung Axis
Microbiota and Metabolites
Lung Microbiota and Metabolites
Increased Permeability
Granuloma
Dysbiosis
Suppression of Immune Responses
(a)
(b)
Induction of Epigenetic Modifications in the Host Genome
miRNA-based regulation
DNA Methylation
Histone Modification (Myeobacterial Protein Methyltransferases)
Phagocytosis
Phagosome
Proliferation and Production of Metabolites
M. tuberculosis
Cytoplasm
Nucleus
Induction of Epigenetic Modifications in the M. tuberculosis Genome by Myeobacterial Methyltransferases
Mycobacterial Protein Methyltransferases
Mycobacterial DNA Methyltransferases
Mycobacterial RNA Methyltransferases
Mycobacterial Mycolic acid Methyltransferases
Other Mycobacterial Methyltransferases
(c)
Immune cells
M cell
Gut Microbiota and Metabolites
Dysbiosis
Homeostasis
Regulation of Epithelial cell Proliferation
Epithelial cell
HDAC
DNAme of ISC-related genes
DNAme of Ifn-4 gene
Suppression of Immune Responses
T CD4+
Treg
Histone Acetylation of FoxP3
Formation of Normal Microbiota Community
Butyrate

beyond the capabilities of the host genome. It is also well known that the natural microbiota plays an important role in regulating the gut immune system [37]. As mentioned, the mass production of biologically active molecules as a result of direct production and a digestive process is known as one of the hallmarks of the gut microbiota. These by-products are crucial for maintaining the interaction between the microbiota community and host cells. These types of molecules include but are not restricted to neurotransmitters, chemical moieties, SCFAs, and simple gases, which act as proven epigenetic mediators [33, 37]. In other words, the microbiota can affect epigenetic regulation of host cells by producing metabolites directly or indirectly by stimulating signaling cascades that cause epigenetic alterations in different genes. In the natural human gut, dominated bacterial phylae include Proteobacteria, Firmicutes, Actinobacteria, and Bacterioidetes that are involved in the several metabolites production. SCFA (propionate, acetate, and butyrate) are some of the end by-products of gut microbiota fermentation in the presence of complex carbohydrates or other indigestible nutrients [41]. Among them, butyrate is very important for inducing epigenetic alterations (Fig. 1). Butyrate induces H3 histone acetylation at the *Foxp3* gene locus. Foxp3 chiefly regulates differentiation pathways of naïve CD4 + T cells into Treg cells which are important for regulating gut immunity [42]. On the other hand, intestinal stem cells (ISCs) are situated between intestinal epithelial cells that regulate epithelial cell differentiation and proliferation by ISC-related genes. Commensal gut microbiota helps suppress ISC-related genes by inducing DNA methylation. Intestinal epithelial cells inherently produce several histone deacetylases (HDACs) to adjust the epithelial composition and gut barrier function. In addition, the normal microbiota epigenetically suppresses the epithelial TLR4 expression to provide an opportunity for better microbiota community formation under normal conditions [37, 43]. However, current knowledge is still limited and only a small amount of information on the full spectrum of microbiota's effects on the human body has been investigated. Indeed, the study of epigenetic alterations in the immune response to *M. tb* and microbiota can solve an important mystery about host susceptibility.

3 Microbiota, Epigenetics, and Tuberculosis

The examples above indicated that numerous produced metabolites by microbiota, mainly gut microbiota, can stimulate epigenetic mechanisms and affect the immune responses [44]. This means that microbiota can potentially cause several disorders by inducing these epigenetic mechanisms [13, 45]. Like many other pathogens, *M. tb* has the potential to modify the host epigenome [46]. Modifying the host transcriptional profile may be induced by altering the epigenome in TB infection [6], but the involved epigenetic mechanisms are not yet completely clarified [35]. Recently, the crosstalk between the epigenetic mechanisms and TB infection has been extensively depicted [6]. This paper has reviewed the knowledge on the histone epigenetic alterations and DNA methylation, as well as miRNA-based

regulation occurring in TB infection. Examining the relationships between different epigenetic mechanisms in the progression of TB may help reveal important data on host susceptibility and the pathogenesis of TB infection. To more clarify these associations, a study has hypothesized that the profile of DNA methylation of the peripheral blood mononuclear cells in active TB patients and treated TB individuals is different from those in the control group. Identification of novel epigenetic alterations related to early infection, infection development, treatment responses, and outcome of active TB infection and developing new epigenetic targets for more efficient diagnosis and treatment have been declared as some of the purposes of this study [12]. However, the various aspects of the relationship between epigenetic mechanisms and TB infection are still unclear. Many epigenetic mechanisms affect the activity of involved genes in the immune responses during TB infection. Most epigenetic mechanisms associated with TB infection immunomodulation include methylation and acetylation of the core histone tails, DNA methylation, and miRNA-based regulation [6]. Although numerous bacterial by-products retain the ability to cause various epigenetic alterations in host cells, only a few amounts of molecules have been identified with epigenetic impacts upon TB infection. Among them, Eis, LpqH, ManLAM, and ESAT-6 have been studied more [35, 47]; their epigenetic functions will be explained in the following paragraphs.

3.1 Histone Epigenetic Alterations in Tuberculosis

Chromatin-remodeling and histone epigenetic alterations are post-translational and reversible epigenetic mechanisms that may interact with important cellular processes [48]. The chromatin structure is highly dynamic because it is involved in several cellular processes (usually transcription and DNA replication) with active regeneration by activating chromatin remodeling and histone-modifying enzymes [49]. Histone-modifying enzymes can direct different covalent alterations like sumoylation, deamination, proline isomerization, ubiquitylation, ADP ribosylation, methylation, acetylation, and phosphorylation, frequently on the protruding N-terminal tails of the core histones. Most of the important histone-modifying enzymes are included, e.g., histone demethylases (HDMs), histone methyltransferases (HMTs), histone deacetylases (HDACs), and histone acetyltransferases (HATs) [35]. The core histones include H2A, H2B, H3, and H4 that form a surrounded octamer by 147 base pairs of DNAs (1.7 turns), identified as a nucleosome. The nucleosome is the major chromatin unit that repeats at approximately every 200 base pairs (bp) of the DNA sequence. In addition, the histone H1 linker fortifies nucleic acid molecules around core histones to compact DNA by circling the nucleosomes on top of each other [50]. Certain changes (such as hyperacetylation or hypomethylation) of alkaline histones neutralize the positive charge of these proteins to some extent, which is essential for interaction with negatively charged strands of DNA, and destabilize the binding of histones to DNA. As a result, these alterations cause the chromatin to relax and increase gene expression. The acetylation of histones by HATs increases space between nucleosomes and therefore

activates chromatin, while the deacetylation of these proteins by HDACs suppresses the gene expression. To generalize, increasing chromatin compaction and suppression of gene expression may have been associated with histone methylation and histone deacetylation. Methylation of histones activates or represses gene expression based on the position of the target amino acid. Several studies evidenced chromatin remodeling and histone epigenetic alterations in TB infection induced by mycobacterial metabolic products to modify transcriptional profiles, possibly reducing innate and adaptive immune responses [6]. The ESAT6 may induce histone acetylation in the *CIITA* promoter I locus and histone methylation at the lysine 4 of the H3 histone site (H3K4me), which leads to regulating *CIITA* and *IFN-γ* expression using chromatin-remodeling complexes [51]. CIITA regulates the expression of different genes involved in the antigen presentation in macrophages, such as the *MHC class II* gene [52]. The majority of studies in the field of histone modification and TB infection have reported differential histone methylation or acetylation on promoters associated with the immunogenic genes [6]. Several mycobacterial proteins with histone-modifying activity, like SET8, SUV39H1, Rv2966c, Rv1988, and Rv1198, have been recognized for inducing histone epigenetic alterations during TB infection [6].

Histone acetylation has also been well defined in TB infection. Acetylation of lysine residues of core histone tails may highly stimulate gene expression [53]. In contrast, *M. tb* uses histone deacetylases and subsequently reduces gene expression [46]. For instance, LpqH acts as a mycobacterial factor and a TLR2 agonist and reduces the presentation of antigens to T cells by *CIITA* histone deacetylation in the promoter region [54]. Moreover, Eis is an acetyltransferase protein that effectively acetylates free histones (such as histones not attached to the nucleosomes) and represses the ERK1/2-JAK pathway in T cells [55]. Overexpression of matrix metalloproteinases (MMPs), in particular MMP-1 and MMP-3, and degradation of the pulmonary extracellular matrix has been described upon TB infection. The regulation of expression of such MMPs is related to histone acetylation [56]. Also, evidence shows mycobacterial suppression of IFN-γ-dependent HLA-DR gene expression by the histone deacetylation complex formation in its promoter [57]. Histone methylation usually modifies arginine or lysine residues, leading to activation or suppression of gene expression, respectively [58]. Its modification also depends on the degree of methylation of the residual lysine. Altogether, histone epigenetic alterations establish the differences in functions and dynamics of the chromatin. For example, tri-methylation (not mono- or di-methylation) of H3 lysine 9 (H3K9me3), H4 lysine 20 (K4K20me3), and H3 lysine 27 (H3K27me3) suppresses gene expression. In contrast, tri-methylation of H3 lysine 4 (H3K4me3) and mono-methylation of H3 lysine 9 (H3K9me1), H3 lysine 27 (H3K27me1), and H4 lysine 20 (K4K20me1) is associated with active chromatin [53, 59].

In addition to evaluating histone epigenetic alterations at various cell lines, some studies have demonstrated acetylation changes in MtHU, histone-like proteins purified from *M. tb* cells [60–62]. Acetylation of MtHU suppresses its ability to bind to DNA and facilitates chromatin in the host cell reprogramming and intracellular bacterial survival [62]. In total, histone epigenetic alterations are very

complex, and the outcome of gene transcription depends on various epigenetic factors that synergistically function with histones and regulate the fate of gene expression [59]. Also, epigenetic modification in *M. tb* cells, besides in host cells, is interesting.

3.2 DNA Methylation in Tuberculosis

Before 1980, Ginder and McGhee demonstrated that DNA methylation is important in regulating gene expression [63]. DNA methylation modulates various physiological cellular processes and multifactorial disorders [64, 65]. DNA methylation is the almost irreversible and more stable epigenetic mechanism, unlike other epigenetic mechanisms. The enzymes involved in this mechanism are the DNA methyltransferases (DNMTs), expressed by host cells or microorganisms [66, 67]. Three families of DNMTs perform DNA methylation in eukaryotic cells: DNMT1, DNMT2, and DNMT3 [68]. Several studies showed the function and the mechanisms of action of eukaryotic DNMTs [68–70]. In brief, DNMT1 is involved in the maintenance of DNA methylation during cell proliferation. DNMT3a and DNMT3b are usual primers of DNA methylation. DNMT3b is also responsible for most non-CpG methylation [70]. Finally, it shows that DNMT2 acts as an RNA-specific methyltransferase and is unable to induce methylation in DNA [71]. Prokaryotic DNMTs are not as complex as eukaryotes. They are almost three times smaller than their eukaryotic homologs. As a matter of fact, more methyltransferases are present in prokaryotes, and it seems that eukaryotic DNMTs are probably reminiscent of ancestral prokaryotic methyltransferase [72]. DNMTs induce methylation at carbon n○ 5 cytosines (C5) by transferring methyl groups (-CH3) from the cofactor S-adenosylmethionine (SAM) [46]. These alterations alter the chromatin's three-dimensional (3D) structure at specific binding sites for several transcription factors and affect gene expression. It is reported that approximately 70–80% of all CpG islands in the human genome are methylated [68]. The *CpG islands* are identified as CpG-rich regions. Nearly 50% of them are around the transcription start sites (TSSs) and in the promoter of most housekeeping genes [73]. Nevertheless, CpG methylation has also been reported from gene promoters lacking the CpG-rich regions, leading to more dynamic methylation variability and less stable gene silencing in these cases [6, 74].

Tarashi et al. reported in vitro and in vivo evaluations about the DNA methylation profile in *M. tb*-infected cells as well as in TB patients compared to the control groups [6]. The results indicated that *M. tb* often causes CpG islands DNA methylation at enhancer sites and promoter of various main genes involved in the immune responses that may enhance the host susceptibility to TB infection. For example, aberrant methylation of the *VDR* gene is well known to increase this host susceptibility [75]. Likewise, the *TLR2* promoter hypermethylation can suppress its expression in TB infection [76]. As aforementioned in the previous section of this chapter, such epigenetic alterations have suppressed *TLR4* by commensal microbiota to form a normal microbiota community. Both these mechanisms result in the

suppression of the immune system, but, despite inducing similar epigenetic alterations, the final effect of *M. tb* is negative while the effect of the microbiota is positive on the human body. Another investigation reported the profile of DNA methylation of inflammatory genes in the cell lines infected with *M. tb* [77]. Activation of inflammasome protein complex NLRP3 is regulated through DNA methylation in TB infections [78]. It is also indicated that dysbiosis during some disorders such as colorectal cancer (CRC) may decrease the function of inflammasomes by DNA methylation and therefore increase inflammation [37]. In addition, CD82 expression is controlled by the hypomethylation promoter in TB infection [79]. CD82 is a significant factor in the intracellular growth and pathogenesis of *M. tb* virulent strains. Moreover, *M. tb* has been shown to induce aberrant methylation throughout the whole host cell genome, especially in the non-promoter regions [80]. In general, it looks that aberrant DNA methylation often occurs in low-density CpG regions such as distal regulatory regions or enhancers in TB infection, rather than high-density CpG regions and promoters [6]. Pacis et al. attained great results by evaluating the DNA methylation profile of the whole genome caused by TB infection in monocyte-derived DCs [81, 82]. These results emphasized the DNA methylation effect on the expression of immune genes and confirmed the theory of trained immunity. This theory points to a stronger and faster response to secondary infections due to the existence of memory cells in the innate immune system [83].

Besides, the role of bacterial DNA methylation in the presentation of particular methylation marks in the genome of bacteria has recently been considered [84]. The association between DNA methylation and inducing antibiotic resistance has been attracted much attention as a new model in the TB research field [85]. Thus, these reports bring new hope for the fight against TB infection. It is probable that an amended assessment of DNA methylation profile can be beneficial for developing well-organized control strategies of TB infection, and evaluation of different DNMTs can offer a great advantage in this manner.

3.2.1 Mycobacterial Methyltransferases

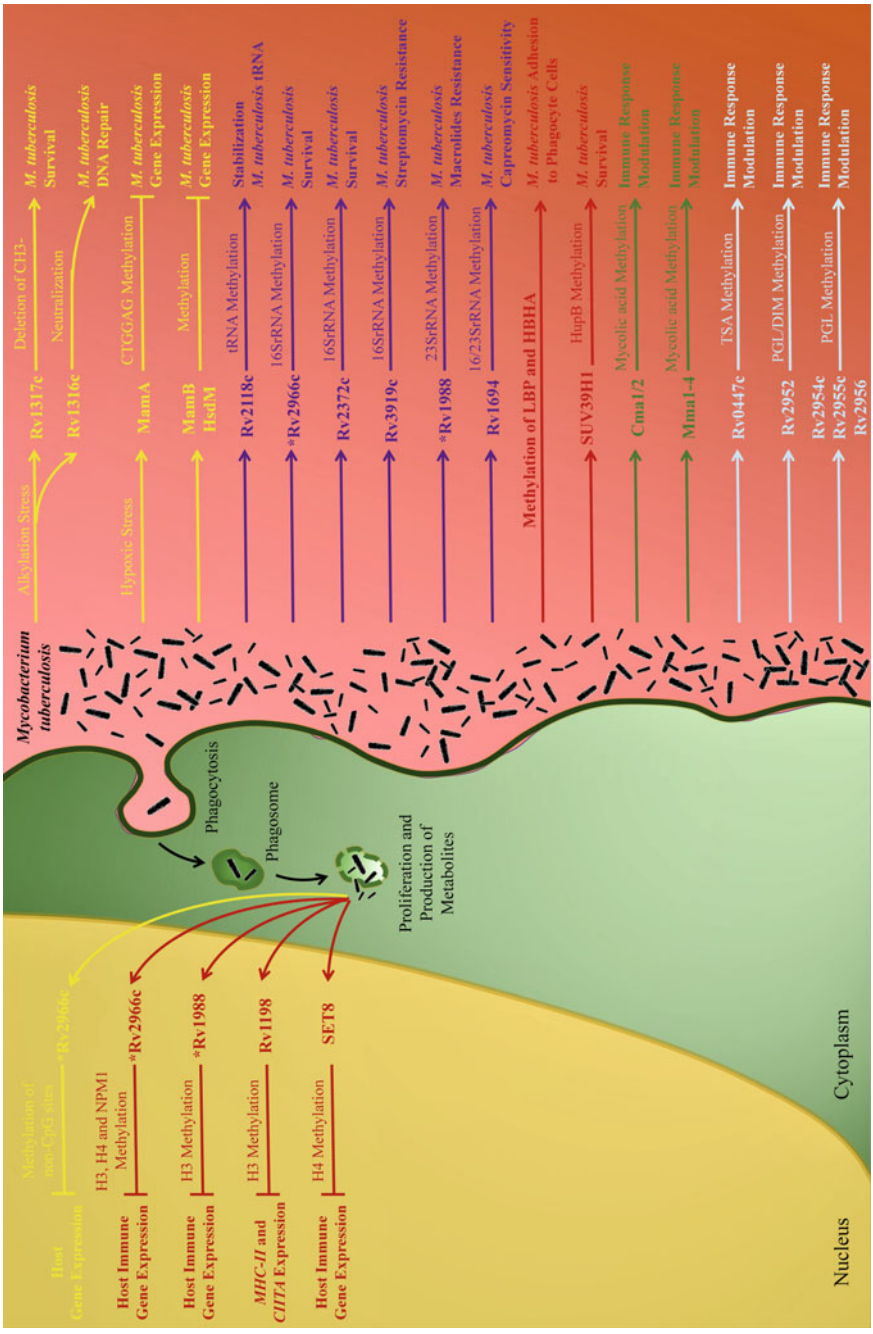
The methylation process regulates numerous cellular processes according to metabolic status. This process is catalyzed by different methyltransferases that transfer a methyl group from SAM to the proteins, lipids, nucleic acids, and secondary metabolites. It is predicted that more than 1% of human genes encode various methyltransferases, while in the *M. tb* genome, despite a genome reduction, this number has increased to 3% [67]. Sharma et al. identified 17 hypothetical genes encoding proteins with methylase activity in *M. tb* [86]. These genes have been identified by sequence analysis of the binding domains of the SAM gene. In general, about 121 different methyltransferases have been coded by the genome of *M. tb* [87], which employs several substrates, including DNA, RNA, protein, mediators of mycolic acid biosynthesis, and other fatty acids. Therefore, mycobacterial methyltransferases can be classified into five categories: DNA methyltransferases (DNMTs), RNA methyltransferases, protein methyltransferases, mycolic acid methyltransferases, and other methyltransferases. Figure 2 presents different

categories of mycobacterial methyltransferases. It is well known that DNMTs and protein methyltransferases may be a part of epigenetic alterations, but there is some evidence that also announced the term of RNA epigenetics. RNA epigenetics refers to the enzymes, including RNA methyltransferases, that post-transcriptionally modify different RNAs [88]. Understanding the function of different mycobacterial methyltransferases might represent a new frontier in TB and epigenetics research.

3.2.2 Mycobacterial DNA Methyltransferases

The GC-rich genome of *M. tb* (about 65%) may be the main reason that DNA methylation is introduced as the only identified mechanism in prokaryotes that heritably regulates expression patterns, whereas there are several mechanisms for this purpose in the case of eukaryotes. DNA methylation in *M. tb* represents a growing area of interest [6, 67]. The adenine and cytosine residues often methylate in the DNA sequence to form N4-methylcytosine (4mC), N6-methyladenine (6 mA), and 5-methylcytosine (5mC). In prokaryotes, 6 mA formation has been confirmed as an important regulatory mechanism of gene expression through epigenetic alterations, but some reports have suggested that cytosine methylation also plays a fundamental role in prokaryotes [89, 90]. Although in most eukaryotes, cytosine methylation is a well-established mechanism of gene expression [91]. Several *M. tb* strains such as H37Rv, H37Ra, and *M. smegmatis* express no DNA adenine methyltransferase (Dam) or DNA cytosine methyltransferase (Dcm) [92]. Dam and Dcm are the main methyltransferases in several prokaryotes [93]. However, analysis of the mycobacterial genome has clarified a significant amount of 6 mA and 5mC, highlighting DNMTs other than Dam and Dcm in *M. tb* [94]. So far, about 121 different mycobacterial methyltransferases have been identified, and five of them are characterized to be mycobacterial DNMTs [87]. The genes coding mycobacterial DNMTs are *Rv1317c*, *Rv1316c*, *Rv3263*, *MamB*, and *Rv2756c*. *Rv1317c* contains two co-operonic DNMTs in *M. tb ada* operon that have the potential to certify the survival of *M. tb* during alkylation stress. The fused protein AlkA and AdaA (*Rv1317c*) and a separate protein AdaB/OGT (*Rv1316c*) are coded by *ada* operon. Exposure to nitrogen and oxygen radicals and alkylation stress are usual in unfavorable surroundings, which may induce devastating effects on the genome. *M. tb* has adopted AdaB/OGT to neutralize O_6-methylation of guanine, one of the major mutagenic lesions in the alkylation stress, and AlkA and AdaA for removal of methyl groups [95]. In addition, AdaB/OGT repairs DNA damage at O_6-alkylated guanine and inhibits mutations associated with O_6-alkylguanine (T15S and R37L) in mycobacterial DNA. These mutations were observed in correlation with defects in alkylated DNA repair [96].

Fig. 2 Mycobacterial methyltransferases. Mycobacterial methyltransferases modify the methylation pattern of the mycobacterial genome (presented on the right side) and host cell (presented on the left side) based on conditions. Different mycobacterial methyltransferases have shown in different colors: mycobacterial DNA methyltransferases in yellow; mycobacterial RNA methyltransferases in purple; mycobacterial Protein methyltransferases in red; mycobacterial mycolic acid methyltransferases in green and other mycobacterial methyltransferases in white. Multifunctional mycobacterial methyltransferases have been specified with a star ▶


Mycobacterium tuberculosis
Alkylation Stress
Hypoxic Stress
Rv1317c
Rv1316c
MamA
MamB
HsdM
Rv2118c
*Rv2966c
Rv2372c
Rv3919c
*Rv1988
Rv1694
SUV39H1
Cma1/2
Mma1-4
Rv0447c
Rv2952
Rv2954c
Rv2955c
Rv2956
Deletion of CH3-
Neutralization
CTGGAG Methylation
Methylation
tRNA Methylation
16SrRNA Methylation
16SrRNA Methylation
16SrRNA Methylation
23SrRNA Methylation
16/23SrRNA Methylation
Methylation of LBP and HBHA
HupB Methylation
Mycolic acid Methylation
Mycolic acid Methylation
TSA Methylation
PGL/DIM Methylation
PGL Methylation
M. tuberculosis Survival
M. tuberculosis DNA Repair
M. tuberculosis Gene Expression
M. tuberculosis Gene Expression
Stabilization M. tuberculosis tRNA
M. tuberculosis Survival
M. tuberculosis Survival
M. tuberculosis Streptomycin Resistance
M. tuberculosis Macrolides Resistance
M. tuberculosis Capreomycin Sensitivity
M. tuberculosis Adhesion to Phagocyte Cells
M. tuberculosis Survival
Immune Response Modulation
Immune Response Modulation
Immune Response Modulation
Immune Response Modulation
Phagocytosis
Phagosome
Proliferation and Production of Metabolites
Cytoplasm
Nucleus
Host Gene Expression
Methylation of non-CpG sites
*Rv2966c
H3, H4 and NPM1 Methylation
Host Immune Gene Expression
*Rv1988
H3 Methylation
Host Immune Gene Expression
Rv1198
H3 Methylation
MHC-II and CIITA Expression
SET8
H4 Methylation
Host Immune Gene Expression

Besides, an interesting point to discuss is the importance of DNA Methylation in epigenetic regulation. The three mycobacterial DNMTs, MamA (*Rv3263*), MamB, and HsdM (*Rv2756c*), are responsible for epigenetic regulation, and none of them are interestingly associated with restriction endonucleases. MamA (mycobacterial adenine methyltransferase) is well characterized, which induces methylation of the CTGGAG sequence in the mycobacterial genome to affect the expression of several genes. It has been highlighted that MamA deletion is related to reducing *M. tb*survival in hypoxic conditions as physiological stress. Therefore, MamA is a critical mycobacterial mediator of adaptation to various physiological stresses [97]. In addition to the identified mycobacterial DNMTs, MTSP11 (*Rv3204*) was also introduced as a possible mycobacterial DNMT. High homology has been identified between *Rv3204* and other bacterial DNMTs and detected in several Mycobacterium lineages other than *M. tb*, such as *M. leprae, M. smegmatis,M. bovis*, and *M. marinum* [98]. Actually, mycobacterial DNMTs play an important role in deceiving the host and warranting its survival even in detrimental conditions.

3.2.3 Mycobacterial RNA Methyltransferases

In total, 17 RNA methyltransferases have been reported from the genome of *M. tb* [87]. These enzymes are involved in the methylation of rRNA, tRNA, and mRNA nucleotides at specific positions. RNA methyltransferases affect translation initiation, post-transcriptional maturation, pre-mRNA processing, and antibiotic resistance [99]. After transcription, the rRNAs modification is a highly conserved phenomenon in almost every kingdom of life, and this modification usually occurs by RNA methylation in prokaryotes. These types of alterations intensify the functional interaction of ribosomes. Rv2118c is a mycobacterial tRNA methyltransferase and forms N_1-methyladenosine of a highly conserved adenine nucleotide at position 58 in the TΨC loop, which helps to stabilize tRNA in *M. tb* [100]. This modification of tRNA acts as a virulence factor and determines growth in pathogens. Another category of RNA methyltransferase is related to the methylation of 16SrRNA in *M. tb*. Rv2966c targets 30S ribosomes near the P-site and methylate guanidine 966 (G966) of the 16S rRNA. In addition, Rv2966c epigenetically affects the genome of host cells and modulates the host machinery. Such a dual nature of some proteins results from the multiple functional domains presence, which can switch their task specifically in special environments. There is such an interesting feature in mycobacterial methyltransferases as well. In order to further elucidate Rv2966c in the host cells, Sharma et al. evaluated the function of Rv2966c as a secretory mycobacterial protein that interacts not only with the host genome but also with the histone proteins, H3 and H4. Its DNA methylation activity is principally included induction of 5mC in non-CpG regions of host cells [86]. Rv2372c methylates the N3 position of uridine1498 (U1498) of the 16S rRNA. The methylated U1498 is present at the ribosomes decoding center, and a mutation in U1498 may inhibit the interaction of the first peptide with ribosomes during translation [87]. Rv3919c (*gidB*), as the other example of 16SrRNA methyltransferase, is associated with streptomycin resistance in *M. tb*.Streptomycin is an aminoglycoside antibiotic that binds to the accuracy center of the small

ribosomal subunit and inhibits protein translation. Rv3919c methylates the N7 position of guanine 518 (G518) of the 16S rRNA and stabilizes the 16S rRNA during protein translation. It is indicated that mutations in *the gidB* gene decrease streptomycin resistance in *M. tb* clinical isolates [101].

Besides Rv3919c, other RNA methyltransferases also play a notable role in the antibiotic resistance of *M. tb*. The ribosomes of the cells are the target of most of the antibiotics, and mutations in these target sites lead to antibiotic resistance. Rv1988 (*erm*) is a significant example of macrolide-lincosamide-streptogramin (MLS) resistance in *M. tb* isolates. Rv1988 has been introduced as a member of the erythromycin resistance rRNA methylase (*erm*) family, which encodes 23SrRNA methyltransferase [102]. In addition, Rv1694 (*tlyA*) is a 23S/16SrRNA methyltransferase with a dual substrate activity (16S and 23S rRNA). Its mutation is interestingly involved in resistance to capreomycin. Capreomycin is a cyclic aminoglycoside-like peptide antibiotic that binds the ribosome at the interface of its small and large subunits. Optimum binding of capreomycin and its activity results from proper methylation by Rv1694. Rv1694 methylates cytosine 1409 (C1409) and cytosine 1920 (C1920). C1409 is located at the decoding center of the 30S subunit of 16S rRNA, and C1920 is in a highly conserved region of the 50S subunit of ribosome 23S rRNA [103].

3.2.4 Mycobacterial Protein Methyltransferases

Protein methylation by adding the methyl group to the amino acid residues of lysine or arginine of a protein sequence is a type of post-translational modification. Methylation of histone proteins by HMTs has been broadly studied, which epigenetically activates or suppresses the expression of particular genes. *M. tb* induces methylation of its own proteins in addition to proteins of host cells. Laminin-binding protein (LBP) and heparin-binding haemagglutinin (HBHA) are mycobacterial adhesives involved in the interaction of *M. tb* with the non-phagocytic cells when are methylated. A complex methylation pattern has been identified in lysines of the HBHA active domain at its C terminal, which suppresses proteolysis and impacts the immune characteristics of this protein [104]. In addition, methylation of mycobacterial histone-like protein HupB, which belongs to MtHUs, at lysine 138 (K138) accrues by mycobacterial histone methyltransferase SUV39H1. This epigenetic modification is involved in the survival of *M. tb* inside the host cells [60].

Finally, mycobacterial protein methyltransferases may methylate proteins of host cells. Rv1988, in addition to the induction of antibiotic resistance by its RNA methyltransferase activity, epigenetically modulates the host cell machinery by methylation of histones. Rv1988, which is present only in the pathogenic species *Mycobacterium*, is part of the secretory mycobacterial virulence factor. This enzymatic protein methylates H3 histone at arginine 42 (H3R42) and then represses the expression of the host genes involved in the immune system to TB infection [105]. Rv2966c is another multifunctional methyltransferase in *M. tb*. After infection, this protein localizes to the host nucleus and interacts with histone H3, H4, and nucleophosmin (NPM1). NPM1 is a histone chaperone and is involved in

transcriptional regulation. The nuclear localization of Rv2966c may result from its interaction with NPM1. Moreover, Rv2966c can interact with specific H3 and H4 histone epigenetic alterations and is associated with chromatin in the genomic regions of ARNT2, GRK5, and H2AFY2. Therefore, it is concluded that the multifaceted nature of Rv2966c allows it to interact with the host chromatin at different levels [86]. Rv1198 has been involved in regulating MHC-II and CIITA expression by inducing H3 lysine 9 methylation (H3K9me) in the *M. tb*-infected macrophages [106]. Finally, overexpression of SET8 as an H4 lysine 20 (H4K20) methyltransferase is reported during TB infection. SET8 involves the modulation of immune evasion strategies [107].

3.2.5 Mycobacterial Mycolic Acid Methyltransferases

Known mycobacterial methyltransferases also methylate other substrates, not necessarily involved in epigenetic mechanisms but involved in the host response. Mycolic acid is a long-chain fatty acid (C60–C90) that shapes mycobacteria's insoluble cell wall skeleton. It has been widely established that mycolic acid is changed by the addition of methyl groups, methoxy groups, ketones, and cyclopropane rings in pathogenic mycobacteria. A family of AdoMet-dependent methyltransferases produces structural variation in mycolic acid. AdoMet-dependent methyltransferases facilitate the transfer of a highly specific methyl group from the SAM to a number of biological targets in the cell [108]. To date, eight distinct mycolic acid methyltransferases have been considered in *M. tb* that can alter mycolic acid. The main mycolic acid methyltransferases include MmaA1 (*Rv0645c*), MmaA2 (*Rv0644c*), MmaA3 (*Rv0643c*), MmaA4 (*Rv0642c*), CmaA1)*Rv3392c*(, CmaA2 (*Rv0503c*), UmaA (*Rv0469*), and PcaA (*Rv0470c* / UmaA2). *cma1* gene in *M. tb* produces cyclopropane mycolic acid synthase (Cmas1), which qualifies *M. smegmatis* to produce mycolic acid-containing cyclopropane in large amounts [109]. A strong homology between known regions for SAM binding has been identified in different SAM-dependent methyltransferases, and the *cma1* gene has been detected [110]. Similarly, the *cma2* gene was revealed based on the *cma1* sequence. Remarkably, four additional genes, including *mma1*, *mma2*, *mma3*, and *mma4*, were identified by the similarity of DNA hybridization to a *cma1* probe in *M. tb* [111]. The *mma1* overexpression in *M. tb* leads to the presence of trans cyclopropanes from nearby methyl branches. The product of the *mma2* gene is similar to cma2, which changes its proximal position by the cyclopropanation enzyme. The purpose of this similar function is ambiguous. The mma4 product adds the hydroxyl group at the distal position of mycolic acid, whereas an enzyme is encoded by *mma3* that methylates the hydroxyl group to form a methyl ether [67]. Generally, various mycolic acid structures organize the characteristic of the thick membrane rich in lipids in *M. tb* that, along with arabinogalactan and peptidoglycan, forms an impermeable cell envelope. Accordingly, this particular type of methylation is involved in modulating the immune response and pathogenesis of *M. tb*.

3.2.6 Other Mycobacterial Methyltransferases

Evidence has highlighted the importance of mycobacterial methyltransferases in the biosynthesis of main components of *M. tb*. For instance, biosynthesis of a constituent lipid of the mycobacterial cell wall, tuberculostearic acid (TSA), is catalyzed by TSA methyltransferase, UfaA (*Rv0447c*). This TSA methyltransferase catalyzes the transformation of a methyl group from SAM during biosynthesis of TSA or, in other words, 10-methylstearic-acid [112]. Phthiotriol/phenolphthiotriol dimycocerosates methyltransferase (*Rv2952*) is another mycobacterial methyltransferase that is involved in the biosynthesis of glycosylated phenolphthiocerol dimycocerosates (PGL) and phthiocerol dimycocerosates (DIM). In addition, methylation of phenolic glycolipids has been induced by *Rv2954c*, *Rv2955c*, and *Rv2956* expression in *M. tb* [113]. Such methylation processes impart a significant pathogenic characteristic during TB infection. In total, the activity of methyltransferases has not been widely considered in *M. tb*, but the available data in the kinds of literature point to the potential roles of different mycobacterial methyltransferases in enabling the *M. tb*survival in various extreme environments and even involving the multidrug resistance development and maintaining the diversity [67].

3.3 MiRNA-Based Regulation During Tuberculosis Infection

miRNAs are part of the non-coding RNA family, commonly thought of as an epigenetic mechanism because they retain the ability to regulate biological processes [46]. These conserved miRNAs were firstly reported in 2001 [59]. Many such regulatory RNAs can bind specifically to different target mRNAs encoded by the host genome and act as an endogenous gene silencer post-transcriptionally [114]. Increasing evidence highlighted the role of commensal microbiota in modulating host gene expression and maintaining homeostasis by miRNAs [115, 116]. It is interesting, if not surprising, that true crosstalk can occur between the host and the commensal microbiota community. Indeed, microRNAs from the host also can selectively control the microbiota function. Regulation of microbiota gene transcription and their growth may result from the entrance of miRNAs to some of them, such as *Fusobacterium nucleatum* and *Escherichia coli*. It is confirmed that the host secreted miRNAs also feedback on the microbiota to maintain the homeostasis [115]. Apart from normal processes, the role of different miRNAs has also been studied in the progression of many infections. Bacteria display some strategies based on the induction of specific miRNA expression to escape from the host immune responses. Focusing on *M. tb*, numerous pieces of evidence confirmed the manipulation of host miRNA profiles to adjust immune genes involved in TB infection [117, 118]. As a matter of fact, identification of miRNAs profile under different conditions (homeostasis by commensal microbiota or TB infection) opens new doors to evaluate potential new biomarkers related to the diagnosis, disease monitoring, and different therapeutic markers in TB infection. Consequently, further evaluations are important to understand better the regulatory role of miRNAs and more mechanisms that may not yet be revealed regarding the relationship between miRNAs in TB infection [119].

4 Conclusion

Nowadays, growing evidence of the importance of human microbiota and epigenetic mechanisms in TB infection is compelling. However, the role of these issues during infection should be better explained. Besides, a huge number of mycobacterial methyltransferases have received considerable attention in the areas of epigenetics and TB infection. In general, commensal microbiota and pathogens play important roles as epigenome regulators, and epigenetic strategies can be characteristically used to fight for survival and maintain homeostasis. Such data may change the current perspective of TB infection and help determine the stratification of the different stages of TB infection and develop useful diagnostic tools for more efficient prevention and treatment. Finally, understanding the potential of the microbiota community and epigenetic mechanisms as a biomarker will enable the development of more effective management strategies for combating TB infection. Therefore, further research is needed to clarify the correlation between microbiota, epigenome, and TB infection.

Core Messages

- TB infection may result from the combined interaction of microbiota and epigenetics, not just an infectious agent effect.
- The term "epigenetic" introduces a biological phenomenon without directly linking a genotype and its phenotype.
- Commensal microbiota and *M. tb* can use epigenetic strategies to fight for survival or maintain homeostasis.
- More than 3% of the *M. tb* genome is predicted to encode methyltransferases.
- The study of the correlation between microbiota and epigenetics can be fundamental for effective TB infection control.

References

1. Chakaya J, Khan M, Ntoumi F, Aklillu E, Fatima R, Mwaba P, Kapata N, Mfinanga S, Hasnain SE, Katoto PD (2021) Global tuberculosis report 2020–reflections on the global TB burden, treatment and prevention efforts. Int J Infect Dis
2. Organization WH (2016) World health statistics 2016: monitoring health for the SDGs sustainable development goals. World Health Organization
3. Murray CJ, Salomon JA (1998) Modeling the impact of global tuberculosis control strategies. Proc Natl Acad Sci 95(23):13881–13886
4. Vesga JF, Hallett TB, Reid MJ, Sachdeva KS, Rao R, Khaparde S, Dave P, Rade K, Kamene M, Omesa E (2019) Assessing tuberculosis control priorities in high-burden settings: a modelling approach. Lancet Glob Health 7(5):e585–e595

5. Organization WH (2019) High-priority target product profiles for new tuberculosis diagnostics: report of a consensus meeting. Geneva, Switzerland: WHO; 2014
6. Tarashi S, Badi SA, Moshiri A, Ebrahimzadeh N, Fateh A, Vaziri F, Aazami H, Siadat SD, Fuso A (2020) The inter-talk between *Mycobacterium tuberculosis* and the epigenetic mechanisms. Epigenomics 12(5):455–469
7. Wang J, Xiong K, Zhao S, Zhang C, Zhang J, Xu L, Ma A (2020) Long-term effects of multi-drug-resistant tuberculosis treatment on gut microbiota and its health consequences. Front Microbiol 11:53
8. Organization WH (2015) The end TB strategy. World Health Organization
9. Hullar MA, Fu BC (2014) Diet, the gut microbiome, and epigenetics. Cancer J (Sudbury, Mass) 20(3):170
10. Yang T, Owen JL, Lightfoot YL, Kladde MP, Mohamadzadeh M (2013) Microbiota impact on the epigenetic regulation of colorectal cancer. Trends Mol Med 19(12):714–725
11. Danjuma L, Ling MP, Hamat RA, Higuchi A, Alarfaj AA, Benelli G, Arulselvan P, Rajan M, Subbiah SK (2017) Genomic plasticity between human and mycobacterial DNA: a review. Tuberculosis 107:38–47
12. Chen Y-C, Hsiao C-C, Chen T-W, Wu C-C, Chao T-Y, Leung S-Y, Eng H-L, Lee C-P, Wang T-Y, Lin M-C (2020) Whole genome DNA methylation analysis of active pulmonary tuberculosis disease identifies novel epigenotypes: pARP9/miR-505/RASGRP4/GNG12 gene methylation and clinical phenotypes. Int J Mol Sci 21(9):3180
13. Tarashi S, Badi SA, Moshiri A, Nasehi M, Fateh A, Vaziri F, Siadat SD (2018) The human microbiota in pulmonary tuberculosis: Not so innocent bystanders. Tuberculosis 113:215–221
14. Lederberg J, McCray AT (2001) Ome sweetomics–a genealogical treasury of words. The scientist 15(7):8–8
15. Integrative H, Proctor LM, Creasy HH, Fettweis JM, Lloyd-Price J, Mahurkar A, Zhou W, Buck GA, Snyder MP, Strauss JF III (2019) The integrative human microbiome project. Natur 569(7758):641–648
16. Gilbert JA, Blaser MJ, Caporaso JG, Jansson JK, Lynch SV, Knight R (2018) Current understanding of the human microbiome. Nat Med 24(4):392–400
17. Au R (2017) Why our microbiome is important to our physiology and diseases. Int J Clin Pharmacol Pharmacother 2:125
18. Tiffany CR, Bäumler AJ (2019) Dysbiosis: from fiction to function. American Journal of Physiology-Gastrointestinal and Liver Physiology 317 (5):G602-G608
19. Osei Sekyere J, Maningi NE, Fourie PB (2020) *Mycobacterium tuberculosis*, antimicrobials, immunity, and lung–gut microbiota crosstalk: current updates and emerging advances. Ann N Y Acad Sci 1467(1):21–47
20. Arnold IC, Hutchings C, Kondova I, Hey A, Powrie F, Beverley P, Tchilian E (2015) Helicobacter hepaticus infection in BALB/c mice abolishes subunit-vaccine-induced protection against *M. tuberculosis*. Vaccine 33 (15):1808–1814
21. Winglee K, Eloe-Fadrosh E, Gupta S, Guo H, Fraser C, Bishai W (2014) Aerosol *Mycobacterium tuberculosis* infection causes rapid loss of diversity in gut microbiota. PLoS ONE 9(5):e97048
22. Zhou Y, Lin F, Cui Z, Zhang X, Hu C, Shen T, Chen C, Zhang X, Guo X (2015) Correlation between either Cupriavidus or Porphyromonas and primary pulmonary tuberculosis found by analysing the microbiota in patients' bronchoalveolar lavage fluid. PLoS ONE 10(5): e0124194
23. Maji A, Misra R, Dhakan DB, Gupta V, Mahato NK, Saxena R, Mittal P, Thukral N, Sharma E, Singh A (2018) Gut microbiome contributes to impairment of immunity in pulmonary tuberculosis patients by alteration of butyrate and propionate producers. Environ Microbiol 20(1):402–419

24. Wipperman MF, Fitzgerald DW, Juste MAJ, Taur Y, Namasivayam S, Sher A, Bean JM, Bucci V, Glickman MS (2017) Antibiotic treatment for tuberculosis induces a profound dysbiosis of the microbiome that persists long after therapy is completed. Sci Rep 7(1):1–11
25. Segal LN, Clemente JC, Li Y, Ruan C, Cao J, Danckers M, Morris A, Tapyrik S, Wu BG, Diaz P (2017) Anaerobic bacterial fermentation products increase tuberculosis risk in antiretroviral-drug-treated HIV patients. Cell Host Microbe 21(4):530–537. e534
26. Hu Y, Kang Y, Liu X, Cheng M, Dong J, Sun L, Zhu Y, Ren X, Yang Q, Chen X (2020) Distinct lung microbial community states in patients with pulmonary tuberculosis. Sci China Life Sci 1–12
27. Danneskiold-Samsøe NB, Barros HDdFQ, Santos R, Bicas JL, Cazarin CBB, Madsen L, Kristiansen K, Pastore GM, Brix S, Junior MRM (2019) Interplay between food and gut microbiota in health and disease. Food Res Int 115:23–31
28. Dang AT, Marsland BJ (2019) Microbes, metabolites, and the gut–lung axis. Mucosal Immunol 12(4):843–850
29. Young RP, Hopkins RJ, Marsland B (2016) The gut–liver–lung axis. Modulation of the innate immune response and its possible role in chronic obstructive pulmonary disease. Am J Respir Cell Mol Biol 54(2):161–169
30. Breithaupt-Faloppa AC, Vitoretti LB, Cavriani G, Lino-dos-Santos-Franco A, Sudo-Hayashi LS, Oliveira-Filho RM, Vargaftig BB, Tavares-de-Lima W (2012) Intestinal lymph-borne factors induce lung release of inflammatory mediators and expression of adhesion molecules after an intestinal ischemic insult. J Surg Res 176(1):195–201
31. Vrancken G, Gregory AC, Huys GR, Faust K, Raes J (2019) Synthetic ecology of the human gut microbiota. Nat Rev Microbiol 17(12):754–763
32. Waddington CH (2012) The epigenotype. Int J Epidemiol 41(1):10–13
33. Miro-Blanch J, Yanes O (2019) Epigenetic regulation at the interplay between gut microbiota and host metabolism. Front Genet 10
34. Grabiec AM, Potempa J (2018) Epigenetic regulation in bacterial infections: targeting histone deacetylases. Crit Rev Microbiol 44(3):336–350
35. Kathirvel M, Mahadevan S (2016) The role of epigenetics in tuberculosis infection. Epigenomics 8(4):537–549
36. Bock C (2009) Epigenetic biomarker development. Epigenomics 1(1):99–110
37. Watson MM, Søreide K (2017) The gut microbiota influence on human epigenetics, health, and disease. In: Handbook of Epigenetics. Elsevier, pp 495–510
38. Younge N, McCann JR, Ballard J, Plunkett C, Akhtar S, Araújo-Pérez F, Murtha A, Brandon D, Seed PC (2019) Fetal exposure to the maternal microbiota in humans and mice. JCI insight 4(19)
39. McDonald B, McCoy KD (2019) Maternal microbiota in pregnancy and early life. Science 365(6457):984–985
40. Neu J (2016) The microbiome during pregnancy and early postnatal life. In: Seminars in fetal and neonatal medicine. vol 6. Elsevier, pp 373–379
41. Wong JM, De Souza R, Kendall CW, Emam A, Jenkins DJ (2006) Colonic health: fermentation and short chain fatty acids. J Clin Gastroenterol 40(3):235–243
42. Round JL, Mazmanian SK (2010) Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc Natl Acad Sci 107(27):12204–12209
43. Obata Y, Furusawa Y, Hase K (2015) Epigenetic modifications of the immune system in health and disease. Immunol Cell Biol 93(3):226–232
44. Orozco-Solis R, Aguilar-Arnal L (2020) Circadian regulation of immunity through epigenetic mechanisms. Front Cell Infect Microbiol 10:96
45. Lu L, Claud EC (2018) Intrauterine inflammation, epigenetics, and microbiome influences on preterm infant health. Curr Pathobiology Rep 6(1):15–21
46. Yadav V, Dwivedi V, Bhattacharya D, Mittal A, Moodley P (2015) Understanding the host epigenetics in *Mycobacterium tuberculosis*. J Infect Dis 2:016

47. Fol M, Włodarczyk M, Druszczyńska M (2020) Host epigenetics in intracellular pathogen infections. Int J Mol Sci 21(13):4573
48. Dong W, Hamon MA (2020) Revealing eukaryotic histone-modifying mechanisms through bacterial infection. Seminars in Immunopathology. Springer, pp 1–13
49. Jerković I, Szabo Q, Bantignies F, Cavalli G (2020) Higher-order chromosomal structures mediate genome function. J Mol Biol 432(3):676–681
50. Margueron R, Reinberg D (2010) Chromatin structure and the inheritance of epigenetic information. Nat Rev Genet 11(4):285–296
51. Kumar P, Agarwal R, Siddiqui I, Vora H, Das G, Sharma P (2012) ESAT6 differentially inhibits IFN-γ-inducible class II transactivator isoforms in both a TLR2-dependent and-independent manner. Immunol Cell Biol 90(4):411–420
52. Dunne J, P, Richard G, Keane J, (2015) Commercially available, FDA-approved epigenetic modifiers as therapeutic agents in bacterial infection. Clin Anti-Inflamm Anti-Allergy Drugs 2(1):79–88
53. Hamon MA, Cossart P (2008) Histone modifications and chromatin remodeling during bacterial infections. Cell Host Microbe 4(2):100–109
54. Pennini ME, Pai RK, Schultz DC, Boom WH, Harding CV (2006) *Mycobacterium tuberculosis* 19-kDa lipoprotein inhibits IFN-γ-induced chromatin remodeling of MHC2TA by TLR2 and MAPK signaling. J Immunol 176(7):4323–4330
55. Kim KH, An DR, Song J, Yoon JY, Kim HS, Yoon HJ, Im HN, Kim J, Lee SJ, Kim K-H (2012) *Mycobacterium tuberculosis* Eis protein initiates suppression of host immune responses by acetylation of DUSP16/MKP-7. Proc Natl Acad Sci 109(20):7729–7734
56. Moores R, Rand L, Elkington P, Friedland J (2012) Matrix metalloproteinase-1 expression in tuberculosis is regulated by histone acetylation: p2151. Clin Microbiol Infect 18:626–627
57. Wang Y, Curry HM, Zwilling BS, Lafuse WP (2005) Mycobacteria inhibition of IFN-γ induced HLA-DR gene expression by up-regulating histone deacetylation at the promoter region in human THP-1 monocytic cells. J Immunol 174(9):5687–5694
58. Shilatifard A (2006) Chromatin modifications by methylation and ubiquitination: implications in the regulation of gene expression. Annu Rev Biochem 75:243–269
59. Singh M, Yadav V, Das G (2018) host epigenetic modifications in *Mycobacterium tuberculosis* infection: a boon or bane. In: The Value of BCG and TNF in autoimmunity. Elsevier, pp 39–55
60. Yaseen I, Choudhury M, Sritharan M, Khosla S (2018) Histone methyltransferase SUV 39H1 participates in host defense by methylating mycobacterial histone-like protein HupB. EMBO J 37(2):183–200
61. Anand C, Garg R, Ghosh S, Nagaraja V (2017) A Sir2 family protein Rv1151c deacetylates HU to alter its DNA binding mode in *Mycobacterium tuberculosis*. Biochem Biophys Res Commun 493(3):1204–1209
62. Ghosh S, Padmanabhan B, Anand C, Nagaraja V (2016) Lysine acetylation of the *Mycobacterium tuberculosis* HU protein modulates its DNA binding and genome organization. Mol Microbiol 100(4):577–588
63. McGhee JD, Ginder GD (1979) Specific DNA methylation sites in the vicinity of the chicken β-globin genes. Nature 280(5721):419–420
64. Zhang G, Pradhan S (2014) Mammalian epigenetic mechanisms. IUBMB life 66(4):240–256
65. Fuso A (2013) The 'golden age'of DNA methylation in neurodegenerative diseases. Clin Chem Lab Med (CCLM) 51(3):523–534
66. Chen Z, Zhang Y (2019) Role of mammalian DNA methyltransferases in development. Ann Rev Biochem 89
67. Grover S, Gangwar R, Jamal S, Ali S, Nisaa K, Ehtesham NZ, Hasnain SE (2019) Mycobacterial methyltransferases: significance in pathogenesis and virulence. In: *Mycobacterium tuberculosis*: molecular infection biology, pathogenesis, diagnostics and new interventions. Springer, pp 103–122

68. Ponnaluri VC, Estève P-O, Ruse CI, Pradhan S (2018) S-adenosylhomocysteine hydrolase participates in DNA methylation inheritance. J Mol Biol 430(14):2051–2065
69. de Mendoza A, Lister R, Bogdanovic O (2020) Evolution of DNA methylome diversity in eukaryotes. J Mol Biol 432(6):1687–1705
70. Fuso A, Ferraguti G, Scarpa S, Ferrer I, Lucarelli M (2015) Disclosing bias in bisulfite assay: methprimers underestimate high DNA methylation. PLoS ONE 10(2):e0118318
71. Jeltsch A, Ehrenhofer-Murray A, Jurkowski TP, Lyko F, Reuter G, Ankri S, Nellen W, Schaefer M, Helm M (2017) Mechanism and biological role of Dnmt2 in nucleic acid methylation. RNA Biol 14(9):1108–1123
72. Sandoval J, Heyn H, Moran S, Serra-Musach J, Pujana MA, Bibikova M, Esteller M (2011) Validation of a DNA methylation microarray for 450,000 CpG sites in the human genome. Epigenetics 6(6):692–702
73. Illingworth RS, Bird AP (2009) CpG islands–'a rough guide.' FEBS Lett 583(11):1713–1720
74. Fuso A, Ferraguti G, Grandoni F, Ruggeri R, Scarpa S, Strom R, Lucarelli M (2010) Early demethylation of non-CpG, CpC-rich, elements in the myogenin 5'-flanking region: a priming effect on the spreading of active demethylation? Cell Cycle 9(19):3965–3976
75. Wang M, Kong W, He B, Li Z, Song H, Shi P, Wang J (2018) Vitamin D and the promoter methylation of its metabolic pathway genes in association with the risk and prognosis of tuberculosis. Clin Epigenetics 10(1):118
76. Chen Y-C, Hsiao C-C, Chen C-J, Chao T-Y, Leung S-Y, Liu S-F, Wang C-C, Wang T-Y, Chang J-C, Wu C-C (2014) Aberrant Toll-like receptor 2 promoter methylation in blood cells from patients with pulmonary tuberculosis. J Infect 69(6):546–557
77. Zheng L, Leung ET, Wong H, Lui G, Lee N, To K-F, Choy K, Chan RC, Ip M (2016) Unraveling methylation changes of host macrophages in *Mycobacterium tuberculosis* infection. Tuberculosis 98:139–148
78. Wei M, Wang L, Wu T, Xi J, Han Y, Yang X, Zhang D, Fang Q, Tang B (2016) NLRP3 activation was regulated by DNA methylation modification during *Mycobacterium tuberculosis* infection. BioMed Res Int
79. Koh H-J, Kim Y-R, Kim J-S, Yun J-S, Kim S, Kim SY, Jang K, Yang C-S (2018) CD82 hypomethylation is essential for tuberculosis pathogenesis via regulation of RUNX1-Rab5/22. Exp Mol Med 50(5):1–15
80. Sharma G, Sowpati DT, Singh P, Khan MZ, Ganji R, Upadhyay S, Banerjee S, Nandicoori VK, Khosla S (2016) Genome-wide non-CpG methylation of the host genome during *M. tuberculosis* infection. Sci Rep 6:25006
81. Pacis A, Tailleux L, Morin AM, Lambourne J, MacIsaac JL, Yotova V, Dumaine A, Danckaert A, Luca F, Grenier J-C (2015) Bacterial infection remodels the DNA methylation landscape of human dendritic cells. Genome Res 25(12):1801–1811
82. Pacis A, Mailhot-Léonard F, Tailleux L, Randolph HE, Yotova V, Dumaine A, Grenier J-C, Barreiro LB (2019) Gene activation precedes DNA demethylation in response to infection in human dendritic cells. Proc Natl Acad Sci 116(14):6938–6943
83. van der Heijden CD, Noz MP, Joosten LA, Netea MG, Riksen NP, Keating ST (2018) Epigenetics and trained immunity. Antioxid Redox Signal 29(11):1023–1040
84. Sánchez-Romero MA, Cota I, Casadesús J (2015) DNA methylation in bacteria: from the methyl group to the methylome. Curr Opin Microbiol 25:9–16
85. Warrier T, Kapilashrami K, Argyrou A, Ioerger TR, Little D, Murphy KC, Nandakumar M, Park S, Gold B, Mi J (2016) N-methylation of a bactericidal compound as a resistance mechanism in *Mycobacterium tuberculosis*. Proc Natl Acad Sci 113(31):E4523–E4530
86. Sharma G, Upadhyay S, Srilalitha M, Nandicoori VK, Khosla S (2015) The interaction of mycobacterial protein Rv2966c with host chromatin is mediated through non-CpG methylation and histone H3/H4 binding. Nucleic Acids Res 43(8):3922–3937

87. Grover S, Gupta P, Kahlon PS, Goyal S, Grover A, Dalal K, Ehtesham NZ, Hasnain SE (2016) Analyses of methyltransferases across the pathogenicity spectrum of different mycobacterial species point to an extremophile connection. Mol BioSyst 12(5):1615–1625
88. He C (2010) Grand challenge commentary: RNA epigenetics? Nat Chem Biol 6(12):863–865
89. Sánchez-Romero MA, Casadesús J (2019) The bacterial epigenome. Nat Rev Microbiol 1–14
90. Campbell JL, Kleckner N (1990) E. coli oriC and the dnaA gene promoter are sequestered from dam methyltransferase following the passage of the chromosomal replication fork. Cell 62 (5):967–979
91. Singal R, Ginder GD (1999) DNA methylation. Blood J Am Soc Hematol 93(12):4059–4070
92. Hemavathy KC, Nagaraja V (1995) DNA methylation in mycobacteria: absence of methylation at GATC (Dam) and CCA/TGG (Dcm) sequences. FEMS Immunol Med Microbiol 11(4):291–296
93. Palmer BR, Marinus MG (1994) The dam and dcm strains of Escherichia coli—a review. Gene 143(1):1–12
94. Srivastava R, Gopinathan K, Ramakrishnan T (1981) Deoxyribonucleic acid methylation in mycobacteria. J Bacteriol 148(2):716–719
95. Yang M, Aamodt RM, Dalhus B, Balasingham S, Helle I, Andersen P, Tønjum T, Alseth I, Rognes T, Bjørås M (2011) The ada operon of *Mycobacterium tuberculosis* encodes two DNA methyltransferases for inducible repair of DNA alkylation damage. DNA Repair 10 (6):595–602
96. Miggiano R, Casazza V, Garavaglia S, Ciaramella M, Perugino G, Rizzi M, Rossi F (2013) Biochemical and structural studies of the *Mycobacterium tuberculosis* O6-methylguanine methyltransferase and mutated variants. J Bacteriol 195(12):2728–2736
97. Shell SS, Prestwich EG, Baek S-H, Shah RR, Sassetti CM, Dedon PC, Fortune SM (2013) DNA methylation impacts gene expression and ensures hypoxic survival of *Mycobacterium tuberculosis*. PLoS Pathog 9(7):e1003419
98. Agus R, Hidayah N (2019) Isolation and cloning of Rv3204 of *Mycobacterium tuberculosis* to Escherichia coli BL21 as vaccines tuberculosis: a preliminary study. J Phys Conf Ser 2:022010. IOP Publishing
99. Shatkin AJ, Manley JL (2000) The ends of the affair: capping and polyadenylation. Nat Struct Biol 7(10):838–842
100. Varshney U, Ramesh V, Madabushi A, Gaur R, Subramanya H, RajBhandary U (2004) *Mycobacterium tuberculosis* Rv2118c codes for a single-component homotetrameric m1A58 tRNA methyltransferase. Nucleic Acids Res 32(3):1018–1027
101. Wong SY, Lee JS, Kwak HK, Via LE, Boshoff HI, Barry CE (2011) Mutations in gidB confer low-level streptomycin resistance in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 55(6):2515–2522
102. Buriánková K, Doucet-Populaire F, Dorson O, Gondran A, Ghnassia J-C, Weiser J, Pernodet J-L (2004) Molecular basis of intrinsic macrolide resistance in the *Mycobacterium tuberculosis* complex. Antimicrob Agents Chemother 48(1):143–150
103. Witek MA, Kuiper EG, Minten E, Crispell EK, Conn GL (2017) A novel motif for S-adenosyl-L-methionine binding by the ribosomal RNA methyltransferase TlyA from *Mycobacterium tuberculosis*. J Biol Chem 292(5):1977–1987
104. Pethe K, Aumercier M, Fort E, Gatot C, Locht C, Menozzi FD (2000) Characterization of the heparin-binding site of the mycobacterial heparin-binding hemagglutinin adhesin. J Biol Chem 275(19):14273–14280
105. Yaseen I, Kaur P, Nandicoori VK, Khosla S (2015) Mycobacteria modulate host epigenetic machinery by Rv1988 methylation of a non-tail arginine of histone H3. Nat Commun 6 (1):1–13

106. Sengupta S, Naz S, Das I, Ahad A, Padhi A, Naik SK, Ganguli G, Pattanaik KP, Raghav SK, Nandicoori VK (2019) Withdrawal: Mycobacterium tuberculosis EsxL inhibits MHC-II expression by promoting hypermethylation in class-II transactivator loci in macrophages. J Biol Chem 294(5):1632
107. Singh V, Prakhar P, Rajmani R, Mahadik K, Borbora SM, Balaji KN (2017) Histone methyltransferase SET8 epigenetically reprograms host immune responses to assist mycobacterial survival. J Infect Dis 216(4):477–488
108. Klimašauskas S, Weinhold E (2007) A new tool for biotechnology: AdoMet-dependent methyltransferases. Trends Biotechnol 25(3):99–104
109. Yuan Y, Lee RE, Besra GS, Belisle JT, Barry C (1995) Identification of a gene involved in the biosynthesis of cyclopropanated mycolic acids in *Mycobacterium tuberculosis*. Proc Natl Acad Sci 92(14):6630–6634
110. Wang AY, Grogan DW, Cronan JE Jr (1992) Cyclopropane fatty acid synthase of *Escherichia coli*: deduced amino acid sequence, purification, and studies of the enzyme active site. Biochemistry 31(45):11020–11028
111. Yuan Y, Barry CE (1996) A common mechanism for the biosynthesis of methoxy and cyclopropyl mycolic acids in *Mycobacterium tuberculosis*. Proc Natl Acad Sci 93 (23):12828–12833
112. Meena LS, Kolattukudy PE (2013) Expression and characterization of R v0447c product, potentially the methyltransferase involved in tuberculostearic acid biosynthesis in *Mycobacterium tuberculosis*. Biotechnol Appl Biochem 60(4):412–416
113. Simeone R, Huet G, Constant P, Malaga W, Lemassu A, Laval F, Daffe M, Guilhot C, Chalut C (2013) Functional characterisation of three o-methyltransferases involved in the biosynthesis of phenolglycolipids in *Mycobacterium tuberculosis*. PLoS ONE 8(3):e58954
114. Bentwich I, Avniel A, Karov Y, Aharonov R, Gilad S, Barad O, Barzilai A, Einat P, Einav U, Meiri E (2005) Identification of hundreds of conserved and nonconserved human microRNAs. Nat Genet 37(7):766–770
115. Liu S, da Cunha AP, Rezende RM, Cialic R, Wei Z, Bry L, Comstock LE, Gandhi R, Weiner HL (2016) The host shapes the gut microbiota via fecal microRNA. Cell Host Microbe 19(1):32–43
116. Masotti A (2012) Interplays between gut microbiota and gene expression regulation by miRNAs. Front Cell Infect Microbiol 2:137
117. Wang C, Yang S, Sun G, Tang X, Lu S, Neyrolles O, Gao Q (2011) Comparative miRNA expression profiles in individuals with latent and active tuberculosis. PLoS ONE 6(10): e25832
118. Chakrabarty S, Kumar A, Raviprasad K, Mallya S, Satyamoorthy K, Chawla K (2019) Host and MTB genome encoded miRNA markers for diagnosis of tuberculosis. Tuberculosis 116:37–43
119. Pedersen JL, Bokil NJ, Saunders BM (2019) Developing new TB biomarkers, are miRNA the answer? Tuberculosis 118:101860

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The Pathogenesis and Progression of Sarcoidosis from the Standpoint of Tuberculosis

48

Yoshinori Kawabata

The important thing is not to stop questioning; curiosity has its own reason for existing.

Albert Einstein

Summary

Tuberculosis (TB) is a systemic necrotizing granulomatous disease caused by *Mycobacterium tuberculosis* that mainly affects the lungs. Sarcoidosis is a systemic non-necrotizing granulomatous disease that also mainly affects the lungs and intrathoracic lymph nodes. In this review, the pathogenesis and progression of sarcoidosis are discussed from the standpoint of TB. Both diseases have similar pathogenesis: epithelioid cell granuloma is formed by activated macrophage function and T helper type 1 cell activity. TB shows early- and late-onset disease after latent infection. Large amounts of persuasive data from human and animal experiments support *Propionibacterium acnes* (*P. acnes*) as the causative agent of sarcoidosis. Drug-related sarcoidosis and sarcoidosis seen in patients with acquired immunodeficiency syndrome during active antiretroviral therapy are also reviewed. The author understands sarcoidosis to be an aerogenous infection caused by *P. acnes,* for which cellular immunity is necessary to control, as with TB. This understanding is different from the hypothesis of Eishi in which granuloma formation causes the disease (endogenous allergic infection). Antibiotic therapy may be an additional choice for patients with unresolved sarcoidosis in the near future.

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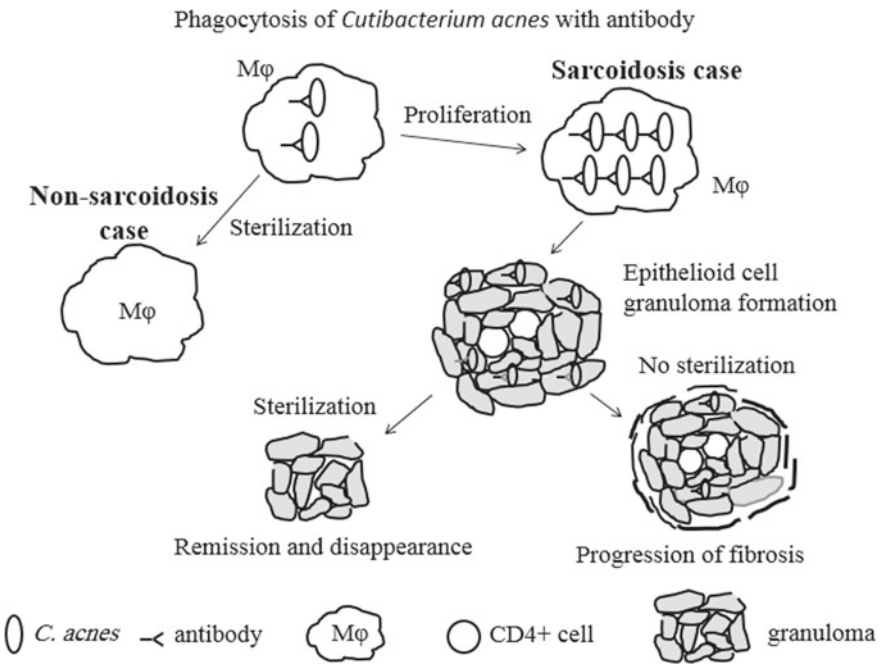
Graphical Abstract



Simplified schema of sarcoidosis pathogenesis. Ordinarily, macrophages can control antibody-attached *Propionibacterium acnes(P. acnes)* by phagocytosis. However, macrophages in sarcoidosis cases cannot control antigen-attached *P. acnes*, and the microorganisms proliferate in the macrophages. Mainly with the help of CD 4+ cells, macrophages phagocytosing *P. acnes* transfer into epithelioid cell granulomas (ECGs). When *P. acnes* is sterilized by these ECGs, the granulomas finally disappear. If ECGs cannot sterilize *P. acnes*, fibrosis progresses around these granulomas

Keywords

Cellular immunity · Pathogenesis · Sarcoidosis · Tuberculosis

1 Introduction

Epithelioid cell granuloma formation is the pathological feature shared by tuberculosis (TB) and sarcoidosis, although TB also causes caseous necrosis. Both diseases present with lympho-hematological dissemination into the thoracic lymph nodes and systemic organs. The cause and pathogenesis of TB are clear, even if the exact mechanism requires further investigation, while those of sarcoidosis are not well elucidated yet. This review attempts to explain the cause, pathogenesis, and progression of sarcoidosis compared to that of TB.

2 Pathogenesis of Tuberculosis

2.1 Human Pathology

TB is caused by *Mycobacterium tuberculosis (M. tb)* inhalation, and less than 10% of infected persons develop TB. The pathology of TB was mostly established by the 1950s [1–4]. Following primary infection, a continuous process occurs in which alveolar macrophages (A Mφs) engulf *M. tb*, but *M. tb* can proliferate in AMφs, which results in cell death (limit is up to 40 *Mtbs* per one Mφ) [5], and this process continues. AMφs engulfing *M. tb* move to the intrathoracic lymph nodes and sometimes enter the bloodstream (lympho-hematological dissemination). Cellular immunity develops around six weeks after primary infection; the tuberculin reaction and interferon-gamma release assay (IGRA) become positive, and a few patients show the early onset of TB [1–4, 6]. The primary focus (with/without subsequent nearby, aerogenous foci) and intrathoracic lymph node lesions (the primary complex) suddenly undergo caseous necrosis following exudation in which necrotic lung tissue structure is preserved as confirmed by elastic tissue staining or reticulin fiber staining. The caseous necrosis is soon surrounded by an epithelioid cell layer and later by fibrosis, and progression of the tuberculous lesions stops [1–4, 7]. Large numbers of *M. tb* are contained in this exudative type of caseous necrosis [2, 3, 8]. Lympho-hematological dissemination also ceases at this time. These phenomena are thought to be mainly due to T helper type 1 (Th1) cell activity and activated Mφ function [9–11]. After establishing cellular immunity, *M. tb* bacteria spilling out from areas of caseous necrosis are engulfed by activated AMφs. They can be contained in epithelioid cell granulomas without further *M. tb* growth [2].

There are two types of TB: early-onset and late-onset. Early-onset disease indicates:

- the failure of containment at the primary focus with cavitation;
- intrathoracic lymph node swelling;
- lympho-hematological dissemination called early miliary tuberculosis; and
- pleuritis.

The ratio of each lesion reported among patients in Japan and Sweden was 47% and 62% for intrathoracic lymph node swelling, 36% and 25% for intrathoracic lymph node swelling + pulmonary lesions, and 17% and 3% for pulmonary lesions only, respectively [6, 12] (Table 1). The patients in Sweden suffered from a concentrated infection with symptomatic onset.

Except for the small percentage of patients with early-onset TB, the remaining infected persons develop the latent infection. Most incidences of chronic onset are of chronic pulmonary TB. Previously, the original location of chronic pulmonary TB was thought to be the primary focus [2–4, 13] or the hematogenously disseminated focus [14–16] in the lung apex. The reason for this apical predisposition has been discussed for quite some time, but it remains unresolved [9]. Iwasaki

Table 1 Initial radiological changes of early-onset tuberculosis

	Japan	Sweden
Subjects	2328	65
Discovery method	Health screening	Fever onset
Tuberculin reaction	All positive	All positive
Incubation period	Within three months	31–46 days
Fever	–	65 (100%)
Lymph node swelling	3.6% (47%)	34 (62%)
Lymph node swelling + Lung infiltration	2.8% (36%)	16 (25%)
Lung infiltration	1.3% (17%)	2 (3%)
Erythema nodosum	–	12 (18%)

stated that the primary focus in the upper lobe did not heal easily and showed concentric enlargement (due to necrosis following productive reaction, which means the fibrous capsule undergoes necrosis) and aerogenous spreading (due to lysis of necrotic material) to the nearby lung [2].

Presently, latent infection is thought to be the static state worldwide. However, these theories were recently questioned because of positive IGRA testing performed at the time of latent infection. To explain positive IGRA results and the protective effect of preventative anti-tuberculosis drugs for latent infection cases, two new theories were proposed [17, 18]. Both theories emphasize that *M. tb* resides in foamy AMφs without being attacked by cellular immune functions, and these foamy AMφs somehow move to the apex and subsequently form tuberculous lesions. Hunter et al. proposed a three-act play (caseous pneumonia to cavitation) [17], and Cardona proposed a dynamic reinfection hypothesis (repeated endogenous reinfection with final necrotizing granuloma formation at the apex and cavitation) [18]. Up to now, Hunter et al.'s theory has been supported [19], but how can foamy A Mφs containing *M. tb* survive, and how can *M. tb* proliferate at the apex under preserved cellular immunity? These questions need to be explained in both theories. Recently, a hypothesis of necrosis-associated extracellular clusters was proposed [20]. Meanwhile, I proposed dynamic reactivation of the primary focus or an aerogenously spreading focus due to repeated softening (liquefaction, lysis) and necrosis following a proliferative/productive reaction (Fig. 1a–d) referring to Iwasaki's observation [2], and finally, this enlarged necrotizing granuloma reaches membranous bronchioles with subsequent softening and cavitation (Fig. 1e–g) [21]. Softening begins with the infiltration of neutrophils [2–4], but the cause of this softening is unclear. Focal, repeated reactivation, as in my theory, stimulates cellular immunity, which causes the IGRA test to remain positive. Table 2 provides comparative data to explain the above hypotheses.

Whatever the pathogenesis, cavitation is the beginning of most chronic pulmonary TB at the apex, followed by intrapulmonary aerogenous spreading. Other types of late-onset TB are extrapulmonary and late-onset miliary TB. Both result from reactivation of old, latently infected, necrotizing granulomas originally disseminated

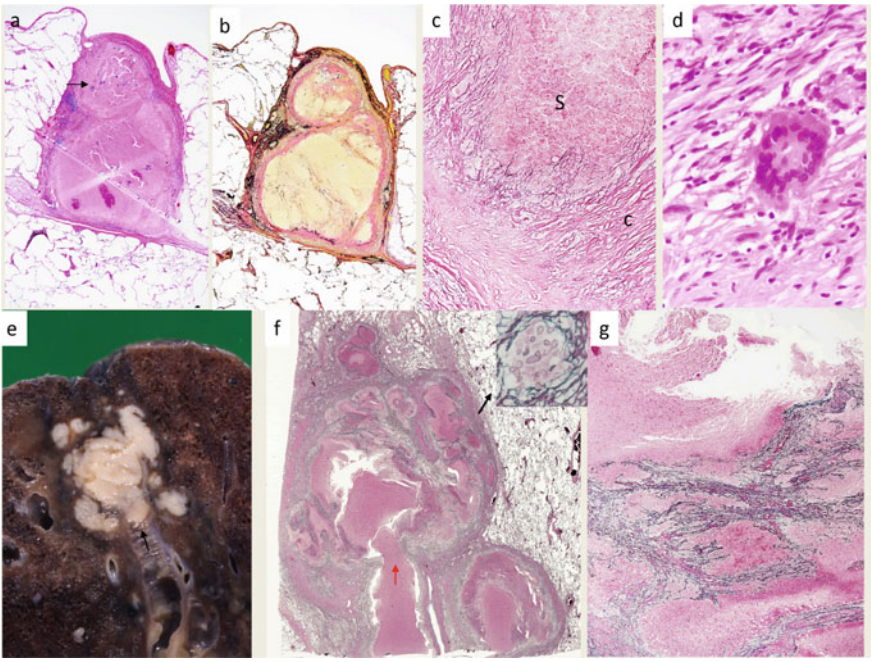


Fig. 1 Pathological findings of pulmonary tuberculosis: **a**, a 5-mm-sized encapsulated caseous lesion found by chance at the time of lobectomy for lung cancer. Deposition of calcification was noted in various forms (arrow). Hematoxylin–eosin (HE) staining, × 20; **b**, a fibrous capsule around snowman-shaped, fused caseous lesions underwent necrosis (called necrosis following productive reaction), forming a 5-mm-sized enlarged lesion. Elastica van Gieson stain, × 20; **c**, multiple areas of caseous necrosis showing disruption of reticulin fibers (s: softening and lysis) surrounded by dense collagen and increased reticulin fibers (c). Reticulin fiber staining, × 200; **d**, remaining Langerhans-type giant cell in the fibrotic capsule (wall). HE staining, × 800; **e**, a macroscopic feature of an approximately 17-mm-sized caseous lesion communicating with one bronchiole (arrow); **f**, a panoramic microscopic feature of (e) showing the discharge of necrotic material into the draining bronchiole. The red arrow indicates the orifice of the bronchiole, and the black arrow in the inset indicates the epithelioid cell granuloma of the capsule. Reticulin fiber staining; g, loss of lung tissue in the area of softening and increased collagen fibers and reticulin fibers in necrosis (necrosis following productive reaction, part of the image in f). Reticulin fiber staining, × 40

hematogenously at the time of the primary infection, and miliary TB is suspected to result from direct invasion of reactivated lesions into the bloodstream.

Patients with a marked decrease of CD4 + cells, such as that caused by advanced acquired immunodeficiency syndrome (AIDS), cannot produce well-formed epithelioid cell granulomas or caseous necrosis, and large numbers of *M. tb* increase in the lesions [22, 23]. There are many reports of anti-tumor necrosis- α (TNF-) treatment reactivating latent (TB) infection [24–28]. Especially, Gómez-Reino et al. reported that the estimated incidence of TB associated with infliximab in rheumatoid arthritis patients was 1893 per 100,000 in the year 2000, whereas the annual incidence of TB

Table 2 Comparative data concerning sources of chronic pulmonary tuberculosis

Origin	Location	Size (diameter)	Numbers of *M. tuberculosis*	Progression Apical predisposition
Foamy cells	Foamy cells	15 μm	Up to 40	Granuloma or lipid pneumonia to caseous pneumonia with subsequent cavitation
Hematogenous apical spread	Granuloma ± necrosis	300 μm	Small numbers	
Primary focus	Necrotizing granuloma	3 mm	10^4 CFU (primates)	Enlargement by repeated lysis and necrosis with subsequent cavitation

in inhabitants and rheumatoid arthritis is much lower [26]. The subject of exogenous reinfection tuberculosis will be omitted.

In this review, I will emphasize the role of Th1 cells and activated Mφs for protective immunity. However, there are other modifiers of the inflammatory process, such as Th 17 cells, T helper type 2 (Th2) cells, Th1/Th2 balance, CD8 cell subset, regulatory T cells, innate immunity (including Mφ differentiation to M1 and M2), and dendritic cells, which are not addressed in this review.

2.2 Animal Experiments in Tuberculosis

Animal experiments using primates opened a new era in understanding human TB. Various results were reported using cynomolgus macaques infected with small numbers of *M. tb* introduced via the airway. Caseous necrosis occurred quickly during granuloma formation at four weeks after infection together with the development of cellular immunity typical for the production of IFN-γ and also when the numbers of *M. tb* rose to around 10^4 per one focus [29]. This model resulted in active TB, latent TB infection, and endogenous reactivation [30], and it could also reproduce the immune-depleted state [31, 32]. The character of the second infection was different from that of the first infection, as cellular immunity was already established [33].

3 Causes, Pathogenesis, and Progression of Sarcoidosis

Many recent review papers [34–37], except for one review in 2017 [38], have considered sarcoidosis to be an immune-inflammatory granulomatous disease of unknown etiology, rather excessive immune response to unknown antigens or an autoimmune disease. Sarcoid granuloma development has been linked to an immune response triggered by microorganisms or products, according to a study by Inaoka et al. [36].

3.1 Causative Agents

The histological feature of sarcoidosis is well-formed epithelioid cell granulomas similar to one type of tuberculous lesions. *M. tb* has been suspected of being the causative agent for quite some time now [39, 40]. Meta-analysis has also supported *M. tb* as a candidate for the causative microorganism [41, 42]. However, the essential weak points in these suppositions are that *M. tb* remains to be cultured from sarcoid lesions, and the presence of *M. tb* causes TB, not sarcoidosis.

In contrast, *Propionibacterium acnes* (*P. acnes*) has been cultured from sarcoidosis patients' lymph nodes at a high incidence (76–90%) and high concentration. However, *P. acnes* was also cultured from non-sarcoidosis patients' lymph nodes at a low incidence (20–57%) and low concentration in 1978 and 1984, respectively, in Japan [43, 44]. *P. acnes*, a ubiquitous gram-positive anaerobe found across body sites, is considered an indigenous bacterium (i.e., normal bacterial flora) [45, 46], and the numbers of *C. acne* in hair follicles increase rapidly around puberty and peak at age 15–19 years [47]. *P. acnes* is suspected of causing acne vulgaris [48]. Because of its presence in the normal bacterial flora and because lymph nodes in non-sarcoidosis cases contain *P. acnes*, this microorganism has not been accepted as a causative agent of sarcoidosis until recently.

When a specific microorganism such as *P. acnes* is considered to be the cause of sarcoidosis, two questions have to be answered:

i. why do only limited numbers of people get sarcoidosis?
ii. is granuloma formation a form of protective immunity as in tuberculosis and leprosy or an allergic reaction as in berylliosis?

3.2 *P. Acnes* and Culture with Inflammatory Cells

Cell cultures have shown that human polymorphonuclear leukocytes cannot kill *P. acnes* in any incubation mixture but that human monocytes reduce *P. acnes* viability in the incubation of serum from patients with acne vulgaris [49]. Another study showed that *P. acnes* can survive in human Mφs but cannot replicate or escape [50]. These results suggest that most human Mφs can kill *P. acnes* or control its infection with the help of the antibody to *P. acnes,* whereas polymorphonuclear leukocytes cannot kill *P. acnes* even with the help of the antibody when *P. acnes* infects or invades the lungs.

3.3 Comparison of *P. Acnes* in Sarcoidosis Patients and Non-sarcoidosis Cases

I compared various cases of sarcoidosis and non-sarcoidosis to determine whether *P. acnes* is the causative bacterium (Table 3).

Table 3 Comparative data between sarcoidosis patients and non- sarcoidosis cases

	Culture of *P. acnes*	*P. acnes* DNA	Antibody	Cellular immunity
Sarcoidosis patients	High incidence and high numbers	High incidence and high amount	100% and high titer	Positive
Non-sarcoidosis cases	Low incidence and small numbers	Low incidence and a small amount	High % and low titer	Negative

The Eishi group vigorously studied the relationship between *P. acnes* and sarcoidosis following previous Japanese reports that *P. acnes* was cultured from lymph nodes in patients with sarcoidosis, as stated previously [43, 44]. Ishige et al. reported that *P. acnes* DNA was found in 80% of sarcoidosis patients' lymph nodes and in 20% of non-sarcoidosis lymph nodes [51]. They extended these findings and also studied European patients. In patients from three European countries, a high incidence (82–100%) of *P. acnes* DNA was found in the sarcoidosis patients, whereas a low incidence (19–66%) of *P. acnes* DNA was found in the control cases, and the amount of DNA was statistically higher (greater than $\log^1$) in the sarcoidosis patients than in the control cases [52]. Hiramatsu et al. reported that *P. acnes* DNA was found in bronchoalveolar lavage (BAL) cells from 70% of sarcoid patients and from 23% of controls by polymerase chain reaction (PCR), and *P. acnes* DNA was found in the cytoplasm of 0.2–2.8% of AMφs from three sarcoidosis patients [53]. In BAL cells from sarcoidosis patients, the mean amount of *P. acnes* DNA was substantially greater than that found in controls, according to Ichikawa et al.'s research [54]. Ichikawa et al. used a more sensitive method than Hiramatsu et al. [53, 54]. The lung is at the frontline of infection, and *P. acnes* begins to increase in the AMφs. So, it is reasonable for there to be around a three-fold difference (60:21 genomes) between patients and normal persons in the AMφs as frontline cells and a more than $\log^1$ difference between the internal organs (mediastinal lymph nodes).

Around puberty, most young people suffer from acne vulgaris. An antibody against *P. acnes* was observed in 100% of young patients with acne vulgaris and in 40% of young normal controls. Patients with acne vulgaris positive for *P. acnes* showed a significantly higher titer than negative patients, and the titer generally correlated with the severity of the acne vulgaris [55]. Schupp et al. reported that *P. acnes*-IgGs but not that of the anti-*P. acnes*-IgAs were significantly increased in the BAL fluid of sarcoidosis patients compared with that of healthy volunteers [56]. Cellular immunity to *P. acnes* was noted only in sarcoidosis patients [57, 58]. Meanwhile, Ishige et al. reported that *P. acnes* was cultured from the lung (50%), mediastinal lymph nodes (73%), gastric lymph nodes (50%), and intestinal lymph nodes (25%) from non-sarcoidosis cases, but the numbers cultured were small compared with those of sarcoidosis patients [59]. Recently, a meta-analysis has also supported *P. acnes* as a candidate for the causative microorganism rather than *M. tb* [42].

In summary, the antibody and *P. acnes* itself (whether revealed by culture or DNA testing) were found at a high incidence and high titer, numbers, or amounts in sarcoidosis patients, and at a low incidence and low titer, numbers, or amounts in non-sarcoidosis cases, and cellular immunity against *P. acnes* was only noted in sarcoidosis patients (Table 3). How can these results be explained? I propose a simplified schema of sarcoidosis pathogenesis in Graphical Abstract to explain the two previous questions. I suppose the essential difference between sarcoidosis and non-sarcoidosis cases might lie in the ability of Mφ. Mφ in non-sarcoidosis cases can control *P. acnes* inhaled into the lung through the assistance of the antibody to *P. acnes*. In some cases, *P. acnes* can survive in Mφ as a latent infection in various organs, including the lymph nodes. Even with repeated inhalation, Mφ of non-sarcoidosis cases can control *P. acnes*. Contrastingly, Mφ of sarcoidosis patients cannot control *P. acnes* and frequently permit its proliferation, which is why the high antibody titer and high antigen numbers persist in sarcoidosis patients. This may be related to some deficiency of innate immunity (mainly Mφ function). Sarcoidosis patients need to develop cellular immunity with the help of CD4 + cells and enclose proliferating *P. acnes* by forming epithelioid cell granulomas. Although most patients can sterilize *P. acnes* in the granulomas, a small number of patients cannot do this, thus leading to the subsequent progression of fibrosis around granulomas and new granuloma development.

3.4 Animal Experimental Data

Extrapulmonary sensitization of *P. acnes* in mice led to pulmonary granulomas, which were treated with antibiotics, according to Nishiwaki et al. [60]. Werner and colleagues found that intratracheal instillation of *P. acnes* into mice caused granulomatous lesions. There was an increase in the frequency and size of granulomatous lesions in mice lacking in the adaptor of innate immunity [61]. Intravenous *P. acnes* injection into sensitized rabbits generated extensive and widespread lung granulomatous nodular lesions in only three days, according to Ichiyasu et al.'s study [62]. It is clear that *P. acnes* can cause granulomatous lesions somewhat resembling the epithelioid cell granulomas seen in sarcoidosis and that antibiotics can alleviate them. Unfortunately, no animal experiments have succeeded in producing continuous epithelioid cell granuloma formation or latent infection with endogenous reactivation [63, 64].

3.5 Genetic Predisposition

The incidence and mortality of sarcoidosis vary [65–67]. Genetic predispositions, including human leucocyte antigen (HLA), were studied vigorously [68–72]. Among sarcoidosis patients, genetic differences were found between patients with Löfgren's syndrome and non-Löfgren sarcoidosis and between patients with Löfgren's syndrome [73–76].

Nucleotide-binding oligomerization domain (NOD) 1 plays a role as a pattern recognition receptor for bacterial peptidoglycan. Allelic discrimination of NOD1 was found in Japanese sarcoidosis patients [77]. This variation causes recognition impairment of intracellular *P. acnes*. Another genetic risk factor for sarcoidosis was also reported [78].

3.6 Histological Findings of Sarcoidosis and Correlation with *P. Acnes*

The unit lesion of epithelioid cell granuloma is about 300 μm in size and is a well-defined nodule composed of epithelioid cells and lymphocytes. Ultra-structurally, the not mature epithelioid cell is rich in the rough endoplasmic reticulum, whereas the mature epithelioid cell is rich in Golgi apparatuses and storage vesicles [79]. CD4 + cells are located rather diffusely in the nodule, small numbers of CD8 + cells are located at its periphery, and B cells are scant around the nodule [80, 81] (Fig. 2a–e). CD4 + cells are mainly of the Th1 subtype and produce interleukin-2 and interferon-γ [82]. Reticulin fibers proliferate around and, in the granuloma, and the degree depends on the stage of the granuloma (Fig. 2f). The fate of granulomas alternates between hyalinization and resolution [83]. Granulomas are present in lymphatic routes of the lung—around bronchovascular structures, bronchial submucosal tissues, interlobular septa, and the visceral pleura —and tend to conglomerate in the lungs [81, 83–87].

The presence of *P. acnes* in the lesions was found pathologically. Negi et al. investigated this immunohistochemically using specific antibodies for *P. acnes* (PAB antibody and TIG antibody) [88]. Immunohistochemistry using the PAB antibody revealed a high percentage of small round bodies within sarcoid granulomas of the lungs and lymph nodes examined from two countries. Many small round bodies were observed in the immature granulomas, whereas only a few or none were present in the mature granulomas (Fig. 3a). In non-granulomatous areas, many small round bodies were found in AMφs (Fig. 3b) and Mφs of paracortical lymph nodes from sarcoidosis patients and some from non-sarcoidosis cases. Brown-colored, large, spheroidal, acid-fast Hamazaki-Wesenberg (HW) bodies were noted in 50% of the sarcoid and 15% of the non-sarcoid lymph nodes. Both PAB and TIG antibodies reacted with HW bodies in the sarcoid lymph nodes. Electron microscopy showed that HW bodies had a single bacterial structure and lacked a cell wall suggestive of the bacterial L form [88]. Immunohistochemical proof of *P. acnes* was also reported in the granulomas of many extra-thoracic organs [89–92].

Recently, Suzuki et al. reported that small round bodies attached to IgA and IgM were abundantly detected at a high percentage in sinus Mφs of sarcoid lymph node samples compared with non-sarcoid samples [93]. They speculated that:

- *P. acnes* are endogenously reactivated microbiota in the lung and lymph nodes;
- *P. acnes* proliferate (the L form produces many round bodies) in Mφs and perforate the Mφs;

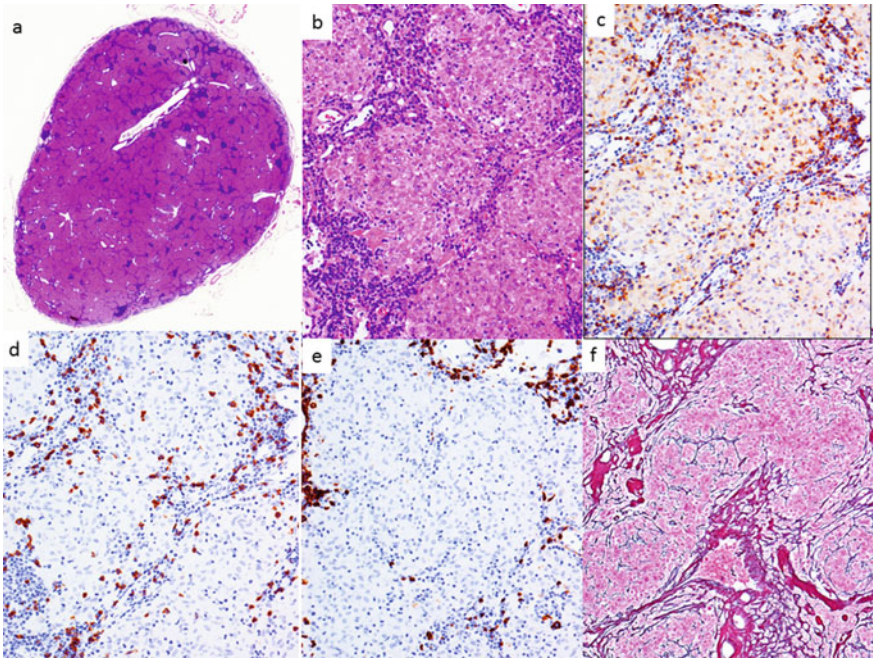


Fig. 2 Histological features of sarcoidosis in the lymph node: **a**, the lymph node is filled with granulomas. Hematoxylin–eosin staining (HE), panoramic view; **b**, each epithelioid cell granuloma is well-formed and composed of epithelioid cells with an unclear border. HE, × 200; **c**, diffuse infiltration of CD4 + cells into the granulomas. Immunostaining using an anti-CD4 antibody. × 200; d, peripheral infiltration of CD8 + cells in the granulomas. × 200; **e**, Infiltration of CD20 cells only around the granulomas. × 200; **f**, granulomas are surrounded by fine reticulin fibers and mild reticulin fibers in the granulomas (mainly proliferative-stage granuloma) using reticulin staining, × 100

- IgA and IgM attach to the surface of round bodies; and
- then the round bodies are engulfed by Mφs.

Due to delayed-type hypersensitivity, epithelioid cell granulomas are formed to control *P. acnes*. Eishi stated that sarcoidosis is an endogenous allergic infection due to *P. acnes* [94], and the above data support this hypothesis. Regardless of the different viewpoints concerning the role of granuloma formation, *P. acnes is* closely correlated with sarcoidosis. Leheste et al. support *P. acnes* as a causative agent of sarcoidosis with reservation [95]. I support the etiological role of *P. acnes*. Still, I speculate that only sarcoidosis patients have to drive cellular immunity to control the proliferation of *P. acnes* because even with the help of antibodies and complements, Mφs cannot control engulfed *P. acnes*, as stated in Section "Comparison of *P. Acnes* in Sarcoidosis Patients and Non-sarcoidosis Cases". I understand that sarcoidosis is an exogenous infection due to *P. acnes*, which requires cellular

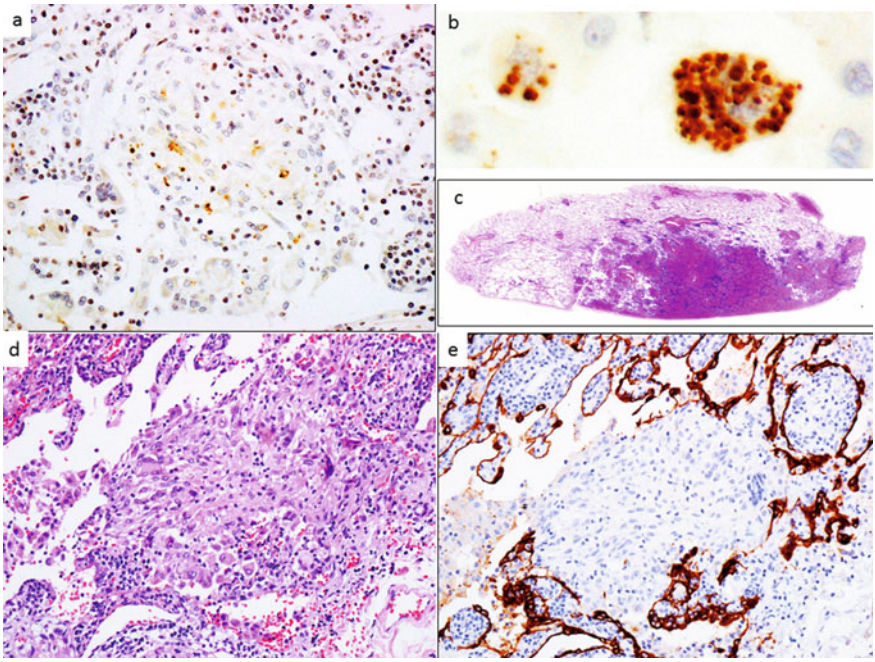


Fig. 3 Intra-alveolar granulomas and proliferation of *P. acnes* in the lung as evidenced in a patient with drug-induced sarcoidosis [120]: **a**, small numbers of round bodies are noted in the immature epithelioid cell granuloma. Immunostaining using a specific antibody for *P. acnes* (PAB antibody stain by Dr. Eishi), × 200; **b**, macrophages are studded with many small round bodies. Immunostaining using PAB antibody, × 600; **c**, nodular-shaped conglomerated epithelioid cell granulomas in the lung. Hematoxylin–eosin staining (HE), panoramic view; **d**, one granuloma located at the periphery is clearly located in the alveolar lumen. HE, × 200; **e**, the location of this granuloma in the alveolar lumen was confirmed by immunostaining using an anti-keratin antibody, × 200

immunity, as in TB. It is outside the scope of this review to consider the immunopathogenesis of sarcoidosis, including various inflammatory cells, gene expression, cytokines, and others.

3.7 Early-Onset Sarcoidosis Following Primary Infection and Disease Patterns

Intrathoracic sarcoidosis is grouped or staged as follows:

i. stage 1, swelling of bilateral hilar and mediastinal lymph nodes (BHL);
ii. stage 2, BHL + the lungs;
iii. stage 3, the lungs only without fibrosis; and
iv. stage 4, the lungs with fibrosis with/without non-fibrous shadows in 1961 [96]

As indigenous bacteria, *P. acnes* is a microbe residing on the skin and in other areas, including the mouth [50, 95]. As stated earlier, *P. acnes* was cultured from the lungs of non-sarcoidosis cases (50%) [59], and thus, *P. acnes* might be repeatedly inhaled. The initial age of primary infection is unclear, but I speculate that it might be around puberty. Many children suffer from acne vulgaris around puberty [55], and they will burst pimples or scratch hair dandruff containing many *P. acnes* bacteria, thus releasing them into the environment. Of course, there are many chances to inhale *P. acnes* in crowded public spaces as their incidence increases in certain seasons [73, 97].

Iwai et al. reported that epithelioid cell granulomas were only found in the lungs and intrathoracic lymph nodes in 23 of 320 sarcoidosis autopsy cases. Some showed granulomas at an early, proliferative stage in the bilateral lungs and intrathoracic lymph nodes [98]. These cases were speculated to have early onset of sarcoidosis. Their findings indicated that the causative agent (*P. acnes*) first entered the lungs, where AMφs engulfed it and then spread to the intrathoracic lymph nodes, similar to the primary infection with *M. tb* except for the bilateral involvement.

There is no definite concept of early-onset sarcoidosis yet. However, I suppose that early-onset sarcoidosis corresponds to stage 1 (BHL), roughly to stage 2 (BHL + the lungs), and a part of stage 3 (the lungs only) (Fig. 4). Health screening data can be used to detect early-onset sarcoidosis. Nagai et al. reported that people in their 20 s had the greatest incidence (> 50%) of BHL ± the lungs compared with other ages, and in the 1960s, health screening revealed an incidence of 55% in 20-year-olds and 36% in 10-year-olds [99]. However, the incidence in 10-year-olds gradually decreased by age, which was somewhat related to a decrease in school mass screenings. Izumi reported on the incidence of radiological patterns of symptom-free sarcoidosis found by health screenings, in which BHL was 77%; BHL + the lungs was 18.4%; and the lungs only was 4.5% (these ratios somewhat resembled those of early-onset TB shown in Table 1), and found that spontaneous remission of BHL occurred within five years in 97% of those screened [100]. It is not clear from Izumi's paper whether the lung-only cases showed an alveolar pattern or reticular pattern along the lymphatics. Nevertheless, it is speculated that most early-onset sarcoidosis cases showed only BHL in Japan. Nowadays, no such data are available because Japan stopped health screening in youth because of radiation damage and the overall decrease in intrathoracic TB. In Sweden, mainly through health screening (57% by health screening and 10% by chance), BHL was detected in 73%, BHL + the lungs in 21%, and the lungs only in 6% of the subjects aged 14–44 years, and spontaneous remission of BHL had occurred in 82% within five years [101]. Shigematsu et al. compared 20 cases of pulmonary acinar shadows with BHL with 20 cases of reticulonodular shadows without BHL. Age was not statistically different between the two groups. The former group showed almost total spontaneous regression in 67% within nine months, and 100% had intra-alveolar granulomas (Fig. 3c–e) [102]. This group can be considered to have early-onset sarcoidosis because the granulomas were located in the alveolar space (aerogenous spreading) and not in the lymphatic routes. Battesti et al. also reported

33 cases of sarcoidosis (mean patient age, 28 years) showing infiltrative shadows and 27 also showing BHL. The infiltrative shadows always disappeared, with or without steroid treatment [103]. They concluded that alveolar sarcoidosis (granulomas in the alveolar space) is a distinct, acute form of sarcoidosis [102, 103]. These reports can be considered indicative of the early-onset form of pulmonary sarcoidosis following primary infection. Quite rarely, acute onset showing alveolar shadows and BHL with hypoxia was reported [104, 105], but it is not clear whether these persons inhaled large amounts of causative agents.

BHL is one of the features of Löfgren's syndrome, and the prognosis is good even if patients present with the symptoms of fever, erythema nodosum, and polyarthralgia. Löfgren's syndrome also seems to be a form of the early-onset of sarcoidosis, even if the mean age of the patients is 30 to 40 years old, that shows somewhat excessive cellular immunity (a lung accumulation of CD4 + T cells expressing a particular T-cell receptor for an antigen) and relation to a genetic factor (HLA-DRB1*03 positivity) [73–75, 97, 106]. Authors reporting on Löfgren's syndrome speculated that the incubation period might be around one month [73, 97]. Some hypersensitivity reactions might be related to acute alveolar sarcoidosis and Löfgren's syndrome because they both present similar symptoms and show marked improvement with steroid therapy. Iwai et al. also reported cases of early-stage systemic dissemination [98], which may be a counterpart of early-onset miliary TB.

In summary, patients with a disease pattern of BHL only, BHL + the lungs with infiltrative shadows (with intraluminal granulomas), and infiltrative lung shadows seem to have early-onset sarcoidosis and have a good prognosis.

3.8 Chronic Sarcoidosis, Mainly Pulmonary Sarcoidosis

There is no concept of late-onset sarcoidosis similar to that of TB, and the relative ratio of late-onset sarcoidosis compared with early-onset sarcoidosis is presently unclear as health screenings are no longer performed anymore. Izumi reported that among sarcoidosis cases found from 1963 through 1986, 60% were asymptomatic cases found during health screening, 3% were found by chance during examinations for other unrelated diseases, and 37% were found based on symptoms [100]. Most incidences of stage 3 and all of stage 4 might correspond to chronic pulmonary sarcoidosis (Fig. 4). Here, chronic pulmonary sarcoidosis is discussed as the main candidate of late-onset, chronic sarcoidosis. Patients with chronic pulmonary sarcoidosis showing extensive reticulonodular shadows are generally seriously ill. Pathological findings of end-stage pulmonary sarcoidosis showed moderate to severe fibrosis mainly along lymphatic routes with upper lobe shrinkage, focal vascular stenosis, bronchial stenosis, bronchial dilatation, and even honeycombing or multiple cysts [86, 87, 107–109]. Histologically, epithelioid cell granulomas remained in the areas of fibrosis, but mostly, granulomas underwent atrophy or hyalinosis, and the degree of interstitial inflammation was mild to moderate [98, 107–109]. It is thought that sarcoidosis fibrosis originates in a state of chronic,

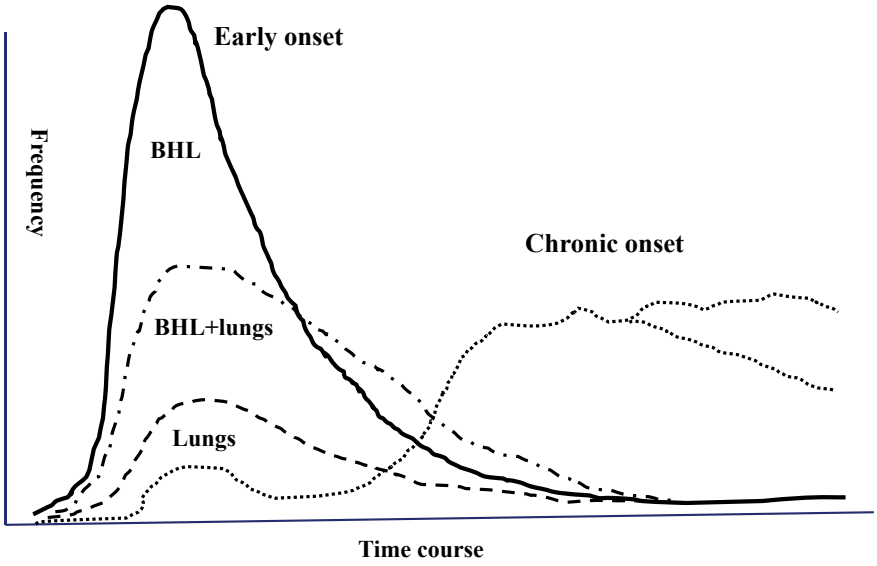


Fig. 4 Speculated natural courses of sarcoidosis. Analogous to tuberculosis, sarcoidosis also can be divided into early-onset and chronic onset. Bilateral lymph node (BHL)-only cases show good prognosis as with Löfgren's syndrome, whereas BHL + the lungs cases show a somewhat protracted course, but cases associated with infiltrative pulmonary shadows have a good prognosis. Lungs-only cases that show infiltrative shadows also have a good prognosis. Whether a BHL stage is present in the natural history of chronic onset has not been well studied, but the clinical course is chronic and generally protracted with progressive fibrosis, and pulmonary shadows are characterized by diffuse reticulonodular shadows along lymphatic routes

uncontrolled inflammation and is helped by pro-fibrotic genetic characteristics and immunological responses [110, 111].

There are many unanswered areas concerning the development of chronic pulmonary sarcoidosis. It is unclear whether endogenous reactivation occurs sometime after latent infection or gradual, ongoing progression occurs from the beginning of infection with or without a mild, unrecognized BHL stage (Fig. 4). The presence of HW bodies was reported in the lymph nodes of sarcoidosis patients, as stated earlier [88, 94], so some sarcoidosis patients do appear to go through a stage of latent infection. It is well known that the main location of the granulomas in the lungs is along the lymphatic routes, but the essential point is how this lymphatic spreading takes place. In *M. tb* infection, lymphatic spreading stops after the development of cellular immunity [1, 2]. Further, the intrapulmonary lymphatic route is used only temporarily as a route of passage, and usually, no granulomas are noted in the pulmonary lymphatic system in chronic pulmonary tuberculosis. So, do A Mφs that engulf *P. acnes* remain for a long time in the lymphatics without the development of cellular immunity, or are there repeated airway inhalation with subsequent lymphatic spreading? Then, following the proliferation of *P. acnes* and the development of cellular immunity, are epithelioid cell granulomas formed at these

locations due to endogenous reactivation, or can the AMφs engulfing *P. acnes* move into the lymphatics even after cellular immunity develops (secondary complex formation)? Under normal conditions of cellular immunity, small numbers of *M. tb* are contained and killed in the granulomas, and they usually regress without a trace [2]. Spontaneous regression of BHL in sarcoidosis patients might follow the same process. Fibrosis around granulomas is considered wall formation to prevent the proliferation of microorganisms in the granuloma. Future studies will need to investigate why some patients show a continuous progression of fibrosis and structural remodeling of the lungs even after granuloma formation and to elucidate the genetic factors that result in the inability to kill *P. acnes* and stop inflammation (Graphical Abstract).

3.9 Drug-Related Sarcoidosis and Sarcoidosis in Treated Cases of AIDS

There are many review articles on drug-induced sarcoidosis, which is generally considered a paradoxical reaction or the development of autoimmune disease [112–115]. There are many reports of sarcoid-like granulomatosis in patients treated with anti-TNF-α [116–118]. Daïen et al. reported that the median time between anti-TNF-α introduction and granulomatosis diagnosis was 18 (range 1–51) months and that lymph node and/or lung involvement was observed by computed tomography scanning of the chest in eight of ten patients, with improvement in all patients after discontinuation of drugs with or without steroids [116]. They also reported that *P. acnes,* but not *M. tb*, was cultured in one case and speculated that the incidence of sarcoid-like granuloma was 1/2800 (35.7/100,000) among treated patients. Isshiki et al. also reported the immunohistochemical presence of *P. acnes* in the granuloma [118]. Anti-TNF-α treatment significantly affects both innate and adaptive immune responses to various pathogens [119]. So, as another explanation, due to the suppression of CD4 cells but also Mφ function, patients become susceptible either to early-onset sarcoidosis or to endogenous reactivation of *P. acnes* in Mφs. Eishi also speculated that latently infected *P. acnes* might be reactivated by anti-TNF-α treatment, resulting in sarcoidosis in certain susceptible subjects among those treated [94]. Figure 3 shows bilateral nodular shadows in a patient undergoing anti-TNF-α treatment. Clear intra-alveolar growth of granulomas was noted (Fig. 3d, e), with numerous round bodies embedded in the Mφs (Fig. 3b) and small numbers of round bodies in the granuloma (Fig. 3a) as shown by anti-PAB antibody staining (this case was reported without immunostaining [120]). This might be a case of early-onset sarcoidosis following anti-TNF-α treatment.

Judson et al. reported a high incidence of sarcoidosis (154/100,000) following anti-CD25 antibody therapy [121]. In addition, sarcoidosis was induced by immune checkpoint inhibitors and by interferon-α and β treatment [122–125]. These drugs are not supposed to reduce Mφ function or Th1 cell function, so investigation of the causative mechanisms is mandatory.

The development of sarcoidosis was also reported after antiretroviral therapy in AIDS patients [126–130], which, fortunately, can now be controlled by antiretroviral therapy. Approximately 75% of the human immunodeficiency virus (HIV)-infected patients showed sarcoidosis have CD4 cell counts above 200 cells/mL. Most show a significant decrease of HIV viral load and radiological findings similar to those of non-AIDS sarcoidosis cases [129]. The association of sarcoidosis is interpreted as immune reconstitution inflammatory syndrome [126, 129, 130]. HIV can infect both CD4 + cells and Mφs and causes immunodeficiency of both cell types [131–133]. I speculate that with some functional recovery of CD4 + cells and Mφs, the significant proliferation of *P. acnes* in Mφs can be controlled through epithelioid cell granuloma formation. Diffuse histiocytosis containing high numbers of non-tuberculous mycobacteria is a well-known pathological feature of the immunodeficiency state [134–137].

In both of the above instances, I would hope that immunostaining using an anti-PAB antibody can be performed to confirm whether *P. acnes* is present in the granuloma. Then, we can settle the issue of whether secondary sarcoidosis is either of infectious origin or is a paradoxical immunological reaction or an immune reconstitution inflammatory syndrome.

3.10 Sarcoidosis Therapy

P. acnes has been the major antigen for sarcoidosis development [35, 36, 56, 93, 94, 138–141]. First published in 1999, it indicated that the symptoms and/or evidence for corticosteroid therapy are controversial [142]. Systemic therapy is less evident in pulmonary and extrapulmonary diseases. To treat pulmonary sarcoidosis, experts recommend 20–40 mg prednisolone per day [142, 143]. Various immunosuppressive and cytotoxic agents, including anti-TNF-α have been used with varying degrees of improvement, but they are complicated by side effects [144–149] and even failure [150]. The above concept might continue to be accepted for some time. Recently, Le et al. explained that targets could be extended to prevent fibrosis (M2 macrophage polarization) and not just suppress Th1-type inflammation [151].

In cutaneous sarcoidosis, minocycline treatment resulted in a good response (improvement in 20/27 and 10/12 patients, respectively), and an immunomodulating effect or an anti-infectious effect was speculated [152, 153]. In addition, there are anecdotal reports of antibiotic therapy for sarcoidosis resulting in improvement [154–156]. The Japanese Antibacterial Drug Management for Cardiac Sarcoidosis (J-ACNES) trial was recently started in 2018 [157]. Yamaguchi et al. reported that minocycline therapy was less effective than doxycycline therapy for sarcoidosis (skin: 18.8% vs. 30.6%, systemic symptoms: 18.2% vs. 34.8%) and showed more complications (40.3% vs. 25.5%) [158, 159]. From a different standpoint, Drake et al. reported effective antimycobacterial therapy for sarcoidosis [160, 161], but there have been no subsequent reports. I speculate antimycobacterial therapy might also be effective for *P. acnes*.

I think that *P. acnes* is the causative agent of sarcoidosis based on the data from the Eishi group, and I also think that granuloma formation is a protective reaction—not a hypersensitivity or allergic reaction—because granuloma formation reduces the numbers of *P. acnes* [88]. The Eishi group also indicated some protective role of granuloma formation [94]. Only limited, small numbers of infected patients progress to disease, mainly presenting as BHL. Without health screening, most cases at the BHL stage might be overlooked. When BHL + pulmonary shadows or pulmonary shadows alone do not regress during follow-up, prompt, appropriate antibiotic therapy might be another treatment choice as antibiotics can kill or reduce the numbers of *P. acnes* with subsequent disappearance of granulomas. When the pulmonary shadows become fibrotic and irreversible, antibiotic therapy might not be effective. However, I would not deny using prednisolone, immunosuppressive drugs, or antifibrotic drugs as supplementary drugs.

3.11 Remaining Subjects

It is suspected that patients with sarcoidosis have some defect of Mφ function [77], and I speculate this is so, but it needs to be confirmed by a cell culture system (using control blood monocytes and patient blood monocytes co-cultured with *P. acnes* with/without antibody to *P. acnes* and complement). As *M. tb* antigen was previously confirmed from sarcoidosis lesions [40–42], the reason for this finding needs to be clarified in the future. An animal model that presents active disease, latent infection and re-exacerbation is needed, as it can be used to examine drug effects and understand pathogenesis, among others. As the locations of chronic pulmonary sarcoidosis are predominantly via lymphatic routes, the reason why cellular immunity cannot prevent lymphatic spreading needs to be clarified. Unfortunately, the data on anti-*P. acnes* antibiotic therapy are scant, and wide-ranging trials are indicated using patients with unresolved lesions and not end-stage lesions to confirm whether antibiotics should be the drug of the first choice.

4 Conclusion

The etiology of sarcoidosis still has not been clarified. However, from the ample confirmative evidence presented by the Eishi group, we must accept that the etiology of sarcoidosis is *P. acnes*. The mechanism of epithelioid cell granuloma formation might be similar to that of *M. tb* infection. Still, there are some essential differences between *M. tb* infection and *P. acnes* infection. *M. tb* infection causes a primary complex with caseous necrosis in almost all those infected and leaves them IGRA positive. In contrast, histological traces of *P. acnes* infection are not clear, and there is no cellular immunity to *P. acnes* except in sarcoidosis patients. Chronic pulmonary TB generally begins with cavity formation at the apex and spreads

aerogenously, whereas chronic pulmonary sarcoidosis predominantly shows a generalized spread along lymphatic routes. I hope antibiotic therapy may help prevent deaths from uncontrolled sarcoidosis in the near future.

Core Messages

- It is unique to think about the pathogenesis of sarcoidosis from the viewpoint of tuberculosis.
- Evidence shows *Propionibacterium acnes* as the causative agent of sarcoidosis.
- Antibiotic therapy can be considered as a treatment for progressive sarcoidosis.

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References

1. Rich AR (1951) The pathogenesis of tuberculosis, 2nd edn. Charles C Thomas, Springfield, IL
2. Iwasaki T (1951) Pathology of tuberculosis. Hoken-doujin-sha, Tokyo (In Japanese)
3. Canetti G (1955) The tubercle bacillus in the pulmonary lesion of man. Springer Publishing Company, New York, Histobacteriology and its bearing on the therapy of pulmonary tuberculosis
4. Medlar EM (1955) The behavior of pulmonary tuberculous lesions. a pathological study. Am Rev Tuberc 71(Suppl. 1):1–244
5. Repasy T, Lee J, Marino S, Martinez N, Kirschner DE, Hendricks G, Baker S, Wilson AA, Kotton DN, Kornfeld H (2013) Intracellular bacillary burden reflects a burst size for *Mycobacterium tuberculosis* in vivo. PLoS Pathog. https://doi.org/10.1371/journal.ppat.1003190
6. Poulsen A (1950) Some clinical features of tuberculosis I. Incubation period. Acta Tuberc Scand 24(3–4):311–346
7. Grosset J (2003) *Mycobacterium tuberculosis* in the extracellular compartment: an underestimated adversary. Antimicrob Agents Chemother 47:833–836. https://doi.org/10.1128/aac.47.3.833-836.2003
8. Iida T, Uchida K, Lokman N, Furukawa A, Suzuki Y, Kumasaka T, Takemura T, Kawachi H, Akashi T, Eishi Y (2014) Calcified granulomatous lung lesions contain abundant *Mycobacterium tuberculosis* components. J Mycobac Dis 4:142. https://doi.org/10.4172/2161-1068.1000142
9. Cardona PJ (2015) The key role of exudative lesions and their encapsulation: lessons learned from the pathology of human pulmonary tuberculosis. Front Microbiol 6:612. https://doi.org/10.3389/fmicb.2015.00612
10. Modlin RL, Bloom BR (2013) TB or not TB: that is no longer the question. Sci Transl Med 5(213):213sr6. https://doi.org/10.1126/scitranslmed.3007402

11. Cadena AM, Flynn JL, Fortune SM (2016) The importance of first impressions: early events in *Mycobacterium tuberculosis* infection influence outcome. MBio 7(2):e00342-e416. https://doi.org/10.1128/mBio.00342-16
12. Chiba Y, Tokorozawa M (1948) Clinical research of tuberculous primary infection (developing mechanism of tuberculosis). Hoken-doujin-sha, Tokyo (In Japanese)
13. Medlar EM (1948) The pathogenesis of minimal pulmonary tuberculosis: a study of 1225 necropsies in cases of sudden and unexpected death. Am Rev Tuberc 58(6):583–611. https://doi.org/10.1164/art.1948.58.6.583
14. Huebschmann P (1928) Pathologische anatomie der tuberculose. Julius Springer, Berlin
15. Balasubramanian V, Wiegeshaus EH, Taylor BT, Smith DW (1994) Pathogenesis of tuberculosis: pathway to apical localization. Tuber Lung Dis 75(3):168–178. https://doi.org/10.1016/0962-8479(94)90002-7
16. Lucas SB (1998) Histopathology. In: Davies PDO (ed) Clinical tuberculosis. Chapman & Hall, London, pp 113–127
17. Hunter RL, Jagannath C, Actor JK (2007) Pathology of postprimary tuberculosis in humans and mice: contradiction of long-held beliefs. Tuberculosis (Edinb) 87(4):267–278. https://doi.org/10.1016/j.tube.2006.11.003
18. Cardona PJ (2009) A dynamic reinfection hypothesis of latent tuberculosis infection. Infection 37(2):80–86. https://doi.org/10.1007/s15010-008-8087-y
19. Riaz SM, Bjune GA, Wiker HG, Sviland L, Mustafa T (2020) Mycobacterial antigens accumulation in foamy macrophages in murine pulmonary tuberculosis lesions: association with necrosis and making of cavities. Scand J Immunol 91(4):e12866. https://doi.org/10.1111/sji.12866
20. Wong KW, Jacobs WR Jr (2016) Postprimary tuberculosis and macrophage necrosis: is there a big connection? MBio 7(1):e01589-e1615. https://doi.org/10.1128/mBio.01589-15
21. Kawabata Y (2017) Pathology of Tuberculosis. Kekkaku 92:647–660
22. Ottenhoff TH, Kumararatne D, Casanova JL (1998) Novel human immunodeficiencies reveal the essential role of type-1 cytokines in immunity to intracellular bacteria. Immunol Today 19(11):491–494. https://doi.org/10.1016/s0167-5699(98)01321-8
23. Havlir D, Barnes P (1999) Tuberculosis patients with human immunodeficiency virus infection. N Eng J Med 340(5):367–373. https://doi.org/10.1056/NEJM199902043400507
24. Keane J (2005) TNF-blocking agents and tuberculosis: new drugs illuminate an old topic. Rheumatology 44(6):714–720. https://doi.org/10.1093/rheumatology/keh567
25. Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, Siegel JN, Braun MM (2001) Tuberculosis associated with infliximab a tumor necrosis factor alpha neutralizing agent. N Engl J Med 345(15):1098–1104. https://doi.org/10.1056/NEJMoa011110
26. Gómez-Reino JJ, Carmona L, Valverde VR, Mola EM, Montero MD; BIOBADASER Group (2003) Treatment of rheumatoid arthritis with tumor necrosis factor inhibitors may predispose to significant increase in tuberculosis risk: a multicenter active-surveillance report. Arthritis Rheum 48(8):2122–2127. https://doi.org/10.1002/art.11137
27. British Thoracic Society Standards of Care Committee (2005) BTS recommendations for assessing risk and for managing *Mycobacterium tuberculosis* infection and disease in patients due to start anti-TNF-alpha treatment. Thorax 60(10):800–805.https://doi.org/10.1136/thx.2005.046797
28. Tubach F, Salmon D, Ravaud P, Allanore Y, Goupille P, Bréban M, Pallot-Prades B, Pouplin S, Sacchi A, Chichemanian RM, Bretagne S, Emilie D, Lemann M, Lortholary O, Mariette X; Research Axed on Tolerance of Biotherapies Group. (2009) Risk of tuberculosis is higher with anti-tumor necrosis factor monoclonal antibody therapy than with soluble tumor necrosis factor receptor therapy: the three-year prospective french research axed on tolerance of biotherapies registry. Arthritis Rheum 60(7):1884–1894.https://doi.org/10.1002/art.24632

29. Lin PL, Pawar S, Myers A, Pegu A, Fuhrman C, Reinhart TA, Capuano SV, Klein E, Flynn JL (2006) Early events in *Mycobacterium tuberculosis* Infection in cynomolgus macaques. Infect Immun 74(7):3790–3803. https://doi.org/10.1128/IAI.00064-06
30. Lin PL, Rodgers M, Smith L, Bigbee M, Myers A, Bigbee C, Chiosea I, Capuano SV, Fuhrman C, Klein E, Flynn JL (2009) Quantitative comparison of active and latent tuberculosis in the cynomolgus macaque model. Infect Immun 77(10):4631–4642. https://doi.org/10.1128/IAI.00592-09
31. Lin PL, Rutledge T, Green AM, Bigbee M, Fuhrman C, Klein E, Flynn JL (2012) CD4 T cell depletion exacerbates acute *Mycobacterium tuberculosis* while reactivation of latent infection is dependent on severity of tissue depletion in cynomolgus macaques. AIDS Res Hum Retroviruses 28(12):1693–1702. https://doi.org/10.1128/IAI.00592-09
32. Lin PL, Myers A, Smith L, Bigbee C, Bigbee M, Fuhrman C, Grieser H, Chiosea I, Voitenek NN, Capuano SV, Klein E, Flynn JL (2010) Tumor necrosis factor neutralization results in disseminated disease in acute and latent *Mycobacterium tuberculosis* infection with normal granuloma structure in a cynomolgus macaque model. Arthritis Rheum 62(2):340–350. https://doi.org/10.1002/art.27271
33. Cadena AM, Hopkins FF, Maiello P, Carey AF, Wong EA, Martin CJ, Gideon HP, DiFazio RM, Andersen P, Lin PL, Fortune SM, Flynn JL (2018) Concurrent infection with *Mycobacterium tuberculosis* confers robust protection against secondary infection in macaques. PLoS Pathog 14(10):e1007305 https://doi.org/10.1371/journal.ppat.1007305
34. Korsten P, Tampe B, Konig MF, Nikiphorou E (2018) Sarcoidosis and autoimmune diseases: differences, similarities and overlaps. Curr Opin Pulm Med 24(5):504–512. https://doi.org/10.1097/MCP.0000000000000500
35. Bennett D, Bargagli E, Refini RM, Rottoli P (2019) New concepts in the pathogenesis of sarcoidosis. Expert Rev Respir Med 13(10):981–991. https://doi.org/10.1080/17476348.2019.1655401
36. Inaoka PT, Shono M, Kamada M, Espinoza JL (2019) Host-microbe interactions in the pathogenesis and clinical course of sarcoidosis. J Biomed Sci 26(1):45. https://doi.org/10.1186/s12929-019-0537-6
37. Starshinova AA, Malkova AM, Basantsova NY, Zinchenko YS, Kudryavtsev IV, Ershov GA, Soprun LA, Mayevskaya VA, Churilov LP, Yablonskiy PK (2020) Sarcoidosis as an autoimmune disease. Front Immunol 10:2933. https://doi.org/10.3389/fimmu.2019.0293
38. Ichikawa H, Mori Y, Kataoka M, Nakata Y (2017) Is *Propionibacterium acnes* a probable causative infectious agent in the pathogenesis of sarcoidosis? Int Med Rev 3(11):1–18
39. Agrawal R, Kee AR, Ang L, Tun Hang Y, Gupta V, Kon OM, Mitchell D, Zierhut M, Pavesio C (2016) Tuberculosis or sarcoidosis: opposite ends of the same disease spectrum? Tuberculosis (Edinb) 98:21–26. https://doi.org/10.1016/j.tube.2016.01.003
40. Celada LJ, Hawkins C, Drake WP (2015) The etiologic role of infectious antigens in sarcoidosis pathogenesis. Clin Chest Med 36(4):561–568. https://doi.org/10.1016/j.ccm.2015.08.001
41. Fang C, Huang H, Xu Z (2016) Immunological Evidence for the Role of Mycobacteria in Sarcoidosis: A Meta-Analysis. PLoS ONE 11(8):e0154716. https://doi.org/10.1371/journal.pone.0154716
42. Esteves T, Aparicio G, Garcia-Patos V (2016) Is there any association between sarcoidosis and infectious agents?: a systematic review and meta-analysis. BMC Pulm Med 16(1):165. https://doi.org/10.1186/s12890-016-0332-z
43. Homma JY, Abe C, Chosa H, Ueda K, Saegusa J, Nakayama M, Homma H, Washizaki M, Okano H (1978) Bacteriological investigation on biopsy specimens from patients with sarcoidosis. Jpn J Exp Med 48(3):251–255
44. Abe C, Iwai K, Mikami R, Hosoda Y (1984) Frequent isolation of *Propionibacterium acnes* from sarcoidosis lymph nodes. Zentralbl Bakteriol Mikrobiol Hyg [A] 256(4):541–547. https://doi.org/10.1016/s0174-3031(84)80032-3

45. Funke G, Renaud FN, Freney J, Riegel P (1997) Multicenter evaluation of the updated and extended API (RAPID) Coryne database 2.0. J Clin Microbiol 35(12):3122–3126
46. Cogen AL, Nizet V, Gallo RL (2008) Skin microbiota: a source of disease or defence? Br J Dermatol 158(3):442–455. https://doi.org/10.1111/j.1365-2133.2008.08437.x
47. Miura Y, lshige I, Soejima N, Suzuki Y, Uchida K, Kawana S, Eishi Y (2010) Quantitative PCR of *Propionibacterium acnes* DNA in samples aspirated from sebaceous follicles on the normal skin of subjects with or without acne. J Med Dent Sci 57(1):65–74
48. Tanghetti EA (2013) The role of Inflammation in the pathology of acne. J Clin Aesthet Dermatol 6(9):27–35
49. Webster GF, Leyden JJ, Musson RA, Douglas SD (1985) Susceptibility of *Propionibacterium acnes* to killing and degradation. Infect Immun 49(1):116–121
50. Fischer N, Mak TN, Shinohara DB, Sfanos KS, Meyer TF, Brüggemann H (2013) Deciphering the intracellular fate of *Propionibacterium acnes* in macrophages. Biomed Res Int 2013:603046. https://doi.org/10.1155/2013/603046
51. Ishige I, Usui Y, Takemura T, Eishi Y (1999) Quantitative PCR of mycobacterial and propionibacterial DNA in lymph nodes of Japanese patients with sarcoidosis. Lancet 354 (9173):120–123. https://doi.org/10.1016/S0140-6736(98)12310-3
52. Eishi Y, Suga M, Ishige I, Kobayashi D, Yamada T, Takemura T, Takizawa T, Koike M, Kudoh S, Costabel U, Guzman J, Rizzato G, Gambacorta M, du Bois R, Nicholson AG, Sharma OP, Ando M (2002) Quantitative analysis of mycobacterial and propionibacterial DNA in lymph nodes of Japanese and European patients with sarcoidosis. J Clin Microbiol 40(1):198–204. https://doi.org/10.1128/jcm.40.1.198-204.2002
53. Hiramatsu J, Kataoka M, Nakata Y, Okazaki K, Tada S, Tanimoto M, Eishi Y (2003) *Propionibacterium acnes* DNA detected in bronchoalveolar lavage cells from patients with sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 20(3):197–203
54. Ichikawa H, Kataoka M, Hiramatsu J, Ohmori M, Tanimoto Y, Kanehiro A, Nakata Y, Tanimoto M (2008) Quantitative analysis of propionibacterial DNA in bronchoalveolar lavage cells from patients with sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 25(1):15–20
55. Basal E, Jain A, Kaushal GP (2004) Antibody response to crude cell lysate of *propionibacterium acnes* and induction of pro-inflammatory cytokines in patients with acne and normal healthy subjects. J Microbiol 42(2):117–125
56. Schupp JC, Tchaptchet S, Lützen N, Engelhard P, Müller-Quernheim J, Freudenberg MA, Prasse A (2015) Immune response to Propionibacterium acnes in patients with sarcoidosis–in vivo and in vitro. BMC Pulm Med 15:75. https://doi.org/10.1186/s12890-015-0070-7
57. Ebe Y, Ikushima S, Yamaguchi T, Kohno K, Azuma A, Sato K, Ishige I, Usui Y, Takemura T, Eishi Y (2000) Proliferative response of peripheral blood mononuclear cells and levels of antibody to recombinant protein from *Propionibacterium acnes* DNA expression library in Japanese patients with sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 17(3):256–265
58. Teirstein AS (1998) Kveim antigen: what does it tell us about causation of sarcoidosis? Semin Respir Infect 13(3):2206–2211
59. Ishige I, Eishi Y, Takemura T, Kobayashi I, Nakata K, Tanaka I, Nagaoka S, Iwai K, Watanabe K, Takizawa T, Koike M (2005) *Propionibacterium acnes* is the most common bacterium commensal in peripheral lung tissue and mediastinal lymph nodes from subjects without sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 22(1):33–42
60. Nishiwaki T, Yoneyama H, Eishi Y, Matsuo N, Tatsumi K, Kimura H, Kuriyama T, Matsushima K (2004) Indigenous pulmonary *Propionibacterium acnes* primes the host in the development of sarcoid-like pulmonary granulomatosis in mice. Am J Pathol 165 (2):631–639. https://doi.org/10.1016/S0002-9440(10)63327-5
61. Werner JL, Escolero SG, Hewlett JT, Mak TN, Williams BP, Eishi Y, Núñez G (2017) Induction of pulmonary granuloma formation by *Propionibacterium acnes* Is regulated by MyD88 and Nox2. Am J Respir Cell Mol Biol 56(1):121–130. https://doi.org/10.1165/rcmb.2016-0035OC

62. Ichiyasu H, Suga M, Matsukawa A, Iyonaga K, Mizobe T, Takahashi T, Ando M (1999) Functional roles of MCP-1 in *Propionibacterium acnes*-induced, T cell-mediated pulmonary granulomatosis in rabbits. J Leukoc Biol 65(4):482–491. https://doi.org/10.1002/jlb.65.4.482
63. Jeny F, Pacheco Y, Besnard V, Valeyre D, Bernaudin JF (2016) Experimental models of sarcoidosis. Curr Opin Pulm Med 22(5):492–499. https://doi.org/10.1097/MCP.0000000000000295
64. Hu Y, Yibrehu B, Zabini D, Keubler WM (2017) Animal models of sarcoidosis. Cell Tissue Res 367(3):651–661. https://doi.org/10.1007/s00441-016-2526-3
65. Rybicki BA, Major M, Popovich J Jr, Maliarik MJ, Iannuzzi MC (1997) Racial differences in sarcoidosis incidence: a 5-year study in a health maintenance organization. Am J Epidemiol 145(3):234–241. https://doi.org/10.1093/oxfordjournals.aje.a009096
66. Cozier YC (2016) Assessing the worldwide epidemiology of sarcoidosis: challenges and future directions. Eur Respir J 48(6):1545–1548. https://doi.org/10.1183/13993003.01819-2016
67. Mirsaeidi M, Machado RF, Schraufnagel D, Sweiss NJ, Baughman RP (2015) Racial difference in sarcoidosis mortality in the United States. Chest 147(2):438–449. https://doi.org/10.1378/chest.14-1120
68. Berlin M, Fogdell-Hahn A, Olerup O, Eklund A, Grunewald J (1997) HLA-DR predicts the prognosis in Scandinavian patients with pulmonary sarcoidosis. Am J Respir Crit Care Med 156(5):1601–1605. https://doi.org/10.1164/ajrccm.156.5.9704069
69. Rossman MD, Thompson B, Frederick M, Maliarik M, Iannuzzi MC, Rybicki BA, Pandey JP, Newman LS, Magira E, Beznik-Cizman B, Monos D, ACCESS Group (2003) HLA-DRB1*1101: a significant risk factor for sarcoidosis in blacks and whites. Am J Hum Genet 73(4):720–735.https://doi.org/10.1086/378097
70. Zhou H, Diao M, Zhang M (2016) The association between ANXA11 gene polymorphisms and sarcoidosis: a meta-analysis and systematic review. Sarcoidosis Vasc Diffuse Lung Dis 33(2):102–111
71. Sato H, Woodhead FA, Ahmad T, Grutters JC, Spagnolo P, van den Bosch JM, Maier LA, Newman LS, Nagai S, Izumi T, Wells AU, du Bois RM, Welsh KI (2010) Sarcoidosis HLA class II genotyping distinguishes differences of clinical phenotype across ethnic groups. Hum Mol Genet 19(20):4100–4111. https://doi.org/10.1093/hmg/ddq325
72. Sauer WH, Stern BJ, Baughman RP, Culver DA, Royal W (2017) High-Risk Sarcoidosis. Current Concepts and Research Imperatives. Ann Am Thorac Soc 14(Supplement_6):S437–S444 https://doi.org/10.1513/AnnalsATS.201707-566OT
73. Grunewald J, Eklund A (2009) Lofgren's syndrome: human leukocyte antigen strongly influences the disease course. Am J Respir Crit Care Med 179(4):307–312. https://doi.org/10.1164/rccm.200807-1082OC
74. Grunewald J, Brynedal B, Darlington P, Nisell M, Cederlund K, Hillert J, Eklund A (2010) Different HLA-DRB1 allele distributions in distinct clinical subgroups of sarcoidosis patients. Respir Res 11(1):25. https://doi.org/10.1186/1465-9921-11-25
75. Grunewald J, Idali F, Kockum I, Seddighzadeh M, Nisell M, Eklund A, Padyukov L (2010) Major histocompatibility complex class II transactivator gene polymorphism: associations with Löfgren's syndrome. Tissue Antigens 76(2):96–101. https://doi.org/10.1111/j.1399-0039.2010.01476.x
76. Rossides M, Grunewald J, Eklund A, Kullberg S, Di Giuseppe D, Askling J, Arkema EV (2018) Familial aggregation and heritability of sarcoidosis: a Swedish nested case-control study. Eur Respir J 52(2):1800385. https://doi.org/10.1183/13993003.00385-2018
77. Tanabe T, Ishige I, Suzuki Y, Aita Y, Furukawa A, Ishige Y, Uchida K, Suzuki T, Takemura T, Ikushima S, Oritsu M, Yokoyama T, Fujimoto Y, Fukase K, Inohara N, Nunez G, Eishi Y (2006) Sarcoidosis and NOD1 variation with impaired recognition of intracellular *Propionibacterium acnes*. Biochim Biophys Acta 1762(9):794–801. https://doi.org/10.1016/j.bbadis.2006.07.006

78. Fischer A, Ellinghaus D, Nutsua M, Hofmann S, Montgomery CG, Iannuzzi MC, Rybicki BA, Petrek M, Mrazek F, Pabst S, Grohé C, Grunewald J, Ronninger M, Eklund A, Padyukov L, Mihailovic-Vucinic V, Jovanovic D, Sterclova M, Homolka J, Nöthen MM, Herms S, Gieger C, Strauch K, Winkelmann J, Boehm BO, Brand S, Büning C, Schürmann M, Ellinghaus E, Baurecht H, Lieb W, Nebel A, Müller-Quernheim J, Franke A, Schreiber S; GenPhenReSa Consortium (2015) Identification of immune-relevant factors conferring sarcoidosis genetic risk. Am J Respir Crit Care Med 192(6):727–736.https://doi.org/10.1164/rccm.201503-0418OC
79. De Vos R, De Wolf-Peeters C, Facchetti F, Facchetti F, Desmet V (1990) Plasmacytoid monocytes in epithelioid cell granulomas: ultrastructural and immunoelectron microscopic study. Ultrastruct Pathol 14(4):291–302. https://doi.org/10.3109/01913129009032244
80. van Maarsseven AC, Mullink H, Alons CL, Stam J (1986) Distribution of T-lymphocyte subsets in different portions of sarcoid granulomas: immunohistologic analysis with monoclonal antibodies. Hum Pathol 17(5):493–500. https://doi.org/10.1016/s0046-8177(86)80040-5
81. Ma Y, Gal A, Koss MN (2007) The pathology of pulmonary sarcoidosis: update. Semin Diagn Pathol 24(3):150–161. https://doi.org/10.1053/j.semdp.2007.06.002
82. Bäumer I, Zissel G, Schlaak M, Müller-Quernheim J (1997) Th1/Th2 cell distribution in pulmonary sarcoidosis. Am J Respir Cell Mol Biol 16(2):171–177. https://doi.org/10.1165/ajrcmb.16.2.9032124
83. Gal AA, Koss MN (2002) The pathology of sarcoidosis. Curr Opin Pulm Med 8(5):445–451 https://doi.org/.1097/00063198-200209000-00018
84. Carrington CB (1976) Structure and function in sarcoidosis. Ann N Y Acad Sci 278:265–283. https://doi.org/10.1111/j.1749-6632.1976.tb47038.x
85. Rosen Y (2007) Pathology of sarcoidosis. Semin Respir Crit Care Med 28(1):36–52. https://doi.org/10.1055/s-2007-970332
86. Corrin B, Nicholson AG (2011) Diffuse parenchymal disease of the lung. Pathology of the lungs, 3rd edn. Churchill Livingstone, Edinburgh, pp 266–326
87. Flieder DB, Sander A, Koss MN (2013) Sarcoidosis. In: Hasletone P, Flieder DB (eds) Spencer's pathology of the lung, 6th edn. Cambridge University Press, Cambridge, pp 475–511
88. Negi M, Takemura T, Guzman J, Uchida K, Furukawa A, Suzuki Y, Iida T, Ishige I, Minami J, Yamada T, Kawachi H, Costabel U, Eishi Y (2012) Localization of *Propionibacterium acnes* in granulomas supports a possible etiologic link between sarcoidosis and the bacterium. Mod Pathol 25(9):1284–1297. https://doi.org/10.1038/modpathol.2012.80
89. Satoh F, Morita H, Tayama H, Inoue Y, Eishi Y, Yoshimura A (2013) Renal sarcoidosis with limited lung manifestations expressing *Propionibacterium acnes* antigens in the affected tubulointerstitium. Am J Med Sci 346(3):250–252. https://doi.org/10.1097/MAJ.0b013e31828bdf9f
90. Asakawa N, Uchida K, Sakakibara M, Omote K, Noguchi K, Tokuda Y, Kamiya K, Hatanaka KC, Matsuno Y, Yamada S, Asakawa K, Fukasawa Y, Nagai T, Anzai T, Ikeda Y, Ishibashi-Ueda H, Hirota M, Orii M, Akasaka T, Uto K, Shingu Y, Matsui Y, Morimoto SI, Tsutsui H, Eishi Y (2017) Immunohistochemical identification of *Propionibacterium acnes* in granuloma and inflammatory cells of myocardial tissues obtained from cardiac sarcoidosis patients. PLoS ONE 12(7):e0179980. https://doi.org/10.1371/journal.pone.0179980
91. Nagata K, Eishi Y, Uchida K, Yoneda K, Hatanaka H, Yasuhara T, Nagata M, Sotozono C, Kinoshita S (2017) Immunohistochemical detection of *Propionibacterium acnes* in the retinal granulomas in patients with ocular sarcoidosis. Sci Rep 7(1):15226. https://doi.org/10.1038/s41598-017-15710-0
92. Yang G, Eishi Y, Raza A, Rojas H, Achiriloaie A, De Los RK, Raghavan R (2018) *Propionibacterium acnes*-associated neurosarcoidosis: a case report with review of the literature. Neuropathology 38(2):159–164. https://doi.org/10.1111/neup.12411

93. Suzuki Y, Uchida K, Takemura T, Sekine M, Tamura T, Furukawa A, Hebisawa A, Sakakibara Y, Awano N, Amano T, Kobayashi D, Negi M, Kakegawa T, Wada Y, Ito T, Suzuki T, Akashi T, Eishi Y (2018) *Propionibacterium acnes*-derived insoluble immune complexes in sinus macrophages of lymph nodes affected by sarcoidosis. PLoS ONE 13(2): e0192408. https://doi.org/10.1371/journal.pone.0192408
94. Eishi Y (2013) Etiologic aspect of sarcoidosis as an allergic endogenous infection caused by *Propionibacterium acnes*. Biomed Res Int 2013:935289. https://doi.org/10.1155/2013/935289
95. Leheste JR, Ruvolo KE, Chrostowski JE, Rivera K, Husko C, Miceli A, Selig MK, Brüggemann H, Torres G (2017) *P. acnes*-driven disease pathology: current knowledge and future directions. Front Cell Infect Microbiol 7:81. https://doi.org/10.3389/fcimb.2017.00081
96. Scadding JG (1961) Prognosis of intrathoracic sarcoidosis in England. A review of 136 cases after five years' observation. Br Med J 2(5261):1165–1172. https://doi.org/10.1136/bmj.2.5261.1165
97. Grunewald J, Eklund A (2007) Sex-specific manifestations of Löfgren's syndrome. Am J Respir Crit Care Med 175(1):40–44. https://doi.org/10.1164/rccm.200608-1197OC
98. Iwai K, Takemura T, Kitaichi M, Kawabata Y, Matsui Y (1993) Pathological studies on sarcoidosis autopsy. II. Early change, mode of progression and death pattern. Acta Pathol Jpn 43(7–8):377–385. https://doi.org/10.1111/j.1440-1827.1993.tb01149.x
99. Nagai S (1998) Sarcoidosis in Japan and sarcoidosis in the world. Kokyuu to Zyunkann 46 (1):5–12 (In Japanese)
100. Izumi T (1988) Sarcoidosis in Kyoto (1963–1986). Sarcoidosis 5(2):142–146
101. Hillerdal G, Nöu E, Osterman K, Schmekel B (1984) Sarcoidosis: epidemiology and prognosis: a 15-year European study. Am Rev Respir Dis 130(1):29–32. https://doi.org/10.1164/arrd.1984.130.1.29
102. Shigematsu N, Matsuba K, Takahashi T (1978) Clinicopathologic characteristics of pulmonary acinar sarcoidosis. Chest 73(2):186–188. https://doi.org/10.1378/chest.73.2.186
103. Battesti JP, Saumon G, Valeyre D, Amouroux J, Pechnick B, Sandron D, Georges R (1982) Pulmonary sarcoidosis with an alveolar radiographic pattern. Thorax 37(6):448–452. https://doi.org/10.1136/thx.37.6.448
104. Gera K, Gupta N, Ahuja A, Shah A (2014) Acute alveolar sarcoidosis presenting with hypoxaemic respiratory failure. BMJ Case Rep 2014:bcr2013202247. https://doi.org/10.1136/bcr-2013-202247
105. Ansari-Gilani K, Yang M, Ramaiya NH (2019) Alveolar sarcoidosis with intense FDG uptake, mimicking multi-focal pneumonia and infiltrative lung malignancy. Clin Nucl Med 44(8):653–654. https://doi.org/10.1097/RLU.0000000000002637
106. Tejera Segura B, Holgado S, Mateo L, Pego-Reigosa JM, Carnicero Iglesias M, Olivé A (2014) Löfgren syndrome: a study of 80 cases. Med Clin (Barc) 143(4):166–169. https://doi.org/10.1016/j.medcli.2014.02.029
107. Shigemitsu H, Oblad JM, Sharma OP, Koss MN (2010) Chronic interstitial pneumonitis in end-stage sarcoidosis. Eur Respir J 35(3):695–697. https://doi.org/10.1183/09031936.00150609
108. Xu L, Kligerman S, Burke A (2013) End-stage sarcoid lung disease is distinct from usual interstitial pneumonia. Am J Surg Pathol 37(4):593–600. https://doi.org/10.1097/PAS.0b013e3182785a2d
109. Zhang C, Chan KM, Schmidt LA, Myers JL (2016) Histopathology of explanted lungs from patients with a diagnosis of pulmonary sarcoidosis. Chest 149(2):499–507. https://doi.org/10.1378/chest.15-0615
110. Bonham CA, Strek ME, Patterson KC (2016) From granuloma to fibrosis: sarcoidosis associated pulmonary fibrosis. Curr Opin Pulm Med 22(5):484–491. https://doi.org/10.1097/MCP.0000000000000301

111. Patterson KC, Hogarth K, Husain AN, Sperling AI, Niewold TB (2012) The clinical and immunologic features of pulmonary fibrosis in sarcoidosis. Transl Res 160(5):321–331. https://doi.org/10.1016/j.trsl.2012.03.005
112. Ramos-Casals M, Perez Alvarez R, Diaz-Lagares C, Cuadrado MJ, Khamashta MA, BIOGEAS Study Group (2010) Autoimmune diseases induced by biological agents: a double-edged sword? Autoimmun Rev 9(3):188–193.https://doi.org/10.1016/j.autrev.2009.10.003
113. Pérez-De-Lis M, Retamozo S, Flores-Chávez A, Kostov B, Perez-Alvarez R, Brito-Zerón P, Ramos-Casals M (2017) Autoimmune diseases induced by biological agents. A review of 12,731 cases (BIOGEAS Registry). Expert Opin Drug Saf 16(11):1255–1271. https://doi.org/10.1080/14740338.2017.1372421
114. Decock A, Van Assche G, Vermeire S, Wuyts W, Ferrante M (2017) Sarcoidosis-Like lesions: another paradoxical reaction to Anti-TNF therapy? J Crohns Colitis 11(3):378–383. https://doi.org/10.1093/ecco-jcc/jjw155
115. Cohen Aubart F, Lhote R, Amoura A, Valeyre D, Haroche J, Amoura Z, Lebrun-Vignes B (2019) Drug-induced sarcoidosis: an overview of the WHO pharmacovigilance database. J Intern Med. https://doi.org/10.1111/joim.12991
116. Daïen CI, Monnier A, Claudepierre P, Constantin A, Eschard JP, Houvenagel E, Samimi M, Pavy S, Pertuiset E, Toussirot E, Combe B, Morel J, Rhumatismes C, Inflammation (CRI) (2009) Sarcoid-like granulomatosis in patients treated with tumor necrosis factor blockers: 10 cases. Rheumatology 48(8):883–886. https://doi.org/10.1093/rheumatology/kep046
117. Petrovici A, Kaiser MJ, Louis R, Dang DN (2016) Sarcoid-like granulomatosis in patients treated with anti-TNFα. Rev Med Liege 71(3):124–128
118. Isshiki T, Matsuyama H, Sakamoto S, Honma N, Mikami T, Shibuya K, Eishi Y, Homma S (2019) Development of *Propionibacterium acnes*-associated sarcoidosis during etanercept therapy: a case report. Intern Med 58(10):1473–1477. https://doi.org/10.2169/internalmedicine.2086-18
119. Harris J, Keane J (2010) How tumour necrosis factor blockers interfere with tuberculosis immunity. Clin Exp Immunol 161(1):1–9. https://doi.org/10.1111/j.1365-2249.2010.04146.x
120. Ishiguro T, Takayanagi N, Kurashima K, Matsushita A, Harasawa K, Yoneda K, Tsuchiya N, Miyahara Y, Yamaguchi S, Yano R, Tokunaga D, Saito H, Ubukata M, Yanagisawa T, Sugita Y, Kawabata Y (2008) Development of sarcoidosis during etanercept therapy. Intern Med 47(11):1021–1025. https://doi.org/10.2169/internalmedicine.47.0602
121. Judson MA, Elicker BM, Colby TV, Kwon S, de Windt E, Chalkias S, Prada C, Smirnakis K, Singhal P (2019) The development of sarcoidosis in patients receiving daclizumab: a case series from multiple clinical trials. Respir Med 149:23–27. https://doi.org/10.1016/j.rmed.2019.01.015
122. Gkiozos I, Kopitopoulou A, Kalkanis A, Vamvakaris IN, Judson MA, Syrigos KN (2018) Sarcoidosis-like reactions induced by checkpoint inhibitors. J Thorac Oncol 13(8):1076–1082. https://doi.org/10.1016/j.jtho.2018.04.031
123. Cornejo CM, Haun P, English J 3rd, Rosenbach M (2019) Immune checkpoint inhibitors and the development of granulomatous reactions. J Am Acad Dermatol 81(5):1165–1175
124. Chakravarty SD, Harris ME, Schreiner AM, Crow MK (2012) Sarcoidosis triggered by interferon-beta treatment of multiple sclerosis: a case report and focused literature review. Semin Arthritis Rheum 42(2):206–212. https://doi.org/10.1016/j.semarthrit.2012.03.008
125. López V, Molina I, Monteagudo C, Jordá E (2011) Cutaneous sarcoidosis developing after treatment with pegylated interferon and ribavirin: a new case and review of the literature. Int J Dermatol 50(3):287–291. https://doi.org/10.1111/j.1365-4632.2010.04728.x
126. Naccache JM, Antoine M, Wislez M, Rosenbach M (1999) Sarcoidosis-like pulmonary disorder in human immunodeficiency virus-infected patients receiving antiretroviral therapy. Am J Respir Crit Care Med 159(6):2009–2013. https://doi.org/10.1016/j.jaad.2018.07.051

127. Morris DG, Jasmer RM, Huang L, Gotway MB, Nishimura S, King TE Jr (2003) Sarcoidosis following HIV infection: evidence for CD4+ lymphocyte dependence. Chest 124(3):929–935. https://doi.org/10.1378/chest.124.3.929
128. Foulon G, Wislez M, Naccache JM, Blanc FX, Rabbat A, Israël-Biet D, Valeyre D, Mayaud C, Cadranel J (2004) Sarcoidosis in HIV-infected patients in the era of highly active antiretroviral therapy. Clin Infect Dis 38(3):418–425. https://doi.org/10.1086/381094
129. Crothers K, Huang L (2009) Pulmonary complications of immune reconstitution inflammatory syndromes in HIV-infected patients. Respirology 14(4):486–494. https://doi.org/10.1111/j.1440-1843.2008.01468.x
130. Miranda EJ, Leite OH, Duarte MI (2011) Immune reconstitution inflammatory syndrome associated with pulmonary sarcoidosis in an HIV-infected patient: an immunohistochemical study. Braz J Infect Dis 15(6):601–606. https://doi.org/10.1590/s1413-86702011000600018
131. Baldwin GC, Fleischmann J, Chung Y, Koyanagi Y, Chen IS, Golde DW (1990) Human immunodeficiency virus causes mononuclear phagocyte dysfunction. Proc Natl Acad Sci U S A 87(10):3933–3937. https://doi.org/10.1073/pnas.87.10.3933
132. Cox RA, Anders GT, Cappelli PJ, Johnson JE, Blanton HM, Seaworth BJ, Treasure RL (1990) Production of tumor necrosis factor-alpha and interleukin-1 by alveolar macrophages from HIV-1-infected persons. AIDS Res Hum Retroviruses 6(4):431–441. https://doi.org/10.1089/aid.1990.6.431
133. Stevenson M (2017) HIV persistence in macrophages. Nat Med 23(5):538–539. https://doi.org/10.1038/nm.4337
134. Bültmann BD, Flad HD, Kaiserling E, Müller-Hermelink HK, Kratzsch G, Galle J, Schachenmayr W, Heimpel H, Wigger HJ, Haferkamp O (1982) Disseminated mycobacterial histiocytosis due to M. Fortuitum associated with helper T-lymphocyte immune deficiency. Virchows Arch A Pathol Anat Histol 395(2):217–225. https://doi.org/10.1007/BF00429614
135. Burmester GR, Gramatzki M, Müller-Hermelink HK, Solbach W, Djawari D, Kalden JR (1984) Dissociation of helper/inducer T-cell functions: immunodeficiency associated with mycobacterial histiocytosis. Clin Immunol Immunopathol 30(2):279–289. https://doi.org/10.1016/0090-1229(84)90062-x
136. Corrin B, Nicholson AG (2011) Chronic bacterial infection. Pathology of the lungs, 3rd edn. Churchill Livingstone, Edinburgh, pp 197–221
137. King YA, Hu CH, Lee YJ, Lin CF, Liu D, Wang KH (2017) Disseminated cutaneous *Mycobacterium kansasii* infection presenting with Rosai-Dorfman disease-like histological features in a patient carrying anti-interferon-γ autoantibodies. J Dermatol 44(12):1396–1400. https://doi.org/10.1111/1346-8138.13973
138. Modlin RL, Hofman FM, Meyer PR, Sharma OP, Taylor CR, Rea TH (1983) In situ demonstration of T lymphocyte subsets in granulomatous inflammation: leprosy, rhinoscleroma and sarcoidosis. Clin Exp Immunol 51(3):430–438
139. Broos CE, van Nimwegen M, Hoogsteden HC, Hendriks RW, Kool M, van den Blink B (2013) Granuloma formation in pulmonary sarcoidosis. Front Immunol 4:437. https://doi.org/10.3389/fimmu.2013.00437
140. Broos CE, Hendriks RW, Kool M (2016) T-cell immunology in sarcoidosis: disruption of a delicate balance between helper and regulatory T-cells. Curr Opin Pulm Med 22(5):476–843. https://doi.org/10.1097/MCP.0000000000000303
141. Patterson KC, Chen ES (2018) The pathogenesis of pulmonary sarcoidosis and implications for treatment. Chest 153(6):1432–1442. https://doi.org/10.1016/j.chest.2017.11.030
142. ATS, ERS, WASOG Committee (1999) Statement on sarcoidosis. Am J Respir Crit Care Med 160(2):736–755. https://doi.org/10.1164/ajrccm.160.2.ats4-99
143. Iannuzzi MC, Rybicki BA, Teirstein AS (2007) Sarcoidosis. N Engl J Med 357(21):2153–2165. https://doi.org/10.1056/NEJMra071714

144. Paramothayan S, Lasserson TJ, Walters EH (2006) Immunosuppressive and cytotoxic therapy for pulmonary sarcoidosis. Cochrane Database Syst Rev (3):CD003536 https://doi.org/10.1002/14651858.CD003536.pub2
145. Murray PI, Bodaghi B, Sharma OP (2011) Systemic treatment of sarcoidosis. Ocul Immunol Inflamm 19(2):145–150. https://doi.org/10.3109/09273948.2010.542870
146. Baughman RP, Drent M, Kavuru M, Judson MA, Costabel U, du Bois R, Albera C, Brutsche M, Davis G, Donohue JF, Müller-Quernheim J, Schlenker-Herceg R, Flavin S, Lo KH, Oemar B, Barnathan ES, Investigators S (2006) Infliximab therapy in patients with chronic sarcoidosis and pulmonary involvement. Am J Respir Crit Care Med 174(7):795–802. https://doi.org/10.1164/rccm.200603-402OC
147. Denys BG, Bogaerts Y, Coenegrachts KL, De Vriese AS (2007) Steroid-resistant sarcoidosis: is antagonism of TNF-alpha the answer? Clin Sci (Lond) 112(5):281–289. https://doi.org/10.1042/CS20060094
148. Jamilloux Y, Cohen-Aubart F, Chapelon-Abric C, Maucort-Boulch D, Marquet A, Pérard L, Bouillet L, Deroux A, Abad S, Bielefeld P, Bouvry D, André M, Noel N, Bienvenu B, Proux A, Vukusic S, Bodaghi B, Sarrot-Reynauld F, Iwaz J, Amoura Z, Broussolle C, Cacoub P, Saadoun D, Valeyre D, Sève P, Groupe Sarcoïdose Francophone (2017) Efficacy and safety of tumor necrosis factor antagonists in refractory sarcoidosis: A multicenter study of 132 patients. Semin Arthritis Rheum 47(2):288–294.https://doi.org/10.1016/j.semarthrit.2017.03.005
149. James WE, Baughman R (2018) Treatment of sarcoidosis: grading the evidence. Expert Rev Clin Pharmaco 11(7):677–687. https://doi.org/10.1080/17512433.2018.1486706
150. James P, Utz JP, Limper AH, Kalra S, Specks U, Scott JP, Vuk-Pavlovic Z, Schroeder DR (2003) Etanercept for the treatment of stage II and III progressive pulmonary sarcoidosis. Chest 124(1):177–185. https://doi.org/10.1378/chest.124.1.177
151. Le V, Crouser ED (2018) Potential immunotherapies for sarcoidosis. Expert Opin Biol Ther 18(4):399–407. https://doi.org/10.1080/14712598.2018.1427727
152. Bachelez H, Senet P, Cadranel J, Kaoukhov A, Dubertre L (2001) The use of tetracyclines for the treatment of sarcoidosis. Arch Dermatol 137(1):69–73. https://doi.org/10.1001/archderm.137.1.69
153. Steen T, English JC (2013) Oral minocycline in treatment of cutaneous sarcoidosis. JAMA Dermatol 149(6):758–760. https://doi.org/10.1001/jamadermatol.2013.2977
154. Baba K, Yamaguchi E, Matsui S, Niwa S, Onoe K, Yagi T, Hattori T, Ozawa H, Hara K (2006) A case of sarcoidosis with multiple endobronchial mass lesions that disappeared with antibiotics. Sarcoidosis Vasc Diffuse Lung Dis 23(1):78–79
155. Takemori N, Nakamura M, Kojima M, Eishi Y (2014) Successful treatment in a case of *Propionibacterium acnes*-associated sarcoidosis with clarithromycin administration: a case report. J Med Case Rep 8:15. https://doi.org/10.1186/1752-1947-8-15
156. Sheu J, Saavedra AP, Mostaghimi A (2014) Rapid response of tattoo-associated cutaneous sarcoidosis to minocycline: case report and review of the literature. Dermatol Online J 20 (8):13030/qt6dd1m2j9
157. Ishibashi K, Eishi Y, Tahara N, Asakura M, Sakamoto N, Nakamura K, Takaya Y, Nakamura T, Yazaki Y, Yamaguchi T, Asakura K, Anzai T, Noguchi T, Yasuda S, Terasaki F, Hamasaki T, Kusano K (2018) Japanese antibacterial drug management for cardiac sarcoidosis (J-ACNES): a multicenter, open-label, randomized, controlled study. J Arrhythm 34(5):520–526. https://doi.org/10.1002/joa3.12084
158. Yamaguchi T, Zaima M, Yamada, Y, Tanaka K, Urushiyama H, Narita Y, Kono C (2008) Tetracycline treatment of sarcoidosis. JJSOG 28:41–47. (In Japanese with English abstract)
159. Yamaguchi T, Yamaguchi Y, Suzuki M, Kono C, Yamada Y (2014) Treatment of sarcoidosis by the use of doxycycline. JJSOG 34:31–33 (In Japanese)
160. Drake WP, Richmond BW, Oswald-Richter K, Yu C, Isom JM, Worrell JA, Shipley GR (2013) Effects of broad-spectrum antimycobacterial therapy on chronic pulmonary sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 30(3):201–211

161. Drake WP, Oswald-Richter K, Richmond BW, Isom J, Burke VE, Algood H, Braun N, Taylor T, Pandit KV, Aboud C, Yu C, Kaminski N, Boyd AS, King LE (2013) Oral antimycobacterial therapy in chronic cutaneous sarcoidosis: a randomized, single-masked, placebo-controlled study. JAMA Dermatol 149(9):1040–1049. https://doi.org/10.1001/jamadermatol.2013.4646

Kawabata Yoshinori bone in Japan in 1945, graduated from Kanazawa University Faculty of Medicine, worked as a physician for the first five years, later as a chest physician (five years) and a pulmonary pathologist at the Research Institute of Tuberculosis (1974–1996), ended up as a pulmonary pathologist at the Saitama Cardiovascular and Respiratory Center (1996–2010), followed by as a part-time physician in this Center up to know (2010–2022). His main areas of clinicopathological study are tuberculosis, sarcoidosis, various kinds of diffuse lung diseases, including asbestosis. It was a nice memory that studied pulmonary pathology under the late Dr. CB Carrington at Stanford University in 1982-1983 (one year).

Tuberculosis: A Historical and Global Bioethical Perspective

49

Kirubel Manyazewal Mussie

MEDICINE means Mercy - Empathy - Dare - Integrity - Care - Ingenuity - Nobility - and Ethics.

Abhijit Naskar

Summary

Tuberculosis (TB) is an ancient disease that has been a global health problem for centuries. Why and how such a curable disease has been allowed to exist for many centuries and to damage billions of lives and to overwhelm economies and health systems in many countries is a question worth asking from an ethical point of view–especially from a global bioethical framework which engages itself with, among other things, the moral understanding and description of complex elements within global health catastrophes such as TB. To contribute towards such a broader inquiry, this chapter briefly historicizes TB and discusses the factors, treatment, and control of TB from a bioethical perspective. TB has always been intertwined with wider socioeconomic, cultural, ethical, and political facets of human living. In addition to patient-level ethical questions such as liberty and human rights, a moral inquiry of TB extends to including a critical analysis of policy and program-level interventions. Such a global and complex pandemic deserves much attention from bioethics. The overwhelmingly strong and hence agonizing bond between medicine and the market should be challenged by any means. The greed of big, profit-driven drug research

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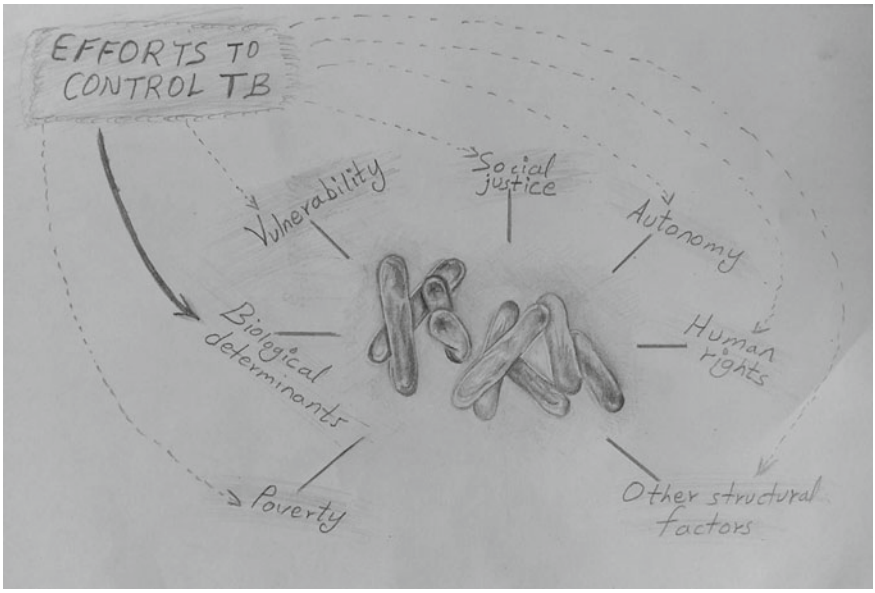
institutions and pharmaceutical companies remains a challenge to global bioethics and a reminder of one of humanity's failures to make the universe a fair place for its inhabitants, especially for the marginalized and vulnerable. To lessen such seemingly inevitable problems, a global sense of ethics and morality should be mainstreamed in as many corners of the world as possible.

Graphical Abstract



Efforts to control tuberculosis (TB)

Keywords

Tuberculosis · History of TB · DOT · Ethical issues · Global bioethics · Poverty

1 Introduction

TB, an airborne disease caused by *Mycobacterium tuberculosis (M. tb)*, is pointed out as a threat to global health security [1], causing nearly ten million illnesses and 1.6 million deaths annually [2]. Global interest to address TB has remained high ever since the World Health Organization (WHO) declared a global emergency of TB in 1993 [3]. Through and with other organizations such as the International

Union Against Tuberculosis and Lung Diseases (IUATLD) or the Global Fund, the WHO has been working to address TB as a public health problem. This has initiated global cooperation to fight the disease. WHO reports a 47% decline in TB mortality rate since 1990 [4]. Nevertheless, many have been suffering from a curable disease.

The WHO adopted the ambitious "End TB Strategy," which envisions a world free of TB by 2035 [5]. However, previous global TB strategies tracked a lesson that implementations were insufficient for an effective outcome. Following the 1993's TB as a global health emergency, the WHO invented a TB management strategy called directly observed therapy short-course (DOTS). This strategy adheres to:

> political commitment; microscopy services; drug supplies; surveillance and monitoring systems and use of highly efficacious regimens; and direct observation of treatment [6].

Even though the practicability and effectiveness of the whole DOTS strategy have always been controversial [7, 8], the last component in particular, directly observed therapy, has always been a topic of discussion in connection with ethics, morality, and human rights [9, 10]. TB, in general, is one of the most important but, at the same time, neglected global health topics in bioethics [11]. This chapter contributes to addressing this gap. It begins by briefly presenting a historical perspective on TB, emphasizing those historical events that can lay the ground to better understand the ethical aspects. Then, it discusses the various ethical problems in connection with the risk factors, treatment, and control of TB.

2 A Historical Perspective

TB has historically been one of the worst health catastrophes the human species has suffered from. Archaeological evidence shows that illness from TB among human beings dates back at least 5000 B.C. The number of deaths it resulted in the past two centuries is only around one billion [11]. Such a long historical account of TB provides a broader perspective compatible with its complex nature. This is especially important if one wants to draw a global bioethical perspective, which global pandemics like TB necessitate.

In her book *Spitting Blood: The History of Tuberculosis*, Helen Bynum argues that the root of today's TB is some 300 million years [12]. She discusses the two major categories in the group of *Mycobacterium tuberculosis* complex (MBTC), which namely are *M. tb* and *M. bovis*. Human beings host the earlier while the latter dwells among animals. Even though *M. tb* is older than *M. bovis*, horizontal transmission of TB occurred when ancient communities consumed *M. bovis* from animals through milk, meat, and other animal products [12].

Therefore, the long-lasting interaction between animals and human beings is a crucial event in the history of TB. According to [13], archaeological studies show that TB was also present amongst Egyptians 5500 years ago. It had lived with the ancient Israelites when they were under the Egyptians 3700–3300 years ago. A review by Daniel [14] on the history of TB shows that east Africa was.

the ancestral home of both tubercle bacilli and its human hosts.

Beginning with the Hippocratic School, which considered TB as a hereditary instead of infectious disease, the process of formulating "pulmonary phthisis" as a clinical issue passed through the period of Aristotle, the Indian literature in 1500 BC, and ancient Rome medicine in the fifth century AD [12]. TB has lived in our society for a long time, hence connected to different aspects of human life. This old but 'ever-fresh' disease is deeply rooted in the history of human living.

The term "tuberculosis" came into existence not before the ancient world exerted efforts to describe the disease using various words and concepts. The biblical perspective, for instance, used the word "consumption" in explaining TB amongst the Israelites living in the land of Ancient Egypt [13]. The reason why TB is not clearly stated in both the Old and New Testament books is, according to what Herzog [15] tells us in a thematic review series of the history of TB, because the bible deals mainly with rural and sparsely-settled populations, whereas TB is a disease of big and crowded cities. However, there existed different names and ways to describe the disease. Hippocrates (460–370 BC), for example, discusses the archaic term "phthisis"–the Greek word for "consumption" [15]. "Phthisis" was seen as a common but deadly disease. The following quote from Herzog's work illuminates this:

> Hippocrates also wrote something that no physician would dare to write today: he warned his colleagues against visiting consumptives in an advanced stage of the disease, since the patient would in any case die, thereby damaging the physician's reputation [15].

Although TB was a tragic disease that caused many deaths, society romanticized it, and people attached aesthetic images to it. The TB patient with a pale face was seen as an attractive person–described as a fortunate "consumptive" with bright eyes, clean skin tone, and a delicate body [14, 16]. It was a matter of interest for some people to be bestowed with the "beauty" of TB and die of it. Symptoms of the disease were attached to beauty and attractiveness.

Major achievements in developing TB diagnosis started to emerge in the nineteenth century. Robert Koch (1843–1910) laid the ground for the bacteriology of *M. tb* [17]. He unveiled the mysterious phantom; he found out the bacteria that caused the disease with different names: consumption, phthisis scrofula, and the white plague, to mention some. In 1882, Koch concluded that there was one cause for this disease, and he named this cause "tuberculosis bacillus" [14, 17]. His work not only added a new word–"tuberculosis bacillus"–into the medical vocabulary but also marked a transition from "consumption" to "tuberculosis." In 1895, 13 years after Koch introduced his TB bacillus, another scientific news came from the world of physics. The German mechanical engineer Wilhelm Conrad von Röntgen (1845–1923) discovered X-rays which later made radiography of the chest and other body parts of TB patients possible [15]. Later in 1921 followed the development of TB vaccines (Bacillus Calmette-Guérin, or BCG) by the French bacteriologists Albert Calmette and Camille Guérin [14].

One of the major developments in the treatment of TB was sanatorium care. Such institutional care was designed to isolate TB patients by keeping them in a place to receive accommodation and medical treatment. Some sanatoria were built in higher landscapes [15], and some were luxurious, looking like "hospital-hotels" but depressing for the patients [12]. Providing a good diet and care in the sanatoria helped the patients recover quickly. Despite these benefits, however, such institutional care had some drawbacks. Many patients discharged from the sanatoria died of the disease in a few years, and the long-term results of the sanatoria were depressing [15]. In addition, there was carelessness towards patients' feelings. A doctor's word was much more important than that of the patient's, which resulted in considering those patients who do not comply with doctors' commands as having deviant behavior [12]. Some sanatoria were built in "immune places" in Asia–places where consumption does not exist–and this is considered one of the reasons for a high TB burden in the region [15].

In the era of the WHO, especially around the end of the twentieth century, interest to address TB globally increased. Through and with other organizations, the WHO started to address TB as a public health problem, which initiated global cooperation in fighting the disease. One event after the other emerged; for example, the invention of DOTS in 1995, the Stop TB Initiative in 2000, and the End TB Strategy in 2014 [18]. This resulted in the development of projects, procedures, declarations, and work coalitions, all aiming at systematically addressing a disease that challenged health systems in the world, particularly the fragile ones in resource-scarce countries.

Attention towards drug resistance (DR) in TB treatment grew stronger in the 1940s [19]. Studies concerning anti-TB drug resistance and its factors started to emerge. In not more than two decades after the introduction of the first effective anti-TB drug in 1943,

> the advent of every new drug led to the selection of mutations conferring resistance to it [19].

The WHO launched the DOTS (Directly Observed Treatment, short-course) in 1995 as a solution to stop TB. This new tool was supposed to help address the problem of DR by increasing patient compliance to TB treatment. Thus, DOT 'became the new mantra' which countries were to incorporate into their TB programs in particular and health policies in general [12]. Today, nearly 25% of the world's population is infected with latent TB [20], and TB is reported as the world's ninth biggest killer [21]. Either because of treatment interruption or by acquiring DR strains directly from a DR-TB patient, the bacteria become resistant to first-line drugs and thus require time-consuming and costly treatment procedures [20, 22–24].

As can be drawn from a historical perspective on TB, the wider socioeconomic and cultural factors that ignite today's TB in the developing world are similar to those that were prevalent many decades ago. Human history depicts factors facilitating the spread of TB: for example, living in a confined and crowded space, burial practices, and human migration and settlement [12, 15]. TB has never been confined to conventional medicine; it has never been a matter of clinical concern

only. It has always been involving the world in its entirety, connecting with many, if not all, aspects of life and living. Why and how such a curable disease has been allowed to exist for many centuries and damage billions of lives and households, and to overwhelm economies and health systems in many countries is a question worth asking from an ethical point of view–especially from a global bioethical framework which engages itself with, among other things, the moral understanding and description of complex elements in global health catastrophes such as TB.

3 TB Through the Lens of Global Bioethics

Bioethics as a concept has existed for several thousands of years. Ten Have claims that informal bioethics emerged with the human race [25]. Elaborating this, Leonard Chuwa writes:

> the notion of bioethics has existed for as long as the art of medicine has existed. …Among indigenous peoples all over the world, bioethics has always been part and parcel of their daily lives [26].

However, only after the 1970s, bioethics appeared as a discipline when V. R. Potter introduced the term in his work *Bioethics: Bridges to the Future,* published in 1971 [27]. The term "bioethics," however, was coined in 1926 by Fritz Jahr, after the two Greek words *bios* (meaning *life*) and *ethos* (meaning *behavior*) [28]. Bioethics can simply be understood as a study of the ethical implications of health-related life sciences. The definition and scope of bioethics have been improving since its inception through the influences of ethical and philosophical perspectives and disciplines. Today, bioethics is mostly perceived as a field of study dealing with ethical and legal issues relating to healthcare provision and biomedical research [27], particularly among vulnerable populations groups such as, for example, children [29], older patients [30, 31], and refugees [32, 33].

A few years after the formal genesis of bioethics, global concerns about ethical issues started to emerge. This led to the birth of global bioethics. Global bioethics cannot, and should not, be understood as the globalization and international application of a sole normative framework and set of moral principles. One of the most important aims of global bioethics is rather, as Leonard Chuwa asserts, the cultivation and recognition of indigenous cultures and social values [26]. As a traditional bioethical perspective started to stumble in the face of growing cultural pluralism and complexities in the life sciences, the need for a broader and synthetic global ethical approach grew stronger. In addition to global phenomena such as colonization, the two world wars, globalization, and international trades, global pandemics such as TB laid the basis for the genesis of global bioethics [26].

TB is often spoken of from the pulpit of biomedicine as an airborne disease caused by bacteria that transmits from one person to another when people do not heed preventive measures such as isolation of TB patients and proper sanitization of hands and equipment. In the case of DR-TB, much emphasis to seek explanation is

given to patient adherence to medication. However, on the other hand, several scholars assert that TB is a disease of poverty [34–38]. Poor living conditions take the greatest share from the factors of TB. Good evidence for this fact is the TB trend among the once less developed and, as a result, TB challenged communities and countries. For example, due to crowded and poor living conditions, people in Liverpool suffered greatly from TB towards the end of the twentieth century [39]. Similarly, Lawn and Zumla write:

> Tuberculosis continued to cause many deaths in London during the nineteenth century and accounted for up to 25% of deaths in Europe. The death toll from TB began to fall as living standards (housing, nutrition, and income) improved early in the twentieth century, well before the advent of antituberculosis drugs [40].

Thus, the inevitable vulnerability of poor populations to TB is an ethical concern, especially if seen from a global bioethics viewpoint that advocates for fair life and the wellbeing of the inhabitants of the universe. In the words of Leonard Chuwa,

> the vicious circle of poverty and disease, which dehumanize the poor, is doubly unethical [26].

Another factor exacerbating human vulnerability to TB is the disease being one of the most underfunded but deadliest global pandemics. In its *Ethics guidance for the implementation of the End TB Strategy* developed in 2017, the WHO asserts that eliminating TB from the world needs US$ 2 billion every year globally [41]. However, TB research remains underfunded (always below US$ 700 million per year), and the slow progress in TB research raises ethical and human rights concerns which the international community should act on [41].

Infectious diseases such as TB do not get the needed attention from pharmaceuticals, thereby leaving the poor–which constitute the majority of the world's population–for more ostracism and marginalization [26]. Such a lack of political commitment increases and complicates the risk factors of TB. In the same document, the WHO adds that the role of the international community and aid organizations should go beyond providing TB drugs and should also include supporting local governments with strengthening TB programs and health systems [41].

One of the principles of the End TB Strategy is.

> protection and promotion of human rights, ethics, and equity [5].

Thus, treatment and control of TB have ethical implications. Treatment of TB does not involve clinical interventions only. It also involves public health interventions such as isolating the TB patient and enforcing direct health worker observation of treatment adherence. Isolating TB patients is known for positively impacting the fight against TB [42]. Thus, as Michael Selgelid asserts,

> we should not overestimate the importance of biomedical or 'narrow public health' interventions such as isolation, but we should not underestimate their importance either [11].

Regardless, such TB treatment and control mechanisms have always been ethically questionable, especially in terms of social justice and patient autonomy. Forcing the TB patient to isolate, especially in the case of DR-TB, can easily be

seen as an act of violating human rights. Additionally, if we know that TB is a disease of poverty and that the poor constitute the majority of the world's population, TB control measures such as quarantine and isolation are simply acting of protecting and favoring the privileged few at the expense of the many poor.

Another important TB treatment measure is directly observed therapy (DOT), which can simply be defined as an act of observing a TB patient while s/he swallows TB drugs [43]. When patients are diagnosed with TB, they start a treatment course (DOTS), which involves treating patients with first-line TB drugs for six to nine months. TB patients in this treatment course are expected to commute to TB clinics every day for at least two months and swallow TB drugs in the presence of a healthcare provider. If the given health system has enough resources, then DOT will take place at the TB patient's living place, or the TB patient will be hospitalized during the entire treatment course. This incurs high costs and creates ethical dilemmas within low-income countries. For example, in Ethiopia, a country identified by the WHO as one the high TB burden countries globally [20], this challenge is reflected upon in the country's national TB care and control training manual. Stating that TB patients in their initial treatment phase must take TB drugs with the presence of a healthcare provider, the document further notes that there would be a need to hospitalize some patients who cannot visit TB clinics on a daily basis but the cost of this is beyond the country's capacity [44].

The main purpose of DOT is to ensure that patients adhere to TB treatment, thereby preventing the development of DR and other complications that could result from non-adherence. Arguments supporting DOT are built based on the idea that ensuring public health is critical when finding solutions to diseases. From this point of view, nudging and even coercion are justifiable if doing so can protect society from health threats [45]. Nonetheless, such measures appear ethically questionable from a global bioethical perspective. For TB, it is not patient adherence that is most important. It is rather distributive justice [11]. Before requiring patients their full adherence, there are unmet challenges and needs, whether due to negligence or resource scarcity. For example, diagnostic tools have always been scarce [46], and the health side effects of anti-DR-TB drugs have always been tremendous [47]. In addition, the long-lasting treatment courses (two years in the case of DR-TB, for example) burden patients with psychosocial and economic challenges [48]. Given the lack of funding and cooperation from the international community to address TB, it is plausible to claim that the possibilities for a less burdensome treatment course and less toxic drugs have not been widely explored. Research on TB drugs is slow–only two new TB drugs have been approved in the last 40 years–and this slow development has caused TB patients to experience lengthy treatment courses, side effects from toxic drugs, and other problems that complicate patient adherence [41].

Thus, from a social justice perspective, the current TB treatment strategies appear questionable as they stand. When it comes to treating TB patients, especially implementing the DOTS strategy, strategies should be context-sensitive. This can partly be done by healthcare workers who interact with TB patients. Improvising such strategies can help address ethical questions. Not only when there is resource limitation, as Livingston [49] and Prince, Otieno [50] write, do health workers

improvise; but also, if they intend to provide quality care [51]. Mol et al. [52] use the term tinkering and believe in "good care" as a

> persistent tinkering in a world full of complex ambivalence and shifting tensions.

Literature shows that health workers compromise the strict implementation of protocols and guidelines for different reasons [53]. In relation to this, Molterer et al. [54] mention that tinkering helps create a balance between obeying rules and obeying context, thereby providing flexibility within guidelines. This implies the need to wipe the lens through which we define care ethics and to examine the idolatry towards TB treatment strategies.

We find a similar direction from Beauchamp and Childress's *Principles of Biomedical Ethics*. The authors assert that rules need continuous arrangements and contextualization–a process called 'progressive specification'–to handle ethical dilemmas [55]. Such a process may require justification. In the case of TB patients, there are more than enough reasons to justify this. TB treatment strategies burden TB patients who are mostly in poverty. More than 95% of TB cases and deaths occur in low and middle-income countries [11]. Going to TB clinics every day requires, for example, a good transportation facility and stable employment that permits employees in such cases. Additionally, the medications require that patients follow a nutritious diet–a lifestyle that hardly co-exists with poverty. Thus, it is worth noting that the very clinical practice context that TB strategies serve can (perhaps should) shape their rules and principles. After all,

> principles, rules, obligations, and rights are not wooden standards that disallow compromise [55].

Regarding patient autonomy, the context we refer to when we raise ethical questions is important. TB is mostly a problem of the South and particularly sub-Saharan Africa [56]. Thus, the ethical question that we raise in connection with TB within, for example, an African context, should depend on Ubuntu ethics, referred to as".

> a set of values central among which are reciprocity, common good, peaceful relations, emphasis on human dignity, and the value of human life as well as consensus, tolerance, and mutual respect [57].

Thus, the claim that a TB patient is autonomous and can decide for her/himself can be compromised even when the patient is capable of decision-making. As Chikezie Onuoha argues,

> many of the assumptions implicit in the Western framework that make claim to universal validity may not be shared by non-western cultures:

for example,

> within most western societies, the principle of autonomy sometimes implies that every person has an atomistic right to self-determination' whereas this is different in most African culture where 'the person is viewed as a relational self' and therefore, 'inter-dependence rather than individualism provides the basis for moral decisions [58].

This is not to mean that TB treatment measures should be practiced in a way that suppresses individual freedom and disrespects persons. It is rather to mean that TB programs should become comprehensive–that they should place the TB patient in his/her environment and collaborate more with other bodies to raise the needed resources. TB strategies are resource-demanding for both TB control programmers and patients. Once communal welfare is achieved, and the health workforce in resource-scarce settings is strengthened [59–61], TB patient autonomy will be much easier to deal with within a non-Western context.

4 Conclusion

TB is one of the contemporary global bioethical problems. In today's increasingly interconnecting and globalizing world, analyzing such an ancient and global problem using local, exclusive norms is insufficient. Instead, broader transnational perspectives and solutions are needed. Speaking from a strict global bioethical perspective, the international community ought to create and maintain a global platform for discussing, budgeting, and acting to fight TB. Biomedical interventions appear insufficient to address such a complex and global problem as TB. Interventions should extend to socioeconomic and political facets of life. The international community and pharmaceutical companies have the ethical obligation to fight TB even though TB is not a problem of the influential and privileged few. The overwhelmingly strong and hence agonizing bond between medicine and the market should be challenged by any means. The greed of pharmaceutical companies remains a challenge to global bioethics and a reminder of one of humanity's failures to make the universe a fair place for its inhabitants. To abate such seemingly inevitable problems, a global sense of ethics and morality should be mainstreamed in as many corners of the world as possible.

To the ignorant human, the vulnerability of the poor is a sign of an individual or a specific health system's failure. To the humane human, it is rather a sign of humanity's failure to maintain wellbeing within the global family–universe.

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Core Messages

- TB is a disease that is thousands of years old but continues to kill millions of lives every year.
- TB demands more than biomedical interventions.
- TB is a poorly discussed topic in bioethics, even though it is one of the deadliest global pandemics.
- The solution for diseases of poverty such as TB is also found within the true sense of global bioethics.

References

1. Sakamoto H, Lee S, Ishizuka A, Hinoshita E, Hori H, Ishibashi N, Komada K, Norizuki M, Katsuma Y, Akashi H, Shibuya K (2019) Challenges and opportunities for eliminating tuberculosis—leveraging political momentum of the UN high-level meeting on tuberculosis. BMC Public Health 19(1). https://doi.org/10.1186/s12889-019-6399-8
2. WHO (2019) Ten threats to global health in 2019. WHO. https://www.who.int/vietnam/news/feature-stories/detail/ten-threats-to-global-health-in-2019
3. TB: A Global Emergency (1994) World Health Organization, Geneva
4. WHO (2015) Global tuberculosis report 2015. World Health Organisation, Geneva, Switzerland
5. World Health Organization (2015) The End TB strategy: global strategy and targets for tuberculosis prevention, care and control after 2015. Switzerland, Geneva
6. Davies P (2003) The role of DOTS in tuberculosis treatment and control. Am J Respir Med 2 (3):203–209. https://doi.org/10.1007/BF03256649
7. Van Rie A, Warren R, Mshanga I, Jordaan AM, van der Spuy GD, Richardson M, Simpson J, Gie RP, Enarson DA, Beyers N, van Helden PD, Victor TC (2001) Analysis for a limited number of gene codons can predict drug resistance of *Mycobacterium tuberculosis* in a high-incidence community. J Clin Microbiol 39(2):636
8. Laing RO, McGoldrick KM, Laing RO (2000) Tuberculosis drug issues: prices, fixed-dose combination products and second-line drugs. Int J Tuberc lung Dis Official J Int Union Against Tuberc Lung Dis 4(12 Suppl 2):S194–S207
9. Mussie KM, Gradmann C, Manyazewal T (2020) Bridging the gap between policy and practice: a qualitative analysis of providers' field experiences tinkering with directly observed therapy in patients with drug-resistant tuberculosis in Addis Ababa. Ethiopia. BMJ open 10 (6):e035272. https://doi.org/10.1136/bmjopen-2019-035272
10. Mussie KM, Elger BS, Kaba M, Pageau F, Wienand I (2022) Bioethical implications of vulnerability and politics for healthcare in Ethiopia and the ways forward. J Bioeth Inq. https://doi.org/10.1007/s11673-022-10210-x
11. Selgelid MJ (2008) Ethics, Tuberculosis and Globalization. Public Health Ethics 1(1):10–20. https://doi.org/10.1093/phe/phn001
12. Bynum H (2012) Spitting blood: The history of tuberculosis. Oxford University Press, Oxford
13. Daniel VS, Daniel TM (1999) Old Testament biblical references to tuberculosis. Clin Infect Dis 29(6):1557–1558. https://doi.org/10.1086/313562
14. Daniel TM (2006) The history of tuberculosis. Respir Med 100(11):1862–1870. https://doi.org/10.1016/j.rmed.2006.08.006
15. Herzog BH (1998) History of tuberculosis. Respiration 65(1):5–15. https://doi.org/10.1159/000029220
16. Sagbakken M (2010) Tuberculosis as a global challenge : a qualitative study of patients' and health workers' perception and management of tuberculosis in Ethiopia and Norway.
17. Gradmann C (2009) Laboratory disease : Robert Koch's medical bacteriology. Krankheit im Labor Robert Koch und die medizinische Bakteriologie. Johns Hopkins, Baltimore
18. Zumla BMA (2013) History of tuberculosis and drug resistance. N Engl J Med 368(1):88–90. https://doi.org/10.1056/NEJMc1212308
19. Keshavjee S, Farmer PE (2012) Tuberculosis, drug resistance, and the history of modern medicine. N Engl J Med 367(10):931–936. https://doi.org/10.1056/NEJMra1205429
20. WHO (2019) Global tuberculosis report 2019. World Health Organisation, Geneva, Switzerland
21. WHO (2017) Global tuberculosis report 2017
22. CDC (2017) Tuberculosis: Drug-Resistant TB. Centers for Disease Control and Prevention https://www.cdc.gov/tb/topic/drtb/default.htm.
23. IUATLD (2010) Management of tuberculosis. In: Disease IUATaL (ed) A guide to the essentials of good practice

24. Noe A, Ribeiro RM, Anselmo R, Maixenchs M, Sitole L, Munguambe K, Blanco S, Souef PL, García-Basteiro AL (2017) Knowledge, attitudes and practices regarding tuberculosis care among health workers in Southern Mozambique. BMC Pulm Med 2017 (2012). https://doi.org/10.1186/s12890-016-0344-8
25. Ht H (2013) GLOBAL BIOETHICS: TRANSNATIONAL EXPERIENCES AND ISLAMIC BIOETHICS. Zygon 48:600–617
26. Chuwa LT (2014) African indigenous ethics in global bioethics : interpreting Ubuntu. Advancing global bioethics. Springer, Berlin
27. Sinaci M (2016) The possibility of global bioethics in a globalized World. In. https://doi.org/10.22618/TP.PCMS.20164.349028
28. Sass H-M (2007) Fritz Jahr's 1927 concept of bioethics. Kennedy Inst Ethics J 17(4):279–295. https://doi.org/10.1353/ken.2008.0006
29. Bartholdson C, Lützén K, Blomgren K, Pergert P (2015) Experiences of ethical issues when caring for children with cancer. Cancer Nurs. https://doi.org/10.1097/NCC.0000000000000130
30. Mussie KM, Setchell J, Elger BS, Kaba M, Memirie ST, Wangmo T (2022) Care of older persons in eastern Africa: a scoping review of ethical issues. Front Public Health. https://doi.org/10.3389/fpubh.2022.923097
31. Mussie KM, Pageau F, Merkt H, Wangmo T, Elger BS (2021) Challenges in providing ethically competent health care to incarcerated older adults with mental illness: a qualitative study exploring mental health professionals' perspectives in Canada. BMC Geriatr. https://doi.org/10.1186/s12877-021-02687-9
32. Ozgumus AM, Ekmekci PE (2019) Refugee health: a moral discussion. J Immigr Minor Health. https://doi.org/10.1007/s10903-018-0762-1
33. Pérez-Molina JA, Álvarez-Martínez MJ, Molina I (2016) Medical care for refugees: a question of ethics and public health. Enferm Infecc Microbiol Clin. https://doi.org/10.1016/j.eimc.2015.12.007. Epub 2016 Jan 19
34. Carter DJ, Glaziou P, Lönnroth K, Siroka A, Floyd K, Weil D, Raviglione M, Houben RMGJ, Boccia D (2018) The impact of social protection and poverty elimination on global tuberculosis incidence: a statistical modelling analysis of sustainable development goal 1. Lancet Glob Health 6(5):e514–e522. https://doi.org/10.1016/S2214-109X(18)30195-5
35. Dara M, Zachariah R (2018) Hunger and tuberculosis: two sides of the same coin. Int J Tuberc Lung Dis 22(6):592. https://doi.org/10.5588/ijtld.18.0279
36. Shete PB, Reid M, Goosby E (2018) Message to world leaders: we cannot end tuberculosis without addressing the social and economic burden of the disease. Lancet Glob Health 6(12): e1272–e1273. https://doi.org/10.1016/S2214-109X(18)30378-4
37. Mussie KM, Yimer SA, Manyazewal T, Gradmann C (2019) Exploring local realities: perceptions and experiences of healthcare workers on the management and control of drug-resistant tuberculosis in Addis Ababa. Ethiopia. PLoS ONE 14(11):e0224277. https://doi.org/10.1371/journal.pone.0224277
38. Mussie KM, Gradmann C, Yimer SA, Manyazewal T (2021) Pragmatic management of drug-resistant tuberculosis: a qualitative analysis of human resource constraints in a resource-limited country context-Ethiopia. Int J Public Health. https://doi.org/10.3389/ijph.2021.633917
39. Spence DPS, Hotchkiss J, Williams CSD, Davies PDO (1993) Tuberculosis and poverty. BMJ 307(6907):759
40. Lawn SD, Zumla AI (2011) Tuberculosis. The Lancet 378(9785):57–72. https://doi.org/10.1016/S0140-6736(10)62173-3
41. World Health O (2017) Ethics guidance for the implementation of the End TB strategy. vol WHO/HTM/TB/2017.07. World Health Organization, Geneva
42. Fairchild AL, Oppenheimer GM (1998) Public health nihilism vs pragmatism: history, politics, and the control of tuberculosis. Am J Public Health 88(7):1105–1117. https://doi.org/10.2105/AJPH.88.7.1105

43. Organization WH (1999) What is DOTS? A guide to understanding the WHO-recommended TB Control Strategy Known as DOTS. WHO, Geneva, Switzerland
44. FMoH (2016) National comprehensive tuberculosis, leprosy and TB/HIV training manual for health care workers. Ethiopian Federal Ministry of Health (FMoH), Addis Ababa, Ethiopia
45. Singh JA, Upshur R, Padayatchi N (2007) XDR-TB in South Africa: no time for denial or complacency. PLoS Med 4(1):e50. https://doi.org/10.1371/journal.pmed.0040050
46. Gupta R, Espinal M (2003) A prioritised research agenda for DOTS-Plus for multidrug-resistant tuberculosis (MDR-TB). Int J Tuberc Lung Dis Official J Int Union Against Tuberc Lung Dis 7(5):410
47. Nathanson E, Gupta R, Huamani P, Leimane V, Pasechnikov AD, Tupasi T, Vink K, Jaramillo E, Espinal MA (2004) Adverse events in the treatment of multidrug-resistant tuberculosis: results from the DOTS-Plus initiative. Int J Tuberc Lung Dis 8(11):1382–1384
48. Sterling TR, Lehmann HP, Frieden TR (2003) Impact of DOTS compared with DOTS-plus on multidrug resistant tuberculosis and tuberculosis deaths: decision analysis. Brit Med J Publishing Group. https://doi.org/10.1136/bmj.326.7389.574
49. Livingston J (2012) Improvising medicine : an African oncology ward in an emerging cancer epidemic. Duke University Press, Durham, N.C.
50. Prince RJ, Otieno P (2014) In the Shadowlands of Global Health: observations from health workers in Kenya
51. McKenna K, Leykum LK, McDaniel RR (2013) The role of improvising in patient care. Health Care Manage Rev 38(1):1. https://doi.org/10.1097/HMR.0b013e31823ea9c7
52. Mol A, Moser I, Pols J (2010) Care in practice : on tinkering in clinics, homes and farms, vol, vol 8. Transcript Verlag, Bielefeld, Perspectives from empirical science studies
53. Horn R (2014) "I don't need my patients' opinion to withdraw treatment": patient preferences at the end-of-life and physician attitudes towards advance directives in England and France. A European Journal 17(3):425–435. https://doi.org/10.1007/s11019-014-9558-9
54. Molterer K, Hoyer P, Steyaert C (2019) A Practical Ethics of Care: tinkering with different 'goods' in residential nursing homes. J Bus Ethics. https://doi.org/10.1007/s10551-018-04099-z
55. Beauchamp TL, Childress JF (2009) Principles of biomedical ethics, 6th edn. Oxford University Press, Oxford
56. Aliyu G, El-Kamary SS, Abimiku Al, Blattner W, Charurat M (2018) Demography and the dual epidemics of tuberculosis and HIV: Analysis of cross-sectional data from Sub-Saharan Africa.(Research Article)(Report). PLoS ONE 13 (9):e0191387. https://doi.org/10.1371/journal.pone.0191387
57. Ujomudike PO (2016) Ubuntu Ethics. In: ten Have H (ed) Encyclopedia of Global Bioethics. Springer International Publishing, Cham, pp 2869–2881. https://doi.org/10.1007/978-3-319-09483-0_428
58. Onuoha C (2007) Bioethics across borders : an African perspective. Uppsala UniversitetUppsala
59. Manyazewal T, Woldeamanuel Y, Oppenheim C, Hailu A, Giday M, Medhin G, Belete A, Yimer G, Collins A, Makonnen E, Fekadu A (2022) Conceptualising centres of excellence: a scoping review of global evidence. BMJ Open. https://doi.org/10.1136/bmjopen-2021-050419
60. Makuku R, Mosadeghrad AM (2022) Health workforce retention in low-income settings: an application of the root stem model. J Public Health Pol 43, 445–455. https://doi.org/10.1057/s41271-022-00361-x
61. Manyazewal T, Oosthuizen MJ, Matlakala MC (2016) Proposing evidence-based strategies to strengthen implementation of healthcare reform in resource-limited settings: a summative analysis. BMJ Open. https://doi.org/10.1136/bmjopen-2016-012582

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The Problem of Tuberculosis: Myths, Stigma, and Mimics

50

Alisha Kamboj, Michael Lause, and Kamal Kamboj

The biggest disease today is not leprosy or tuberculosis, but rather the feeling of being unwanted.

Mother Teresa

Summary

Tuberculosis (TB) is a major global public health problem. TB is caused by *Mycobacterium tuberculosis (M. tb)*. Although the most common organ infected are the lungs, it can infect all body tissues and organs. As per World Health Organization (WHO) data from 2018, *M. tb* infected ten million people and was the cause of mortality for about 1.5 million people worldwide. It is often termed the "disease of the poor," and low-income nations bear a significant disease burden. The disease burden is greatly compounded by rampant misconceptions and significant stigma associated with this entity. These factors, in addition to TB's notorious role as the "second great imitator"–for frequently mimicking or masquerading a variety of infectious and non-infectious medical conditions–may contribute to delays in care-seeking, diagnosis, management, and adherence to the therapeutic regimen. Herein, we dispel commonly held myths, discuss the

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stigma linked with the disease and its psychosocial consequences, and delve into the many mimics that complicate the evaluation of TB. Ultimately, our goal is that this discussion may enable individuals, communities, and systems to impart improved care for patients with TB worldwide.

Graphical Abstract

Common myths about tuberculosis (TB)

Keywords

Autoimmune disorders · Infectious diseases · Malignancies · Mimics · Myths · Non-tuberculous mycobacteria · Stigma · Tuberculosis

1 Introduction

Tuberculosis (TB) is an infectious illness that is a significant public health issue as well as a leading cause of morbidity and death globally. According to the World Health Organization (WHO), in 2018, TB infected nearly ten million people and caused death in about 1.5 million cases. Moreover, nearly 1.7 billion people–amounting to one-quarter of the global population either have TB infection or are at risk of acquiring active infection. Twenty-two countries account for 80% of all TB cases, and 17 of these are resource-poor nations [1, 2]. These nations face real challenges in the control and containment of the disease. The link between disease and poverty is well-established, and TB is often called the "disease of the poor." Poor living conditions and overcrowding promote the transmission of the disease [3]. TB is an opportunistic infection, and the risk of acquiring the disease is about 20–30 times higher in people with human immune deficiency (HIV) infection than those without HIV. The two potentiate each other leading to increased mortality. Additional risk factors include malnutrition, diabetes, and tobacco use.

The causative organism of TB is *Mycobacterium tuberculosis (M. tb)*. The most common organ infected is the lungs, leading to a pulmonary form of the disease (PTB). However, besides the lungs, the disease can manifest in almost any body organ and cause extrapulmonary TB (EPTB).

Lack of education and awareness about the cause of infection has led to many misconceptions and myths about TB. Further, the stigma associated with the disease often hinders individuals from seeking early diagnosis and proper treatment, which affects their quality of life and aids in the unchecked spread of infection. The existence of TB with other medical comorbidities like diabetes or HIV infection adds to human suffering and mortality [4–7]. Both HIV and diabetes impair the immune response and propagate latent TB infections.

Clinically, TB may mimic other infections and disease conditions, posing a real challenge for the clinicians to impart proper disease treatment and management. While it is now mostly a treatable and curable disease, non-compliance with drug therapy and the use of spurious or low-quality drugs has led to the surfacing of drug-resistant (DR), multidrug-resistant (MDR), and extensive drug-resistant (XDR) strains of TB worldwide, posing new challenges in the WHO's strategy of controlling and finally eradicating this disease. Anti-TB drugs may exhibit adverse reactions, and drug-drug interactions are also seen in patients receiving treatment for other ailments. Improved living conditions and increased education about TB, along with concerted public health measures, will be crucial to help people get over the fear of TB and help eradicate this disease.

2 Tuberculosis-Related Myths

TB is commonly referred to as a "bad" disease that brings shame and misery to the community. Several myths are associated with this disease; these myths are often encountered more frequently in low socioeconomic communities. Lack of education, poor health knowledge and awareness, and inadequate understanding of the disease have led to these myths. People who believe in these myths rely on traditional remedies instead of established treatment practices [8–13] (Graphical Abstract).

2.1 Tuberculosis Is a Curse

TB is often considered a "curse" on a family. In many settings, individuals believe it is caused by witchcraft or the breaking of cultural rules and beliefs. The disease often affects multiple generations of the same family, propagating this belief. However, ever since the famous discovery by Dr. Robert Koch, it has been established that TB is caused by *M. tb*.

2.2 Tuberculosis Is Hereditary

As the disease commonly affects multiple members of the same family, there is a common belief that TB is acquired genetically. The truth is that the spread of TB mostly occurs via inhalation of the bacterium, and families living close to one another have a greater chance of getting exposed to and acquiring the infection.

2.3 Tuberculosis Is Incurable

The scare behind the word "tuberculosis" has led to the misconception that there is no treatment for this disease, and it will eventually lead to death. This is a misunderstanding as there are multiple medications that can prevent and treat TB very effectively. A better understanding of the disease process and current therapeutic agents has made this task possible.

2.4 Tuberculosis Is a Disease of the Lungs

For the general public, the most common symptom associated with TB is "coughing." This has led to another myth that TB is a disease only of the lungs. It is true that TB of the lungs, i.e., PTB, is the most common clinical manifestation of this disease; nevertheless, TB can cause extrapulmonary manifestations and affect almost any organ or tissue in the body. The extrapulmonary manifestations of TB

are most commonly noted in the brain, kidney, spine, skin, lymph nodes, gastrointestinal and genitourinary tract, and bones. The bacteria can also find their way into the bloodstream and get disseminated into multiple organs, causing "military" TB, a potentially fatal form of the disease.

2.5 Tuberculosis Can Be Acquired Through Kissing or Sexual Contact

A common misconception is that one may contract TB by kissing or engaging in sexual activity with an infected individual. However, TB is primarily acquired via inhalation of tubercle bacilli via aerosol infection. When a person with TB speaks, coughs, sneezes, the tubercle bacilli are released into the air, which may persist for many hours. Inhalation of these bacteria by other people leads to infection. The spread of TB has not been shown to occur through touching, kissing, sexual intercourse, sharing bedding, toilet seats, or sharing toothbrushes. The same aerosol dissemination of TB is seen in drug-susceptible and DR forms.

2.6 Tuberculosis Is Spread Through Sharing Utensils or Food

Some individuals assume that this illness may be transferred by exchanging utensils or food with an infected person due to a lack of knowledge of the infectious process. The myth relates to the fear associated with the disease, and there is no scientific basis for this belief. The fact is that TB is not spread by sharing utensils, drinking containers, or touching bed linens.

2.7 Tuberculosis Is Spread by Mosquitoes

Like many infectious diseases, people sometimes think that mosquitoes carry *M. tb* and transmit the infections. However, there is no rationale for this misconception. Unlike many parasitic, bacterial, and viral infections transmitted by mosquitoes and other arthropod vectors, the fact is that *M. tb* infection is not acquired through a mosquito bite.

2.8 All Patients with Tuberculosis Are Infectious

The scare of TB has led many in the general public to believe that all patients diagnosed with TB are infectious and thus capable of spreading the disease. It is true that most people with active PTB are responsible for transmitting the disease as *M. tb* released in the air while coughing and sneezing can be inhaled by those close to the infected individual. However, persons suffering from EPTB are less likely to transmit infections.

2.9 One Can Get Tuberculosis Only Once

Many people think that once a person acquires a TB infection, he/she becomes immune to subsequent exposures. However, exposure to the disease does not provide lifelong immunity to the infected individuals. Malnutrition and a depressed immune system are major factors that put an individual at a greater risk of re-infection or reactivation of latent TB.

2.10 Tuberculosis Infection Always Leads to Disease

There is a common misconception that TB infection always leads to symptomatic disease. However, many individuals infected with TB may remain asymptomatic. The immune system of the person exposed to *M. tb* determines if there will be overt TB infection or not. This is seen in latent TB infections, which may never manifest as active infections in immunocompetent individuals.

2.11 Tuberculosis Is a Problem in Developing Countries

People living in developed nations often think that TB is a disease in underdeveloped nations. While it is true that developing nations account for the majority of TB cases, the disease remains a cause of concern in developed countries as well. Often, travelers and immigrants from endemic regions constitute the most positive cases in developed nations. These infected individuals present a source of infection to the general public. However, such nations can control and contain the spread of TB by following better infection control methods, implementing sensitive diagnostic tools, and augmenting treatment strategies.

3 Tuberculosis-Related Stigma

There is often a strong stigma associated with TB, which can have widespread psychosocial consequences. TB is stigmatized because of its association with poverty, low social class, and malnutrition. This stigma has a tremendous impact on the overall health and well-being of the infected individuals. Persons having TB can develop feelings of shame, disgust, and guilt for no fault of their own. These feelings can lead to stress, anxiety, and depression and compel them to undertake risky behavior. Infected individuals are often deemed undesirable or disvalued by society and face social discrimination. Persons diagnosed with TB may have a fear of losing their job and suffer from a lack of intimate relationships with their partners. Women may fall victim to the unique stigma associated with TB [14]. They face the wrath of society in addition to obstacles with marriage prospects,

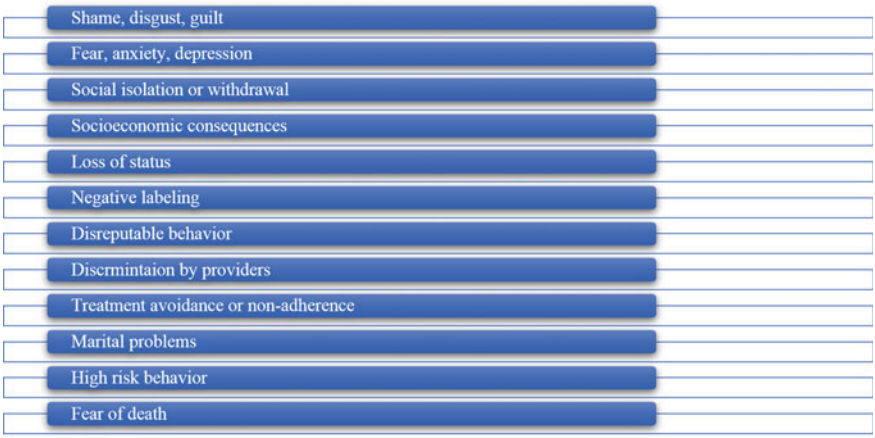


Fig. 1 Different forms of tuberculosis-associated stigma

employment opportunities, and marital issues, including rejection by their partners. They are often negatively labeled by society for suspected disreputable behavior and infidelity as the cause of the disease [15–19] (Fig. 1).

The stigma associated with TB greatly contributes to TB diagnosis and treatment delays and hinders efforts to control this disease. Infected individuals try to conceal the disease by delaying seeking evaluation focused on diagnosis and management of the disease. Such individuals may also not be adherent to the treatment regimen and follow-up. TB stigmatization contributes to the spread of the disease. Non-adherence to drug therapy is responsible for DR. Individuals with DR-TB have an even higher risk of associated stigmatization. Migrants and refugees in developed countries are often denounced as labeled disease carriers. TB is also stigmatized because of its close link to HIV, particularly in HIV endemic areas [20, 21]. Increasing awareness about the cause of TB and longitudinal support programs for individuals with TB and at-risk community members may help reduce TB-related stigma.

4 Conditions that Mimic Tuberculosis

TB is the "second great imitator" after syphilis, as it can mimic or masquerade various infectious and non-infectious medical conditions. TB can imitate diseases caused by many non-tuberculous mycobacteria (NTM) and bacterial, fungal, parasitic, and viral pathogens. In addition, some autoimmune disorders and malignancies share common symptoms and are often confused with TB, complicating management and care [22] (Table 1).

Table 1 Diseases that mimic tuberculosis

Diseases	Examples
Non tuberculous Mycobacteria	*Mycobacterium avium complex*, *M. chelonae*, *M. fortuitum*, *M. gordonae*, *M. kansasii*, *M. marinum*, and *M. xenopi*
Bacterial Infections	Leprosy, cat scratch disease, syphilis, tularemia, nocardiosis, melioidosis, brucellosis, Mycoplasma pneumonia
Fungal Infections	Histoplasmosis, blastomycosis, coccidioidomycosis, paracoccidioidomycosis, aspergillosis
Parasitic Infections	Paragonimiasis, hydatidosis, dirofilariasis, ascariasis, toxocariasis, hookworm infections, schistosomiasis, strongyloidiasis, filarial infections, leishmaniasis, and amebiasis
Viral Infections	Severe acute respiratory syndrome coronavirus 2 (COVID-19), congenital cytomegalovirus
Autoimmune Disorders	Sarcoidosis, granulomatosis with polyangiitis, Crohn's disease, rheumatoid arthritis
Malignancies	Lymphoma, ovarian cancer, lung cancer, colon cancer, ocular cancer

4.1 Non-tuberculous Mycobacteria

NTM constitute a vast group of organisms within the genus *Mycobacterium*. These are ubiquitous microorganisms in the environment that can cause infections in humans. Several NTMs, including *M. avium complex*, *M. chelonae*, *M. fortuitum*, *M. gordonae*, *M kansasii*, *M marinum*, and *M. xenopi,* may present with some of the classic symptoms associated with PTB infection, namely cough, fever, weight loss, anorexia, night sweats, hemoptysis, fatigue, and loss of appetite [23]. In addition, clinical manifestations that mimic TB may be seen with non-tuberculous mycobacterial infections of bone, skin and soft tissue, and other organs in the body [24, 25]. Accurate identification of NTMs is crucial for decreasing morbidity and mortality. However, it is seldom undertaken in resource-poor settings. The problem is compounded by the false positivity of:

- tuberculin skin test in infections with *M. avium* complex, *M. kansasii*;
- gamma interferon assays in infections with *M. kansasii* and *M. marinum* [26]; and
- genetic probes in infections with *M. celatum, M. kansasii*,*M. avium,* and *M. riyadhense* [27–29].

NTMs are often resistant to traditional anti-TB drugs, which can lead to devastating consequences in the setting of misdiagnosed TB infections.

4.2 Bacterial Infections

Some bacterial diseases can be clinically confused with TB due to similarities in presenting signs and symptoms. These include leprosy, cat scratch disease (CSD), syphilis, tularemia, nocardiosis, and melioidosis. The causative organism for leprosy is *Mycobacterium leprae*. Patients with leprosy who are on treatment with glucocorticoids are at an increased risk of acquiring TB of the lungs. Therefore, these patients should be carefully monitored for developing TB [30]. Systemic CSD is caused by a gram-negative bacterium called *Bartonella henselae*. CSD may present with granulomatous manifestations that can mimic TB. *Francisella tularensis*, a gram-negative coccobacillus, is the causative agent for tularemia. Subacute and chronic stages of this infection can present with cough, fever, hemoptysis, weight loss, and mediastinal lymphadenopathy, thus often mimicking TB. Nocardiosis is caused by *Nocardia*, a gram-positive bacillus. Clinical symptoms and radiologic findings of pulmonary, cutaneous, and disseminated nocardiosis can include cavitary lesions that are difficult to differentiate from TB. Melioidosis, a disease caused by *Burkholderia pseudomallei,* may present radiographic findings similar to TB, including small irregular disseminated nodular densities mostly in the upper lobes. *Treponema pallidum*, a gram-negative bacterium, is the causative agent of syphilis. Soft tissue pulmonary nodules (gummas) seen with endemic syphilis present a diagnostic challenge because of their close resemblance to TB. Brucellosis is caused by a gram-negative coccobacillus *Brucella* spp. Both TB and brucellosis are granulomatous diseases and mimic one another [31]. Mycoplasma pneumonia is caused by the bacterium *Mycoplasma pneumoniae,* a small rod that lacks a cell wall. Pleural effusions seen during this infection closely resemble those seen with TB [32].

4.3 Fungal Infections

Fungal infections are a cause of concern, especially in immunocompromised patients. Disease conditions caused by some fungal infections can imitate TB and vice versa. These diseases include those caused by dimorphic fungi (histoplasmosis, blastomycosis, coccidioidomycosis, and paracoccidioidomycosis) and aspergillosis. *Histoplasma capsulatum* is the causative organism for histoplasmosis, a disease endemic in Ohio and Mississippi river valleys. Chronic cavitary pulmonary histoplasmosis with preferential involvement of upper lobes may be confused with TB. Likewise, gastrointestinal histoplasmosis, an uncommon disease, may be misdiagnosed as TB [33–35]. Blastomycosis is common in Ohio and Mississippi river valleys and is caused by *Blastomyces dermatitidis.* The clinical presentation with chronic pulmonary blastomycosis includes chronic cough, weight loss, hemoptysis, and night sweats, which are typical PTB symptoms [36]. Further, upper lobe cavitation seen in the two diseases may be clinically indistinguishable. Coccidioidomycosis is caused by *Coccidioides immitis.* This disease is prevalent in diverse areas worldwide. Radiological findings of pulmonary coccidioidomycosis include

pneumonia, cavitary lesions, and pulmonary nodule, which can be confused with TB [37, 38]. Paracoccidioidomycosis is common in Central and South America. It is caused by *Paracoccidioides brasiliensis*. Chronic pulmonary or disseminated *paracoccidioidomycosis may mimic TB* [39]. Aspergillosis is caused by the fungus *Aspergillus* which has a ubiquitous presence. Radiological findings observed with invasive pulmonary aspergillosis and TB, especially in immunocompromised patients, are often indistinguishable and can present a diagnostic challenge even for the experienced radiologist [40]. The course of treatment for fungal infections and TB is drastically different. Hence, an incorrect diagnosis can result in significant morbidity and mortality.

4.4 Parasitic Infections

Parasitic infections have a worldwide occurrence and are seen both in immunocompetent and immunocompromised hosts. Parasitic diseases that can often be confused with TB and vice versa include paragonimiasis (*Paragonimus westermani*), hydatidosis (*Echinococcus granulosus*), dirofilariasis (*Dirofilaria immitis*), ascariasis (*Ascaris lumbricoides*), toxocariasis (*Toxocara canis*), hookworm infections (*Ancylostoma duodenale* and *Necator americanus*), schistosomiasis (*Schistosoma* spp.), strongyloidiasis (*Strongyloides stercoralis*), filarial infections (*Wuchereria bancrofti* and *Brugia malayi*), amebiasis (*Entamoeba histolytica*), and leishmaniasis (*Leishmania* spp.). Pulmonary manifestations of many of these parasitic infections present with cough, fever, and hemoptysis, symptoms that closely resemble those of PTB [41–45]. Symptoms resulting from infections in sites other than the lungs may resemble EPTB or miliary TB. A classic example is amebic colitis being confused with gastrointestinal TB [46].

4.5 Viral Infections

Severe acute respiratory syndrome coronavirus-2 (COVID-19) presents with cough, fever, increased sputum production, dyspnea, hemoptysis, and nausea and can sometimes be confused with TB. COVID-19 is also associated with a significant amount of stigma. A slight delay in proper diagnosis can have serious implications. While TB has a longer and slower incubation period, COVID-19 is a rapidly progressive infection, particularly among the elderly and immunocompromised individuals [47, 48]. Another example of a viral mimicker is congenital cytomegalovirus (CMV), which can present in a similar way as congenital TB [22].

4.6 Malignancies

Several types of malignancies can often be confused with TB. While management of the malignancy varies depending on cancer type and organ involved, mainstays

of treatment include radiation and chemotherapy. These therapies can cause immunosuppression and drastic structural changes in the lungs. Lymphoma is a malignancy of the lymphatic tissue. The clinical symptoms of lymphoma overlap with TB, i.e., loss of appetite, weight loss, night sweats, and low hemoglobin. The two diseases also share common radiological features [49]. PTB and lung cancer often coexist in areas where TB is endemic. Chronic inflammation processes in the lungs that occur during TB infection increase the risk of lung cancer. Both diseases exhibit common radiological findings like pulmonary cavitary and nodules [50]. Differential diagnoses of the two diseases can sometimes be challenging, and an accurate diagnosis is warranted for proper disease management. Gastrointestinal TB may mimic peritoneal cancer, while TB involving the genitourinary tract can mimic ovarian cancer [51, 52]. Ocular TB can masquerade as an ocular tumor [53, 54]. TB can also masquerade as squamous cell carcinoma and colon cancers [55–57].

4.7 Autoimmune Disorders

Sarcoidosis, a chronic inflammatory multi-organ medical condition of unclear etiology, presents with the appearance of immune system cells forming granulomas in a variety of organs and most commonly involves the lungs. Clinical symptoms associated with sarcoidosis include fever, anorexia, weight loss, fatigue, and anorexia, and patients with this condition may be misdiagnosed as TB initially. Furthermore, the two diseases share many radiological findings. The overlapping clinical manifestations and imaging findings present a diagnostic challenge [58–60].

Wagener's granulomatosis is a rare autoimmune condition characterized by anti-neutrophil cytoplasm antibodies that lead to vasculitis of the small and medium-sized blood vessels. Granulomatosis with polyangiitis and TB exhibit a considerable overlap of features, often leading to misdiagnosis [61, 62].

As seen in Crohn's disease, Inflammatory bowel conditions may present with clinical symptomatology similar to that seen in gastrointestinal TB and hence is an important differential diagnosis. Differentiating these conditions is very important as immunosuppressive drugs that may be used to treat Crohn's disease can activate TB [63, 64].

Rheumatoid arthritis is an autoimmune disease that primarily affects the joints but can also cause damage to other body organs. Arthritis associated with TB and rheumatoid arthritis follow the chronic disease course and can have similar clinical and radiological findings, making distinguishing these two conditions difficult [65, 66].

5 Conclusion

TB continues to cause immeasurable human suffering, particularly among people with low socioeconomic status worldwide. The disease also greatly impacts national economies worldwide. Despite global initiatives undertaken by the WHO

toward eradicating this disease, we remain a long way away from this goal. As poverty and TB are closely associated, eliminating the former would facilitate the eradication of not only TB but many other infectious diseases. Stigmatization is a great barrier to the containment of many diseases, and TB is no exception. Stigmatization of TB patients carries vast impacts on the psychological health and quality of life of affected individuals [67]. Public health initiatives towards decreasing stigmatization and discrimination of TB patients are warranted. Further, national and international efforts towards imparting awareness about TB, especially among poor and backward communities, will go a long way towards achieving control of this human health menace.

Social injustices and inequalities are the root causes for all human miseries, and tuberculosis is no exception.

Alisha Kamboj, Michael Lause, Kamal Kamboj.

Core Messages

- TB remains a major global public health problem.
- Myths and stigma associated with TB greatly impact its control.
- TB and its mimics pose a diagnostic challenge for clinicians.

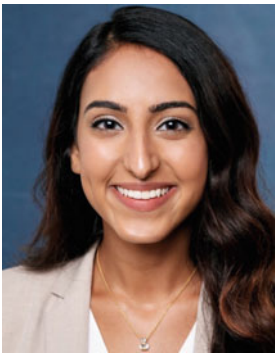
References

1. World Health Organization (2005) Addressing poverty in TB control. Options for National TB control programs. WHO/HTM/TB/2005.352
2. Emmanuelli X, Grosset J (2003) Tuberculosis and poverty. Rev Mal Respir 20(2):169–171. http://www.ncbi.nlm.nih.gov/pubmed/12844011. Accessed 7 Sept 2020
3. World Health Organization (2015) Global tuberculosis report 2015, 20th ed. World Health Organization. https://apps.who.int/iris/handle/10665/191102
4. Bruchfeld J, Correia-Neves M, Källenius G (2015) Tuberculosis and HIV coinfection. Cold Spring Harb Perspect Med 5(7):a017871
5. Kamboj A, Lause M, Kamboj K (2021) Tuberculosis. Int J Child Health Hum Dev 14(3)
6. Cadena J, Rathinavelu S, Lopez-Alvarenga JC, Restrepo BI (2019) The re-emerging association between tuberculosis and diabetes: lessons from past centuries. Tuberculosis (Edinb) 116S:S89–S97
7. Procop GW (2017) HIV and mycobacteria. Semin Diagn Pathol 34(4):332–339
8. Center for Disease Control and Prevention (2015) TB. Get the facts about tuberculosis disease. https://www.cdc.gov/tb/publications/pamphlets/TB_disease_EN_rev.pdf?s_cid=cs_284. Accessed 4 Sept 2020
9. Sowards W (2020) Common myths and facts about TB. https://www.passporthealthusa.com/2017/12/common-myths-and-facts-about-tuberculosis/. Accessed 5 Sept 2020
10. Lanphear BP, Snider DE (1991) Myths of tuberculosis. J Occup Med 33(4):501–504. http://www.ncbi.nlm.nih.gov/pubmed/1645402. Accessed 5 Sept 2020

11. Musuka G, Teveredzi V, Mutenherwa F, Chingombe I, Mapingure M (2018) Tuberculosis knowledge, misconceptions/myths in adults: findings from Lesotho, Malawi, Namibia and Zambia Demographic Health Surveys (2013–2016). BMC Res Notes 11(1):778
12. Gil N, López L, Rodríguez D, Rondón M, Betancourt A, Gutiérrez B, Rueda ZV (2018) Myths and realities about knowledge, attitudes and practices of household contacts of tuberculosis patients. Int J Tuberc Lung Dis 22(11):1293–1299
13. Mathew AS, Takalkar AM (2007) Living with tuberculosis: the myths and the stigma from the Indian perspective. Clin Infect Dis 45(9):1247
14. Long NH, Johansson E, Diwan VK, Winkvist A (1999) Different tuberculosis in men and women: beliefs from focus groups in Vietnam. Soc Sci Med 49(6):815–822
15. Juniarti N, Evans D (2011) A qualitative review: the stigma of tuberculosis. J Clin Nurs 20 (13–14):1961–1970
16. Craig GM, Daftary A, Engel N, O'Driscoll S, Ioannaki A (2017) Tuberculosis stigma as a social determinant of health: a systematic mapping review of research in low incidence countries. Int J Infect Dis 56:90–100
17. Cremers AL, de Laat MM, Kapata N, Gerrets R, Klipstein-Grobusch K, Grobusch MP (2015) Assessing the consequences of stigma for tuberculosis patients in urban Zambia. PLoS One 10(3):e0119861 (Kumar A, ed)
18. Daftary A, Mitchell EMH, Reid MJA, Fekadu E, Goosby E (2018) To end TB, first-ever high-level meeting on tuberculosis must address stigma. Am J Trop Med Hyg 99(5):1114–1116
19. Maleche A, Citro B, Tisile P, Abdullaev T (2017) Measuring TB-related stigma. Int J Tuberc Lung Dis 21(11):4–5
20. Courtwright A, Turner AN (2010) Tuberculosis and stigmatization: pathways and interventions. Public Health Rep 125 Suppl 4(Suppl 4):34–42
21. Wouters EW, Sommerland N, Masquillier C, Rau A, Engelbrecht M, Van Rensburg AJ, Kigozi G, Ponnet K, Van Damme W (2020) Unpacking the dynamics of double stigma: how the HIV-TB co-epidemic alters TB stigma and its management among healthcare workers. BMC Infect Dis 20(1):106
22. Herchline TE (2020) Tuberculosis (TB) Differential diagnoses. Medscape web source. https://emedicine.medscape.com/article/230802-differential. Accessed 25 Aug 2020
23. Gomathy NS , Padmapriyadarsini C, Silambuchelvi K , Nabila A, Tamizhselvan M, Banurekha VV, Lavanya J , Chandrasekar C (2019) Profile of patients with pulmonary non-tuberculous mycobacterial disease mimicking pulmonary tuberculosis. Indian J Tuberc 66 (4):461–467
24. Kumar M, Verma S, Dhole TN, Kumar R (2016) Non-tuberculous mycobacteria mimic of MDR-TB infection in Pott's disease. BMJ Case Rep 016
25. Metta H, Corti M, Brunzini R (2008) Disseminated infection due to *Mycobacterium chelonae* with scleritis, spondylodiscitis and spinal epidural abscess. Braz J Infect Dis 12(3):260–262
26. Arend SM, van Meijgaarden KE, de Boer K, Cerdá de Palou E, van Soolingen D, Ottenhoff THM, van Dissel JT (2002) Tuberculin skin testing and in vitro T cell responses to ESAT-6 and culture filtrate protein 10 after infection with *Mycobacterium marinum* or *M. kansasii*. J Infect Dis 186(12):1797–1807
27. Gildeh E, Abdel-Rahman Z, Sengupta R, Johnson L (2016) A case of false-positive *Mycobacterium tuberculosis* caused by *Mycobacterium celatum*. Case Rep Infect Dis. 2016:1761923
28. Jorgensen JH, Salinas JR, Paxson R, Magnon K, Patterson JE, Patterson TF (1999) False-positive Gen-Probe direct *Mycobacterium tuberculosis* amplification test results for patients with pulmonary M. kansasii and M. avium infections. J Clin Microbiol. 37(1):175–178
29. van Ingen J , Al-Hajoj SAM, Boeree M, Al-Rabiah F, Enaimi M, de Zwaan R, Tortoli E, Dekhuijzen R, van Soolingen D (2009) Mycobacterium riyadhense sp. nov., a non-tuberculous species identified as *Mycobacterium tuberculosis* complex by a commercial line-probe assay. Int J Syst Evol Microbiol. 59(Pt 5):1049–1053

30. Mangum L, Kilpatrick D, Stryjewska B, Sampath R (2018) Tuberculosis and leprosy coinfection: a perspective on diagnosis and treatment. Open forum Infect Dis 5(7):ofy133
31. Sfairopoulos D, Tsiara S, Barkas F, Margariti PN, Agouridis AP, Tsioutis C, Ntzani EE, Rizos EC (2020) Is brucellosis a great mimic of tuberculosis? A case report. Eur J Clin Microbiol Infect Dis 39(9):1711–1715
32. Yaguchi D, Ichikawa M, Shizu M, Inoue N, Kobayashi D, Imai N, Ito M (2018) Tuberculous pleurisy mimicking Mycoplasma pneumoniae infection in a previously healthy young adult: a case report. Medicine (Baltimore) 97(20):e10811
33. Qureshi A (2008) A case of histoplasmosis mimicking tuberculosis. J Pak Med Assoc. 58 (8):457–458. http://www.ncbi.nlm.nih.gov/pubmed/18822647. Accessed 7 Sept 2020
34. Ramesh V, Narreddy S, Gowrishankar S, Barigala R, Nanda S (2018) A challenging case of pyrexia of unknown origin: adrenal histoplasmosis mimicking tuberculosis in a patient with chronic hepatitis C. Trop Doct. December 49475518819622
35. Ai XB, Wang ZJ, Dong QC, Lin X, Chen YP, Gong FY, Liang H (2018) Ileum histoplasmosis mimicking intestinal tuberculosis and Crohn's disease. Case Rep Gastroenterol 12(1):63–68
36. Koroscil MT, Skabelund A (2018) Chronic pulmonary blastomycosis mimicking pulmonary tuberculosis. Mil Med 183(7–8):e332–e333
37. Cadena J, Hartzler A, Hsue G, Longfield RN (2009) Coccidioidomycosis and tuberculosis coinfection at a tuberculosis hospital: clinical features and literature review. Medicine (Baltimore) 88(1):66–76
38. Capoor MR, Sen B, Varshney P, Verghese M, Shivaprakash MR, Chakrabarti A (2014) Coccidioidomycosis masquerading as skeletal tuberculosis: an imported case and review of coccidioidomycosis in India. Trop Doct 44(1):25–28
39. Quagliato Júnior R, Grangeia T de AG, Massucio RA de C, De Capitani EM, Rezende S de M, Balthazar AB (2007) Association between paracoccidioidomycosis and tuberculosis: reality and misdiagnosis. J Bras Pneumol 33(3):295–300
40. Kim SH, Kim MY, Hong SI, Jung J, Lee HJ, Yun SC, Lee SO, Choi SH, Kim YS, Woo JH (2015) Invasive pulmonary aspergillosis-mimicking tuberculosis. Clin Infect Dis 61(1):9–17
41. Kunst H, Mack D, Kon OM, Banerjee AK, Chiodini P, Grant A (2011) Parasitic infections of the lung: a guide for the respiratory physician. Thorax 66(6):528–536
42. Schaberg T, Eller J, Wernitz S, Küstner U, Lode H (1991) Pulmonary parasitoses as differential diagnosis of lung tuberculosis. Z Arztl Fortbild (Jena) 85(19):937–942. http://www.ncbi.nlm.nih.gov/pubmed/1755243. Accessed 7 Sept 2020
43. Aronson N, Herwaldt BL, Libman M, Pearson R, Lopez-Velez R, Weina P, Carvalho EM, Ephros M, Jeronimo S, Magill A (2016) Diagnosis and treatment of leishmaniasis: clinical practice guidelines by the infectious diseases society of America (IDSA) and the American society of tropical medicine and hygiene (ASTMH). Clin Infect Dis 63(12):e202–e264
44. Schaberg T, Rahn W, Racz P, Lode H (1991) Pulmonary schistosomiasis resembling acute pulmonary tuberculosis. Eur Respir J 4(8):1023–1026. http://www.ncbi.nlm.nih.gov/pubmed/1783077. Accessed 7 Sept 2020
45. Lall M, Sahni AK, Rajput AK (2013) Pleuropulmonary paragonimiasis: mimicker of tuberculosis. Pathog Glob Health 107(1):40–42
46. Pai SA (2009) Amebic colitis can mimic tuberculosis and inflammatory bowel disease on endoscopy and biopsy. Int J Surg Pathol 17(2):116–121
47. Akbar H, Kahloon R, Akbar S, Kahloon A (2020) Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection mimicking as pulmonary tuberculosis in an inmate. Cureus 12(6):e8464
48. PAHO (2020) COVID-19: considerations for tuberculosis (TB) care services. https://www.paho.org/hq/index.php?option=com_content&view=article&id=15759:tuberculosis-and-covid-19-what-health-workers-and-authorities-need-to-know&Itemid=1926&lang=en. Accessed 4 Sept 2020

49. Dres M, Demoule A, Schmidt M, Similowski T (2012) Tuberculosis hiding a non-Hodgkin lymphoma "there may be more to this than meets the eye." Respir Med Case Rep 7:15–16
50. Tamura A (2016) Tuberculosis and lung cancer. Kekkaku 91(1):17–25. http://www.ncbi.nlm.nih.gov/pubmed/27192776. Accessed 8 Sept 2020
51. Hasanzadeh M, Naderi HR, Hoshyar AH, Shabane S, Shahidsales S (2014) Female genital tract tuberculosis presenting as ovarian cancer. J Res Med Sci 19(2):184–189. http://www.ncbi.nlm.nih.gov/pubmed/24778675. Accessed 7 Sept 2020
52. Piura B, Rabinovich A, Leron E, Yanai-Inbar I, Mazor M (2003) Peritoneal tuberculosis—an uncommon disease that may deceive the gynecologist. Eur J Obstet Gynecol Reprod Biol 110 (2):230–234
53. Demirci H, Shields CL, Shields JA, Eagle RC (2004) Ocular tuberculosis masquerading as ocular tumors. Surv Ophthalmol 49(1):78–89
54. Sassalos TM, Rao RC, Demirci H (2018) Ocular tuberculosis masquerading as a tumor. Lancet Infect Dis 18(8):924
55. Arora KS, Garg S, Kaur P, Mohapatra S (2018) Primary oral tuberculosis on the tongue mimicking squamous cell carcinoma. Indian J Tuberc 65(1):84–86
56. Kumar A, Patodia M, Pandove P (2012) Sharda V (2012) Colonic tuberculosis masquerading as colon cancer. J Surg Case Rep 5:10
57. Powell J, Bath M, Joshi H, Machesney M (2020) Case of extrapulmonary tuberculosis Mycobacterium mimicking a colon cancer. BMJ Case Rep 13(5):e235486
58. Vadala R, Bhat MNM, Rabindrarajan E, Ramakrishnan N (2019) Concomitant presentation of sarcoidosis and pulmonary tuberculosis with ARDS: a diagnostic dilemma and therapeutic challenge. Indian J Tuberc 66(2):314–317
59. Mandal SK, Ghosh S, Mondal SS, Chatterjee S (2014) Coexistence of pulmonary tuberculosis and sarcoidosis: a diagnostic dilemma. BMJ Case Rep 2014(dec19 1):bcr2014206016-bcr2014206016.
60. Hansen KC, Jensen-Fangel S, Hønge BL (2019) Contribution to differential diagnosis of sarcoidosis and disseminated tuberculosis. BMJ Case Rep 12(11):e230652
61. Mahmood FS, Schwatz E, Kurrup S, Sharp C, Hands G, Moody A (2013) A diagnostic dilemma: differentiating between granulomatosis with polyangiitis and tuberculosis. Clin Med 13(4):411–413
62. Molinari L, Melamud JI, Ferrari L, Landi P, Semeniuk G, Quadrelli SA (2009) Wegener's granulomatosis and tuberculosis. A bad combination. Medicina (B Aires) 69(6):640–642. http://www.ncbi.nlm.nih.gov/pubmed/20053604. Accessed 8 Sept 2020
63. Almadi MA, Ghosh S, Aljebreen AM (2009) Differentiating intestinal tuberculosis from Crohn's disease: a diagnostic challenge. Am J Gastroenterol 104(4):1003–1012
64. Rafael MA, Martins Figueiredo L, Oliveira AM, Nuno Costa M, Theias Manso R, Martins A (2020) Gastrointestinal tuberculosis mimicking Crohn's disease. GE Port J Gastroenterol 27 (4):278–282
65. Latief W, Asril E (2019) Tuberculosis of the wrist mimicking rheumatoid arthritis—a rare case. Int J Surg Case Rep 63:13–18
66. Teo S, Teh K, Azura L, Ng Y (2011) The great mimic again? A case of tuberculosis knee. Malaysian Orthop J 5(3):32–34
67. Aggarwal AN (2019) Quality of life with tuberculosis. J Clin Tuberc Other Mycobact Dis 17:100121



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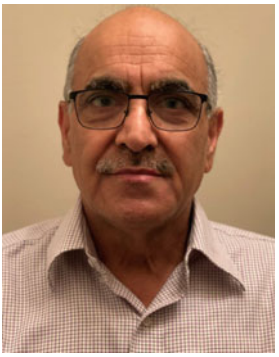

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To eliminate tuberculosis and its related suffering, integrated studies are essential. No grandchild should miss her grandmother, who died of tuberculosis, and hearing her voice while telling the story, nor should she miss her father's grief that he did not have the opportunity to meet his mother for the last time.

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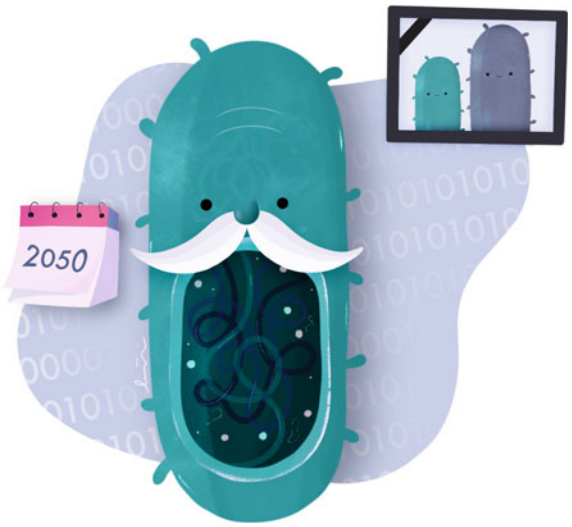
Summary

The authors of *Tuberculosis: Integrated Studies for a Complex Disease* were asked how they would see the future of their field 30 years later. This Chapter presents the authors' views on the situation of tuberculosis in 2050.

Graphical Abstract



2050' TB. (*Adapted with permission from the Association of Science and Art (ASA), Universal Scientific Education and Research Network (USERN); Made by Nastaran-Sadat Hosseini*)

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1 Introduction

Tuberculosis (TB) has been a disease for centuries with various challenges [1]. Like other places where challenges and opportunities come together, TB challenges were the inspiration for the scientific community to mobilize different groups for the purpose of interest. For example, with the emergence of drug resistance, there has been a huge volume of research on the discovery of new medicines and drug delivery methods and the repurposing of old drugs [2, 3]. Moreover, to enhance the capacity to detect TB cases, studies have sought diagnostics and biomarkers, with much hope recently expressed in the direction of point-of-care tests [4].

Despite all such efforts as being highlighted in 50 Chapters of this volume, we are still writing about TB and thinking about how to fight this old disease–implying that the problem of TB might be complex, so calling the need for an integrated science to deal with multiple dimensions in a simultaneous and effective manner. We are not the first one; there have been proposed integrated platform for TB research, integrated prevention services, integrated models for drug screening, integrated imaging protocol, integrated understanding of the disease pathogenesis, integrated control models, integrated mapping of the genome of the pathogen, etc. [5–12], to name some.

These integrated jobs date back decades ago. So, a question arises: why is there a disease named TB yet? It might be due to the fact that this integration has happened to a scale that is not global, and so TB remains to be a problem, especially in resource-limited settings.

Hope *Tuberculosis: Integrated Studies for a Complex Disease* helps to globalize the integrated science of TB.

2 TB and Related Conditions

2.1 TB and Tobacco Use

There have been many achievements in the field of TB over the last three decades. However, the TB organism is changing its genetic nature due to our lack of sustained efforts. Moreover, the unprecedented times of the COVID-19 pandemic have stalled the gains made over the last years in reducing the TB disease burden around the world. Further, tobacco users are more susceptible to the severity and progression of TB, more so among individuals suffering from COVID-19. However, despite the existence of a TB-tobacco framework, integrated interventions are finite, and their implementation is limited. The countries need to tackle TB-tobacco for

synergistic outcomes and upscale the integrated framework in maximum settings. This could give momentum to the endgame of two major public health concerns resulting in achieving the goals of SDGs (Sonu Goel, Garima Bhatt 2020).

2.2 TB and Drug Use

There has been a long history of the adverse health consequences of criminalized psychotropic drugs, including links between such drug use and a range of infections transmitted through nonsterile acts of drug use (e.g., HIV and hepatitis C) and through overlaps between drug use and adverse social and structural contexts and policies (e.g., TB). TB, drug use, and associated adverse consequences (e.g., overdose) and other linked infectious epidemics continue to cause significant and preventable morbidity and mortality. Advances in integrated science, medicine, and public health bring together the insights of diverse yet complementary disciplines to enrich the study and understanding of multi-level fundamental causes of illness and disease. Integrated science, medicine, and public health also create the tools and the knowledge base to move the world from a predominate focus on efficacious interventions to the expanded implementation of interventions with population-level effectiveness needed to reach the goals of the elimination of TB, the elimination of HIV and the elimination of the harms and adverse consequences of criminalized psychoactive drug use (Ashly E Jordan, David C Perlman 2020).

2.3 TB and Cancer

When we look at cancer cases with a projection after 30 years, we can predict that there will be a much greater increase in cancer frequency because the average life span and the ratio of chronic diseases in cancer patients will increase as a result of the development of diagnosis and treatment methods in cancer patients. Therefore, mortality and morbidity associated with TB will increase. We believe that the aging world population, many environmental factors, viruses full of new and atypical unknowns, and stress may be the most important reasons for the increase in cancer prevalence. At the same time, new agents to be used in cancer patients will cause more immunosuppression, so this will increase the incidence of TBC in this population. These negative thoughts can perhaps be turned into positive ones to some extent. How does? There is no doubt that these increased morbidity and mortality rates can be reduced with the developments in TB treatment and extended screening programs. With rapidly advancing technology, however, advances in molecular biology and genetics and bioengineering innovations, many new agents will come up with better results in the clinic, considering that we have achieved longer lifetimes in metastatic disease in many cancers. However, we think that full control of cancer can be achieved with genetic engineering, epidemiology, and prevention studies. Again, environmental problems and poor use of environmental resources may create a very troublesome process for TB, which continues to be an important

health problem. If patients with immunosuppressed cancer are affected by TB and the frequency of TB increases, the development of lung cancer on the basis of TB may form the basis of a future health problem in a vicious circle. In our opinion, adding many other scientific fields to the multidisciplinary understanding of medicine at the same time can minimize the incidence of TB and cancer and the negativities they will create together (Yaşar Barış Turgut, Alican Tahta, Özgür Tanrıverdi 2020).

3 Resistance and Drug Discovery

Thirty years later (Integrated Science TB 2050), there might be a huge advancement in technologies and strategies to overcome the problem of resistance. Doctors and clinicians may be able to better schedule the anti-TB treatment (ATT). It might be possible that the ATT will become short, affordable, and less toxic. Advancements in computational approaches may also accelerate the discovery and development process of new anti-TB drugs. The mortality rate owing to TB may decrease, and a smaller number of cases might be observed (Himanshu Verma, Shalki Choudhary, Om Silakari 2020).

3.1 A New Vaccine

According to the United Nations Agenda, the world should be free of TB by 2050. However, global leadership and adequate economic investment are needed to eradicate TB and other infectious diseases that threaten and will threaten humanity. This purpose is only possible through adequate universal health coverage, especially in third world countries, which, in turn, can protect nations from potentially catastrophic health costs produced by drug-resistant TB. In the next decades, there will surely be enough research and development to design novel anti-TB drugs that control active infection, including latent infection, which have been the bottleneck in the fight against TB in the last 50 years. However, during this time, the tubercle bacillus will most likely generate resistance against those new anti-TB compounds, which will force this effort to start again. The greatest hopes for TB control might lie in the development of a new prophylactic vaccine, which will only be possible if there is a true political will to stop TB and other neglected diseases, and it is not a profit opportunity for pharmaceutical companies (Paola Santos, Milena Maya-Hoyos, Marcela López-R, Cristian Rosales, Vanessa Vásquez, Andrés Varón, Bibiana Chavarro-Portillo, Nelson E. Arenas, Carlos Y. Soto 2020).

3.2 De Novo Drug Deisgn

De novo drug design has established itself as an efficient method for the development of potent and selective inhibitors for different classes of biological targets. The ever-growing wealth of structural data on therapeutic targets will certainly further enhance the importance of de novo design for the drug discovery process. Advancements in structural biology techniques such as cryo-electron microscopy for the high-resolution structure of *M. tb* therapeutic targets with the inhibitors, along with molecular simulations, will definitely play a crucial role in drug designing in the near future.

3.3 OMICs

The ambitious plans to eradicate TB will demand continuous efforts for the next 30 years, mainly due to the limited access to medicines in developing countries. Future challenges to be overcome include the development of new drugs active against resistant strains and latent TB, new therapeutic regimens shorter than the current treatment, design of new personalized therapies that consider bio-psycho-social issues of each patient, mainly in the presence of co-infections and/or comorbidities. In the upcoming years, advances in host-direct therapy will allow improvement of the immune response against the bacilli. This approach will work synergically with the novel drugs in order to mitigate the infection. With the OMICs development, novel targets will be described and validated, bringing a horizon of possible pharmacological interventions. The therapeutic arsenal will be constituted by distinct options with high specificity and low toxic profile; however, the access to these new advances will not be able to reach all those patients in the developing countries; being necessary to establish new approaches not only to guarantee the use of these new drugs but also to allow the right use and the monitoring of those new drugs (João Lucas Prates, Guilherme Felipe dos Santos Fernandes, Cristhian N. Rodríguez-Silva, Jean Leandro dos Santos 2020).

3.4 A New Targeted Therapy

A deadly disease like TB has been afflicting mankind for thousands of years, and despite so much progress made in the field of medical sciences, we have not succeeded in eradicating the scourge of the disease from our society. The main culprit is the biological makeup of the bacteria which can devise safety mechanisms to evade anti-TB drugs and survive in a dormant state for a very long time. Developing new therapeutics for curing the disease by circumventing the resistance-developing mechanisms or novel molecules with entirely new mechanisms of action are the only options available to medicinal chemists. Drug-resistant TB, especially in immunocompromised patients, is a big challenge. The ultimate goal is the complete eradication of the disease by the year 2050. DprE1 is a new

druggable target present in the bacterium which has no parallels in the human host. A substantial quantum of research work has been done to develop DprE1 inhibitors as potential anti-TB drugs. Some molecules have already cleared the pre-clinical stages of drug development and entered into different phases of clinical trials. Artificial intelligence (AI) and simulation techniques can play important roles in new drug discoveries for treating TB. DprE1 inhibitors provide a ray of hope for achieving the laid down target of eradicating the deadly disease in the near future (Mange Ram Yadav, Prashant R. Murumkar, Monica Chauhan, Rahul B. Ghuge, Rahul R. Barot 2020).

4 Pathogenesis

4.1 Lung Microbiota

Studying how *Mycobacterium tuberculosis* (*M. tb*) infection and its treatment alter the lung's microbiota could open the door for a potential role of probiotics in the treatment and prevention of TB. New information on the role of the lung microbiota, its interaction with the alveolar epithelial cells, and the innate immune responses and subsequent adaptive immune responses could provide clues on the pathogenesis of *M. tb* infection and prompt new therapies for protection. In the future, characterization of the microbiome will not only be extremely fast but probably very inexpensive. This will lead to accessible and affordable personalized medicine, where the use of probiotics or bacteria-derived products will be part of new and better therapeutic strategies to treat and prevent diseases like TB (Jorge Cervantes 2020).

4.2 Mutagenesis

Niels Bohr is believed to have said: "Prediction is very difficult, especially if it's about the future." Still, it can be helpful to think about existing problems that are either clamoring for a solution or showing great promise. It is highly desirable to extrapolate mutation rates of *M. tb* living in the human body from those measured in vitro. Ingenious mathematical models should one day be invented that are based on human physiology and microbiology. It would also be satisfying and of practical importance that mathematical methods are found that allow the fluctuation protocol and sequencing-based methods to complement each other. Finally, it is expected that biological technology advances will continue to stimulate innovative investigators to devise mathematical models that address what is missing in the existing models according to the distinctive purposes and characteristics of an investigation (Qi Zheng 2020).

4.3 Epigenetics

Epigenetic mechanisms play an important role in regulating gene expression and maintenance of normal cellular processes. These mechanisms alter gene expression levels without inducing genetic mutations. Microorganisms target some of the epigenetic processes to attenuate the host immune system and promote the emergence of drug-resistant pathogenic strains. Therefore, an appropriate host mRNA transcription profile is essential for maintaining regular gene expression and protein synthesis levels. Accordingly, therapeutic molecules that target epigenetic mechanisms such as DNA methylation, acetylation, and phosphorylation are critical for restoring and maintaining normal gene expression and protein production levels. It is envisioned that further research in the field of epigenetics will, in the future, unravel new vital cellular pathways and essential substrate molecules thereof. Notably, the discovery of new pathways and molecular targets such as DNA, RNA, and virulent proteins may be applied as markers that may be employed diagnostically and therapeutically. Ultimately, this is pivotal for disease detection and treatment as well as for preventing the emergence of drug-resistant microbial pathogens (Musa Marimani, Aijaz Ahmad, Adriano Duse 2020).

In the past decade, clinical bacteriology has undergone important changes with the introduction of "human microbiota" and "epigenetic modifications." In the future, introducing an effective biomarker for rapid prognosis, diagnosis, treatment, and control strategies will be an urgent need to cope with new threats from infectious microorganisms and emerging pathogens. Hence, the goal of many scientists is to understand the importance of human microbiota and epigenetic modifications better so that they improve human health; but different aspects of their association are not fully understood. Many studies have shown that certain infectious agents and commensal microbiota can change the epigenetic status of their own genome as well as mammalian host cells during infection and symbiosis. Epigenetic mechanisms are pivotal in regulating gene expression during cellular response to extracellular stimuli. Bacterial infections have a profound effect on the host epigenome, which triggers susceptibility to diseases. Nonetheless, the human microbiota and epigenetic modifications era bring exciting discoveries and challenges that can potentially be included in health programs. Improvement of multidisciplinary collaborations such as the cooperation of physicians, clinical microbiologists, and scientists of other research fields may lead to profound changes in this regard. Besides, the development of related technologies such as whole-genome sequencing (WHS) of microbiome and epigenome on a large scale and bioinformatics techniques can be advantageous to reach the goal of human health improvements (Samira Tarashi, Mir Davood Omrani, Arfa Moshiri, Abolfazl Fateh, Seyed Davar Siadat, Andrea Fuso 2020).

5 Diagnosis

Since the goal is to achieve full elimination of TB by 2050, defined as less than 1 case per million people per year, designing new tools for accurate, rapid diagnosis of both active and latent TB is critical. Moreover, effective new vaccines are needed to achieve the long-term vision of TB elimination, particularly in areas of high HIV/AIDS prevalence with a high rate of drug resistance. The 'Test and Treat' strategy is a key element of the End TB Strategy. We need better drugs to deal with the TB threat, and more commercial incentive to develop new drugs is needed. Furthermore, discovering better technologies for preventing TB and speeding the process of diagnosis as well as monitoring treatment is highly required. Microfluidics might be an important tool for commercial product development in TB diagnostics. Microfluidic approaches based on molecular or cellular biomarkers using microchip diagnostics might meet the requirement of TB diagnosis (Shima Mahmoudi, Babak Pourakbari, Setareh Mamishi 2020).

Quick and effective identification of TB effusions is still a challenge. ADA has stood the test of time, but it is plausible that in geographical areas where this assay is not available, alternative biomarkers with similar accuracy, such as interleukin 27 (IL27) or unstimulated interferon-gamma (IFNγ), might find a place for diagnostic purposes. More studies on the potential advantages of combining biomarkers (e.g., ADA plus IL27), particularly in older populations, are needed. However, the best hope is the improvement of rapid molecular assays that also help to detect mutations associated with drug resistance. Their application on sputum and pleural fluid samples will become routine, even though the low bacillary burden of TB effusions will presumably prevent the development of extremely sensitive NAAT. Randomized controlled trials on shorter anti-TB regimens and the role of therapeutic thoracenteses are expected to become available in the near future (José M. Porcel, Laura Porcel 2020).

TB has been a burden to mankind for millennia and remains the top infectious killer worldwide. In the last few years, momentum has been created to End TB (e.g., End TB strategy, 2018 UN High-Level Meeting). However, the progress is recently threatened by the COVID-19 pandemic, which takes much of the attention and resources. As a result, the COVID-19 response efforts have exerted clear drawbacks on TB control. Modeling studies suggest that a few months of lockdown can interrupt services for many more months and throw back the entire TB control efforts by years. Our work highlights how innovation is used to improve medical diagnostics and its impact in TB high burdened countries. Thus, we can safely anticipate that scent- or volatile organic compound (VOC)-based tests can play a greater role in TB and other diseases detection in the future. However, a technology alone unlikely makes a difference as it needs an entire ecosystem of services to make use of innovation in the field and to move forward from innovation to application, validation, and impact. Future focus, in the light of not only COVID-19 but also other health challenges, is needed to combine services for various diseases, make them truly patient-centered, and be instrumental in reaching universal health

coverage for all (Negussie Beyene, Georgies Mgode, Robert Burny, Cynthia D. Fast, Christophe Cox, Lena Fiebig 2020).

We see an increasing importance of the role of diagnostic microdevices in the fight against TB. It can be expected that in 30 years, the diagnostic process in microdevices will be fully automatic, free from human intervention and its associated risks. The tendency we observe towards the use of low-cost, widely available, and portable components such as lab-on-paper platforms or mobile phone cameras will be fully established in the future. Its combination will enable the use of these technologies in the point-of-care of low-income countries, where they are needed the most. The automatization in the manufacturing process of these microdevices is also of great importance in order to upscale the production of sufficient chips to cover the diagnostic needs. During the next years, the industry will approach an automatization of chip manufacturing, allowing a mass-production (Marina Cañadas-Ortega, Clara Gómez-Cruz, Juan José Vaquero, Arrate Muñoz-Barrutia 2020).

6 Extrapulmonary TB

6.1 Tubercular Uveitis

Uveitis consists of more than 30 entirely different disease entities that could not be differentiated by a single diagnostic test. Diagnosis is derived from a combination of good history taking, general physical and ophthalmological examinations, and staged and tailored workup that consists of multimodal ancillary tests. A useful parameter to determine the natural clinical course and the treatment response are also not available. It is even trickier in tubercular uveitis (TBU), where obtaining *M. tb* from ocular tissue as a gold-standard diagnosis is challenging to do. Consequently, diagnosing TBU could be untimely because TBU is mainly diagnosed by excluding the other disease entities. Further, it needs four to six weeks of response to ATT to corroborate the diagnosis. We need well-defined diagnostic tests to confirm a TBU promptly to be confident and quick in starting the ATT and whether it is necessary to add steroid or immunosuppressive treatment. It is essential because of the potential for hepatotoxicity and drug resistance of ATT. Clinically, TBU may be a spectrum of a disease caused by a direct infection at one end (e.g., choroidal granuloma with good response to ATT alone) and immune response at the other end (e.g., serpiginous-like choroiditis that needs immunosuppression). Immunologically, the increased expression of particular type 1 IFN genes might represent the direct infection spectrum, while the increased production of IFNγ and tumor necrosis factor-alpha (TNFα) might represent the immune response spectrum. These immune responses in all levels of biological processes are consequently potential to become diagnostic biomarkers. They should be explored from intraocular fluid or tears (less invasive) or peripheral blood to recognize TB as the cause, understand the clinical course, and predict response to ATT (Rina La Distia Nora, Wandya Hikmahwati, Ikhwanuliman Putera 2020).

7 Elimination

To achieve TB elimination globally, increasing awareness and training for healthcare providers about collaborative approaches with TB patients will be indispensable. To fully maintain TB patients in their treatment plan, improving their understanding of the disease at the beginning of ATT and increasing support from their peers and family members will be very imperative. In every solution, vulnerable populations such as imprisoned and migrants must be considered to decrease the spread of drug-resistant TB. A comprehensive family-based approach is required to improve the screening of children for TB. To effectively control TB in children, new drugs with proven efficacy and reduced toxicity, new diagnostic techniques, and effective vaccines are needed. For the worldwide elimination of TB, the multiple stakeholders of the international community should work together in the selection of the best and complete care for TB patients. Furthermore, a strong commitment of all government officials will be needed to support any research projects which are targeted at the development of new diagnostic tools, efficacious vaccines, and better anti-TB drug regimen options. At the patient level also, every patient needs to consider these conditions to control TB, take all their medication as ordered, attend all their appointments, always cover their mouth with a tissue when they cough or sneeze, and wash their hands after coughing or sneezing (Mohammed Assen Seid 2020).

TB is continuously presenting new challenges as a global health hazard. In present times, health designs focus on the diagnosis and control of active disease, which is the "tip of the iceberg." Co-infection with HIV, the emergence of multidrug resistance, and diabetes are deterrents to effective disease control. However, rapidly evolving molecular diagnostics and genomic sequencing of the mycobacterium have helped in a better understanding of the biological behavior and epidemiology of this daunting ailment. Further research to develop tools that can reliably identify latent infections and predict their future course is prudent to rationalize preventive therapy. Rapid development in the field of genomics can help to master the epidemiologic evolution and heterogeneity in response and host-pathogen interaction. A multidisciplinary approach to developing efficient vaccines, effective treatment, and global commitment toward socioeconomic support, strengthening health facilities, and improving public awareness can promise the elimination of the disease worldwide by 2050 (Richa Sinha, Rahul 2020).

The search for new drugs targeting TB has witnessed a phenomenal change in recent times, which resulted in multiple drugs entering the clinical pipeline.[1] This strengthened the internationally agreed target for TB elimination by 2050, where the annual incidence would be less than one case per million population. However, it also means a $\sim$ 1000-fold reduction in incidence in about 30 years, corresponding to a $\sim$ 20% annually. The current global TB incidences are falling at about 2% per year, and the projected aim may seem unrealistic. However, the scientific community is working towards finding drugs that should treat TB in ten days, similar to the

[1] https://www.newtbdrugs.org/pipeline/clinical.

treatment of other bacterial infections. Ideally, these drugs should be equally effective against multidrug-resistant and extensively drug-resistant TB, have no drug-drug interaction issues, be effective with once-a-day oral dosage, and have good safety and tolerability. We believe that in addition to a better vaccine than BCG that works in only 15–40% of children and possibly not at all in adults, the following will bring us closer to the TB elimination target by 2050:

i. high-quality early diagnosis and proper treatment in line with the Stop TB Strategy;
ii. implementation of both national and international health-system policies; and
iii. investment in promotion and intensification of both drug and vaccine research (Vinayak Singh, Nicole Cardoso, Stanislav Huszár 2020).

Detecting and treating latent TB infection combined with highly potent prophylactic vaccines will ultimately be needed to eradicate TB. TB control currently depends on the early detection of individuals that spread the disease in the community–individuals with pulmonary TB that have high bacillary loads in aerosols expelled into the air by their cough, sneeze, and even their exhaled breath. Sputum specimens–even a set of a spot and early morning specimen–may be the best option to be used as a gold standard to test novel strategies and technologies, but they are cumbersome and problematic in their own right, as they are cooperation-dependent. Tongue scrapings potentially provide full identification of *M. tb* with molecular methods-based drug susceptibility testing through next generation sequencing. Point-of-care breath tests help in the screening process but fail this full identification that is necessary to confirm the diagnosis and tailor treatment. With potentially toxic drugs like the injectable amikacin and linezolid, therapeutic drug monitoring using limited sampling remains necessary to optimize outcomes and reduce toxicity, but these potentially toxic compounds should be replaced by equally effective but less toxic compounds. Inhaled TB drugs should be further tested and added to reduce the period of time that patients need to be isolated. Duration of therapy will not be likely to be reduced below six months of treatment, but relapses may be further reduced by adding therapeutic vaccinations. Testing vaccines in the context of multidrug-resistant TB provides a platform to bring novel (therapeutic, as well as pre- and post-exposure prophylactic) highly effective vaccines to the market. With timely diagnosis by active case finding, adjuvant surgical treatment will eventually become redundant, but host-directed therapy will continue to be beneficial, based on the detection of genetic polymorphisms that predict an exacerbated, paradoxical inflammatory response, as reported for *Mycobacterium ulcerans* infection. In 30 years from now, we can and will defeat TB, the captain of all men of death (Tjip S van der Werf, Yvette A de Reus, 2020).

Limiting TB incidence, mortality, and the socioeconomic burden has become more challenging with the emerging threats of TB, HIV co-infection, multidrug-resistant TB, and extensively drug-resistant TB. These challenges require sustainable financing, health system strengthening, research and innovations, and leadership to develop a collaborative and accountable environment at all tiers (Sobia Faisal 2020).

Global initiatives in controlling the TB epidemic by employing the revolutionary strategy of directly observed treatment, short-course (DOTS) developed during the latter half of the last millennium have proven instrumental in saving millions of lives worldwide. High TB burden countries, including India and Kenya, have utilized this strategy to reverse the trend in the progression of the epidemic and bring about a significant reduction in the incidence and mortality of the disease. The international initiatives in health since the last decade have maintained a firm focus on social determinants of health, equity, and universal access, which is a promising sign. We envision that the nations of the world which continue to bear the brunt of this age-old epidemic would resolve to translate this international agenda into action by introducing a paradigmatic shift in their TB action plan–from a disease control model to a health promotion model. This could only be achieved through initiatives to address the fundamental health determinants in the population, including poverty eradication, ensuring food security, and provision of better living and working conditions for all, especially the poor and the marginalized. Such a shift would provide the much-needed impetus toward realizing our dream of a TB-free world (GK Mini, Sapna Mishra, Jinbert Lordson, Malu Mohan 2020).

By 2050, the world should have eliminated (less than one case per million population) neglected diseases such as TB according to the sustainable development goals. From the current progress, this seems unlikely, but some progress was in place before the COVID-19 pandemics. Current progress indicates that by 2050, an effective vaccine to prevent new TB infections, much shorter regimens for TB prevention, and treatment of susceptible and drug-resistant TB will be available. Likewise, point-of-care tests for risk of progression from infection to active disease in those born before the vaccine will be accessible to all contacts and other vulnerable populations. M-health applications will be widely used for the prevention of TB reemergence. An effective, short, and safe regimen for drug-resistant TB will be available. Computational intelligence will be used to predict disease and prognosis and interpret and integrate clinical and laboratory information. Researchers will concentrate their efforts on developing innovative health-promoting technologies, and governments will warrant universal access to all innovative technologies. This may include precision medicine approaches, such as genetic evaluation of hosts and pathogens. As shown by the COVID-19 pandemic, continual funding for research and implementation must be a priority (Anete Trajman 2020).

TB is a curable disease, but the problem is the persistence of a huge pool of infected persons, some of which will develop the disease. Prevention has long been neglected under the assumption that the mere treatment of patients with active disease would be sufficient to control the number of cases. It appears that screening exposed persons for infection and preventive treatment of infected persons is a part of the global strategy for the control of TB and will contribute to the expected decline of TB worldwide. New tools for the detection of infection and shorter and more efficient preventive treatment are needed to achieve this in the future (Jean-Pierre Zellweger 2020).

M. tb might be controlled to a great extent, about one case per million that too among the people below the poverty line or with an impaired immune system. It would be possible as:

i. the people shall be aware of the dreadful virulence of *M. tb* and stabilizing the immunity to combat TB by taking healthy foods;
ii. the integrated ligand and structure-based drug design will definitely be useful in developing some potential chemotherapeutics to combat TB including multidrug-resistant and extensively drug-resisatnt TB;
iii. there would be the availability of early detection of TB testing kit and a shorter duration treatment to eliminate dormant bacilli for major impact on TB control;
iv. an effective vaccine for both pre- and post-exposure patients, including those who are HIV-positive, may be available to radically change TB's profile at the mass population-level; and
v. the situations arising out of pandemic COVID-19 shall be well addressed to ensure and safe guard the TB prevention (Sisir Nandi, Mridula Saxena, Anil Kumar Saxena 2020).

8 Challenges

Based on the World Health Organization (WHO) TB control programs and technology developments, as well as gene therapy, there is hope that this historically and cruel illness will be brought under control. But still being researched, the literature has a number of limitations either on disease diagnosis, progression, early diagnosis, treatment, and ideal treatment outcomes with survival. At present, there are challenges to recognize the pathological agent by routine screening. PCR, which is currently being used and has been giving reliable results in clinical samples from suspected TB persons collected in a non-invasive manner. The molecular test, despite being negative for blood, was positive in bone aspirate and urine. Though there are many diagnostic options, the early detection of disease and referral to a specialist service for rapid diagnostic investigation are measures that help with patient prognosis. Moreover, there is lacking consensus, based on well-controlled trials, about dealing with a child with TB (spondylitis, Pott's disease, spinal arthritis, etc.). There are debatable concerns such as the length of therapy, the role of corticosteroids, and when surgery should be recommended (Amer Hayat Khan 2020).

8.1 Pediatric TB

TB has been with humanity since its inception. Over time, there have been advances in the knowledge of pathophysiology, diagnosis, and treatment. On the other hand, new challenges have arisen, including the emergence of AIDS at the end of the twentieth century, the appearance of multidrug-resistant strains of *M. tb* in addition to the huge population contingent most vulnerable to TB who live in

conditions of poverty. The WHO proposes strategies for disease control by 2035 published in the document TB End Strategy. And what to expect from science for the next 30 years, especially in pediatric TB? The emphasis on research involving children is fundamental. The development of a vaccine that prevents all forms of TB would be welcome, but it does not seem easy. It would be useful to have simple and low-cost diagnostic tests that can be highly sensitive to diagnose the paucibacillary forms of active TB that commonly affect children. However, even with the advancement of science, I consider the training and qualification of professionals essential for the rapid diagnosis of the disease, in addition to the urgent need to reduce social inequalities around the world. In this sense, the effective and universal application of health actions that are already known today for the control of TB should deserve attention from all countries (Christiane Mello Schmidt, Claudete Aparecida Araújo Cardoso, Rafaela Baroni Aurílio, Maria de Fátima Pombo Bazhuni Sant´Anna, Clemax Couto Sant´Anna 2020).

8.2 TB—An Opportunistic Infection

With the introduction of more biological agents and their increasing use for a variety of inflammatory conditions, further study is needed to identify and characterize the risks of opportunistic infections like TB. The use of biologics that increase the risk of reactivation of TB, like TNFα antagonists, can hinder efforts to eliminate TB, particularly in more developed countries where its usage is more common. Reducing this risk through improved latent TB infecion diagnostics and testing strategies remains an important goal (Rachel K. Lim, Dina A. Fisher, Stephen K. Field 2020).

8.3 Failure to Meet Commitments by 2035

Despite the tremendous success in TB control, around three million incident TB cases are missed (either undetected or unreported) each year. These missed cases not only increase the existing pool of the diseased but also remain a source of TB transmission in the community. “Find treat and cure” all TB victims is the key to halting the epidemic along with other required preventive approaches. During the past two decades, TB remained on the priority agenda of global public health advocates, which resulted in an average decline of 1.6% per annum in TB incidence from 2000 to 2018. Although the declining trend gives hope, the pace of decline is insufficient to meet the End TB targets and commitments by 2035.

8.4 Non-tuberculous Mycobacteria

Non-tuberculous mycobacteria are an emerging class of uber-resistant pathogens causing high morbidity and mortality worldwide. As the number of clinical TB

infections continues to decrease, their increasing identification is alarming clinicians across the spectrum. The following are fervent wishes pertaining to NTMs:

- Notifiable infections across healthcare systems;
- Urgent discovery, development, and rapid deployment of accurate, sensitive diagnostic tests; and
- Urgent discovery, development, and rapid deployment of potent antimicrobials specific to NTM

These wishes, if implemented, would entail a possible control of the NTM infections, without which these bacteria possess the potential to rapidly overwhelm healthcare systems worldwide (Mohammad Naiyaz Ahmad, Satyaveni Malasala, Nanduri Srinivas, Arunava Dasgupta, Sidharth Chopra 2020).

8.5 Medical Ethics

Informal bioethics was born together with the human race long before the birth of formal medicine. We can see that bioethics, as a formal discipline, has gone through a tremendous evolution: from narrow medical ethics to a broader, global sense of ethics, from the narrow "I" to the broad "we." Traditional bioethics is known for its overwhelming focus on (individual) autonomy. But this has constantly been proved insufficient to meet the health needs of the universe. A broader sense of bioethics has been advocated for since the birth of global bioethics as a topic towards the end of the twentieth century. Therefore, this positive development will continue, and the increasing cultural pluralism will give more opportunities for bioethics to broaden and advance. Instead of contextualizing and globalizing dominant ethical principles, future bioethics will (and must) engage with exploring and illuminating indigenous ethical values. Reasoning based on principles should continue to grow weaker, and relationships should grow to matter. This will give more opportunities for learning and acknowledging others' perspectives. Future bioethics should continue to stimulate interdisciplinary and new ways of thinking about health and illness. This will help the discipline address at least one risk: the risk of collapsing back to medical ethics (Kirubel Manyazewal Mussie 2020).

8.6 Unhealthy Lifestyle

I am not optimistic about the future. Science has to benefit the people. Nowadays, we are suffering from new microorganisms such as COVID-19, global warming complicated by sea-level rise, severe rainstorms, intense typhoons and a significant increase in forest fires, and destruction of the earth, including that of tropical rain forests and coral reefs, among other threats. We have to stop neoliberalism as the

final stage of capitalism. Only when we can change our economic system for people so that they can coexist communally with the earth and its creatures will we see a future for human beings. We can live without consuming so much energy, take advantage of local production for local consumption, and eat more vegetables. Then, we can be healthy without succumbing to adult diseases and cancers and can die peacefully without medicine except for those who really need it. My hope in the future is that we will be able to diagnose diseases without invasive procedures (Yoshinori Kawabata 2020).

8.7 Myths and Stigma

The mission of the WHO to reduce the burden of TB by 95% by the year 2035 is both promising as well as challenging. The next 30 years are likely to see the development of faster diagnostic tests and newer and maybe more potent drugs to treat TB. However, despite this progress, it is unlikely there will be much progress towards the eradication of poverty, social injustice, inequality, and illiteracy. Furthermore, myths and stigma about TB will still be there in one form or another, and so will be the problem of non-compliance and drug resistance. Due to the complexities of issues involved, it seems rather unlikely that TB will be totally eradicated during the next three decades (Alisha Kamboj, Michael Lause, Kamal Kamboj 2020).

Imagine a patient coming from a rural area of some developing country and suspected of suffering from TB. This patient provides a sputum sample to the medical staff, the respective diagnosis is made by WGS, and the presence of *M. tb* is confirmed. In addition, the presence of mutations that confer resistance to rifampicin and isoniazid is identified. This is a new patient but carrier of a multidrug-resistant *M. tb* strain and diagnosed in less than a week. Based on this diagnosis, the patient is provided with individualized treatment, which consists of a cocktail of a new set of antibiotics administered through a controlled-release epidermal patch. The patient is expected to be cured in less than four to six weeks.

Undoubtedly, the integration of the knowledge generated in the last years in the different fields related to the fight against TB and drug-resistant TB will allow the development of significant and innovative advances and the establishment of optimistic scenarios in the medium and long term, as shown in the previous example. The only remaining challenge is to bring these scientific advances closer to the poor, who are the most affected by this disease. Such is the magnitude of the challenge that will face the attention and resolution of this disease, which has accompanied humanity practically since its debut as a society (Damián Pérez-Martínez, Paulina Mejía-Ponce, Cuauhtémoc Licona-Cassani, Everest de Igartua, Gustavo Bermúdez, Diana Viveros, Roberto Zenteno- Cuevas 2020).

9 Treatment

9.1 Inhalation Techniques

For long of its existence, humanity has been plagued with TB. Since the discovery of antibiotics, there has been a reprieve because a cure for latent TB infection and active TB disease was available to patients from all walks of life. As antibiotic resistance has grown in recent decades, the drug-resistant forms of TB are now becoming a bigger problem, along with the co-infection with other diseases such as HIV, HPV, and malaria. The regimens for drug-resistant forms of TB can involve significant doses and harsh side effects. The use of an inhalation technique not only directly treats a pulmonary infection but also can result in a rapid increase in serum. By lowering doses significantly, there exists a chance to use other species, such as the copper ion, to treat the infection. We anticipate that these notions in delivery and composition will evolve over the next many decades (Thomas Manning, Jenu Thomas-Richardson, Courtney Johnson, Krupesh Patel, Yatri Thaker, Govind Thomas-Richardson, Dennis Philips, Greg Wylie 2020).

9.2 Nutrition

TB spread will gradually decline if the proper concentration is given to the nutritional status. And due to the current pandemic situation, the usage of face masks has become an essential aspect of daily life. This face mask usage and proper personal hygiene may alleviate the spread of TB within 2050. Furthermore, nutritional packages and supplementation with vitamins will enhance the immunity status among the susceptible population. Respective authorities must implement strategies to overcome malnutrition in vulnerable populations such as infants, children, and pregnant women. A healthy, nourished status will be ideal for overcoming such infectious diseases. Hence by 2050, there must be an extremely significant level of decrease in TB spread (Vijey Aanandhi Muthukumar, Praveen Devanandan, Ranadheer Chowdary Puvvada 2020).

9.3 Bronchoscopy

Interventional bronchoscopy has been under exponential growth of new ideas, technologies, and inventions for the last two decades. Research progress in this particular area is moving so fast that in the near future, we can expect further development in different directions, including:

i. Endobronchial ultrasound will increase its abilities for precise diagnostics of mediastinal and pulmonary lesions, moving to more and more therapeutic indications for TB and lung cancer;

ii. AI systems, which are now on preliminary clinical assessment, will become irreplaceable assistants for bronchologist, improving diagnostic yield and making the investigation more comfortable both for patient and operator; and
iii. High-definition bronchoscopy systems will be replaced by systems of even higher resolutions (4K-6K-8K or even more) with multiple additional image filters, allowing doctors to detect even the smallest changes in the bronchial mucosa.

Along with an increase in the optical resolution of bronchoscopes, their maneuverability will significantly increase, mostly due to a decrease in the diameter of the endoscope, which will "shed light" on previously inaccessible areas of the bronchial tree and thus further expand possibilities for endoscopic diagnostics and treatment (Ilya Sivokozov, Atadzhan Ergeshov 2020).

9.4 Precision Medicine

The past several years have seen advancements in population pharmacokinetics software and the advent of precision medicine. As technology continues to evolve, a more individualized approach to medical treatment is becoming a reality—particularly in more developed countries with large resources. In the near future, a person's genetics, environmental factors, as well as socioeconomic factors will be combined with her or his clinical attributes to inform clinical judgment for the purposes of customizing a patient's drug treatment. Utilizing a precision medicine approach, combining more precise drug dosing with more efficient drug resistance prediction models will lead to fewer adverse events and an increase in efficacy. The timeframe in which technology will allow for a truly individualized approach depends on a number of unknowns, but the future is promising (Ashlan J. Kunz Coyne, Anthony M. Casapao, Eric F. Egelund 2020).

9.5 Surgery

TB may remain to persist in the new form after three to four decades. The current form of infection may get extinguished; however, newer forms of multidrug-resistant and extensively drug-resistant forms may emerge as the immunosuppressants are used for varied pathologies. Surgery may continue to play a role in the therapy of severe cases; however, minimally invasive modalities of surgical operations may take over the conventional forms of surgery. The ever-advancing pharmaceutical industry and research may bring about a wonder drug that might revolutionize the management of this infection (Ashish Gupta 2020).

9.6 Adjunctive Immunotherapy

We believe that TB will remain one of the major public health problems worldwide due to the high prevalence of drug-resistant TB forms and the HIV/AIDS pandemic. Humanity will search for novel approaches in the development of new anti-TB drugs with bacteriostatic and bactericidal effects on *M. tb*. As a result of their action, super drug-resistant *M. tb* forms will appear. Novel treatment regimens will develop against the background of the emergence of new anti-TB drugs. They will include diverse combinations of anti-TB drugs and various durations of treatment. Such changes in the treatment approaches will be reflected in WHO guidelines and national recommendations in countries where the TB burden remains high. It can be assumed that national guidelines in each country will take into consideration national features of TB epidemiology and personalized approaches to each and every TB patient in combination with effective medical staff training and TB treatment using the most innovative strategies. We are of the opinion that adjunctive immunotherapy can be one of such innovative approaches, which will provide the opportunity to survive patients with severe TB forms and increase treatment effectiveness for TB patients (Dmytro Butov, Valeriy Myasoedov, Anton Tkachenko, Tetiana Butova 2020).

10 Animal TB

For decades, bovine TB eradication efforts have been largely successful in most developed countries. However, significant obstacles threaten to hinder many efforts. Wildlife reservoirs, which transmit *Mycobacterium bovis* to cattle, represent an immense impediment. Eliminating a disease from a wild population presents social and scientific challenges. It is likely that addressing wildlife-cattle transmission will require a vaccine. In view of the vast resources that have been expended in the search for a new human TB vaccine to replace the century-old BCG strain, an efficacious vaccine for either wildlife or cattle is a demanding challenge. However, with ever-expanding vaccine platforms and high technology delivery systems, the challenge is likely surmountable. During the early years of eradication, disease prevalence was high, and most cattle herds were less than 100 head. Today, disease prevalence is low, and herds may number in the tens of thousands. Diagnostic assays and herd disposition practices that worked in the early years are not practical or effective today. The preferred herd disposition (whole herd depopulation) is not financially possible in herds of 20,000 heads or more. Diagnostic tests (or combinations of tests) which are both highly sensitive and highly specific, thus allowing for the removal of all infected animals (and only infected animals) with confidence, will be required. Even today, we see advances in bovine immunology and bacterial genomics that will identify novel targets for creative diagnostic platforms. Improved understanding of disease pathogenesis, host immune response, and pathogen genomics promise to one day bring bovine TB eradication efforts to a

successful conclusion (Mitchell V Palmer, Carly Kanipe, Jason E Lombard, Paola M Boggiatto 2020).

The point-of-care tests ia subject that will undergo extensive development and, in the near future, can bring us very useful analytical tools for clinical practice. The development of new, simple, portable, and accurate diagnostic methods to be used near the patient (in a vet clinic or on a remote livestock farm), in addition to conventional analyzes, can increasingly reduce the time for clinical decision-making. Further up, new and more effective vaccines, individualized treatments and new therapeutic solutions, nanotechnology, robotics, and "big data" software in support (or making) the diagnosis and treatment will be part of the daily life of veterinary medicine. The human veterinarian of the future will have new and fascinating challenges (Hélder Quintas, Justina Prada, Maria da Conceição Fontes, Ana Cláudia Coelho, Isabel Pires 2020).

11 Integrated Science

The initiative taken by Prof. Nima Rezaei, in the form of the Integrated Science book series published by Springer, is a really commendable and a great way of portraying love for science. The work of students, researchers, clinicians, and academicians exhibited in this book series will inspire many more and bring them closer. This book series is a great platform that is growing day by day and eventually will reach great heights. Its success will be considered a yardstick to measure the success of similar ventures by others in coming years. The book series will showcase some of the groundbreaking research by great minds and raise the standards of the Integrated Science book series. The Integrated Science book series will act as a medium of convergence of different workforces from different disciplines to one point of bright illumination, giving away the secrets of science. This is an unstoppable universal force and a boon for the medical fraternity (Sagar Mali, Anushka V Devnikar, Arvind Natarajan 2020).

11.1 Future Epidemics

Although mathematical models can provide some useful predictions relating to new infections and new epidemics, the future remains essentially unpredictable. We have to rely on real-time identification of events, be able to track their course precisely, and respond in real-time. This requires widespread and thorough surveillance systems, global organization, and coordination of local, national, and international health systems. Clinical practice and treatment have to develop new approaches in real-time. The feasibility of a large public infrastructure for pharmaceutical and related biomedical innovations, across the entire drug cycle, through research, development, production, and distribution must be established. There is a worldwide decrease in the number of health professionals. Notably, there are fewer

and fewer experts in infectious diseases. This is particularly true in low and middle-income countries. The teaching of medical microbiology and immunology has to be improved, incorporating advances in education and learning technologies to rekindle interest in the field (Gianluca Quaglio, Damiano Pizzol, Giovanni Putoto 2020).

11.2 Implementation Research

Implementation research (IR) is one of the growing and interesting fields of research because of its important role in knowing the reason behind the success and failure (bottlenecks) of any program. Findings from IR are now used by various programs to effectively implement the interventions and for further scale-up. Many programs implemented either by national and international development partners as well as government bodies are now interested in including IR along with their new programs to know the bottlenecks during implementation and also for exploring the barriers and facilitators for scaling up of the program. To achieve the ambitious goal of controlling TB globally, the importance of conducting IR in the TB program will further increase with time. Thirty years later, IR will be one of the important parts not only of the national TB program but also of all health and public health programs across the globe (Basant Joshi 2020).

11.3 Ophthalmology

The future of ophthalmology is expected to be different from the current landscape. Ophthalmologists will face significant challenges in the way they practice. The current era of technology and innovations will dominate and influence the ophthalmology practice. As expected, tiny, well-designed, and connected instruments and the accompanying apps may make it possible to undertake eye examinations anywhere in the world. It would not be surprising very soon to see unprecedented advances in diagnostic and intervention techniques. Advances in imaging modalities may make it possible to diagnose and treat diseases that were tough to address in the past (Samir S. Shoughy, Khalid F. Tabbara 2020).

11.4 Neurosurgery

The future of neurosurgery will be defined by advanced imaging, neuronavigation with heads-up display, and functional neurosurgical procedures. The degree of spatial understanding and localization of pathology will be much more precise than at present and will create opportunities for decreased disruption of normal tissue with complete eradication of oncologic pathologies. Neuromodulation of key targets in the brain will define neurosurgery, treating many more diseases than are thought possible today. Currently, there are very few targets within the brain for

neuromodulation, with several promising avenues now being developed for neurological disorders. Patients suffering from stroke or spinal cord injury may soon experience neurological recovery of their bodily functions through neurostimulation (Alexander E Braley, Walter A Hall 2020).

11.5 Genomics and Molecular Epidemiology

In the last decade, the use of genomics and molecular epidemiology as a tool to understand the biology of the pathogen and to trace transmission across the host population has been rising exponentially, partially the result of constantly decreasing sequencing prices. It is foreseen that this decrease will continue for the next few years, and in three decades, it is expected that WHS will become a standard approach for any pathogenic agent in public health. This outstanding increment of genomic data conjugated with improved bioinformatics tools will allow, in real-time, to sequence pathogen genomes, trace transmission chains and detect the emergence of strains with specific features such as enhanced virulence and drug resistance. A glimpse of such an approach is visible already, directed at the fight against the current COVID-19 pandemic where, by the time of this writing, the mark of two million sequenced genomes worldwide of the virus responsible for the disease is being achieved. In the case of Mycobacteria-related diseases in a 30 years period, while the control measures of these pathogens pioneered several genomic approaches, they will likely be fully eradicated before the full potential of genomics applied to pathogenic agents is on the verge of being achieved (Teresa Rito, Osvaldo Inlamea, Olena Oliveira, Raquel Duarte, Pedro Soares, Margarida Correia-Neves 2020).

11.6 Integrated Computational Approaches

Integrated computational approaches such as in silico selection of putative metabolite and substrate mimics and further hit selection for designing the inhibitors based on the in vitro activity and transcriptional profiling [13] can accelerate the process. Combining bioinformatics data from databases like TBCyc, SRI's BioCyc collection [14], and Pathway Logic models [15] for *M. tb* are useful to identify new molecules. One of the bottlenecks in drug discovery is the occurrence of drug resistance in *M. tb*. The advent of machine learning approaches and WGS would help in identifying mutations and predicting antimicrobial resistance [16]. Machine learning models use single-point data and dose response data and further combine bioactivity and cytotoxicity data to assist in drug designing of *M. tb*. These methods will also predict the sensitivity and specificity of the designed drugs [17].

Apart from various new technologies, innovative diagnostic tools will also be helpful in the rapid diagnosis of TB infection. The inception of novel treatments like phage therapy due to the fast increase of multidrug-resistant TB could eradicate this deadly pathogen. Phage therapy can be considered as an adjunct to drug

treatment alone or in combination for drug-resistant TB [18] (Ankit Ganeshpurkar, Ravi Singh, Meenakshi Singh, Ashok Kumar, Sushil Kumar Singh 2020).

11.7 AI

The rapid advances in high-throughput tech opened up the integration of a large amount of multiomics data on a single-cell level. Since Alexander Flemings (1928) found the first antibiotic from fungus, nature has become fond of antimicrobial agents. In the past decades, a handful number of new antibiotics have been introduced in the TB field because traditional drug screening methods are extremely labor- and time-consuming. In the future, computer-based in silico simulation and machine learning algorithms will become a popular method to screen novel antibiotics. AI-based drug screen and lead compound designing will be of great potential. Recently, the Collins group announced powerful deep learning in silico-based drug screening platform by which they identified one compound with an unconventional mode of action that fights infections with drug-resistant bugs [19]. The drug screening platform is now experiencing a paradigm shift, aiming at AI-based in silico simulation that will replace in vitro testing and time-consuming high-throughput assays. Ultimate success in the TB drug screening field requires a complete picture of the *M. tb* multiomics makeup. Thus, an AI-mediated drug screening platform necessitates careful integration of multilayers of information, multiomics measurements of the transcriptome, proteome, and metabolome, as well as comprehensive information on available nutrients, lifecycle, and environments. The translation of the *M. tb*-specific dataset into multiomic integration will allow secure data sharing in large databases and strong computational infrastructure, serving as a basis of AI-mediated drug screening platform (Jae Jin Lee, Philip Sell, Hyungjin Eoh 2020).

12 Conclusion

This Chapter presented the authors' views on the situation of TB in relation to tobacco use, drug use, and cancer, along with thoughts about the problem of resistance and drug discovery, highlighting the need for a new vaccine, de novo drug design, OMICs, and a new targeted therapy; pathogenesis involving lung microbiota, mutagenesis, and epigenetics; diagnosis; extrapulmonary TB; prevention, elimination, and control; challenges, such as pediatric TB, TB as an opportunistic infection, failure to meet commitments by 2050, non-tuberculous mycobacteria, medical ethics, unhealthy lifestyles, myths and stigma; treatment options, e.g., inhalation techniques, nutritional strategies, bronchoscopy, surgery, precision medicine, and adjunctive immunotherapy; animal TB; integrated science of TB, comprising future TB-related thoughts of epidemics, implementation research, genomics and molecular epidemiology, integrated computational approaches, and AI.

Core Messages

- TB is a complex disease.
- Integrated studies are required to tackle the challenge of TB in relation to tobacco use, drug use, cancer, and HIV.
- Integrated studies can promote drug discovery and understanding the TB pathogenesis using OMICS technologies.
- Integrated studies should be globalized to increase the chance to meet commitments to eliminate TB by 2050.
- Integrated studies of TB mainly involve implementation research, genomics and molecular epidemiology, and computational approaches.

References

1. Tripathi RP, Tewari N, Dwivedi N, Tiwari VK (2005) Fighting TB: an old disease with new challenges. Med Res Rev 25(1):93–131
2. Patil K, Bagade S, Bonde S, Sharma S, Saraogi G (2018) Recent therapeutic approaches for the management of TB: challenges and opportunities. Biomed Pharmacother 99:735–745
3. Ginsberg AM, Spigelman M (2007) Challenges in TB drug research and development. Nat Med 13(3):290–294
4. McNerney R, Maeurer M, Abubakar I, Marais B, McHugh TD, Ford N et al (2012) TB diagnostics and biomarkers: needs, challenges, recent advances, and opportunities. J Infect Dis 205(suppl_2):S147–S158
5. Philipp WJ, Poulet S, Eiglmeier K, Pascopella L, Balasubramanian V, Heym B et al (1996) An integrated map of the genome of the tubercle bacillus, Mycobacterium TB H37Rv, and comparison with *Mycobacterium leprae*. Proc Natl Acad Sci 93(7):3132–3137
6. Porter JDH, Ogden JA, Pronyk P (1999) The way forward: an integrated approach to TB control. In: TB: an interdisciplinary perspective, pp 359–378
7. Bottasso O, Bay ML, Besedovsky H, del Rey A (2009) Immunoendocrine alterations during human TB as an integrated view of disease pathology. NeuroImmunoModulation 16(2):68–77
8. Zhang L, Xing W, Zhou J, Zhang R, Cheng Y, Li J et al (2020) Characteristics of TB patients in the integrated TB control model in Chongqing, China: a retrospective study. BMC Infect Dis 20(1):1–8
9. Kim YK, Lee KS, Kim BT, Choi JY, Kim H, Kwon OJ et al (2007) Mediastinal nodal staging of nonsmall cell lung cancer using integrated 18F-FDG PET/CT in a TB-endemic country: diagnostic efficacy in 674 patients. Cancer: Interdisc Int J Am Cancer Soc 109(6):1068–1077
10. Gupta A, Bhakta S (2012) An integrated surrogate model for screening of drugs against Mycobacterium TB. J Antimicrob Chemother 67(6):1380–1391
11. Belani H, Chorba T, Fletcher F, Hennessey K, Kroeger K, Lansky A et al (2012) Integrated prevention services for HIV infection, viral hepatitis, sexually transmitted diseases, and TB for persons who use drugs illicitly: summary guidance from CDC and the US Department of Health and Human Services. Morb Mortal Wkly Rep 61(5):1–43
12. Reddy TBK, Riley R, Wymore F, Montgomery P, DeCaprio D, Engels R et al (2009) TB database: an integrated platform for TB research. Nucleic Acids Res 37(suppl_1):D499–D508
13. Ekins S, Madrid PB, Sarker M, Li S-G, Mittal N, Kumar P et al (2015) Combining metabolite-based pharmacophores with Bayesian machine learning models for mycobacterium TB drug discovery. PLoS ONE 10(10):e0141076

14. Karp PD (2001) Pathway databases: a case study in computational symbolic theories. Science 293(5537):2040–2044
15. Talcott C (2006) Symbolic modeling of signal transduction in pathway logic. IEEE, pp 1656–1665
16. Deelder W, Christakoudi S, Phelan J, Benavente ED, Campino S, McNerney R et al (2019) Machine learning predicts accurately Mycobacterium TB drug resistance from whole genome sequencing data. Front Genet 922
17. Kouchaki S, Yang Y, Walker TM, Sarah Walker A, Wilson DJ, Peto TEA et al (2019) Application of machine learning techniques to TB drug resistance analysis. Bioinformatics 35 (13):2276–2282
18. Dedrick RM, Guerrero-Bustamante CA, Garlena RA, Russell DA, Ford K, Harris K et al (2019) Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant *Mycobacterium abscessus*. Nat Med 25(5):730–733
19. Stokes JM, Yang K, Swanson K, Jin W, Cubillos-Ruiz A, Donghia NM et al (2020) A deep learning approach to antibiotic discovery. Cell 180(4):688–702

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