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Infectious Diseases-Drug Delivery Systems

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Editors

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Preface

The Washington Post of 2016 stirred an intense discussion between the public, scientific communities, and health authorities. How many diseases are precisely known to humankind? At the moment, scientist estimates the presence of more than 10,000 human diseases and only fewer available treatments that too for major diseases.[1]

In 2022, the scenario is not far different considering the deliberate speed of academic/industry research, economic up-downs, tougher regulatory policies, complex clinical trial setups, the impact of the Covid-19 pandemic in slowing processes, businesses, and changing world political dynamics and policies. The same question on "availability of effective treatment" is valid now and maybe even for the next 2–3 decades.

Diseases can be genetic or caused by environmental factors (mainly known as infectious diseases). Human infectious diseases are typically classified according to the source of infection as anthroponoses (human–human transmission), zoonoses (animal–human transmission), and sapronoses (abiotic decaying substrate—human). These infectious diseases contribute to the enormous financial burden on the country's economy. By 2001, around 1415 species of organisms had been recorded known to be pathogenic to humans, mainly comprised of bacteria, viruses/prions, fungi, protozoa, and helminths.

This book is a trivial attempt to compile all possible and available information on etiology, pathology, current therapy options available for a wide spectrum of diseases, the role of drug delivery sciences, advances in new techniques, diagnostic tools, and new drug research of various infectious diseases.

Total **four volumes** are compiled to accommodate vast available information.

Volume 1—Malarial drug delivery systems (MDDS)
Volume 2—Tubercular drug delivery systems (TDDS)
Volume 3—Viral drug delivery systems (VDDS)
Volume 4—Infectious diseases-drug delivery systems (IDDS)

[1] Are there really 10,000 diseases and just 500 'cures'? – The Washington Post. https://www.orpha.net/

Volume 1: MDDS

Malaria is a disease caused by the parasite Plasmodium. The parasite spread to humans through the bites of infected mosquitoes causing high fever, nausea, vomiting, diarrhea, body pain, rapid heart rate, and shaking chills. Each year millions of people get infected by malaria, and many hundred-thousand people die. Some of the most significant risk areas include Sub-Saharan Africa, South and Southeast Asia, Pacific Islands, Central America, and Northern South America. The treatment of malaria mainly comprises the most common antimalarial drugs like chloroquine, primaquine, etc. In the case of drug resistance, artemisinin-based combination therapies (ACTs) are preferred. ACT is an amalgamation of two or more drugs that work against the malaria parasite using a different mechanism of action.

Volume 2: TDDS

Tuberculosis (TB) is a potentially severe infectious disease that affects the lungs and, in some cases, the kidney, spine, and brain. *Mycobacterium* causes tuberculosis via air route. As a result, two TB-related scenarios are possible: latent TB infection (LTBI) and TB disease. If not treated properly, TB disease can be fatal. TB bacteria usually grow in the lungs (pulmonary TB). The typical test used to diagnose TB is the Mantoux tuberculin skin test (TST). The medications used to treat latent TB infection include Isoniazid, Rifapentine, and Rifampin. Classically, the patient may undergo several treatment regimens (1st/2nd /3rd line) recommended as per disease condition and health policy of that specific country. TB treatment can take 4, 6, or 9 months depending on the regimen.

Volume 3: VDDS

Viruses are very tiny infectious germs, which cause infectious diseases such as the common cold, flu, and wart to severe illnesses such as HIV/AIDS, Ebola, and Covid-19 (which caused the recent pandemic where millions of people lost life). They invade living, normal cells and use those cells as host. Depending upon the type of virus, the target body cells are different. Virus infections and diseases are categorized under ten other groups, i.e., contagious, respiratory, gastrointestinal, exanthematous, hepatic, transmission, cutaneous, hemorrhagic, neurologic, and rest of the viruses not in these categories. All viruses have a protein coat and a core of genetic material, either RNA or DNA; unlike bacteria, viruses can't survive without a host. The diagnosis of viral diseases/infections can be performed by viral culture, serological tests, virus antigen detection, and viral nucleic acid or antibody detection. The treatment of viral diseases/infections depends on the type of viral infection. Antibiotics

do not work for viral infections. FDA has already approved several antiviral medicines for the treatment of certain illnesses.

Volume 4: IDDS

Each infectious disease has its specific signs and symptoms. Diagnosis of infectious diseases needs lab testing. Samples of body fluids, e.g., blood, urine, saliva, etc., can reveal evidence of the particular microbe that is causing the illness. While imaging, scans using X-rays, computerized tomography, and magnetic resonance imaging can help pinpoint disease states. Often, local tissue biopsies provide helpful information on the state of infection and adverse observations of disease (if any). This volume is focused on diagnosis, detection, disease models, the link between two or multiple infectious diseases, and vaccine development for the treatment of infectious diseases

This book series compiles all the new treatment avenues that have been explored to treat malaria, tuberculosis, viral infections, and other infectious diseases like Ebola and hepatitis. This series covers various aspects of drug delivery advances for disease targeting, new drug molecules, analysis of currently ongoing clinical trials, vaccine development, and availability of disease models to evaluate drug performance. Dedicated chapters are included on herbal treatment opportunities for each disease. In addition, readers can refer to information on global disease health scenarios, cellular pathophysiology, and drug resistance, full coverage on polymeric nanoparticles, solid lipid nanoparticles, dendrimers, liposome, and micro/nanoemulsions as drug delivery carriers.

Experts from all over the world have shared their knowledge to generate this one-stop resource. This book series is destined to fill the knowledge gap through information sharing and organized research compilation between the diverse area of pharma, medicine, clinical, chemist, and academics to fulfill following specific objectives:

- To discuss opportunities and challenges in the treatment of infectious diseases
- To enlist current efforts by researchers and experts
- To facilitate the insight and knowledge sharing
- To highlight innovative, cutting-edge micro and nanotechnology research
- To establish collaborations between academic scientists, industrial, and clinical researchers

In summary, we are sure this book series will provide you great insights into drug delivery sciences (conventional, micro-nanomedicines, upcoming drug delivery trends) along with updates on clinical and chemical drug research for the treatment of infectious diseases.

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New Malarial Drugs in Synthesis Research

Phoebe Gallagher and Charles Preuss

Abstract Malaria, caused by infections of the *Plasmodium* parasite, has increased in incidence, particularly in southeast Asia and Africa, due to the development of increased resistance against artemisinin drugs. Prior to the discovery of artemisinin-based drugs, chloroquine was the top choice for the treatment of the disease. However, mutations within the parasite have allowed it to develop several mechanisms that reduce the effectiveness of this drug, along with many of the newer drugs. By studying the parasite's internal processes, life cycle, and resistance mechanisms, researchers have discovered different drug targets to combat the disease. Several types of active compounds are currently being studied for their effect against different *Plasmodium* strains, including synthetic drugs and natural drugs. Plants and marine species are the main sources of naturally derived antimalarial compounds explored in this chapter. Many of these compounds show significant activity against different drug targets and have been determined to show potential for the formation of novel antimalarial drugs.

Keywords Marine-derived compounds · Terrestrial antimalarials · *Plasmodium falciparum* resistance · Synthetic antimalarial compounds

1 Introduction: The *Plasmodium* spp. Parasite

Malaria is a parasitic disease caused by six species of the *Plasmodium* spp. parasite including *P. falciparum*, *P. vivax*, *P. ovale wallikeri*, *P. ovale curtisi, P. malariae*, and *P. knowlesi* [1]. Understanding the life cycle of the parasite is vital to

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understanding how to treat it. Within an infected *Anopheles* mosquito, male and female gametocytes produce gametes which allow for sexual reproduction and the formation of an oocyst which then releases thousands of sporozoites into the saliva of the mosquito. Upon receiving a bite from an infected mosquito, the sporozoites enter hepatocytes of the vertebrate and replicate as schizonts, after which they are released into the bloodstream where they invade erythrocytes. Within the red blood cells, they undergo asexual replication to form mature schizonts that rupture and release merozoites which go on to infect other red blood cells [2]. *P. falciparum* is the strain that causes most human infections and death; however, *P. vivax* and *P. ovale* have a dormant hypnozoite stage in the liver which puts those infected with these species at risk for relapsing malaria [1]. In the past, chloroquine was a common drug used to treat malaria, but as resistance to the drug has increased, movement toward artemisinin-based therapy (ACTs) has been favored [1]. These drugs have many different possible mechanisms of action including activation by heme to produce free radicals, inhibition of phosphatidylinositol-3-kinase, and upregulation of the unfolded protein pathway. These all act to either inhibit growth of the parasite by altering signaling/protein pathways or by damaging the cells of the parasite directly [3]. More recently, artemisinin resistance has risen particularly in southeast Asia and Africa, which has pushed researchers toward the pursuit of novel alternative treatments for the disease [1]. Figure 1 depicts the life cycle of the malarial parasite in humans (Adapted from [4]).

2 Search for Innovative Antimalarial Drugs

New antimalarial drugs were initially being pursued for their ability to be a single-dose cure for the disease. Since many tropical areas have high rates of malarial transmission, many patients are diagnosed with malaria several times in their lifetimes. Because of this, single-dose drugs that are safe for repeated dosing are preferred. For novel drugs to be deemed effective for the treatment of the disease, their pharmacokinetic properties must also be ideal. A drug with good absorption and slower elimination is favored since slowly eliminated drugs can yield a prophylactic effect post-treatment. Ideally, a drug should be active orally against the *Plasmodium* spp. parasite for 1–3 days [1].

In addition to the above qualities, drugs that are cost-effective and maintain good safety profiles are being developed, especially for pregnant women and children. Because this is an important aspect in the search for antimalarial treatment, many existing drugs that hold good safety profiles are being repurposed for prevention or treatment of the disease. Antimicrobials like sulfamethoxazole/trimethoprim (also known as Cotrimoxazole), typically used in HIV patients to prevent *Pneumocystis jirovecii* infections, are given as a prophylactic antimalarial treatment for pregnant women and children due to its safe and effective nature. While the development of

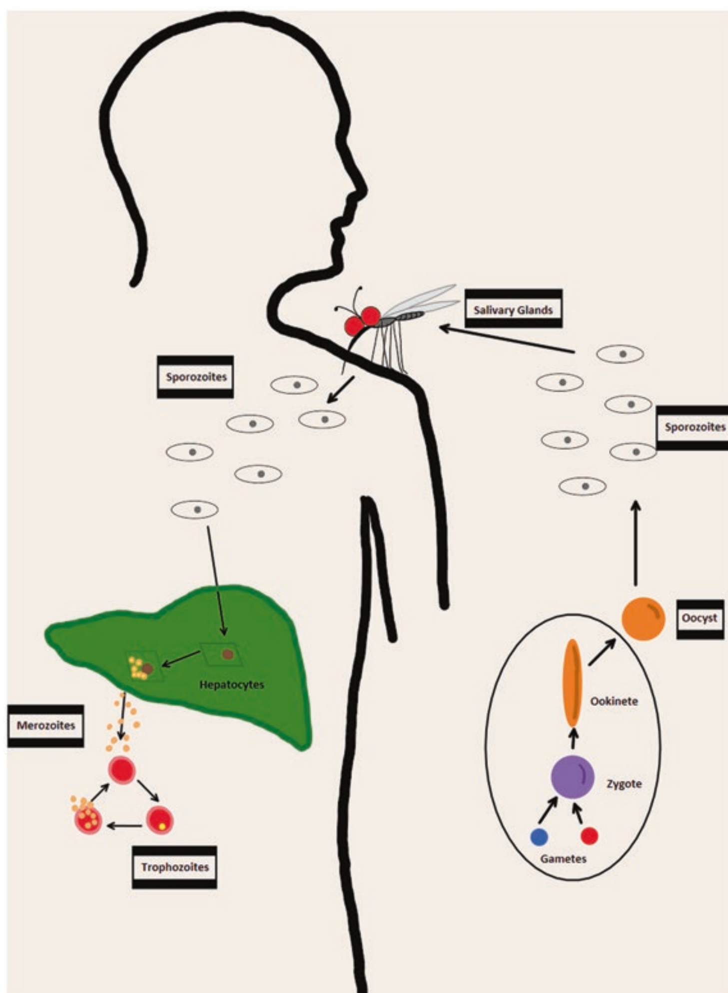

Fig. 1 The Malaria Life Cycle. (Adapted from [4])

novel drugs that can treat already infected individuals is a major goal for researchers, novel prophylactic drugs are also being developed with DSM-265 being an example of one discussed later in this chapter.

Drugs that can inhibit multiplication and affect multiple stages of the *Plasmodium* spp. life cycle are viewed as favorable for development since killing the *Plasmodium* spp. parasite at multiple stages can reduce infection and killing hypnozoites, which

cause relapse infections and can help further reduce the patient's risk for relapse. One such drug that has been co-administered as a single dose with other treatment drugs to reduce relapse is tafenoquine, an 8-aminoquinoline which works against the asexual and hypnozoite stages of *P. vivax* to prevent relapse. However, the search for relapse-preventing drugs has been made difficult due to the difficulty in finding appropriate models that can accurately measure hypnozoite susceptibility to different drugs.

Due to malarial drug resistance, more emphasis has been given to drugs that can prevent initial disease and relapse while also avoiding resistance. Resistance-proofing novel drugs has not only been performed through the selection process by choosing targets that have slower mutation rates, but also by combining drugs to further minimize risk of resistance. Drug combinations ideally have fixed dose combinations and pediatric dosage formulations. Additionally, they ideally have similar half-lives so that the slower-eliminated drug is not left unprotected from resistance mechanisms [1]. Combining drugs can also assist with delivering the drug to the target molecule by overcoming resistance mechanisms to increase the effectiveness of the treatment [3]. By combining two drugs with different mechanisms of action, the probability of developing resistance for either drug is reduced should a parasite resist treatment and survive. Since the probability of a genetic mutation selecting for resistance to both mechanisms together is significantly reduced, this decreases the likelihood that resistance will develop for either. For this reason, ensuring that these drugs have similar pharmacokinetics and elimination half-lives is important to ensure that they stay protected from resistance [4]. Similarly, the formation of hybrid molecules is another process used to combat resistance which involves combining two synergistic molecules that have different targets [1].

When it comes to finding novel drugs, researchers must investigate molecules that can target certain biological processes to kill the *Plasmodium* spp. parasite. Quinine, the first molecule discovered to treat malaria, was originally discovered from the bark of the Cinchona tree (found in South America) and the synthetic derivatives of this molecule were then created to form more quinoline drugs. Synthetically derived drugs are a potential solution to the discovery of novel antimalarials but are limited in the amount of existing synthetic libraries and combinations of chemical reactions required to make new potential drugs. As a result, the production of synthetic drugs is viewed less favorably compared to using biological molecules derived from existing organisms instead. Discussed later in this chapter, many marine animals provide a source for these potentially useful biological molecules [5].

3 Resistance Mechanisms

The need for novel drugs stems from the fact that many current antimalarial drugs that have been successful in the past have fallen victim to resistance mechanisms by the *Plasmodium* spp. parasite [6]. With chloroquine being one of the first

synthetically derived alkaloids that was used for the treatment of malaria, it soon became one of the first to display resistance in *Plasmodium falciparum.* Because of this increase in resistance, measures were taken to develop alternative antimalarial drugs including artemisinin, sulfadoxine-pyrimethamine, and mefloquine. However, several strains of the parasite have eventually developed resistance to these alternative treatments and combination drugs have been recommended for treatment [7].

Understanding resistance mechanisms is important since it allows scientists to find alternative treatments that can bypass these mechanisms and assert their effects. Drug-resistant strains are determined by measuring the shift in their IC_{50}, i.e., the drug concentration necessary to inhibit parasitic activity by 50% and comparing it to sensitive strains. Typically, there is a 20-fold increase in the IC_{50} of resistant strains compared to sensitive strains. Currently, there are only a few resistance mechanisms involving genetic mutations that are known to researchers. Among these include mutations in antifolate targets such as dihydropteroate synthase (*pfdhps*), dihydrofolate reductase (*pfdhfr*) along with electron transport inhibitors such as cytochrome bc1 (*pfcytb*). Additionally, genetic mutations led to the formation of transporters such as the chloroquine transporter (*pfcrt*) and multidrug resistance 1 (*pfmdr1*) which have also allowed for resistance to form to certain antimalarial drugs, including chloroquine which is thought to prevent heme detoxification. Mutations in dihydrofolate reductase have allowed for the parasitic folate pathway to progress in the presence of antifolate drugs pyrimethamine and cycloguanil while mutations in dihydropteroate synthase have a similar effect on sulfadoxine.

Different strains of *Plasmodium* spp. exhibit different genetic mutations that have led to the formation of these resistance mechanisms. The V1/S strain has mutations in *pfdhfr* that leads to high resistance against cycloguanil and pyrimethamine. Strains Dd2 and FCB have genetic mutations that have allowed for increased copy numbers of *pfmdr1* which has made it easy for develop resistance for mefloquine and artemisinin-derived drugs [6]. The following strains of *P. falciparum* are frequently used in drug discovery research to determine the potential of new drugs: the chloroquine-sensitive strains TM4, D10, 3D7, PoW, and D6 along with the chloroquine-resistant strains W2, Dd2, FcB1, and FCR-3. Lastly, the multidrug-resistant K-1 strain is also used to determine the effectiveness of new potential drugs [8]. Figure 2 highlights mechanisms of resistance to antimalarials (Reproduction permission [6]).

4 Potential Drug Targets

Molecules that target different aspects of the *Plasmodium* spp. life cycle are being looked at as possible drugs to combat malaria. Specifically, proteases responsible for playing a vital role in the *Plasmodium* spp. life cycle are being targeted. These include cysteine proteases, aminopeptidases, and aspartic proteases (also known as plasmepsins). Current research is looking into plasmepsin V inhibitors along

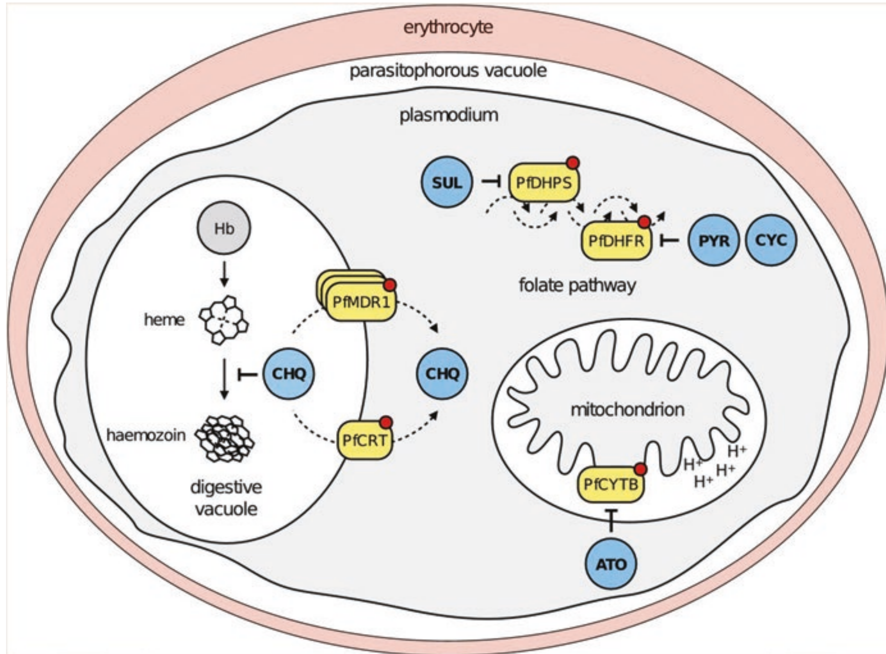

Fig. 2 Resistance mechanisms to antimalarials [6]

with drugs that target plasmepsins IX and X, whose functions in the *Plasmodium* spp. life cycle have recently been discovered. Another potential drug target that is being investigated is the Na + -ATPase 4, which has been the target for drugs like cipargamin, SJ557733, and 21A092. Inhibitors to other enzymes relevant to *Plasmodium* spp. processes including phenylalanyl-tRNA synthetase, histone deacetylase, hypoxanthine-guanine-(xanthine) phosphoribosyltransferase, and N-myristoyltransferase are also being investigated [1].

Replication inhibitors are a popular target for drug development and include ozonides like OZ439, imidazolopiperazines like KAF156, and spiroindolone cipargamin such as KAE609. Inhibition of pyrimidine synthesis is a popular target for drug development. DSM-265, a drug currently in Phase 2 development, acts as a dihydroorotate dehydrogenase inhibitor in both the blood and liver stages of the *Plasmodium* spp. life cycle in humans [1].

Antimalarial drugs can target an assortment of different targets in the *Plasmodium* spp. parasite. One of these is elongation factor 2 (EF-2), which is expressed in many of the life cycle stages of the parasite. It is involved in the translocation of tRNA within the ribosome during protein synthesis. M5717 is a new drug discovered which inhibits PfeEF2; however, resistance has been found in strains 3D7, 7G8, and Dd2 due to mutations in the genes responsible for encoding elongation factor-2 [3]. This drug also has the potential to affect the parasite at different stages, including its blood stages, pre-erythrocytic stages, and female and male mature gametocytes.

Along with these characteristics, it is also a single-dose treatment that costs less than $1 (US dollar) which makes it a promising drug currently in development [1].

The *Plasmodium* spp. parasite requires a low intracellular sodium concentration to survive. To help it maintain these low sodium concentrations, it requires a P-type ATPase transporter that pumps sodium out of the cell. By inhibiting this transporter, it causes a build-up of sodium within the cell which kills the parasite. (+)-SJ733 and KAE609 both inhibit the PfATP4 transporter responsible for this function, although there is no evidence that these compounds bind to the transporter themselves. The sodium-proton antiporter is another transporter that is responsible for decreasing the sodium concentration within the cell; however, its mechanism causes it to bring in protons as a result. However, in order for the pH to remain a consistent 7.3 within the parasite, the V-type H+-ATPase is necessary for removing excess protons. MMV253 is a drug that targets this V-type H+ ATPase and inhibits it, causing the *Plasmodium* spp. parasite to die [3]. Currently, its goal is to become a single-dose radical cure [1].

Phosphatidylinositol 4-kinase (PI4-K) is another possible drug target since it works to regulate intracellular signaling and trafficking by phosphorylating lipids. UCT943 and MMV048 both inhibit PI4K by interfering with its ATP binding region and disrupting the intracellular distribution of PI4-phosphate; however, MMV048 was disqualified as a candidate due to its toxicity. UCT943 drug is more useful in targeting early parasite development but has decreased effectiveness in late-stage parasite development [3].

P. falciparum requires pyrimidines to replicate. Therefore, it requires oxidized dihydroorotate to make orotate as a precursor to the formation of pyrimidines. The enzyme responsible for this step is known as dihydroorotate dehydrogenase, which can be inhibited to stop replication of the parasite within erythrocytes. DSM265 is a promising drug in development that targets this enzyme and is even capable of working on chloroquine-resistant strain D10. Another requirement for parasite replication is folate. *Plasmodium* spp. has the dihydrofolate reductase enzyme (pfDHFR) that helps recycle folate which helps synthesize purines, thymidylate, and methionine. P218 is a new drug in development that, like cycloguanil and pyrimethamine, acts to target and inhibit pfDHFR [3]. Figure 3 highlights the mechanism of action of antimalarials (Reproduction permission, [1]).

5 Synthetic Drugs in Development

M5717, developed in 2015, can affect the *Plasmodium* spp. parasite through a new mechanism of action. The identification of a novel M1 compound led to the development of the improved M2 and M3 precursors, which were eventually modified into their most potent form known as M5717. By modifying the precursors through replacement with a fluorine group, ethylpyrrolidine, and a morpholine fragment, the compound was engineered to have activity against the 3D7 strain in addition to other drug-resistant strains at equal potency against all life cycles of the *Plasmodium*

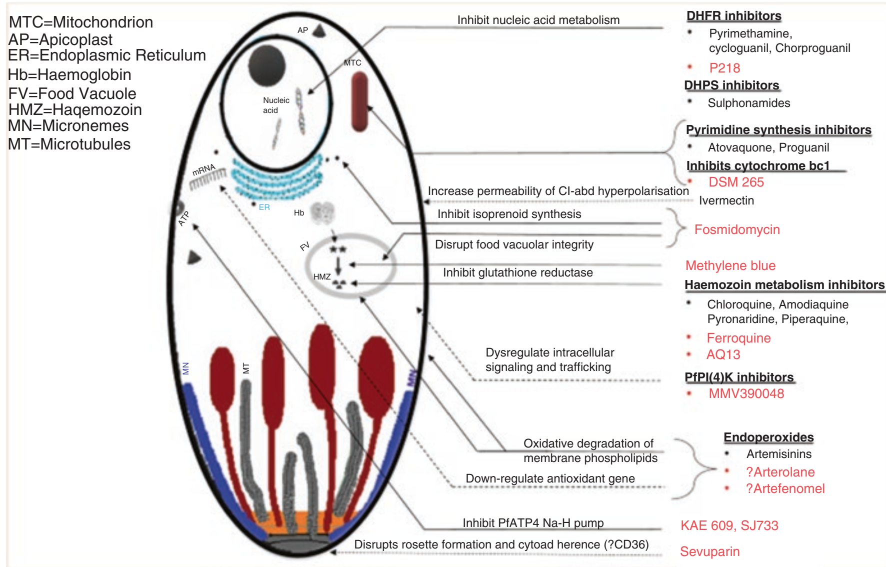

Fig. 3 Antimalarial mechanisms of action [1]

spp. It works through inhibition of PfoEF2. It has been deemed to be as effective as current antimalarial drugs after trials with *Plasmodium berghei* mouse model using dosages 120 mg/kg p.o. q.d. It can clear the blood stage completely and it can be used in a long duration in combination with other fast-acting compounds. It also shows promising results with chemoprotection and late-stage transmission.

MMV253, a new triaminopyrimidine (TAP), has demonstrated viral potency in the past as well as potential efficacy as an antimalarial. It originated by M'1 an identified TAP that is formed from inhibition of herG which was then optimized to increase solubility and potency, thus allowing it a 36-hour half-life. The mechanism of action of this drug is through inhibition of pfATP4.

From the class of 2-aminopyrazine comes UCT943, a potential antimalarial drug with a single-dose curing ability at a 4 × 10 mg/kg dosage with ED90, i.e., the dose that produces an antimalarial effect in 90% of the experimental population, of 1 mg/kg. The original 3,5-diaryl-2-aminopyridine series yielded the U1 compound which was potent against the NF54 strain, but due to low solubility and high metabolic clearance, needed to be altered. Through the replacement of the hydroxy and methoxy groups with a trifluoromethyl group, the U2 version was formed. However, this change led to further decreases in the solubility and thus the mesyl group was replaced with piperidine carboxamide to form the U3 compound. To further improve it to its final form (UCT943), a nitrogen atom was added to the pyridine ring. This potential drug is potent across the many life stages of both *P. vivax* and *P. falciparum* and works by targeting its phosphatidylinositol-4-OH kinase (pfPI4K).

AN13762 comes from the benzoxaborole class of antimalarials and has a multi-strain efficacy. It was initially derived from AN3661 which was discovered to be a

potent compound against the 3D7 strain by targeting its pfCPSF3. To increase its potency, its alkylcarboxylic acid chain was moved and pyrazine ether was put in its place which gave rise to A2. However, this alteration resulted in an increased clearance rate. By replacing the ester with an amide group, the A3 form was produced which had increased stability and bioavailability but reduced the potency. By converting the primary amide into a tertiary cyclic amide and adding a methyl group on benzoxaborole, the final form (AN13762) was formed which allowed it to maintain its metabolic stability while increasing the potency. Despite the familiarity with the mechanism of action for the AN3661 form, AN13762's mechanism is still unknown. Due to its lack of cytotoxicity to humans up to 100 mM, it shows good drug potential and has now entered the preclinical phase of drug development [3].

SC83288, an amicarbalide derivative, is a fast-acting treatment given via IV or through parenteral administration for severe malaria that is highly potent against drug-resistant strains at an $IC_{50} < 20$ nM. It also works against early stages of gametocytes at an $IC_{50} = 199$ nM; however, it is not effective against late stages of gametocytes. It was identified as a good alternative for artemisinin since it has no cross resistance with those drugs and can be used in conjunction with slow-acting drugs for better results [3]. In fact, it was found to have a 10-fold reduction in *Plasmodium* spp. parasite per life cycle compared to parenteral administration of artesunate [1]. It was initially discovered that amicarbalide (S1) can dock into the lactate dehydrogenase of *P. falciparum* which made it highly potent against Dd2 strains. Through replacement of the amidine group with a sulfonamide linker, DNA binding effects were minimized and improvement in solubility and metabolic stability resulted to produce S2 version. To increase potency and solubility, the second amidine group was converted into a piperazine ring which further increased potency and solubility but resulted in poor permeability. Lastly, SC83288 was produced by converting the butyl chain into an acetyl group to make this final form more permeable and effective. This new compound was shown to not exhibit cytotoxicity, genotoxicity, or hERG binding when tested on the P. vinckei malarial mouse model, allowing it to be safe and effective for curing these infections at a dose of 80 mg/kg. Unfortunately, it is inactive against *P. berghei*. Its mechanism of action is unknown, but it is hypothesized that pfATP6 is indirectly associated and that PFMDR2 plays a role in the resistance mechanism for this parasite [3].

Chloroquine-resistant *P. falciparum* strains have been difficult to treat due to mutation in PfCRT, a transporter which pumps chloroquine out of the parasite. Chloroquine typically works by binding heme and inhibiting beta-hematin formation. Reversed chloroquine class drugs combine a chloroquine core with a reversal agent such as imipramine and have shown promising results for overcoming this chloroquine resistance. Imipramine and other reversal agents serve to inhibit this transporter so that the parasite cannot pump out the chloroquine. Of this class, new drug DM1157 has been developed to overcome this resistance. Initially, its precursor, D2, was developed by combining a chloroquine core with imipramine to make the first agent in this class of drugs. However, D2 suffered from poor bioavailability and stability. By substituting imipramine with 1-(2,2-diphenylmethyl) piperazine, the resulting product was made to be more stable. However, this change resulted in

a high partition coefficient which reduced its solubility in water. Converting the piperazine into aminopiperidine and the two phenyl groups into pyridines led to a more potent and hydrophilic compound, DM1157, which binds more strongly to heme to inhibit beta-hematin formation. DM1157 can be administered orally or subcutaneously, with an effective oral dose of 4 × 30 mg/kg when used in mice which lead to two-thirds of the mice being cured within 30 days of infection. It has been proven effective in multidrug-resistant strains of *P. falciparum* and *P. vivax* in Indonesia [3].

Discussed previously, SJ733, a single-dose dihydroisoquinoline that functions to inhibit PfATP4, can affect the blood schizont stage of both *Plasmodium falciparum* and *P. vivax* while also inhibiting gametocytogenesis. With a half-maximal effective concentration in the 10–60 nM range and no toxicity seen in animal models, even with a given dose 43 times the effective dose, it is being considered as a promising antimalarial drug candidate [1].

Also discussed in the above section are KAF156 and KAE609 whose synthesis is described here. The compound K1 was initially discovered as a compound with antimalarial potential that contained benzodioxole and phenyl groups that were changed to 4-fluorobenzene to form the compound K2 to increase the compound's potency. The addition of a dimethyl group and removal of the glycine residue from the piperazine ring on K2 created the new compound KAF156 which had significantly more potency and efficacy compared to the original compound, K1. In vivo studies with mice infected with *P. berghei* showed that the compound blocked transmission of the parasite when mice were given an oral dose of 10 mg/kg prior to infection. Another promising compound identified was K'1, a spiroazepineindole that had efficacy against the K1 and NF54 strains of the parasite. Being a racemic compound, K'1 underwent chiral separation and further studies discovered that the 1R, 3S enantiomer was significantly more potent than the other enantiomer. Named K'2, this enantiomer underwent further alterations, including the reduction of its ring size and a Pictet–Spengler reaction, which yielded the final product: KAE609. This compound was noted to be as effective at treating *P. falciparum* and *P. vivax* as artesunate, while maintaining low cytotoxicity [3]. Table 1 summarizes the characteristics of synthetic antimalarial compounds.

6 Naturally Derived Antimalarials

The synthetic library is one of the major ways in which antimalarial compounds are produced for the purpose of drug development. However, many of these substances require many steps for their synthesis which further complicates their creation and increases the cost of development. Many researchers have therefore turned toward nature in their search for antimalarial compounds since many plants, animals, and bacteria produce substances with antimalarial properties which could make them easier and cheaper to mass produce.

Table 1 Synthetic antimalarial compounds

Drug name	Drug type	Mechanism of action	Stage specificity	Plan for development	Side effects	Clinical status
M5717	Quinoline-4-carboxamide	Inhibits EF-2	Multi-stage	Single-dose treatment	–	Preclinical
UCT943	2-Aminopyrazine	PfPI(4)K inhibitor	Blood schizont	Treatment and prophylaxis	–	Preclinical
AN13762	Benzoxaborole	Unknown	Blood schizont	Single-dose cure; prophylaxis	–	Preclinical
SC83288	Amicarbalide derivative	Hypothesized association with pfATP6	Blood schizont	Treatment for severe malaria	–	Preclinical
DM1157	Reversed chloroquine	Hemozoin metabolism inhibitors	Blood schizont	Single-dose cure	–	Preclinical
SJ733	Dihydroisoquinoline	Inhibits PfATP4	Blood schizont and inhibits gametocytogenesis	Single-dose cure	–	Clinical
KAE609	Spiroindolone	PfATP4 inhibitor	Blood schizont	Combine with long-acting drug that is not susceptible to PfATP4 mutation	Hepatotoxicity	Clinical
MMV253	Triaminopyrimidine	ATPase inhibitor	Blood schizont	Single dose	–	Preclinical
KAF156	Imidazolopiperazine	Unknown	Multi-stage	Single dose with lumefantrine	Mild transaminitis; asymptomatic bradycardia	Clinical

6.1 Marine-Derived Antimalarials

Marine invertebrates such as sponges, soft corals, and tunicates may hold the potential for the discovery of antiplasmodial drugs through their secondary metabolites. Additionally, marine invertebrate harbor microorganisms that are being investigated for their potential in contributing to the secondary metabolites that may play a role in drug development. A significant advantage to these antimalarial compounds is that due to their biologically derived nature, large-scale gene cloning is an option for mass production of these antimalarial metabolites [5].

6.1.1 Marine-Derived Isonitriles

Isonitrile-containing compounds from marine invertebrates and other biological sources have been studied and determined to contain moderate to high antimalarial activity with minimal cytotoxicity. Isolated from the marine sponge *Axinella cannabina*, axisonitrile-1, along with its formamide-containing derivatives, which includes axisonitrile-3, have been evaluated as potential active compounds against malaria. Years after the discovery of these compounds, axisonitrile-3 was also able to be isolated from *Acanthella klethra* and experiments found that this isolate demonstrated antimalarial activity against both chloroquine-resistant and chloroquine-sensitive *P. falciparum* while maintaining no cytotoxicity toward KB cells, a human epidermal cancer cell line. Research into the secondary metabolites associated with *Cymbastela hooperi*, another marine sponge, allowed for the discovery of amphilectane, isocycloamphilectane, and neoamphilectane compounds all of which have some isonitrile, isothiocyanate, or isocyanate functional groups within them. Notably, scientists were able to isolate kalihinane dipertenoids from a Japanese sponge known as *Acanthella sp.* These compounds have previously demonstrated antifungal, antihelminthic, and antifouling activity but have also been found to have one of the most potent antiplasmodial effects with an IC_{50} of 0.4 ng/mL. These compounds all demonstrated selective antimalarial activity when administered in vitro at a low nM concentration with very minimal cytotoxicity associated with them. Further research into these compounds compared the IC_{50} between the isonitrile, isothiocyanate, and isocyanate compounds to determine the activity levels for each group. Findings concluded that the isonitrile functional group appeared to be the best candidate compounds since they had antimalarial activity at lower concentrations compared to the other studied compounds. Additionally, when comparing isonitrile-containing derivatives, the carbon skeletons from the structurally similar compounds differed in their IC_{50} which suggests that the location of the functional group can also play a role in the compound's potency.

As discussed in a previous section, the *Plasmodium* spp. parasite requires that the heme within the host's erythrocytes be destroyed for its survival. Isonitriles provide an antiplasmodial effect by preventing heme detoxification. By interacting with the free heme and forming a pharmacophore, isonitriles inhibit the formation

of heme into β-hematin thus preventing the formation of hemozoin, a compound that neutralizes free heme within the cell. In doing so, the parasite is susceptible to the toxic effects of free heme and cannot survive. Additionally, isonitriles prevent peroxidative and glutathione-mediated heme destruction which further promotes the death of the parasite [5].

6.1.2 Marine-Derived Alkaloids

Alkaloids, such as chloroquine, are a valuable source for antimalarial compounds. Manzamines, a polycyclic alkaloid first derived from a marine sponge from the genus *Haliclona,* are a very promising source for antimalarial drugs. Since the discovery of the first alkaloid, the discovery of similar manzamine alkaloids has been found in many different genus and orders of sponges suggesting that the compounds produced result from a bacterium that shares a symbiotic relationship with these different sponges. Further research discovered the *Micronosphora spp.* bacteria were the source of these manzamines and that there are many derivatives of these manzamine-based alkaloids. These manzamine-type alkaloids have been shown to potentially inhibit the growth of *P. falciparum.* When comparing the different derivatives of these manzamine-type alkaloids, it was discovered that several types play no significant role in antimalarial activity. By comparing these structures to those that do display antimalarial activity, it was discovered that structural differences in the 8-membered ring play a role in the compound's effectiveness, specifically that the carbon 34 methine, the carbon 12 hydroxyl, or ring configuration are the determinant factors.

Marine-derived alkaloids are especially useful in being potential alternative uses for chloroquine-resistant strains of *P. falciparum.* Homofascaplysin A, an alkaloid derived from *Hyrtios erecta,* contains a β-carboline group that shows activity against chloroquine-resistant *P. falciparum* strains of the parasite but unfortunately also leads to myoblast toxicity. Similarly, decahydroquinoline derivatives known as lepadins also display antimalarial activity in some analogs and are derived from sponges *Clavelina lepadiformis* and *Didemnum spp.*. Related to lepadins are phloeodictynes, derived from the genus *Oceanapia,* whose bicyclic structure with long alkyl chains allows it to have activity against the chloroquine-resistant FGB1 strain. Another potential alkaloid is heptyl prodigiosin, a pigment from the α-proteobacteria living among a marine tunicate. Despite its activity against chloroquine-sensitive strain 3D7 of *P. falciparum* and *P. berghei,* in vivo studies on mice demonstrated sclerosed lesions at the site of injection. For all the alkaloid compounds described above so far, the mechanism of action has yet to be elucidated. However, for the better known antiplasmodial compounds, oroidin and salinosporamide A, the mechanisms of action have been determined and well documented. Oroidin and its derivatives work to inhibit the enoyl-ACP reductase in *Plasmodium falciparum,* thus inhibiting fatty acid synthesis. A γ-lactam alkaloid known as salinosporamide A, obtained from the marine bacterium *Salinispora sp.,* acts as a proteasome inhibitor.

Both compounds successfully reduce malarial infection and research continues to investigate the potential for the derivatives of these compounds in the treatment of malaria [5].

6.1.3 Marine-Derived Endoperoxides

Endoperoxides, such as artemisinin, contain a 1,2,4-trioxane moiety and other complex structural features that have rendered them a difficult group of drugs to synthesize in the lab. Therefore, the discovery and research of marine-derived endoperoxides provide a valuable source to produce endoperoxides with the potential to become an alternative to artemisinin-based drugs. Endoperoxides like artemisinin rely on an endoperoxide linkage for their function. During the digestion of hemoglobin, the heme iron (II) is released which allows the endoperoxide to interact with it and leads to the peroxide's cleavage. This causes C-centered radicals to form which alkylate-sensitive macromolecular targets, such as the *Plasmodium*'s calcium-dependent ATPase (PfATP6). Currently, there are two sources of endoperoxides from a biogenetic origin, polyketides and terpenoids, which are undergoing further research into their potential as antimalarials [5].

Polyketides, an endoperoxide derivative, known as plakinidae consist of five- or six-membered 1,2-dioxygenated rings and sometimes contain an additional peroxyketal group due to a 3-methoxy substitution. Originally, these substances were isolated from *Plakortis halichondroides* but have recently been isolated from *Plakortis simplex*, a sponge found in the Caribbean. This same sponge also contains plakortin analogs known as plakortide Q, 3-epiplakortin, and dihydroplakortin that act in a similar effective fashion against the D10 and W2 chloroquine-resistant strains of the *Plasmodium spp.* parasite without cytotoxicity [5]. Plakortide Q demonstrated the highest inhibition against both the drug-resistant and sensitive strains of the parasite with an IC_{50} = 0.52–1 μM which indicates the importance of the structural C3 configuration on its activity [9]. However, despite similarity in structure, not all plakortins isolated from sea sponges have antimalarial activity. Plakortide L, isolated from *Plakortis sp.,* and Plakortide O and P, both isolated from *Plakortis halichondrioides*, have shown to have very low *in vitro* activity against the *Plasmodium spp.* parasite. Mentioned earlier, derivatives of these compounds isolated from *Plakortis sp.* include the peroxyketals which contain a 3-alkoxy-1,2-dioxane group that mimics the function of the 1,2,4-trioxane ring belonging to artemisinin. Peroxyplakoric acids A3 and B3 look promising for use as antimalarial substances against *P. falciparum* due to their good selective toxicity and have an IC_{50} = 50 ng/ml [5].

Not only do these compounds provide a hopeful source for effective antimalarials but can provide further insight on the antimalarial mechanisms by which different functional groups play. Plakortin contains a simple structure that can help researchers further elucidate the relationship between structure and activity, especially the role played by the alkyl side chain and the dioxane ring on the heme. It is hypothesized that the differences in structure surrounding the dioxane ring and/or

alkyl side chain in these compounds are likely responsible for the differences in antimalarial activity between these similar compounds. The peroxyketal derivatives of these compounds have given researchers further insight on the importance of the side chain on antimalarial activity. In fact, research on these compounds indicated increased potency of the compound when converting the ester into an amide group. By comparing the differences in structure and function between these similar compounds, research has concluded that the unsaturated bond within the backbone of these peroxyplakoric acids and their derivatives plays an essential role in its antimalarial activity [5].

Terpenoids, another group of endoperoxide 1,2-dioxane derivatives undergoing testing for their potential as antimalarials, have only had limited research performed toward revealing their potential. Two terpenoids identified to have some activity against *P. falciparum* include sigmosceptrellin A and sigmosceptrellin B, its carbon 3 epimer. Despite their near-identical stereochemistry, the difference in one carbon significantly reduces the activity of the C-3 epimer and further reveals the role of stereochemistry in compound potency. Isolated from the sponge *Diacarnus levii*, the norditerpene derivative methyl-3-epinuapapuanoate has been shown to have some activity (IC_{50} = 1.2 μg/mL) against the chloroquine-resistant strains of *P. falciparum* and a 56% growth inhibition toward the *P. berghei* species of the parasite at a concentration of 25 mg/kg [5].

6.1.4 Marine-Derived Quinones and Phenols

Ilimaquinone, a quinone obtained from the marine sponge *Dactylospongia elegans,* reveals a similar structure, function, and potency to the previously revealed substance xestoquinone. These compounds act as a protein kinase inhibitor to *P. falciparum's* Pfnek-1, a never in mitosis/*Aspergillus*-related protein kinase involved in eukaryotic cell division [5]. Xestoquinone demonstrated antiplasmodial activity through this mechanism of action with an IC_{50} = 3 μM [9]. Like these compounds comes another group of quinone derivatives known as alisiaquinones that were isolated from a New Caledonian sponge and exhibits similar activity against Pfnek-1 as both ilimaquinone and xestoquinone. Of these compounds, alisiaquinone C has an even greater potency against *P. falciparum* and other plasmodial strains with an IC_{50} = 0.1 μM. Not only does this compound work just as well as the others at inhibiting Pfnek-1, but it also works by inhibiting the parasite's farnesyl transferase which allows it to work more effectively than the other quinones studied [5]. The *Plasmodium spp.* parasites farnesyl transferase serves to promote protein–protein interactions and protein–membrane interactions by post-translationally modifying adding an isoprenyl group to a cysteine residue on the protein. Inhibiting this enzyme is toxic to the *Plasmodium sp.* parasite and results in its death [10].

Phenols demonstrating effective antimalarial activity have been obtained from marine sponges that belong to the *Didiscus* and *Hyrtios* genus. A sesquiterpene phenol, (S)-curcuphenol isolated from the *Didiscus* genus sponge, has demonstrated effective activity against the W2 and D6 strains of *P. falciparum*, with minimum

inhibitory concentration of 3.6 μg/mL and 1.8 μg/mL, respectively. 15-Oxopuupehenol, the compound obtained from sponges of the *Hyrtios* genus, has demonstrated activity against the D6 strain (minimum inhibitory concentration of 2.0 μg/mL) and W2 strain (minimum inhibitory concentration of 1.3 μg/mL) of *P. falciparum* as well [5].

6.1.5 Marine-Derived Peptides

Other promising molecules that have shown antimalarial activity come in the form of peptides isolated from marine cyanobacteria. Venturamide, a modified cyclic hexapeptide isolated from the species *Oscillatoria,* shows good selectivity and moderate activity against *P. falciparum* with an IC_{50} = 6–7 μM. Another linear alkynoic lipopeptide, derived from *Lyngbya majuscula,* demonstrates a similar activity against the parasite. Lastly, gallinamide A, isolated from the *Schizothrix* species of cyanobacterium, has shown moderate antimalarial activity (IC_{50} = 8.4 μM) due to its unique structure containing 2-E-pentenoic acid and a dimethyl isoleucine terminus [5].

6.1.6 Marine-Derived Polyethers

Polyethers are another class of compounds that have previously demonstrated effective antimalarial activity. The polycyclic alkyl skeleton of these compounds makes them lipophilic, and their terminal carboxyl group provides them with an oxygen-rich cavity that facilitates their ability to bind metal cations such as K^+, Na^+, and Ca^{2+} [11]. Due to their lipophilic structure, they can interact with the cell membrane of the infected red blood cells and are hypothesized to act as ionophores, making the membrane permeable to certain ions. The *Streptomyces sp.* derived from several marine sources creates a substance known as H668 which has shown to have a significant activity against the parasite with an IC_{50} = 150 ng/mL [5].

The marine bacterium *Streptomyces hygroscopicus* is known to produce a monoglycosidic polyether substance known as K-41 that has been shown to have some antiplasmodial effects [11]. Specifically, this compound has demonstrated *in vitro* antimalarial effects for the multidrug-resistant K1 strain and drug-sensitive FCR3 strain of *P. falciparum,* but with an IC_{50} value 3.6 times more potent for the K1 strain compared to FCR3. For the K1 strain, it demonstrated similar activity to that of the pyrimethamine, artemisinin, and chloroquine drugs. Additionally, in vivo testing with oral administration of K-41 to rodents demonstrated a more potent effect against the N strain of *P. berghei* compared to artemether and artesunate antimalarial drugs. Unfortunately, one drawback to this compound is its cytotoxicity for MRC-5, a human diploid embryonic cell line [12].

6.1.7 Other Marine-Derived Compounds

Callophycus serratus, red algae plant found in Fiji, have contributed to the isolation of macrolide diterpene-benzoate compounds known as bromophycolides A, D, E, H, and M which have IC_{50} values of 0.9, 0.3, 0.8, 0.9, and 0.5 μM, respectively. Unfortunately, several of these compounds demonstrated cytotoxicity against human breast cancer cell lines [8]. Pycnidione, a compound derived from the *Phomas* species of marine fungus, has a similar structure to atovaquone which allows it to have antiplasmodial activity with an IC_{50} value in the 0.15 to 0.4 μM range [9]. Table 2 summarizes characteristics of marine antimalarial compounds.

6.2 Terrestrial-Derived Antimalarial Compounds

Plants have provided a source medicine for humans for thousands of years. The Chinese herb *Artemisia annua* was used as early as 243 A.D. in ancient China as a antimalarial remedy before its active ingredient, artemisinin, was isolated and used as a treatment in modern medicine. Many countries still rely on ethnopharmacological remedies to this day since they are affordable and widely available. In fact, plants provide the major source of treatment for malarial infections in sub-Saharan Africa currently. However, the specific plant-derived sources for medicine differ by environment, population, and region. Because of the potential of these plant sources, researchers have been looking into isolating antimalarial compounds from different plant sources in hope of discovering novel drugs to treat multidrug-resistant forms of the parasite [8].

Isolates from *Friesodielsia discolor*, from the Annonaceae family of plants, gave rise to three flavonoid isolates showing antimalarial activity against the K1 strain: 3′-formyl-2′,4′-dihydroxy-6′-methoxychalcone (IC_{50} = 9.2 μM), 8-formyl-7-hydroxy-5-methoxyflavanone (IC_{50} = 9.3 μM), and tectochrysin (IC_{50} = 7.8 μM). Unfortunately, the isolates also demonstrated cytotoxicity toward KB cells and MCF-7 cells, both human cancer cell lines at an IC_{50} between 13.9–34.5 μM. The isolates from the root extract of another member of the Annonaceae family, *Mitrephora diversifolia*, gave rise to 5-hydroxy-6-methoxyonychine which demonstrated inhibitory action against the 3D7 (IC_{50} = 9.9 μM) and Dd2 (IC_{50} = 11.4 μM) strains of *P. falciparum*. The last potential compound isolated from this family of plants was Miliusacunines A and B, isolated from *Miliusa cuneatus*, which showed inhibitory activity toward the TM4 and K1 strains of the parasite with IC_{50} values of 19.3 μM and 10.8 μM, respectively. Fortunately, neither of these compounds demonstrated any cytotoxicity to Vero cells which is a human kidney epithelial cell line [8].

Another family of plants being investigated for antimalarial potential is the Araceae family. Extracts from the organic matter of *Rhaphidophora decursiva* revealed seven compounds with antimalarial action toward the W2 and D6 parasitic strains. Of these compounds, polysyphorin and rhaphidecurperoxin both showed

Table 2 Marine antimalarials

Type	Compound	Mechanism of action	Origin	Effectiveness	Cytotoxicity
Isonitriles	Axisonitrile-3	Prevent heme detoxification	*Acanthella klethra*	Effective against both chloroquine-resistant and sensitive strains	None toward KB cells
	Amphilectane; Isocycloamphlectane; Neoamphilectane	Unknown	*Cymbastela hooperi*	Low IC_{50}	Low
Alkaloids	Homofascaplysin A	Unknown	*Hyrtios erecta*	Effective toward chloroquine-resistant P. falciparum	Myoblast toxicity
	Lepadins	Unknown	*Clavelina lepadiformis and Didemnum*	Chloroquine-resistant strains	–
	Phloeodictynes	Unknown	*Oceanapia*	Chloroquine-resistant FGB1 strain	–
	Heptyl prodigiosin	Unknown	*α-proteobacteria*	3D7 strains of *P. falciparum* and *P. berghei*	Sclerosed lesions
	Oroidin	Inhibit enoyl-ACP reductase	*Agelas* genus	Reduced malarial infection	–
	Salinosporamide A	Proteasome inhibitor	*Salinispora*	Reduced malarial infection	–
Endoperoxides (polyketides)	Plakinidae	Unknown	*Plakortis halichondroides; Plakortis simplex*	D10 and W2 chloroquine-resistant strains	None
	Plakortide Q	Unknown	*Plakortis sp.*	Inhibition toward resistant and sensitive strains highest	–
	Plakortide O and P	Unknown	*Plakortis halichondrioides*	Low *in vitro* activity against parasite	–
	Peroxyplakoric acids A3 and B3	Unknown	*Plakortis sp.*	Selective toxicity toward resistant and sensitive strains at $IC_{50} = 50$ ng/ml	Low
Endoperoxides (Terpenoids)	Sigmosceptrellin A and B	Unknown	*Diacarnus levii*	Chloroquine-resistant strains of *P. falciparum* at $IC_{50} = 1.2$ µg/mL) and *P. berghei* growth inhibition	–

Quinones	Ilimaquinone	Inhibit Pfnek-1	*Dactylospongia elegans*	Antiplasmodial at $IC_{50} = 3\ \mu M$	–
	Alisiaquinone C	Inhibit Pfnek-1 and farnesyl transferase	New Caledonian Sponge	Potency against *P. falciparum* and other strains at $IC_{50} = 0.1\ \mu M$	–
Phenols	(S)-curcuphenol	Unknown	*Didiscus* genus	Potency against W2 and D6 strains with MIC = 3.6 and 1.8 μg/mL	–
	15-Oxopuupehenol	Unknown	*Hyrtios* genus	Activity against D6 and W2 with MIC = 2.0 and 1.3 μg/mL	–
Peptides	Venturamide	Unknown	*Oscillatoria sp.*	Selective with moderate activity toward *P. falciparum* $IC_{50} = 6–7\ \mu M$	–
	Gallinamide A	Unknown	*Schizothrix sp.*	Moderate antimalarial activity with $IC_{50} = 8.4\ \mu M$	–
Polyethers	H668	Affect membrane permeability by acting as ionophores	*Streptomyces sp.*	Significant activity against parasite with $IC_{50} = 150$ ng/ml	–
	K-41	Act as ionophores	*Streptomyces hygroscopicus*	Antimalarial effect for K1 and FCR1 strains	Cytotoxicity for MRC-5
Other	Bromophycolides A, D, H, E, and M	Unknown	*Callophycus serratus*	Effective toward parasite with IC_{50} values between 0.5–0.9 μM	–
	Pycnidione	Cytochrome bc1 inhibitor	*Phomas sp.*	Antiplasmodial at $IC_{50} = 0.15–0.4\ \mu M$	–

IC_{50} values in the 1.4–1.8 μM range against the parasite but with a cytotoxicity against KB cells with an effective dose (ED_{50}) of 8.3–13.1 μM. The compounds rhaphidecursinols A and B, grandisin, and decursivine also demonstrated activity against the D6 and W2 strains of the parasite with IC_{50} values in the 3.4–12.9 μM range. Another compound, epigrandisin, showed activity against the W2 strain but not the D6 strain at a concentration of 23 μM. These last five compounds all showed cytotoxicity toward KB cells at ED_{50} = 23.9–37.0 μM. One last compound extracted from this species of plant was roridin E, a trichothecene compound. This compound showed potential due to its ability to inhibit parasitic growth at sub-nanomolar concentrations. Unfortunately, it also demonstrated significant cytotoxicity against KB cells which has excluded it from further research into its potential. However, a similar trichothecene known as verrucarin L acetate was later isolated from *F. fitulosa*, a species from the Moraceae family, that retained the same potency of antimalarial activity with significantly less cytotoxicity toward KB cells with an ED_{50} = 0.2 μM [8].

Isolates from *Gongronema napalense*, a member of the Asclepiadaceae family of plants, gave rise to a steroidal glycoside whose activity against *P. falciparum* has been investigated. The compound, gongroneside A, has demonstrated antimalarial activity against the D6 (IC_{50} = 1.6 μM) and W2 (IC_{50} = 1.4 μM) strains of the parasite with no cytotoxicity toward KB cells at 13.7 μM [8].

The Asteraceae family of plants yielded two flavonoid glycoside compounds, apigenin 7-O-glycoside and luteolin 7-O-glycoside, from *Achillea millefolium*. These compounds demonstrated antimalarial activity against the W2 and D10 strains with IC_{50} = 15.3–62.5 μM range. *Carpesium divaricatum*, another plant belonging to this family, produced a compound known as 2-isopropenyl-6-acetyl-8-methoxy-1,3-benzodioxin-4-one that also showed inhibitory action toward the D10 strain with an IC_{50} = 2.3 μM. Another plant of interest from this family is the *Microglossa pyrifolia* due to its use as an herbal antimalarial remedy in Ghana. Two diterpene compounds were extracted from this species, E-phytol and 6E-geranylgeraniol-19-oic acid. E-phytol demonstrated inhibition toward the PoW strain with an IC_{50} = 8.5 μM and the Dd2 strain with an IC_{50} = 11.5 μM. 6E-geranylgeraniol-19-oic acid also showed inhibitory action against the PoW strain (IC_{50} = 12.9 μM) and the Dd2 strain (IC_{50} = 15.6 μM). One last species from this family of plants that was investigated for antimalarial potential was that of *Echinops hoehnelii*. Two acetylenic thiophene compounds were isolated from its roots: 5-(penta-1,3-diynyl)-2-(3,4-dihydroxybut-1-ynyl)-thiophene and 5-(penta-1,3-diynyl)-2-(3-chloro-4-acetoxy-but-1-yn)-thiophene which were used for *in vivo* studies on *Plasmodium berghei*-infected mouse models. The first compound demonstrated parasitic growth suppression by 50.2% at 100 mg/kg while the latter showed a growth suppression of 32.7% at 100 mg/kg. Neither of these compounds demonstrated any oral toxicity [8].

Buxus sempervirens, from the Buxaceae family of plants, yielded eight lupine triterpene compounds which exhibited antimalarial activity toward the multidrug-resistant HB3 strain with an IC_{50} in the 0.5–3.0 μM range. Of these, 23-O-(trans)-feruloyl-23-hydroxybetulin appeared to be the most promising since it was 75 times more discriminating toward the parasite than to HeLa cells when compared to the others [8].

Interest in the *Cecropia pachystachya* species, belonging to the Cecropiaceae family, was due to its long-time use in Brazil as a medicinal plant. In vivo and *in vitro* studies of this plant material on both *P. falciparum* and *P. berghei* resulted in a 35–66% reduction of parasitic infection. This initial study led to the isolation of two compounds within this plant, β-sitosterol and tormentic acid, which both demonstrated antimalarial activity. However, only tormentic acid exhibited antimalarial action toward the chloroquine-resistant W2 strain with an IC_{50} in the 19.0–25.2 μM range [8].

The Chloranthaceae family of plants yielded several lindenane-type sesquiterpenoids with good antimalarial potential against the Dd2 strain of the parasite, each having IC_{50} values below 11.4 μM. Of these compounds, the most promising were Fortunilide A (IC_{50} = 5.2 nM) and sarglabolide J (IC_{50} = 7.2 nM), isolated from the species *C. fortune*, and chlorajaponilide C (IC_{50} = 1.1 nM), isolated from *C. serratus* and *C. spicatus*. In addition to their low IC_{50} values, they all had values over 500 for their selectivity index. The selectivity index is the ratio of antimalarial activity to cytotoxicity, indicating that these compounds appeared to be favorable as potential novel drugs [8].

Kaurene diterpene lactone isolates from *Parinari capensis*, a member of the Chrysobalanaceae family of plants, include 13-hydroxy-9-methyl-15-oxo-20-norkaur-16-en-18-oic acid γ-lactone, 10-hydroxy-13-methoxy-9-methyl-15-oxo-20-norkaur-16-en-18-oic γ-lactone, and 10-hydroxy-9-methyl-15-oxo-20-norkaur-16-en-18-oic acid γ-lactone. Experiments on these compounds to study their action against the FCR-3 strain of *P. falciparum* determined they had good activity against the parasite, and each had IC_{50} values of 1.7, 1.9, and 5.0 μM, respectively. However, these compounds also demonstrated cytotoxicity toward Graham cells which are human embryonic kidney cells also known as HEK 293, in the ED_{50} = 3.2–9.2 μM range which excluded them as potential drug candidates. Despite this, they could still be used as a basis for chemical alteration to reduce cytotoxicity while maintaining good antimalarial activity [8].

Another plant family of interest is the Clusiaceae family, specifically the species *Garcinia mckeaniana*. Isolates from this species yielded three xanthone compounds that each contain two isoprene units with antimalarial potential known as mckeanianones A-C and bannaxanthones I and E. These isolates demonstrated IC_{50} values between 6.0–8.5 μM for the mckeanianones and 3.6–7.3 μM for the bannaxanthones against the TM4 and K1 strains of the parasite. However, mckeanianones B and C as well as the bannaxanthone compounds all demonstrated cytotoxicity against Vero cells at IC_{50} = 12.6–29.5 μM [8].

Three compounds with antimalarial activity against the D6 and W2 strains were isolated from *Rourea minor*, a species of the Connaraceae family. Two of these compounds were neolignane glycosides and one was the previously known compound, 1-(26-hydroxyhexacosanoyl)-glycerol which exhibited an IC_{50} value of 9.5 μM against the D6 strain and 12.7 μM against the W2 strain. Rourinoside, one of the neolignane glycosides, exhibited IC_{50} values of 3.7 μM against the D6 strain and 2.1 μM against the W2 strain. The other neolignane glycoside isolated, rouremin, exhibited IC_{50} values of 5.1 μM against the D6 strain and 4.5 μM against the

W2 strain. None of these compounds demonstrated any cytotoxicity against KB cells at 20 μg/mL [8].

Ergosta-4,6,8,22-tetraene-3-one, 3-epideoxyflindissol, and 3β-O-*cis*-coumaroyl betulinic acid and 3β-O-*trans*-coumaroyl betulinic acid were isolated from the species *Cornus florida*, a member of the Cornaceae family of plants. These compounds demonstrated inhibition toward the D10 strain of the parasite with IC_{50} values of 61, 128, 10.4, and 15.3 μM, respectively [8].

In Brazzaville Congo, the plant species *Cogniauxia podolaena* has been traditionally used as a treatment for malaria. Because of this, researchers investigated the active compounds in the plants as a source of potential antimalarials against the FcM29 strain and isolated cucurbitacins B and D along with 20-epibryonolic acid, three bioactive triterpenes. Compounds cucurbitacin B (IC_{50} = 2.9 μM) and cucurbitacin D (IC_{50} = 7.8 μM) displayed high cytotoxicity toward KB cells with a 95% inhibition at 1 μg/mL. 20-epibryonolic acid (IC_{50} = 4.4 μM) only demonstrated 20% inhibition toward KB cells which makes it a more favorable compound in the search for novel antimalarials [8].

Muang Kout (*Diospyros quaesita* Thw.), a plant found in Laos, is a member of the Ebenaceae family of plants whose isolates yielded a compound known as betulinic acid 3-caffeate. This compound showed antimalarial activity against the D6 strain with an IC_{50} = 1.4 μM and the W2 strain with an IC_{50} = 0.98 μM. Unfortunately, this substance demonstrated cytotoxicity against KB cells with an ED_{50} = 4.0 μM [8].

Not all naturally derived compounds are effective by themselves, the natural product-based synthetic compound library holds many compounds derived from natural compounds that have been modified to produce a desired effect. Jatrophone diterpene derivatives isolated and modified from *Jatropha isabelli*, a member of the Euphorbiaceae family, have demonstrated inhibitory action toward the 3D7 and K1 strains of the *P. falciparum* parasite. These two diterpene derivatives have demonstrated IC_{50} values of 5.7 and 5.9 μM, respectively, against the 3D7 strain, and 5.9 and 6.1 μM, respectively, against the K1 strain of the parasite. They were evaluated for cytotoxicity against the HepG2, RAJI, BJ, and HEK293 human cell lines which resulted in low toxicity to these cells with an ED_{50} = 26 μM. Isolates from *Strophioblachia fimbricalyx*, another member of this family, known as 9-O-demethyltrigonostemon and 3,6,9-trimethoxyphenanthropolone demonstrated inhibitory activity against the K1 strain. The former compound had an IC_{50} value of 8.7 μM while the latter had an IC_{50} value of 9.9 μM [8].

Cajanus cajan L., a member of the Fabaceae family, is a plant that has been used as an antimalarial in Nigeria. A cajachalcone compound was isolated from this species known as 2′,6′-dihydroxy-4-methoxy chalcone that exhibited activity against the K1 strain of the parasite with an IC_{50} value of 7.4 μM. Another member of this family found in Madagascar, *Piptadenia pervillei*, provided two isolates: (+)-catechin 5-gallate and (+)-catechin 3-gallate. Both exhibited inhibitory activity against the FcB1 strain of *P. falciparum* with (+)-catechin 5-gallate having an IC_{50} value of 1.2 μM and (+)-catechin 3-gallate having an IC_{50} value of 1.0 μM. Neither of these compounds exhibited cytotoxicity toward MRC-5, human embryonic lung cells, at a concentration of 75 μM. One last species investigated from this family was a

specific variety of *Prosopis glandulosa* which yielded two isolates, prosopilosidine and isoprosopilosidine. Prosopilosidine exhibited an IC_{50} value of 0.1 μM toward the D6 strain and 0.3 μM toward the W2 strain with low cytotoxicity against KB cells at an ED_{50} of 20.2 μM. Isoprosopilosidine exhibited an IC_{50} = 0.1 μM against the D6 strain and 0.3 μM against the W2 strain and had a low cytotoxicity toward KB cells at an ED_{50} = 18.8 μM [8].

Quercus laceyi, a species from the Fagaceae family of plants, isolated four kaempferol-3-O-glucosides which showed activity against the HB3 strain of the parasite at an IC_{50} value in the 0.6–2.1 μM range. These compounds, however, also demonstrated cytotoxicity toward HeLa cells at <3 μM [8].

Another family of plants which had several species with antimalarial potential was that of the Hypericaceae family. An isolate from *Vismia orientalis*, a plant used medicinally in Tanzania, known as vismione D demonstrated activity against the K1 strain of the parasite with an IC_{50} = 2.4 μM but showed cytotoxicity against L6 cells, human muscle cells, at IC_{50} = 10 μM. Researchers also investigated *Psorospermum glaberrimum*, another species of this family, and were able to isolate 3-geranyloxyemodin anthrone and acetylvismione D from its stems. These isolates exhibited activity against the W2 strain with an IC_{50} = 1.7 μM for 3-geranyloxyemodine anthrone and IC_{50} = 0.1 μM for acetylvismione D [8].

Luteolin 7-O-β-D-glucopyranoside and chrysoeriol 7-O-β-D-glucopyranoside, both types of flavonoid glycosides obtained from *Phlomis brunneogaleata*, a member of the Lamiaceae family, demonstrated inhibitory action toward the K1 strain of the parasite with IC_{50} = 5.4 μM for lutein 7-O-β-D-glucopyranoside and IC_{50} = 12.7 μM for chrysoeriol 7-O-β-D-glucopyranoside. Several *Salvia* species of plants within this family have been used in South Africa for medicinal purposes and researchers wanted to investigate the isolates of these strains to determine antimalarial potential. *S. radula* isolates, betulafolientriol oxide and salvigenin, demonstrated the most potent antimalarial activity with IC_{50} = 10.4 and 75 μM, respectively [8].

Species within the Loganiaceae family and Lythraceae family were also investigated for their antimalarial potential. Isolates from *Strychnos icaja*, a member of the Loganiaceae family, included 15-hydroxyvomicine and N-methyl-sec-iso-pseudostrychnine which demonstrated activity against the 3D7 strain at IC_{50} = 101.0 μM for the former and IC_{50} = 110.6 μM for the latter monomer. In China and India, members of the genus *Ammannia*, within the Lythraceae family, are frequently used for treatment of diseases and researchers further investigated the isolates of several species from this genus of plants. *Ammannia multiflora* yielded two compounds with activity against the NF-54 strain of *P. falciparum*, 4-hydroxy-α-tetralone, and tetralone-4-O-β-D-glucopyranoside which demonstrated IC_{50} values of 194 and 124 μM, respectively. Another species within this genus, *Ammannia baccifera* yielded the compound ammaniol, which demonstrated an IC_{50} value of 88.3 μM against this same strain [8].

A benzylisoquinoline alkaloid, isolated from *Doryphora sassafras*, a member of the Monimiaceae family, demonstrated antimalarial activity against the 3D7 strain (IC_{50} = 3.0 μM) and the Dd2 strain (IC_{50} = 4.4 μM). This compound, known as

1-(4-hydroxybenzyl)-6,7-methylenedioxy-2-methylisoquinolinium trifluoroacetate, also demonstrated no cytotoxicity against human HEK293 cells at 120 μM. Another species of this family, *Glossocalyx brevipes*, yielded methyl 2-(1′β-geranyl-5′β-hydroxy-2′-oxocyclohex-3′-enyl) acetate and methyl 2-(1′β-geranyl-5′β-hydroxy-2′-oxocyclohex-3′-enyl) acetic acid. These two compounds were tested against the D6 and W2 strains of the parasite and demonstrated IC_{50} values of 2.2 and 6.6 μM, respectively, against the D6 strain. For the W2 strain, methyl 2-(1′β-geranyl-5′β-hydroxy-2′-oxocyclohex-3′-enyl) acetate had activity against it at an IC_{50} = 4.8 μM and methyl 2-(1′β-geranyl-5′β-hydroxy-2′-oxocyclohex-3′-enyl) acetic acid had antimalarial action at an IC_{50} = 8.3 μM [8].

The Moraceae family, discussed earlier as contributing a trichothecene compound as an alternative to roridin E, also has another species known as *Ficus septica* which has contributed three phenanthroindolizine alkaloids with antimalarial activity. These compounds, dehydrotylophorine, dehydroantofine, and tylophoridicine D, were tested for inhibitory action on the 3D7 strain of the parasite and demonstrated activity against the parasite at IC_{50} values between 0.03 and 0.4 μM. Dehydroantofine and tylophoridicine D demonstrated no cytotoxicity against L929, mouse fibroblast cells, at 50 μM; however, dehydrotylophorine did at an IC_{50} = 8.2 μM [8].

The Malvaceae family and the Myristicaceae family were investigated for compounds with antimalarial activity. (R)-(-)gossypol, isolated from the twigs of *Thespesia danis*, a member of the Malvaceae family, demonstrated antiplasmodial activity at IC_{50} = 4.5 μM despite its enantiomer being inactive at concentrations up to 20 μM. This sheds further light on the importance of stereochemistry on compound activity. Malabaricone A, a compound isolated from *Knema glauca* of the Myristicaceae family, was found to be an active compound against the K1 strain of the parasite at IC_{50} = 8.5 μM. Unfortunately, it was found to be cytotoxic toward KB cells at an ED_{50} greater than 60 μM and was therefore ruled out as a potential therapeutic agent [8].

Two compounds isolated from *Piper sarmentosum* from the Piperaceae family of plants demonstrated potential as a therapeutic agent. Many species of plants within this family have been used traditionally in Thailand for treatment of diseases and researchers were hopeful to find compounds within this family with antimalarial activity. Sarmentine (IC_{50} = 85.5 μM) and 1-piperettyl pyrrolidine (IC_{50} = 21.9 μM) showed inhibitory action toward the K1 strain of the parasite. Through another species from this family, *Piper tricuspe*, three more compounds were isolated with antiplasmodial potency. Dictyochromenol, 3-farnesyl-ρ-hydroxy benzoic acid, and 2′E,6′E-2-farnesyl hydroquinone all demonstrated activity within the 1.4–29.8 μM range despite demonstrating cytotoxicity. With these IC_{50} values being within the ED_{50} range, it suggests that their antimalarial action is due to their cytotoxicity [8].

Antiparasitic action toward the D6 and W2 strains of the parasite was evaluated for four compounds isolated from *Nauclea orientalis*, a member of the Rubiaceae family. The first compound isolated from this species, Naucleaorine, demonstrated activity against the D6 strain with an IC_{50} = 6.9 μM and the W2 strain with an IC_{50} = 6.0 μM. However, this compound demonstrated cytotoxicity against KB cells

at ED_{50} = 38 μM. The next isolate, epimethoxynaucleaorine, showed activity against the D6 strain with an IC_{50} = 12.4 μM and against the W2 strain with an IC_{50} = 13.2 μM with a cytotoxicity toward KB cells at ED_{50} = 37.9 μM. The third isolate from this species, 3α,23-dihydroxyurs-12-en-28-oic acid, showed activity against the D6 strain with an IC_{50} = 9.7 μM and toward the W2 strain with an IC_{50} = 12.7 μM. Unfortunately, it also showed cytotoxicity toward KB cells at ED_{50} = 42.2 μM. Lastly, oleanolic acid demonstrated activity against the D6 strain with an IC_{50} = 4.6 μM and toward the W2 strain with an IC_{50} = 5.1 μM and a cytotoxicity toward KB cells at ED_{50} = 46 μM [8].

Several compounds of interest were isolated from members of the Rutaceae family of plants due to their medicinal properties and use in Uganda against different diseases. Extracts from the species *Citropsis articulata* revealed two alkaloids, 5-hydroxynoracronycine and 1,5-dihydroxy-2,3-dimethoxy-10-methyl-9-acridone, which demonstrated inhibition toward growth of the parasite. 5-hydroxynoracronycine demonstrated activity at IC_{50} = 2.8 μM and 1,5-dihydroxy-2,3-dimethoxy-10-methyl-9-acridone at IC_{50} = 10 μM. Unfortunately, both exhibited cytotoxicity toward Vero cells at ED_{50} = 28.8 μM for the former compound and ED_{50} = 101 μM for the latter one. *Zanthoxylum chiloperone*, another species of this family, has traditionally been used for its medicinal properties to combat malaria along with other diseases. Researchers isolated two canthinone alkaloids, canthin-6-one and 5-methoxycanthin-6-one, in addition to pyranocoumarin *trans*-avicennol. These compounds were tested against the F32, K1, PFB, and FcB1 strains of the parasite and demonstrated activity toward them at the IC_{50} value range between 1.4–41.6 μM. Unfortunately, two of these compounds, pyranocoumarin *trans*-avicennol (ED_{50} = 12.8 μM) and canthin-6-one (ED_{50} = 42.7 μM), demonstrated cytotoxicity toward the human fibroblast cell line, MCR5 [8].

Several potential compounds were isolated from different species of the Simaroubaceae family of plants. Isolates from *Eurycoma longifolia* revealed the compounds eurycomanone and pasakbumin B which were tested against the W2 and D6 strains of the parasite. The IC_{50} value of eurycomanone for the W2 strain was 0.04 μM and 0.06 μM for the D6 strain. Pasakbumin B also showed activity against the W2 strain (IC_{50} = 0.05 μM) and the D6 strain (IC_{50} = 0.08 μM). Both compounds also demonstrated high cytotoxicity against MCF-7, human breast cancer cells, and A549, human lung cancer cells, at low concentrations. The roots and stems of *Picrolemma sprucei* isolated a quassinoid neosergeolide which demonstrated activity against the K1 strain of the parasite at IC_{50} values of 0.002 μM. The indole alkaloid isolated from *Aspidosperma vargasii*, ellipticine, demonstrated inhibitory activity against K1 at IC_{50} = 0.07 μM. Another indole alkaloid isolated from another species within this family, *A. Desmanthum*, known as aspidocarpine also demonstrated inhibitory activity against K1 with an IC_{50} = 0.02 μM. *Pothomorphe peltata* yielded a compound known as 4-nerolidylcatechol which demonstrated antimalarial activity at an IC_{50} = 0.7 μM [8].

The Platanaceae, Theaceae, and Verbenaceae families of plants were explored for potential antimalarial compounds. *Platanus occidentalis*, of the Platanaceae family, yielded four kaempferol 3-O-rhamnosides which were tested against the HB3 strain of the parasite. The IC_{50} values for these substances toward that drug-resistant strain varied between 0.5 and 1.8 μM. Unfortunately, cytotoxicity against the HeLa cells ranged between 9.3 and 20 μM. Studies on an extract from the leaves of *Camellia sinensis*, of the Theaceae family, revealed gallocatechin, a flavonoid with stronger antimalarial potency compared to mefloquine, a commonly used antimalarial drug. Lastly, lippialactone, an α-pyrone isolated from *Lippia javanica* of the Verbenaceae family, demonstrated activity against the D10 strain of the parasite with IC_{50} = 23.8 μM [8].

The last family of plants investigated for antimalarial activity was those from the Tiliaceae family. Several compounds isolated from *Grewia bilamellata* demonstrated antimalarial activity toward the D6 and W2 strains of the parasite. These compounds, 3α, 20-lupandiol, grewin, nitidanin, 2α,3β-dihydroxyolean-12-en-28--oic acid, and 2,6-dimethoxy-1-acetonylquinol, had IC_{50} values in the 5.5–42.2 μM range and demonstrated no cytotoxicity toward KB cells at 50 μM [8]. Table 3 summarizes characteristics of terrestrial antimalarial compounds.

Table 3 Lead terrestrial antimalarial compounds

Compound	Source	Plant family origin	Effectiveness	Cytotoxicity
Roridin E	*Rhaphidophora decursiva*	Araceae	IC_{50} = 0.4 nM against D6 strain; IC_{50} = 1 nM against W2 strain	High toward KB cells at ED_{50} = 0.4 nM
Verrucarin L acetate	*Ficus fistulosa*	Moraceae	IC_{50} = 0.4 nM against D6 strain; IC_{50} = 1 nM against W2 strain	Low toward KB cells at ED_{50} = 200 nM
Dehydroantofine	*Ficus septica*	Moraceae	IC_{50} = 30 nM against 3D7 strain	No cytotoxicity at 50 μM
Tylophoridicine D	*Ficus septica*	Moraceae	IC_{50} = 60 nM against 3D7 strain	No cytotoxicity at 50 μM
Fortunilide A	*C. fortune*	Chloranthaceae	IC_{50} = 5.2 nM against Dd2	Low cytotoxicity toward WI-38 cells at IC_{50} = 8.8 μM
Sarglabolide J	*C. fortune*	Chloranthaceae	IC_{50} = 7.2 nM against Dd2	Low cytotoxicity toward WI-38 cells at IC_{50} = 4.0 μM
Chlorajaponilide C	*C. serratus/C. spicatus*	Chloranthaceae	IC_{50} = 1.1 nM against Dd2	Low cytotoxicity toward WI-38 cells at IC_{50} = 5.4 μM
Fortunilide E	*C. fortune*	Chloranthaceae	IC_{50} = 43 nM	No cytotoxicity at 100 μM

7 Conclusion

Both synthetic and naturally derived compounds have been discovered to show activity against many different drug targets to bypass the resistance mechanisms that have developed over time within the *Plasmodium* parasite. Additional changes to these pre-existing molecules hold potential for further increasing their effectiveness while minimizing cytotoxicity to the host cell. These, along with other components, are all factors that must be considered when developing effective antimalarial drugs. Further research into these compounds will further elucidate different parasitic processes and identify potential drug targets for the development of novel drugs. Researchers are optimistic that the development of novel drugs from these different compounds can help us combat the malaria pandemic.

References

1. Ashley EA. Drugs in development for malaria. CrossMark. 2018:861–79.
2. Giulia Siciliano PA. Enlightening the malaria parasite life cycle: bioluminescent plasmodium in fundamental and applied research. Front Microbiol. 2015;6:391.
3. Tse EG. The past, present and future of anti-malarial medicines. Malar J. 2019;18:1–21.
4. White NJ. Antimalarial drug resistance. J Clin Invest. 2004;113:1084–92.
5. Ernesto Fattorusso OT-S. Marine antimalarials. Mar Drugs. 2009;7:130–52.
6. Ding XC. A framework for assesssing the risk of resistance for anti-malarials in development. Malar J. 2012;11:1–11.
7. Packard RM. The origins of antimalarial-drug resistance. N Engl J Med. 2014;371:397–9.
8. Wen-Hui Pan X-YX-J. Antimalarial activity of plant metabolites. Int J Molecular Sciences. 2018;19:1382.
9. Mayer AMS. Marine Pharmacology in 2005-6: marine compounds with antihelmintic, antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, and antiviral activities. Biochim Biophys Acta. 2009;1790:283–308.
10. Eastman RT. Resistance to a protein farnesyltransferase inhibitor in plasmodium falciparum. J Biol Chem. 2005;280:13554–9.
11. MinKyun Na DA. A new antimalarial polyether from a marine Streptomyces sp. H668. Tetrahedron Lett. 2009;49:6282–5.
12. Kazuhiko Otoguro AI. In vitro and in vivo antimalarial activities of the monoglycoside polyether antibiotic, K-41 against drug resistant strains of plasmodia. J Antibiot. 2002;55:832–4.

New Drugs in Synthesis Research for Tuberculosis

Ivie Patino and Charles Preuss

Abstract *Mycobacterium tuberculosis* is the bacteria which causes tuberculosis. It is a significant public health concern worldwide. An estimated ten million new cases and 1.5 million deaths were estimated in 2020. Tuberculosis is the second leading cause of mortality due to an infectious agent after the COVID-19 virus at its peak. Patients with an active tuberculosis infection can present with signs and symptoms of fever, chills, productive cough, loss of appetite, weight loss, and night sweats. The initial treatment of tuberculosis consists of the four-drug combination of rifampin, isoniazid, pyrazinamide, and ethambutol. A major concern with the current pharmacotherapy is drug resistance. Hence, the need to discover new drug treatments to stay one step ahead of *Mycobacterium tuberculosis*. Several bacterial efflux pump inhibitors of plant origin have been studied. Examples are reserpine, piperine, farnesol, luteolin, and biochanin A. The phenothiazines are a class of antipsychotic drugs which have antimycobacterial activity. Thioridazine is a promising lead compound. Its antimycobacterial activity is thought to be by inhibition of the activity of efflux pumps and enhancement of macrophage killing activity through the inhibition of potassium and calcium channels, causing the activation of bacterial hydrolases.

Keywords Tuberculosis · Antimycobacterials · Reserpine · Thioridazine

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1 Tuberculosis New Drug Discovery: Background and Significance

Although *Mycobacterium tuberculosis (Mtb)*, the etiological agent of tuberculosis (TB), was identified over a century ago in 1882, TB remains a threat to public health to this day. With an estimated ten million new cases and 1.5 million deaths in 2020, TB is the second leading cause of mortality due to an infectious agent after the COVID-19 virus [1, 2]. TB can exist as either an active infection or as a latent infection. Individuals with an active TB infection may spread the bacillus to others, whereas latent TB infections are non-communicable. Latent TB infections are also asymptomatic, while active TB infections are consistent with symptoms of fever, chills, productive cough, loss of appetite, weight loss, and night sweats [3].

Despite the dissemination of the bacilli Calmette-Guérin (BCG) vaccine against *Mtb* to neonates and infants in high-burden regions, and the existence of successful combination drug therapies to treat the infectious disease, it is estimated that one-quarter of the population is latently infected with TB [1]. The persistence of TB remains a global crisis; however, low-income countries are particularly vulnerable and are often areas of high burden. These high endemic countries are disproportionally affected by TB due to several socioeconomic factors, including poverty, overcrowding, air pollution, malnutrition, and co-epidemics like HIV [2, 4]. Controlling the prevalence of TB has proven difficult as there are many challenges surrounding our understanding and management of the disease. One such challenge includes the "missing" individuals, estimated at three million, who have developed active TB but have been "missed" in the health system or remain undiagnosed. These "missed" individuals perpetuate transmission, as persons living with TB typically infect up to 10 to 15 people with whom they have close contact with in just a single year [8]. This population of individuals is "missed" for various reasons, including limited access to public health. If these individuals are members of certain groups, like children or persons living with HIV, they may be "missed" due to difficulty with traditional diagnostics. Traditional diagnostics like sputum smear microscopy does not perform as well in these groups, so even if a TB infection is present, the patient may fail to be diagnosed accordingly [8].

These determinants, pertaining to both the host and environmental factors, evoke conditions that favorably select for the emergence of drug-resistant strains, which enervates prospects for controlling TB infection across the globe [4]. The ongoing COVID-19 pandemic has further undermined efforts to reduce the prevalence and transmission of TB. The lockdowns and guidelines imposing restrictions on face-to-face activities, and healthcare personnel facing overwhelming workloads due to the influx of COVID patients, impede TB treatment efforts. Many protocols require quotidian monitoring by healthcare professionals while patients take their medications. Further, fewer cases of TB have been reported in high-burden countries since the onset of the COVID-19 pandemic. Since 2019, the number of TB cases reported to the World Health Organization has dropped 30% in high-burdened countries like India, Indonesia, and the Philippines [7]. It is estimated that if reported case

numbers drop by 25–50%, undetected TB patients may go without treatment and perpetuate transmission, leading to a potential rise in TB deaths by 400,000 [6]. Patients with TB are also at high risk for COVID-19; the comorbidity of pulmonary tuberculosis, along with sequelae from TB treatment, may lead to poor COVID-19 treatment outcomes in this patient population [7].

1.1 First-Line Drugs and their Limitations

The treatment strategy for TB infection has not changed since the discovery of isoniazid six decades ago. Currently, along with isoniazid, three other drugs are most commonly used to treat TB. This four-drug cocktail includes the following: Isoniazid discovered in 1951, pyrazinamide discovered in 1952, ethambutol discovered in 1961, and rifampin discovered in 1966 [2, 5].

Isoniazid may act as either bacteriostatic against slow-growing cells, or bactericidal against quickly growing cells, and is effective against intra- and extracellular organisms. It is a prodrug oxidized to its active radical by the *Mtb* catalase-peroxidase enzyme produced by the Kat G gene. The now-activated isoniazid inhibits an enzyme required for mycolic acid synthesis, enoyl-acyl protein carrier reductase (InhA), which synthesizes long-chain fatty acids. Mycolic acid synthesis is essential to bacterial cell wall synthesis in *Mtb*; thus, this disruption of proper cell wall synthesis, by inhibiting InhA, leads to isoniazid's bactericidal effect against *Mtb* [9] (Development of a Novel Lead that Targets M. tuberculosis Polyketide Synthase 13).

Although the specific mechanism of action of pyrazinamide is unknown, it is believed to inhibit the fatty acid synthetase I (FAS-I) enzyme of *Mtb,* after it has been metabolized into pyrazinoic acid by pyrazinamidase (PZase) in the cytoplasm. The FAS-I enzyme is vital in elongating fatty acids, which may then eventually be utilized to form mycolic acids, an essential component of the *Mtb* cell wall. Pyrazinamide is bacteriostatic and is considered narrow spectrum, as it only exhibits antibacterial effects against *Mtb* and *mycobacterium africanum (Maf)*. Utilizing pyrazinamide in combination with isoniazid to treat TB effectively reduces the treatment time, though treatment regimens for TB are still considerably lengthy [10, 11].

As with pyrazinamide, ethambutol's specific mechanism of action is recondite. Ethambutol is thought to inhibit the three arabinosyltransferase proteins EmbA, EmbB, and EmbC. These enzymes contribute to the formation of the mycobacterial cell wall by first polymerizing arabinose into arabinan, then finally to arabinogalactan. Thus, ethambutol falls into the category of cell wall synthesis inhibitors along with pyrazinamide and isoniazid. Ethambutol is bacteriostatic and narrow spectrum, with antimicrobial activity exhibited only against mycobacteria [12, 13].

Unlike isoniazid, pyrazinamide, and ethambutol, rifampin is not considered a cell wall synthesis inhibitor; instead, rifampin inhibits bacterial transcription [14]. Rifampin is able to inhibit bacterial transcription, without interfering with

eukaryotic transcription, as it specifically binds to the bacterial DNA-dependent RNA polymerase and has no specificity for any of the three eukaryotic RNA polymerases. Rather than inhibiting the enzyme's active site, by binding to the beta subunit of bacterial RNA polymerase, rifampin physically blocks the elongation of mycobacterial RNA. Rifampin acts on both intra- and extracellular bacilli and is broad-spectrum, and because rifampin inhibits effective bacterial protein synthesis, it is considered bactericidal [9] (Fig. 1).

The empiric treatment of sensitive TB infection consists of a two-month perid termed the intensive phase, followed by a continuation phase which may last anywhere from four to seven months. Patients are typically prescribed rifampin, isoniazid, and pyrazinamide in the intensive phase of treatment, then rifampin and isoniazid in the continuation phase of treatment [15]. Though less than ideal, the long duration of antibiotic therapy, typically totaling 6 to 9 months, is necessary for proper TB treatment, which is believed to be attributable to the presence of bacilli existing in a "dormant" state referred to as "persisters" [16]. These persisters display metabolic alterations that enable the mycobacteria to inhibit their growth

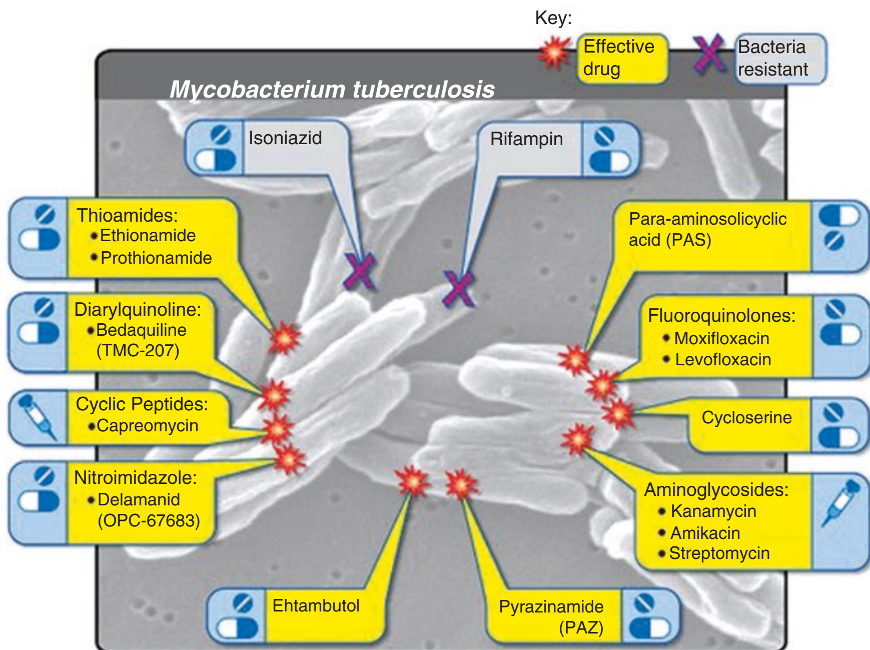

Fig. 1 Effective and resistant drugs against *Mtb*. MDR-TB occurs when a Mycobacterium tuberculosis strain is resistant to isoniazid and rifampin, two of the most powerful first-line drugs. To cure MDR-TB, healthcare providers must turn to a combination of second-line drugs, several of which are shown here. Second-line drugs may have more side effects, the treatment may last much longer, and the cost may be up to 100 times more than first-line therapy. MDR-TB strains can also grow resistant to second-line drugs, further complicating treatment. (Credit: NIAID)

under periods of stress but still remain viable. Though they exhibit drug tolerance, there remains a distinction between persisters and mutant bacilli that have acquired drug resistance; the persisters remain quiescent in the presence of antibiotics, unlike mutants which typically continue to grow [17]. Persisters are not necessarily resistant to antibiotic therapy; however, they can tolerate exposure to these drugs because they have downregulated or inactivated the pathways that are the targets of antibiotics [17].

The current TB treatment regimen has proven successful in the management of TB infection; however, the strategy is no panacea. The protracted course of antibiotic therapy required can lead to drug toxicity and yield undesirable effects. Complications of TB chemotherapy can include GI intolerance, neuropathy, arthralgia, increase in liver enzymes, hepatitis, immune thrombocytopenia, agranulocytosis, hemolysis, renal failure, optic neuritis, and ototoxicity [2]. The prolonged duration and the adverse events associated with anti-TB regimens impede the effective treatment of tuberculosis often by leading to improper medical adherence [16]. The implementation of monitoring protocols, like directly observed therapy, was necessitated due to poor patient compliance and requires a healthcare provider oversee each patient take their medication to ensure treatment adherence [2].

Erratic or incomplete treatment regimens due to personal error, high dose mostly multiple drugs together, other severe side effects lead to relapsed disease and the rise of drug-resistant forms of TB, which are even more challenging to manage, prompting excess morbidity and mortality, as 30% of deaths due to antimicrobial resistance is attributed to drug-resistant TB [4, 16]. Of the ten million annual cases of TB, half a million individuals are estimated to have rifampin-resistant TB, with 78% of rifampin-resistant patients having multidrug-resistant TB (MDR-TB), and 6.2% of the MDR-TB group having extensively drug-resistant TB (XDR-TB) [2]. Drug-resistant TB treatment can take more than two years to complete and is toxic, expensive, and highly ineffective in treating and preventing disease spread. It is projected that in the next 35 years, drug-resistant TB will cost the global economy $16.7 trillion while killing 75 million people in the process https://www.tballiance.org/why-new-tb-drugs/antimicrobial-resistance.

1.2 The Promises and Limitations of Bedaquiline

Bedaquiline is a novel antitubercular agent and the first anti-TB drug approved by the Food and Drug Administration (FDA) since rifampin in the 1960s (Bedaquiline for the treatment of resistant tuberculosis: Promises and pitfalls). Bedaquiline, a diarylquinoline, exhibits a unique mechanism of action despite its close relation to fluoroquinolones. Unlike fluoroquinolones, bedaquiline does not inhibit DNA gyrase. Instead, bedaquiline is the only FDA-approved agent that has been found to target mycobacterial ATP production by inhibiting the *Mtb* ATP synthase proton pump with a >20,000-fold lower affinity for the human mitochondrial ATP synthase

enzyme (Bedaquiline: Current status and future perspectives). The robust bactericidal action of bedaquiline leads to its accelerated approval by the FDA for the treatment of MDR-TB and became the first agent specifically approved for MDR-TB management.

There were several studies conducted that evinced bedaquiline's potential therapeutic benefits but it was two studies in particular, C208 and C209, that led the FDA to grant bedaquiline's accelerated approval. The first stage of trial C208 conducted by Diacon et al. found quicker sputum culture conversion (hazard ratio, 2.253; 95% confidence interval, 1.08–4.71; $p = 0.031$) and a higher culture conversion rate (17/21, 81% vs. 15/23, 65%, $p = 0.242$) in the bedaquiline group when compared with the placebo group at week 24. C208 Stage 2 substantially improved the culture conversion rate (52/66, 79% vs. 38/66, 58%, $p = 0.008$) and the cure rate (38/66, 58% vs. 21/66, 32%, $p = 0.003$) at week 24 and week 120, respectively, compared to the control group receiving placebo treatments (bedaquiline and delamanid in the treatment of multidrug-resistant tuberculosis: Promising but challenging). To evaluate the safety and efficacy of bedaquiline, researchers applied the methods used in trial C208 to a larger population of patients in trial C209. The results of trial C209 were consistent with that of trial C208, in which culture conversion rates at week 24 reached 79.5% (Bedaquiline and delamanid in the treatment of multidrug-resistant tuberculosis: Promising but challenging).

Though bedaquiline has shown promising results for treatment of MDR-TB, it is not without faults. A pitfall of bedaquiline is that despite its recent induction, resistance against the agent has already emerged. The rapid emergence of resistance is likely caused by improper or unregulated use of the drug. The elimination half-life of bedaquiline at 5–6 months is exceptionally longer than other antibiotics or antitubercular agents (Bedaquiline and delamanid in the treatment of multidrug-resistant tuberculosis: Promising but challenging). Sustained exposure of subinhibitory concentrations of bedaquiline as a single drug engenders resistant *Mtb* strains; therefore, treatment regimens must be deliberately designed to avert the drug acting as the sole effective antibiotic in circulation (Bedaquiline for the treatment of resistant tuberculosis: Promises and pitfalls).

There also exist several safety concerns surrounding use of bedaquiline, with the drug even carrying two black box warnings (Bedaquiline for the treatment of resistant tuberculosis: Promises and pitfalls). Concerns associated with bedaquiline treatment include QT interval prolongation, increased prevalence of hepatic disorders, adverse drug interactions, and increased risk of death (Bedaquiline for the treatment of resistant tuberculosis: Promises and pitfalls). There has been scrutiny surrounding bedaquiline's accelerated approval based on surrogate endpoints, as the drug was approved based on sputum culture conversion rates rather than patient clinical outcome. Due to the related adverse events associated with the antitubercular agent, the WHO has advised for pharmacovigilance methods be implemented when administering bedaquiline to facilitate early detection and reporting of adverse events (Bedaquiline for the treatment of resistant tuberculosis: Promises and pitfalls) (Fig. 2).

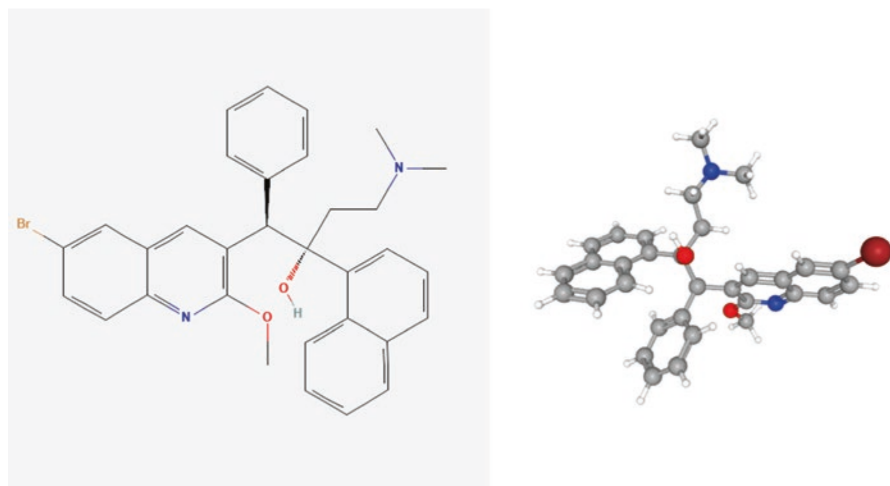

Fig. 2 2-dimensional and 3-dimensional structure of Bedaquiline

2 Identifying Novel Antibiotic Targets Through Enhancing the Biological Understanding of *Mycobacterium Tuberculosis*

Our incomplete knowledge on the inner workings of mycobacterial physiology and metabolism, especially in vivo, has hindered our ability to winnow the TB pandemic. Due to the ambiguity of our understanding of mycobacterial biology, the adaptability of mycobacteria metabolism, and the presence of various host microenvironments during infection, there exist uncertainties regarding the nutrients available to the bacilli [4]. The complexity surrounding host infection with TB cannot efficiently be replicated with standard in vitro and animal studies [4]. Even diagnostic methods face complications, often resulting in misleading results. More insight on the biology, physiology, and metabolism of *Mtb* will illuminate novel targets that may be used to develop improved TB chemotherapeutics (Fig. 3).

2.1 *Role of Rel$_{Mtb}$ in Response to Nutritional Stress*

When exposed to aerosolized droplets infected with the tubercle bacillus, many individuals will present with a latent TB infection. During a latent TB infection, the bacillus resides in phagosomes of resident macrophages in the alveolus. The intracellular expansion of the *Mtb* bacterium spreads to surrounding cells, ultimately triggers the adaptive immune response, and leads to the recruitment of other immune cells. Eventually, the *Mtb* is fettered in a granuloma consisting of impermeable

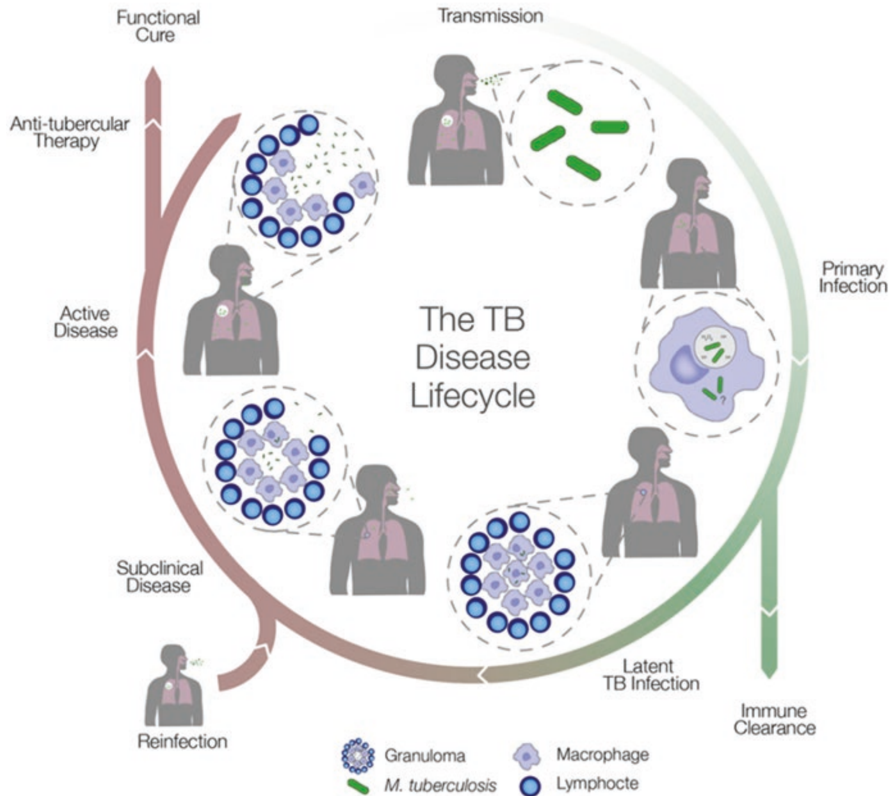

Fig. 3 Pathogenesis of TB [4]

caseous material produced by macrophages and many immune cells, including dendritic cells, B-cells, and T-cells. Within this granuloma, the bacillary population is relegated to a nutrient-limited environment, where they are exposed to hypoxia, antimicrobial reactive oxygen species (ROS), nitrogen intermediates, and restricted access to amino acids [18]. However, the *Mtb* bacterium has adapted various mechanisms to deal with this nutritional stress it experiences during infection of its host.

One of the responses *Mtb* has adapted against nutrient starvation is the stringent response, which allows for the long-term survival of the bacillus, even in this hostile environment. The stringent response is mediated by the Rel A and SpoT proteins [19, 20]. The RelMtb homolog of Rel A and SpoT proteins in mycobacteria produces a hyperphosphorylated guanine ((p)ppGpp) as part of the stringent response to nutrient starvation. The (p)ppGpp acts as a signaling molecule and modulates the expression of mycobacterial genes related to survival under nutrient starvation, allowing the bacilli to slow their growth and metabolism of amino acids, carbohydrates, and phospholipids [21].

In vitro and mouse infection studies have shown that the inactivation of RelMtb enhances the bactericidal effect of isoniazid under nutrient-depleted conditions. This finding is significant, being that isoniazid customarily has poor bactericidal effects against persister cells. Because persisters have restricted their growth, inhibition of mycolic acid biosynthesis by isoniazid does not produce the same bactericidal effect as it does in replicating cells. The promise of RelMtb serving as a potential target for anti-TB therapeutics led researchers, conducting high-throughput screens, to identify a RelMtb synthase inhibitor. Research conducted with the inhibitor has supported the previous claims of isoniazid potentiation but awaits in vivo validation [4].

2.2 Role of RPFs in Response to Nutritional Stress

In response to nutritional stress, *Mtb* may also enter a viable but non-culturable state (VBNC), meaning the bacilli will not propagate on growth media, effectively losing its culturability. This VBNC state of *Mtb* can impair detection methods, which ultimately leads to an underestimation of total viable cells [22, 23]. However, these VBNC mycobacteria may potentially be resuscitated with secretory proteins termed RPFs, short for resuscitation-promoting factors. If the bacilli can be resuscitated in culture, we may refer to them as differentially culturable tubercle bacteria (DCTB). DCTB requires the culture to be supplemented with culture filtrate (CF), which contains RPFs, to emerge [24]. These RPFs are secreted by actively growing bacilli to prompt the growth of those bacilli lying dormant. Mycobacteria have five RPF genes, RPF A-E. These RPFs are transglycosylases that can lyse peptidoglycan, a component of the cell wall. RPFs are dispensable for mycobacterial growth in vitro; however, they are needed for the resuscitation of DCTB [25].

It has been found that, not only does the sputum of TB patients typically contain culturable bacilli, it also contains DCTB which seem to exhibit drug tolerance and requires resuscitation with RPFs [25]. This finding prompted the studies of Wet, Warner, and Mizrahi on RPFs, which yielded “an RPF-null quintuple deletion mutant of *Mtb* that was subsequently used to demonstrate the presence in sputum samples from TB patients of both RPF-dependent and RPF-independent populations of differentially culturable tubercle bacilli” [4]. The findings of their study emphasized the limitations of diagnostic techniques utilized to quantify viable bacillary numbers. Underestimation of the bacterial burden, in turn, results in misjudgment of responses to therapeutics, further compounding the issue of antimicrobial resistance [4]. Gaining erudition on DCTB, and the complexities surrounding bacillary phenotype during infection, is vital to advance the development of novel diagnostic methods and drug regimens desperately needed for TB [25].

2.3 Role of DNA Replication Machinery as Potential Drug Targets

Despite DNA replication serving as arguably one of the most important processes for the survival and pathogenesis of infectious agents, including *Mtb*, relatively few antibiotics target DNA replication machinery compared to other processes such as transcription, translation, and cell wall synthesis. Three families of natural products are known to impede on DNA replication: (1) DNA gyrase inhibitors, (2) sliding clamp inhibitors, and (3) DNA polymerase III inhibitors, only the latter two will be discussed further in this section [26].

Isolated from *Streptomyces griseus,* Griselimycins target the bacterial replisome. Synthetic analogs of the cyclic peptide, Griselimycin, were generated to show improved pharmacokinetic properties and therapeutic potential. The Griselimycin analogs bind to *dna*N-encoded β sliding clamp and abrogate its interaction with DnaE1, ergo interfering with DNA replication [26].

Additionally, the macrolide nargenicin, isolated from *Nocardia sp* ACC18, targets the bacterial replisome. Nargenicin binds to the DnaE polymerase in the presence of DNA, in turn, repressing DNA replication. Significantly, nargenicin was found to have no effect on human DNA polymerases during counter-screening efforts. Nargenicin was recently shown to have bactericidal activity against *Mtb* making the compound a new potential lead for the TB drug pipeline [26].

The discovery of DnaE serving as the target for nargenicin coincided with the validation of the proofreading domain of DnaE utilizing ara-A, a chain terminating adenosine analog. The intrinsic 3′-5′ exonuclease residing in the PHP domain of DnaE was identified as the proof-reading domain for the polymerase which provides replication fidelity in *Mtb* in a study by Rock et al. Utilizing PHP knockouts rendered the mycobacterial cells susceptible to DNA chain termination by ara-A [4].

Interestingly mycobacteria have been found to exhibit a noncanonical system for mismatch repair. This noncanonical mismatch repair system includes the mismatch-specific endonuclease NucS which interacts with the β sliding clamp for proper functioning. Though this discovery of the interaction between NucS and the β sliding clamp was found in actinomyocete studies, if we assume the system operates in a similar fashion in *Mtb*, we can then expect the natural compound griselimycin to interfere with mismatch repair and SOS mutagenesis, as well as DNA replication, by precluding NucS from binding to the β sliding clamp [4]. Together these findings encourage the exploration of DNA replication and metabolism as an area for drug discovery efforts (Fig. 4).

2.4 Role of DNA Metabolism as a Potential Drug Target

During a phenotypic screen, the compound 1-(5- isoquinolinesulfonyl)homopiperazine (Fasudil) was observed to invoke an antitubercular effect through inhibition of GuaB2 [4]. GuaB2 belongs to the mycobacterial inosine 5′-monophosphate

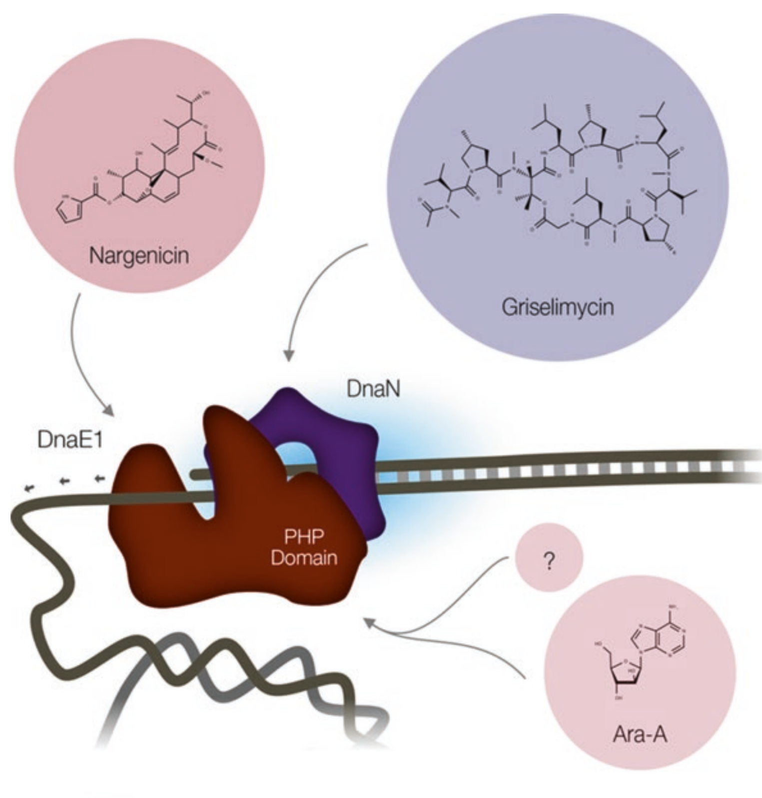

Fig. 4 DNA replication as a Potential Drug target [4]

dehydrogenase (IMPDH) family. The IMPDH enzyme catalyzes the first committed step in the synthesis of guanine nucleotides through the NAD + -dependent conversion of inosine 5′- monophosphate (IMP) to xanthosine monophosphate (XMP) during de novo purine biosynthesis. Inhibition of guanosine nucleotide biosynthesis is calamitous for the pathogen due to its omnipresence within the cellular process including nucleic acid synthesis, cell envelope biogenesis, protein synthesis, cofactor biosynthesis, and the stringent response [4].

To assess the validity of GuaB2 as a lead for the drug pipeline, investigation of the purine salvage pathway was vital. The ability to produce GMP directly from guanine in Mtb, by the enzyme hypoxanthine-guanine phosphoribosyl-transferase, highlights a potential metabolic bypass that may circumvent drug inhibition of GuaB2 [4].

No official consensus on the vulnerability of GuaB2 has been reached to date. Two studies conducted concurrently yielded conflicting results. One study utilizing GuaB2-depleted conditional mutants found that exogenous guanine supplementation was not effective in rescuing the bacilli. The results of this study prompted the researchers to classify GuabB2 as "vulnerable and bactericidal" target [4]. In the

second study however, the combination of the patient's inability to clear the infection coupled with evidence of increased levels of guanine in infected individuals compared to healthy contrast the findings of the first study. The researchers in this study determined that GuaB2 was "essential but not vulnerable" due to the potential for guanine salvage at the concentrations observed in vitro [4].

These results highlight the importance of further investigation into Mtb metabolic adaptability in reference to changing nutrients available to the bacilli in the vast array of host microenvironments during infection. Further insight into the physiology of Mtb host infection engenders novel targets vulnerable to exploitation by antibiotics.

3 In Silico Strategies and Their Potential for Expediting TB Drug Development

The drug discovery process was transformed with high-throughput screening (HTS). HTS is an optimized assay used in the pharmaceutical industry that enables the testing of large compound libraries against a desired target. This method utilizes robotic automation with instruments including liquid handling software and plate readers. The automation of HTS has enhanced compound screening and efficiency, allowing for the expedited identification of compounds for the "hit to lead" drug discovery effort. In addition to HTS, computer-aided drug design (CADD) has also enhanced the drug discovery process thanks to the advancement of data storage capacities, supercomputing powers, and parallel processing.

Computational methods, also known as in silico methods, exploit already available data to gain insight on protein function and potential to serve as a drug target. In silico methods can be used to search for prioritized candidates for drug discovery pipelines. Pragmatically auditing libraries of small compounds elucidates molecules with promising ability to bind to, and modulate the function of target proteins. Computer-aided drug design is divided into two categories based on the availability of structural data for the target: (1) ligand-based drug design (LBDD), and (2) structure-based drug design (SBDD), which will be elaborated on later in this section. The LBDD and SBDD approaches are not mutually exclusive however and are often used together to complement each other [2]. It is also common to utilize these in silico methods in an effort to optimize high-throughput screening through prioritization of prospective hits.

Despite the introduction of innovative techniques like HTS and CADD to the drug developmental process, there have been no recent significant contributions of candidates to the TB drug pipeline. The exigent need for novel anti-TB therapeutics underscores the importance of maximizing the advantages of in silico methods and their application in TB drug discovery.

3.1 Big Data

To maximize the benefits of CADD biological, chemical, and structural data from various open data sources needs to be mined. A passel of data exists for utilization against TB as research has been conducted on the disease for decades. Enhanced biological techniques and technologies led to a burgeoning of various available data points. Protein structures were rendered from X-ray crystallography, nuclear magnetic resonance (NMR), cryo-electron microscopy (EM), homology modeling, and molecular dynamics (MD) simulations, while cost-effective sequencing permitted the study of genomics, transcriptomics, proteomics, metabolomics, lipidomics, and more for many species, *Mtb* included [2]. There are countless useful open access resources to obtain data of particular interest, including some databases specific to information on TB. A broad, and widely applicable database that maybe be of use for TB drug discovery, is Uniprot (https://www.uniprot.org/). Uniprot contains comprehensive data related to protein structure, function, and sequencing. Pertaining more to mycobacteria, the TB Database (http://tbdb.bu.edu/tbdb_sysbio/MultiHome.html) has complied information on mycobacterial genomes, gene expression, gene correlation, gene epitopes, and computationally modeled molecular pathways by integrating transcriptomics, proteomics, metabolomics, and lipidomics. Exclusive to respiratory pathogenic mycobacteria, Mycobrowser (https://mycobrowser.epfl.ch/), can be used to find assimilated data like genomic details, protein information, drug and transcriptome data, mutant and operon annotation, and structural views. Deidentified TB patient clinical data are provided by the TB Portals (https://tbportals.niaid.nih.gov/). Clinical data provided by TB Portals include age of onset, gender, comorbidity, socioeconomic factors, geographic location, treatment regimen, clinical imaging, radiological annotation, patient outcome, and genomic information from drug-sensitive and drug-resistant cases (Table 1).

3.2 Structure-Based Drug Design

Structure-based drug design depends on the availability of three-dimensional structural data for the desired biological target, typically a protein receptor or enzyme. Structural data can be obtained experimentally, via X-ray crystallography and NMR, or generated via computational methods like homology modeling or protein threading [2]. The structure of the target protein is assessed to utilize the binding pocket in virtual screening efforts of molecular databases or for designing novel ligands to generate potential new hits. Moreover, SBDD also requires information on a target's druggability and potential binding sites to obviate endeavors wasted on investigating intractable targets. A target's druggability refers to its capacity to be therapeutically modulated by a small molecule or ligand [2]. Idyllic binding sites are concaves or cavities on the protein surface that possess chemical complementarity to a ligand enabling its effective binding to achieve a desired effect. Druggability

Table 1 Antimycobacterial Drug Discovery Resources

Function	Software/ webserver name	Availability	Website
Protein	UniProt	Free webserver	https://www.uniprot.org/
Genomes	TB Database	Free webserver	http://tbdb.bu.edu/tbdb_sysbio/MultiHome.html
Genomic + proteomic	Mycobrowser	Free webserver	https://mycobrowser.epfl.ch/
Comparative modeling	SWIAA-MODEL	Free webserver	https://swissmodel.expasy.org/
Structural geometry	Robetta	Free webserver	http://new.robetta.org/
Druggability	PockDrug	Free webserver	http://pockdrug.rpbs.univ-paris-diderot.fr/cgi-bin/index.py?page=home
Docking	Dock	Free/open source platform	http://dock.compbio.ucsf.eu/
Molecular dynamics	CHARMMing	Free webserver	https://www.charmming.org/charmming
Molecular descriptors, finger prints, & Quantitative structure–activity relationship	E-Dragon	Free webserver	http://146.107.217.178/lab/edragon/start.html
Pharmacokinetic properties	QikProp	Commercially available through Schödinger	https://www.schrodinger.com/qikprop
Clinical data	TB portals	Open access	https://tbportals.niaid.nih.gov/
Chemical library	Pubchem	97 million compounds	https://pubchem.ncbi.nlm.nih.gov/
Chemical library	GDB-17	166 billion compounds	http://gdb.unibe.ch/
Chemical library	ZINC	230 million compounds	http://zinc.docking.org/

Adapted from Macalino et al. [2]

assessments typically consist of binding site prediction, identification, and discrimination utilizing geometrical considerations and physiochemical features of the binding pocket.

Docking, a virtual screening (VS) method, is frequently used in SBDD to simulate the interaction between potential ligands and the binding pocket of the target [2]. Predicting the preferred orientation of one molecule to another when bound can be used to evaluate the ligand–protein binding affinity using scoring functions. The Druggable Cavity Directory (DCD) is a database that is open for researchers to submit information on druggability and protein pockets which then undergoes verification and becomes available for public consumption. Pharmacophore modeling, another VS technique, may also be used in SBDD. A pharmacophore is an ensemble of chemical and molecular features, like hydrophobicity, aromatic rings, or ionizable groups, required for optimal interactions with a biological target to prompt a biological response [2]. 3D pharmacophore models can be generated and utilized to

probe chemical libraries to generate potential leads for drug pipelines. The sequential application of pharmacophore screening followed by docking methods can provide for a more streamlined process with greater optimization of hit identification and selection.

3.3 Ligand-Based Drug Design

In the absence of structural data for the target protein, LBDD methods can be employed instead. LBDD harnesses knowledge of ligands with known activity to modulate the target to identify novel drug candidates [2]. One essential approach in LBDD is the similarity-based method. Similarity-based searches operate under the assumption that molecules with similarities in chemistry and shape to known active ligands serving as a template have significant probability of eliciting the same desired effect. Parameters for qualifying similarities can be broken down into three categories: (1) one-dimensional comprised of properties like molecular weight, logP, and number of H-bonds, (2) two-dimensional comprised of descriptors including aromaticity and degree of branching, and (3) three-dimensional comprised of characteristics like shape, volume, and surface area [2]. Once an efficient similarity measure has been implemented, a reliable scoring method needs to be established before screening compound libraries. Similarity-based methods can be applied to quantitative structure–activity relationship (QSAR) modeling to identify ligands with improved biological attributes. QSAR modeling establishes a statistical model to quantify the biological activity of a ligand and its structural and physiochemical properties. Because QSAR methods elucidate elements like molecular scaffolds or functional groups that are paramount for desired activity, the method is an advantageous practice in LBDD [2]. Pharmacophores may also be generated in LBDD. Pharmacophores may be generated from a set of diverse ligands that are known to modulate the target to produce the desired outcome. Researchers employ the use of various software programs to generate the pharmacophore by superimposing the ensemble of ligands over one another to distinguish the common ligand substructures required for favorable bioactivity. The pharmacophore may then be utilized in virtual screening of libraries of unexplored molecules for potential hits.

3.4 Integration of Methods

Integration of varying in silico methods employed prior to, or in parallel with, experimental methods like HTS, provides an optimized work flow for discovering novel drug candidates. LBDD may occasionally be applied as a filtration step prior to SBDD to narrow down potential ligands for experimental validation. Alternatively, LBDD and SBDD may yield hits with different scaffolds and these methods can be applied in parallel to maximize the discovery of potential hits. The parallel

application of various in silico methods allows researchers to more efficiently explore the vast chemical and biological space, as the strengths of one method may be used to overcome the weaknesses of another [2]. Assimilation of the methods discussed in this section in a complementary manner creates an efficient work flow leading to prioritized hits that may hopefully increase the success rates of candidates for the TB drug pipeline.

4 Potential Adjunct Therapies: Efflux Pump Inhibitors

The waxy and impermeable cell wall of *Mtb*, coupled with the activity of efflux pumps embedded in the cell membrane, are tools employed by the professional pathogen which allow the bacillus to confer resistance against antibiotic therapy. The uniquely lipid-rich, and complex cell wall contributes to inadequate penetration of many antimicrobial agents, while efflux pumps work to transport substrates, including anti-TB drugs, out of the cell. The work of Srivastava et al. (Efflux-Pump Derived Multiple Drug Resistance to Ethambutol Monotherapy in *Mycobacterium tuberculosis* and Ethambutol Pharmacokinetics-Pharmacodynamics), amongst others, has evinced the development of increased efflux pump activity, upon exposure to subinhibitory concentrations of first-line drugs, as a means of initial drug resistance acquired by *Mtb* before the procurement of chromosomal mutations in drug target genes. Bacilli may be exposed to modicum concentrations of antimicrobial drugs due to the administration of incorrect treatment, poor patient compliance, or lack of drug availability and accessibility seen in many high endemic areas. With this knowledge, it has become a priority for many researchers to gain insight into the mechanisms that underpin drug resistance mediated by increased efflux pump activity, hoping to circumvent this major barrier to effective treatment. The potential use of efflux inhibitors as adjuvant therapy, in conjunction with standard anti-TB therapeutics, may assist in increasing intracellular drug concentrations preventing the bacillus from exposure to subinhibitory drug concentrations that foster the development of resistance and impede on treatment efforts.

4.1 Phenothiazines: Thioridazine as an Adjunct

Phenothiazines, a drug class of tricyclic antipsychotics, was the first drug class developed for the first-line treatment of schizophrenia, bipolar disorder, and other psychotic disorders. Furthermore, phenothiazines have also shown antitubercular activity. The first phenothiazine to display activity against *Mtb* was chlorpromazine, but its pernicious effects have led to its de-prioritization as a potential candidate for the anti-TB pipeline. However, thioridazine has proven to be the most auspicious phenothiazine, exhibiting dual action against *Mtb* through (1) inhibiting efflux pumps; and (2) through augmented destruction of the bacilli by macrophages.

The latter effect of thioridazine against *Mtb* is of substantial value in regards to pulmonary TB, as the disease chiefly consists of intracellular bacillary infection of macrophages. Thioridazine's inhibition of calcium and potassium channels prompts a decrease in pH within the phagolysosome, which in turn evokes the activation of hydrolases, ergo killing the pathogen. The proposed mechanism by which thioridazine inhibits efflux pumps is through inhibition of the type-II NADH menaquinone oxidoreductase (NDH-2). NDH-2 plays an integral role in the generation of ATP through oxidative phosphorylation; therefore, its inhibition depletes the energy necessary for efflux pumps to generate a proton motive force required to transport substrates.

Despite the antitubercular promises of thioridazine, there are concerns regarding the potential development of adverse effects related to the central nervous system and cardiotoxicity with prolonged administration required for TB therapy. In an effort to circumvent potential toxicity, researchers have tried synthesizing analogs for thioridazine and encapsulating it within nano-particles; however, research is still inchoate, and pre-clinical and clinical studies are required. Further insight into thioridazine's mechanism of action and effect on mycobacteria can aid in the development of novel therapeutics, which is vital for improved TB treatments and, in turn, enhanced patient outcomes.

4.2 Phenylalkylamines: Verapamil as an Adjunct

Calcium channel blockers are frequently relied upon to treat several cardiovascular disorders such as angina, hypertension, and arrhythmia. Verapamil, the prototypical phenylalkylamine, a subclass of calcium channel blockers, may also be useful in the treatment of tuberculosis. Verapamil has been found to exhibit numerous effects against mycobacteria including, inhibiting the efflux of ethidium bromide in *Mtb*, moderately reestablishing the activity of first-line anti-TB drugs in resistant strains, and preventing drug tolerance while accelerating clearance of infection in mice treated with the first-line drugs.

Some research determined that the minimum inhibitory concentration (MIC) of bedaquiline and clofazimine was reduced in *Mtb* drug-susceptible and drug-resistant isolates when they were also treated with verapamil. Macrophage-mediated drug tolerance of bedaquiline and moxifloxacin was also decreased in isolates treated with verapamil. Other studies, however, found that though the MIC of bedaquiline and clofazimine was lowered due to the presence of verapamil in vitro, verapamil did not increase any bactericidal effect of the drugs in vivo. Verapamil has exhibited desirable effects for MDR-TB treatment when co-administered with bedaquiline. Not only does verapamil potentiate the effects of bedaquiline, but it also has exhibited the potential to reduce the risk of QT prolongation, one of the toxic effects of bedaquiline treatment. Verapamil is not a direct inhibitor of mycobacterial efflux pumps. Rather verapamil alters the membrane energetics and thus indirectly modifies the activity of efflux pumps. Verapamil exhibits potential as an adjuvant to TB

therapy; however, a barrier does exist as the concentrations needed are likely toxic to humans, and studies must be done to illuminate potential less toxic analogs.

4.3 Protonophores: CCCP as an Adjunct

Efflux pumps rely on the electrochemical gradient to generate a proton motive force (PMF) required to produce the energy needed for their biological activity. Protonophores are another class of compounds that may be used to inhibit mycobacterial efflux pumps. Protonophores, also known as uncouplers, inhibit efflux pumps by dissipating the energy gradient which is required for their proper function. Overexpression of efflux pump genes like Rv1258c caused by point mutations confer antibiotic resistance in mycobacteria through increased expulsion of antimicrobial agents. Carbonyl cyanide m-chlorophenyl hydrazone (CCCP), a protonophore, has been found to induce synergy with various antibiotics. In studies conducted on the effect of CCCP on Rv1258c expressing M. smegmatis strains, CCCP reduced the MIC of tetracycline.

In M. bovis BCG overexpressing Rv1258c, CCCP reduced efflux pump expression and activity. Additionally, in Mtb overexpressing jefA and mmr, two unique efflux pumps, CCCP restored the activity of various antitubercular agents including ethidium bromide, acriflavine, and safranin O. Despite the desirable antitubercular effects of CCCP, it is not without its limitations. Due to concerns regarding cytotoxicity, further studies on CCCP must be conducted for generation of safer analogs or to optimize concentrations required for safety while retaining efficacy.

4.4 Natural Compounds as an Adjunct

The application of natural products can be utilized at various stages of the TB drug discovery process. In addition to their potential role as DNA replication inhibitors discussed previously in this chapter, natural products may also serve as efflux pump inhibitors. Several efflux pump inhibitors of plant origin have been identified including reserpine, piperine, farnesol, luteolin, and biochanin A.

Reserpine, an adrenergic-blocking agent, is derived from the roots of the Rauwolfia serpentine plant (ACS link). In M. smegmatis strains expressing mmpL7, an ABC family transporter, the MIC of isoniazid was abated by reserpine. Also, in this model, reserpine subdued ciprofloxacin resistance mediated by Rv2686. Reserpine elicits increased susceptibility to isoniazid of both M. tuberculosis susceptible and resistant strains. Isoniazid is not the only first-line TB drug potentiated by concomitant administration of reserpine. Mtb is made more vulnerable to pyrazinamide through reserpine's inhibition of pyrazinoic acid efflux pumps.

Derived from Piper nigrum and Piper longum, the organic alkaloid, piperine, has also shown potential as an efflux pump inhibitor. Through inhibition of Rv1258c,

piperine demonstrates synergy with rifampicin and streptomycin. Though synergy was observed, this did not necessarily confer enhanced killing in certain resistant Mtb strains. Further studies need to be conducted on piperine and other natural compounds for their potential uses as adjuvant therapies in the treatment of TB.

5 Promising TB Pipeline Candidates in Research

There is a clamant need to end the pervasive global TB pandemic. The emergence of antibiotic resistance to first-line TB drugs often leaves many patients with no other options. Upon treatment, only 54% of MDR-TB patients and only 30% of XDR-TB patients successfully clear the infection. One in 3 deaths due to antimicrobial resistance is attributed to drug-resistant TB. The impact TB has on reinforcing the cycle of poverty, entrenching communities and in some cases entire countries, further compounds the devastation caused by the disease. The most promising approach to mitigating the global burden of TB is to abridge treatment duration through the development of novel antitubercular agents.

5.1 BRD4592: An Inhibitor of Tryptophan Synthase

Numerous of the newly identified compounds for the TB drug pipeline hit mycobacterial cell wall targets. Disproportionate activity directed against cell wall synthesis leaves libraries replete of potential targets unexplored. In an effort to capitalize on unique targets, researchers performed whole-cell screens of compounds from a diversity-oriented synthetic (DOS) library. Results of the whole-cell screens revealed the bactericidal effect of the compound (2R,3S,4R)-3-(2′-fluoro-[1,1′-biphenyl]-4-yl)-4-(hydroxymethyl)azetidine-2-carbonitrile (BRD4592). BRD4592 was shown to inhibit the mycobacterial enzyme TrpAB. The TrpAB enzyme, encoded by trpA and trpB genes, catalyzes the final steps necessary for L-tryptophan (L-Trp) biosynthesis. The biosynthesis of L-Trp is tightly regulated in *Mtb* because it is the most energetically costly amino acid to synthesize. Regulation of L-Trp biosynthesis occurs at both the transcriptional level and at the enzymatic level through allosteric inhibition of the two subunits of TrpAB.

BRD4592 elucidated a new binding site on the TrpAB enzyme, binding at the protein-protein interface. BRD4592 is thought to inhibit TrpAB through entropy-based stability of vital enzyme states and through stabilizing the enzyme-product complex without altering the structure of the active site. The allosteric inhibition of TrpAB by BRD4592 cannot easily be overcome as multiple conformational states of the enzyme are affected. Another advantage of BRD4592 is its high level of specificity for TrpAB, evoking little to no off-target effects. Metabolic enzymes are difficult drug targets because they typically have active sites that are shallow binding pockets, permitting the binding of polar and hydrophobic substrates which

directly conflict with drug-like properties. However, as demonstrated by BRD4592, allosteric inhibition is an appealing tactic that may be utilized to circumvent issues surrounding direct metabolic enzymatic inhibition.

Coupled with its potential to serve as a future antitubercular agent, BRD4592 can also provide further insight on the complex metabolism of *Mtb* in diverse host microenvironments. Understanding why the starvation of some, but not all, amino acids, leads to death of *Mtb* can provide useful information on how to target *Mtb* metabolism in vivo.

5.2 TAM16: An Inhibitor of Polyketide Synthase

The thick, insoluble cell wall of *Mtb* aids the bacilli in evading destruction by macrophages or antibiotics. Polyketide synthases (PKS) are an enzymatic family that form lipid metabolites utilized in the mycobacterial cell wall. The PKS family encoded by 24 genes has not previously been exploited as a TB drug target and serves as an attractive lead in novel TB drug discovery efforts.

The compound TAM16, a benzofuran, was found to inhibit Pks13, a specific member of the Pks family in *Mtb*. The protein Pks13 performs the final step in mycolic acid synthesis, which requires condensation of two long-chain fatty acids. TAM16 inhibits Pks13 by blocking the active site of the enzyme through binding of the acyl chain-binding groove. There is potential for TAM16 to become a first-line drug for TB treatment as it exhibits activity against MDR-TB and XDR-TB, shows a 100-fold lower frequency of resistance compared to isoniazid, and has promising pharmacokinetic and toxicity profiles. Due to TAM16 disrupting the *Mtb* cell wall, it exhibits synergy with other antitubercular drugs by augmenting their penetration.

By showing efficacious potency when administered as just a single dose, while also potentiating the activity of other antimicrobials, TAM16 has the potential to simplify TB regimens and encourage proper patient compliance.

References

1. World Health Organization. Tuberculosis (TB), 2021, October 14. https://www.who.int/newsroom/fact-sheets/detail/tuberculosis. Accessed 25 Oct 2021.
2. Macalino SJY, Billones JB, Organo VG, Carrillo MCO. In silico strategies in tuberculosis drug discovery. Molecules. 2020;25(3). MDPI AG. https://doi.org/10.3390/molecules25030665.
3. Living with Tuberculosis. American Lung Association, 2020, March 9. https://www.lung.org/lung-health-diseases/lung-disease-lookup/tuberculosis/symptoms-diagnosis.
4. de Wet TJ, Warner DF, Mizrahi V. Harnessing biological insight to accelerate tuberculosis drug discovery. Acc Chem Res. 2019;52(8):2340–8. https://doi.org/10.1021/acs.accounts.9b00275.
5. Finding the missing cases: The role of enhanced diagnostics and CASE-finding in reaching all people with TB; n.d.. https://www.cdc.gov/globalhivtb/who-we-are/resources/keyareafactsheets/finding-the-missing-4-million.pdf.

6. Zumla A, Marais BJ, McHugh TD, Maeurer M, Zumla A, Kapata N, et al. COVID-19 and tuberculosis-threats and opportunities. Int J Tuberc Lung Dis 2020;24(8):757–60. https://doi.org/10.5588/ijtld.20.0387. PMID: 32912377.
7. Tiberi S, Vjecha MJ, Zumla A, Galvin J, Migliori GB, Zumla A. Accelerating development of new shorter TB treatment regimens in anticipation of a resurgence of multi-drug resistant TB due to the COVID-19 pandemic. Int J Infect Dis. 2021;113:S96–9. https://doi.org/10.1016/j.ijid.2021.02.067.
8. History. Centers for Disease Control and Prevention, Centers for Disease Control and Prevention; 2016, December 12. https://www.cdc.gov/tb/worldtbday/history.htm.
9. Drew RH. Isoniazid: an overview. UpToDate, 2020. https://www.uptodate.com/contents/isoniazid-an-overview.
10. Drew RH. Pyrazinamide: an overview. UpToDate, 2021a, November 22. https://www.uptodate.com/contents/pyrazinamide-an-overview.
11. Elad N, Baron S, Peleg Y, Albeck S, Grunwald J, Raviv G, Shakked Z, Zimhony O, Diskin R. Structure of Type-I Mycobacterium tuberculosis fatty acid synthase at 3.3 Å resolution. Nat Commun. 2018;9(1):1. https://doi.org/10.1038/s41467-018-06440-6.
12. Drew RH. Ethambutol: an overview. UpToDate, 2021b. https://www.uptodate.com/contents/ethambutol-an-overview.
13. Ellison RT. Determining the Mechanism of Action of Ethambutol. NEJM Journal Watch: Summaries of and Commentary on Original Medical and Scientific Articles from Key Medical Journals, 2020, May 8. https://www.jwatch.org/na51459/2020/05/08/determining-mechanism-action-ethambutol.
14. Drew RH. Rifamycins (Rifampin, Rifabutin, Rifapentine). UpToDate, 202AD, June 9. https://www.uptodate.com/contents/rifamycins-rifampin-rifabutin-rifapentine.
15. Treatment for TB Disease. Centers for Disease Control and Prevention. Centers for Disease Control and Prevention, 2016, April 5. https://www.cdc.gov/tb/topic/treatment/tbdisease.htm.
16. Dutta NK, Klinkenberg LG, Vazquez M-J, Segura-Carro D, Colmenarejo G, Ramon F, Rodriguez-Miquel B, Mata-Cantero L, Porras-De Francisco E, Chuang Y-M, Rubin H, Lee JJ, Eoh H, Bader JS, Perez-Herran E, Mendoza-Losana A, Karakousis PC. H E A L T H A N D M E D I C I N E Inhibiting the stringent response blocks Mycobacterium tuberculosis entry into quiescence and reduces persistence; 2019. https://www.science.org
17. Torrey HL, Keren I, Via LE, Lee JS, Lewis K. High persister mutants in mycobacterium tuberculosis. PLoS One. 2016;11(5) https://doi.org/10.1371/journal.pone.0155127.
18. Dietrich J, Roy S, Rosenkrands I, Lindenstrøm T, Filskov J, Rasmussen EM, Cassidy J, Andersen P. Differential influence of nutrient-starved Mycobacterium tuberculosis on adaptive immunity results in progressive tuberculosis disease and pathology. Infect Immun. 2015;83(12):4731–9. https://doi.org/10.1128/IAI.01055-15.
19. Avarbock AB, Rubin H, Joshua Wand A, Group Chair G. Functional regulation of RELMTB: the stringent response protein responsible for initiating dormancy in mycobacterium tuberculosis; 2005.
20. Weiss LA, Stallings CL. Essential roles for mycobacterium tuberculosis rel beyond the production of (p)ppGpp. J Bacteriol. 2013;195(24):5629–38. https://doi.org/10.1128/JB.00759-13.
21. Primm TP, Andersen SJ, Mizrahi V, Avarbock D, Rubin H, Barry Iii CE. The stringent response of mycobacterium tuberculosis is required for long-term survival. J Bacteriol. 2000;182(17):4889–98.
22. Li L, Mendis N, Trigui H, Oliver JD, Faucher SP. The importance of the viable but non-culturable state in human bacterial pathogens. Front Microbiol. 2014;5(JUN):1. Frontiers Research Foundation. https://doi.org/10.3389/fmicb.2014.00258.
23. New Evidence for the Complexity of the Population Structure of Mycobacterium tuberculosis Increases the Diagnostic and Biologic Challenges. n.d..
24. Gordhan BG, Peters JS, McIvor A, Machowski EE, Ealand C, Waja Z, Martinson N, Kana BD. Detection of differentially culturable tubercle bacteria in sputum using mycobacterial culture filtrates. Sci Rep. 2021;11(1):6493. https://doi.org/10.1038/s41598-021-86054-z.

25. Chengalroyen MD, Beukes GM, Gordhan BG, Streicher EM, Churchyard G, Hafner R, Warren R, Otwombe K, Martinson N, Kana BD. Detection and quantification of differentially culturable tubercle bacteria in sputum from patients with tuberculosis. Am J Respir Crit Care Med. 2016;194(12):1532–40. https://doi.org/10.1164/rccm.201604-0769OC.
26. DNA Metabolism in Mycobacterial Pathogenesis, Warner DF, Rock JM, Fortune SM, Mizrahi V. DNA replication fidelity in the *Mycobacterium tuberculosis* complex. In: Gagneux S, editor. Strain variation in the mycobacterium tuberculosis complex: its role in biology, epidemiology and control. Advances in Experimental Medicine and Biology, vol. 1019. Cham: Springer; 2017. https://doi.org/10.1007/978-3-319-64371-7_13.

New Drugs in Synthesis Research for Viral Diseases

Justin O. Ortiz, Jeffrey B. Brown, Ajay K. Varadhan, and Charles Preuss

Abstract In 1901, the yellow fever virus was one of the first viruses to be discovered by humans. Since then, over a century of research has been dedicated toward unveiling the viruses that have caused severe human illness. Following these novel discoveries, the field of viral treatments began to spark. Unlike antibiotics, antiviral medications have developed at a much slower pace. In the early 1960s, the first experimental antivirals were developed using trial-and-error methods. Although these methods were laborious and lacked efficiency, the discoveries from this era became the start of the antiviral field. Throughout the years, the complexity of antiviral development has demonstrated that combined therapies involving antiviral drug targets have been one of the most effective approaches in treating viral infections. Overall, this chapter will briefly discuss the pathogenesis of colloquial viruses as well as describe—in detail—the emerging antiviral medications utilized to treat these pathogens.

Keywords Virus · Viral mechanism · Antiviral medications · Emerging pharmacotherapy · Viral pathogenesis

Antiviral drugs are a class of medicines particularly used for the treatment of viral infections. Viruses are among the major pathogenic agents that cause a number of serious diseases in humans, animals, and plants. Viruses cause many diseases in humans, from self-resolving diseases to acute fatal diseases. Developing strategies

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for the antiviral drugs are focused on two different approaches: Targeting the viruses themselves or the host cell factors. Antiviral drugs that directly target the viruses include the inhibitors of virus attachment, inhibitors of virus entry, uncoating inhibitors, polymerase inhibitors, protease inhibitors, inhibitors of nucleoside and nucleotide reverse transcriptase and the inhibitors of integrase. The inhibitors of protease (ritonavir, atazanavir, and darunavir), viral DNA polymerase (acyclovir, tenofovir, valganciclovir, and valacyclovir) and of integrase (raltegravir) are listed among the Top 200 Drugs by sales during the 2010s. Presently, there are a few drugs that treat herpesviruses, many that treat influenza and emerging antiviral drugs that aim to treat hepatitis C infection and HIV. Overall though, effective antiviral drugs have yet to be developed for many viral infections. The action mechanism of antiviral drugs consists of its transformation to triphosphate following the viral DNA synthesis inhibition. An analysis of the action mechanism of known antiviral drugs concluded that they can increase the cell's resistance to a virus (interferons) as well as suppress the virus adsorption in the cell and/or its diffusion into the cell. Antiviral drugs can also inhibit the deproteinization process in the cell (amantadine) and when administered in conjunction with antimetabolites, these drugs can cause the inhibition of nucleic acids synthesis as well.

1 Introduction

Ribonucleic acid (RNA) viruses have been at the top of concern regarding global health for many years. RNA viruses contain ribonucleic acid as their genetic material rather than deoxyribonucleic acid (DNA). RNA viruses, as well as viruses in general, come in all shapes and sizes. The simplest virus contains a single protein capsid that covers their viral genetic material to protect them from harsh environments. Traditionally, viral capsids are one of two shapes. The virus will either be helical or icosahedral. A helical-shaped virus is one that takes the shape of a coil around its viral nucleic acid. An icosahedral, on the other hand, is a 20-sided geometric shape corresponding with an equilateral triangle on each side. Although there are other complex viral capsids, these are the two most common. Viruses that contain RNA as their genetic material rather than DNA are classified as RNA viruses; however, viruses that contain DNA as an intermediate during their replication cycles are classified as retroviruses. One of the most prevalent viruses in this category is the human immunodeficiency virus (HIV). Human retroviruses are divided into three subfamilies: oncoretroviruses, lentiviruses, and spumaviruses. Oncoretroviruses contain human T-cell lymphotropic viruses type 1 and 2 (HTLV-1 and 2). Lentiviruses contain human immunodeficiency viruses type 1 and 2 (HIV-1 and 2), and spumaviruses contain foamy viruses, which have not been found to cause any diseases in vivo. Outside of HIV, the other two retroviruses that can cause human illness are HTLV-1 and HTLV-2. Many of these retroviruses, and viruses in general, have been heavily researched throughout the years; however, vital aspects regarding the pathogenesis of these diseases still remain unclear.

Viral hepatitis, for example, is the eighth highest cause of mortality globally and was responsible for an estimated 1.34 million deaths in 2015, a toll comparable to that of HIV and tuberculosis. Globally, approximately 257 million persons are chronically infected with hepatitis B and 71 million with hepatitis C. At this rate, an estimated 20 million deaths will occur between 2015 and 2030 [86]. There are five types of hepatitis, each being classified as A, B, C, D, or E. The most common types, however, are A, B, and C. For instance, the history of hepatitis C is quite notable. Before the 1980s, researchers were unaware of how hepatitis C was spread through circulation. Thus, during that time, hepatitis C was never screened for in blood transfusions. Accidental infectious needles and transfusions were a common cause of the rapid spread of the virus [33]. Since hepatitis C progresses very slowly, researchers are just now beginning to realize that hepatitis C can directly lead to hepatocellular carcinoma. It has taken decades for science to determine the mechanisms regarding the pathogenesis of this virus. Due to the duration of hepatitis C and possibly many other pathogens, researchers may soon be able to unravel the modalities of much more novel diseases.

This chapter will outline prominent viruses that cause severe human illness, as well as elaborate on the groundbreaking research used to produce potential antivirals that will be able to combat these diseases. As stated previously, viruses are broken down into RNA and DNA viruses. RNA viruses contain RNA in its genetic material and can be first distinguished by its shape. The viral capsid that surrounds the nucleic acid material is most commonly one of two shapes. The viral capsid may be icosahedral or helical. Once separated by their viral capsid shape, the virus can further be differentiated based on if it is enveloped or not. Thus, two RNA viruses can be icosahedral; however, if one is enveloped and the other is naked, they can then proceed to be separated into their respective categories. The last form of separation before the virus is specifically identified by its number of segments, family and virion polymerase, is its genome. RNA viruses can be single-stranded or double-stranded. If the RNA virus is single stranded, it can be a positive-sense or a negative-sense strand. A positive-sense single-stranded RNA virus is similar to mRNA in its structure and thus can be immediately translated by the host cell to cause infection. Negative-sense single-stranded RNA viruses are complementary to mRNA and must first be converted to a positive-sense strand via an RNA polymerase before it is translated. HIV-1, for example, is an enveloped, positive-sense, single-stranded icosahedral RNA virus [54].

2 Picornaviruses and Rotaviruses

Ever wondered what it meant when someone stated that they had the “common cold”? Traditionally, the common cold is caused by a virus and more specifically, picornaviruses! The common cold is a self-limited illness. This refers to any disease whose natural history is to resolve without treatment. Picornaviruses are naked, positive-sense, single-stranded icosahedral RNA viruses. Similar to most positive-sense RNA viruses, the genetic material alone is infectious. These viruses have a

variable host range and typically go through cytoplasmic replication. Some constituents of the family, include enteroviruses, which typically infect the GI tract, and rhinoviruses which typically infect the upper respiratory tract. Rhinoviruses, specifically, are sensitive to low pH's and cannot grow above a temperature of 33 degrees Celsius. This is a major reason why the rhinovirus typically infects the upper respiratory tract due to the lower temperatures present in those areas. Rhinoviruses are also seasonal. These viruses peak during the fall; however, the most severe rhinovirus infections are in the winter. Most picornaviruses such as coronaviruses, parainfluenza viruses, and rhinoviruses can lead to the common cold. Although the common cold may not seem life-threating, there are many viruses in the *Picornaviridae* family that can cause severe morbidity and mortality—such as the poliovirus. Even though polio has been eliminated in the United States, it is still very present in other portions of the world. The poliovirus is a non-enveloped, positive-sense, single-stranded icosahedral RNA virus. Poliovirus typically infects the gut intestinal lining, specifically the gut-associated lymphoid tissue. In more severe cases, poliomyelitis may occur if the virus enters the motor neurons of the body which may lead to paralysis. If poliomyelitis worsens, the nerve responsible for breathing—the phrenic nerve—will be compromised and the paralysis of the diaphragm will eventually lead to death. Presently, the prevention that is used to combat the poliovirus is the current three dose inactivated vaccine, which was approved in the year 2000. When infected with the virus, post-polio syndrome (PPS) can occur decades later. PPS leads to symptoms of pain in the muscles and joints, weakness, and fatigue. PPS is not contagious, although the poliovirus itself certainly is. One emerging treatment tested for PPS has been the use of L-citrulline. L-citrulline is known to change muscular metabolism by increasing nitric oxide synthesis and eventually increasing protein synthesis. If this turns out to be the case, a cost-effective, symptomatic therapy with L-Citrulline could be available for these patients [73]. Although the use of L-citrulline for PPS is in its developmental stages, there are no current pharmaceuticals that can reverse or delay the symptoms of PPS. Picornaviruses, in general, typically have no approved antivirals; however, researchers are actively working toward finding treatments for this family of viruses.

Rotaviruses are non-enveloped, double-stranded icosahedral RNA viruses. There are nine species of rotaviruses: however, only A, B, C, and H infect humans. In respect to these species, Group A dominates the most. Rotaviruses are spread through fecal–oral transmission and typically lead to more severe gastroenteritis than other GI pathogens. Rotaviruses can cause abrupt vomiting, diarrhea, and fever. In developing countries, rotavirus infections can cause serious illnesses and have recently, became one of the leading causes of infant mortalities in these locations. In the early 1990s, the prevention of rotaviruses began with the development of a vaccine. RotaShield was a live attenuated recombinant vaccine that was approved for use in the late 1990s [13]. Unfortunately, the same year that the vaccine was distributed was the same year it was removed from the market due to complications with intussusception. Intussusception occurs when one portion of the intestine slides into an adjacent part of the intestine. This can cut off blood supply, tear the intestines, and lead to necrosis of the bowel tissue. Current research has

shown slight effectiveness with a new antiviral agent—Nitazoxanide—for its potential use against rotavirus infections. As a drug, Nitazoxanide is currently an approved treatment for diarrhea caused by *Cryptosporidium* sp. Nitazoxanide and its active circulating metabolite tizoxanide inhibits simian A/SA11-G3P2 and human Wa-G1P8 rotavirus replication in different types of cells with 50% effective concentrations (EC50s) ranging from 0.3 to 2 μg/ml and 50% cytotoxic concentrations (CC50s) higher than 50 μg/ml [49]. Thiazolides such as nitazoxanide do not impact virus infectivity, binding, entry into target cells or general inhibition of viral protein expression. However, thiazolides do reduce the size, alter the architecture of viroplasms and decrease the rotavirus double-stranded RNA formation. Altogether, research has indicated that thiazolides inhibit rotavirus replication by interfering with viral morphogenesis and may represent a novel class of antiviral drugs effective against rotavirus gastroenteritis [49].

3 Orthomyxoviruses

Unlike icosahedral RNA viruses, all helical RNA viruses are enveloped. Orthomyxoviruses are negative-sense, single-stranded helical RNA viruses that contain the influenza A, B, and C viruses (colloquially known as the flu). Orthomyxoviruses replicate in the nucleus and have a vast host range. Two highly variable structures that are located on the influenza virus allow it to be extremely effective in evading the immune system. These two structures are neuraminidase and hemagglutinin. In order for a cell to be infected by influenza, the virus will attach itself to the sialic acid groups located on the membrane of the cell via hemagglutinin. Once the virus attaches, neuraminidase allows the virus to move from one sialic acid group to another by cleaving these groups until it locates the precise cell surface receptor needed for the virus to enter the cell. Unfortunately, this cell surface receptor still remains unknown. Proceeding this interaction at the level of the plasma membrane, the virus enters the cell through receptor-mediated endocytosis. Once it has entered the nucleus and replicated its viral mRNAs, the viral mRNAs must leave the nucleus and eventually bud off from the plasma membrane. When these viral particles attempt to bud off, hemagglutinin still remains attached to the sialic acid groups on the cell membrane. To prevent self-agglutination, neuraminidase cleaves the interaction between the sialic acid groups which will allow the virus to infect other cells. These two structures located on the virus are prime targets for pharmaceuticals.

Orthomyxoviruses are quite calculated and methodical; however, the human body contains barriers that attempt to prevent the infection. These barriers include, but are not limited to, mucus layers, interferons, and T-cell responses. Unfortunately, reinfection from influenza is quite common due to antigenic drift. Antigenic drift leads to a generation of drift variants due to the vulnerability of inaccurate viral polymerases producing point mutation errors on certain prone regions of the genes [14]. When antigenic drift occurs, the body's immune system may not be able to

recognize and prevent the infection caused by newer flu viruses. As a result, a person becomes susceptible to flu infection again, as antigenic drift has changed the virus' antigenic properties enough that a person's existing antibodies won't recognize and neutralize the newer flu viruses [11]. Due to antigenic drift, there are now 18 subtypes of hemagglutinin proteins and 11 subtypes of neuraminidase proteins. This reassortment is a leading cause for the readjustment of pharmaceuticals used to treat Influenza A. Currently, there are four FDA-approved antiviral drugs to combat influenza. These are peramivir (Rapivab), zanamivir (Relenza), oseltamivir phosphate (Tamiflu), and baloxavir marboxil (Xofluza). Formerly, there were two other drugs that were approved by the FDA, amantadine (Symmetrel) and rimantadine (Flumadine) [15]. Historically, these drugs have been used to treat influenza infection; however, throughout the years, the virus has become resistant to both amantadine and rimantadine due to antigenic drift. Of the four drugs that are currently FDA approved, the most novel antiviral drug is baloxavir marboxil. Baloxavir marboxil works against influenza A and B. Baloxavir marboxil has a mechanism of action that is quite different than the other three drugs. Rather than inhibiting the function of neuraminidase or hemagglutinin, baloxavir marboxil inhibits a subunit of the viral polymerase to prevent the replication of the virus. Since the mechanism of baloxavir marboxil is much different, it may be able to fight against strains of the influenza virus that are already resistant to the other three FDA-approved drugs [48]. Typically, inhaled zanamivir or oseltamivir phosphate is prescribed for 5 days to treat the flu. Another option that decreases the drug dose frequency would be a single dose of intravenous peramivir or oral baloxavir marboxil. Lastly, oseltamivir phosphate is typically used for patients that are hospitalized [15].

4 Rabies

The purpose of this section is to address the potential use of novel antiviral drugs as a treatment for the rabies virus. Currently, the treatment for post exposure prophylaxis to the virus is the human rabies immunoglobulin (HRIG) and rabies vaccine. HRIG can cost up to $10,000 in certain areas of the United States of America. Many individuals exposed to the virus, especially those that are uninsured, can be left with heavy medical debts. The development of the equine rabies immunoglobulin (ERIG) was proposed as a less expensive option; however ERIG is less effective and still unaffordable in many parts of the world. Unfortunately, without HRIG or ERIG, exposure to the virus will most likely to lead to death. The first record of the rabies virus infecting and killing humans was in 2300 B.C. Since then, the exact pathogenesis of the virus still remains unknown. The rabies virus is a helical, enveloped, negative-sense, single-stranded RNA virus. Once contacted with the virus, the disease will lead to a coma and eventually death if not treated immediately. It is speculated that the virus can replicate directly in the muscle tissue—after a bite from an animal for instance—or the virus can rapidly traverse to the peripheral nervous system (PNS) and replicate there. The inability to identify antigens of the rabies

virus in organs during the early stages of infection has led to uncertainties regarding the exact location of replication. Once the virus traverses into the PNS, it rapidly moves into the central nervous system (CNS) and symptoms begin promptly thereafter [40]. If not treated immediately, the virus will lead to encephalomyelitis in the CNS. HRIG is highly effective in post-exposure prophylaxis; however, the major concern lies on the present global shortage for these immunoglobulins. In developing countries, this shortage has led to the majority of deaths after rabies virus exposure. Hence, the discovery for new antiviral drug treatments to combat the virus has been of great interest.

Ribavirin, which is currently used to treat chronic hepatitis C, in conjunction with the use of interferon-alpha, has shown success during in vitro experiments; however, the effectiveness of this combination for in vivo experiments remains unclear. Historically, the two other antivirals that were used to combat the rabies virus were ketamine and amantadine. Ketamine is an NMDA antagonist and amantadine is a drug that has been used to treat influenza A. In 2004, the combination of ketamine, amantadine, and a few other drugs, collectively known as the "Milwaukee Protocol," initially demonstrated promising effects for treating the rabies virus [52]. Unfortunately, it was quickly discovered that the combination of these drugs was ineffective and unreproducible for treating the viral infection. An emerging treatment for rabies —favipiravir—is currently approved to treat the influenza virus in Japan. Favipiravir has been demonstrated to have antiviral effects against the rabies virus in experiments. Although the exact mechanism of the drug is not fully understood, it has shown promising effects in treating the infection when the rabies virus was injected intramuscularly in mice. Notably, favipiravir must be used immediately after initial infection. If the use of the drug was delayed 1–2 days post-infection, it was deemed ineffective. This indicates that the drug is unable to treat the rabies virus once it has already entered the CNS. In order to treat CNS effects caused by the virus, antivirals must be able to access the cerebrospinal fluid and tap into the CNS efficiently—which poses a significant barrier for these drugs. Along with the already present immunoglobulins, antivirals will be a vital addition for treating the rabies virus infection.

5 Human Papillomavirus

Approximately 79 million Americans are currently infected by the human papillomavirus (HPV) and 14 million people are being infected every year [22]. Nearly 80% of sexually active individuals are infected with the virus at some point in their lives; however, most individuals are never aware. The HPV is a naked, double-stranded, icosahedral DNA virus. The HPV is categorized within the *Papillomaviridae* family. The virus uses the host DNA polymerase to replicate in the nucleus of the cell. There are over 200 strains of the virus and approximately 30–40 strains are anogenital while 15–20 strains are oncogenic. Many of the HPV strains lead to genital warts; however, persistent infection of the HPV may lead to certain cancers especially, with strains that are of higher risk.

The HPV grows from the basal layer of the skin and progresses superficially through the epidermis. Beginning at the basal layer, the virus proceeds to the suprabasal layer to maintain the genome and amplify as the cell starts to proliferate. As the virus progresses from the suprabasal layer to the granular layer, it begins to assemble and is eventually released from the granular cell layer. The early genes of the virus are E1-E7, which are heavily present in the basal and suprabasal layer of infection. Particularly, the E6 and E7 proteins have been linked to the malignant transformation of cells. The E5 protein has a role in the pathogenesis of the virus; however, it is not required for the HPV to progress to cancer. The HPV strains are divided into three different categories: low-risk, intermediate-risk, and high-risk. The low-risk strains are solely found in benign lesions and these strains include 6, 11, 42, 43, and 44. The intermediate-risk strains are found in invasive cancers as well as in benign lesions; these strains include 31, 33, 35, 51, 52, and 58. The high-risk strains are nearly always found in carcinomas and these strains include 16, 18, 45, and 56 [54].

The human papillomavirus 9-valent vaccine (Gardasil) is the currently approved HPV vaccine in the United States. The HPV 9-valent vaccine uses the recombinant L1 capsid proteins located on specific HPV strains to protect against the infection. The vaccine is geared toward the HPV 16 and 18 strains, which typically lead to cervical cancer. The vaccine also protects against the HPV 6 and 11 strains, which typically lead to genital warts. Notably, there are no approved antiviral drugs to combat the HPV; however, researchers with San Diego-based Hera Therapeutics presented findings at the 29th Annual International Papillomavirus Conference supporting a potential antiviral treatment for HPV [42]. The emerging drug inhibited the replication of the HPV 16, 18, and 11 strains. Although the specific mechanism of action for the antiviral drug is unknown, the drug essentially inhibits the virus from DNA replication.

6 Human Immunodeficiency Virus and MERS-Cov

One of the leading causes of disease in the United States is HIV. In 2020, there were 37.7 million people living with HIV and 1.5 million people were newly infected that same year [81]. HIV is a retrovirus that infects a variety of species. The three types of retroviruses are oncoretroviruses, lentiviruses, and spumaviruses. HIV-1 and HIV-2 are in the subfamily of lentiviruses. There are three major groups of lentiviruses: HIV-1, HIV-2, and Simian immunodeficiency virus (SIV). These three groups share similar features which include variations in the presentation of the disease as well as following three typical stages of infection. The three stages of infection for lentiviruses include the acute infection, the latent period and a period of high levels of viral replication during opportunistic times. HIV is an enveloped positive-sense RNA virus. It is also classified as a retrovirus due to the production of a DNA intermediate during its replication process. The positive-sense RNA virus must first be transcribed by a viral reverse transcriptase. This will then transition the RNA to a negative-sense single-stranded DNA genome. Once the DNA becomes double-stranded, the HIV genome itself is infectious.

The HIV genome is polycistronic since it covers all three reading frames. Gag, Pol, and Env are the main structural proteins that are responsible for the function of the virus. Gag is responsible for forming the nucleocapsid, the viral matrix as well as the capsid. Pol is responsible for producing the reverse transcriptase and other important enzymes. Env is vital for the production of the envelope protein. The HIV genome contains other regulatory and accessory proteins as well. The regulatory proteins include Tat and Rev, while the accessory proteins include Vpr, Vpu, Vif, and Nef. During the early stages of HIV, Tat, Rev, and Nef are produced. Later in the life cycle, Vpr, Vpu, and Vif are produced after the virus has infected the cell [50]. Once HIV is in the genome, Gag is myristoylated and attaches to the cell membrane. Once Gag is attached to the cell membrane, it recruits 2 copies of viral RNA as well as other proteins. When this process is completed, the virus buds off. Once the virus has budded, the Gag is then cleaved by viral proteases and broken down into several proteins that form the matrix, nucleocapsid, and capsid protein. Essentially, the Gag protein is vital for forming nearly the entire structure of the virus. Opposed to other retroviruses, HIV is unique in its ability to enter and replicate in non-dividing terminally differentiated cells [29]. HIV forms a pre-integration complex (PIC) that allows the virus to enter the nucleus. The size of HIV is too large to pass through the nuclear pores, and thus, the viral capsid must be uncoated in the cytoplasm in order for the viral RNA to enter the nucleus.

The Gag protein is transcribed on the first reading frame, while the Pol protein is transcribed on the third reading frame; however, Gag-Pol precursors are formed 5% of the time. This occurs when the ribosome switches reading frames after it has translated the Gag protein. Thus, the formation of Gag-Pol precursors is 20:1. If a Gag-Pol precursor is produced, it is cleaved by a viral protease and broken down into its respective components. Pol is responsible for forming the enzymes necessary for the pathogenesis of HIV-1. This includes RNase, integrase, protease, and reverse transcriptase. Many of the antivirals used for the treatment of HIV are broken down into categories that can disrupt these enzymes. Nucleoside reverse transcriptase inhibitors (NRTIs) are nucleosides that are missing a 3′ hydroxyl group. When NRTIs are attached to a growing viral RNA, they terminate and interfere with its replication process. These NRTIs can be divided into adenosine, guanosine, cytidine, and thymidine analogs. Current NRTIs used to treat HIV are zidovudine (a thymidine analog) and didanosine (an adenosine analog). Non-nucleoside reverse transcriptase inhibitors (NNRTIs) bind to an area on the reverse transcriptase protein and inhibit the function of the enzyme. Examples of NNRTIs include nevirapine and delavirdine. Researchers have discovered the 3D structure of the viral protease produced by the Pol structural protein which has further led to the discovery of another class of antivirals termed as protease inhibitors. Initially, ritonavir was designed as a protease inhibitor; however, clinical trials have shown that ritonavir has a strong inhibition for the cytochrome P450 3A4 isoenzyme (CYP3A4). Since this isoenzyme is responsible for the degradation of drugs, ritonavir is currently used to enhance the effects of other protease inhibitors [35]. Ritonavir has decreased the amount of drug and drug frequency needed to treat HIV due to its ability to inhibit CYP3A4. Examples of other protease inhibitors include atazanavir and darunavir.

Additionally, a more novel class of drugs that are currently in experimental research includes the portmanteau inhibitors. These drugs inhibit the integrase and reverse transcriptase enzymes. Another structural protein that is highly targeted by antiviral drugs includes Env (gp160). Env is divided into the gp120 and gp41 subunits. These subunits are processed in the Golgi apparatus and the endoplasmic reticulum. The gp120 subunit of Env forms the receptor that is located on the external surface of HIV. The gp41 subunit forms the transmembrane portion responsible for viral entry. The gp120 and gp41 subunits are non-covalently bonded to one another to form a trimer. Once HIV targets a T cell or macrophage, the gp120 subunit binds to the CD4 receptor located on macrophages and T cells. After the gp120 subunit is bound to the CD4 receptor, the V3 loop (also known as the V3 hypervariable region) located on the gp120 subunit is exposed and binds to the CXCR4 receptor on a T cell or the CCR5 receptor on a macrophage. Prior to the interaction between the gp120 subunit and the CD4 receptor, a hydrophobic patch covers the V3 loop [88]. This highly conserved hydrophobic patch has been a prime target for new antiviral drugs. Research studies have shown that targeting certain amino acid residues on this hydrophobic patch have shown an increase in viral gp120 shedding. Notably, adding even a single glycine residue in crucial regions on this hydrophobic patch has demonstrated destabilization of the Env (gp160) trimer. Fostemsavir (Rukobia) is a first-in-class attachment inhibitor that prevents the binding of gp120 with the CD4 receptor. Due to the novel mechanism of action, fostemsavir may be active against HIV strains that have become resistant to the current drugs used to treat HIV [44].

In 2012, the first case of the Middle East respiratory syndrome coronavirus (MERS-Cov) occurred in Saudi Arabia. Since then, the virus has led to at least 2468 cases and 851 deaths globally [76]. MERS-Cov is believed to have originated from dromedary camels and has limited human-to-human transmission. With time; however, possible mutation of the virus can render it highly transmissible. MERS-Cov is a positive-sense, enveloped single-stranded RNA virus that is categorized within the *Coronaviridae* family. MERS-Cov targets a functional receptor—dipeptidyl dipeptidase 4 (DPP4)—which is present on the majority of cells in the body [72]. DPP4 is an enzyme necessary for the conversion of frequent amino acid sequences. DPP4 is predominantly present in the epithelial cells of the lower respiratory tract in humans; however, these receptors are seen more frequently in the upper respiratory tract of dromedary camels. Researchers believe that this makes MERS-Cov less transmissible to humans; however, that has not fully been confirmed. Currently, there are no specific treatments or vaccines used for MERS-Cov; however, a combination of lopinavir, ritonavir, and interferon-beta have shown benefits during in vitro experiments [76]. Lopinavir is an HIV protease inhibitor typically given jointly with ritonavir. Ritonavir increases the half-life of lopinavir and thus has increased the effect of the drug and decreased the necessary dosage. Researchers have also tested the addition of remdesivir to the combination of lopinavir, ritonavir and interferon-beta. Remdesivir is a broad-spectrum antiviral drug and its mechanism of action against MERS-Cov is likely through pretermination of the viral RNA transcription. Although recent meta-analysis and modeling have suggested that interferon treatment may not improve clinical outcomes in MERS-CoV patients, the fixed dose

combination of remdesivir and interferon-beta provides strong antiviral activity against MERS-CoV in vitro and in vivo as compared to the combination without remdesivir. In addition, remdesivir was the only treatment to significantly reduce pulmonary pathology [76].

7 SARS-CoV-2

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has provided for a pandemic involving 118.9 million cases as of March 12, 2021 [47]. Approximately 5% of patients who are infected by CoVs progress to complications including acute respiratory distress syndrome [47]. The coronaviruses (CoVs) include a diverse family of positively charged, single-stranded RNA viruses that are responsible for respiratory infections in human, other mammals, and avian species [84]. Within the *Coronaviridae* family lies the Orthocoronaviridae subfamily, which consists of *α-CoV*, *β-CoV*, *γ-CoV*, and *δ-CoV* [36]. The *γ-CoV* and *δ-CoV* species primarily infect avian species, while the *α-CoV* and *β-CoV* species exclusively infect mammals [36].

Human coronaviruses (HCoVs) have been prevalent for a significant period of time, with the first isolation of strain HCoV-B814 in 1965 [58]. More recently, pathogenic strains including SARS-CoV, Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2 have become of significant concern [19]. Since its emergence in 2019, SARS-CoV-2 has fashioned a devastating spread over the world [34]. As of December 10, 2021, there have been 267,865,289 confirmed cases of COVID-19, including 5,285,888 deaths, reported to World Health Organization [87].

The prominent symptoms of SARS-CoV-2 infection include anosmia, ageusia, and fever [27]. In addition, cough, chest pain, and shortness of breath have been noted as general acute symptoms for patients throughout the course of infection [27]. The CDC classifies the potential symptoms of COVID-19 into mild-to-moderate, severe, and critical symptoms. The mild-to-moderate symptoms include cough, fever, myalgias, and mild pneumonia. Severe symptoms include dyspnea, hypoxia, or more that 50% lung involvement on imaging. Finally, critical symptoms include respiratory failure, shock, or multiorgan system dysfunction. It is important to note that there is a high rate of asymptomatic or pre-symptomatic individuals. A recent trial suggested that 44.2% of randomly selected individuals and 20.2% of nonrandom participants reported a lack of SARS-CoV-2 symptoms within 2 weeks of a positive RT-PCR test [27].

This provides for a discussion of the transmission of SARS-CoV, an area that has become of extreme importance by the Centers for Disease Control and Prevention. The updated CDC guidelines suggest that the primary mode by which people are infected by SARS-CoV2 is via exposure to respiratory fluids that contain traces of the virus [19]. Furthermore, the CDC suggests that exposure can occur in the following three ways: “inhalation of very fine respiratory droplets and aerosol particles, deposition of respiratory droplets and particles on exposed mucous membranes

in the mouth, nose, or eye by direct splashes and sprays, and touching mucous membranes with hands that have been soiled either directly by virus-containing respiratory fluids or indirectly by touching surfaces with virus on them" [19]. Such exposure can be prevented through non-pharmaceutical interventions including masking, hand hygiene, and physical distance from infected people. Social distancing, movement restrictions, and sanitized public transport reflect key public health endeavors to reduce spread of COVID-19 [19]. Pharmaceutical treatments and vaccination are described later in this section.

7.1 Virulence Factors

The mechanism of infection and action of coronavirus has been studied excessively and is summarized here. The pathogen contains a spike (S) protein that binds to cellular entry receptors including human aminopeptidase N, dipeptidyl peptidase 4, and angiotensin-converting enzyme 2 [84]. The spike protein ensures specificity for these entry receptors during viral fusion with this host cell membrane. The envelope and the membrane are structural proteins that are utilized for the incorporation of RNA into the new viral particle during replication. Once entry has been completed, the coronavirus replicates and expresses pathogenic RNA which will be further encompassed in newly replicated viruses. Specifically, the viral genome is categorized by positive-sense, single-stranded RNA that is covered by a nucleocapsid [84].

These nonstructural proteins lead to the formation of the transcription and replication complex of coronavirus. After translation is completed, the structural proteins are translocated to the endoplasmic reticulum (ER) and through the ER-to-Golgi intermediate compartment. Newly formed coronavirus particles are secreted from the infected cell via exocytosis and this process continues [84].

7.2 Current and Future Treatment

The FDA approved remdesivir (Veklury) as a viable treatment for COVID-19 for adults and children who are 12 years and older and weigh at least 40 kg. It is indicated that remdesivir should be administered to patients in a hospital or in a facility that can provide patients with acute care comparable to inpatients hospital treatment. It is important to note that clinical trials regarding the safety and efficacy of remdesivir in the pediatric patient population are still being conducted. Aside from remdesivir, patients with COVID-19 should also make sure to receive supportive care to mitigate symptoms [8].

The CDC suggests that patients with a mild-to-moderate clinical presentation of the disease, which means an absence of viral pneumonia and hypoxia, may be able to manage their symptoms through supportive care at home. Pulse oximetry can be utilized as a tool to gain insight into the patient's oxygenation status. Severe and

clinical presentations of COVID-19 may require hospitalization and support including supplemental oxygen and mechanical ventilatory support. The (FDA) recently expanded the authorization of two monoclonal antibodies for treatment and post-exposure prevention of COVID-19 from only adults to pediatric patients, including newborns [23]. These monoclonal antibodies are bamlanivimab and etesevimab that are targeted against the spike protein of SARS-CoV-2 [23]. These two antibodies are given together and are designed to block viral attachment and cellular entry. It is important to note the potential serious side effects including hypersensitivity and infusion-related reactions of treatment with bamlanivimab and etesevimab. Nausea, dizziness, pruritus, and rash have also been observed in patients [23].

Aside from these previously mentioned treatments for patients infected with COVID-19, vaccinations present a powerful avenue to preventing infection in the future. The CDC does not recommend one of the currently available vaccines over another, but the three vaccines currently utilized have different efficacies. The Pfizer-BioNTech is recommended for people ages 5 or older and consists of two doses given 21 days apart. The Moderna vaccine is recommended for people ages 18 or older and consists of 2 doses given 28 days apart. Both the Moderna and Pfizer-BioNTech vaccines are mRNA vaccines. The Johnson & Johnson's Janssen vaccine is also recommended for people 18 years or older and only consists of 1 dose that contains a modified vector virus as its mechanism of action. It should be indicated that there is significantly increased risk of thrombosis with thrombocytopenia syndrome (TTS) with the use of the Johnson & Johnson's Janssen vaccine [18].

A currently ongoing, double-blind, phase 3 trial is testing the effects of the pan-sarbecovirus monoclonal antibody sotrovimab compared to placebo in symptomatic patients with COVID-19. The study demonstrated that 1% of patients in the sotrovimab group, compared to the 7% of patients in the placebo group, presented disease progression resulting in hospitalization or death ($P = 0.002$) [31]. Sotrovimab represents a viable future treatment to reduce disease progression for high-risk patients with mild-to-moderate COVID-19 [31]. Furthermore, ivermectin, an anti-parasitic, has been extensively studied as a possible treatment in reducing mortality in patients with COVID-19 infection. A recent meta-analysis found that ivermectin reduced risk of death compared to treatment without ivermectin (confidence interval 0.19–0.73) [9]. This provides evidence that ivermectin has strong potential and must be further studied to be utilized to reduce disease progression in the future.

Molnupiravir has recently been noted as an active oral agent that reduces the risk of COVID-19 progression. Molnupiravir is a small-molecule ribonucleoside prodrug of N-hydroxycytidine (NHC) and has activity against a wide range of RNA viruses. NHC is phosphorylated intracellularly and incorporated into viral RNA by viral RNA polymerase leading to deleterious errors in the viral genome. A phase 3, randomized, double-blind, placebo-controlled trial was conducted in non-hospitalized, unvaccinated adults with mild-to-moderate, laboratory-confirmed COVID-19 and at least one risk factor for COVID-19 illness [39]. Over the course of 5 days, participants were either assigned to receive 800 mg of molnupiravir or placebo twice daily. Study results demonstrated that the risk of hospitalization for

any cause or death through day 29 was significantly lower in the molnupiravir group compared to the placebo group (difference, −6.8 percentage points; 95% confidence interval, −11.3 to −2.4; $P = 0.001$). Adverse events were indicated in 33% of the placebo group and 30.4% of the molnupiravir group [39].

8 Hepatitis A

Viral hepatitis is still a prominent public health issue in the United States, accounting for 20,000 deaths each year [65]. The World Health Organization (WHO) suggests that approximately 1.5 million people are infected with hepatitis A virus (HAV) yearly [37]. HAV can be classified as an RNA virus in the Picornaviridae family and is known to be transmitted through the fecal–oral route. This occurs with exposure to contaminated food, water, or contact with another infected person. It is important to note that high-risk groups include men who have sex with men, people traveling to endemic locations, and people who use drugs via injection. The CDC has also suggested that person-to-person transmission is very prevalent among homeless persons due to close quarters and illicit drug use [37]. Historically, the most recent outbreaks of HAV in the United States occurred in 2012–2013 and 2015–2016 [20]. Given this, the incidence of HAV in an acute setting has decreased drastically, approximately reduced by 92% since 1995, since the utilization of the vaccine [59].

The acute symptoms of HAV infection often include nausea, right upper quadrant abdominal discomfort, vomiting, fatigue, malaise, and fever [59]. Furthermore, patients with severe illness may develop dark-colored urine, pale stools, jaundice, pruritus, and icteric sclera. It has been found that most adults are symptomatic but 70% of children under 6 years who are infected with HAV are asymptomatic [37]. It should also be noted that there are several significant values that can be tracked to observe disease progression. These include elevated concentrations of "serum alanine, aminotransferase, aspartate aminotransferase, bilirubin, and alkaline phosphatase" [37]. Clinical suspicion of HAV may also prompt testing for the HAV immunoglobulin M antibody [37]. Lemon S.M. et al. describe five major patterns of infection with regard to HAV that illustrate clearly its clinical presentation: asymptomatic infection, symptomatic infection, cholestatic hepatitis, relapsing infection, and fulminant hepatitis [53].

8.1 Mechanism of Actions

HAV is transmitted via a fecal–oral pathway and gains access to the host bloodstream. The virus enters the liver and possesses a long incubation period lasting approximately 4–6 weeks [37]. As indicated above, an HAV-specific IgM antibody test can be conducted initially, and elevated titers here may be present alongside elevated serum liver antibodies suggesting liver damage. The specific mechanism of

action of liver injury in acute hepatitis A is not fully understood, but there are several pathological and immunological signs that demonstrate acute infection.

There appears to be a reduced induction of type I interferon-stimulated genes in the liver during acute infection which may be due to HAV protease-mediated cleave of mitochondrial antiviral signaling proteins (MAVS) and TIR-domain-containing adaptor-inducing interferon-β (TRIF). MAVS and TRIF serve as adaptor proteins in the cytoplasmic viral RNA sensing pathways, and their cleavage leads to a detrimental type I interferon (IFN) blunting that prolongs HAV survival. The virus acts by stimulating CD8 T cells which are differentiated into effector cytotoxic T cells which causes hepatocyte damage. A significant concentration of IL-15 and IL-19 is present in the serum and in the macrophages and hepatocytes, respectively. IL-18 activates natural killer cells and causes damage to infected and uninfected hepatocytes [10].

8.2 Treatment Options

With respect to acute HAV infection, supportive care is provided as the primary methodology of treatment. Full recovery from symptoms may take several weeks or months, and patients are advised to avoid alcohol or other medications or substances that are considered hepatotoxic. It should be noted that less than 1% of patients develop acute liver failure, a scenario that may require emergency organ transplant [59]. The World Health Organization suggests that food safety, hydration, sanitation, and immunization are strong ways to prevent infection [59].

Within the United States, there is currently a vaccination available against hepatitis A. These are considered inactivated, single-antigen vaccines, and HAVRIX and VAQTA are two currently implemented examples [37]. These vaccinations have demonstrated long-term protection and are highly recommended for high-risk people including travelers to endemic areas and IV drug users. The CDC suggests that it is highly recommended for infants ages 6–11 months who are traveling internationally to receive a vaccine against hepatitis A [59]. Alternatives include a single dose of HAV immune globulin (IG) intramuscularly to provide short-term protection.

9 Hepatitis B Virus

The hepatitis B virus (HBV) is an infectious entity that primarily causes liver disease with both acute and chronic manifestations. HBV contains a double-stranded DNA (dsDNA) genome with 8 genotypes (A-H) and belongs to the Hepadnaviridae family [56]. HBV is a common infectious agent with infection rates in the United States around 4.3% in 2020 [46]. Globally, an estimated 257 million people suffer from chronic HBV infection, and the virus accounts for about 887,000 deaths annually [79]. HBV is transmitted human-to-human via bodily fluids such as blood and

semen, and transmission most commonly occurs via sexual contact, sharing needles, or vaginal childbirth [16].

HBV infection can vary from acute to chronic depending on the viral genotype and the infected individual's strength of immune response and ability to receive treatment. Infantile infection has a significantly higher chance of conferring a chronic infection. While around 90% of infantile cases of HBV become chronic, only 2–6% of infected adults suffer from a chronic infection [16].

Acute HBV infection can be asymptomatic [56] or can present with symptoms from jaundice, fatigue, stomach pain, and nausea to acute hepatic failure [16]. While both acute and chronic HBV infections can cause acute hepatic failure, chronic disease can also cause cirrhosis and hepatocellular carcinoma of the liver [56], representing the majority of morbidity and mortality associated with HBV infection. Extrahepatic manifestations of infection are rare and include systemic, renal, and vascular entities. Some of the most common examples of such entities include a serum sickness-like syndrome, Polyarteritis Nodosa, membranous glomerulonephritis, and Gianotti–Crosti syndrome [56].

Fortunately, HBV infection rates are falling in the United States. One of the biggest reasons for this decline is the development of a vaccine against HBV infection in the early 1980s. Those currently in use are DNA recombinant vaccinations that utilize the hepatitis B surface antigen (HBsAg) to promote an immune response [24]. The vaccination period begins in infancy, and the vaccinated population in the United States is 25.2% [46]. Other means of infection prevention include education about contraception and needle safety.

9.1 Virulence Factors

HBV relies on a couple of key proteins to infect liver hepatocytes and cause disease. HBV attaches to hepatocyte heparan sulfate proteoglycans (poly-l-lysine) and initiates cellular entry via binding of its envelope protein with hepatocyte sodium taurocholate co-transporting polypeptide (NTCP) receptor [26, 45, 71, 74]. Following this binding, the virus is endocytosed into a vesicle where it uncoats and releases its dsDNA. HBV dsDNA is transported to the hepatocyte nucleus where it utilizes host cell DNA polymerase to form covalently closed circular DNA (cccDNA) which acts as the template for all subsequent viral mRNA [79].

HBV utilizes four viral transcripts to build seven proteins. These proteins serve key purposes in the viral life cycle; core proteins combine to build a nucleocapsid, polymerase forms new mRNA from existing cccDNA, and surface proteins coat mature viral capsids that infect new cells [79]. As knowledge of these proteins has grown, emerging drugs aim to target one or multiple proteins to disrupt the HBV viral cycle.

9.2 Current Treatment

HBV treatment varies based on available treatments and the duration of the infection. The mainstay treatment for acute HBV infection is monoclonal antibodies targeting the NTCP receptor on hepatocytes [79]. These neutralizing antibodies, similar to those produced following vaccination, block the ability of the virus to penetrate and infect host cells. The swift administration of antibodies upon HBV exposure is imperative to its efficacy—once the virus has entered the cell, the neutralizing antibodies are unable to prevent infection. Administration of antibodies is particularly important in neonatal cases, as the vast majority of untreated infections will persist to a chronic infection. In addition to antibodies, unvaccinated individuals should also receive a dose of the hepatitis B adult vaccine (*Engerix*-B) for long-term antibody protection.

Chronic infection requires long-term treatment to eradicate or control infection. Current goals of treatment are to reduce serum HBV DNA to undetectable concentrations ($<10^5$ copies/mL), sustained loss of the hepatitis B envelope antigen (HBeAg), and improvement of hepatic and systemic manifestations including normalization of aminotransferases, decrease in hepatic inflammation and tissue damage, and stopped progression of hepatic fibrosis [28].

The primary medications utilized to treat HBV infection include recombinant subcutaneous pegylated interferon-α (PEG-IFN-α) and nucleoside or nucleotide analogs [79]. The complete mechanism of action of PEG-IGN-α is not known; however, it has been shown to decrease viral replication by decreasing RNA transcription via downregulation of cccDNA, resulting from histone hypo-acetylation and repressor protein recruitment [85]. Nucleotide analogs such as tenofovir and adefovir and nucleoside analogs such as lamivudine and entecavir block viral replication and reduce chronic infection complications [25, 28, 79].

9.3 Emerging Treatments

The majority of emerging treatments for HBV focus on chronic infection management. This push is due to the high efficacy of current treatments for acute HBV infection, as well as the high demand for better treatments of chronic disease. Developing medications can be categorized into compounds that directly target the virus, indirectly target the virus through host factor modification, and increasing host immune response via immunotherapy [79]. This section will discuss some developing compounds with an emphasis on those that directly target the virus.

Viral entry blockers aim to disrupt HBV entry into hepatocytes. Heparin, suramin, and synthetic peptides can bind and block initial adhesion of the virus to hepatocyte poly-l-lysine; however, they have not been evaluated in a clinical setting [26, 45, 71, 74]. Additional drugs aim to disrupt viral envelope protein binding to the hepatocyte NTCP receptor. The NTCP receptor is reversibly inhibited by

taurocholate and ezetimibe [61, 69] and irreversibly inhibited by cyclosporine and bulevirtide [70, 82]. Of these compounds, only bulevirtide has been clinically studied.

Another big target of developing drugs is the cccDNA. Many enzymes including endonucleases, zinc-finger nucleases, transcription activator-like effector nucleases, and CRISP/Cas-9 have shown promising results of inhibiting HBV replication in vitro [79]. A different approach is utilizing cytokines to upregulate viral RNA deaminases to degrade cccDNA. IFN-α and lymphotoxin-β may be promising in this regard as in vitro studies have shown some non-toxic degradation cccDNA [62].

A third class of compounds target viral transcripts. Multiple small-interfering RNAs (SiRNAs) have been tested with ARC-520 and ARC-521 showing promising results in clinical trials when used with entecavir [89]. Anti-sense oligonucleotides such as IONIS-HBVRx and IONIS-HBVLRx are in phase 1 trials and aim to inhibit viral RNA via degradation or steric hinderance [32].

Core protein assembly modulators (CpAMs) bind to the HBV capsid and inhibit nucleocapsid assembly and/or encapsidation of RNA, leading to inhibition of dsDNA synthesis. The two classes of CpAMs include heteroaryldihydropyridines which cause the formation of misassembled core proteins or phenylpropanamides which lead to the formation of capsids lacking RNA and HBV polymerase ([79]). These compounds are in various stages of clinical trials.

Additional HBV polymerase inhibitors, nucleoside analogs and nucleotide analogs, are being developed in search of increased efficacy, better oral bioavailability, and better safety as compared to those medications that are currently approved for treatment [43]. Some examples of emerging medications include CMX-157, AGX-1009, lagociclovir, and besifovir ([1, 51, 78]). Of note, besifovir is currently in phase III trials with promising results [89].

The final group of compounds to be discussed are HBsAg release inhibitors. Synthetic nucleic acid polymers (NAPs) have been shown to bind HBsAg and block its release [2]. In particular, REP-2139 and REP-2165 are NAPs in clinical development that are being studied in conjunction with tenofovir and PEG-IFN-2α [7].

10 Hepatitis C Virus

The hepatitis C virus (HCV) is a positive-sense single-stranded RNA virus belonging to the Flavivirus family. HCV has 7 genotypes with 67 subtypes [17]. The CDC estimates that 2.4 million individuals in the United States, about 1% of the population, were infected with HCV between 2013 and 2016. Worldwide, HCV infected approximately 170 million people in 2015, alone [55].

HCV is mainly spread via blood and blood products. Historically, HCV has been spread via blood transfusions; however, this transmission was greatly reduced following the introduction of blood testing prior to transfusion in the early 1970s [75]. The most common routes of viral transmission occur with needle sharing with an infected individual or in infants via vaginal childbirth [17].

HCV infection can have acute and chronic manifestations. However, unlike HBV, an uncured acute HCV infection is much more likely to persist to a chronic infection with 50–80% of all acute infections becoming chronic [55]. Acute infection can cause constitutional symptoms, jaundice, dark urine, clay-colored stool, and acute hepatitis [17]. Chronic complications of HCV infection include chronic hepatitis, cirrhosis, and hepatocellular carcinoma of the liver [17]. Extrahepatic manifestations of chronic HCV infections have been documented and include diabetes mellitus, glomerulonephritis, cryoglobulinemia, porphyria cutanea tarda, and non-Hodgkin's lymphoma [17].

There is no currently approved vaccine against HCV infection [17]. Thus, primary strategies for HCV prevention include testing all blood products for HCV, as well as educating about clean needle use and healthcare safety precautions [12].

10.1 Virulence Factors

HCV targets liver hepatocytes, binding via its envelope proteins, E1 and E2 to hepatocyte high-density lipoprotein receptor scavenger B1, tetraspanin CD81, claudin-1, and occludin [90]. Once bound to one of these targets, HCV is internalized into the cell cytoplasm where the virus uncoats. This releases the HCV RNA which can be translated to make viral protein or reverse-transcribed to yield viral DNA.

Viral proteins such as NS3, NS4B, NS5A, and NS5B are required for viral replication, allowing for sustained cellular infection [55]. Of note, NS5B is the HCV RNA-dependent RNA polymerase, and NS3/4A acts as a serine protease which is crucial for proper viral protein synthesis. The aforementioned proteins are critical for the HCV viral cycle, and thus, they are targeted in current and emerging pharmacotherapies.

10.2 Current Treatment

HCV treatments have made vast improvements in the past decade. In the early 2010s, interferon-α in combination with ribavirin was the mainstay of treatment. In 2014, interferon-free regimens were approved and greatly increased the cure rate of HCV infection to about 95% [83]. Viral cure is defined as undetectable HCV RNA for greater than 6 months. Not only do these regimens have around double the cure rate of the previous interferon-α regimen, but they also have many fewer reported side effects [55].

Current treatments for HCV infection depend on the HCV genotype, previously received antiviral treatment, and hepatic complications such as cirrhosis. First-line medications for treatment-naïve adults without cirrhosis are 8 weeks of glecaprevir/pibrentasvir or 12 weeks of sofosbuvir/velpatasvir [3]. Glecaprevir inhibits the NS3/4A protease, while pibrentasvir inhibits the NS5A protein, required for viral

replication and virion assembly [66]. Conversely, sofosbuvir and velpatasvir inhibit NS5B and NS5A, respectively [67]. Pharmacotherapy for treatment-naïve adults with cirrhosis differs only in individuals infected with HCV genotype 3 who must receive 8 weeks of glecaprevir/pibrentasvir [4].

Guideline therapy for HCV with decompensated cirrhosis is liver transplant [6]. Pharmacotherapy for individuals who cannot receive a transplant or are waiting for transplant includes either daily ledipasvir/sofosbuvir plus ribavirin for genotypes 1, 4, 5, and 6 or daily sofosbuvir/velpatasvir plus ribavirin for all genotypes [5]. Ledipasvir is another NS3/4A protease inhibitor, and ribavirin is a synthetic guanosine analog used for its viral replication inhibition effects [68].

10.3 Emerging Treatments

Investigation of plant-derived compounds has yielded exciting results for future treatment of acute and chronic HCV infection. These compounds are being pursued due to a high chemical diversity, cheaper cost of production, and better safety profile [38]. The compounds have specificity for different HCV genotypes and have been categorized into entry inhibitors, replication inhibitors, and assembly and release blockers.

Griffithsin and scytovirin are proteins isolated from algae that have been shown to block HCV cell entry by targeting envelope glycoproteins E1 and E2 in cell cultures and pseudoparticle assays [38]. SSb2, a *Bupleurum kaoi* root extract, can neutralize free HCV particles by inhibiting viral envelope glycoproteins [57]. BJ486K, a compound isolated from *Marrubium peregrinum*, inhibits an unknown post-attachment step required for cell entry. This compound has been shown to inhibit infection of human hepatocytes by all genotypes of HCV in vitro [38].

A variety of replication inhibitors have also been described in literature. Ethanol extract from mangosteen fruit peels has been shown to have an additive effect with NS5A inhibitors to reduce HCV protein and RNA levels via inhibiting NS5B in vitro *[*21*]*. In addition, animal trials of xanthohumol, found in *Humulus L. Cannabaceae*, have displayed an inhibitory effect on HCV replication [60]. This effect may result from host modulation as opposed to direct effects on the virus. Apigenin, a flavonoid found in many fruits and vegetables, can inhibit maturation of micro-RNA 122, a compound required for HCV stability in hepatocytes [77]. Further, triterpenes from *Cynomorium songaricum* have been shown to inhibit the HCV NS3/4A protease [63].

A currently developing release and assembly inhibitor is naringenin, a flavonoid found in citrus fruit. This compound can inhibit HCV release [64], as well as inhibit the assembly of intracellular HCV particles [30]. The effect of naringenin on HCV viral assembly is likely due to its ability to stimulate PPAR-α, a hepatic transcription factor involved in lipid metabolism that is closely linked with HCV viral assembly. Naringenin has additionally been theorized to inhibit the HCV NS2 protease, but no trials have been completed to confirm this activity [41].

Table 1 Infectious agents, medications, and drug targets

Infection	Medication/Compound	Mechanism of Action
Picornavirus	L-Citrulline	Indirectly increases nitric oxide synthesis
Rotaviruses	Nitazoxamide	Reduces the size and architecuture of viroplasms
Orthomyxovirus	Peramivir	Inhibition of influenza virus neuraminidase
	Zanamivir	Inhibition of influenza virus neuraminidase
	Oseltamivir phosphate	Inhibition of influenza virus neuraminidase
	Baloxavir marboxil	Inhibits viral polymerase subunit to prevent replication
Rhabdovirus	Ribavirin with IFN-α	Guanosine analog used to stop viral RNA synthesis and capping
	Favipiravir	Selectively and potently inhibits the RNA-dependent RNA polymerase
Human Papillomavirus	Gardasil	Prophylactic, induces high initial serum HPV type specific antibodies
Human Immunodeficiency Virus	Zidovudine	Thymidine analogue, blocks viral replication
	Didanosine	Adenosine analogue, blocks viral replication
	Nevirapine	Reverse transcriptase inhibitor
	Delavirdine	Reverse transcriptase inhibitor
	Ritonavir	Cytochrome P450 3A4 isoenzyme inhibitor
	Fostemsavir	Binds to gp120 subunit to inhibit viral interaction with host CD4 receptors
MERS-CoV	Lopinavir, Ritonavir, IFN-β	Protease Inhibitor
	Remdesivir, IFN-β	Pre-termination of viral RNA transcription
SARS-CoV-2	Remdesivir	Pre-termination of viral RNA transcription
	Bamalanivmab	Targets spike protein of SARS-CoV-2
	Etesevimab	Targets spike protein of SARS-CoV-2
	Sotrovimab	Targets spike protein of SARS-CoV-2
	Ivermectin	Inhibitor of nuclear transport mediated by the importin α/β1 heterodimer
	Molnupiravir	Isopropylester cytidine analog
Hepatitis A Virus	HAVRIX	Inactivated, single-antigen vaccine
	VAQTA	Inactivated, single-antigen vaccine
Hepatitis B Virus (HBV)	*Engerix*- B	Production of endogenous anti-Hepatitis B antibodies
	Hepatitis B monoclonal antibiotics	Passive Hepatitis B antibodies, block viral entry into cell
	PEG-IFN-α	Decreases RNA transcription via downregulation of cccDNA
	Tenofovir, Adefovir	Nucleotide analogs, block viral replication
	Lamivudine, Entecavir	Nucleoside analogs, block viral replication
	Heparin, Suramine	Inhibit viral adhesion to hepatocyte poly-l-lysine
	Taurocholate, Ezetimibe	Hepatocyte NTCP receptor inhibitors (reversible)
	Cyclosporine, Bulevirtide	Hepatocyte NTCP receptor inhibitors (irreversible)
	CRISP/Cas-9, Nucleases	Target cccDNA to inhibit replication
	INF-α, Lymphotoxin-β	Degrade cccDNA via upregulation of RNA deaminases
	ARC-520, ARC-521	Small interfering RNAs, viral RNA inhibitors
	IONIS-HBVRx, IONIS-HBVLRx	Anti-sense oligonucleotides, viral RNA inhibitors
	Heteroaryldihydropyridines	Formation of miassembled viral capsid
	Phenylpropenamides	Formation of viral capsids lacking RNA and HBV polymerase
	CMX-157, AG-1009, Lagociclovir, Besifovir	Nucleoside/Nucleotide analogs, block viral replication
	REP-2139, REP-2165	HBsAg release inhibitors
Hepatitis C Virus	Glecaprevir, Ledipasvir	NS3/4A protease inhibitor
	Pibrentasvir, Velpatasvir	NS5A inhibitor, inhibits replication
	Sofosbuvir	NS5B inhibitor, inhibits replication
	Ribavirin	Guanosine analog, inhibits replication
	Griffithsin, Scytovirin (algae)	Target E1 and E2 envelope glycoproteins to inhibit cell entry
	SSb2 (*Bepleurum kaoi)*	Neutralizes free viral particles via inhibiting viral envelope glycoproteins
	BJ486K (*Marrubium peregrinum Lamiacaea)*	Inhibits cell entry via an unknown mechanism
	Ethanol extract (Mangosteen)	NS5B inhibitor, inhibits replication
	Xanthohumol (*Humulus L. Cannabacaea)*	Replication inhibitor via host-modulation
	Apigenin (fruits and vegetables)	Micro-RNA 122 inhibitor leading to viral instability
	Triterpenes (*Cynomoroim songaricum)*	NS3/4A protease inhibitor
	Naringenin (citrus fruits)`	PPAR-α stimulator, inhibits viral release and intracellular assembly

Legend	
Currently Used for Treatment	
In Development	

11 Conclusion

Over the past half a century, there have been significant improvements on antiviral drugs and their overall efficacies. There are; however, viruses that continue to spread globally year after year. Viruses such as the COVID-19, Nipah virus, Ebola virus, and MERS-Cov, have been more difficult to treat with broad or even narrow-spectrum antivirals [80]. Nucleoside analogs such as Ribavirin, have been heavily researched on its potential use for emerging infectious diseases. Ribavirin has activity against broad-spectrum RNA viruses through its inhibition of viral polymerase activity. Since ribavirin inhibits inosine-5′-monophosphate dehydrogenase, it leads to a decrease of intracellular GTP levels which seems promising when combating certain viral infections [80]. Recent reports have also shown that favipiravir (T-705)

may have slight effectiveness against the Ebola virus. Although the mechanism is unknown, T-705 has shown its potential to merit further research. Overall, this chapter has provided a brief overview of the developing therapies used to treat viral infections. This is not an exhaustive list, but the described examples highlight some of the main targets and sources of future therapies regarding infectious diseases. Table 1 is a list of antivirals and their mechanism of action.

References

1. Ahn SH, Kim W, Jung YK, et al. Efficacy and safety of Besifovir Dipivoxil maleate compared with Tenofovir Disoproxil fumarate in treatment of chronic hepatitis B virus infection. Clin Gastroenterol Hepatol. 2019;17(9):1850–1859.e1854.
2. Al-Mahtab M, Bazinet M, Vaillant A. Safety and efficacy of nucleic acid polymers in monotherapy and combined with immunotherapy in treatment-naive Bangladeshi patients with HBeAg+ chronic hepatitis B infection. PLoS One. 2016;11(6):e0156667.
3. American Association for the Study of Liver Diseases. Simplified HCV treatment* for treatment-naive adults without cirrhosis | HCV guidance; 2020a. Retrieved 20 Dec 2021, from: https://www.hcvguidelines.org/treatment-naive/simplified-treatment.
4. American Association for the Study of Liver Diseases. Simplified HCV treatment algorithm for treatment-naive adults with compensated cirrhosis | HCV guidance; 2020b. Retrieved 20 Dec 2021, from: https://www.hcvguidelines.org/treatment-naive/simplified-treatment-compensated-cirrhosis.
5. American Association for the Study of Liver Diseases. Patients with decompensated cirrhosis | HCV guidance; 2021a. Retrieved 20 Dec 2021, from 1: https://www.hcvguidelines.org/unique-populations/decompensated-cirrhosis.
6. American Association for the Study of Liver Diseases. Management of acute HCV infection | HCV guidance; 2021b. Retrieved 20 Dec 2021, from: https://www.hcvguidelines.org/unique-populations/acute-infection.
7. Bazinet M, Pantea V, Placinta G, et al. Preliminary safety and efficacy of REP 2139-Mg or REP 2165-Mg used in combination with tenofovir disoproxil fumarate and pegylated interferon alpha 2a in treatment naïve Caucasian patients with chronic HBeAg negative HBV infection. Hepatology. 2016;66:S256.
8. Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, Hohmann E, Chu HY, Luetkemeyer A, Kline S, Lopez de Castilla D, Finberg RW, Dierberg K, Tapson V, Hsieh L, Patterson TF, Paredes R, Sweeney DA, Short WR, Touloumi G, Lye DC, Ohmagari N, Oh MD, Ruiz-Palacios GM, Benfield T, Fätkenheuer G, Kortepeter MG, Atmar RL, Creech CB, Lundgren J, Babiker AG, Pett S, Neaton JD, Burgess TH, Bonnett T, Green M, Makowski M, Osinusi A, Nayak S, Lane HC, ACTT-1 Study Group Members. Remdesivir for the treatment of Covid-19 – final report. N Engl J Med. 2020;383(19):1813–26. https://doi.org/10.1056/NEJMoa2007764. Epub 2020 Oct 8. PMID: 32445440; PMCID: PMC7262788
9. Bryant A, Lawrie T, Dowswell T, Fordham EJ, Mitchell S, Hill S, Tham TC. Ivermectin for prevention and treatment of COVID-19 infection: a systematic review, meta-analysis, and trial sequential analysis to inform clinical guidelines. Am J Ther. 2021;28(4):e434–60. https://doi.org/10.1097/MJT.0000000000001402.
10. Cao X, Xue YJ, Du JL, Xu Q, Yang XC, Zeng Y, Wang BB, Wang HZ, Liu J, Cai KZ, Ma ZR. Induction and suppression of innate antiviral responses by hepatitis A virus. Front Microbiol. 2018;9:1865. https://doi.org/10.3389/fmicb.2018.01865. PMID: 30174659; PMCID: PMC6107850.
11. Center for Drug Evaluation and Research. "Influenza (Flu) Antiviral Drugs and Related Information." U.S. Food and Drug Administration, FDA, https://www.fda.gov/drugs/information-drug-class/influenza-flu-antiviral-drugs-and-related-information.

12. Center of Disease Control and Prevention. Hepatitis C questions and answers for health professionals; 2021. Retrieved 20 Dec 2021, from: https://www.cdc.gov/hepatitis/hcv/hcvfaq.htm#section1.
13. Centers for Disease Control and Prevention. Vaccines: VPD-VAC/rotavirus/rotashield and intussusception historical info. Centers for Disease Control and Prevention; 2011. https://www.cdc.gov/vaccines/vpd-vac/rotavirus/vac-rotashield-historical.htm.
14. Centers for Disease Control and Prevention. How flu viruses can change: 'Drift' and 'Shift.' Centers for Disease Control and Prevention; 2021a. https://www.cdc.gov/flu/about/viruses/change.htm.
15. Centers for Disease Control and Prevention. What you should know about flu antiviral drugs. Centers for Disease Control and Prevention; 2021b. https://www.cdc.gov/flu/treatment/whatyoushould.htm.
16. Centers for Disease Control and Prevention. Hepatitis B; 2021c. Retrieved 20 Dec 2021, from: https://www.cdc.gov/hepatitis/hbv/index.htm.
17. Centers for Disease Control and Prevention. Hepatitis C; 2021d. Retrieved 20 Dec 2021, from: https://www.cdc.gov/hepatitis/hcv/index.htm.
18. Centers for Disease Control and Prevention. Interim clinical considerations for use of Covid-19 vaccines. Centers for Disease Control and Prevention; 2021e. Retrieved Dec 20, 2021, from: https://www.cdc.gov/vaccines/covid-19/clinical-considerations/covid-19-vaccines-us.html.
19. Centers for Disease Control and Prevention. Scientific brief: SARS-COV-2 transmission. Centers for Disease Control and Prevention; n.d.-a. Retrieved 20 Dec 2021, from: https://www.cdc.gov/coronavirus/2019-ncov/science/science-briefs/sars-cov-2-transmission.html.
20. Centers for Disease Control and Prevention. Disease burden from viral hepatitis A, B, and C in the United States; n.d.-b. www.cdc.gov/hepatitis/statistics/DiseaseBurden.htm.
21. Choi M, Kim Y-M, Lee S, Chin Y-W, Lee C. Mangosteen xanthones suppress hepatitis C virus genome replication. Virus Genes. 2014;49:208–22.
22. Cleveland Clinic. Human Papilloma Virus (HPV): causes, testing, treatment & prevention. https://my.clevelandclinic.org/health/diseases/11901-hpv-human-papilloma-virus#:~:text=Approximately%2079%20million%20Americans%20are,know%20they%20have%20the%20virus.
23. Commissioner, O. of the. FDA expands authorization of two monoclonal antibodies for treatment and post-exposure prevention of Covid-19 to younger pediatric patients, including newborns. U.S. Food and Drug Administration. Retrieved 20 Dec 2021, from: https://www.fda.gov/news-events/press-announcements/fda-expands-authorization-two-monoclonal-antibodies-treatment-and-post-exposure-prevention-covid-19.
24. Das S, Ramakrishnan K, Behera SK, Ganesapandian M, Xavier AS, Selvarajan S. Hepatitis B vaccine and immunoglobulin: key concepts. J Clin Transl Hepatol. 2019;7(2):165–71. https://doi.org/10.14218/JCTH.2018.00037.
25. Dave S, Park S, Murad MH, Barnard A, Prokop L, Adams LA, Singh S, Loomba R. Comparative effectiveness of Entecavir versus Tenofovir for preventing hepatocellular carcinoma in patients with chronic hepatitis B: a systematic review and meta-analysis. Hepatology (Baltimore, Md.). 2021;73(1):68–78. https://doi.org/10.1002/hep.31267.
26. Di Stefano G, Colonna FP, Bongini A, Busi C, Mattioli A, Fiume L. Ribavirin conjugated with lactosaminated poly-L-lysine: selective delivery to the liver and increased antiviral activity in mice with viral hepatitis. Biochem Pharmacol. 1997;54(3):357–63.
27. Dixon BE, Wools-Kaloustian K, Fadel WF, et al. Symptoms and symptom clusters associated with SARS-CoV-2 infection in community-based populations: results from a statewide epidemiological study. Preprint. medRxiv. 2020;2020.10.11.20210922. Published 2020 Oct 22. https://doi.org/10.1101/2020.10.11.20210922
28. D'Souza R, Foster GR. Diagnosis and treatment of chronic hepatitis B. J R Soc Med. 2004;97(7):318–21. https://doi.org/10.1258/jrsm.97.7.318.
29. Fassati A. HIV infection of non-dividing cells: a divisive problem – retrovirology. BioMed Central; 2006. https://retrovirology.biomedcentral.com/articles/10.1186/1742-4690-3-74.
30. Goldwasser J, Cohen PY, Lin W, Kitsberg D, Balaguer P, Polyak SJ, et al. Naringenin inhibits the assembly and long-term production of infectious hepatitis C virus particles through

a PPAR-mediated mechanism. J Hepatol. European Association for the Study of the Liver. 2011;55:963–71.
31. Gupta A, Gonzalez-Rojas Y, Juarez E, Crespo Casal M, Moya J, Falci DR, Sarkis E, Solis J, Zheng H, Scott N, Cathcart AL, Hebner CM, Sager J, Mogalian E, Tipple C, Peppercorn A, Alexander E, Pang PS, Free A, Brinson C, Aldinger M, Shapiro AE, COMET-ICE Investigators. Early treatment for Covid-19 with SARS-CoV-2 neutralizing antibody Sotrovimab. N Engl J Med. 2021;385(21):1941–50. https://doi.org/10.1056/NEJMoa2107934. Epub 2021 Oct 27. PMID: 34706189
32. Han K, Cremer J, Elston R, et al. A randomized, double-blind, placebo-controlled, first-time-in-human study to assess the safety, tolerability, and pharmacokinetics of single and multiple ascending doses of GSK3389404 in healthy subjects. Clin Pharmacol Drug Dev. 2019;8(6):790–801.
33. Hepatitis C Trust. About the hepatitis C virus. http://hepctrust.org.uk/about-hepatitis-c-virus.
34. Hui DS, et al. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health – the latest 2019 novel coronavirus outbreak in Wuhan, China. Intl J Infect Dis. 2020;91:264–6.
35. Hull JS, Montaner MW. Ritonavir-boosted protease inhibitors in HIV therapy. Ann Med. U.S. National Library of Medicine. 2001. https://pubmed.ncbi.nlm.nih.gov/21501034/.
36. International Committee on Taxonomy of Viruses (ICTV). Virus taxonomy: 2018b release; 2019. Available at: https://talk.ictvonline.org/taxonomy/. Accessed 8 Apr 2020. [Google Scholar] [Ref list].
37. Iorio N, John S. Hepatitis A. [Updated 2021 Jul 19]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459290/.
38. Jardim A, Shimizu JF, Rahal P, Harris M. Plant-derived antivirals against hepatitis c virus infection. Virol J. 2018;15(1):34. https://doi.org/10.1186/s12985-018-0945-3.
39. Jayk BA, Gomes da Silva MM, Musungaie DB, Kovalchuk E, Gonzalez A, Delos Reyes V, Martín-Quirós A, Caraco Y, Williams-Diaz A, Brown ML, Du J, Pedley A, Assaid C, Strizki J, Grobler JA, Shamsuddin HH, Tipping R, Wan H, Paschke A, Butterton JR, Johnson MG, De Anda C, MOVe-OUT Study Group. Molnupiravir for oral treatment of Covid-19 in nonhospitalized patients. N Engl J Med. 2021;386:509. https://doi.org/10.1056/NEJMoa2116044. PMID: 34914868.
40. Jochmans, Dirk, and Neyts. The path towards effective antivirals against rabies. Vaccine. Elsevier; 2017. https://www.sciencedirect.com/science/article/pii/S0264410X17318121.
41. Jones CT, Murray CL, Eastman DK, Tassello J, Rice CM. Hepatitis C virus p7 and NS2 proteins are essential for production of infectious virus. J Virol. 2007;81:8374–83.
42. Keshavan M. Startup's antiviral drug combats HPV, new study shows. MedCity News; 2014. https://medcitynews.com/2014/08/san-diego-startups-topical-antiviral-drug-combats-hpv-new-study-shows/.
43. Ko C, Michler T, Protzer U. Novel viral and host targets to cure hepatitis B. Curr Opin Virol. 2017;24:38–45.
44. Kozal M, Aberg J, Pialoux G, Cahn P, Thompson M, Molina JM, Grinsztejn B, Diaz R, Castagna A, Kumar P, Latiff G, DeJesus E, Gummel M, Gartland M, Pierce A, Ackerman P, Llamoso C, Lataillade M. Fostemsavir in adults with multidrug-resistant HIV-1 infection. N Engl J Med. U.S. National Library of Medicine. 2020. https://pubmed.ncbi.nlm.nih.gov/32212519/.
45. Krepstakies M, Lucifora J, Nagel CH, et al. A new class of synthetic peptide inhibitors blocks attachment and entry of human pathogenic viruses. J Infect Dis. 2012;205(11):1654–64.
46. Kruszon-Moran D, Paulose-Ram R, Martin CB, Barker L, McQuillan G. Prevalence and trends in hepatitis B virus infection in the United States, 2015–2018. NCHS Data Brief, no 361. Hyattsville, MD: National Center for Health Statistics; 2020.
47. Kumar SU, Priya NM, Nithya SR, Kannan P, Jain N, Kumar DT, Magesh R, Younes S, Zayed H, Doss CGP. A review of novel coronavirus disease (COVID-19): based on genomic structure, phylogeny, current shreds of evidence, candidate vaccines, and drug repurposing. 3 Biotech. 2021;11(4):198. https://doi.org/10.1007/s13205-021-02749-0. Epub 2021 Mar 27. PMID: 33816047; PMCID: PMC8003899
48. Kuritzkes D. Baloxavir (Xofluza): a new antiviral drug for the flu. Harvard Health; 2019. https://www.health.harvard.edu/blog/baloxavir-xofluza-a-new-antiviral-drug-for-the-flu-2019010215672.

49. La Frazia S, Ciucci A, Arnoldi F, Coira M, Gianferretti P, Angelini M, Belardo G, Burrone OR, Rossignol JF, Santoro MG. Thiazolides, a new class of antiviral agents effective against rotavirus infection, target viral morphogenesis, inhibiting viroplasm formation. J Virol. U.S. National Library of Medicine. 2013. https://pubmed.ncbi.nlm.nih.gov/23926336/.
50. Lalonde MS, Lobritz MA, Ratcliff A, Chamanian M, Athanassiou Z, Tyagi M, Wong J, Robinson JA, Karn J, Varani G, Arts EJ. Inhibition of both HIV-1 reverse transcription and gene expression by a cyclic peptide that binds the Tat-transactivating response element (TAR) RNA. PLoS Pathog. U.S. National Library of Medicine. 2011. https://pubmed.ncbi.nlm.nih.gov/21625572/.
51. Lanier ER, Ptak RG, Lampert BM, et al. Development of hexadecyloxypropyl tenofovir (CMX157) for treatment of infection caused by wild-type and nucleoside/nucleotide-resistant HIV. Antimicrob Agents Chemother. 2010;54(7):2901–9.
52. Ledesma LA et al. Comparing clinical protocols for the treatment of human rabies: the Milwaukee Protocol and the Brazilian Protocol (Recife). Rev Soc Bras Med Trop, Sociedade Brasileira De Medicina Tropical – SBMT. 2020. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7670764/.
53. Lemon SM. Type A viral hepatitis: new developments in an old disease. N Engl J Med. 1985;313:1059–67.
54. Levinson WE. Review of medical microbiology and immunology 15E. McGraw-Hill Education; 2018.
55. Li HC, Lo SY. Hepatitis C virus: virology, diagnosis and treatment. World J Hepatol. 2015;7(10):1377–89. https://doi.org/10.4254/wjh.v7.i10.1377.
56. Liang TJ. Hepatitis B: the virus and disease. Hepatology (Baltimore, Md). 2009;49(5 Suppl):S13–21. https://doi.org/10.1002/hep.22881.
57. Lin L-T, Chung C-Y, Hsu W-C, Chang S-P, Hung T-C, Shields J, et al. Saikosaponin b2 is a naturally occurring terpenoid that efficiently inhibits hepatitis C virus entry. J Hepatol. 2015;62:541–8.
58. Liu DX, Liang JQ, Fung TS. Human Coronavirus-229E, -OC43, -NL63, and -HKU1 (Coronaviridae). In: Encyclopedia of Virology; 2021. p. 428–40. https://doi.org/10.1016/B978-0-12-809633-8.21501-X
59. Loader M, Moravek R, Witowski SE, Driscoll LM. A clinical review of viral hepatitis. JAAPA. 2019;32(11):15–20. https://doi.org/10.1097/01.JAA.0000586300.88300.84.
60. Lou S, Zheng Y-M, Liu S-L, Qiu J, Han Q, Li N, et al. Inhibition of hepatitis C virus replication in vitro by Xanthohumol, a natural product present in hops. Planta Med. 2013;80:171–6.
61. Lucifora J, Esser K, Protzer U. Ezetimibe blocks hepatitis B virus infection after virus uptake into hepatocytes. Antivir Res. 2013;97(2):195–7.
62. Lucifora J, Xia Y, Reisinger F, et al. Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. Science. 2014;343(6176):1221–8.
63. Ma C-M, Wei Y, Wang Z-G, Hattori M. Triterpenes from Cynomorium songaricium—analysis of HCV protease inhibitory activity, quantification, and content change under the influence of heating. J Nat Med. 2009;63:9–14.
64. Nahmias Y, Goldwasser J, Casali M, van Poll D, Wakita T, Chung RT, et al. Apolipoprotein B-dependent hepatitis C virus secretion is inhibited by the grapefruit flavonoid naringenin. Hepatology. 2008;47:1437–45.
65. National Academies of Sciences, Engineering, and Medicine, Health and Medicine Division, Board on Population Health and Public Health Practice, Committee on a National Strategy for the Elimination of Hepatitis B and C. In: Strom BL, Buckley GJ, editors. A national strategy for the elimination of hepatitis B and C: phase two report. Washington, DC: National Academies Press (US); 2017. Summary, Available from: https://www.ncbi.nlm.nih.gov/books/NBK442218/.
66. National Center for Biotechnology Information. PubChem compound summary for CID 66828839, Glecaprevir; 2021a. Retrieved 20 Dec 2021 from: https://pubchem.ncbi.nlm.nih.gov/compound/Glecaprevir.
67. National Center for Biotechnology Information. PubChem compound summary for CID 58031952, Pibrentasvir; 2021b. Retrieved 20 Dec 2021 from: https://pubchem.ncbi.nlm.nih.gov/compound/Pibrentasvir.
68. National Center for Biotechnology Information. PubChem compound summary for CID 37542, Ribavirin; 2021c. Retrieved 20 Dec 2021 from: https://pubchem.ncbi.nlm.nih.gov/compound/Ribavirin.

69. Ni Y, Lempp FA, Mehrle S, et al. Hepatitis B and D viruses exploit sodium taurocholate co-transporting polypeptide for species-specific entry into hepatocytes. Gastroenterology. 2014;146(4):1070–83.
70. Nkongolo S, Ni Y, Lempp FA, et al. Cyclosporin A inhibits hepatitis B and hepatitis D virus entry by cyclophilin-independent interference with the NTCP receptor. J Hepatol. 2014;60(4):723–31.
71. Petcu DJ, Aldrich CE, Coates L, Taylor JM, Mason WS. Suramin inhibits in vitro infection by duck hepatitis B virus, Rous sarcoma virus, and hepatitis delta virus. Virology. 1988;167(2):385–92.
72. Rasmussen DL, Watson SA, Swerdlow AK. Middle East Respiratory Syndrome (MERS). Microbiol Spectr. U.S. National Library of Medicine. 2016. https://pubmed.ncbi.nlm.nih.gov/27337460/.
73. Schmidt S. et al. Treatment with L-citrulline in patients with post-polio syndrome: study protocol for a single-center, randomised, placebo-controlled, double-blind trial. Trials, BioMed Central; 2017. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5343398/.
74. Schulze A, Gripon P, Urban S. Hepatitis B virus infection initiates with a large surface protein-dependent binding to heparan sulfate proteoglycans. Hepatology. 2007;46(6):1759–68.
75. Selvarajah S, Busch MP. Transfusion transmission of HCV, a long but successful road map to safety. Antivir Ther. 2012;17(7 Pt B):1423–9. https://doi.org/10.3851/IMP2459.
76. Sheahan TP, et al. Comparative therapeutic efficacy of remdesivir and combination Lopinavir, Ritonavir, and Interferon Beta against MERS-COV. Nature News. Nature Publishing Group; 2020. https://www.nature.com/articles/s41467-019-13940-6.
77. Shibata C, Ohno M, Otsuka M, Kishikawa T, Goto K, Muroyama R, et al. The flavonoid apigenin inhibits hepatitis C virus replication by decreasing mature microRNA122 levels. Virology. Elsevier. 2014;462–463:42–8.
78. Soriano V, Barreiro P, Benitez L, Pena JM, de Mendoza C. New antivirals for the treatment of chronic hepatitis B. Expert Opin Investig Drugs. 2017;26(7):843–51.
79. Spyrou E, Smith CI, Ghany MG. Hepatitis B: current status of therapy and future therapies. Gastroenterol Clin N Am. 2020;49(2):215–38. https://doi.org/10.1016/j.gtc.2020.01.003.
80. Takizawa N, Yamasaki M. Current landscape and future prospects of antiviral drugs derived from microbial products. Nature News, Nature Publishing Group; 2017. https://www.nature.com/articles/ja2017115.
81. UNAIDS. Global HIV & AIDS statistics – fact sheet. https://www.unaids.org/en/resources/fact-sheet.
82. Urban S, Bartenschlager R, Kubitz R, Zoulim F. Strategies to inhibit entry of HBV and HDV into hepatocytes. Gastroenterology. 2014;147(1):48–64.
83. US Department of Veterans Affairs. Hepatitis C medications: an overview for patients; 2020. Retrieved 20 Dec 2021, from: https://www.hepatitis.va.gov/products/treatment-update.asp.
84. V'kovski P, Kratzel A, Steiner S, Stalder H, Thiel V. Coronavirus biology and replication: implications for SARS-CoV-2. Nat Rev Microbiol. 2021;19(3):155–70. https://doi.org/10.1038/s41579-020-00468-6. Epub 2020 Oct 28. PMID: 33116300; PMCID: PMC7592455
85. Woo A, Kwok R, Ahmed T, et al. Ann Transl Med. 2017;5(7):159. https://doi.org/10.21037/atm.2017.03.69.
86. World Health Organization. Prevention and control of hepatitis; n.d.-a. http://www.emro.who.int/pak/programmes/prevention-a-control-of-hepatitis.html.
87. World Health Organization. Who coronavirus (COVID-19) dashboard. World Health Organization; n.d.-b. Retrieved 20 Dec 2021, from: https://covid19.who.int/.
88. Xiang SH, Finzi A, Pacheco B, Alexander K, Yuan W, Rizzuto C, Huang CC, Kwong PD, Sodroski J. A v3 loop-dependent gp120 element disrupted by CD4 binding stabilizes the human immunodeficiency virus envelope glycoprotein trimer. J. Virol. U.S. National Library of Medicine. 2010. https://pubmed.ncbi.nlm.nih.gov/20089638/.
89. Yuen M, Chan H, Liu K, et al. Differential reductions in viral antigens expressed from cccDNAVS integrated DNA in treatment naive HBeAg positive and negative patients with chronic HBV after RNA interference therapy with ARC-520. J Hepatol. 2016;64(suppl. 2):S390–1.
90. Zhu YZ, Qian XJ, Zhao P, Qi ZT. How hepatitis C virus invades hepatocytes: the mystery of viral entry. World J Gastroenterol. 2014;20:3457–67.

Nanotechnology in Malaria Diagnosis

Anita Patel, Jayvadan Patel, Rutvik Patel, and Vidhyut Patidar

Abstract Malaria has been accountable for the utmost mortality in the majority of malaria-endemic countries. Even after decades of malaria control campaigns, it still continues as a disease of high mortality owing to inappropriate diagnosis and fast-evolving drug-resistant malarial parasites. For well-organized and economical malaria management, WHO suggests that all malaria-suspected patients must get an appropriate diagnosis before administering drugs. In malaria-endemic countries, routine diagnosis is stuck with technical and infrastructural challenges to laboratories. These laboratories are deficient in standard facilities, expertise, or diagnostic supplies; therefore, therapy is administered based on clinical or self-diagnosis. Traditional methods for diagnosing malaria remain problematic; so, it is thus imperative to develop fast, effective, economical, and accurate techniques for the diagnosis of malaria both in symptomatic and asymptomatic individuals. Nanotechnology-based diagnostics methodologies can boost up detection of malaria at lower parasite levels while presenting a speedy, accurate, sensitive, and easy method. As a result, nanotechnology-based diagnosis tool is fascinating lots of research interest, and a small number of researchers have proposed prospective nanotechnology-based tools for malarial diagnostics which can conquer some of the difficulties currently facing a malarial diagnosis. Future innovation will be essential to make possible the appliance of more sensitive and affordable nanotechnology-based methods for malaria diagnosis in resource-limited settings. Finally, the level of malaria endemicity, the necessity of diagnosis, the experience of the general practitioner, the efficiency of healthcare workers, and budget resources are all factors influencing the choice of malaria-diagnostic technique.

Keywords Malaria · Mortality · Diagnosis · Nanotechnology-based diagnostics

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1 Introduction

Malaria is a severe contagious, hematologic disease that has an enormous impact on mankind caused by the Plasmodium genus protozoan. There are five different species of the Plasmodium genus namely *Plasmodium vivax (P. vivax)*, *Plasmodium knowlesi (P. knowlesi)*, *Plasmodium malariae (P. malariae)*, *Plasmodium ovale (P. ovale)*, and *Plasmodium falciparum (P. falciparum)*, which is responsible for causing malaria in human beings, and among them, two species namely *P. falciparum* and *P. vivax* posing the serious risk. *P. falciparum* species is the life-threatening malaria parasite and the most common in the African region. *P. vivax* species is the predominant malaria parasite in the majority of countries outside of Sub-Saharan Africa [1–3].

In 2019, almost half of the world's populace was in danger of malaria, and more than half a million deaths resulting from malaria and its complications are reported each year, making it a significant global health problem. Malaria most likely infects anyone exposed to the parasite. However, a few populace groups are at noticeably greater risk of contractile malaria and developing serious ailment: children and pregnant women are the most affected because of the immature immune systems and lowered immunity, correspondingly [4]. Patients suffering from human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) and people with very low-immune systems shifting to regions with severe malaria transmission like immigrant workers, mobile populaces, and travelers are also at high risk [5].

As stated in the most recent World malaria report, there were 229 million cases of malaria in 2019 in comparison with 228 million cases in 2018. The predictable number of malaria deaths stood at 409000 in 2019 worldwide, compared with 411,000 deaths in 2018. Kids under 5 years of age are the most susceptible group influenced by malaria; in 2019 they reported for 67% (274,000) of all malaria deaths universally. The WHO African area continues to carry an unreasonably high share of universal malaria encumbers. In 2019, the African area was habitat to 94% of all malaria cases and casualties [6].

The malaria parasite life cycle starts with the female Anopheles mosquito carrying the malaria-causing parasite which enters into the human body when the infected mosquito bites and injects the parasites through sporozoites into the blood circulation of the individual. The sporozoites then target and enter the liver (hepatocytes) where it undergoes multiplication asexually and differentiation into merozoites. This phase of the parasite life cycle is named the pre-erythrocytic stage, and this stage is fundamental in establishing malaria infection. During this stage, the infectivity stays symptomless, however, it is difficult for diagnostic tools to detect sporozoites because hepatocytes invasion occurs within 30–45 min after sporozoites are inoculated by the infected mosquito. This short time and low numbers of sporozoites injected leaves little time for their detection [7]. In the erythrocytic stage of infection, the sporozoites then grow and create haploids called merozoites which once more re-enter the bloodstream, beginning a cycle of invasion of red blood cells, asexual reproduction, and release of newly formed merozoites from the red

blood cells repetitively. A few merozoite-infected blood cells depart the cycle of asexual reproduction and grow into sexual forms of the parasite, named male and female gametocytes that circulate in the bloodstream. When a mosquito bites an infected human, it ingests the gametocytes which further develop into gametes, ookinetes, and sporozoites. The cycle of human infection re-starts when the mosquito bites a human, injecting the sporozoites from its salivary glands into the human bloodstream [1, 4, 8].

High fever, headache, shaking chills, and sweating are the first symptoms most commonly appeared 10–15 days after the infective mosquito bite, and might be gentle and hard to rely on these symptoms for early diagnosis of malaria. It is then followed up by more hostile symptoms like severe headache, nausea and/or vomiting, diarrhea, fatigue, jaundice, and later seizures, confusion, and kidney failure. A clinical sign of malaria comprises severe anemia, hypoglycemia, thrombocytopenia, metabolic acidosis, and others [9]. Severe malaria appears when infections are intricate by severe organ collapses or deformities in the patient's blood or else metabolism. The symptoms of severe malaria are as follows: (i) abnormality in blood coagulation; (ii) acute kidney damage; (iii) acute respiratory distress syndrome, an inflammatory response in the lungs that restrains oxygen transfer, which may take place in spite of the parasite loads have declined to respond to the treatment; (iv) cerebral malaria, with unusual behavior, impairment of consciousness, convulsions, unconsciousness, or other neurologic abnormality; (v) hemoglobin in the urine because of hemolysis; (vi) hyperparasitemia, wherein greater than 5% of red blood cells (RBCs) are infected by malaria parasites; (vii) lower blood pressure resulted by cardiovascular failure; (viii) metabolic acidosis (too much acidity in the blood as well as tissue fluids), many times together with; (ix) hypoglycemia, which might also happen in pregnant women with uncomplicated malaria or following therapy with quinine; and (x) severe anemia caused by damage of RBCs. Severe malaria is a case of emergency and should be treated with urgency [10]. If left untreated, *P. falciparum* malaria can step forward to serious illness and death within a period of 24 h.

The malaria parasite relies mainly on iron for growth as several enzymes of the parasite metabolic pathways rely upon iron [11]. Clinical symptoms of malaria appeared in the intra-erythrocytic stage. During this stage, *P. falciparum* degrades greater than 80% of hemoglobin present in the food vacuoles to serve up as a source of amino acids [12]. Free heme or iron protoporphyrin IX together with oxygen gets released as a result of hemoglobin degradation. As this free heme is very noxious to the parasite, *P. falciparum* alters the reactive heme species into a compact, insoluble, weakly magnetic crystal named hemozoin inside host erythrocytes with a diminished pro-oxidant ability which is non-hazardous to the parasite [13]. Hemozoin, also termed as malaria pigment, is a visible marker in identifying malarial parasites chemically as well as structurally identical to a distinguishing hematin pigment, referred to as β-hematin. This β-hematin has been widely investigated as a biomarker for malaria diagnosis [14].

The possibility to detect certain biomarkers for Plasmodium-mediated infection at the early stages of malaria can be crucial for formulating disease management

strategies and choosing the correct prophylaxis for the disease. In general, for the widespread zones of Africa where asymptomatic malaria is uncontrolled, biomarker-based diagnostic methods together with appropriate treatment strategies might notably help lessen human reservoirs of the parasite that often add to the tenacity of malaria spread in such areas. The use of biomarkers provides us with sensitive and effective means for the investigation of disease pathogenesis. Currently, several malaria-associated biomarkers have been utilized for disease identification [15]. Malaria diagnostic devices are mostly contingent on explicit Plasmodium biomarkers, with the exception of clinical judgment, which is dependent on the signs that a patient puts on view. These special biomarkers can be straightforwardly linked with the parasite density the patient is bearing, which can vary from 1 parasite/μL to more than 10,000 parasites/μL [16]. Currently, there are six main Plasmodium biomarkers that serve as targets in the faster and easier diagnosis of malaria. These include unique parasite proteins *P. falciparum* histidine-rich protein 2 (PfHRP-2), *Plasmodium* lactate dehydrogenase (pLDH), *Plasmodium* aldolase, glutamate dehydrogenase (GDH), and hypoxanthine-guanine phosphoribosyltransferase and a pigment marker hemozoin. The utilization of hemozoin is highly suggested as a biomarker in the development of malarial diagnostic tools as it is more affordable, stable, and easily available in comparison to PfHRP-2. It is also recognized that hemozoin is chemically and structurally identical to β-hematin. That is why, the majority of researchers make use of β-hematin to mimic hemozoin in the improvement of malarial sensor devices. They can be detected in a diagnostic test using a recognition element (e.g., antibodies or aptamers), generating a signal that is transduced into an output that can afterward be subject to interpreting [1].

An accurate and well-timed diagnosis of the malarial contagion in human beings is the principal factor in the successful management of this parasitic disease. For effective treatment, early diagnosis is needed to reduce the morbidity and mortality occurring from malaria. Accustomed distinctive tools requisite for diagnosis of malaria, comprise the aptitude to establish the presence or absence of infection, determination of the malarial species present, quantify parasitemia (i.e., parasite/μL of blood or % RBCs get infected), identify low-level parasitemia, and permit monitoring of response to antimalarial therapy (including detection of recrudescence or relapse). To date, not a single malaria diagnostic tool has been developed that meets all of these criteria [8]. Therefore in this chapter, we mainly focus on current conventional and novel diagnostic technologies and their challenges of detecting the malaria parasite and nanotechnology-based methods which have been developed for simple and accurate diagnosis of malaria.

2 Diagnosis of Malaria

The number of malaria cases worldwide seems to be greater than before, due to increased transmission risk in areas where malaria control has declined, the rising prevalence of drug-resistant strains of parasites, and in a comparatively few cases,

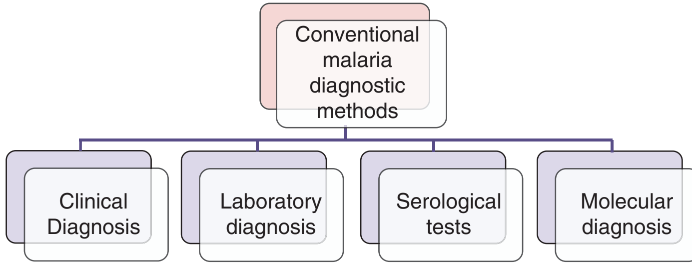

Fig. 1 Conventional malaria diagnostic methods

considerable increases in worldwide travel as well as migration. The need for effective and practical diagnostics for global malaria control is increasing since effectual and accurate diagnosis reduces both complications and mortality from malaria. The universal impact of malaria has urged attention in developing effective diagnostic strategies for not only resource-limited regions where malaria is a great encumber on society but also in developed countries, where malaria diagnostic expertness is often deficient. Malaria diagnosis entails detecting malaria parasites or antigens/products in the patient's bloodstream. Though this might look simple, the diagnostic effectiveness is dependent on many factors. The diverse forms of the five malarial species, such as the different stages of erythrocytic schizogony; the endemicity of different varieties; the interrelationship among levels of spread, drug resistance, inhabitants movement, immunity, parasitemia, and signs and symptoms; the difficulties of recurrent malaria, persisting viable or non-viable parasitemia, and sequestration of the parasites in the deepest tissues; and the utilization of chemoprophylaxis or even presumptive management on the basis of clinical judgment, can all affect the detection as well as interpretation of malaria parasitemia in a diagnostic test [17, 18]. A number of conventional methods are used for the diagnosis of malaria presented in Fig. 1.

2.1 Clinical Diagnosis of Malaria

Clinical identification of malaria is long-established among general practitioners. This technique is the least expensive and most commonly practiced. Clinical identification is based on the patient's signs and symptoms, and on physical findings at an examination. The earliest symptoms of malaria are very non-specific and varying, and consist of fever, headache, weakness, myalgia, chills, dizziness, abdominal pain, diarrhea, nausea, vomiting, and anorexia. Despite its gigantic burden, with 40% of the world's population at risk of infection, the clinical diagnosis of malaria is often not straightforward fairly because non-specific nature of signs and symptoms which bear a resemblance to those of other common or life-threatening diseases, e.g., usual viral or bacterial infections and other feverous diseases. The

overlapping of malaria symptoms with other diseases weakens diagnostic specificity. For this reason, the accurateness of malaria diagnosis can be significantly enhanced by combining clinical and parasite-based findings. The usual distinctive tools necessary for malaria diagnosis include the ability to establish the presence or absence of infection, determination of the malarial species present, quantify parasitemia, and detect low-level parasitemia. To date, not a single malaria diagnostic tool has been developed that meets all of these criteria [18, 19].

2.2 Laboratory Diagnosis of Malaria

Fast and efficient malaria diagnosis not merely eases suffering but also reduces community transmission. The unclear nature of the clinical signs as well as symptoms of malaria might give rise to over-treatment of malaria or non-treatment of other illnesses in malaria-endemic regions, and misdiagnosis in non-endemic regions. In this section, various laboratory methods for the diagnosis of malaria with their advantages and shortcomings have been described [20].

For a long time, microscopy analysis was the standard process to diagnose malaria, especially in developing countries. This technique required only a microscope and a drop of blood to test the malaria-induced parasites. Malaria is diagnosed by staining thick and thin blood films on a glass slide to visualize malaria parasites microscopically. The ample acceptance of this technique by laboratories all around the world can be because of its ease, low cost, its ability to identify the presence of parasites, the infecting species, and measure parasite density-all considerations valuable for the management of malaria. Despite that, this method has numerous downsides for the detection of malarial infection. The staining and interpretation procedures are labor-intensive, time-consuming, and need considerable expertise and skilled healthcare workers specifically for diagnosing species accurately at low parasitemia. It is also difficult to distinguish different species of malarial parasites without skillful technicians. The most important shortcomings of microscopic examination are its relatively low sensitivity, particularly at low-parasite levels as low concentrations of parasites can be extremely hard to diagnose. Even though the expert microscopist can identify up to 5 parasites/μL, the common microscopist identifies only 50–100 parasites/μL. This is most likely resulted in understanding malaria infection rates, especially cases with low parasitemia and asymptomatic malaria. The ability to uphold the required levels of malaria diagnostics expertise is challenging. Microscopy is laborious and ill-suited for high-throughput use and species identification at low-parasite density is still challenging [21–23].

The quantitative buffy coat (QBC) technique was designed to improve the microscopic detection of parasites and simplify malaria diagnosis. QBC technique entails staining parasite deoxyribonucleic acid (DNA) in micro hematocrit tubes using fluorescent dyes like acridine orange and its subsequent finding by epi-fluorescent microscopy. This method is a quick and responsive test for diagnosing malaria in

several laboratories setting. Though the QBC technique is easy, trustworthy, and user-friendly, it necessitates specialized instrumentation, is more expensive than usual light microscopy, and is inadequate at determining species as well as a number of parasites [23, 24].

A rapid diagnostic test (RDT) is an alternate way of quickly establishing the diagnosis of malaria infection by detecting specific malaria antigens in a person's blood. Unlike usual microscopic identification by staining thin and thick peripheral blood coats, and the QBC method, RDTs are all dependent on the same principle and identify malaria antigen in blood flowing the length of a membrane having specific anti-malaria antibodies; they do not have need of electrical energy or specific laboratory instrumentation. Most products target a *P. falciparum*-specific protein, e.g., pLDH or PfHRP-2. Although most RDTs are suitable for *P. falciparum* malaria diagnosis, some also claim that they can efficiently and rapidly diagnose *P. vivax* malaria. RDTs present a probability to lengthen the advantages of parasite-based identification of malaria outside the confines of light microscopy, with potentially noteworthy benefits in the managing of feverish disease in isolated malaria-endemic regions. RDT performance for diagnosis of malaria has been reported as excellent; however, some reports from remote malaria-endemic areas have shown wide variations in sensitivity. Overall, RDTs appear a highly valuable, rapid malaria-diagnostic tool for healthcare workers; however, they must currently be used in conjunction with other methods to confirm the results, characterize infection, and monitor treatment. In malaria-endemic regions where light microscopy facility not available that may benefit from RDTs, upgrading is essential for affordability, ease of use, sensitivity for non-falciparum infection, and stability. The WHO is at the present developing guidelines to make sure lot-to-lot quality control, which is indispensable for the community's self-confidence in this novel diagnostic device [17, 18].

2.3 Serological Tests

The serological tests are considered as the benchmark for malarial serology testing which is generally on the basis of detecting antibodies against asexual blood-stage malaria parasites. The immunofluorescence antibody testing (IFA) is considered to be a trustworthy serologic test for malaria. Even though IFA is time-consuming and subjective, it is a very much sensitive and precise technique. IFA is helpful in epidemiological surveys, for screening prospective blood donors, and periodically for providing proof of recent infection in non-immunes. As of the time, it was a validated technique for identifying *Plasmodium*-specific antibodies in a variety of blood bank units, which was helpful for screening potential blood donors, so circumventing transfusion-transmitted malaria. This technique cannot be automated, which restricts the number of sera that can be studied every day. It also has the need for fluorescence microscopy and qualified technicians; readings can be affected by the level of training of the technologist, predominantly for those serum samples which have low-antibody titers. Furthermore, the need for IFA reagent standardization

puts it impractical for everyday use in blood-transfusion centers, and for harmonizing inter-laboratory outcomes [18, 25].

2.4 Molecular Diagnosis Methods

As mentioned above, conventional malaria diagnostic techniques stay put problematical. New laboratory diagnostic techniques that put on view higher sensitivity as well as higher specificity, exclusive of subjective variation, are immediately required in different laboratories. Latest advances in molecular biological methodologies, e.g., polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP), laser desorption mass spectrometry (LDMS), and flow cytometric (FCM) assay techniques, have allowed comprehensive characterization of the malaria parasite and are creating new approaches for malaria diagnosis.

Over the past few decades, PCR was considered to be the most specific as well as a sensitive diagnostic technique for the identification of malaria with low parasitemia or else mixed infection. For authentication of malaria infection, follow-up therapeutic response, and discovery of the drug resistance, the PCR technique was extensively utilized. Even if PCR emerges to have conquered the two major difficulties of malaria diagnosis-sensitivity and specificity, the usefulness of PCR is restricted by complex methodologies, pricey instrumentation, and the requirement for specifically trained technicians. For that reason, PCR is not routinely put into practice in developing countries on account of the complication of the testing and the inadequacy of resources to carry out these tests satisfactorily and routinely. Quality control in addition to equipment maintenance is as well indispensable for the PCR method; therefore, it might not be appropriate for malaria diagnosis in isolated rural regions or even in regular clinical diagnostic settings. The identification techniques like culture and microscopy, PCR strategies have appreciably favored the identification of communicable diseases and significantly promoted the prevention and management of malaria, but these methods are time-consuming, costly, inaccurate, and need skilled technicians [21, 26].

The LAMP system is identified as a trouble-free and economical molecular malaria-diagnostic test. It is a very sensitive as well as specific methodology, not only for *P. falciparum* but also for *P. vivax*, *P. ovale*, and *P. malariae*. LAMP is a relatively newer technique for nucleic-acid amplification first time described in 2000, and further customized for ease of visualization of amplified product with a fluorescent or colorimetric dye. The limit of detection by the LAMP technique is much the same as that of the PCR technique as they are both in the range of 0.5–5 parasites/μL of blood. It is quicker than the PCR technique and the results can be evaluated visually with no need for any costly thermocycler. The method has a need for a moderately skilled workforce and has a multifaceted primer design [27, 28].

LDMS is speedy, high throughput, and automatic. As compared with the microscopic method, which needs a skilled microscopist and up to 30–60 min to check each peripheral blood smear, LDMS can examine a sample in <1 min. Though, the remote rural areas devoid of electricity are unfriendly for existing high-tech mass

Table 1 Currently used diagnostic test around the world [17, 18, 21–23, 26]

Method for malaria diagnosis	Principle technique & interpretation	Sensitivity	Specificity	Detection time	Advantages
Microscopy	Morphologic interpretation; quantitative	Up to 5 parasites/μl (the expert microscopist) 50–100 parasites/μl (the common microscopist)	High (unless for *P. knowlesi*), difficult to distinguish mix and single infection	Up to 60 min	Low cost, can be stored for a long time
RDT	Antigen and antibody binding; qualitative	50–250 parasites/μl	Moderate (limited to *P. falciparum and P. vivax)*, cannot identify *P. ovale*, *P. malariae*, and *P. knowlesi*	10–20 min	Simple, fast, more practical, and applicable method
PCR	DNA amplification; quantitative and qualitative	Below 5 parasites/μl	High, can identify and differentiate among species	2–8 h	Requiring only a small sample

spectrometers. Future upgrading in instrumentation and techniques must make this technique more realistic [29].

FCM technique has putatively been utilized for malaria diagnosis. This technique may offer a sensitivity of 49–98% and a specificity of 82–97%, for malarial identification, and is feasibly functional for diagnosing clinically unsuspected malaria. The drawbacks are its labor intensiveness, the requirement for trained technicians, expensive diagnostic instruments, and false positives that may take place with other bacterial or else viral infections. So, this technique should be painstaking a screening tool for malaria [18, 30] (Table 1).

3 Novel Malaria Diagnostic Methods Under Development

Novel malaria diagnostic methods were identified as nucleic acid sequence-based amplification (NASBA), isothermal thermophilic helicase-dependent amplification (tHDA), saliva-based test for nucleic-acid amplification, saliva-based test for Plasmodium protein detection, and urine malaria test (UMT) (Fig. 2).

3.1 Nucleic Acid Sequence-Based Amplification (NASBA)

This is a diagnostic method for malaria that involves the use of three enzymes, such as reverse transcriptase, T7 Ribonucleic acid (RNA) polymerase, and Ribonuclease H, to amplify RNA targets in a double-stranded DNA background. The RNA target,

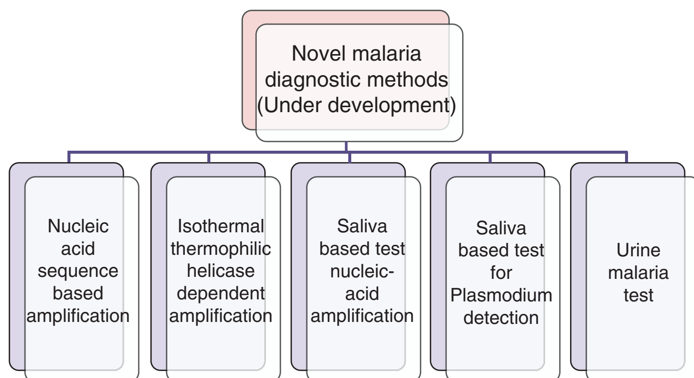

Fig. 2 Novel malaria diagnostic methods under development

such as 18S RNA, is copied into complementary DNA with reverse transcriptase and, afterward, the complementary DNA is amplified using T7 RNA polymerase. It does not require a thermocycler as the reaction can be carried out at 41 °C resulting in more than 108-fold amplification of the target RNA sequence. The sensitivity of the technique, when compared to the microscopy method, ranges from 97.40% to 100% while the specificity ranges from 80.90% to 94%. The limit of detection is 0.01–0.1 parasites/μL of blood. The test is approximated to take about 1 h to complete. NASBA, like the LAMP technique, does not require a thermocycler and has a very lower limit of detection. However, it necessitates extensive training of personnel to make sure the trustworthiness of the results and the cost per test is much higher than other methods [28, 31].

3.2 *Isothermal Thermophilic Helicase-Dependent Amplification (tHDA)*

In this novel malaria diagnostic technique, the double-stranded DNA is separated by helicase, and single-stranded DNA-binding proteins are attached to the separated strands. Specific primers attach to the strands and DNA polymerase synthesizes new strands, and the test is carried out at 65 °C in about 2 h. In the appliance of tHDA for malaria detection, the 18S Ribosomal RNA gene is amplified from whole blood directly with no heat denaturation or else nucleic acid amplification. Probes labeled with either fluorescein or digoxigenin hybridize to the amplicon and the amplification product is sensed with a lateral-flow strip that has anti-fluorescein or anti-digoxigenin antibodies. The sensitivity and specificity of the tHDA method are 96.6% and 100%, respectively, in that order when microscopy is utilized as the gold standard. The limit of detection is 200 parasites/μL of blood and the results can be achieved within 1–2 h. It does not necessitate the exploit of a thermocycler so the expenditure may be more reasonable than the PCR technique. Besides its more

reasonable cost, whole blood can be utilized straightforwardly without any treatment, hence making the method simpler. However, the limit of detection is higher than any other nucleic acid-based techniques and it requires modestly trained workers, the higher limit of detection is not appropriate to identify malaria in patients with low-parasitic load [31, 32].

3.3 Saliva-Based Test with Nucleic-Acid Amplification

This saliva-based malaria diagnosis technique entails the identification of a Plasmodium gene, 18S ribosomal RNA, or *P. falciparum* dihydrofolate reductase gene in saliva with a nested polymerase chain reaction (nPCR). The nPCR requires a similar procedure as conventional PCR but exploits two primer sets and has two consecutive PCR steps. The product obtained from the first PCR reaction stands for the template for the second reaction. A thermocycler is essential for the procedure, and it is mandatory to extract nucleic acid first from the saliva sample previous to nPCR is performed. The sensitivity and specificity of this technique range from 86.36% to 95%, respectively. The specificity varies from 93% to 98.4% in relation to microscopy. The limit of detection is 1–10 parasites/μL of blood for this technique. Saliva-based tests are non-invasive and need minor training for health personnel for the collection of the sample. However, the technique still entails the utilization of PCR, and health personnel will require sophisticated training on the actual PCR protocol. The procedure acquires approximately 6 h to finish, so this can be a most important obstruction for its implementation. It can be expected that the charge of the test will be similar to that of a blood-based PCR test as it needs two PCR reactions using a thermocycler [28, 33].

3.4 Saliva-Based Plasmodium Protein Detection

This saliva-based test finds out the presence of specific Plasmodium proteins in the saliva of an infected person even before actual symptoms initiate. The sensitivity of the test using the whole saliva was 77.9–97.2% and a specificity of 95.4% in comparison with the microscopy method. The limit of detection for this technique is higher at about 1000 parasites/μL of blood. The limit of detection ranges from 1 to 10 gametocytes/μL of blood. The test can be finished between 3 and 30 min. For this technique, the estimated sensitivity in symptomatic patients was found to be 83% when compared to the PCR technique. This method of diagnosis is non-invasive and the same as that of blood-based RDTs. The sensitivity differs and test results are required to be confirmed using microscopy technique, the limit of detection is very high, and more investigation requires to be put into effect to improve it to an acceptable range. It has the prospective to be employed not only for the identification of symptomatic patients but also for asymptomatic individuals. This may be

particularly beneficial for control programs to recognize and treat carriers of the parasite favorable for malaria transmission. The sensitivity of the test is as good as current RDTs. Even if this test is not yet commercialized, it can be expected that the price will be much the same as that of existing blood-based RDTs. Similar to blood-based RDTs, this method of diagnosis is not quantitative and will not be practical for determining the percentage of parasitemia in patients [28, 34].

3.5 Urine-Based Malaria Test

Urine-based malaria diagnostic tests involve the detection of PfHRP-2. An economically available test, named the urine malaria test (UMT), includes dipping the test strip into a urine sample for about 2 min, succeeded by incubation for 20 min. Similar to the frequently used RDTs, a positive result is acknowledged by dark-colored lines on the test strip. Nevertheless, the differentiating factor is that the UMT technique does not entail a finger stick and can be conducted non-invasively. The limit of detection for the UMT test is 125 parasites/μL. The UMT test is somewhat reasonably priced and does not need costly instrumentation or highly trained technician. The limitation of the UMT test is that it only detects PfHRP-2 from *P. falciparum* parasites [28, 35, 36].

Even if undoubtedly novel, these aforementioned techniques of malaria diagnosis still require establishing their practicality for point of care in low-resource settings and show a clinically appropriate limit of detection. Hence, there is a vital requirement to grow novel technologies that facilitate early diagnosis and detection, with their higher sensitivity [21].

4 Nanotechnology in Malaria Diagnosis

Attributable to the unique properties of nanomaterials in optical, mechanical, magnetic, catalytic, and electrical perspectives [37], advancement in nanotechnology has seen many applications especially in biomedical applications such as tissue engineering, drug delivery, bioimaging, and nanodiagnostics. The inimitable properties of nanomaterials or nanostructures present the nano-diagnostic platforms on the aptitude of fast and real-time diagnosis of diseases by only using very few volumes of samples from patients. Nanodiagnostics appliances have drawn increasing interest for the diagnosis of infectious diseases like malaria owing to their unique characteristics in early detection and higher sensitivity and their potential to offer affordability, portability, and robustness.

World Malaria Day has functioned under the theme “Invest in the future: defeat malaria,” and nanotechnology is one among the weapons in this fight against malaria since 2013. The utilization of nanoparticles in the RDTs was first introduced in the 1990s and has since perked up our perception of the burden of the disease. Increased

admittance to those tests, which give an easy yes/no answer, has also improved the speed of therapeutic intervention. In addition, nanostructured surfaces can be customized with polymers or else other functional groups to get better nanostructure monodispersity or lessen non-specific binding of environmental or biological contaminants (e.g., serum proteins). Surfaces can also be personalized with molecules such as aptamers, antibodies, or peptides which allocate nanoparticles to target a particular gene, protein, cell, or organ in vivo. Thus far, researchers have first and foremost exploited nanostructures in malaria diagnosis. An imperative area of focus for recent and future nanotechnology research is the growth of nanotechnology-based molecular diagnostic platforms. Although merely a handful of nanotechnology investigations are focused on malaria diagnosis, many existing nanotechnology platforms could be adapted for malaria diagnostics [4].

Many of these RDTs are based on antibody-labeled gold nanoparticles that are specific for lysed malarial antigens. If the malaria parasites are present, the labeled nanoparticles get attached to malaria parasites and are caught on the test strip, generating a band that is visible by the naked eye and representing a positive result. The attractiveness of these tests is their ease. Unlike the current standard test for malaria (microscopic examination of blood films) very little sample is required – just a drop of blood that can be taken from a finger prick. Additionally, the tests have need of little specialist training, chemicals, or equipment, and therefore the results are achieved in minutes, as contrasting to days. Although, these tests are not perfect, and the discrepancies in their manufacturing can cause inconsistencies in sensitivity and false positives. This represents that the WHO on a regular basis estimates these RDTs to notify users of the consistency of their performance.

Nanotechnology has aided in identifying as well as fully characterizing malaria biomarkers in the development of novel sensors for diagnostic tests with the objective of improving quality, reproducibility, and sensitivity while lessening the associated expenditures. Nanotechnology also can be functional to perk up diagnostic tools already in exploitation. The core element of nearly all RDTs is immunosensors, which make use of antibodies for the detection of biomarkers. Adding to these platforms nanomaterials, for example, gold nanoparticles (AuNPs) [38] or designing multiplex immunoassays with the capacity to detect multiple biomarkers at the same time [39], can improve the sensitivity as well as the overall functioning of sensors, resulting in a superior and more accurate diagnosis. Molecular methods for malaria diagnosis based on nucleic-acid amplification have also been improved through nanotechnology. Taken as a whole, it is very important that the attack on malaria stays constant; the aptitude of the disease to propagate, mainly during the rainy season, can speedily overturn any progress. This needs real solutions to difficulties such as the logistics of giving out interventions and constant funding for research into an efficient technique for the diagnosis of malaria. It seems as though researchers that nanotechnology has a role to play in defeating malaria [40].

Populaces suffering from the primary stage of malaria step forward to cerebral malaria even if systematic treatment was followed indicating that advanced and precise ground-breaking diagnosis techniques required to be urbanized and diagnosed the infection at a primary phase that may improve their care. It is very

imperative to broaden an easy, convenient, and economical system that can be function outside the laboratory by inexperienced individuals for diagnosis of the primary stage of malaria. This diagnosis methodology ought to be reasonably priced and beneficial to the large section of occupants in developing states.

Nanotechnology-based diagnostics methodologies can boost up detection of malaria at lower parasite levels while presenting a speedy, accurate, and easy method. Therefore, nanotechnology-based diagnosis tool is fascinating lots of research interest, and a small number of researchers have proposed prospective nanotechnology-based tools for malarial diagnostics which can conquer some of the difficulties currently facing a malarial diagnosis. In this section, we focus on various nanotechnologies-based methods which have been developed for simple and accurate diagnosis of malaria.

4.1 Colorimetric Detection Using AuNPs

A quick, precise, sensitive single-stranded DNA aptamer-based technique for malaria diagnosis has been productively developed by Jeon and co-workers. The technique is on the basis of interaction among the *Plasmodium* lactate dehydrogenase (pLDH), which is a biomarker for malaria, and pL1 aptamer against *P. vivax* lactate dehydrogenase and *P. falciparum* lactate dehydrogenase. Additionally, the cationic polymers, poly(allylamine hydrochloride) and poly(diallyldimethylammonium chloride), aggregate AuNPs that should be probable to monitor the alteration in color from red to blue, which rely upon pLDH concentration. AuNPs have been employed in biosensors owing to distinctive characteristics, for example, larger surface area, excellent biocompatibility, effortless conjugation of biomolecules, and higher absorption coefficient. Therefore, these types of biosensors have been studied for the detection of nucleic acids as well as small molecules. The pLDH proteins were successfully detected using this aptasensor with little detection limits. In addition, the specificity test confirmed that the aptasenor is incredibly precise in targeting proteins over other interfering proteins. Thus, the AuNPs have obtained great attention as colorimetric reporters, due to their high-extinction coefficients, ease of synthesis, and strongly distance-dependent optical characteristics. Colorimetric AuNPs aptasensors have gained considerable attention for the detection of different targets because the molecular recognition events can be easily detected by the naked eyes without the need for any sophisticated equipment. Based on all that has been said, colorimetric AuNPs aptasensors have great potential as commercial diagnostic tools. By using this system, the *P. vivax* and *P. falciparum* were determined [41, 42].

A colorimetric assay using AuNPs and Merozoite Surface Protein 10 (MSP10) DNA detection in urine can offer quick, simple, safe, and inexpensive identification of *P. vivax*. The assessment exhibited good sensitivity (84%), high specificity (97%), and only mild cross-reactivity with *P. falciparum* (21%). It is easy to use, with an

observable color change that counteracts the requirement for a spectrometer, making it appropriate for exploitation in serious conditions. Using urine gets rid of the need for finger-prick, rising both the safety profile as well as patient acceptance of this model. Inventive use of MSP10 as a marker for *P. vivax* has potential for global appliance in mass screening programs [43].

4.2 Aptamer-Conjugated Nanoparticles

Aptamers are oligonucleotide sequences that are fabricated to distinctively bind desired targets, basically DNA-based antibodies. The stability, as well as economical manufacturing of short DNA sequences, produces aptamers a feasible alternative to antibodies in malaria diagnosis (aptamers are generally <50 bases in length). The most common malaria antigens which are identified by the majority of malaria diagnosis blood tests are PfHRP-2 and pLDH. Aptamers for these antigens have been urbanized, characterized, and established to combine with nanomolar affinities. Conjugation of these aptamers to AuNPs as well as silver nanoparticles has enabled for visual detection of PfHRP-2 and pLDH in solution, and the next step of this research is to include such aptamer-functionalized nanoparticles into a paper-based lateral flow device for future sensitive, economical, and user-friendly malaria diagnostics [44].

4.3 AuNP-Based Fluorescence Immunoassay

One more AuNPs-based simple and susceptible immunoassay described by Guirgis and co-workers productively identifies malaria antigens in infected blood cultures. In this technique, AuNPs functionalized with an antiHsp70 monoclonal antibody attached with cyanine 3B-labeled recombinant *P. falciparum* heat shock protein 70. The cyanine 3B-labeled recombinant *P. falciparum* heat shock protein 70 is released after competition with the free antigen, to the solution which increases the fluorescence intensity. In this study, two types of AuNP-antibody conjugates were exploited as probes, first acquired by electrostatic adsorption of the antibody on the surface of AuNPs and the second acquired by covalent attachment with protein cross-linking agents. With reference to the cross-linked antibodies, electrostatic adsorption of the antibodies to the surface of AuNPs produced conjugates with augmented activity in addition to the linearity of response, in a range of antigen concentrations 8.2–23.8 μg/mL. The approximated limit of detection and limit of quantification for the assay are 2.4 μg/mL and 7.3 μg/mL, respectively. This AuNPs-based fluorescence immunoassay was fruitfully employed for the diagnosis of antigen in malaria-infected human blood cultures at about 3% parasitemia level and is supposed to sense parasite densities as low as 1000 parasites/μL [45].

4.4 Indocyanine Green Liposomal Formulation

Cerebral malaria is a severe form of malaria which is caused by the *P. falciparum*. Misdiagnosis or delayed diagnosis usually delays the treatment that causes death. Therefore, to conquer these problems, Portnoy et al. have developed a nanometer-sized liposomal formulation containing an FDA-approved fluorescent dye indocyanine green for the detection of cerebral malaria in the murine model. Emission intensities of the liposomal formulation were observed high as compared to the free indocyanine green. Liposomal formulation loaded with indocyanine green showed higher emission in the brain of the infected mice as opposed to the control mice as well as drug-treated mice. Histopathological examination showed that the buildup of liposomal formulation in the vasculature of the brain is attributable to the extensive uptake of liposomes with the activated macrophages. Therefore, liposomal formulation loaded with indocyanine green can be a helpful diagnostic tool and biomarker for the early detection of cerebral malaria [46].

4.5 Nanosensors

Portable diagnostics nanosensor device with β-hematin for malaria diagnosis was developed by Armani et al. With the device, the researchers established its impending exploit in the early point-of-care diagnosis of malaria by identifying β-hematin in whole rabbit blood. The limit of detection attained was less than 8.1 ng/mL in 500 μL of blood which is comparable to less than 26 parasites/μL [47].

A recent investigation demonstrated the use of gold (Au) electrodes modified with copper oxide (CuO) nanoparticles, to develop electrochemical sensor devices for the detection of β-hematin in the serum of infected mice as well as human sera identified with malaria parasite with square wave voltammetry method. The process followed the standard addition method, and the consequences pointed out that the β-hematin peak was experiential at around −0.80 V in animal serum and −0.91 V in human serum. The β-hematin peak was not present in animal serum that was not contaminated with malaria parasite revealed as control serum. After pointing the contaminated serum samples with a standard concentration of β-hematin, their outcomes showed a current response raise with rising β-hematin concentration. These outcomes indicated percentage recoveries within the established recovery range (75–110%) for a consistent analytical tool. Therefore, researchers established that the nanosensors would quantitatively detect malaria parasites in human serum inside acceptable limits [14]. Regardless of their enormous perspective in the production of point-of-care diagnostic devices, malaria nanosensors have not been very much investigated. A lot of research is so desirable to perk up the established nanosensors and upscale their applicability further than the laboratories to the hospitals.

4.6 Magnetic Nanoparticles

In recent times, the Raman spectroscopy technique was found to be able to detect hemozoin, produced by the malaria parasite. Raman spectroscopy technique offers a prospective substitute for malaria diagnosis. However, Raman signals can be weak in this technique attributable to the considerably little concentration of hemozoin particularly in the early stage of malaria infection [48]. Therefore, Liu and Yuen suggested the utilization of magnetic nanoparticles for surface-enhanced Raman spectroscopy (SERS) for sensitive as well as rapid detection of enriched β-hematin, whose spectroscopic properties are equivalent to hemozoin. Magnetic nanoparticles can be helpful for the attainment of a few orders of magnitude enrichment in the Raman signal of β-hematin. Additionally, the effect of the magnetic field on SERS augmentation is explored. Briefly, the magnetic nanoparticles with silver shell can augment the Raman signal of β-hematin beneath the influence of a magnetic field, presenting the enormous potential for exploitation in malaria diagnosis [49].

4.7 Transdermal Hemozoin-induced Vapor Nanobubbles

In this method, the detection of hemozoin-generated vapor nanobubbles is done using an ultrasound sensor. A very short pulse of laser administered to blood vessels through the skin localizes heat and evaporates the liquid around the hemozoin crystals. This evaporation of liquid forms expanding and collapsing small-sized vapor nanobubbles inside the malarial parasites. Following the laser is activated, the probe is able to sense acoustic pulse and generates an electrical signal as an acoustic trace. A sufferer with confirmed malaria was checked with the prototype, and hemozoin-generated vapor nanobubbles were identified. The short laser pulse administered is safe to skin and the test is expected to take only a few seconds to carry out and is proficient to detect 0.00034% parasitemia. Since hemozoin clears from the blood within 9 days, in comparison to PfHRP-2 which is cleared only after several months, this technique is estimated to generate few false-positive results. Researchers reported studies of hemozoin-induced vapor nanobubbles in water, whole blood, and individual human RBCs infected with *P. falciparum* and evaluated their non-invasive transdermal detection in *P. yoelii* infected mice. The most important benefits of this method are that it is non-invasive, requires no reagents, and the results can be obtained within seconds. The method has a very low limit of detection and may potentially detect subclinical carriers which are useful for disease surveillance. Highly trained personnel will be needed for the initial deployment of the method in both clinical and field settings. Color of skin has also been found to affect the results of the test and more investigations are considered necessary to address this concern. In addition, there is a need for further studies to determine the safety of extended laser application, even at low pulses, to the skin [50].

4.8 Multi-Walled Carbon Nanotube–Zinc Oxide Nanofiber

A flexible, lightweight, and disposable multi-walled carbon nanotube (MWCNT)–zinc oxide (ZnO) nanofiber-based chemiresistive biosensor for label-free detection of the malaria biomarker, PfHRP-2, was developed by Paul et al.. The sensing platform is formed by depositing nanofibers in between the source and drain electrodes patterned on a thin, flexible polyethylene terephthalate substrate. The MWCNT–ZnO nanofibers are produced by the use of the electrospinning technique followed by a calcination procedure. This approach creates functional groups on the nanofiber surface that are used for the one-step immobilization of PfHRP-2 antibodies without further surface modification. The device exhibits good sensitivity and a wide detection range of 10 fg/mL to 10 ng/mL, and it is specific to the targeted PfHRP-2 biomarker. The fabricated chemiresistive biosensor was reviewed for the detection of the malaria biomarker and can be extended in the future to several other biomarker detection systems towards the smart point of care diagnostics [51].

4.9 Screen-Printed Electrodes Modified with Gold Nanoparticles and MWCNT

A specific and more sensitive screen-printed electrode (SPE) modified with gold nanoparticles and multiwall carbon nanotubes (Nano-Au/MWCNT/SPE) was developed for detection of PfHRP-2 in human serum by an amperometric method by Sharma et al. Nano-Au/MWCNT/SPE immunosensor demonstrated a high level of sensitivity for the detection of PfHRP-2 over those of the bare SPE (by 10 times), the Nano-Au/SPEs, and the MWCNT/SPE. The current Nano-Au/MWCNT/SPE can be packaged without difficulty into a handheld potentiostat device for the identification of PfHRP-2 under field conditions. The materials necessary to perform the immunosensor technique in the field are disposable SPEs as a sensing part, biomolecules like antibodies and alkaline phosphatase conjugate, a battery-operated potentiostat, and a magnetic stirrer. All these items are easily manageable, and experiments can be carried out in the field. In this setting, the potentiostat is not susceptible to any environmental condition. Still, safety measures should be taken with the modified SPE (coated with capturing antibodies) and biomolecules. These modified SPE and biomolecules should be preserved at 2–8 °C [52].

4.10 Nanowire-Based Biosensor

The nanotechnology community is also functioning to counterbalance the rise in antimalarial resistance by improving the accurateness of the diagnosis. By means of components bought on eBay, Jonathan O'Halloran urbanized a nanowire-based biosensor that can read DNA directly from blood samples. Afterward, the company

namely Quantu MDX was set up and, over the past 6 years, has functioned to build up the technology into a handheld point of care device for comprehensive malaria diagnostics. This methodology needs a drop of blood laden into a cassette that segregates the malarial DNA. The malarial DNA is afterward amplified and sent to a biosensor array having 100–1000 nanowire field-effect transistors, each one functionalized to sense different malarial strains, subtypes as well as resistance markers. The device presents readout within 15 min, detailing the species of parasite and its prospective for drug resistance. This information can then be utilized to generate a course of therapy that is individually tailored to counter the resistance. Additionally, the data generated can be shared using a mobile data connection system, favorable for the epidemiological profile of the disease and its strains [40].

4.11 Magneto Immunoassays

Magneto immunoassay-based strategies for the detection of PfHRP-2 for diagnosis of malaria are described by Castilho et al. using magnetic nanoparticles. This immunoassay-based technique is fast, sensitive, inexpensive, and user-friendly with respect to classical assays for the malaria diagnosis based on the detection of PfHRP-2 in serum. The assay depends for the first time on a sandwich magneto immunoassay among PfHRP-2 in the sample and two commercially accessible monoclonal antibodies that aim at two diverse epitopes of the antigen, one of them covalently tied to magnetic nanoparticles and the other labeled with the enzyme horseradish peroxidase (HRP) to provide the electrochemical signal. The customized magnetic nanoparticles are then captured with the magnetic field on the magneto electrode. This electrochemical magneto immunosensor attached with magnetic nanoparticles has revealed superior analytical performance with regard to the limit of detection (0.36 ng/mL), which is much lesser than the limit of detection reported by other techniques. In addition, at a lower level of PfHRP-2 concentration of 31.0 ng/mL, a signal of 15.30 μA was attained with a cutoff value of 0.34 μA, granting an obvious positive outcome with a non-specified adsorption ratio of 51. The magneto immunosensor is competent to present a quantitative result for the PfHRP-2 protein related to malaria in serum samples with a minimum pretreatment. Also, because of high sensitivity, this novel method offers an enormous guarantee for fast, simple, cost-effective, and on-site detection of *P. falciparum* malaria in patients and also be valuable to screen out at-risk blood samples for the avoidance of transfusion-transmitted malaria [39].

4.12 Integrated Magnetic Bead—Quantum Dot Immunoassay

Kim et al. developed an integrated magnetic bead—quantum dot immunoassay technique in which immunoassay using magnetic beads was utilized for capture and quantum dots utilized for detection of PfHRP-2. Conventional immunoassays, such

as enzyme-linked immunoassay, and molecular analysis tools, such as PCR, are complicated to put into practice in low-resource settings. For that reason, to offer a proof-of-principle of translation of this assay to low-resource settings, researchers demonstrated PfHRP-2 detection in an automated droplet-based microfluidic device. The exploitation of magnetic beads and quantum dots permits comparatively speedy and easy detection of malaria antigen with an overall assay time of about 1.5 h. This assay with an optimized pair of capture in addition to detection antibodies permits quantitative and sensitive measurement of the target antigen. Translation of the vial assay to an automated droplet-based tool will possibly put forward a user-friendly diagnostic tool for malaria that need minimum training and enabling testing in low-resource settings [53].

5 Conclusion and Future Prospects

Accurate and efficient diagnosis is the initial step to further carry-on efforts to eliminate and diminish the universal burden of malaria by 90% in 2030. Conventional microscopic testing of peripheral thick and thin blood smears stays the gold standard for malaria diagnosis. Even though this technique needs a skilled microscopist, sensitivity and specificity show a discrepancy compared with the latest technical advances, it is inexpensive and trustworthy. Rapid and convenient RDTs are presently implemented in various remote settings, but are expensive and require improved quality control. Serological tests are valuable for epidemiological surveys, but not appropriate for the identification of acute malaria. Molecular-biological techniques are suitable for research laboratories; they can be utilized to recognize the development of drug resistance; they are functional for species detection and also for quantifying parasite density with low parasitemia. These conventional diagnostic techniques can identify malaria symptomatic infections but frequently miss out on asymptomatic cases. Innovative technologies are desirable to make novel advances and allow rapid diagnosis, an area in which nanotechnology could play a leading role. The increase in the proportion of asymptomatic infections in low-transmission regions calls for nanotechnology-based diagnostic tests that can identify the hidden parasite reservoir. Through the exploitation of AuNPs, AuNPs-based fluorescence immunoassay, magnetic nanoparticles, magneto immunoassays, nanosensors, nanowire, transdermal vapor nanobubbles are already in the development for malaria diagnosis.

While the growth in research, as well as feasibility demonstrations of nanotechnology-based diagnostics for malaria, has been fast, the commercialization and translation are simply beginning to turn out to be a reality. Challenges still comprise the cost of manufacturing, production, and scale up itself and the consequent business models for economic success. Continued charitable sources and financial support from the developed world would be importantly desired to continue further investments for commercialization of the hopeful nanotechnology-based approaches for malaria diagnosis. The prospective for impact is unlimited as millions of

populaces can benefit if these technologies can be commercialized and brought into the hands of the health care providers in the world's poorest areas that are excessively impacted by malaria.

Finally, the level of malaria endemicity, the necessity of diagnosis, the experience of the general practitioner, the efficiency of healthcare workers, and budget resources are all factors influencing the choice of malaria-diagnostic technique.

References

1. Ragavan K, Kumar S, Swaraj S, Neethirajan S. Advances in biosensors and optical assays for diagnosis and detection of malaria. Biosens Bioelectron. 2018;105:188–210.
2. Perez-Guaita D, Marzec KM, Hudson A, Evans C, Chernenko T, Matthaus C, et al. Parasites under the spotlight: applications of vibrational spectroscopy to malaria research. Chem Rev. 2018;118(11):5330–58.
3. Mathison BA, Pritt BS. Update on malaria diagnostics and test utilization. J Clin Microbiol. 2017;55(7):2009–17.
4. Hauck TS, Giri S, Gao Y, Chan WC. Nanotechnology diagnostics for infectious diseases prevalent in developing countries. Adv Drug Deliv Rev. 2010;62(4–5):438–48.
5. WHO. Malaria – World Health Organization; 2021. Available at https://www.who.int/news-room/fact-sheets/detail/malaria
6. WHO. World malaria report 2020; 2020. Available at https://www.who.int/publications/i/item/9789240015791
7. Mawson AR. The pathogenesis of malaria: a new perspective. Pathog Glob Health. 2013;107(3):122–9.
8. Baruah UK, Gowthamarajan K, Vanka R, Karri VVSR, Selvaraj K, Jojo GM. Malaria treatment using novel nano-based drug delivery systems. J Drug Target. 2017;25(7):567–81.
9. Pérez-Tris J, Hasselquist D, Hellgren O, Krizanauskiene A, Waldenström J, Bensch S. What are malaria parasites? Trends Parasitol. 2005;21(5):209–11.
10. Centers for Disease Control and Prevention. About Malaria. Available online: https://www.cdc.gov/malaria/about/biology/index.html
11. Weinberg ED, Moon J. Malaria and iron: history and review. Drug Metab Rev. 2009;41(4):644–62.
12. Goyal M, Alam A, Bandyopadhyay U. Redox regulation in malaria: current concepts and pharmacotherapeutic implications. Curr Med Chem. 2012;19(10):1475–503.
13. Oliveira MF, Kycia SW, Gomez A, Kosar AD, Bohle DS, Hempelmann E, et al. Structural and morphological characterization of hemozoin produced by *Schistosoma mansoni* and *Rhodnius prolixus*. FEBS Lett. 2005;579(27):6010–6.
14. Obisesan OR, Adekunle AS, Oyekunle JAO, Sabu T, Nkambule TTI, Mamba BB. Development of electrochemical nanosensor for the detection of malaria parasite in clinical samples. Front Chem. 2019;7:89.
15. Mathema VB, Na-Bangchang K. A brief review on biomarkers and proteomic approach for malaria research. Asian Pac J Trop Med. 2015;8(4):253–62.
16. Koepfli C, Nguitragool W, Hofmann NE, Robinson LJ, Ome-Kaius M, Sattabongkot J, et al. Sensitive and accurate quantification of human malaria parasites using droplet digital PCR (ddPCR). Sci Rep. 2016;6:39183.
17. Murray CK, Gasser RA Jr, Magill AJ, Miller RS. Update on rapid diagnostic testing for malaria. Clin Microbiol Rev. 2008;21(1):97–110.
18. Tangpukdee N, Duangdee C, Wilairatana P, Krudsood S. Malaria diagnosis: a brief review. Korean J Parasitol. 2009;47(2):93–102.

19. Mwangi TW, Mohammed M, Dayo H, Snow RW, Marsh K. Clinical algorithms for malaria diagnosis lack utility among people of different age groups. Tropical Med Int Health. 2005;10(6):530–6.
20. Chotivanich K, Silamut K, Day NPJ. Laboratory diagnosis of malaria infection-a short review of methods. Aust J Med Sci. 2006;27(1):11–5.
21. Hikal W, Bratovcic A, Baeshen R, Tkachenko K, Said-Al AH. Nanobiotechnology for the detection and control of waterborne parasites. Open J Ecol. 2021;11(03):203–23.
22. Hawkes M, Kain KC. Advances in malaria diagnosis. Expert Rev Anti-Infect Ther. 2007;5(3):485–95.
23. Gavhane J, Thamke R, Pandey A, Sherdiwala F, Yadav R, Kadam N. Recent advances in diagnosis of malaria. New Indian J Pediatr. 2015;4(2):98–107.
24. Barman D, Mirdha BR, Samantray JC, Kironde F, Kabra SK, Guleria R. Evaluation of quantitative buffy coat assay and polymerase chain reaction for diagnosis of malaria. J Commun Dis. 2003;35(3):170–81.
25. She RC, Rawlins ML, Mohl R, Perkins SL, Hill HR, Litwin CM. Comparison of immunofluorescence antibody testing and two enzyme immunoassays in the serologic diagnosis of malaria. J Travel Med. 2007;14(2):105–11.
26. Hanscheid T, Grobusch MP. How useful is PCR in the diagnosis of malaria? Trends Parasitol. 2002;18(9):395–8.
27. Aonuma H, Suzuki M, Iseki H, Perera N, Nelson B, Igarashi I, et al. Rapid identification of Plasmodiumcarrying mosquitoes using loop-mediated isothermal amplification. Biochem Biophys Res Commun. 2008;376(4):671–6.
28. Mbanefo A, Kumar N. Evaluation of malaria diagnostic methods as a key for successful control and elimination programs. Trop Med Infect Dis. 2020;5(2):102.
29. Scholl PF, Kongkasuriyachai D, Demirev PA, Feldman AB, Lin JS, Sullivan DJ Jr, et al. Rapid detection of malaria infection in vivo by laser desorption mass spectrometry. Am J Trop Med Hyg. 2004;71(5):546–51.
30. Shapiro HM, Mandy F. Cytometry in malaria: moving beyond Giemsa. Cytometry A. 2007;71(9):643–5.
31. Oriero E, Jacobs J, Van Geertruyden J, Nwakanma D, D'Alessandro U. Molecular-based isothermal tests for field diagnosis of malaria and their potential contribution to malaria elimination. J Antimicrob Chemother. 2015;70(1):2–13.
32. Li Y, Kumar N, Gopalakrishnan A, Ginocchio C, Manji R, Bythrow M, et al. Detection and species identification of malaria parasites by isothermal tHDA amplification directly from human blood without sample preparation. J Mol Diagn. 2013;15(5):634–41.
33. Mfuh KO, Tassi YS, Esemu LF, Bekindaka ON, Yonga J, Djontu JC, et al. Detection of Plasmodium falciparum DNA in saliva samples stored at room temperature: potential for a non-invasive saliva-based diagnostic test for malaria. Malar J. 2017;16(1):434.
34. Tao D, McGill B, Hamerly T, Kobayashi T, Khare P, Dziedzic A, et al. A saliva-based rapid test to quantify the infectious subclinical malaria parasite reservoir. Sci Transl Med. 2019;11(473):eaan4479.
35. Oyibo WA, Ezeigwe N, Ntadom G, Oladosu OO, Rainwater-Loveth K, O'Meara W, et al. Multicenter pivotal clinical trial of urine malaria test for rapid diagnosis of Plasmodium falciparum malaria. J Clin Microbiol. 2016;55(1):253–63.
36. Oguonu T, Shu E, Ezeonwu BU, Lige B, Derrick A, Umeh RE, et al. The performance evaluation of a urine malaria test (UMT) kit for the diagnosis of malaria in individuals with fever in south-east Nigeria: cross-sectional analytical study. Malar J. 2014;13:403.
37. Patel JK, Patel A, Bhatia D. Introduction to nanomaterials and nanotechnology. In: Patel JK, Pathak YV, editors. Emerging technologies for nanoparticle manufacturing. Cham: Springer; 2021. p. 3–23.
38. Hemben A, Ashley J, Tothill IE. Development of an immunosensor for Pf HRP 2 as a biomarker for malaria detection. Biosensors. 2017;7(3):28.

39. De Souza CM, Laube T, Yamanaka H, Alegret S, Pividori MI. Magneto immunoassays for Plasmodium falciparum histidine-rich protein 2 related to malaria based on magnetic nanoparticles. Anal Chem. 2011;83(14):5570–7.
40. Investingin the future. Nature Nanotech. 2014; 9:241. https://doi.org/10.1038/nnano.2014.74.
41. Jeon W, Lee S, Manjunatha DH, Ban C. A colorimetric aptasensor for the diagnosis of malaria based on cationic polymers and gold nanoparticles. Anal Biochem. 2013;439(1):11–6.
42. Yang C, Wang Y, Marty JL, Yang X. Aptamer-based colorimetric biosensing of ochratoxin A using unmodified gold nanoparticles indicator. Biosens Bioelectron. 2011;26(5):2724–7.
43. Alnasser Y, Ferradas C, Clark T, Calderon M, Gurbillon A, Gamboa D, et al. Colorimetric detection of plasmodium vivax in urine using MSP10 oligonucleotides and gold nanoparticles. PLoS Negl Trop Dis. 2016;10(10):e0005029.
44. Dirkzwager RM, Cheung YE, Tanner JA. The use of aptamer-conjugated nanoparticles in novel malaria diagnosis platforms. In: The 2013 Hong Kong Inter-University Biochemistry Postgraduate Symposium, Hong Kong, China, 2013; abstract no. P29.
45. Guirgis BSS, eCunha CS, Gomes I, Cavadas M, Silva I, Doria G, et al. Gold nanoparticles-based fluorescence immunoassay for malaria antigen detection. Anal Bioanal Chem. 2012;402(3):1019–27.
46. Portnoy E, Vakruk N, Ameer Bishara MS, Magdassi S, Golenser J, Eyal S. Indocyanine green liposomes for diagnosis and therapeutic monitoring of cerebral malaria. Theranostics. 2016;6(2):167–76.
47. Armani AM, Chen D, McBirney SE, Kaypaghian K, Huber H, Ameri H. Portable optical diagnostics for early malaria detection. In: Proceeding of the Frontiers in Biological Detection: From Nanosensors to Systems XI, vol. 10895. San Francisco, CA: SPIE BiOS; 2019. p. 1089508.
48. Moore LR, Fujioka H, Williams PS, Chalmers JJ, Grimberg B, Zimmerman PA, et al. Hemoglobin degradation in malaria-infected erythrocytes determined from live cell magnetophoresis. FASEB J. 2006;20(6):747–9.
49. Liu Q, Yuen C. Effect of magnetic field in malaria diagnosis using magnetic nanoparticles. In: Ramanujam N, Popp J, editors. Proceeding of the Clinical and Biomedical Spectroscopy and Imaging II, Proceedings of SPIE-OSA biomedical optics, vol. 8087. Munich; 2011. paper 80870E.
50. Lukianova-Hleb EY, Campbell KM, Constantinou PE, Braam J, Olson JS, Ware RE, et al. Hemozoin-generated vapor nanobubbles for transdermal reagent- and needle-free detection of malaria. Proc Natl Acad Sci U S A. 2014;111(3):900–5.
51. Paul KB, Kumar S, Tripathy S, Vanjari SRK, Singh V, Singh SG. A highly sensitive self assembled monolayer modified copper doped zinc oxide nanofiber interface for detection of Plasmodium falciparum histidine-rich protein-2: targeted towards rapid, early diagnosis of malaria. Biosens Bioelectron. 2016;80:39–46.
52. Sharma MK, Rao VK, Agarwal GS, Rai GP, Gopalan N, Prakash S, et al. Highly sensitive amperometric immunosensor for detection of Plasmodium falciparum histidine-rich protein 2 in serum of humans with malaria: comparison with a commercial kit. J Clin Microbiol. 2008;46(11):3759–65.
53. Kim C, Hoffmann G, Searson PC. Integrated magnetic bead-quantum dot immunoassay for malaria detection. ACS Sens. 2017;2(6):766–72.

Nanotechnology in TB Diagnosis

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Abstract Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis* (Mtb), is still a significant worldwide health issue, and it is the second largest cause of mortality among all infectious diseases (Mtb). TB is most usually associated with the lungs, although it can also damage the kidneys, brain, and spine. The detection of latent tuberculosis infection (LTBI), extrapulmonary tuberculosis (EPTB), drug-resistant tuberculosis (DR-TB), Human immune deficiency virus (HIV)-associated tuberculosis (TB), and pediatric tuberculosis remains difficult in poor countries. This is mostly due to tuberculosis that has been delayed or misdiagnosed, which is feeding the global epidemic. Because an adequate screening test is currently unavailable, tuberculosis must be diagnosed using traditional methods, despite their limited diagnostic capacity. Nanoparticles have exhibited promising features in improving pharmaceutical distribution, reducing treatment frequency, and improving disease diagnosis. Scientific world believe that nanotechnology has proposed new ways to address residual scientific concerns for TB. Nanotechnology-based concepts have significant potential for diagnosing, treating, and preventing tuberculosis. The creation of antigen/antibody nanocarriers is an intriguing new frontier in the field of diagnostics, with the potential to pave the way for better TB diagnostics. This chapter reviews the current diagnostic techniques for tuberculosis and emphasizes recent advances in nanotechnology-based *Mycobacterium tuberculosis* detection methods.

Keywords Nanotechnology · Diagnosis · Tuberculosis

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1 Introduction

It is an infectious disease that is caused by the tubercle bacillus, *Mycobacterium tuberculosis* [1]. During the eighteenth and nineteenth centuries, tuberculosis reached near-epidemic proportions in the rapidly urbanizing and industrializing societies of Europe and North America. Indeed, "consumption", as it was then known, was the leading cause of death for all age groups in the Western world from that period until the early twentieth century, at which time improved health and hygiene brought about a steady decline in mortality rates [2]. Today, in less-developed countries where population is dense and hygienic standards poor, tuberculosis remains a major fatal disease. The prevalence of the disease has increased in association with the HIV/AIDS epidemic; an estimated one out of every four deaths from tuberculosis involves an individual co-infected with HIV [3].

Multidrug-resistant (MDR) *Mycobacterium tuberculosis* strains, which are resistant to both isoniazid (INH) and rifampicin (RIF), as well as extensively Drug-Resistant Tuberculosis (XDR-TB) strains, which are MDR TB strains plus resistance to any fluoroquinolone and at least one of three injectable second-line drugs (amikacin, kanamycin, or capreomycin) that is virtually chronic. However, antibiotics used to treat TB have several modes of action through various metabolic levels including DNA/RNA/protein synthesis and ability to interfere or inhibit the growth of a specific microorganism [4].

Between 2000 and 2020, it was anticipated that almost a billion people will contract tuberculosis for the first time. Tuberculosis will have killed 1.5 million people by 2020 (including 214,000 people with HIV). TB is the world's 13th top cause of death and the second most deadly infectious disease (after HIV/AIDS). By 2020, an estimate of ten million people will have contracted tuberculosis (TB). In which, there are 5.6 million men, 3.3 million women, and 1.1 million children. MDR-TB, or multidrug-resistant tuberculosis, is still a public health concern and a security threat. In 2020, just around one-third of patients with drug-resistant tuberculosis received treatment [5].

Nanotechnology, also known as general-purpose technology, makes use of nanoscale molecules with diameters ranging from 1 to 100 nanometers. The notion of nanotechnology has been used to produce biomedical applications such as tissue engineering, medication delivery, bioimaging, and nanodiagnostics for several decades. Nanodiagnostics-based fast tests for infectious diseases have gotten a lot of attention because of their unique properties in early detection with high sensitivity and specificity [6].

2 Pathogenesis

The cycle of TB infection begins with the dispersion of *Mycobacterium tuberculosis* aerosols. In the patient's lung, the bacilli are phagocytized by alveolar macrophage cells, which then invade the underlying epithelium. Here, monocytes

from the nearby blood vessels form the beginning of a granuloma. Within the granuloma, a core of infected macrophages is surrounded by foamy macrophages, mononuclear phagocytes, and lymphocytes. The result is a fibrous capsule with increased foamy macrophages, presumed to create the typical caseous debris (necrotic tissue resembling cheese) in the center of the granuloma. The cycle is complete as the damaged lungs produce a cough that, once again, contains the highly transmissible infectious droplet nuclei. Infected macrophages may be carried by the lymphatic system to the lungs, lymph nodes, kidneys, epiphyses of the long bones, and other areas of the body. In general, hypersensitivity develops during the three-to-eight-week period after infection, signaling the action of cellular immunity and control of the infection. However, as previously stated, in high-risk groups, the progression of disease to cavitation in the lung and hematogenous dissemination are likely to occur [7].

3 Diagnosis

Sputum microscopy with a standard light microscope is the predominant approach for diagnosing pulmonary tuberculosis in low- and middle-income countries, where the majority of tuberculosis cases occur. Conventional microscopy has a low sensitivity when compared to culture, which is especially problematic in HIV-positive patients. In high-income countries, fluorescence microscopy, instead of conventional microscopy, is the standard diagnostic procedure. Fluorescence microscopy is associated with improved sensitivity as well as reduced work effort.

Different microscopic alternatives such as fluorescent microscopy, Kinyoun staining, and modified carbol-fuchsin staining were also tried for the diagnosis of TB. As per a systematic review of 45 relevant studies, fluorescent microscopy has been found to be on an average 10% more sensitive than conventional microscopy (95% CI: 5–15%) and almost 98% specific [8].

However, initially, fluorescent microscopy was not widely implemented due to high cost, frequent burn-out of expensive mercury vapor lamps, continuous power supply necessity, and the need for a dark room. The advent of light emitting diode (LED)-based fluorescent microscopes has overcome these short-comings and are now being increasingly used [9].

Different staining techniques like Ziehl–Neelsen staining (ZN), fluorescent Auramine-O staining (AO), and culture on modified Lowenstein–Jensen media (gold standard) were employed for the detection of *Mycobacterium tuberculosis* in the conventional microscopy technique [10].The three tiers of the network of TB laboratories and the responsibilities and the tests offered at each level (Table 1) [11] and the list of diagnostic tests/kits are tabulated in Table 2 [12].

Table 1 Three tiers of the network of TB laboratories and the responsibilities and the tests offered at each level

Sr. No.	Levels	Tests
1	Peripheral (Community level)	Smear microscopy (ZN/LED), LAM, and Digital X-ray
2	Intermediate (Regional, district, and sub-district level)	Smear microscopy(ZN / LED), LAM, Xpert MTB RIF, Xpert MTB RIF ULTRA, and Solid culture (including drug-susceptibility testing (DST))
3	Referral (Reference level)	Smear microscopy, LAM, Xpert MTB RIF, Xpert MTB RIF ULTRA, Solid and liquid culture media,Drug-susceptibility testing (DST) first and second line, NAAT, and Rapid speciation tests

Adapted from WHO [11]

3.1 Smear Sputum Microscopy

It is a simple, quick, and economical approach that is very specific in places where tuberculosis is prevalent.

It also recognizes the most infectious patients and can be used in a wide range of communities and socioeconomic levels. As a result, it has become an important part of the global TB control strategy. Sputum smear microscopy, on the other hand, has severe limits in terms of performance. When the bacterial load in a sputum sample is fewer than 10,000 organisms/ml, the sensitivity is severely impaired [13].

It also has a bad track record in patients with extrapulmonary tuberculosis, pediatric tuberculosis, and HIV-TB co-infection.

Because serial sputum examinations are required, some patients who do not return for additional sputum testing are referred to as “diagnostic defaulters.” A personal observation revealed that limited resources, high quantities of samples, and all of these factors combined often cut the observation time per slide to less than 60 seconds, lowering the test’s sensitivity [14].

3.2 Fluorescence Microscopy

It was first used in the 1930s to improve the results of smear microscopy. The smear is stained with fluorochrome dyes. To excite and fluoresce the dye, a halogen or high-pressure mercury vapor lamp is traditionally utilized. A meta-analysis of studies comparing fluorescence and conventional microscopy indicated that fluorescence microscopy’s sensitivity was 10% higher than conventional microscopy, and that it remained high even after the samples were concentrated [15].

The sensitivity of low-grade smear positive sputum was shown to be higher. The estimates of specificity, on the other hand, were comparable to conventional microscopy, while turnaround times were lower. According to the findings of this

Table 2 List of diagnostic tests/kits for TB

Sr. No	Diagnostic tests	Manufacturers	Methods	Detection limit/ sensitivity (%)
1	Conventional solid media	Various	Growth-based detection	1000–10,000 CFU/ml 28–75%
2	Automated liquid culture systems	BD, bioMe'rieux, Trek	Growth-based detection	81.5%
3	Phage-based detection	Biotec	Growth-based detection	100 CFU/mL
4	Conventional microscopy with acid-fast staining	Various	Direct visualization	20–43%
5	Fluorescent microscopy with nonspecific cell-wall staining	Various	Direct visualization	52–97%
6	Detection of diagnostic antibody responses to TB	Various	Antibody detection	89.4%
7	Automated, nonintegrated NAAT	GenProbe, Roche, BD, many others	Molecular detection	97.7%
8	Luminescent probe of culture isolate	GenProbe	Species identification	<100 CFU/ml
9	Reverse hybridization line probe from culture isolates	Innogenetics, Hain	Species identification	2×10^3 CFU/ml
10	Tuberculin skin test with PPD	Various	LTBI detection	87–98%
11	Whole-blood IFN-γ release assay	Cellestis	LTBI detection	75–84%
12	ELISPOT IFN-γ release assay	Oxford Immunotech	LTBI detection	97%
13	Abbott RealTime MTB Amplification Reagent Kit	Abbott GmbH & Co KG, Wiesbaden, Germany	Molecular detection	17 CFU/ml 93%
14	BD MAX™ MDR -TB	Becton Dickenson & Co, BD Bioscience	Molecular detection	100%
15	Truenat™ MTB	Molbio Diagnostics Pvt. Ltd. Goa, India	RTPCR	95–99% (29.2 cells/ml)
16	T-SPOT®.TB 8 with T-Cell Select	Oxford Immunotec Ltd	Antibody detection	98.8%
17	STANDARD™ E TB-Feron ELISA	SD Biosensor Inc	Antibody detection	98.03%

LTBI latent TB infection, *IFN* interferon (Modified from Refs. [12, 24])

meta-analysis, successful and broad application of fluorescence microscopy could improve case detection by increasing sensitivity and reducing time spent on microscopic examination. With fluorescence microscopy, cost is a big concern. This can be avoided by using light-emitting diodes (LEDs), which cost less than a tenth of the price of a mercury vapor lamp [16].

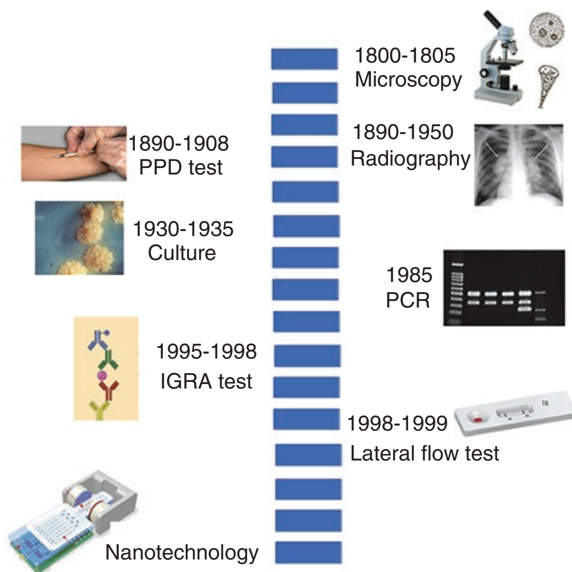

Fig. 1 Timeline of the evolution of tuberculosis diagnostic assays

3.3 Rapid Culture-Based Methods

This comprises fast automated liquid culture, which can produce results in a matter of weeks, thin layer agar culture, which takes an average of 11.5 days, and the Microscopic Observation Drug-Susceptibility Assay (MODS), which takes an average of 9.2 days [17] (Fig. 1).

4 Immunological Methods

4.1 Tuberculin Skin Test

The Tuberculin Skin Test (TST) is also known as the Mantoux Test. It is a skin test to detect if you have been infected with TB bacteria. The tuberculosis skin test is another name for the tuberculin test or PPD test. The PPD test determines if someone has developed an immune response to the bacterium that causes tuberculosis (TB). The skin test is the preferred test in children under 5 years of age. The standard recommended tuberculin test is the Mantoux test, which is administered by injecting 0.1 mL of liquid containing 5 TU (tuberculin units) PPD (purified protein derivative) into the top layers of the skin of the forearm. The basis of the reading of the skin test is the presence or absence and the amount of induration (localized swelling). A negative test does not always mean that a person is free of tuberculosis.

The tuberculin skin test is based on the fact that infection with *Mycobacterium tuberculosis* bacterium produces a delayed-type hypersensitivity skin reaction to

certain components of the bacterium. Reaction in the skin to tuberculin PPD begins when specialized immune cells, called T cells, sensitized by prior infection, are attracted by the immune system to the skin site where they release chemical messengers called lymphokines. These lymphokines induce induration (a hard, raised area with clearly defined margins at and around the injection site) through local vasodilation (expansion of the diameter of blood vessels) leading to fluid deposition known as edema, fibrin deposition, and attraction of other types of inflammatory cells to the area [18].

4.2 ELISA

The ELISA test for the diagnosis of tuberculosis using highly purified A 60 antigen extracted from mycobacteria was developed by Anda Biologicals, France, during the late 1980s. It is claimed to have about 95% sensitivity and specificity. Both IgG and IgM antibodies can be separately tested. IgM antibodies appear early in the disease and IgG appears later. The test is negative in healthy, normal subjects and is not related to the tuberculin test or BCG vaccination status. The A 60 antigen is common to many mycobacteria including *Mycobacterium tuberculosis*, *Mycobacterium leprae, Mycobacterium bovis*, and *Mycobacterium avium*. Hence, clinical, radiological, and other laboratory data must be considered along with the results of the Elisa test for final diagnosis.

The *Mycobacterium tuberculosis* antigen is bound on the surface of the microtiter strips. Diluted patient serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized *Mycobacterium tuberculosis* antigen takes place. After 1 hour of incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of IgG antibodies is directly proportional to the intensity of the color [19].

4.3 Interferon Gamma Determination

B assay is a new test to diagnose tuberculosis infection by measuring in vitro T-cell interferon gamma release in response to two *Mycobacterium tuberculosis*-specific antigens: early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10).IGRAs are in vitro blood tests of cell-mediated immune response; they measure T-cell release of IFN-γ following stimulation by antigens specific to the

Mycobacterium tuberculosis complex (with the exception of BCG substrains), i.e., early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10). These antigens are encoded by genes in the *Mycobacterium tuberculosis* genome's region of difference 1 (RD1) locus [20].

They are more specific than the purified protein derivative (PPD) for *Mycobacterium tuberculosis* because they are not encoded in the genomes of any BCG vaccine strains or most species of nontuberculous mycobacterium (NTM), other than *Mycobacterium marinum, Mycobacterium kansasii, Mycobacterium szulgai,* and *Mycobacterium flavescens.*

However, not all nontuberculous mycobacteria (NTMs) have been studied for cross-reactivity. There is some evidence of cross-reactivity between ESAT-6 and CFP-10 of *M. tuberculosis* and Mycobacterium leprae but the clinical significance of this in settings where leprosy and TB are endemic (e.g., India and Brazil) is poorly characterized. Two commercial IGRAs are available in many countries: the QuantiFERON-TB Gold In-Tube (QFT) assay (Cellestis/Qiagen, Carnegie, Australia) and the T-SPOT.TB assay (Oxford Immunotec, Abingdon, United Kingdom). Both tests are approved by the U.S. Food and Drug Administration (FDA) and Health Canada and are CE (Conformité Européenne) marked for use in Europe [21].

The QFT assay is an enzyme-linked immunosorbent assay (ELISA)-based, whole-blood test that uses peptides from the RD1 antigens ESAT-6 and CFP-10 as well as peptides from one additional antigen (TB7.7 [Rv2654c], which is not an RD1 antigen) in an in-tube format. The result is reported as the quantification of IFN-γ in international units (IU) per milliliter. An individual is considered positive for *Mycobacterium tuberculosis* infection if the IFN-γ response to the TB antigens is above the test cutoff (after subtracting the background IFN-γ response of the negative control) [22].

The T-SPOT.TB assay is an enzyme-linked immunosorbent spot (ELISPOT) assay performed on separated and counted peripheral blood mononuclear cells (PBMCs) that are incubated with ESAT-6 and CFP-10 peptides. The result is reported as the number of IFN-γ-producing T cells (spot-forming cells). An individual is considered positive for *Mycobacterium tuberculosis* infection if the spot counts in the TB antigen wells exceed a specific threshold relative to the negative-control wells [23].

4.4 FDA Guidelines on the Diagnostic Test

In order to provide reasonable assurance of the safety and effectiveness of nucleic acid-based in vitro diagnostic devices for the detection of the MTB-complex in respiratory specimens, FDA concludes that special controls, when combined with the general controls of the Federal Food, Drug, and Cosmetic Act, are required. Before marketing a device of this type, a manufacturer must (1) comply with the Federal Food, Drug, and Cosmetic Act's general controls, including the premarket

notification requirements described in 21 CFR 807 Subpart E, (2) address the specific safety and effectiveness issues identified in this guideline, and (3) obtain a substantial equivalence determination from FDA.

The classification regulation for nucleic acid-based in vitro diagnostic tools for the detection of MTB-complex in respiratory specimens is identified in this guideline. Other sections of this guidance include health hazards and mitigation actions that, if implemented by manufacturers and combined with general controls, will address the risks associated with these devices and, in most cases, will result in a prompt premarket notification [510(k)] review. This document will be used in conjunction with other FDA publications to address the particular content requirements of a premarket notification submission for nucleic acid-based in vitro diagnostic instruments for the detection of the MTB-complex in respiratory specimens. Furthermore, the performance of devices for other intended uses, such as the existence of mutations associated with MTB-complex medication resistance that may be paired with MTB-complex detection, is not addressed by this guidance [25].

5 Novel Diagnostic Methods

5.1 Nucleic Acid Amplification Test

To improve the diagnosis of *Mycobacterium tuberculosis* complex (MTBC), various nucleic acid amplification tests (NAATs) have been developed over the past few decades.

Most NAATs are based on the detection of multicopy insertion sequences (ISs), which is expected to increase the sensitivity of the tests. IS986, IS987, IS1081, and IS6110 have long been used as NAAT targets for the diagnosis of MTBC.

In nature, ISs, which exhibit high transposition ability, play a role in the major regions of bacterial repetitive elements; these sequences are therefore often used for typing different species and strains and can help their host adapt to the environment [26].

In the application of NAATs, ISs, especially IS6110, have been employed in multiplex PCR for the identification of MTBC in various types of clinical specimens, including pulmonary and extrapulmonary specimens, as such sequences exhibit high copy numbers in most MTBC strains (up to 25 copies per genome) although strains with only a single copy or no copies have also been identified in rare cases, as found in *Mycobacterium bovis* and its substrains, such as *Mycobacterium bovis* BCG.

One NAAT that has the potential to be approachable and cost-effective is loop-mediated isothermal amplification (LAMP). For the identification of pulmonary tuberculosis, LAMP is being tested as a point-of-care (POC) diagnostic. The general performance parameters of LAMP and fluorescent smear microscopy appear to

be similar. The performance of LAMP in smear negative samples, on the other hand, was not fully adequate [27].

The commercial Xpert MTB/RIF Ultra kit (Sunnyvale, USA, Ultra), which targets IS6110 and IS1081 for detection, is recommended by the WHO as the initial diagnostic test for all adults and children with the signs and symptoms of MTBC.

It is one of the most widely used automated, integrated, cartridge-based molecular assay systems for NAATs [28].

5.2 Amplified Mycobacterium Tuberculosis Direct Test

The Amplified Mycobacterium Tuberculosis Direct (AMTD) test detects *Mycobacterium tuberculosis* rRNA. By using the culture of *Mycobacterium tuberculosis* as a gold standard, a number of different diagnostic indices were examined in an attempt to determine the diagnostic performance of the AMTD test and demonstrate how it might usefully be interpreted during the early management of disease.

The Amplified Mycobacterium Tuberculosis Direct test (AMTD test; Gen-Probe, Inc., San Diego, Calif.) can be used to detect *Mycobacterium tuberculosis* rRNA in respiratory specimens (sputum, broncho-alveolar lavage [BAL], and bronchial and tracheal secretions) and can be performed in approximately 3.5 h. With this test, specific RNA amplification products are hybridized to complementary acridinium-ester-labeled DNA probes. Subsequent degradation of the acridinium-ester probes results in luminescence that is measured in relative light units (RLU).

According to the manufacturers, an RLU reading of >30,000 signifies a positive test, but they recommend repeating the tests at between 30,000 and 100,000 RLU. Sensitivities and specificities of the test at other RLU values, in comparison with the clinical diagnosis of tuberculosis, have been reported with the results of around 90% for each [29].

To qualitatively detect *Mycobacterium tuberculosis* complex ribosomal ribonucleic acid (rRNA), the MTD test employs Transcription-Mediated Amplification (TMA) and the Hybridization Protection Assay (HPA2). Both cultivable and non-cultivable organisms will be detected by the MTD test. *Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium bovis* BCG, *Mycobacterium africanum, Mycobacterium microti*, and *Mycobacterium canetti* are all members of the *Mycobacterium tuberculosis* complex. All organisms in the *Mycobacterium tuberculosis* complex will be detected by the MTD test. M. microti, on the other hand, only infects animals; *Mycobacterium bovis* is rarely passed from sick animals to humans; and *Mycobacterium africanum* develops pulmonary disease in humans in tropical Africa. *Mycobacterium tuberculosis* is by far the most widespread member of the tuberculosis complex, which causes human disease all throughout the world. The Centers for Disease Control and Prevention (CDC) has reported an increase in tuberculosis infections associated with AIDS, foreign-borne cases, and increased transmission in higher-risk populations [30].

Traditional tuberculosis culture methods can identify tuberculosis growth in as little as 1 week, but it can take up to 8 weeks. The MTD test, on the other hand, detects *Mycobacterium tuberculosis* complex rRNA within 2.5–3.5 hours of starting the test procedure. While the MTD test cannot determine medication susceptibility, it can be used to diagnose *Mycobacterium tuberculosis* quickly and accurately. This could lead to more appropriate utilization of isolation facilities, earlier discovery and containment of infected contacts, and more suitable therapy initiation [31].

5.3 Multiplex Polymerase Chain Reaction (PCR)

Amplification techniques like various types of PCR have attracted considerable interest in the diagnosis of tuberculosis, particularly extrapulmonary tuberculosis (EPTB), with the hope of shortening the time required for detection. Insertion sequence, IS6110, is the most commonly used target due to its multiple-copy presence in the genome of *Mycobacterium tuberculosis*. Most studies using IS6110 as a single target reported variable success.

More importantly, studies have shown that IS6110 may be absent in around 10–15% isolates of *Mycobacterium tuberculosis* in India. Studies that have evaluated a combination of target genes have reported higher sensitivity and specificity [32].

Few studies have reported a promising role of Protein b, MPB64, and IS6110 genes when used in combination to confer a higher sensitivity and specificity for EPTB. Thus, it is logical to hypothesize that a multiple PCR using multiple targets may provide better sensitivity for the diagnosis of EPTB. A large number of sequences of mycobacterial genome like IS6110, IS986, 65 kDa, and 38 kDa antigens have been used as targets in the polymerase chain reaction (PCR) test.

Multiplex PCR was standardized, and the DNA equal to 2–3 organisms was found to have quantitative sensitivity. It tested positive for *Mycobacterium tuberculosis* H37RV, a common tuberculosis strain. The results of each independent MPCR experiment were compared to those of one positive and one negative control. The positive control included was the DNA of H37Rv and the negative control included was the PCR grade water. The identification of M. tuberculosis was done using a specific pair of primers designed to amplify IS6110 and Protein b in the *Mycobacterium tuberculosis* complex and the expected band size was about 123 bp for IS6110 and 419 bp for Protein b [33].

5.4 LAMP Test

TB-LAMP is a manual assay that requires less than 1 hour to perform and requires minimal instrument in the form of a heating block, and generates a fluorescent result that can be detected with the naked eye.

WHO recommends that TB-LAMP can be used as a replacement for microscopy for the diagnosis of pulmonary TB in adults with the signs and symptoms of TB. It can also be considered as a follow-on test to microscopy in adults with signs and symptoms of pulmonary TB, especially when further testing of sputum smear-negative specimens is necessary [34].

Additionally, it has the potential for higher throughput as it can test up to 14 samples per test run (max. 70 samples per day). TB-LAMP, which is a simple, accurate, robust, and affordable test, has been developed for use in high burden and resource limited settings. The TB-LAMP assay includes loop primers for a total of six primers binding to 8 locations. This requirement for homogeneous sequence at multiple binding-sites preserves the specificity of the assay even in the absence of a probe. The LAMP method is relatively insensitive to the accumulation of DNA and DNA by-products (pyrophosphate salts), so the reaction proceeds until large amounts of amplicon are generated. This feature makes visible detection of successful amplification possible by using dsDNA-binding dyes such as SYBR green, by detecting turbidity caused by precipitating magnesium pyrophosphate, or by using a non-inhibitory fluorescing reagent that is quenched in the presence of divalent cations [35].

5.5 Real-Time PCR

In recent years, PCR technologies have improved markedly with the development of RT-PCR for the detection of mycobacterial infection.

This method has the advantage over conventional PCR in speed, automation, high sensitivity and specificity, and a low risk of cross-contamination. The RT-PCR technique is widely used to assess whether MTB DNA or a sequence is present in a sample, and it detects amplified DNA in real time as the reaction advances. It uses complementary primers to monitor the amplification of a targeted DNA/RNA molecule throughout PCR amplification, rather than at the end, as in conventional PCR. The amount of DNA/RNA contained in the sample is proportional to the cycle of the PCR technique in which considerable fluorescence emerges. Cycle threshold (Ct) or cycle quantification is the name given to this number (Cq). RT-PCR is sensitive, specific, and repeatable, and automating the operation lowers hands-on time and cross-contamination risk [36]. Within 2 hours, RT-PCR-based approaches have been proven to identify MTB directly from positive cultures or clinical specimens with greater sensitivity and specificity.

In comparison to smear microscopy, which requires 5000–10,000 bacilli/ml, it requires approximately 6 copies/ml of MTB DNA. Smear microscopy sensitivity has been shown to increase by up to 39% for concentrated samples like sputum. To produce a positive culture, which is the gold standard, at least 100 live bacilli are required, with a turnaround time of 2–10 weeks [37].

5.6 Xpert MTB/RIF Assay

The Xpert MTB/RIF assay is an automated, cartridge-based nucleic acid amplification test that uses the multi-disease GeneXpert platform. The assay can be performed directly on sputum, on processed sputum sediments, and on selected extrapulmonary specimens, from both adults and children.

The Xpert MTB/RIF assay simultaneously detects *Mycobacterium tuberculosis* and rifampicin resistance in less than 2 hours. The sensitivity of the Xpert MTB/RIF assay for detecting TB is superior to that of microscopy and comparable to that of solid culture, along with high specificity. The biosafety precautions required for Xpert MTB/RIF are similar to those for smear microscopy and allow the use of the assay outside of conventional laboratories. Training requirements are minimal, which allows testing by non-laboratory staff. WHO recommends the use of Xpert MTB/RIF as the initial diagnostic test in patients with suspected TB meningitis, instead of conventional microscopy, phenotypic culture and DST; treatment should follow immediately if the result is positive; additional testing is needed if the initial Xpert MTB/RIF result is negative [38].

5.7 PCR Restriction-Enzyme Analysis

PCR restriction analysis (PRA), a simple, easy-to-read, reproducible, and rapid molecular technique, has been used in recent years for the identification of *Mycobacterium* species. PRA techniques have been developed for several mycobacterial genes, such as *hsp65* the 16S–23S rRNA gene spacer and *rpoB* [39].

However, most of these techniques require the use of an algorithm to identify the *Mycobacterium* species. Also, the formation of short restrictions or small differences between bands requires the use of NuSieve or MetaPhor agarose, both of which are expensive, or polyacrylamide gel, which is difficult to handle, thus making the assay technically demanding.

PRA is simple to perform, easy to read, and reproducible and is used in several laboratories to identify NTM. Most PRA techniques used, however, are impeded by difficulties such as minor differences in band sizes between species, the occurrence of new patterns which have not been reported earlier, and the need to use elaborate algorithms to identify species [40].

5.8 Lab-on-Chip

Lab-on-Chip devices are promising solutions for closing the diagnosis gap in low-income countries because they combine multiple laboratory components on a single chip, decreasing infrastructure and technological requirements while maintaining

analytical capabilities. Other important advantages of Lab-on-Chips are operating speed, simplicity of customization (adding/removing probes), the capacity to run multiplex testing, and the flexibility to scale-down costs. Micro-Electro-Mechanical-Systems (MEMS) and microfluidics are used in the Lab-on-Chip technology to merge multiplexed DNA amplification with microarray detection for the rapid, cost-effective, and accurate study of biological materials.

Multiple disorders can be discovered in a single test thanks to the technology. Mycobacterium Tuberculosis Complex (TB) and its drug-resistant mutant forms, as well as nine additional non-TB mycobacterium diseases that are frequently mistaken as TB due to similar symptoms, may all be detected quickly with Lab-on-Chip. In less than 3 hours, all of these strains and mutations can be identified using a single test from direct sputum or cultured samples [41].

6 Diagnostic Gaps Between Existing Technologies and Its Unmet Clinical Need

Mycobacterium tuberculosis was discovered more than a century ago, yet diagnosis remains a serious healthcare issue in the developing world due to a number of obstacles, which are outlined below. For starters, *Mycobacterium tuberculosis* cannot provide assistance for on-site patient care because it is a slow-growing bacterium. Second, Pulmonary Tuberculosis (PTB) patients do not develop symptoms until later in the infection, delaying medical treatment. Third, even active PTB infections frequently have low sputum bacteria counts, making smear microscopy and other POC diagnostic procedures difficult to detect in the developing world. Fourth, existing processes make it more difficult to identify tuberculosis from sputum and other invasive bodily fluids than from blood and urine samples [42].

The lack of specific and confirmed biomarkers (for Active TB and LTBI infection) derived from either the host or the pathogen stems from a lack of understanding of the host–pathogen interaction, pathogenesis, and guarded immune response triggered by *Mycobacterium tuberculosis* during infection, which limits the utility of rapid TB diagnostic tests. Although Xpert MTB can diagnose tuberculosis on the same day, its application is limited due to its high cost and low detection rate in extrapulmonary tuberculosis (EPTB) [43].

As a result, there is an urgent need for a low-cost tuberculosis diagnostic test for resource-constrained situations in order to miniaturize tuberculosis detection, which can be accomplished utilizing a revolutionary nanotechnology method.

7 Role of Nanotechnology in the Diagnosis of TB

7.1 Nanoparticles

The use of nanotechnology in diagnostic applications, known as nano diagnostics, has been extensively researched for the development of high-sensitivity diagnostic tests that can be used before infection is detected. Because of the small size of nanoparticles and their great surface-to-volume ratio, this field is unique and significant in a wide range of human activities. Nanomaterials or nanostructures have unique features that influence nanodiagnostic platforms and the capacity to detect small amounts of clinical samples quickly [44].

The technology itself is diverse, with various possibilities such as nanosuspensions, nanoemulsions, and niosomes (nonionic surfactant-based vesicles). As a result, nanodiagnostic techniques have a high likelihood of becoming cost-effective, user-friendly, and durable.

Nanotechnology has made significant development in the last two decades, revealing its extensive potential and useful applications in biomedicine, biotechnology, human and animal health, as well as nanodiagnostics and nanomedicines. Although the majority of nanodiagnostic work has been done in the field of cancer diagnostics, this approach has also proven to be useful in the diagnosis of a variety of infectious disorders [45].

Certain infectious disease-producing organisms, such as bacteria (*Mycobacterium tuberculosis*), viruses (SARS), and fungi, are capable of causing epidemic outbreaks, which increase morbidity and death. As a result, building nano-based diagnostic systems in a clinical setting is becoming more crucial. This is owing to nanodiagnostics' ability to use diverse body fluids from patients, such as blood, sputum, or urine samples, to acquire consistent and rapid results using simple and movable tools [6].

Furthermore, high-potential highly sensitive nanodiagnostics platforms must be stable, cost-effective, reproducible, and might be extremely valuable in the diagnosis of infectious diseases, especially in resource-constrained areas of developing countries (Fig. 2).

7.2 Gold Nanoparticles Based Diagnostics for TB

AuNPs have distinct physiochemical (inert and nontoxic) and optical features, making them useful for clinical diagnosis, treatments, and other cross-disciplinary studies. The potential of AuNPs to interact optically with antibodies, antigens, and other biomolecules allows them to be used in the diagnosis of a variety of infections. Furthermore, even after antigen immobilization, AuNPs had little effect on functional activity. The surface functionalization of gold nanoparticles enhances the antibody–antigen response, enhancing immunoassay signals and, as a result, test

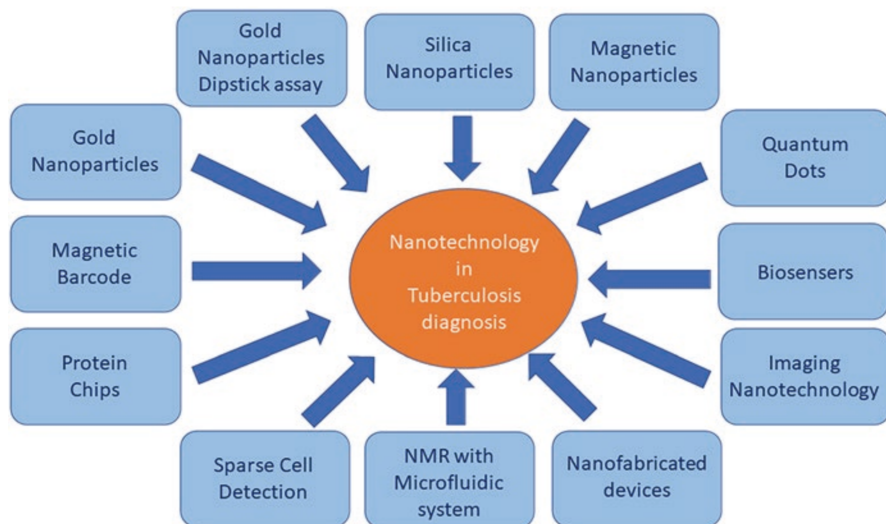

Fig. 2 Outline of nanotechnology-based approach for diagnosis of tuberculosis

sensitivity. It provides a simple, low-cost assay that permits multiple samples to be tested at the same time. Even with a small amount of mycobacterial DNA, the assay was found to be very specific and generate trustworthy findings. AuNP probes (thiol-linked single-stranded DNA, or ssDNA, modified gold nanoparticles) provide a low-cost option for the colorimetric detection of target genes/sequences from test DNA samples [46].

Initially, researchers who employed DNA probes (oligonucleotides produced from the gene sequence of the *Mycobacterium tuberculosis* RNA polymerase component) in combination with AuNPs for *Mycobacterium tuberculosis* colorimetric detection, described the use of AuNPs in TB diagnosis. When complementary DNA is present, the nanoprobe solution remains pink (no DNA probe aggregation) at wavelength 526 nm; however, when complementary DNA is absent, the solution turns purple (due to nanoprobe aggregation at a high NaCl concentration).When compared to other diagnostic procedures, such as InnoLiPA-Rif-TB, which had 100% concordance, the method is more accurate. The technique has proven to be more sensitive than smear microscopy and may be conveniently observed for detection. The main advantage of this process is that it has a low risk of contamination (since it is performed in a single tube), and it is rapid (takes approximately 15 min per sample).

Following that, the activity of this method was compared to that of an automated liquid culture system (BACTECTM MGITTM) and semi-nested PCR revealing that the test has higher sensitivity and specificity in the detection of *Mycobacterium tuberculosis* complex [47]. Microfluidics technology, which utilized calorimetric detection of AuNPs in combination with the *Mycobacterium tuberculosis* insertion sequence (IS6110), was also used to boost the sensitivity of this test [48]. The

unique metal gold (Au), which gives the AuNPs colloid a red color, has gotten a lot of attention thanks to Surface Plasmon Resonance (SPR). The method is based on continuous monitoring of changes in the refractive index of the surface, which are caused by the molecules' affiliation or dissociation with the sensor [49].

The optical sensor sensitivity of the SPR-based test allows it to identify even minute amounts of disease-specific analyte from complicated fluid without requiring any special procedures. SPR has evolved as a potent optical instrument that can provide valuable data in the examination of biological and chemical investigations as a result of these advantages. In clinical samples, the SPR-based CFP-10 antigen detection system was created, and it proved to be reliable in TB diagnosis [50].

AuNPs modified indium tin oxide (ITO) electrodes were developed for the detection of *Mycobacterium tuberculosis* directly from clinical samples utilizing genomic DNA (gDNA) [51]. The approach used two probes: a capture probe and a gold nanoprobe, both of which were combined with the alkaline phosphatase (ALP) enzyme as the detection probe. The ITO probe is first triggered with a capture probe, and then immersed in gDNA-containing hybridization buffer to create double-strand DNA (dsDNA) through hybridization of the probe and target nucleotide sequence. Finally, to create a hybridization sandwich, ITO is put as an electrode in the buffer containing the detection probe. Voltammetry is then used to record the electric signals.

7.3 Gold Nanoparticles Dipstick Assay

Alkanethiol derivatives and anti-MTB rabbit antibodies were used to coat colloidal AuNPs with the *Mycobacterium tuberculosis* antigen. In this test, the antigen-coated AuNPs serve as a counter or detector reagent. The *Mycobacterium tuberculosis* antigen coated on AuNPs binds to blood samples or antibodies immobilized on nitrocellulose (NC) membranes. The ensuing binding could be observed with the naked eye due to the development of the red color caused by the gold nanoparticles on the nitrocellulose membrane (NC) [52].

7.4 Silica Nanoparticles Based Detection

Mesoporous silica nanoparticles (SiO_2NPs) have been used in a variety of applications, including imaging, drug delivery, and biosensors. For the detection of *Mycobacterium tuberculosis*, indirect immunofluorescence microscopy has been devised using nanoparticles linked with fluorescent dye [53].SYBR Green I-mediated assay stained only bio-conjugated fluorescent silica nanoparticles, according to the technology. The intensity of fluorescent signals is five times higher than that of a standard FITC-based detection approach. This assay produces

encouraging results in less than 2 hours, making it a viable approach for detecting *Mycobacterium tuberculosis* quickly [54].

7.5 Magnetic Nanoparticles Based Detection

Nature contains magnetic nanoparticles (MNPs), which are nanoscale-sized molecules. They have properties that make them suitable for use in nano-biomedicine, specifically imaging treatment [55]. MNPs may be easily changed on the surface with recognition moieties, such as antibodies, antibiotics, and carbohydrates, allowing them to be used for bacterial detection. The super paramagnetic iron oxide nanoparticles (IONPs), which are made up of magnetite [Fe_3O_4] or maghemite [-Fe_2O_3] nanoparticles, are widely employed in drug therapy, cell tracking, and drug delivery via magnetic resonance imaging (MRI). Using IONPs in combination with IgG has dramatically increased the detection limit (104 CFU/mL) of bacterial cells when using nano-MALDI platforms. Several studies have used diagnostic magnetic resonance (DMR) in combination with iron oxide nanoparticles to detect *Mycobacterium tuberculosis* DNA [56].

Engstrom and his colleagues created a new platform for detecting rifampicin mutations in *Mycobacterium tuberculosis* rpoB gene using streptavidin-tagged magnetic nanobeads labeled with biotin. The test consisted of 11 padlock probes (PLPs) that targeted *Mycobacterium tuberculosis*'23S ITS region. One probe was designed to identify MTBC, another PLP for wild-type, and the remaining nine PLPs were designed to detect a common mutation in the RRDR-rpoB gene. The signal is detected using AC susceptometry and is based on the Brownian relaxation principle [57].

The effectiveness of super-paramagnetic iron oxide (SPIO) nanoparticles in improving the sensitivity and specificity of MRI systems in TB diagnosis has also been tested [58]. This approach is more effective at diagnosing tuberculosis at the molecular level, and it also serves as a useful tool for analyzing antibody-antigen and parasite-host interactions. In order to generate conjugates, SPIO nanoparticles are activated with an anti-MTB surface antibody. The conjugate was then incubated with mycobacterium before being imaged with MRI, which reduces signal intensity and demonstrates specific target identification. For the detection of EPTB, this approach is more specific (Musculoskeletal TB, Central nervous system TB, abdominal TB).

7.6 Quantum Dots Based Detection System

Quantum dots, or semiconductor nanocrystals, have remarkable optical and physical characteristics that make them excellent for medical study [59]. Quantum dots have a number of advantages over alternative fluorescence-based approaches,

including broad absorption spectra, limited emission spectra, slow excited-state decay rates, and broad absorption cross-sections. It can also identify numerous targets at the same time, making it a popular application for detecting many diseases in a single clinical sample.

For TB detection, the hybrid detection method (Quantum dots and magnetic beads) employs *Mycobacterium tuberculosis*-specific molecular probes. The first probe detects the mycobacterium's 23S rRNA gene with extreme precision, whereas the second probe recognizes the IS900 conserved sequence in mycobacterium treated with sulphurous acid chromium quantum dots with extreme precision. After hybridization with target gene sequences of mycobacterium DNA extracted from suspected TB patient samples, a sandwich is created. After that, the quantum dots and magnetic bead conjugates are subjected to UV radiation, which causes red fluorescence. (This can be seen with the naked eye).

These conjugate detection approaches are also very versatile molecular probes that can be easily tailored to suit diagnostic requirements. The approach can directly identify *Mycobacterium tuberculosis* complex unamplified DNA from clinical samples [60].

Liandris and his colleagues used the quantum dots of $CdSeO_3$ in conjugation with streptavidin and species-specific probes to detect the surface antigen of mycobacterium species in a similar investigation. Sandwich hybridization was used to target mycobacterial gDNA, which is composed of two biotinylated probes that acknowledged and detected the target DNA selectively. The detection limit of the sample is approximately 104 cells/mL [61].

7.7 Magnetic Barcode Assays

Magnetic barcode (MB) assays work in a similar way to QDs in terms of principle. As probes for TB detection, the test utilized unique complementary DNA sequences of *Mycobacterium tuberculosis*. The most notable difference is the need for DNA extraction and PCR amplification, which is not, required in quantum dots tests. After probes grab DNA, complementary magnetic nanoparticle probes label the resulting conjugate, which is subsequently identified using nuclear magnetic resonance (NMR) techniques [62].

7.8 Biosensors Based Detection System

A biosensor is an analytical system that incorporates a bio-recognition element to detect the presence or absence of a specific biological analyte (transduction system, amplifiers, and display unit). The biosensor is made up of an analytical equipment and biological analytes that detect physiological and chemical changes in the sensing area [63]. The biosensor works by detecting short *Mycobacterium tuberculosis*

DNA nucleotide sequences. The following categories of TB biosensors exist: mass/piezoelectric, biochemical, electrical, and optical sensors. These sensing platforms employ antibody-antigen interaction, nucleic acid hybridization, and entire mycobacterium bacilli to detect antigen-antigen interactions [64].

7.9 Protein Chips

Proteomics plays a significant role in illness diagnosis and drug development. Protein chips can be treated with TB bacilli tiny modular protein components that can attach to proteins with a specific biochemical or structural motif [65].

7.10 Imaging Nanotechnology

Labelling of targeted TB-bacilli molecules with quantum dots or synthetic chromophores such as fluorescent proteins that will facilitate direct investigation of intracellular signalling complex by optical techniques, for examples: confocal fluorescence microscopy or correlation imaging [66].

7.11 Sparse Cell Detection

This approach can take use of sparse cells' distinctive features, which are exhibited in variances in internal TB bacilli deformation. In normal physiological settings, sparse cells are both rare and physiologically distinct from their surrounding cells. Identifying and then isolating these sparse cells are difficult [65].

7.12 NMR with Microfluidic System

Iron-based magnetic nanoparticles tagged with antibodies are used for binding the *Mycobacterium tuberculosis* bacilli while the microfluidic system deliver *Mycobacterium tuberculosis* and buffer solutions. Concentrating the specimens with the membrane filter can markedly improve the detection sensitivity. Magnetic nanoparticles have high sensitive detection of Bacillus Calmette-Guerin. Integration with quantum dots can detect not only *Mycobacterium tuberculosis* but also *Mycobacterium avium* subspecies "paratuberculosis" [66].

Super paramagnetic NPs are made of different magnetic materials such as nickel, iron, cobalt etc., which are pushed for biomedical applications because they act non-magnetically when they are not under the influence of an external magnetic field, avoiding undesirable self-magnetic agglomeration [67].

7.13 Nano-Fabricated Devices

Nano-fabricated devices are ideal for point-of-care application in the detection of *Mycobacterium tuberculosis* because of the reduced costs of the automated sensitive detection and ability of nanostructured zinc-oxide-films detection of genomic target up to 100 pM in clinical specimens [66].

8 Conclusion and Future Perspectives

Approximately 40–50% of tuberculosis infections are now undiscovered, either due to a lack of diagnostic services, a lack of public awareness, or a lack of tubercle bacilli in clinical samples. Due to their low detection sensitivity, assays targeting the whole bacillus in clinical samples miss many cases in such circumstances. This demonstrated that detecting bacillary by-products or caused changes in the host-immune response might be used as an alternative tuberculosis diagnostic tool (TB). There have been several experiments in this area, but none have shown to be therapeutically useful.

Nanodiagnostics for tuberculosis within hours are clearly advantageous. However, only a tiny percentage of these have been translated into clinical TB molecular diagnostics, and there are only a few approaches that can be used directly in respiratory samples.

Nanotechnology is a rapidly evolving field that attracts multi-disciplinary teams to address numerous healthcare concerns such as infectious disease diagnostics and therapy, cancer treatment, and cardiovascular disease treatment. These technologies have made a substantial contribution to the diagnosis of bacterial and viral illnesses. Nano-based technologies, in particular, aid in the miniaturization of diagnostic instruments and implants. In the field of tuberculosis, which is a significant killer disease, nano-biotechnology can aid TB management with the added benefits of speed, convenience of administering tests, and lower costs, which is especially beneficial for resource-constrained countries.

Nanotechnology is advancing as a technique for targeted medicine delivery and disease detection around the world. In compared to traditional microbiological and molecular biology approaches, nanotechnology-based diagnostic schemes have the ability to produce results in hours, with enhanced sensitivities and specificities for a fraction of the cost. Nanotechnology has the ability to enable a local response to various issues in an area where many infectious illnesses exist, such as tuberculosis detection, treatment, and prevention. Future advances in nano diagnostics will enable non-specialized health staff to use them by miniaturizing biochip technology to the nano-scale range for point-of-care diagnostics using a specimen-in answer-out strategy.

References

1. Kerantzas CA, Jacobs WR. Origins of combination therapy for tuberculosis: lessons for future antimicrobial development and application. MBio. 2017;8(2):e01586–16.
2. Rohde K, Yates RM, Purdy GE, Russell DG. Mycobacterium tuberculosis and the environment within the phagosome. Immunol Rev. 2007;219(1):37–54.
3. Jalhan S, Jindal A, Aggarwal S, Gupta A, Hemraj. Review on current trends and advancement in drugs trends and drug targets for tuberculosis therapy. Int J Pharm Bio Sci. 2013;4(1):320–33.
4. Shubladze N, Tadumadze N, Bablishvili N. Molecular patterns of multidrug resistance of Mycobacterium tuberculosis in Georgia. Int J Mycobact. 2013;2(2):73–8.
5. World Health Organization 2021. Tuberculosis – key facts. World Health Organization, viewed 21 Nov 2021. https://www.who.int/news-room/fact-sheets/detail/tuberculosis.
6. Wang Y, Yu L, Kong X, Sun L. Application of nanodiagnostics in point-of-care tests for infectious diseases. Int J Nanomedicine. 2017;12:4789–803.
7. Sharma D, Sarkar D. Pathophysiology of tuberculosis: an update review. PharmaTutor. 2018;6(2):15–21.
8. Kivihya-Ndugga LEA, Van Cleeff MR, Githui WA, Nganga LW, Kibuga DK, Odhiambo JA, Klatser PR. A comprehensive comparison of Ziehl-Neelsen and fluorescence microscopy for the diagnosis of tuberculosis in a resource-poor urban setting. Int J Tuberc Lung Dis. 2003;7:1163–71.
9. Singhal R, Myneedu VP. Microscopy as a diagnostic tool in pulmonary tuberculosis. Int J Mycobacteriol. 2015;4(1):1–6.
10. Bennedsen J, Larsen SO. Examination for tubercle bacilli by fluorescence microscopy. Scand J Respir Dis. 1966;47:114–20.
11. World Health Organization. Implementing the End TB strategy: the essentials. Geneva; 2015. http://www.who.int/tb/publications/2015/The Essentials to End TB/en/.
12. Perkins MD, Cunningham J. Facing the crisis: improving the diagnosis of tuberculosis in the HIV era. J Infect Dis. 2007;196(Suppl 1):S15–27.
13. Cambanis A, Ramsay A, Wirkom V, Tata E, Cuevas LE. Investing time in microscopy, an opportunity to optimize smear-based case detection of tuberculosis. Int J Tuberc Lung Dis. 2007;11(1):40–5.
14. Steingart KR, Ng V, Henry M, Hopewell PC, Ramsay A, Cunningham J, Urbanczik R, Perkins MD, Aziz MA, Pai M. Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis. 2006;6(10):664–74.
15. Bell WJ, Brown PP. Fluorescence microscopy in the laboratory diagnosis and assessment of pulmonary tuberculosis. Cent Afr J Med. 1962;8:4–9.
16. Somlo AM, Black TC, Somlo LI. The value of fluorescence microscopy in the detection of acid-fast bacteria. Tech Bull Regist Med Technol. 1969;39:51–4.
17. Hung NV, Sy DN, Anthony RM, Cobelens FG, Van Soolingen D. Fluorescence microscopy for tuberculosis diagnosis. Lancet Infect Dis. 2007;7:238–9.
18. Snider DE. The tuberculin skin test. Am Rev Respir Dis. 1982;125(3P2):108–18.
19. Daftary VG, Banker DD, Daftary GV. ELISA test for tuberculosis. Indian J Med Sci. 1994;48(2):39–42.
20. Pai M, Denkinger CM, Kik SV, Rangaka MX, Zwerling A, Oxlade O, Metcalfe JZ, Cattamanchi A, Dowdy DW, Dheda K, Banaei N. Gamma interferon release assays for detection of Mycobacterium tuberculosis infection. Clin Microbiol Rev. 2014;27(1):3–20.
21. Sorensen AL, Nagai S, Houen G, Andersen P, Andersen AB. Purification and characterization of a low-molecular-mass T-cell antigen secreted by Mycobacterium tuberculosis. Infect Immun. 1995;63:1710–7.
22. Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. Lancet. 2000;356:1099–04.
23. Geluk A, Van Meijgaarden KE, Franken KL, Subronto YW, Wieles B, Arend SM, Sampaio EP, De Boer T, Faber WR, Naafs B, Ottenhoff TH. Identification and characterization of the

ESAT-6 homologue of Mycobacterium leprae and T-cell cross-reactivity with Mycobacterium tuberculosis. Infect Immun. 2002;70:2544–8.
24. The Global Fund 2021. List of TB Diagnostic test kits and equipments classified according to the Global Fund Quality Assurance Policy, viewed 20 Nov 2021. https://www.theglobalfund.org/media/9461/psm_productsdiagnosticstb_list_en.pdf
25. U.S. Food & Drug Administration. Nucleic acid-based in vitro diagnostic devices for the detection of mycobacterium tuberculosis complex in respiratory specimens – class II special controls guideline for industry and Food and Drug Administration Staff. 27 Feb 2018. https://www.fda.gov/medical-devices/guidance-documents-medical-devices-and-radiation-emitting-products/nucleic-acid-based-in-vitro-diagnostic-devices-detection-mycobacterium-tuberculosis-complex-respiratory.
26. Caws M, Wilson SM, Clough C, Drobniewski F. Role of IS6110-targeted PCR, culture, biochemical, clinical, and immunological criteria for diagnosis of tuberculous meningitis. J Clin Microbiol. 2000;38:3150–5.
27. Alonso H, Samper S, Martin C, Otal I. Mapping IS6110 in high-copy number Mycobacterium tuberculosis strains shows specific insertion points in the Beijing genotype. BMC Genomics. 2013;14:422–7.
28. Dorman SE, et al. Xpert MTB/RIF Ultra for detection of Mycobacterium tuberculosis and rifampicin resistance: a prospective multicentre diagnostic accuracy study. Lancet Infect Dis. 2018;18(1):76–84.
29. Alcala L, Ruiz-Serrano MJ, Hernangomez S, Marin M, de Viedma DG, San Juan R, Bouza E. Evaluation of the upgraded amplified Mycobacterium tuberculosis direct test (Gen-Probe) for the direct detection of Mycobacterium tuberculosis in respiratory and non-respiratory specimens. Diagn Microbiol Infect Dis. 2001;41:51–6.
30. Bradley ST, Reed SL, Catanzaro A. Clinical efficacy of the amplified Mycobacterium tuberculosis direct test for the diagnosis of pulmonary tuberculosis. Am J Respir Crit Care Med. 1996;153:1606–10.
31. Pitchenik AE, Fertel D, Block AB. Mycobacterial disease: epidemiology, diagnosis, treatment, and prevention. Clin Chest Med. 1988;9:425–41.
32. Sekine K, Nagata N, Shindo T, Morino E, Shimbo T, Akiyama J, Mizokami M, Uemura N. Combined identifying granuloma and biopsy culture is useful for diagnosing intestinal tuberculosis. Int J Color Dis. 2015;30:939–45.
33. Chauhan DS, Sharma VD, Parashar D. Molecular typing of Mycobacterium tuberculosis isolates from different parts of India based on IS6110 element polymorphism using RFLP analysis. Indian J Med Res. 2007;125(4):577–81.
34. Boehme CC, Nabeta P, Henostroza G, Raqib R, Rahim Z, Gerhardt M, Sanga E, Hoelscher M, Notomi T, Hase T, Perkins MD. Operational feasibility of using loop-mediated isothermal amplification for diagnosis of pulmonary tuberculosis in microscopy centers of developing countries. J Clin Microbiol. 2007;45:1936–40.
35. Yadav R, Sharma N, Khaneja R, Agarwal P, Kanga A, Behera D, Sethi S. Evaluation of the TB-LAMP assay for the rapid diagnosis of pulmonary tuberculosis in Northern India. Int J Tuberc Lung Dis. 2017;21:1150–3.
36. Espy MJ, Uhl JR, Sloan LM, Buckwalter SP, Jones MF, Vetter EA, Yao JD, Wengenack NL, Rosenblatt JE, Cockerill FR 3rd, Smith TF. Real-time PCR in clinical microbiology: applications for routine laboratory testing. Clin Microbiol Rev. 2006;19(1):165–56.
37. Palomino JC. Nonconventional and new methods in the diagnosis of tuberculosis: feasibility and applicability in the field. Eur Respir J. 2005;26(2):339–50.
38. Rachow A, Zumla A, Heinrich N, Rojas-Ponce G, Mtafya B, Reither K, Ntinginya EN, O'Grady J, Huggett J, Dheda K, Boehme C, Perkins M, Saathoff E, Hoelscher M. Rapid and accurate detection of Mycobacterium tuberculosis in sputum samples by Cepheid Xpert MTB/RIF assay-a clinical validation study. PLoS One. 2011;6:e20458.

39. Kim BJ, Lee KH, Park BN, Kim SJ, Bai GH, Kook YH. Differentiation of mycobacterial species by PCR-restriction analysis of DNA (342 base pairs) of the RNA polymerase gene (rpoB). J Clin Microbiol. 2001;39:2102–9.
40. Lee H, Park HJ, Cho SN, Bai GH, Kim SJ. Species identification of mycobacteria by PCR restriction fragment length polymorphism of the *rpoB*gene. J Clin Microbiol. 2000;38(8):2966–71.
41. Cabibbe AM, Miotto P, Moure R, Alcaide F, Feuerriegel S, GianniPozzi VN, Drobniewski F, Niemann S, Reither K, Cirillo DM. A lab-on-chip based platform for fast molecular diagnosis of multi-drug resistant tuberculosis. J Clin Microbiol. 2015; https://doi.org/10.1128/JCM.01824-15.
42. Sharma S, Zapatero RJ, Estrela P, O'Kennedy R. Point-of-care diagnostics in low resource settings: present status and future role of microfluidics. Biosensors. 2015;5:577–601.
43. Rufai SB, Singh S, Singh A, Kumar P, Singh J, Vishal A. Performance of Xpert MTB/RIF on ascitic fluid samples for detection of abdominal tuberculosis. J Lab Physicians. 2017;9:47–52.
44. Azzazy HME, Mansour MMH, Kazmierczak SC. Nanodiagnostics: a new frontier for clinical laboratory medicine. Clin Chem. 2006;52:1238–46.
45. Kumar A, Mazinder Boruah B, Liang XJ. Gold nanoparticles: promising nanomaterials for the diagnosis of cancer and HIV/AIDS. J Nanomater. 2011:1–17.
46. Kim J, Oh SY, Shukla S, Hong SB, Heo NS, Bajpai VK, Chun HS, Jo CH, Choi BG, Huh YS, Han YK. Heteroassembled gold nanoparticles with sandwich-immunoassay LSPR chip format for rapid and sensitive detection of hepatitis B virus surface antigen (HBsAg). Biosens Bioelectron. 2018;107:118–22.
47. Cordeiro M, Ferreira Carlos F, Pedrosa P, Lopez A, Baptista PV. Gold nanoparticles for diagnostics: advances towards points of care. Diagnostics. 2016;6:1–20.
48. Tsai TT, Huang CY, Chen CA, Shen SW, Wang MC, Cheng CM, Chen CF. Diagnosis of tuberculosis using colorimetric gold nanoparticles on a paper-based analytical device. ACS Sens. 2017;2(9):1345–54.
49. Khan I, Saeed K, Khan I. Nanoparticles: properties, applications and toxicities. Arab J Chem. 2019;12:908–31.
50. Masson JF. Surface plasmon resonance clinical biosensors for medical diagnostics. ACS Sens. 2017;2:16–30.
51. Zhu L, Song L, Liu W, Yan X, Xiao J, Chen C. Gold nanoparticles-modified indium tin oxide microelectrode for in-channel amperometric detection in dual-channel microchip capillary electrophoresis. Anal Methods. 2017;29:4319–26.
52. Stephen BJ, Singh SV, Datta M, Jain N, Jayaraman S, Chaubey KK, Gupta S, Singh M, Aseri GK, Khare N, Yadav P, Dhama K, Sohal JS. Nanotechnological approaches for the detection of mycobacteria with special references to Mycobacterium avium subspecies paratuberculosis (MAP). Asian J Anim Vet Adv. 2015;10:518–26.
53. Sun R, Wang W, Wen Y, Zhang X. Recent advance on mesoporous silica nanoparticles-based controlled release system: intelligent switches open up new horizon. Nano. 2015;5(4):2019–53.
54. Qin D, He X, Wang K, Zhao XJ, Tan W, Chen J. Fluorescent nanoparticle-based indirect immunofluorescence microscopy for detection of Mycobacterium tuberculosis. J Biomed Biotechnol. 2007;2007(7):1–9.
55. Akbarzadeh A, Samiei M, Davaran S. Magnetic nanoparticles: preparation, physical properties, and applications in biomedicine. Nanoscale Res Lett. 2012;7:144.
56. Vallabani NVS, Singh S. Recent advances and future prospects of iron oxide nanoparticles in biomedicine and diagnostics. Biotech. 2018;8(6):1–23.
57. Engström A, De la Torre TZG, Strømme M, Nilsson M, Herthnek D. Detection of rifampicin resistance in Mycobacterium tuberculosis by padlock probes and magnetic nanobead-based readout. PLoS One. 2013;8:e62015.
58. Sabale S, Kandesar P, Jadhav V, Komorek R, Motkuri RK, Yu XY. Recent developments in the synthesis, properties, and biomedical applications of core/shell superparamagnetic iron oxide nanoparticles with gold. Biomater Sci. 2017;5:2212–25.

59. Kairdolf BA, Smith AM, Stokes TH, Wang MD, Young AN, Nie S. Semiconductor quantum dots for bioimaging and biodiagnostic applications. Annu Rev Anal Chem (Palo Alto Calif). 2013;6:143–62.
60. Gazouli M, Lyberopoulou A, Pericleous P, Rizos S, Aravantinos G, Nikiteas N, Anagnou NP, Efstathopoulos EP. Development of a quantum-dot-labelled magnetic immunoassay method for circulating colorectal cancer cell detection. World J Gastroenterol. 2012;18:4419–26.
61. Liandris E, Gazouli M, Andreadou M, Sechi LA, Rosu V, Ikonomopoulos J. Detection of pathogenic mycobacteria based on functionalized quantum dots coupled with immunomagnetic separation. PLoS One. 2011;6:e20026.
62. Chen YT, Kolhatkar AG, Zenasni O, Xu S, Lee TR. Biosensing using magnetic particle detection techniques. Sensors. 2017;17(10):1–36.
63. Mehrotra P. Biosensors and their applications – a review. J Oral Biol Craniofac Res. 2016;6:153–9.
64. Lim JW, Ha D, Lee J, Lee SK, Kim T. Review of micro/nanotechnologies for microbial biosensors. Front Bioeng Biotechnol. 2015;3:1–13.
65. Fakruddin MD, Hossain Z, Afroz H. Prospects and applications of nanobiotechnology: a medical perspective. J Nanobiotechnol. 2012;10(4):31.
66. Veigas B, Doria G, Baptista PV. Nanodiagnostics for tuberculosis. In: Cardona P-J, editor. Understanding tuberculosis: global experiences and innovative approaches to the diagnosis. Rijeka: InTech; 2012. p. 257–76.
67. Saraf S. Applications of novel drug delivery system for herbal formulations. Fitoterapia. 2010;81(7):680–9.

Nanotechnology Based Vaccination Approach in Malarial Infection

Priya Patel, Mansi Faladia, Pranav Shah, and Jayvadan Patel

Abstract Malaria afflicted an estimated 219 million people in 2017, killing 435,000 people worldwide. This morbidity and mortality burden is the consequence of more than a century of worldwide effort and research aimed at improving malarial prevention, diagnosis, and treatment. Malaria is the most frequent illness in Africa and certain Asian nations, accounting for many indigenous cases. The global malaria fatality rate ranges from 0.3% to 2.2%, and in cases of severe malaria in tropical climatic zones, the rate rises from 11% to 30%. Malaria is caused by a tiny protozoon belonging to the Plasmodium species group, which includes various subspecies. Plasmodium species can cause illness in humans. Plasmodium is a kind of amoeboid intracellular parasite that accumulates malaria pigment (an insoluble metabolite of haemoglobin). Parasites are present in several animals; some in red blood cells and others in tissues. Five Plasmodium species can infect humans out of 172 species. These are *Plasmodium malariae*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium knowlesi*. The zoonotic malaria *P. knowlesi* has been identified in South-East Asia. Malaria symptoms often occur 10 days to 1 month after infection. Mild symptoms are possible. Some people do not feel ill for up to a year after being bitten by a mosquito. Parasites can survive in the body for years without creating any symptoms. Malaria treatment should begin as soon as feasible. To cure malaria, your doctor will prescribe medications that destroy the parasite. Malaria medications are not effective against some parasites. The kind of medication and duration of therapy is determined by the parasite causing your symptoms. Due to the shortcomings of conventional malaria therapy, a nanotechnology approach has been implemented with vaccine development in various phase trials.

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1 Introduction

Malaria is an infectious illness spread by mosquitos which affects people and other animals. It is a potentially fatal disease caused by parasites transferred to humans by the bites of infected female Anopheles mosquitoes [1]. It is both preventable and treatable. There were an estimated 229 million cases of malaria globally in 2019 with the fatalities of 409,000. Children under the age of five are the most vulnerable group to malaria, accounting for 67% (274,000) of all malaria fatalities globally in 2019. The World Health Organization (WHO) has certified ten nations as having eradicated malaria. However, in many Sub-Saharan African nations, where malaria remains a major cause of mortality and serious disease in children, progress has halted in recent years. Because of this slowing, the WHO has established a 'High Burden-High Impact' initiative aimed at India and the 10 African nations with the greatest malaria load. In these countries, novel malaria control strategies are necessary [2, 3].

The African Region of the WHO bears a disproportionately large percentage of the global malaria burden. In 2019, the region accounted for 94% of malaria cases and fatalities. Plasmodium parasites produce malaria. The parasites are transmitted to humans by the bites of infected female Anopheles mosquitos, sometimes known as "malaria vectors" [4]. Malaria is caused by five parasite species, two of which – *P. falciparum* and *P. vivax* – represent the greatest threat. *P. falciparum* was responsible for 99.7% of projected malaria cases in the WHO African Region in 2018. The WHO South-East Asia Region had 50% of the cases, the Eastern Mediterranean had 71%, and the Western Pacific had 65%. P. vivax is the most common parasite in the WHO Region of America, accounting for 75% of malaria cases [5].

2 Malaria Causes

Malaria is caused by a tiny protozoon belonging to the Plasmodium species group, which includes various subspecies. Plasmodium species can cause illness in humans. Plasmodium is a malarial pigment-accumulating amoeboid intracellular parasite. Parasites are present in several animals; some in red blood cells and others in tissues. Five Plasmodium species can infect humans out of 172 species. These are *Plasmodium malariae, Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, and Plasmodium knowlesi*. *P. knowlesi*, a zoonotic malaria, has been identified in South-East Asia. Other species infect people only seldom. All of the Plasmodium species described above are responsible for the sickness known as malaria. The Plasmodium life cycle is quite complicated and is divided into two stages: sexual and asexual, vector insects and vertebrate hosts. The sexual phase of the parasite's life cycle occurs in the vectors, mosquitoes. Humans, the intermediate

host for malaria, go through the asexual phase of the life cycle. The female mosquitos of the genus Anopheles are the only vectors of human malaria [2, 3].

3 Challenges to Conventional Malaria Treatment

Plasmodium vivax is now well-acknowledged as a cause of severe malaria and mortality. For decades, one of the most significant challenges in the fight against malaria has been medication resistance. Drug resistance has been documented in three of the five Plasmodium species that cause human malaria, namely *P. falciparum, P. vivax*, and *P. malariae*. WHO defined drug resistance in 1967 as the capacity of a parasite strain to live or reproduce independent of medication administration and absorption when given in dosages equivalent to or greater than those generally indicated but within the tolerance range of a particular person. Drug resistance often results in a delay or failure to discharge asexual parasites from the peripheral circulation, allowing gametocytes to be produced, which are responsible for the transmission of the resistant genotype. Antimalarial medicines are now available to work on a restricted number of biological targets. As a result, the next task is to create breakthrough technologies that can successfully remove the parasite with maximum precision, reducing the possibility of medication resistance. The primary disadvantages of traditional malaria treatment are the emergence of multiple drug resistance and the non-specific targeting of intracellular parasites, which results in high dosage needs and eventual unacceptable damage.

Nanosized carriers have received considerable attention in order to reduce the negative effects of pharmacological treatment, such as low bioavailability and drug selectivity. Several nanosized delivery technologies have previously been shown to be successful in animal models for malaria treatment and prevention. Several ways are being discovered for delivering antimalarials via nanocarriers, as well as the processes that allow them to be targeted to Plasmodium spp.-infected cells [6].

4 Nano-biotechnology Approach for Malaria Treatment

The final answer to the parasite illness malaria is nano-biotechnology. Nano-biotechnology aids in the eradication of malaria by providing effective treatment methods for vector removal as well as directly attacking the parasite. Some nano-biotechnological approaches that aid in the simple and safe treatment of malaria are listed below (shown in Fig. 1). The capacity of a nanocarrier to remain in the bloodstream for an extended length of time in order to increase contact with the infected red blood cells (RBCs) and parasite membranes is the most essential attribute of a nanocarrier in the context of malaria. Other intriguing qualities include medication stability protection, cell adhesion capabilities, and the capacity to be surface-modified by the conjugation of particular ligands. Many of these potential

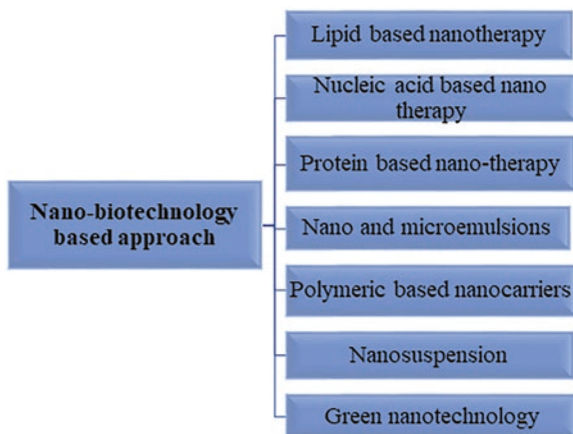

Fig. 1 Different nano-biotechnology approaches for malaria treatment

benefits can be realised in the treatment of cerebral malaria by colloidal nanocarriers that are suitable for intravenous delivery. Non-parenteral methods are favoured in uncomplicated malaria, but they limit the range of options for the use of drug nanocarriers [7].

Over 20 years ago, the use of liposomes in the treatment of malaria was studied. In general, liposomal formulation appears to be preferable to free medication therapies [8]. As a result, employing nanotechnology can reduce the toxicity of medicinal compounds. The injection of an antimalarial medication with liposomes tailored to infected RBCs and a tagged antibody against infected erythrocyte surface antigens on chloroquine liposomes against drug-resistant *Plasmodium berghei* resulted in a cure rate of 75–90% in infected animals. Artesunate liposomes were employed in a malaria-resistant therapy to minimise dosage frequency by employing a modest release for 24 hours and with encapsulated beta-artemether. Artemether and lumefantrine co-loaded into tiny lipid nano-drops have better effectiveness and can reach the target location more quickly [9].

Different types of liposomes, either encapsulated or linked with the recombinant human tumour necrosis factor, were also demonstrated to be effective against experimental cerebral malaria (ECM) generated in *P. berghei* K 173-infected mice (shown in Fig. 2). According to this analysis, liposomes combined with a human necrosis factor were more effective than their free form in avoiding ECM-associated mortality by lowering mouse parasitemia [10].

Liposomes containing a Plasmodium amino acid sequence were shown to be efficient against *P. berghei*-infected mice. The peptide, which contained a conserved region I as well as a consensus heparin sulphate proteoglycan-binding sequence, was attached to the distal end of a lipid Y polyethylene glycol. Furthermore, animals immunised with RTS, S malaria antigen encapsulated in liposomes containing lipid A produced much higher levels of antibody and cytotoxic T cell immune response than non-fusion RTS, S.40 A vaccine based on an RTS, S antigen has recently been used to successfully prevent malaria in African children [11].

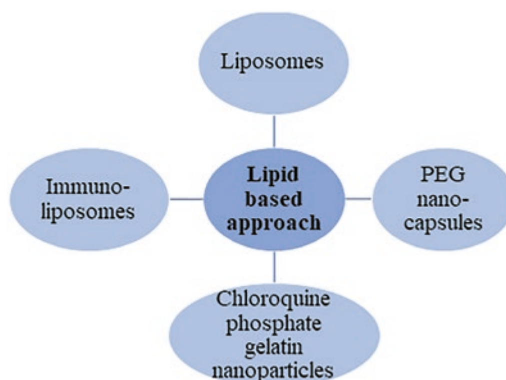

Fig. 2 Different lipid based approaches of nano-biotechnology

PEG-coated halofantrine-loaded poly-d,l-lactic acid (PLA) nano-capsules were tested against malaria (shown in Fig. 2), and their cardiotoxicity was assessed in mice infected with *P. berghei*. In the investigation, the NPs of primaquine on poly (diethyl methylidene malonate) were tested in mice infected with *P. berghei*, demonstrating that primaquine-loaded NPs have a higher enhanced life span (ILS) index. For liver targeting, the identical chemical was encapsulated in albumin and gelatin NPs of varying sizes 47. Primaquine was recently synthesised as a lipid nano-emulsion (10–200 nm) and has shown to be particularly efficient as an antimalarial drug against *P. berghei* infection in Swiss albino mice [12].

Chloroquine phosphate, an antimalarial medication, was encapsulated in gelatin nanoparticles and swelling-controlled distribution was proven in a physiological medium (pH 7.4), with a reduced release reported in the acidic pH range [13]. The ability of transferrin-conjugated solid lipid NPs (SLNs) to penetrate the brain for cerebral malaria therapy was investigated. The brain absorption of quinine was dramatically increased in this research when compared to unconjugated SLNs or drug solutions. Currently, violacein's anti-plasmodial (antimalarial) biological activities have been verified in vitro and in vivo [14, 15].

The effects of antimalarial medicines loaded into immunoliposomes targeted with a packed red blood cell (pRBC)-specific monoclonal antibody are currently being investigated. Recently, researchers encapsulated chloroquine and fosmidomycin in liposomes, and discovered that immunoliposome encapsulation of chloroquine and fosmidomycin improved antimalarial medication effectiveness tenfold [16].

5 Nucleic Acid-Based Nano-therapy of Malaria

MicroRNAs are short regulatory noncoding RNAs that play a role in gene targeting and silencing. Although these microRNAs are physiological regulators, they can be employed as therapeutic agents for a variety of disorders. These microRNAs have

proven to be effective in malaria prevention. An effort has been made to demonstrate that *P. falciparum* is susceptible to antisense oligonucleotide nucleotide NPs (ODN-NS) [17]. This intriguing approach typically employs antisense oligodeoxy (OD) N-chitosan particles of 50 mm in size. These particles promote the uptake of antisense ODN by *P. falciparum*-infected erythrocytes via erythrocyte permeation routes that target the Plasmodium topoisomerase II gene. In comparison to sense sequence controls, ODN chitosan NPs with both negatively and positively charged surfaces, as well as free antisense ODNs in a concentration of 0.5 M, displayed sequence-specific inhibition. The main distinction between ODN–chitosan NPs and free ODNs is specificity. The antisense impact of ODN–chitosan NPs was shown to be more sequence specific than that of free ODNs. When compared to free ODNs, ODN–chitosan NPs were revealed to be more sequence-specific inhibitors in their antisense impact. Similarly, the negatively charged surface of ODN–chitosan NPs inhibited *P. berghei* growth by around 87%, while the positively charged surface inhibited growth by 74%, and free ODNs inhibited growth by 68%. The vulnerability of *P. falciparum* to these microRNAs was established for the first time [18].

6 Protein-Based Nano-therapy for Malaria

Currently, research is being conducted to develop protein-based nanocarriers for antimalarial medication delivery. Until now, the only option has been to use gelatin, a collagen-denatured, biodegradable, and biocompatible protein [19]. Gelatin is a substance in which amide groups seldom cause the gelatin to be positively charged with an isoelectric point. Furthermore, due to its safety record, gelatin is a pharmaceutical adjuvant and an authorised plasma expander. Gelatin, one of the natural polymers employed in pharmaceutical nanotechnology research, plays an important part in the nano-delivery system for bioactive chemicals. The loading of pharmaceuticals is subsequently accomplished by polyion complexation between drug molecules and surface-charged groups on gelatin. The gelatin NPs were created using a twofold dissolving method and can subsequently be stabilised with a suitable cross-linking agent utilised for optimum chloroquine administration at physiological pH. However, no adequate antimalarial activity or safety has been observed [20].

7 Vaccine for Malaria

Malaria vaccinations are viable, according to several research. To begin, vaccination with irradiated sporozoites prevents or partially protects rats, monkeys, and humans from sporozoite infection. In humans, vaccination with the bites of over 1000 irradiated, infected mosquitos, administered in batches of a few hundred bites over many

months, provides sterilising protection against sporozoite challenge in more than 90% of vaccine recipients [21, 22]. Second, those who are frequently infected with malaria develop 'naturally acquired immunity' (NAI), which protects them against clinical sickness. However, if their parasites are eradicated by severe medication treatment, these people can become infected again, indicating that NAI does not entail absolute antiinfection immunity. If we could re-create NAI using a vaccine, we could protect patients from malaria [23, 24]. Third, research on vaccination demonstrates that vaccines currently on hand can protect against malaria infection in animal models and in humans. Notably, naïve people injected with a recombinant anti-sporozoite vaccine based on the circumsporozoite protein – the primary surface antigen of sporozoites — showed persistent protection against experimental sporozoite exposure. Protection has been obtained on a regular basis in new world monkeys [25].

8 Strategies for Vaccine Design

To begin, choose antigens that match the desired requirements. If they are immune-accessible, they generate protective immune responses in animal models, and either lack or have limited antigenic diversity. Antigens with significant variability yet potential as excellent vaccine targets, such as PfEMP1, will necessitate a method to overcome that diversity. Important candidates include parasite receptors that facilitate the binding of merozoites to erythrocytes or infected cells to endothelial cells [26]. Various vaccine candidates along with their antigens and primary mechanisms are shown in Table 1.

Second, mix antigens from various levels. Because of the stage specificity of antigen expression, even modest numbers of parasites escaping into the blood of a non-immune person following vaccination with a pre-erythrocytic-stage vaccine would result in clinical malaria. However, if a pre-erythrocytic vaccination was paired with a second line of defence — specifically, a component tailored to inhibit the growth of asexual blood stages — vaccinated persons with break-through blood-stage infection may lower the chance of major clinical disease even more. A combination of an anti-mosquito-stage vaccination with a blood- or pre-erythrocytic-stage vaccine, for example, would reduce the likelihood of resistance clones emerging in vaccinated hosts spreading across the population [31].

Third, combine several antigens from a single stage. If the protection generated by an antigen is independent of that provided by its companions, and if each is unable to elicit sterile immunity on its own, the efficacy of the vaccination will grow as additional antigens are added. Many leading vaccine candidates (MSP1 and apical membrane antigen1(AMA1)) are polymorphic, and several forms of each antigen maybe required to provide protection against parasite diversity. Because each person's immune response is unique (due to polymorphisms in HLA, other genetic traits, and previous exposure), antigen mixtures may be required to ensure that each

Table 1 Candidate vaccine antigens

Stage and process	Antigens	Primary mechanism
Sporozoite hepatocyte invasion	CSP73, TRAP/SSP273, STARP74, SALSA74	Antibody binding to antigens expressed on the surface of sporozoite: Prevent binding interactions required for invasion. Enhance splenic clearance or complement mediated lysis [27]
Hepatic stages growth, schizogony	CSP73, TRAP/SSP273, LSA173,TEXP173, LSA375,STARP74, SALSA74	T-cell recognition of antigens expressed on the surface of infected hepatocyte73: Release soluble immune mediators (IFN-g) resulting in intracellular parasite death lyse infected hepatocyte directly (CTL) [28]
Merozoite erythrocyte invasion	MSP120, MSP230, MSP376, MSP477, PfEBA17567, PvDABP78, AMA179, PfEBA17567, PvDABP78, AMA179, SERA80, GLURP28, Pf155/RESA30, RAP181, RAP281	Antibody binding to parasite antigens: Agglutinate or prevent the release of merozoites Block invasion into erythrocytes Induce monocytes to release soluble immune mediators, killing parasites (antibody dependent cellular inhibition) facilitate phagocytosis [29]
Erythrocyte stages (asexual) growth, sequestration	PfEMP16,85, rifins24, Pf33224	Antibody binding to antigens expressed on the surface of infected erythrocyte: Enhance splenic clearance or complement mediated lysis interfere with parasite nutrition and growth induce antibody-dependent cellular inhibition prevent or reverse endothelial adherence, thereby enhancing splenic clearance and reversing a key pathogenic mechanism [30]
Erythrocyte rupture	Glycosyl phosphatidylinositol	Antibody binding to GPI resulting in prevention of toxic effects [30]
Mosquito stages fertilization, oogenesis	Pfs255,86,Pfs285,26, Pfs48/45	Antibody binding to parasite antigens: Inhibit exflagellation and fertilization complement induced lysis of gametes and zygotes neutralize ookinete function [30]

person has a reasonable chance of generating a sufficient response, particularly for genetically restricted T-cell responses [32]. Fourth, immunizations must be kept as basic as feasible. Optimizing the immunogenicity of individual components in a combination is likely to be challenging, especially as the mixture becomes more complicated. The total quantity of protein that may be injected in one vaccination for protein-based vaccines is restricted by the formulation and probably by the incidence of adverse effects; also, malaria vaccines must be affordable, and the cost grows rapidly with complexity [31, 32].

9 Challenges in Vaccine Development

Pharmaceuticals have been created using a procedure that entails screening a large number of compounds for activity and selecting lead candidates for further development. For malaria, we currently have at least 5000 candidate proteins, many of which have numerous variations, although we have in vitro and animal models that indicate the relevance of a candidate [33]. There are several constraints to screening anti-morbidity vaccines in human populations in endemic locations. To begin with, creating several test vaccinations is a significant undertaking. Second, these vaccinations are primarily intended for children, and employing children as a major efficacy screen adds to the trial's complexity. Finally, decisions must be taken at the trial's end point. Surrogate efficacy indicators may be employed for ethical reasons as well as to reduce sample size requirements. In theory, mosquito-stage vaccines should have the easiest development route since there is a very simple test for detecting the influence of vaccine-induced antibodies on parasite transmission to mosquitoes, enabling down-selection to occur earlier in development. The final demonstration that such a vaccine is successful in the field would need big trials in which a considerable proportion of the population is vaccinated and whole villages are randomly assigned to treatment groups [33].

10 Different Malaria Vaccines

An efficient malaria vaccine would be a great tool for reducing the disease burden and might help malaria eradication in some parts of the world. Current malaria vaccine candidates are focused against human and mosquito phases of the parasite life cycle, although few proteins have been examined for possible vaccine development thus far (shown in Fig. 3). RTS,S, the most advanced vaccine candidate, provided moderate protection against malaria in phase II clinical trials and is presently being examined in a phase III study in Africa [34]. To increase the odds of creating a highly efficient malaria vaccine, new vaccine targets must be found.

11 Pre-erythrocytic Vaccines

Pre-erythrocytic vaccines (PEV) target antigens from Plasmodium sporozoite and liver stages, which are clinically silent forms of Plasmodium that cause human infection once sporozoites are inoculated into the skin by a mosquito. PEV is designed to elicit either (1) antibodies against surface antigens that remove sporozoites from the skin or circulation or inhibit their invasion of hepatocytes, or (2) T cell responses that target infected hepatocytes. The vaccine's protective efficacy was first demonstrated in a human in the 1970s, using radiation-attenuated WSV

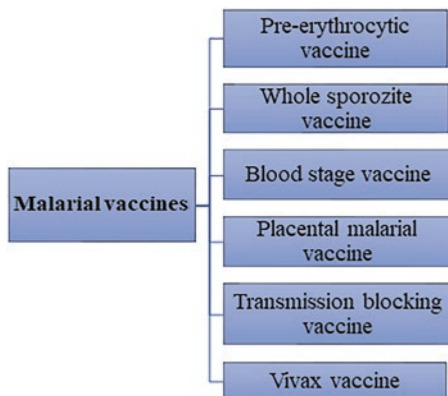

Fig. 3 Different malaria vaccines targeting at different stages of malaria

delivered through hundreds of mosquito bites; the vaccine protected from subsequent challenge with homologous (i.e., identical strain) and heterologous *P.falciparum* sporozoites (PfSPZ), but not from challenge with homologous blood-stage parasites. PEV with high activity can totally eliminate pre-erythrocytic parasites before they are released into circulation, and they are also known as anti-infection vaccines (AIV) [34, 35].

12 RTS, S and CSP-Based Vaccines

The discovery that WSV causes sterilising immunity in humans occurred at the same time as the invention of genetic engineering technologies. The first malaria gene to be cloned encodes the circumsporozoite protein, or CSP5, a significant surface antigen of sporozoites that is now a primary target of vaccine research. RTS,S, the most advanced PEV, combines a P. falciparum CSP fragment with a central repeat (thus “R”) and C terminal sections (carrying T cell epitopes, so “T”) linked to hepatitis B surface antigen (“S”), or “RTS” [36]. RTS is produced in yeast that also contains hepatitis B “S” expression cassettes, resulting in the production of S and RTS polypeptides that spontaneously co-assemble into mixed lipoprotein particles (or “RTS,S”) with the CSP fragment on their surface [36].

13 Whole Sporozoite Vaccines

Despite evidence that WSV confers sterilising immunity against sporozoite challenge in humans since the 1970s, WSV was not pursued as a product due to the notion that producing irradiated sporozoites was unfeasible for a vaccine [37]. Sanaria proposed a platform method in 2010 that involves extracting PfSPZ from

the salivary glands of aseptic mosquitos infected with cultivated laboratory parasites, followed by purification, vialing, and cryopreservation in the liquid nitrogen vapour phase [38]. PfSPZ-based vaccine that provides high-grade, durable protection against infection with Pf malaria, including radiation attenuation (called PfSPZ Vaccine), chemoattenuation achieved in vivo by the concomitant administration of antimalarial drugs [39] (called PfSPZ-CVac for chemoprophylaxis vaccination), and genetic attenuation by deletion of genes required to complete the liver-stage development (called PfSPZ-GA1 for the first genetically attenuated malaria vaccine). To induce sterile protection against sporozoite challenge, the PfSPZ vaccine requires direct venous injection. Implementing WSV will provide logistical and financial obstacles, including (1) liquid nitrogen cold chain, (2) intravenous inoculation, and (3) manufacturing scale-up [39–41].

WSV has been shown to be effective in people, although it is crucial to note that this efficacy is dose-dependent. The amount and duration of protection from homologous or heterologous sporozoite challenge in malaria-naive adults depend on dosage and regimen with either PfSPZ Vaccine or PfSPZ-CVac, both of which have demonstrated high levels of sterile homologous immunity [13, 42]. Protection against heterologous CHMI and protection beyond a few months have yet to be thoroughly explored. Five administrations of the PfSPZ Vaccine to adult residents in a malaria transmission area in Mali reduced the risk of new *P. falciparum* infection by 52% in a time-to-event analysis over the 24 weeks after the last dose, and reduced the proportion of people infected across the transmission season by 29% [35]. The reported time-to-event effectiveness for RTS,S in adults with AS02 or AS01 adjuvants appears to be greater [13, 42, 43].

PfSPZ-CVac utilising chloroquine gave significant levels of sterile immunity against homologous sporozoite challenge that lasted up to 2 years in malaria-naive people, but only a minority of vaccines produced sterile heterologous immunity. PfSPZ-CVac field trials have been finished or are underway, but the findings have not yet been published. The PfSPZ-CVac methods are an important translational research paradigm for studying human sterile immunity. To achieve complete chemoattenuation in vivo, development as a feasible vaccine approach will necessitate safe and dependable administration, such as coformulation of non-attenuated extremely sensitive sporozoites and long-lived chemoprophylactic drugs. GAP vaccines are being investigated for safety, immunogenicity, and protective effectiveness in malaria-naive individuals [44].

14 Blood-Stage Vaccines

BSV attacks the asexual parasite forms that cause sickness and death in erythrocytes through recurrent multiplicative cycles. The length between fevers, or periodicity, varies amongst malaria parasite species and is determined by cycle duration: 1 day for *P. knowlesi*, 2 days for *P. falciparum, P. vivax*, and *P. ovale*, and 3 days for *P. malariae*. At the end of each cycle, a brood of 1–2 dozen progeny (called

merozoites) egress from host erythrocytes, and within seconds, each merozoite has invaded a new erythrocyte to begin another round of multiplication (and a subset of invasive merozoites commit to generate the sexual forms that will infect mosquitos) [45].

Blood-stage parasites are an appealing target since this is the stage of development that causes disease, and because the passive transfer of IgG purified from semi-immune African adults was demonstrated to eliminate parasitaemia from African toddlers 6 decades ago, and subsequently in Thai adults. Immunization with complete parasite preparations rich in merozoites protected monkeys from *P. falciparum* infection in subsequent experiments, focussing vaccine makers' attention on merozoite invasion in the years afterward [46–48].

The difficulties in developing anti-merozoite vaccines include (1) the short time (seconds) that merozoites pass between erythrocytes and are accessible to antibodies, (2) antigenic polymorphism, (3) redundant invasion pathways, and (4) the large number of parasites that must be targeted in comparison to the numerical bottlenecks attacked by PEVs and TBVs. Over 30 BSV studies were performed between 2000 and 2015, with the vast majority targeting the antigens MSP1 and AMA1 and a minority targeting additional antigens such as EBA175 and MSP3. In general, these studies aimed to elicit high titre antibodies against merozoite surface antigens, which would reduce parasite invasion or, in the instance of MSP3, would induce antibody-dependent cellular inhibition [49]. Finally, the findings revealed no indication of protection against either controlled human infection or spontaneously occurring illness. In two studies, AMA-1 candidates produced high titre antibodies that were functional in vitro but did not show effectiveness against controlled infection with the homologous parasite. Only GMZ2 (composed of conserved domains of GLURP and MSP3) demonstrated statistically significant, albeit low (14%) effectiveness against spontaneously acquired infection in a pre-specified investigation [49–51].

Following these setbacks, the focus shifted to discovering novel BSV antigens or improving the approach to current targets. PfRH5 and the AMA1-RON2 complex [52] are two vaccine candidates that aim to solve the issue of duplicate invasion routes.

15 Placental Malaria Vaccines

PMV target parasites that bind to chondroitin sulphate A (CSA) and sequester in the placenta; hence, PMV offer a separate BSV strategy. While vaccinations that protect the entire population, such as PEV and BSV candidates, may assist pregnant women, naturally acquired protection against placental malaria provides a specialised vaccine strategy [53]. Natural antibodies against CSA binding parasites are related to placental malaria protection and are developed throughout consecutive pregnancies when women in endemic regions develop placental malaria resistance [54]. Placental parasites produce the unique PfEMP1 family member VAR2CSA, which binds

CSA72; recombinant VAR2CSA generates antibodies that inhibit parasite attachment to CSA. VAR2CSA is a complicated target with a large (>300 kD) extracellular domain with six DBL domains and additional interdomain sections, and recent research discovered functional atypical VAR2CSA with seven or eight DBL domains in some field isolates [55].

Over the last 5 years, the first studies of VAR2CSA-based vaccines have been undertaken. Because of its huge size, VAR2CSA vaccine research has concentrated on single domains or domain combinations. Two candidates with strong binding affinity for CSA based on N-terminal VAR2CSA segments have completed first-in-human trials. The PAMVAC produced by Drosophila cells was evaluated in several human adjuvants and found to be safe, well tolerated, and capable of inducing functionally active antibodies against homologous parasites. PAMVAC will be tried next in malaria-exposed nulligravidae. PRIMVAC, a second VAR2CSA candidate, completed a first-in-human study in France and Burkina Faso, demonstrating that the vaccine was safe, immunogenic, and elicited functional antibodies against the homologous VAR2CSA variant expressed by NF54-CSA infected erythrocytes. Cross-reactivity towards heterologous VAR2CSA variants, on the other hand, was minimal and only seen in the higher dosage group. Researchers expected that changing the vaccination timing, antigen dosage, and combining the vaccine with additional VAR2CSA-based vaccines would increase cross-reactivity against heterologous VAR2CSA variants [56, 57].

16 Transmission-Blocking Vaccines

TBV incorporates mosquito/sexual-stage antigens (gametes and zygotes) to produce antibodies that destroy parasites in the mosquito bloodmeal and stop parasite transmission through the vector. Monoclonal antibodies produced in rats against gamete/zygote preparations recognised target antigens and prevented mosquito infection [58, 59]. The four leading candidates have been classified as gamete surface proteins that are first expressed by gametocytes in the human blood, such as Pfs230 and Pfs48/45 of *Plasmodium falciparum*, and zygote surface proteins that are only expressed post-fertilization in the mosquito host, such as Pfs25 and Pfs28 [59–61]. These antigens are cysteine-rich, with many 6-cys or epidermal growth factor (EGF)-like domains, and they have proved difficult to generate as well folded recombinant protein. Different stages of Plasmodium and vaccine candidates, which are targeted at different stages are shown in Fig. 4.

Pfs25 was the first TBV candidate to be developed as a recombinant protein. Pfs25 candidates demonstrated equivalent or more serum transmission-blocking activity in animal experiments than other antigens or antigen combinations, hence Pfs25 has been the focus of clinical trials published so far. Currently, studies are being conducted to assess the efficacy of Pfs230 vaccine candidates. Pfs230 antibodies produced in animals had lytic activity against *P. falciparum* gametes in the presence of complement, suggesting that human Pfs230 antisera may have comparable activity [62–65].

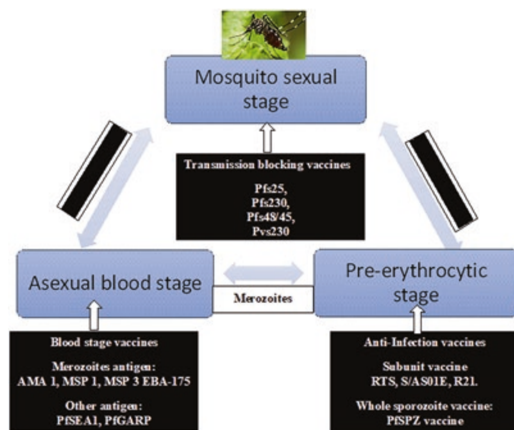

Fig. 4 Different stages of Plasmodium and vaccine candidates that target each stage

As monomers, both Pfs25 and Pfs230 recombinant antigens demonstrated weak immunogenicity. To improve immunogenicity, our research creates protein-protein conjugate vaccines by chemically connecting Pichia-expressed Pfs25 to carriers like ExoProtein (EPA) to create nanoparticles, which are then formulated in adjuvants. While previous trials of Pfs25 candidates failed to induce adequate antibody responses or were overly reactogenic in human vaccines, a Pfs25-EPA conjugate formulated with Alhydrogel® was reported in 2016 to be well-tolerated and to induce functional antibodies in humans that block *P. falciparum* transmission to mosquitos in membrane feeding assays, and this activity correlated with titers. However, most vaccines required four doses to achieve functional activity, and antibody titers and activity declined fast. Ongoing experiments (NCT02334462) compare and combine Pfs25 and Pfs230 vaccine antigens utilising Pichia-expressed Pfs230 domain. These trials are also evaluating the advantages of different adjuvants, such as the GSK adjuvant AS01, which is used in the RTS, S vaccine [66, 67].

17 Vivax Vaccines

P. vivax is the major cause of malaria in Asia and Latin America, causing an estimated 14.3 million cases per year. Although previously classified as benign tertian malaria, *P. vivax* is now widely recognised as a public health hazard that causes high morbidity and mortality 96. In addition, sterile heterologous immunity to *P. vivax* has been established [68]. Despite this, *P. vivax* research suffers from a lack of resources since malaria research funds—which are not proportionate to the extent of the issue in any case— are mostly directed to *P. falciparum* research. This lack of investment is especially short-sighted because vaccines may disproportionately

Table 2 List of vaccines in clinical trials

Antigen	Vaccine platform	References
P. falciparum genetically-attenuated sporozoites	Whole organism	[73]
P. falciparum CSP (RTS,S)	VLP used in adjuvant	[74, 75]
P. falciparum CSP	Ad35 vectored	[76, 77]

Table 3 Summary of stage 1 PfSPZ vaccine clinical trials [78]

Country	Protocol name	Primary performing institutions	ClinTrials.gov Identifier
USA	VRC 314	NIAID Vaccine Research Centre, Centre for Vaccine Development University of Maryland Baltimore	NCT02015091
Tanzania	BSPZV1	Ifakara Health Institute	
		Swiss Tropical and Public Health Institute	NCT02132299
USA	WRAIR 2080	Naval Medical Research Centre	
		Walter Reed Army Institute of Research	NCT02215707

benefit *P. vivax* control: dormant liver forms called hypnozoites produced by *P. vivax* (but not by *P. falciparum*) allow the parasite to relapse repeatedly over months or years, thwarting efforts to control or eliminate this species, hence the benefit of vaccines conferring durable immunological protection [68, 69].

Vaccine development for *P. vivax* has largely followed that of *P. falciparum*. Orthologous (PvCSP and Pvs25, respectively) of the PEV (PfCSP) and TBV (Pfs25) candidates that have grabbed the most attention for *P. falciparum* have been tested in humans. However, the PvCSP vaccine prepared as a monomer in GSK's AS01 adjuvant failed to induce sterile protection against challenge with P. vivax sporozoites 98, and Pvs25 expressed in *S. cerevisiae* formulated in Montanide ISA 51 caused systemic reactogenicity, resulting in the clinical trial's termination. Notably, Pvs25 was well-tolerated when formulated in Alhydrogel®, and the antibody responses, albeit moderate, were significant, demonstrating effective transmission-blocking action in mosquito feeding experiments that were proportional to antibody concentration. Based on the clinical success of the *P. falciparum* candidate Pfs230D1-EPA, the *P. vivax* candidate Pvs230D1-EPA is now being made in preparation for trials that might begin in 2021 [70–72] (Tables 2 and 3).

18 Conclusion

Malaria seems to be a challenging problem for current researchers to solve. Pharmaceutical nanotechnology has the potential to improve the efficacy of presently used antimalarial medications as well as new chemical entities with low solubility, chemical instability, a poor bioavailability profile, and toxicity. It is critical to bring together highly interdisciplinary experts with skills in parasitology,

biomaterials, polymer science, nanotechnology, pathology, toxicology, and cellular immunology to successfully walk this long road. There has been a surge in the use of nanomaterials as vaccinations. Multiple nanomaterial platforms, when combined with parallel breakthroughs in antigen design, may eventually be capable of recognizing and reliably raising the defined, designed immune responses required for the protection against malaria. It is expected that this review will eventually lead to a better understanding of malaria disease and provide insights into new strategies for developing a vaccination-based approach to treat malaria-infected patients.

References

1. Dahalan FA, Churcher TS, Windbichler N, et al. The male mosquito contribution towards malaria transmission: Mating influences the Anopheles female midgut transcriptome and increases female susceptibility to human malaria parasites. PLoS Pathog. 2019;15(11):1–15.
2. Kantele A, Jokiranta TS. Review of cases with the emerging fifth human malaria parasite, Plasmodium knowlesi. Clin Infect Dis. 2011;52:1356–662.
3. Pasvol G. Management of severe malaria: interventions and controversies. Infect Dis Clin N Am. 2005;19:211–40.
4. Caraballo H, King K. Emergency department management of mosquito-borne illness: malaria, dengue, and West Nile virus. Emerg Med Pract. 2014;16(5):1–23.
5. WHO. World Malaria Report 2020. Switzerland: World Health Organization; 2020. ISBN 978-92-4-001579-1
6. van Hensbroek MB, Onyiorah E, Jaffar S, et al. A trial of artemether or quinine in children with cerebral malaria. N Engl J Med. 1996;335(2):69–75.
7. Gilbert K. Ongoing challenges in the management of malaria. Malar J. 2009;8(1):1–12.
8. Esperance S, Raquel G. Current knowledge and challenges of antimalarial drugs for treatment and prevention in pregnancy. Tandfoline. 2010;11(8):1277–93.
9. Owais M, Varshney GC, et al. Chloroquine encapsulated in malaria-infected erythrocyte-specific antibody-bearing liposomes effectively controls chloroquine-resistant Plasmodium berghei infections in mice. Antimicrob Agents Chemother. 1995;39(1):180–4.
10. Gabriëls M, Plaizier-Vercammen J. Physical and chemical evaluation of liposomes, containing artesunate. J Pharm Biomed Anal. 2003;31(4):655–67.
11. Parashar D, Aditya NP, Murthy RS. Development of artemether and lumefantrine co-loaded nanostructured lipid carriers: physicochemical characterization and in vivo antimalarial activity. Drug Deliv. 2016;23(1):123–9.
12. Richards RL, Rao M, Wassef NM, et al. Liposomes containing lipid A serve as an adjuvant for induction of antibody and cytotoxic T-cell responses against RTS,S malaria antigen. Infect Immun. 1998;66(6):2859–65.
13. Seder RA, et al. Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. Science. 2013;341:1359–65.
14. Mbela TKM, Poupaert JH, Dumont P. Poly(diethylmethylidene malonate) nanoparticles as primaquine delivery system to liver. Int J Pharm. 1992;79(1–3):29–38.
15. Singh KK, Vingkar SK. Formulation, antimalarial activity and biodistribution of oral lipid nanoemulsion of primaquine. Int J Pharm. 2008;347(1–2):136–43.
16. Bajpai AK, Choubey J. Design of gelatin nanoparticles as swelling controlled delivery system for chloroquine phosphate. J Mater Sci Mater Med. 2006;17(4):345–58.
17. Gupta Y, Jain A, Jain SK. Transferrin-conjugated solid lipid nanoparticles for enhanced delivery of quinine dihydrochloride to the brain. J Pharm Pharmacol. 2007;59(7):935–40.
18. Rahman K, Shah AA, Khan MH, et al. In silico profiling of regulatory MicroRNA targets in GJB3 gene. Global J Biotechnol Biochem. 2014;9(2):41–9.

19. Gujjar R, Marwaha A, El Mazouni F, et al. Identification of a metabolically stable triazolopyrimidine-based dihydroorotate dehydrogenase inhibitor with antimalarial activity in mice. J Med Chem. 2009;52(7):1864–72.
20. James TY, Pelin A, Bonen L, et al. Shared signatures of parasitism and phylogenomics unite Cryptomycota and microsporidia. Curr Biol. 2013;23(16):1548–53.
21. Mishra A, Kaushik NK, Sardar M, Sahal D. Evaluation of antiplasmodial activity of green synthesized silver nanoparticles. Colloids Surf B Biointerfaces. 2013;111:713–8.
22. Marimuthu S, Rahuman AA, Rajakumar G, et al. Evaluation of green synthesized silver nanoparticles against parasites. Parasitol Res. 2011;108(6):1541–9.
23. Egan JE, et al. Humoral immune responses in volunteers immunized with irradiated Plasmodium falciparum sporozoites. Am J Trop Med Hyg. 1993;49:166–73.
24. Rieckmann KH, Beaudoin RL, et al. Use of attenuated sporozoites in the immunization of human volunteers against falciparum malaria. Bull World Health Organ. 1979;57(1):261–5.
25. Baird JK. Host age as a determinant of naturally acquired immunity to Plasmodium falciparum. Parasitol Today. 1995;11:105–11.
26. Hoffman SL, et al. Naturally acquired antibodies to sporozoites do not prevent malaria: vaccine development implications. Science. 1987;237:639–42.
27. Cheng Q, et al. Measurement of plasmodium falciparum growth rate in vivo: a test of malaria vaccines. Am J Trop Med Hyg. 1997;57:495–500.
28. Bolad A, Berzins K. Antigenic diversity of Plasmodium falciparum and antibody-mediated parasite neutralization. Scand J Immunol. 2000;52:233–9.
29. Hoffman SL, Franke ED, Hollingdale M, et al. In: Hoffman SL, editor. Malaria Vaccine Development: A Multi-immune Response Approach. Washington: ASM; 1996. p. 35–75.
30. Perlaza BL, et al. Immunogenicity of four Plasmodium falciparum pre-erythrocytic antigens in Aotus lemurinus monkeys. Infect Immun. 1998;66:3423–8.
31. Kester KE, et al. Efficacy of recombinant circumsporozoite protein vaccine regimens against experimental Plasmodium falciparum malaria. J Infect Dis. 2001;183:640–7.
32. Gamain B, Miller LH, et al. The surface variant antigens of Plasmodium falciparum contain cross-reactive epitopes. Proc Natl Acad Sci U S A. 2001;98:2664–9.
33. Wang L, Richie TL, Stowers A, Nhan DH, et al. Naturally acquired antibody responses to Plasmodium falciparum merozoite surface protein 4 in a population living in an area of endemicity in Vietnam. Infect Immun. 2001;69:4390–7.
34. Stowers AW, Miller LH. Are trials in new world monkeys on the critical path for blood-stage malaria-vaccine development? Trends Parasitol. 2001;17:415–9.
35. Heppner DG, et al. New world monkey efficacy trials for malaria vaccine development: critical path or detour? Trends Parasitol. 2001;17:419–25.
36. Clyde DF, Most H, et al. Immunization of man against sporozite-induced falciparum malaria. Am J Med Sci. 1973;266:169–77.
37. Dame JB, et al. Structure of the gene encoding the immunodominant surface antigen on the sporozoite of the human malaria parasite Plasmodium falciparum. Science Direct. 1984;225:593–9.
38. Cohen J. Vaccine composition against malaria. USA patent (1996).
39. Smithkline Beecham BiologicalsWilde, M. D. Hybrid protein between CS from Plasmodium and HBsAG. (1991).
40. Hoffman SL, et al. Development of a metabolically active, non-replicating sporozoite vaccine to prevent Plasmodium falciparum malaria. Hum Vaccin. 2010;6:97–106.
41. Mueller AK, Labaied M, Kappe SH, Matuschewski K. Genetically modified Plasmodium parasites as a protective experimental malaria vaccine. Nature. 2005;433:164–7.
42. Richie TL, et al. Progress with Plasmodium falciparum sporozoite (PfSPZ)-based malaria vaccines. Vaccine. 2015;33:7452–61.
43. Epstein JE, et al. Protection against Plasmodium falciparum malaria by PfSPZ vaccine. JCI Insight. 2017;2:e89154.

44. Epstein JE, et al. Live attenuated malaria vaccine designed to protect through hepatic CD8(+) T cell immunity. Science Direct. 2011;334:475–80.
45. Ishizuka AS, et al. Protection against malaria at 1 year and immune correlates following PfSPZ vaccination. Nat Med. 2016;22:614–23.
46. Roestenberg M, et al. Protection against a malaria challenge by sporozoite inoculation. N Engl J Med. 2009;361:468–77.
47. Cohen S, Mc G. Gamma-globulin and acquired immunity to human malaria. Nature. 1961;192:733–7.
48. Edozien JC, Gilles HM, et al. Adult and cord-blood gammaglobulin and immunity to malaria in Nigerians. Lancet. 1962;280:951–5.
49. Sabchareon A, et al. Parasitologic and clinical human response to immunoglobulin administration in falciparum malaria. Am J Trop Med Hyg. 1991;45:297–308.
50. Siddiqui WA. An effective immunization of experimental monkeys against a human malaria parasite, Plasmodium falciparum. Science. 1977;197:388–9.
51. Sirima SB, Cousens S, Druilhe P. Protection against malaria by MSP3 candidate vaccine. N Engl J Med. 2011;365:1062–4.
52. Payne RO, et al. Demonstration of the blood-stage Plasmodium falciparum controlled human malaria infection model to assess efficacy of the P. falciparum apical membrane antigen 1 vaccine, FMP2.1/AS01. J Infect Dis. 2016;213:1743–51.
53. Spring MD, et al. Phase 1/2a study of the malaria vaccine candidate apical membrane antigen-1 (AMA-1) administered in adjuvant system AS01B or AS02A. PLoS One. 2009;4:e5254.
54. Sirima SB, et al. A phase 2b randomized, controlled trial of the efficacy of the GMZ2 malaria vaccine in African children. Vaccine. 2016;34:4536–42.
55. Salanti A, et al. Selective upregulation of a single distinctly structured var gene in chondroitin sulphate A-adhering Plasmodium falciparum involved in pregnancy-associated malaria. Mol Microbiol. 2003;49:179–91.
56. Fried M, Duffy PE. Designing a VAR2CSA-based vaccine to prevent placental malaria. Vaccine. 2015;33:7483–8.
57. Doritchamou JYA, et al. Placental malaria vaccine candidate antigen VAR2CSA displays atypical domain architecture in some Plasmodium falciparum strains. Commun Biol. 2019;2:457–65.
58. Mordmuller B, et al. First-in-human, randomized, double-blind clinical trial of differentially adjuvanted PAMVAC, a vaccine candidate to prevent pregnancy associated malaria. Clin Infect Dis. 2019;69:1509–16.
59. Sirima SB, et al. PRIMVAC vaccine adjuvanted with Alhydrogel or GLA-SE to prevent placental malaria: a first-in-human, randomised, double-blind, placebocontrolled study. Lancet Infect. 2020;3099(19):307–39.
60. Carter R, Chen DH. Malaria transmission blocked by immunisation with gametes of the malaria parasite. Nature. 1976;263:57–60.
61. Gwadz RW. Successful immunization against the sexual stages of Plasmodium gallinaceum. Science. 1976;193:1150–1.
62. Carter R, Kaushal DC. Characterization of antigens on mosquito midgut stages of Plasmodium gallinaceum. III. Comparison of surface antigens of male and female gametes and zygotes. Mol Biochem Parasitol. 1984;13:235–41.
63. Duffy PE, Pimenta P, Kaslow DC. Family of epidermal growth factor-like antigens that are targets of malaria transmission-blocking antibodies. J Exp Med. 1993;177:505–10.
64. Grotendorst CA, Kumar N, Carter R, Kaushal DC. A surface protein expressed during the transformation of zygotes of Plasmodium gallinaceum is a target of transmission-blocking antibodies. Infect Immun. 1984;45:775–7.
65. Barr PJ, et al. Recombinant Pfs25 protein of Plasmodium falciparum elicits malaria transmission-blocking immunity in experimental animals. J Exp Med. 1991;174:1203–8.
66. Kapulu MC, et al. Comparative assessment of transmission-blocking vaccine candidates against Plasmodium falciparum. Sci Rep. 2015;5:11193.

67. Menon V, et al. Assessment of antibodies induced by multivalent transmissionblocking malaria vaccines. Front Immunol. 2017;8:1–10.
68. Read D, et al. Transmission-blocking antibodies against multiple, non-variant target epitopes of the Plasmodium falciparum gamete surface antigen Pfs230 are all complement-fixing. Parasite Immunol. 1994;16:511–9.
69. Radtke AJ, et al. Adjuvant and carrier protein-dependent T-cell priming promotes a robust antibody response against the Plasmodium falciparum Pfs25 vaccine candidate. Sci Rep. 2017;7:40312.
70. Baird JK. Evidence and implications of mortality associated with acute Plasmodium vivax malaria. Clin Microbiol Rev. 2013;26:36–57.
71. Wu Y, et al. Phase 1 trial of malaria transmission blocking vaccine candidates Pfs25 and Pvs25 formulated with montanide ISA 51. PLoS One. 2008;3:26–36.
72. Malkin EM, et al. Phase 1 vaccine trial of Pvs25H: a transmission blocking vaccine for Plasmodium vivax malaria. Vaccine. 2005;23:3131–8.
73. Spring M, Murphy J, Nielsen R, et al. First-in-human evaluation of genetically attenuated Plasmodium falciparum sporozoites administered by bite of Anopheles mosquitoes to adult volunteers. Vaccine. 2013;31(43):4975–83.
74. Stoute JA, Slaoui M, Heppner DG, et al. A preliminary evaluation of a recombinant circumsporozoite protein vaccine against Plasmodium falciparum malaria. RTS,S Malaria Vaccine Evaluation Group. N Engl J Med. 1997;336:86–91.
75. The Rts SCTP. Efficacy and safety of the RTS,S/AS01 malaria vaccine during 18 months after vaccination: a phase 3 randomized, controlled trial in children and young infants at 11 African sites. PLoS Med. 2014;11:e1001685.
76. Creech CB, Dekker CL, Ho D, et al. Randomized, placebo-controlled trial to assess the safety and immunogenicity of an adenovirus type 35-based circumsporozoite malaria vaccine in healthy adults. Hum Vaccin Immunother. 2013;9:2548–57.
77. Ouédraogo A, Tiono AB, Kargougou D, et al. A phase 1b randomized, controlled, double-blinded dosage-escalation trial to evaluate the safety, reactogenicity and immunogenicity of an adenovirus type 35 based circumsporozoite malaria vaccine in burkinabe healthy adults 18 to 45 years of age. PloS One. 2013;8:e78679.
78. Richie TL, Billingsley PF, Sim BK, et al. Progress with Plasmodium falciparum sporozoite (PfSPZ)-based malaria vaccines. Vaccine. 2015;33(52):7452–61.

Nanotechnology-Based Vaccination for TB

Anwesha Barik and Sanjay Dey

Abstract With a projected global target to end the tuberculosis epidemic by the year 2030, United Nations termed this as an important Sustainable Development Goal (SDG). Following regular BCG vaccination regime since birth especially in tuberculosis-endemic regions and proper utilization of world-wide annual funding for prevention, diagnosis, care and treatment of Tuberculosis has caused the cumulative reduction in the incidence to about 11% between 2015 and 2020 which is still half way to reach the expectation in the same duration of the time. Inappropriate dosing regimen, poor quality of anti-tuberculosis drugs, medication non-adherence, in addition resulted in emerging cases of multi-drug resistant tuberculosis strains which made the therapy more extensive and limited. It further increases the economic burden especially in the developing countries. Prevention of the eruption as well as reactivation of the disease may be ensured by suitable vaccines which are expected to elicit the immunity superior to that caused by natural infection. Practical experiments since last few years already resulted in several attenuated or subunit vaccines alternative to BCG, all of which are currently at different stages at clinical trials. Nanocarrier-based vaccines, specifically have garnered attention to ensure protection of antigens from proteolytic degradation, controlled release of antigens, uptake, and processing by antigen presenting cells with improved sensitivity and low immunogenicity of the carriers itself. This chapter has emphasized the updated status of the development strategy of nanocarrier-based vaccines and its future indication to be the potential alternative to regular BCG vaccination to combat tuberculosis.

Keywords Tuberculosis · Vaccine · Nanoparticle · Clinical trials · Cellular immunity · Protective immunity · Bacille Calmette Guerin (BCG) · Adjuvant

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1 Introduction

Immunology has evolved a lot to understand the mechanism following which the immunity is attributed either naturally or with the aid of vaccines. Although being one of the greatest discoveries in the modern healthcare, majority of the vaccines were designed years ago empirically without implementing the complete knowledge of immunology and the knowledge was not even developed in some of the cases. The vaccines usually induce the rapid, long-lasting immunity similar to that elicited by the live infection. It all starts with activation of dendritic cells of innate immune system by pattern recognition receptors including mostly Toll like receptors, some being through C type lectin like receptors, cytosolic nod like receptors after presentation of protein antigens as well as adjuvant present in the vaccines. The peptide components, in addition, also activate T cells after attachment on Major Histocompatibility Complex (MHC) molecules of dendritic cells. Different subsets of the helper T cells, such as Th1, Th2, and Th17, arise in exposure to several pathogens which, especially the follicular T helper cells, further secrete Interleukin 21. Those activated T cells start developing B cells in lymph node, which further leads to production of short-lived plasma cells resulting in secretion of different isotopes of antibodies. Memory B cells are found to be generated as well which elicit antigen-antibody response on further exposure of infection [1]. The central memory may also be contributed by the differentiating CD4+ T cells and CD8+ T cells apart from those B cells. This huge complex correlated mechanism indicates that there are various factors to be considered in the rational development of vaccines especially in the pandemics. These factors include but not limited to corresponding activity of adjuvant along with vaccines to stimulate innate as well as adaptive immunity, modulation in differentiation of T cells and B cells in regulation of quality and coverage of protective immunity conferred by vaccines, effect of varied range of age, genetic information, nutrition status in human volunteers in clinical trial to understand regulation of immune response in large heterogeneous population.

Tuberculosis (TB) has already been termed as “Critical” according to World Health Organization (WHO) among all other infectious diseases. It has also been included in the list of priority research which might lead to the development of cost-effective vaccines to combat not only latent tuberculosis or antimicrobial resistant tuberculosis (AMR strains) but also may broaden the protective coverage against more threatening multidrug-resistant tuberculosis (MDR strains), extensively drug-resistant tuberculosis (XDR strains) as well [2]. Despite the effectiveness of Bacille Calmette Guerin (BCG) vaccine in controlling mortality among vaccinated children since birth while acting against tuberculosis, WHO suggests that the vaccine’s ability to induce non-specific immune sensitization to other pathogens to generate “trained immunity” also needs to be considered while developing novel vaccine alternative to BCG. It may further help to reduce prevalence of anti-microbial resistant forms of other infectious diseases including malaria as well. Several advantages, such as the huge surface area of the sub-micron sized particles, increased interaction of the vaccine with huge surface area of respiratory mucosa, enhanced

penetration into bacilli loaded granulomas attributed by nanotechnology-based approaches, may benefit in targeting especially the most prevalent respiratory forms of tuberculosis in which BCG seems to be ineffective. Previous studies showed that the antigens embedded on nano-particulate platforms have the capacity to enhance immunogenic response to other pathogens causing other infectious diseases, which indicates the same potential for tuberculosis vaccines as well, once developed [3, 4].

2 Need of Vaccines in Infectious Diseases

Management of infectious diseases by means of vaccination at early age has already been found to play pivotal role in prevention since the pioneering work accomplished by Dr. Jenner around 200 years ago. Incidence of infectious diseases such as human immunodeficiency virus (HIV), TB, and malaria has been continuously declining the quality of life and the rate of societal development across the developing countries, especially in Africa and South-east Asia. Development of those vaccines is still ongoing, which makes to estimate the potential advantages difficult outweighing the risks associated. Ever after the developmental period, maximum benefit may be achieved only if the well-planned long-term global surveillance can be ensured to detect new strains of infective microorganism as upcoming threats and all other emerging resistant strains resulting from concurrent mutations which pose huge threat to the established routine vaccination regime. Brian Greenwood reviewed the contribution of vaccination to the healthcare system for infectious as well as non-infectious diseases and raised a reasonable doubt regarding interrelation between vaccination of infective diseases and management of non-infective chronic conditions such as cardiovascular problems, diabetes, and cancer [5]. Therapeutic vaccines for targeting some of the cancers such as prostate cancer is commercially available as Sipuleucel T; though complexity and financial burden associated with immunotherapy, tailored for the patient specifically seems to be unaffordable even in the resource-rich countries. Introduction of clean water and sanitation along with routine vaccination nevertheless brought a great decline in child morbidity due to infection diseases (small pox, measles, etc.), when compared to the deaths at pre-vaccination period. Eradication of two infectious diseases has already been officially recognized by WHO, latest being the rinderpest virus in 2011 apart from the smallpox caused by variola virus [6]. Polio virus, on the other side, has almost been eradicated except a few outbreaks in some sparse regions throughout the World including Afghanistan, Pakistan, and some countries in African Region as recently documented by WHO (https://www.who.int/emergencies/disease-outbreak-news/item/circulating-vaccine-derived-poliovirus-type-2-global-update). The low-vaccination coverage at those regions is one of the underlying causes behind this. Hidden subclinical features, sometimes asymptomatic, presumably make the eradication of polio even more challenging. Development of vaccine by attenuation of the causative microorganism did not only induce strong and effective immunization for poliovirus but also for other viruses and bacteria such as rubella, influenza,

tuberculosis, typhoid, rotavirus, rabies, and measles. Induction of long-term growth of those causative organisms in tissue culture reduces the virulence while maintaining its viability; therefore, the presence of those inactivated live bacterial cell or viral components easily spread to the non-vaccinated persons, broadening the coverage of immunized population. These live strains may rarely cause the reversion of the virulence, through concurrent mutations. Although the incidences, namely paralysis are rarely observed in immune-compromised patients especially who are administered with Oral polio Vaccine (OPV) or anti-tuberculosis vaccine BCG [7]. Residual pathogenic features in those inactivated components are sometimes responsible for those adverse events, albeit small whereas non-live vaccines do not pose such threats to those immune-deficient individuals.

3 Nanotechnology-Based Vaccination and Immune Response

The vaccine delivery system is designed by following a basic framework which includes an antigen, delivery vehicle, and adjuvants which possibly stimulates to produce stronger immune response while improving the durability of the immunogenicity. Despite following the standard procedure to develop the vaccines such as live-attenuated, killed, inactivated pathogens, challenges are often faced primarily due to weak immunogenicity and increased risk of undesirable effects caused by purified antigens. Inadequate antibody titers even after repeated vaccination and failure to provide protection against variants/serotypes are also some of the major drawbacks with the conventional formulations. Translation in terms of vaccine development strategy has been encountered through discovery of human hepatitis B vaccines at late twentieth century. One of those involved extraction of surface antigens from the plasma of human carriers of the infection and other one marked the first ever commercialized recombinant-expressed vaccine [8]. Recombinant technology, in brief, deals with the insertion of deoxyribonucleic acid (DNA) encoding surface antigens/membrane proteins of the pathogenic microorganisms into yeast, mammalian, or bacterial cells to express the protein redundantly. Human papilloma virus infection also found to be prevented using the vaccines developed similarly as well. Assembly of those expressed proteins form viral-like particles in vitro lead to induce in vivo immunogenicity without causing virulence. The cell-mediated and humoral immune response in exposure to nanotechnology-based antigen carriers is highlighted in Fig. 1.

Viral-like particle (VLP)-based vaccines have shown effectiveness against chronic diseases such as hypertension, drug addiction, rheumatoid arthritis, Alzheimer's disease reaching even through the pre-clinical and clinical evaluation [9]. These particles are basically versatile nanoparticles (20–100 nm in diameter), developed either by genetic modification of the viral capsid subunit followed by conjugation with antigenic peptides or other bioactive cell mediators such as Toll-like receptors (TLR) [10]. Smaller VLPs can cross the tissue barriers itself and reach lymph nodes directly to be up-taken preferentially by antigen presenting cells

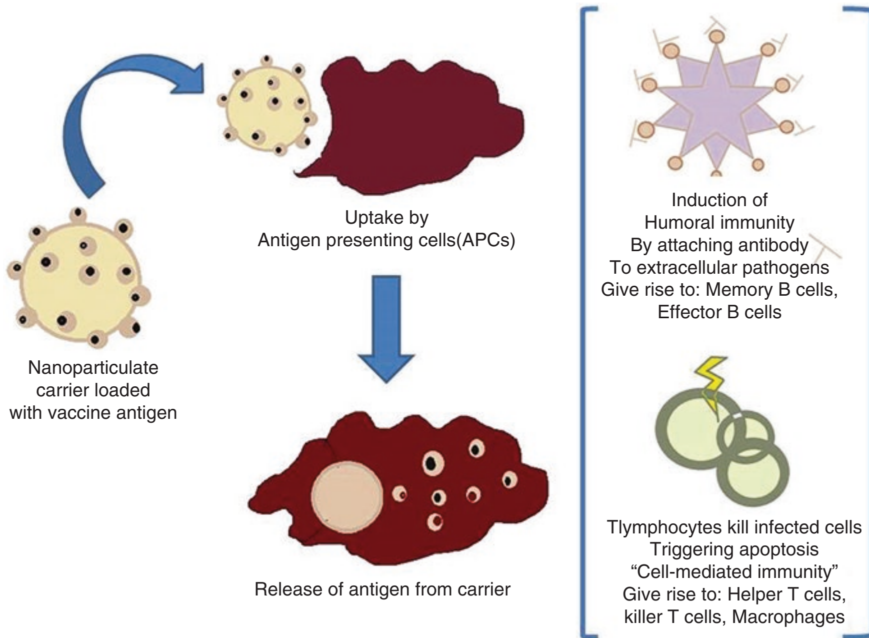

Fig. 1 Representation of cell-mediated and humoral immune response in exposure to nanotechnology-based antigen carriers, i.e., vaccines

(APCs), whereas the larger ones are carried by dendritic cells (DCs) to be transported to lymph nodes. Although securing an excellent safety record since the induction of this technology in prophylactic use, concerns are raised regarding occurrence of autoimmune diseases such as, rheumatoid arthritis, Guillain–Barré syndrome, and multiple sclerosis following the vaccination [11]. Structural stabilization of protein antigens inside VLPs has been made feasible through combination of synthetic nanoparticle with lipid moieties which further aid in viral membrane fusion due to viral glycoprotein recognition [12]. Extensive use of recombinant DNA technology or purification of proteins may also be avoided herewith.

Fabrication of clinically safe, biocompatible, and biodegradable nanoformulations incorporating antigens enhance rapid uptake and recruitment of DCs to produce high titers of humanized DC specific antibodies in comparison to that produced by the antigen itself. Sustained release of granulocyte/macrophage colony-stimulating factor (GM-CSF) in response of administration of hepatitis B surface antigen (HBsAg) encapsulated in modified Poly-lactic co-glycolic acid (PLGA) based thermosensitive hydrogel matrix has been observed to promote strong induction of immune response similarly resulting in improved T cell response followed by antibody production which is not typical with direct administration of low concentration of HBsAg [13]. Nanoemulsions, cationic liposomes, self-assembled peptide nanoparticles are also evaluated as vaccine delivery modules as evident from the literature [14].

4 Pathogenesis and Role of Vaccines in TB

The development of TB starts from inhalation of mycobacteria laden infectious droplet spread through sputum of active pulmonary TB patient. The overall development of the disease follows three more steps onwards. First step involves the phagocytosis of the aerosol born tubercle bacilli inside the alveolar macrophages in the lung where a major portion of the bacilli get destroyed. A few bacilli being able to bypass the process reach to the remote sites of the body through lymphatic channels or bloodstream especially the organs where the disease is prone to be developed including regional lymph nodes, kidney, brain, and bone. The bacilli are ingested by the macrophages there to form granuloma which is somehow able to keep the infection under control. Considering the granuloma development as one of the hallmarks of active mycobacterial infection, it can be categorized in several classes either based on pathological appearance (e.g., necrosis) or based on the degree of liquefaction. Solid necrotic granula has been observed to be having less viable mycobacteria resulting in efficient containment of the infection, whereas comparatively large granulomas may drain the bacilli to the bronchial tree which may further aspirated by the lungs, have possibility to be coughed up and transmitted thereafter. The reason behind lack of development of TB in majority of the infected persons might be the successful containment of the bacilli within granuloma without interfering with their activity. The granulomas are assumed to be advantageous in preventing the bacterial spread, but they can start their multiplication again once the patient become immune-compromised in future or with the age. These phenomena often rise to the development of asymptomatic disease or latent tuberculosis infection (LTBI).

Depending on the time of exposure and pathologic pattern, TB may be classified as primary and post-primary. Children suffer from primary infection where the host is protected by effective immunity system by the formation of Ghon complex resulting in an accompaniment of small subpleural granuloma with granulomatous hilar lymph node infection. This primary TB can result in a plethora of clinical spectrum such as disseminated TB in immunologically naïve patients with acquired immunodeficiency syndrome (AIDS), meningitis where in immune-competent patients these nodes get dissolved with time preventing further spread of the microorganism [15]. Reactivation of this previous infection in immune-compromised adult patients leads to the spreading of granulomatous inflammation. Combination product of long-secreted mycobacterial antigens with host lipid is entrapped within foamy alveolar macrophages infiltrating to the obstructed bronchioles covering a large area of lungs. Necrotic caseous pneumonia develops and expelled into cavities where microorganism proliferate in a huge number while distorting the systemic immunity. This may take a duration of as long as 6–8 weeks to be detected via skin tuberculin test after getting first exposure to infection provided strong immune response is elicited [16].

Infection due to exposure to *Mycobacterium tuberculosis* can only be controlled by *Mycobacterium bovis BCG*, the licensed vaccine available till date. BCG vaccine has been found to shorten the gap between infection and induction of adaptive

immune response by stimulation of both CD4+ and CD8+ antibodies through the generation of long-term immune memory. This phenomenon further efficiently controls the rapid spreading of granulomas to other organs [17]. Newly developed vaccines often need to be evaluated by means of some in vivo biomarkers (often known as immune correlates of protection; COP). In lieu of this, animal studies after pulmonary vaccination of BCG have showed that the airway trafficking of CD4+ cells of T helper 17 cell subtype generates T helper 1 cells which together leads to cytokine storm (giving rise to interferon γ, tumor necrosis factor, interleukin 2 and 17). This further activates mycobacteria-infected macrophages to restrict bacterial growth and recruit more immune cells to mediate protection to further exposure to the causative microorganism. In human, contradictory results were evident due to lack of correlation between BCG-induced T helper cells, cytokine storm, and protection against tuberculosis [18]. Expression of CD153 by *Mycobacterium tuberculosis*, on the other hand, acted as a potential biomarker to mediate protection as evident in animal as well as human [19]. Highly polyfunctional *Mycobacterium tuberculosis* specific CD4+ cells seem to be the only source of this surface molecule belonging to tumor necrosis factor (TNF) superfamily, whereas the vaccine-induced CD8+ cells have no relation with the secretion. Several studies in human and murine models suggest that CD8+ T cells have failed to recognize the *Mycobacterium tuberculosis* infected macrophages thereby compromising the immune control [20, 21]. The protective role of those cells remains unresolved although the response of CD8+T cells seems to show progression kinetics with bacterial burden [22]. Elevated level of natural mycobacteria-specific immunoglobulin type A (IgA) due to BCG vaccination especially in broncho-alveolar fluid also seems to attribute to the extended protection against further tubercular infection [23]. Some novel vaccine candidates are still in several stages of clinical trial in spite of having the best clinical response of BCG. Those are supposed to be used in prophylactic, post-exposure, and therapeutic purpose in children, adolescents, and adults to broaden the coverage of protective immunity against all possible tuberculosis specific antigens. Some of them are live attenuated type (MTBVAC), recombinant BCG vaccine (VPM1002), some contains killed whole mycobacteria cells (RUTI, *Mycobacterium vaccae*-based vaccines and the *Mycobacterium obuense*-based DAR-901 vaccine). Some utilizes adjuvants (H4:IC31, H56:IC31, ID93 + GLA-SE and M72/AS01$_E$) to elevate response against recurrent infections also where as some uses non-replicating viral vectors (MVA85A and Ad5Ag85A) to genes encoding antigens of interest to increase antigen production in host cells thereby boosting the innate immunity primed by BCG vaccination [17, 24].

5 Nanotechnology-Based Vaccination Strategies

Like all other infective and non-infective diseases, tuberculosis treatment also found its remarkable feature through nanotechnology while ensuring slow sustained release of drug for long time with causing minimalistic side effects and improved

bioavailability. Multiple drugs (mostly antibiotics) together can be encapsulated in those drug delivery modules and there is a possibility to explore variable routes of administration as well. Those include niosome, liposome, solid lipid nanoparticle, nanobeads, and microemulsion-based anti-tuberculosis drug delivery systems. Similar to those, nanotechnology is being equally suitable to address unique challenges posed by a pathogen strengthening the possibility of global implementation of novel cost-effective vaccination strategy. Biocompatible, non-toxic approaches such as nanoparticles, liposomes, dendrimers, VLPs, virosomes have shown their potential experimentally in ensuring long-term immunity. Additional use of adjuvant either initiates the innate immunity or acts as a delivery vehicle to target the antigen directly to APCs which further results in induction of cell-mediated and humoral immunity both by major histocompatibility complex (MHC) class of molecules [25]. The several types of vaccine candidates are summarized herewith.

5.1 Viral Vector-Based and DNA-Based Vaccines

Avirulent viral carriers (replication-deficient influenza virus, fowl pox virus, chimpanzee adenovirus, etc.) express the potential antigen of the pathogen (secretory antigenic protein 85A, ESAT-6, Ag85A, etc.) resulting in mediating cell-mediated and humoral immune responses. Vaccines under development stage and vaccines showing immunogenicity against latent TB as well as active TB were in different stages of the clinical trial as reported by Ojha et al. previously [25]. According to the current status updated at clinicaltrials.gov, ChAdOx1 85A Vaccine is under a brief study comparing immunogenicity of the vaccine with that of BCG on the basis of age de-escalation and dose-escalation. On review of safety and efficacy data of this study, the vaccine will be subjected to a comparison study between groups treated by ChAdOx1 85A, ChAdOx1, MVA85A, and BCG. Subunit protein antigens when mixed with toxoid adjuvant may sometimes extend further protection to the subjects irrespective of their BCG vaccination or tuberculosis infection status. Safety and efficacy study of Ag85B-ESAT-6 + IC31, LTK63 adjuvant mixed Tuberculosis Nasal Subunit Vaccine (Ag85B-ESAT6), Ag85B-ESAT-6 + IC31 mixed with KLK and ODN1a, Ag85B-ESAT-6 mixed with Liposomal Adjuvant System, CAF01 individually concluded the well tolerance for almost all the cases with a few instances of local or systemic side effects. The adjuvanted tuberculosis subunit vaccine H1/IC31 has even showed stronger immunogenic response to the tubercular antigens in tubercular naïve patients when compared to previously tuberculosis-infected patients [26]. A recent study performed an in silico analysis to design "a novel multi-epitope subunit vaccine" while using an adjuvant griselimycin as a vaccine construct [27]. A series of experimentally validated tubercular antigens, Rv2608, Rv2684, Rv3804c (Ag85A), and Rv0125 (Mtb32A), were included to predict the efficacy by scores of allergenicity, molecular docking, solubility, and antigenicity. In silico cloning in *E. coli* has also been studied to confirm the expression of antigens. The prediction of long-lasting immunity was pretty consistent with typical

immune response against TB which may lead to universal, widely effective vaccine, once translated in vivo.

The pathogen can also be expressed in animal models through intramuscular or intradermal administration of plasmid vectors cloned with respective DNA. The potential of using antigens such as Ag85A, Ag85B, ESAT6, and hsp65 has been evaluated in preclinical models and it showed robust immune responses in vivo while expressing interferon-gamma, CD4+Th1, interleukin 2 at high level [28]. Lu et al. developed a novel recombinant DNA-based BCG prime boost approach with recombination plasmid pcDNA3.1-Rv1769 or pcDNA3.1-Rv1772 [29]. The two neglected pathogens Rv1772 and Rv1769 were hypothesized to be excellent subunit to strengthen the pre-existing immunogenicity primed by BCG. It resulted in potent immune response (CD4+ and CD8+ both) in BALB/c mouse primed with either BCG-Pasteur or BCG-China. Similar regimen has been followed to evaluate immunogenicity in female BALB/c by administering Poly (lactic-co-glycolic acid) nanoparticles encapsulated with DNA (pcDNA3.1-Mtb72F) using either TB10.4 or CpG as adjuvant [30]. When compared with the naked DNA itself with or without adjuvant, the optimized DNA- and nanoparticle-loaded vaccine produced higher IFN gamma/IL4 ratio. The outcome corroborated with a somehow similar experiment published previously although without developing the nanoparticles as carrier [31]. Comparing with BCG and some other DNA vaccines, intramuscular administration of a novel DNA vaccine expressing all the antigens Ag85B, ESAT-6, Rv2660c, and fusion BER together through electroporation (pBER/EP), produced T cell responses abundantly even more than the DNA/EP prime and vaccinia-85B boost immunization as claimed by Tang et al. [32]. DNA vaccines have advantage over majority of the vaccines against mycobacterium tuberculosis as it induces MHC Class I restricted Cytotoxic T lymphocytes and MHC class II restricted Th1 immunity and low cost associated with this. Whereas BCG vaccine has a poor CD8+ T cell responses. The disadvantage of DNA vaccines relies on poor immunogenicity due to broad immune responses in exposure to dominant and subdominant epitopes of several antigens as observed in the studies conducted in several preclinical models. Some of those problems have been overcome at the stage of design and formulation of DNA vaccines as reported elsewhere [33].

5.2 Nanoparticle/Nanobead-Based Vaccines

Nanoparticles may be classified in several types, namely nanospheres, nanocapsules, and nanomicelle which is mostly differentiated by the degree of porosity being governed by varying extent of intramolecular cross-linking. The nanoparticulate anti-tuberculosis vaccine delivery systems with size range of 300–600 nm may be up-taken efficiently by APCs and induce Th1 response, requisite against TB infection whereas the micron-sized particles induce Th2 immune responses [34]. The advantages of using such delivery modules are quite a lot to enlist. The high-encapsulation efficiency, ability to present multiple antigens directly to APCs while

preventing early enzymatic, endosomal degradation even from the degradation at high temperature which ultimately enables the antigens with adjuvants reach into the cytosol to initiate signaling cascades by Toll-like receptors (TLRs). This probably justifies the immediate immuno-stimulation by nanoparticle-based vaccines according to the reported literature [35]. It has been observed that Ag85A, tuberculosis-specific antigen when fused with heparin binding hemagglutinin adhesion protein (HBHA) and further encapsulated in nanoparticles formulated from carnauba wax, induced mycobacterial adherence and increased contact with pulmonary alveolar cells. Mice models already vaccinated with BCG received this novel vaccine as a prime-boost approach and showed vigorous immuno-modulation while presenting the nanoparticles themselves as the microorganism *Mycobacterium tuberculosis* on entering the host system [36]. Another group developed one fusion protein comprising three anti-tubercular antigens (Acr, Ag85B, and HBHA) on the surface of carnauba wax-based nanoparticles to develop novel mucosal vaccine to target TB (Nano-FP1). Drastic improvement in bacterial control was found with significant induction of cellular immune response, i.e., CD4+ and CD8+ T cell, resident memory T cells in lungs as well as humoral immune response by elevating the generation of antigen specific IgA and IgG [37]. Negligible production of harmful inflammatory mediators in this approach may indicate towards improved vaccine efficacy. Curcumin nanoparticles, in another study, have been found to increase the central long-lasting memory pool of CD4+ and CD8+ T cells especially in spleen and lungs when studied in BCG-vaccinated mice challenged by *M. tuberculosis* infection; although the particles are not used as vaccine delivery modules or adjuvants [38]. Pathogenesis of the disease was reduced as well. Carbon, silica, and gold-based nanoparticles also have been explored for its potential to deliver anti-TB vaccines. Although not for delivering vaccines or subunit antigens, mesoporous silica nanoparticles were developed where polyethyleneimine coating effectively released rifampicin and pH-controlled release of isoniazid took place from dissolving of cyclodextrin plugs at acidic environment [39]. These particles are observed to be engulfed efficiently by the macrophages, then being directed towards acidic endosomes thereby causing intracellular drug release at a high amount. This approach might be effective in vaccine delivery as the pathogenesis of *M. tuberculosis* at the acidified phagosomes may be correlated with the mechanism of acid-resistance of the said pathogen. Literature suggests aerosol route of administration to be more suitable for delivery of nanoparticle-based TB vaccines compared to other routes, whereas microparticles showed more effectiveness than their nano-counterpart [40].

Natural protein-based polymers including collagen, albumin, and gelatin also have the potential to be used as adjuvant/vaccine delivery systems to target TB due to their intrinsic property of mucoadhesion [41]. Nanoparticles formulated from cationic polymers or having positive surface charge tend to interact electrostatically with the anionic cell membranes thereby improving the uptake of antigens to macrophages and DCs especially for micron size particles. Smaller nanoparticles do not seem to be impacted more. Administration of anti-TB DNA vaccine tagged with Fe_3O_4-Glu-polyethyleneimine nanoparticle in tuberculosis-infected mice has been

found to lead to durable, potent, long-lasting immune response when compared to that exerted by naked DNA vaccines [42]. Chitosan and its derivatives also being positively charged polymers facilitate in efficient uptake of antigens into the cell while elevating levels of anti-TB antibody, humoral and cellular immunity, secretion of interferon-gamma on administration of nanoparticles via parenteral and nasal mode [43]. Chitosan, however, may induce hemolysis and other cellular toxicity due to enhanced cellular interaction which might be reduced by coating with other negatively charged compounds such as alginate. Alginate nanoparticles themselves showed higher chemotaxis by bone marrow dendritic cells and presentation of antigen to the T cells which was validated by decreased load of bacilli in spleen and lung with a little damage to the respiratory system [44]. PLGA may be assumed as the mostly used biodegradable synthetic polymers among many for the same purpose. A newly published study evaluated the activity of aerosolized mycobacterial antigen (H4, fusion of antigens Ag85B and TB10.4, and H28, fusing the H4 backbone with Rv2660c, additional TB-latency antigen) self-assembled into polyester nanoparticles exerted specific immune response without inducing cross-reactive antibodies to host cell protein impurities as well as causing no potent safety issues in tuberculosis infected mice [45]. The literature showed that PLGA nanoparticles have not only exhibited structural diversity, biocompatibility but also improved antigen uptake by DCs followed by tunable release of antigens leading to sustained high-immune Th1 and Th2 responses [46]. Either on surface modification of PLGA nanoparticles with various biomaterials such as mannose, cationic lipids, or use of the adjuvants has shown improved stability and efficacy in terms of antigen presentation to DCs; although encapsulation efficiency, morphology, and yield have been compromised [47, 48]. Antigen encapsulated within polylactic acid (PLA) microparticles exhibited high immunogenicity and induced cellular immune responses [49]. Non-biodegradable synthetic polymers have not been used for the same purpose except a few instances involving poloxamers [41].

5.3 Liposome-Based Vaccines

Liposomes are spherical concentric vesicles formulated from amphiphilic bilayers usually ranged between 20 nm and 10 μm. The diversity of liposomes as vaccine delivery modules lies in the embedding of hydrophobic compounds in lipid bilayer, whereas the hydrophilic compounds may be trapped within aqueous core of the liposomes. The liposomes are reportedly eliminated by the reticulo-endothelial system (RES) thereby reducing drug efficacy which might be improved by PEGylation of liposomes to reduce interaction with RES and increase circulation of those in blood. Liposomes have the ability to interact with the immunostimulatory Toll-like receptors and to release antigens gradually from a depot-like system. Diogo et al. developed phosphatidylserine liposome-based vaccine delivery system to enhance BCG-primed protection against tuberculosis infected mice [50]. Phosphatidylserine usually is present in the inner surface of cell membrane but appears on the outer

surface of early apoptotic cell membrane. This phenomenon has been mimicked in this formulation where this typical feature of the dying cells supposed to help to be recognized by the APCs and antigens may be engulfed by macrophages and DCs. T cell responses were exhibited thereafter. Among all different types, the cationic liposomes, which are formulated from neutral and cationic lipids induce strong Th1 immune response requisite to target tuberculosis [51]. Dimethyldioctadecylammonium (DDA) is one of the extensively used cationic liposome adjuvants, however it shows insufficient immune response when used alone with TB subunit vaccines [52]. DDA in combination with other cationic liposome adjuvant such as MPL, α,α′-trehalose 6,6′-dibehenate (TDB) leads to improved interaction with Toll like Receptors, stimulation of APCs, differentiation of T helper cell lineage to Th1, Th2, Th17 cells, induction of both humoral and cellular immunity (CD4+ and CD8+ both) at a high level. CAF01(DDA-TDB) containing the antigens Ag85A and ESAT-6 has validated this claim and appears to be the only one cationic liposome based TB subunit vaccine reached up to the clinical trial [53]. Liu et al. also constructed a novel subunit vaccine to target tuberculosis by combining cholesterol, DDA, and poly I:C to form cationic liposome adjuvant [54]. Poly I:C improved the stability of the vaccine as well as it, being a ligand to Toll like receptor type 3 helped to release antigens ESAT6-Ag85B-MPT64(190–198)-Mtb8.4-Rv2626c (LT70) encapsulated in the form of fusion protein. Ko and group observed a similar outcome with another liposome-based TB subunit vaccine made up of de-O-acylated lipooligosaccharide (dLOS)/DDA which induced Th1 immune response and increased the protective efficacy against the infection caused by *Mycobacterium tuberculosis* as well [55]. Despite the possibilities to be a potential adjuvant, cationic liposomes are reported to have cytotoxicity which may further compromise the in vivo stability and capacity to exhibit strong immune responses especially Th1 [56].

5.4 Dendrimer-Based Vaccines

Dendrimers may be defined as spherical compact nanosized macromolecules with a highly branched three-dimensional architecture. There are exponential number of branches of hydrophilic or hydrophobic moieties which can be seen radiating out from a central core. The sizes and shape of the dendrimers can be controlled according to the specification desired. Therapeutically active molecules may be attached to the terminal branches by means of chemical bond formation or complexation. The mostly used type of dendrimers is polyamidoamine (PAMAM) which has a potential to bind to multiple receptors simultaneously enabling them to activate several complex biological processes. Modified dendrimers, i.e., conjugated with mannose have exhibited potent immunogenic responses whiling rising the level of CD4 and CD8 T cells [57]. Several other types of dendrimers are commercially available, namely L-DOPA (3,4-dihydroxy-l-phenylalanine), PPI [Poly (propyleneimine)] and PEHAM [poly(etherhydroxylamine)], registered by different companies. Dendrimes are found to be used to deliver vaccines to treat cancer, AIDS, foot and

mouth disease, MAPs (multiple antigen peptides) are the similar macromolecules with radiating peptides and used extensively in serological assays. The only MAP-based vaccine against tuberculosis, which has been reported to date generated significant immune response while releasing ESAT-6, the TB-specific peptide antigen and demonstrated protection against tuberculosis infection [25].

5.5 Nanosuspensions and Nanoemulsion-Based Vaccines

The drug molecules with high-molecular weight and limited aqueous solubility often lead to insufficiencies in target receptor binding. Excessively high-surface area offered by nanosized particles of the same therapeutically active molecules may overcome the issue thereby enhancing the solubility and bioavailability. These nanosuspension and nanoemulsions are nanosized heterogeneous dispersions of polymeric or lipoidal carriers of drugs (here, antigens in case of vaccines) stabilized by suitable surfactants. Flexibility in selection of routes of administration even by intranasal or directly into respiratory tract results into high-lung deposition and coverage due to large surface area of the formulation. The early analysis of bacterial lipopolysaccharides leads to the development of potent adjuvant monophosphoryl lipid A or MPL while filtering off the toxic effects. Comparison between different commercial vaccines formulated from the same adjuvant such as saponin-based liposomes, squalene-oil-in-water emulsions, nano-suspensions when conjugated with aluminum hydroxide revealed comparable immunogenic responses between nanoemulsion and liposome [58]. The nanoemulsion exhibited more protection against further exposure to *Mycobacterium tuberculosis*, induced Th1 specific CD4+ T cells as well as specific antibody titers at a high level against the same antigen according to the data acquired from randomized, double blind Phase 2 trial [59]. The similar trend has also been observed when compared between the same formulations prepared using GLA (glucopyranosyl lipid adjuvant), another synthetic adjuvant with TLR4 agonistic activity which paved the way for commercialization of vaccines for tuberculosis and malaria in the near future. On conjugation with different compounds, GLA has also been found to stimulate cytokine production similar to MPL in response to exposure to specific antigen by means of screening of biological responses via physicochemical characterization [60]. Investigation via placebo-controlled Phase 1 clinical trial of ID93 (comprising *Mycobacterium tuberculosis* specific antigen Mtb72f) conjugated with GLA-based squalene-oil-in-water emulsion not only proved the safety among individuals but the Phase 2a study also demonstrated rise in multi-functional antibody titers, robust CD4+ T cell immune responses while broadening the BCG protection coverage [61]. The outcome was found to be somehow different in the in vitro experiments, where aqueous nanosuspension showed dramatically high titer of cytokine as well as antibody. Screening of the adjuvants and variety in the formulation by means of Limulus Amoebocyte Lysate Assay and Human macrophage cellular cytokine production assay concluded that the potent immune system activators may not be the same both

in in vitro and in vivo. The nano suspension elicited the remarkable reduction in the antigen-specific Th1 responses even in the preclinical models so as in the clinical trials [62].

5.6 Virus-Like Particle (VLP) Based Vaccines

As continuation to the virus-like particles as discussed in a previous section of the same article, these presumably can act as the adjuvant not only by enhancing the antigen delivery but also by improving the presentation of the antigens to the T cells, macrophages. Being in nanometer range, VLPs show improved stability, potential to cross biological barriers ensuring targeted intracellular uptake, potent CD4, CD8, Th1 immune responses once the antigens are processed. Dhanasooraj et al. fused the gene of cfp-10, a potent mycobacterial antigen on non-infectious hepatitis-B core (HBc) virus nano-sized viral-like particles, and improved the intracellular uptake of the antigens in to antigen presenting cells [63]. This culture filtrate protein (cfp 10), as a T cell antigen, is recognized to be an early marker of tuberculosis and it has well-characterized epitopes sufficient to raise the Th1 immune response as well as release of interferon-gamma. This study corroborated with the outcome of another previous study which experimentally showed fusion of ESAT-6 antigen-specific gene on the major immunodominant portion of same HBc VLPs high magnitude of CD4+/ CD8+ T cell responses along with rise in ESAT-6 specific antibodies [64].

5.7 Virosome-Based Vaccines

Virosomes are biodegradable unilamellar nanosized (60–200 nm approximately) vesicles surrounded by phospholipids with glycoprotein epitopes interspersed either on the viral surface or into the hollow cores. These are often termed as “artificial virus” although being non-replicative in nature and may interact with the target cells due to similarity with mammalian cell membrane thereby releasing the antigens inside the cells. Pathogenic antigens may be captured within lumen of virosomes or adsorbed on the surface by means of chemical cross-linking. These antigens are effectively phagocytosed by the APCs and processed via MHC class 1 antigen processing pathway to elicit potent immunogenicity thus making virosome a suitable vaccine delivery platform. In addition to that, hydrophobic drugs may be directly incorporated into phospholipid membrane of the virosome, whereas the hydrophilic drug moieties can be carried inside the core. Coating of the virosomes with hydrophilic polymers such as poly-(ethylene glycol) is found to enhance circulation time while escaping early uptake by reticuloendothelial system (RES) [65]. The only study found in the literature worked on Sendai virosome modified from

hemagglutinating virus of Japan [66]. Increased Th1 specific cytokine release activated cytotoxic T lymphocytes along with rising levels of Natural Killer cells were observed after intramuscular administration of vaccines made up of Sendai virosomes encapsulated with Ag85A antigen fused pAAVCMV plasmid. This study somehow showed the potential of virosome as an efficient carrier to deliver therapeutic agents/antigens to target tuberculosis.

5.8 Immune-Stimulating Complex (ISCOM)-Based Vaccines

Immune stimulating complexes (ISCOMs) can be briefly described as self-assembled complex formed as a result of micelles typically arranged in a shape of pentagonal dodecahedron. These colloidal formulations comprised micelles having approximately 40–60 nm in size, formulated from the combination of cholesterol, phospholipids (usually either phosphatidylcholine or phosphatidylserine), and saponin in the presence of a non-ionic surfactant. The chemical hydrophobic interaction between the lipids and cholesterol as well as the negative charge at the surface of the micelles together ensures this unique structural organization, albeit stable. A wide variety of pathogens, antigenic peptides may be encapsulated in these complexes including, but not limited to hepatitis B, herpes simplex virus, influenza. Sustained delivery of the antigens has been observed on intradermal or mucosal administration of the vaccine and cell mediated as well as humoral immunity has been induced once the antigens or proteins are released from the hollow center of the complexes. Andersen et al. developed an intranasal vaccine while combining a tuberculosis specific fusion protein Ag85B-ESAT-6 and an adjuvant vector CTA1-DD conjugated with ISCOMs, which resulted in effective Th1 specific immunogenic activities. It also led to the secretion of interferon gamma at high amount, and CD4+ T cell responses were observed thereafter [67]. Concluding with the in vivo responses in the experimental animals, huge localized antibody responses at the infected region especially at the animals primarily boosted with BCG vaccine were found along with instances of causing fewer burdens of tubercle bacilli in the lungs of the same group of animals when compared with the non-vaccinated animals. Another more recent approach involved the formulation of ISCOMs comprising tuberculosis-specific antigen Ag85A following the process of ultrasonication [68]. Determination of antibody titer and immunoglobulin isotyping from samples of mice revealed that antigens were presented and processed by APCS to secrete several cytokines required to produce humoral and cellular immunity. Despite numerous advantages, no ISCOM-based tuberculosis vaccine still has reached to clinical trials to be a potential alternative to BCG possibly due to its extremely hydrophobic nature. It seems to be quite challenging to encapsulate the hydrophilic antigens into the core of those micelles [69].

6 Nanotechnology-Based Vaccine Clinical Trials

BCG vaccination is a part of childhood immunization regime as recommended by WHO, and the coverage of the vaccines was found to be about 90% across more than hundred countries by the year 2018 [70]. Although TB poses to be a major threat to infants especially in the countries where incidence of tuberculosis is high, unvaccinated as well as tuberculin negative adults exposed to multidrug resistant TB patient and patient infected with LTBI can also be vaccinated with BCG. BCG is observed to secrete specific antibodies, to activate B cells, to induce protective immune responses while helping in the proliferation of Th1 type CD4+/ CD8+ T cells and memory T cells. In addition to that, correlation if the infection with secretion of IgA as well as Type I/II interferon has also been found [71]. A number of preclinical evaluations has been performed either by changing the routes of administration of BCG vaccine namely, intravenous, intradermal, aerosol or by genetic modification of BCG to design a mutant sub-strain to overcome the lack of protection against respiratory tuberculosis by means of conventional BCG vaccination [23, 72, 73]. Unclear mechanism of protection from further exposure to *Mycobacterium tuberculosis* after vaccination and lack of specific immune markers to track the progress of the prevention makes the conduction of Phase IIb and Phase III of clinical trials quite challenging in spite of having data of safety and immunogenicity. About 14 new vaccines are awaiting at different stages of clinical trials according to the current status [74].

Among all, seven vaccines are either live attenuated vaccines or inactivated mycobacteria. VPM1002, being a recombinant BCG vaccine candidate, is currently in Phase III study entitled as priME study (NCT04351685) and the findings indicate that this can be a potential alternative to BCG vaccination at birth. It is said to contain a protein listeriolysin O, extracted from *Listeria monocytogenes* and the protein along with Urease C gene is found to improve the availability and recognition of TB antigens to the T cells of the immune system. Other inactivated whole cell mycobacteria candidates (RUTI, Vaccae, MIP, DAR-901) are, in general, found to overexpress antigens at multistage of the infection including latency antigens against latent tuberculosis infection with a broad protection coverage in neonates, adolescents, young adults and even in immuno-compromised patients [75]. RUTI, especially being comprised of liposomes containing stress cultured tuberculosis bacilli, subjected to further fragmentation and detoxification by means of removal of glycolipids from cell wall improves the presentation of the antigens and elicits strong Th1, Th2, Th3 responses [76]. Status of the clinical trials of some of the subunit as well as adjuvant vaccines has already been discussed in previous sections. Ad5HuAg85A, ChAdOx85A/MVA85A, and TB/FLU-04 L all these vaccines are developed by recombinant viral vectors and successfully proved their respective safety and immunogenicity according to Phase I study. The evaluation of protective efficacy covered by the vaccines in BCG-naïve as well as BCG-immunized individuals as booster regimen or as an individual protective therapy seems to be started [74]. The phase I and phase II data of the adjuvanted vaccine candidates (D93 +

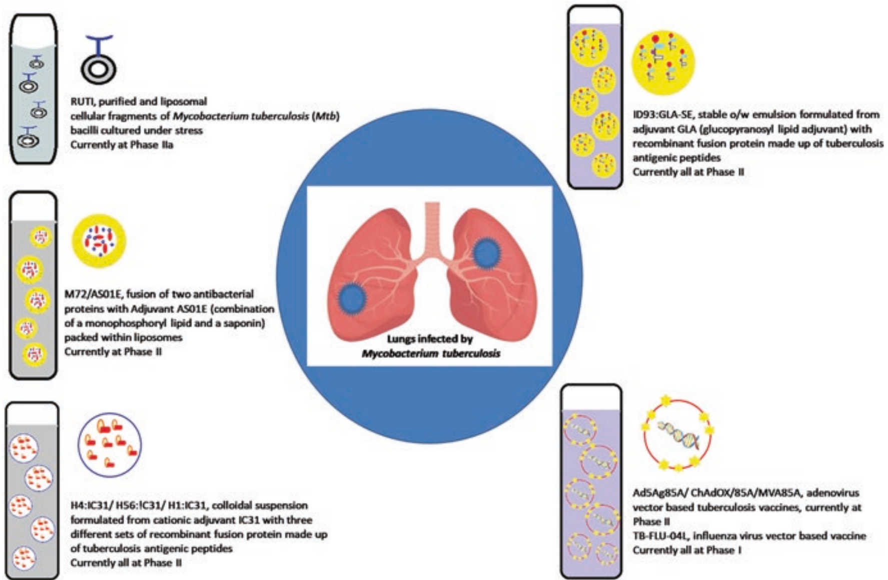

Fig. 2 Brief representation of nanotechnology-based vaccines to target tuberculosis at different stages of clinical trials till date

GLA-SE), H56:IC31, M72/AS01E, GamTBvac also showed adequate safety, tolerability, and immunogenicity against tuberculosis infection. All the above-mentioned vaccines are considered mostly as pre- and post-exposure vaccines which, all over, made a significant progress through clinical trial and at least one is expected to complete the hurdles to make an effective vaccine for all individuals even against latent tuberculosis. The current status of the nanovaccines against tuberculosis has been represented in Fig. 2.

7 Conclusion

The current status of vaccine candidates in clinical trial or in vitro experiments as published in several journals strongly indicates some of the challenges in development of TB vaccine right after BCG. While prioritizing a safe, affordable, effective vaccines alternative to BCG according to WHO, specific populations should also be targeted to achieve protection coverage in neonates and to halt transmission of tuberculosis among adolescents as well as adults. This population-based factor came to notice once the clinical trial data of MVA5A in infant population suggested that the effectiveness of that particular vaccine candidate might be more suitable in adults to reduce transmission rate especially in high-burden TB endemic zones since the disease transmission in infants or neonates is not commonly observed

[77]. Considering all the factors such as age-structured models, different epidemiological zones, design of trials, BCG sensitization, and mathematical modeling studies were performed to evaluate unbiased impact and mechanism of vaccines, and it was found that trial follow-up periods heavily rely on distinguishing delay in incubation of causative pathogen from prevention of rapid progression of disease. Modeling studies on impact evaluations may be combined therefore with the trial data to improve the immunity conferred by vaccines on various populations which might help in taking decisions by funding agencies. Although the subunit vaccines offer protective immune response primed with prior BCG vaccination, whole cell vaccines irrespective of live or attenuated ones are hypothesized to have a broad range of antigen including lipid, carbohydrates which may or may not be antigenic. It may result in unrestricted immune responses via inducing T-cells, B-cells, natural killer cells and lead to long-lasting immune memory thereafter. Advanced development of novel vaccines also needs sufficient knowledge in the mechanism of complex immunity in response to infection caused by *Mycobacterium tuberculosis*. Discovery of novel biomarker for tracking the effectiveness of tuberculosis vaccine candidates at different stages of the disease might help in better understanding of the immunity enhancement strategies and lead the way in development of immunodominant antigens to elicit specific immune responses.

References

1. Pollard AJ, Bijker EM. A guide to vaccinology: from basic principles to new developments. Nat Rev Immunol. 2021;21:83–100.
2. Bloom DE, Black S, Salisbury D, Rappuoli R. Antimicrobial resistance and the role of vaccines. Proc Natl Acad Sci. 2018;115:12868–71.
3. Collins KA, Snaith R, Cottingham MG, Gilbert SC, Hill AVS. Enhancing protective immunity to malaria with a highly immunogenic virus-like particle vaccine. Sci Rep. 2017;7:46621.
4. González-Miró M, Rodríguez-Noda LM, Fariñas-Medina M, Cedré-Marrero B, Madariaga-Zarza S. Bioengineered polyester beads co-displaying protein and carbohydrate-based antigens induce protective immunity against bacterial infection. Sci Rep. 2018;8:1888.
5. Greenwood B. The contribution of vaccination to global health: past, present and future. Philos Trans R Soc Lond Ser B Biol Sci. 2014;369:20130433.
6. Youde J. Cattle scourge no more. The eradication of rinderpest and its lessons for global health campaigns. Politics Life Sci. 2013;32:43–57.
7. Vos A, Neumann G, Hundt B, Neubert A. Chapter twenty two - Attenuated vaccines for veterinary use. In: Rupprecht C, Nagarajan T, editors. Current laboratory techniques in rabies diagnosis, research and prevention. Amsterdam: Academic; 2014. p. 237–44.
8. Gerety RJ, Ellis RW. Plasma-derived vs recombinant hepatitis B vaccine. JAMA. 1987;258:1474.
9. Jennings GT, Bachmann MF. Immunodrugs: therapeutic VLP-based vaccines for chronic diseases. Annu Rev Pharmacol Toxicol. 2009;49:303–26.
10. Smith DM, Simon JK, Baker JR Jr. Applications of nanotechnology for immunology. Nat Rev Immunol. 2013;13:592–605.
11. Martínez-Sernández V, Figueiras A. Central nervous system demyelinating diseases and recombinant hepatitis B vaccination: a critical systematic review of scientific production. J Neurol. 2013;260:1951–9.

12. Scherer EM, Leaman DP, Zwick MB, McMichael AJ, Burton DR. Aromatic residues at the edge of the antibody combining site facilitate viral glycoprotein recognition through membrane interactions. Proc Natl Acad Sci U S A. 2010;107:1529–34.
13. Mundargi RC, Babu VR, Rangaswamy V, Patel P, Aminabhavi TM. Nano/micro technologies for delivering macromolecular therapeutics using poly(D,L-lactide-co-glycolide) and its derivatives. J Control Release. 2008;125:193–209.
14. Raman S, Machaidze G, Lustig A, Aebi U, Burkhard P. Structure-based design of peptides that self-assemble into regular polyhedral nanoparticles. Nanomedicine. 2006;2:95–102.
15. Hunter RL. The pathogenesis of tuberculosis: the early infiltrate of post-primary (adult pulmonary) tuberculosis: a distinct disease entity. Front Immunol. 2018;9:2108.
16. Kumar P. Adult pulmonary tuberculosis as a pathological manifestation of hyperactive antimycobacterial immune response. Clin Transl Med. 2016;5:38.
17. Andersen P, Scriba TJ. Moving tuberculosis vaccines from theory to practice. Nat Rev Immunol. 2019;19:550–62.
18. Kagina BM, Abel B, Scriba TJ, Hughes EJ, Keyser A, Soares A, et al. Specific T cell frequency and cytokine expression profile do not correlate with protection against tuberculosis after bacillus Calmette-Guérin vaccination of newborns. Am J Respir Crit Care Med. 2010;182:1073–9.
19. Sallin MA, Kauffman KD, Riou C, Du Bruyn E. Host resistance to pulmonary Mycobacterium tuberculosis infection requires CD153 expression. Nat Microbiol. 2018;3:1198–205.
20. Yang JD, Mott D. Mycobacterium tuberculosis-specific CD4+ and CD8+ T cells differ in their capacity to recognize infected macrophages. PLoS Pathog. 2018;14:e1007060.
21. Nyendak M, Swarbrick GM, Duncan A, Cansler M, Huff EW, Hokey D, et al. Adenovirally-induced polyfunctional T cells do not necessarily recognize the infected target: lessons from a phase I trial of the AERAS-402 vaccine. Sci Rep. 2016;6:36355.
22. Lin PL, Flynn JL. CD8 T cells and Mycobacterium tuberculosis infection. Semin Immunopathol. 2015;37:239–49.
23. Dijkman K, Sombroek CC, Vervenne RAW, Hofman SO, Boot C, Remarque EJ, et al. Prevention of tuberculosis infection and disease by local BCG in repeatedly exposed rhesus macaques. Nat Med. 2019;25:255–62.
24. Nasiruddin M, Neyaz MK, Das S. Nanotechnology-based approach in tuberculosis treatment. Tuberc Res Treat. 2017;2017:4920209.
25. Ojha R, Pandey RK, Prajapati VK. Chapter 5 - Vaccine delivery systems against tuberculosis. In: Kesharwani P, editor. Nanotechnology based approaches for tuberculosis treatment. London: Academic; 2020. p. 75–90.
26. Hussein J, Zewdie M. A phase I, open-label trial on the safety and immunogenicity of the adjuvanted tuberculosis subunit vaccine H1/IC31® in people living in a TB-endemic area. Trails. 2018;19:24.
27. Bibi S, Ullah I, Zhu B, Adnan M, Liaqat R, Kong W-B, et al. In silico analysis of epitope-based vaccine candidate against tuberculosis using reverse vaccinology. Sci Rep. 2021;11:1249.
28. Lowrie DB. DNA vaccines against tuberculosis. Curr Opin Mol Ther. 1999;1:30–3.
29. Lu M, Xia ZY, Bao L. A *Mycobacterium bovis* BCG-naked DNA prime-boost vaccination strategy induced $CD4^+$ and $CD8^+$ T-cell response against *Mycobacterium tuberculosis* immunogens. J Immunol Res. 2014;2014:395626.
30. Dalirfardouei R, Tafaghodi M, Meshkat Z, Najafi A, Gholoobi A, Nabavinia M, et al. A novel formulation of Mtb72F DNA vaccine for immunization against tuberculosis. Iran J Basic Med Sci. 2020;23:826–32.
31. Rashidian S, Teimourpour R, Meshkat Z. Designing and construction of a DNA vaccine encoding tb10.4 gene of Mycobacterium tuberculosis. Iran J Pathol. 2016;11:112–9.
32. Tang J, Liang J, Cai Y, Zhou J, Tan Z, Tang X, et al. A novel DNA vaccine against *Mycobacterium tuberculosis* infection (VAC4P.1109). J Immunol. 2015;194:72.14.
33. Bruffaerts N, Huygen K, Romano M. DNA vaccines against tuberculosis. Expert Opin Biol Ther. 2014;14:1801–13.

34. Tafaghodi M, Khademi F, shiehzadeh F, Firouzi Z. Chapter 8 - Polymer-based nanoparticles as delivery systems for treatment and vaccination of tuberculosis. In: Kesharwani P, editor. Nanotechnology based approaches for tuberculosis treatment. London: Academic; 2020. p. 123–42.
35. Pati R, Shevtsov M, Sonawane A. Nanoparticle vaccines against infectious diseases. Front Immunol. 2018;9:2224.
36. Stylianou E, Diogo GR, Pepponi I, van Dolleweerd C, Arias MA, Locht C, et al. Mucosal delivery of antigen-coated nanoparticles to lungs confers protective immunity against tuberculosis infection in mice. Eur J Immunol. 2014;44:440–9.
37. Hart P, Copland A, Diogo GR, Harris S, Spallek R, Oehlmann W, et al. Nanoparticle-fusion protein complexes protect against Mycobacterium tuberculosis infection. Mol Ther. 2018;26:822–33.
38. Ahmad S, Bhattacharya D, Kar S, Ranganathan A, Van Kaer L, Das G. Curcumin nanoparticles enhance Mycobacterium bovis BCG vaccine efficacy by modulating host immune responses. Infect Immun. 2019;87:e00291–19.
39. Clemens DL, Lee B-Y, Xue M, Thomas CR, Meng H, Ferris D, et al. Targeted intracellular delivery of antituberculosis drugs to Mycobacterium tuberculosis-infected macrophages via functionalized mesoporous silica nanoparticles. Antimicrob Agents Chemother. 2012;56:2535–45.
40. Muttil P, Wang C, Hickey AJ. Inhaled drug delivery for tuberculosis therapy. Pharm Res. 2009;26:2401–16.
41. Khademi F, Derakhshan M, Yousefi-Avarvand A, Tafaghodi M. Potential of polymeric particles as future vaccine delivery systems/adjuvants for parenteral and non-parenteral immunization against tuberculosis: a systematic review. Iran J Basic Med Sci. 2018;21:116–23.
42. Yu F, Wang J, Dou J, Yang H, He X, Xu W, et al. Nanoparticle-based adjuvant for enhanced protective efficacy of DNA vaccine Ag85A-ESAT-6-IL-21 against Mycobacterium tuberculosis infection. Nanomedicine. 2012;8:1337–44.
43. Khademi F, Taheri R-A, Yousefi Avarvand A, Vaez H, Momtazi-Borojeni AA, Soleimanpour S. Are chitosan natural polymers suitable as adjuvant/delivery system for anti-tuberculosis vaccines? Microb Pathog. 2018;121:218–23.
44. Nagpal PS, Kesarwani A, Sahu P, Upadhyay P. Aerosol immunization by alginate coated mycobacterium (BCG/MIP) particles provide enhanced immune response and protective efficacy than aerosol of plain mycobacterium against M.tb. H37Rv infection in mice. BMC Infect Dis. 2019;19:568.
45. Chen S, Quan DH, Wang XT, Sandford S, Kirman JR, Britton WJ, et al. Particulate mycobacterial vaccines induce protective immunity against tuberculosis in mice. Nano. 2021;11:2060.
46. Khademi F, Yousefi-Avarvand A, Derakhshan M, Abbaspour MR, Sadri K, Tafaghodi M. Formulation and optimization of a new cationic lipid-modified PLGA nanoparticle as delivery system for Mycobacterium tuberculosis HspX/EsxS fusion protein: an experimental design. Iran J Pharm Res. 2019;18:446–58.
47. Khademi F, Derakhshan M, Yousefi-Avarvand A, Najafi A, Tafaghodi M. A novel antigen of Mycobacterium tuberculosis and MPLA adjuvant co-entrapped into PLGA:DDA hybrid nanoparticles stimulates mucosal and systemic immunity. Microb Pathog. 2018;125:507–13.
48. Khademi F, Yousefi A, Derakhshan M, Najafi A, Tafaghodi M. Enhancing immunogenicity of novel multistage subunit vaccine of Mycobacterium tuberculosis using PLGA:DDA hybrid nanoparticles and MPLA: subcutaneous administration. Iran J Basic Med Sci. 2019;22:893–900.
49. Carpenter ZK, Williamson ED, Eyles JE. Mucosal delivery of microparticle encapsulated ESAT-6 induces robust cell-mediated responses in the lung milieu. J Control Release. 2005;104:67–77.
50. Diogo GR, Hart P, Copland A, Kim M-Y, Tran AC, Poerio N, et al. Immunization with Mycobacterium tuberculosis antigens encapsulated in phosphatidylserine liposomes improves protection afforded by BCG. Front Immunol. 2019;10:1349.

51. Hu Y, Ehrich M, Fuhrman K, Zhang C. In vitro performance of lipid-PLGA hybrid nanoparticles as an antigen delivery system: lipid composition matters. Nanoscale Res Lett. 2014;9:434.
52. Christensen D, Korsholm KS, Rosenkrands I, Lindenstrøm T, Andersen P, Agger EM. Cationic liposomes as vaccine adjuvants. Expert Rev Vaccines. 2007;6:785–96.
53. Christensen D. Chapter 17 - Development and evaluation of CAF01. In: Schijns VEJC, O'Hagan DT, editors. Immunopotentiators in modern vaccines. Second ed. Amsterdam: Academic; 2017. p. 333–45.
54. Liu X, Da Z, Wang Y, Niu H, Li R, Yu H, et al. A novel liposome adjuvant DPC mediates mycobacterium tuberculosis subunit vaccine well to induce cell-mediated immunity and high protective efficacy in mice. Vaccine. 2016;34:1370–8.
55. Ko A, Wui SR, Ryu JI, Lee YJ, Hien DTT, Rhee I, et al. Potentiation of Th1-type immune responses to Mycobacterium tuberculosis antigens in mice by cationic liposomes combined with De-O-Acylated lipooligosaccharide. J Microbiol Biotechnol. 2018;28:136–44.
56. Fan Y, Sahdev P, Ochyl LJ, Akerberg J, Moon JJ. Cationic liposome-hyaluronic acid hybrid nanoparticles for intranasal vaccination with subunit antigens. J Control Release. 2015;208:121–9.
57. Sheng KC, Kalkanidis M, Pouniotis DS, Esparon S, Tang CK, Apostolopoulos V, et al. Delivery of antigen using a novel mannosylated dendrimer potentiates immunogenicity in vitro and in vivo. Eur J Immunol. 2008;38:424–36.
58. Kester KE, Cummings JF, Ofori-Anyinam O, Ockenhouse CF, Krzych U, Moris P, et al. Randomized, double-blind, phase 2a trial of falciparum malaria vaccines RTS,S/AS01B and RTS,S/AS02A in malaria-naive adults: safety, efficacy, and immunologic associates of protection. J Infect Dis. 2009;200:337–46.
59. Leroux-Roels I, Forgus S, De Boever F, Clement F, Demoitié MA, Mettens P, et al. Improved $CD4^{+}$ T cell responses to Mycobacterium tuberculosis in PPD-negative adults by M72/AS01 as compared to the M72/AS02 and Mtb72F/AS02 tuberculosis candidate vaccine formulations: a randomized trial. Vaccine. 2013;31:2196–206.
60. Dowling QM, Sivananthan SJ, Guderian JA, Moutaftsi M, Chesko JD, Fox CB, et al. Modulating potency: physicochemical characteristics are a determining factor of TLR4-agonist nanosuspension activity. J Pharm Sci. 2014;103:879–89.
61. Penn-Nicholson A, Tameris M, Smit E, Day TA, Musvosvi M, Jayashankar L, et al. Safety and immunogenicity of the novel tuberculosis vaccine ID93 + GLA-SE in BCG-vaccinated healthy adults in South Africa: a randomised, double-blind, placebo-controlled phase 1 trial. Lancet Respir Med. 2018;6:287–98.
62. Misquith A, Fung HW, Dowling QM, Guderian JA, Vedvick TS, Fox CB. In vitro evaluation of TLR4 agonist activity: formulation effects. Colloids Surf B Biointerfaces. 2014;113:312–9.
63. Dhanasooraj D, Kumar RA, Mundayoor S. Vaccine delivery system for tuberculosis based on nano-sized hepatitis B virus core protein particles. Int J Nanomedicine. 2013;8:835–43.
64. Yin Y, Li H, Wu S, Dong D, Zhang J, Fu L, et al. Hepatitis B virus core particles displaying Mycobacterium tuberculosis antigen ESAT-6 enhance ESAT-6-specific immune responses. Vaccine. 2011;29:5645–51.
65. Asadi K, Gholami A. Virosome-based nanovaccines; a promising bioinspiration and biomimetic approach for preventing viral diseases: a review. Int J Biol Macromol. 2021;182:648–58.
66. Kaneda Y, Nakajima T, Nishikawa T, Yamamoto S, Ikegami H, Suzuki N, et al. Hemagglutinating virus of Japan (HVJ) envelope vector as a versatile gene delivery system. Mol Ther. 2002;6:219–26.
67. Andersen CS, Dietrich J, Agger EM, Lycke NY, Lövgren K, Andersen P. The combined CTA1-DD/ISCOMs vector is an effective intranasal adjuvant for boosting prior Mycobacterium bovis BCG immunity to mycobacterium tuberculosis. Infect Immun. 2007;75:408–16.
68. Pabreja S, Garg T, Rath G, Goyal AK. Mucosal vaccination against tuberculosis using Ag85A-loaded immunostimulating complexes. Artif Cells Nanomed Biotechnol. 2016;44:532–9.
69. Cruz-Bustos T, González-González G, Morales-Sanfrutos J, Megía-Fernández A, Santoyo-González F, Osuna A. Functionalization of immunostimulating complexes (ISCOMs) with

lipid vinyl sulfones and their application in immunological techniques and therapy. Int J Nanomedicine. 2012;7:5941–56.
70. Zwerling A, Behr MA, Verma A, Brewer TF, Menzies D, Pai M. The BCG World Atlas: a database of global BCG vaccination policies and practices. PLoS Med. 2011;8:e1001012-e.
71. Jacobs AJ, Mongkolsapaya J, Screaton GR, McShane H, Wilkinson RJ. Antibodies and tuberculosis. Tuberculosis (Edinb). 2016;101:102–13.
72. Darrah PA, Zeppa JJ, Maiello P, Hackney JA, Wadsworth MH, Hughes TK, et al. Prevention of tuberculosis in macaques after intravenous BCG immunization. Nature. 2020;577:95–102.
73. Sander P, Clark S, Petrera A, Vilaplana C, Meuli M, Selchow P, et al. Deletion of zmp1 improves Mycobacterium bovis BCG-mediated protection in a guinea pig model of tuberculosis. Vaccine. 2015;33:1353–9.
74. Martin C, Aguilo N, Marinova D, Gonzalo-Asensio J. Update on TB vaccine pipeline. Appl Sci. 2020;10:2632.
75. Scriba TJ, Kaufmann SHE, Henri Lambert P, Sanicas M, Martin C, Neyrolles O. Vaccination against tuberculosis with whole-cell mycobacterial vaccines. J Infect Dis. 2016;214:659–64.
76. Cardona PJ. RUTI: a new chance to shorten the treatment of latent tuberculosis infection. Tuberculosis (Edinb). 2006;86:273–89.
77. Tovar M, Arregui S, Marinova D, Martín C, Sanz J, Moreno Y. Bridging the gap between efficacy trials and model-based impact evaluation for new tuberculosis vaccines. Nat Commun. 2019;10:5457.

Disease Models in Malarial Research

Priyanka Banerjee and Saptarshi Sanyal

Abstract Malaria is a significant reason of thriving illness and death, and until now no vaccine is available. Various studies have utilized the animal models to understand the pathogenesis of infection, and a huge amount of data on parasite biology, regulation of immune system and disease methods have been achieved from these studies. Moreover, these standards have been used for pre-clinical testing of various drugs and vaccines. The highlights to study the disease models of malaria and pre-clinical vaccines evaluation conclude that the animal models have an intricate role that is used for testing which gets reflected in the environment of the infected individuals. The key mathematical models provide an unambiguous structure for understanding malaria transmission undercurrents in human population for over many years. With the impromptu threatening of the disease as a major source of death and disability, due to the modified environmental and socio-economic conditions, it is obligatory to make a critical valuation of the existing models and study their evolution and efficacy in describing the host–parasite mechanistic approach. The models that are developed, beginning from the basic, following an underlying hierarchical structure lands up to the different levels of complexity. The objective is intricating usage of some of the mathematical models, to describe malaria frequency, by involving the critical features of the host–vector–parasite interactions. Highlighting the evolution of the compartment models with the approach to summarize the modelling activity helps to reach a wider range of researchers working on epidemiology, transmission and other aspects of malaria which may simplify to additionally develop the apt models germane to the present scenario and aid to espouse better considerate of the modelling strategies to control the disease.

Keywords Malaria · Pathogenesis · Pre-clinical · Hierarchical · Evolution · Models · Control · Strategies

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1 Introduction

Malaria is an antiquated mortal and morbid disease having an enormous social, economic and health burden. It is largely present in the tropical countries. Even though malaria has been probed for hundreds of years, it is still considered as a major public health problem, acknowledged as endemic in 2008. The cases obtained were for children, primarily under 5 years of age [1]. With no efficient vaccines closely obtained, the anti-malarial drugs were losing its effectivity due to the parasites' evolution and resistance towards the drugs. Malaria has also expanded notoriety in current times, due to the geographical intervention. The unpredicted changes have effects on both the vector and parasite life cycle. The change in climate can affect the prevalence of the scattered growth that is responsible for spreading disease and producing less immunity. Malaria is produced by the protozoan parasite, Plasmodium. In humans, it is caused by *Plasmodium falciparum (P. falciparum*), *Plasmodium malariae (P. malariae)*, *Plasmodium ovale (P. ovale)* and *Plasmodium vivax (P. vivax)*. Among all the species, *P. falciparum* is the deadliest and the most common cause of infection. The parasite generally requires two hosts to complete its life cycle—the vector female Anopheles mosquito and human. The name malaria was given back in the year 1740. Malaria is both preventable and treatable. As per the World Health Organization (WHO) [1], the most effected region is India. The female mosquitos are accountable for spreading the Plasmodium parasite from one another between the twilight and dawn. The red blood corpuscles are damaged which is observed under the microscope, considered as a gold standard technique for diagnosing. The deferral value is obtained when observed under 100X visual focal point, using Giemsa. The visual observations of a patient suffering from malaria include positive symptoms of the disease, like, high temperature, cold, headache, vomiting, pain in muscles and fatigue. The pathogenesis needs to be studied or larger data needs to be collected to gather more information regarding the various animal models or mathematical models to develop. There are various models where pre-clinical interventions are needed. The approach of developing newer drug molecules or vaccine supplements could be obtained using various rodents' models. In such a case, the favoured experimental conditions reflect the role and studies of various organs that are sequestered by the parasite for identification of adaptive or innate immunity and leading to the development of newer approaches.

There have been numerous reasons to encourage the use of animal models to study malaria. Firstly, the models acknowledge investigation into the progress of the disease, which is more challenging in humans. It also enables studies of organs to which the parasite beholds, such as the spleen, lungs and brain. The use of non-lethal parasites in mice has identified the role of adaptive and innate immunity in intervening clearance and survival from infection. Finally, these studies have led to the development of novel vaccine approaches [2–4]. An approach is needed where the frequent terms used as severe and cerebral comes in conjunction and has an interchangeable approach, bears heterogenicity and severity like profound metabolic derangement, multi-organ failure, oedema, thrombocytopenia and anaemia. Adhesion-related pathology in malaria is distinct in placental infections of pregnant

women. A crucial feature of the parasite variant is their capability to bind with Chondroitin sulphate A (CSA) in the placenta [5], where they are believed to induce fatal disease, yet unknown inflammatory pathways [6]. The need for more accurate clinical and pathological findings of severe malaria is important. Direct conception of microcirculatory obstruction and associated signs has helped to deliver improved specificity in making a diagnosis [7–11]. A key point in analysing these findings is nonetheless the whereabouts and location of the tissue burden of sequestered parasites in severe disease and the immune mechanisms that attenuate the disease. A crucial characteristic of malaria mosquito that has not been always completely realized is the discrepancy of diseases in humans (Figs. 1 and 2).

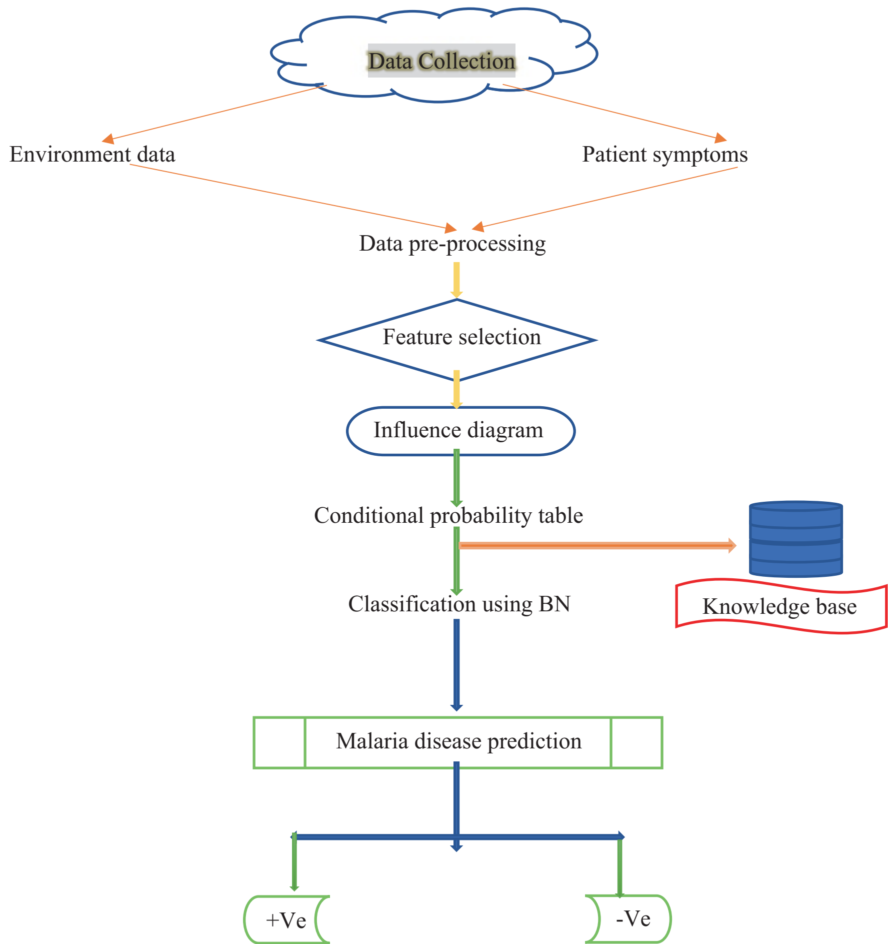

Fig. 1 A framework for malaria disease prediction

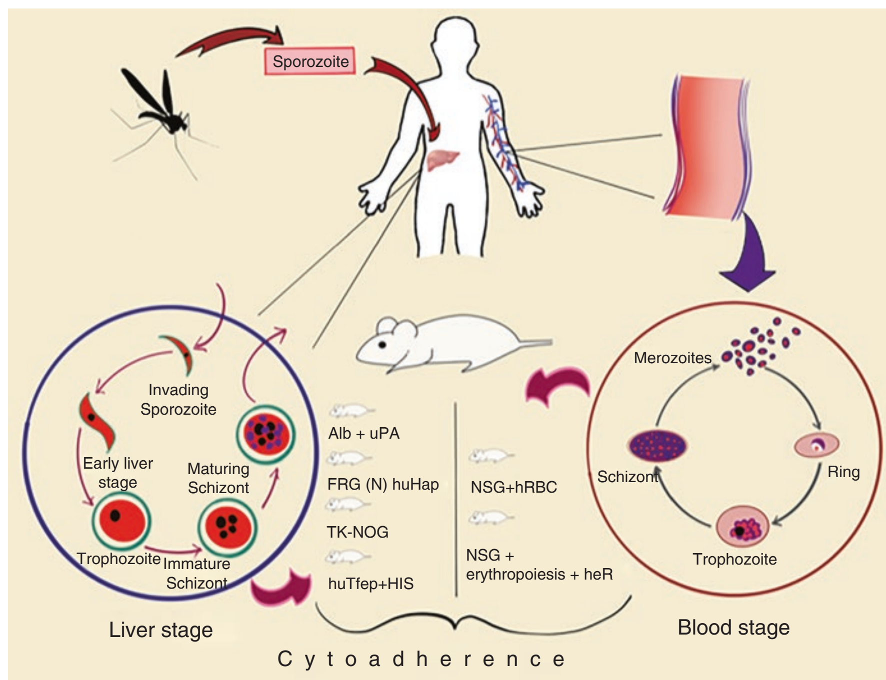

Fig. 2 Disease model and transmission of malaria and cytoadherence of infected RBCs to endothelial cells and formation of rosettes with uninfected RBCs

2 Models to Study the Disease Prevalence

2.1 In-Vitro Models

2.1.1 Isolation of Cord Blood Mononuclear Cells

Ten samples of umbilical cord blood of normal full-term new-borns following the informed consent approved by Ethical Committee of Research on human beings were used to isolate HSCs. Between 50 and 70 ml of cord blood was collected into blood bags containing 30 ml of CPDA-1 anticoagulant. Cord blood samples were overlaid on Lympho-prep solution at a 1:1 ratio by volume and centrifuged at 1200 × g, 20 °C for 30 min. The interface layer containing the cord blood mononuclear cells was collected and washed twice with cold phosphate buffer saline containing 2 mM EDTA. Viability of the isolated cells was assessed using the trypan blue exclusion method. Isolation of CD34+ haematopoietic stem cells was isolated from cord blood mononuclear cells with a CD34 isolation kit and Mini-MACS columns according to the protocol. 1 × 108 cord blood mononuclear cells were suspended in 500 μl cold PBS containing 2 mM EDTA and 0.5% foetal calf serum. The

cell suspensions were incubated at 4 °C, for 30 min with 100 μl of blocking reagent and anti-CD34 antibody-conjugated magnetic microbeads. Subsequently, the cells were washed with cold PBS buffer to remove unbound anti-CD34 antibodies and loaded onto an LS column. The column was rinsed with PBS buffer to remove non-specific cells. The retained CD34+ cells were eluted from the column with PBS buffer and centrifuged at 800 g for 10 min at 4 °C, and the resulting cell pellet was re-suspended in Stemline II medium, cells were counted and cultured [12, 13].

2.1.2 Cultivation and Differentiation of HSCs Derived from CBMNs

The isolated CD34+ haematopoietic stem cells were cultured. Briefly, 5 × 105 cell/ml of CD34+ cells were cultured in 12-well tissue culture plates using Stemline II medium supplemented with 50 ng/ml of stem cell factor, 10 ng/ml of IL-3, 100 μg/ml transferrin and 100 μg/ml of Humulin. CD34+ HSCs were incubated at 37 °C in a humidified atmosphere with 5% CO_2, and half volumes of medium were replaced with fresh complete medium every 3 days. Cell number and viability were assessed after 5 and 10 days of cultivation by the trypan blue exclusion method. On tenth day of cultivation, cell surface markers of all mononuclear cells were determined by flow cytometric analysis, and cells were morphologically examined after Giemsa staining. Ten days old HSC-derived MNCs were used in co-cultivation with malaria antigens in all assays [12, 13].

2.1.3 Cultivation of Parasites and Antigen Preparations

Antigens used in this study were prepared from two sources of human malaria parasites. *P. falciparum* parasites were obtained from in vitro cultivation of laboratory strain maintained in blood group O human erythrocytes from healthy donors by in vitro cultivation, using RPMI-1640 medium supplemented with 10% human serum. The parasites were incubated at 37 °C in a humidified atmosphere with 5% CO_2, starting from ring stages until most of the *P. falciparum* parasites entered mature schizont stages. *P. vivax* parasites were isolated from blood of 10 *P. vivax*-infected patients. The blood samples were filtered to deplete leukocytes. The resulting *P. vivax*-infected blood were washed twice with McCoy 5A medium by centrifugation at 800 × g, 4 °C for 5 min and cultured at 5% haematocrit in McCoy's 5A medium supplemented with 25% human AB-serum. The parasites were kept at 37 °C in a humidified atmosphere with 5% CO_2 for 24–30 h, until most of the *P. vivax* parasites entered the matured schizont stages. Two types of antigen preparations, intact infected erythrocytes and parasitized cell lysates were prepared from both the species. Schizont stage parasites were isolated by gradient centrifugation at 1200 × g, 4 °C for 30 min to enrich the parasite. *P. falciparum*-infected erythrocytes and *P. vivax*-infected erythrocytes in the interface layer between medium were collected and washed twice by centrifugation at 800 × g, 4 °C for 5 min with cold

RPMI-1640. The resultants were counted and used to stimulate the HSC-derived MNCs. For the parasite lysates, the same batch pellets were stored at −70 °C without any cryo-preservative agent and twice freeze thawed for use as whole malaria-infected erythrocyte lysates. Uninfected erythrocyte controls were prepared from the erythrocytes of the healthy donor which are used in the *P. falciparum* parasite culture by repeating the same protocol as that used for the infected erythrocytes [12, 13].

2.1.4 Stimulation of HSC-Derived MNCs with MalariaAntigens

On tenth day of the cultivation, HSC-derived MNCs from each cord blood sample were individually co-cultured with the different malaria antigens and whole parasite infected erythrocyte lysates from both *P. falciparum* and *P. vivax* at a concentration of (MNCs: antigens = 1:5). Stimulation with intact uninfected erythrocyte (1:5) and whole uninfected erythrocyte lysate (1:5) were used as baseline controls for the leukocyte response. Phytohemagglutinin-A, 5 μg/ml was used for validating the activity of T lymphocytes for mitogenic response. On second to fourth day after stimulation, the HSC derived MNCs were harvested for phenotypic characterization by flow cytometry [12, 13].

2.1.5 Phenotypic Characterization and Expression of Death Receptor

Phenotyping of the cells was performed by three-colour flow cytometry. Stimulated HSC-derived MNCs from each condition were labelled with fluorescent, dye-conjugated monoclonal antibodies to define various populations of cells including T lymphocytes, dendritic cells, B lymphocytes, NK and NKT cells, HSCs and expression of death receptor. After staining, the cells were washed with PBS, pH 7.4 and fixed with 1% paraformaldehyde in PBS. The analysis was performed using the Cell-Quest software The mononuclear cells were gated for exclusion of debris and dead cells from the analysis.

Statistical Analyses The data are represented as fold change of population and analysed by the GraphPad Prism programme. Statistical significance was determined by one-way analysis of variance (ANOVA). The Mann Whitney test for non-parametric data was used for statistical analysis between the fold changes of population of each condition. The results were considered significant at $P < 0.05$, where:

$$\text{Fold change of population} = \text{Percentage population of each condition} / \text{Percentage population of media}\left[62,63\right].$$

These findings suggest that immunosuppression by malaria infection possibly takes place at an early stage of lymphocyte maturation. Additionally, the use of naive mononuclear cells derived from HSC as an in vitro model for research in immunity can be easily implemented and would reduce the discrepancies of mononuclear cell quality in different studies.

2.2 Cell Line Models

2.2.1 Stem Cell Derived Erythrocytes [12, 13]

The lack of blood supply in blood banks that rely on constant blood donations lead many researchers to look for alternative solutions to produce erythrocytes for transfusion. The first report of the production of human erythrocytes from hematopoietic stem cells using a liquid system has been described. The isolated mononuclear cells from peripheral blood of a patient with thalassemia, in which a defect in the chain of haemoglobin, cause an erythropoiesis increase, using a two-step protocol, it was observed that the erythroid cells when cultured in the presence of erythropoietin. However, ethical concerns of using blood from a thalassemia patient presenting a defect in haemoglobin still remains. Following this study, many protocols have been developed in order to generate erythrocytes from HSC. After isolation of HSC from diverse origins through a magnetic assorted cell sorting selection based on the CD34+ expression, cells were cocultured with mouse stromal cells. The cells were cultured in the presence of a cocktail of specific growth factors to allow a correct differentiation toward erythroid commitment: interleukin 3, hydrocortisone, stem cells factor and EPO. After 20 days in culture, pure population of erythrocytes could be isolated from the supernatant. Nonetheless, production of erythrocytes from HSC faced some difficulties that limited the amount of cells which are produced as well as the ability to produce mature red blood cells, as the haemoglobin isoforms remain at foetal state. The stem cell-derived erythrocytes have been intensively used in the malaria to solve the challenging in vitro culture of *P vivax*. Unlike *P. falciparum* that can invade erythrocytes of all ages, *P. vivax* shows a preference for invading immature erythrocytes. This reticulocyte invasion makes use of peripheral blood as a source of cells to culture parasites in vitro nearly impossible as reticulocyte are only 0.5–1% of erythrocytes in the blood stream and their lifespan prior to maturation is only 24 h. Thus, a reticulocyte-enriched source of blood is needed in order to grow *P. vivax* in vitro. Early studies used several methodologies to concentrate reticulocytes from blood by ultra-centrifugation. However, more studies demonstrated the preference of *P. vivax* for reticulocytes revealing the possibility of using stem cell derived reticulocytes. The first report attempting to establish an in vitro culture of *P. vivax* using HSPC derived reticulocytes showed that parasites could be maintained in culture for more than 50 days using stem cell-derived reticulocytes. However, conditions still need to be optimized as reticulocyte production were only 0.5%, after 14 days, and the parasitaemia reached very low levels below

0.0013%. In a more recent study, after 14 days of culture, up to 18% of reticulocytes were permissive to *P. vivax* invasion. They were also able to successfully cryopreserve reticulocytes in order to create a stock of cells to provide to *P. vivax* at each schizogony cycle. Nevertheless, the amount of reticulocytes generated remained extremely low and the parasite could still not multiply in vitro. Before HSPC derived reticulocytes can be used for successful *P. vivax* in vitro culture, the problems of low-reticulocyte yield and the lack of intra erythrocyte development of the parasite were addressed. As a source of reticulocytes, the differentiated CD34+ cells were reintroduced into the reticulocytes using the previously described protocol and interestingly purified the reticulocyte population passing the cells through leukocyte reduction filters to get rid of nucleated cells. Alternatively, they also concentrated reticulocytes from the peripheral blood and umbilical cord blood. They also tested different culture media supplemented with different serum concentrations. The parasites could be maintained in vitro for 26 months, though the parasite density dramatically dropped from the first day to an almost undetectable level after the second day. The parasite's ability to invade and replicate within reticulocytes generated from HSPC is a precondition for the establishment of in vitro culture that relies on stem cells as a source. The earliest erythroid stage, which is permissive to *P. falciparum* invasion, was quite understood. After CD34+ isolation, HSPCs were cultured for 8 days in the presence of IL-3, EPO and SCF with medium refreshments at days 3 and 6 without IL-3 and decreased SCF concentrations. A selection was done after 7 days by FACS sorting based on CD71+ expression, and the cells were cultured with only EPO supplementation for an additional 10 days. It was found that the polychromatic erythroblasts were poorly invaded, while ortho erythroblasts could be invaded and allowed for parasite intracellular maturation, indicating the permissiveness of the erythroid progenitors to *P. falciparum*.

Notably, the 3T3 cells were used instead of the more commonly used MS5 cells as a layer for differentiating erythroid progenitors. They could reach a significant yield of reticulocytes and observed the presence of adult haemoglobin in reticulocytes derived from PB and BM. However, no information was given about the level of parasitaemia post-invasion. It was observed that better enucleation happens in PB source vs. BM. All the sources that have been tested had similar permissiveness and better invasion rates compared to reticulocyte-enriched blood leading to the hypothesis that *P. vivax* prefers immature reticulocytes. A reverse genetics approach to investigate the role of RBC receptors are involved in *P. falciparum* invasion. Using lenti-viral shRNA delivery, the gene knockdown of different genes encoding for potential receptors in erythroid cells has been calibrated. They differentiated those that were genetically modified erythroid progenitors on stromal cell layer to obtain enucleated cells used further for *P. vivax* invasion assays. The authors observed a dramatic invasion decrease in CD55 as well as in CD44 RBCs. This work was the first to highlight the possibility of using genetically modified erythrocytes to study Plasmodium biology. Significant blood haemolysis was reported during malaria infection, and thus, HSPC are also of particular interest to study erythropoiesis impairment that leads to anaemia during malaria episodes. Several studies tried to investigate the mechanism by which Plasmodium infection causes erythropoiesis

impairment. In this scope, Hemozoin attracted particular interest. Hz is produced by the parasite when it metabolizes heme in its food vacuole. Starting from UCB, they differentiated CD34+ cells based on a 3-step process, in the presence of Fms-like tyrosine kinase 3 and thrombopoietin, with an addition of insulin growth factor-1 and finally without SCF. It was found that the main reasons for haemolysis are soluble mediators stem cell derived cells *P. falciparum* HSPC-derived erythrocytes. It appears that *P. falciparum* infection significantly influences transcription in erythroid progenitors. It is observed that an up-regulation of 35 genes in polychromatophilic erythroblasts and 609 regulated genes in ortho-erythroblasts were obtained. These results may indicate a negative effect of *P. falciparum* on erythropoiesis. *P. vivax* infection has a similar effect on erythroid development. Later on, it was observed that a decrease of the erythroid multiplication and development in the presence of the infected reticulocytes lend support to the idea that the *P. vivax* might have a negative effect on erythropoiesis. The recent important development of stem cell research contributed to the production of stem cell derived erythrocytes and enabled testing the use of human embryonic stem cells or human-induced pluripotent stem cells. Indeed, recent findings have demonstrated that those pluripotent cells can be maintained and expanded in vitro prior to differentiation into specific lineage. It was developed as a protocol to produce enucleated red blood cells from ESC. The differentiation was initiated by dispensing hESC as erythroid bodies in the presence of bone morphogenetic protein 4, vascular endothelial growth factor 165, and beta fibroblast growth factor to induce mesodermal commitment. The second step, which is the erythropoiesis leading to the last stages of the blood production, was performed in coculture with OP9 cells or human mesenchymal stem cells in the presence of IL-3, HDS, EPO and SCF. The development of such techniques and the possibility to permanently edit the genome of erythroid cells will make a great contribution for a deeper study of intra erythrocyte parasite biology. Studying the liver stage of Plasmodium parasites is of great importance for understanding the establishment of infection and for immunogenic and therapeutic purposes. In addition, *P. vivax* can produce dormant forms known as hypnozoites in the liver. These hypnozoites are responsible for its ability to maintain long-term infections and relapsing episodes that contribute to the difficulties of eradicating *P. vivax*.

An immortalized HepG2 cell line has been extensively used as a starting material to investigate the exo-erythrocytic cycle of *P. berghei*, *P. vivax*, *P. falciparum and P. gallinaceum*. However, even though they were able to infect liver cells with *P. falciparum* sporozoites, it is still difficult to get these parasites to successfully complete the cycle and infect RBCs. The hepatocytes were isolated from a hepatoma patient which were cultivated with insulin, epidermal growth factor, thyrotropin releasing factor, HDS, glucagon, nicotinamide, linoleic acid, L-glutamine, pyruvic acid and essential amino acids at 37 °C and 5% CO_2. Interestingly, the levels of enzyme activities and protein secretions were higher than the ones observed in HepG2 cell lines. These cells were infected with *P. falciparum* and *P. vivax* sporozoites and when RBCs were added to the culture, blood stage parasites were observed after 7 and 10 days for *P. falciparum* and *P. vivax*, respectively. One of the drawbacks of using immortalized cell lines is that the metabolism of those cells

might differ from the in vivo ones. They found that *P. vivax* parasites were able to develop, and after 10 days, rings could be observed in reticulocytes added in co-culture. Using primary cells for parasite cultures required the fresh cells which could be constantly available. The previously frozen primary hepatocytes that remained permissive to Plasmodium sporozoites in a microsystem surrounded by fibroblast stromal cells were preserved. Primary hepatocytes were used to evaluate the effect of a drug on the liver stage. Primary hepatocytes were isolated from the rhesus which were infected with the Plasmodium sporozoites in the presence or absence of the compound. The development was well established in vitro, but the incubation was shown to inhibit the formation of liver schizonts as well as hypnozoites. Similarly, *P. berghei* liver stages could develop within murine primary hepatocytes in vitro but their ability to infect RBCs remain unexplored in this study. The TRAP-based vaccine in the presence of CD8+ enriched splenocytes inhibits the parasite development in the liver. This method was proposed as an in vitro system to screen possible vaccine candidates but its suitability to human Plasmodium vaccines needs further investigation. Surprisingly, stem cell derived hepatocytes have not been used widely for malarial research. Nonetheless, hepatocyte like cells originating from the human foreskin fibroblasts were permissive to different Plasmodium species including *P. falciparum, P. vivax, P. berghei* and *P. yoelii*. The hepatocyte differentiation protocol was adapted in which the ESCs were cultivated for the first 5 days in the presence of activin A to induce endodermal commitment. The following 10 days led to hepatoblast formation through hypoxia culture condition and was divided into two steps, the first 5 days in the presence of BMP-4 and FGF-2 and the last 5 days in the presence of hepatocyte growth factor. The iPSC-derived HLCs have low levels of enzymes that can metabolize drugs as they remain immature hepatocytes and thus are not optimal for antimalarial drug screen. However, one should note that different protocols seem to create a variety of HLCs with different characteristics. A general scheme can be drawn with a 4-step protocol: mesodermal differentiation in presence of activin A, hepatic specification, hepatoblastoma expansion and hepatic maturation. For each of these four steps, growth factor concentrations as well as the time of exposure remain variable between different studies. Optimizing the generation of stem cell derived hepatocytes, which are more similar to the adult hepatocytes, would have great impact on understanding the biology of Plasmodium stages and lead to improved testing of potential antimalarial drugs and vaccine candidates.

2.3 Animal Models

2.3.1 Anti-PE Plasmodium Immunity and Vaccine Development: From Traditional Mouse Models to Human Clinical Trial

The distinct human host cell of malarial parasite that infect the generations of humans impedes infection of the traditional immunology; that is, the in-bred mouse. Thus, the utilization of rodent parasites like *P. yoelii* and *P. berghei* allowed a

careful assessment for immunity. Thus, there is contribution towards protection against contagious sporozoite, after whole attenuated sporozoites vaccination. Attenuation was originally spawned by gamma irradiation [14] but can now be accomplished through genetic engineering with the precise removal of genes from the parasite genome [15, 16] or by treatment of an infectious sporozoite immunization with drugs that prevent the infection [17, 18]. For the last decade ever, the whole sporozoite of the mice have been identified for providing humoral immunity. Earlier thought to be immunologically subdued, liver stage Plasmodium infection was able to induce an innate immune response [19–22]. The pathways by which this innate immune response is provoked and the effect it has on the resulting adaptive immune response were the major areas of active investigation. Although nowadays there is a chance of developing humanized mouse models for the frontiers of malaria, compounding it with a significant deviation and extrapolation of the accurate results between the rodents and the human cell. The host–parasite interactions and the immunity produced generally develop fully within 3 days. Although traditional rodent models of infection have been useful, differences in rodent and human Plasmodium species compounded with significant divergence in rodent and human hosts presents significant implications for the extrapolation of results achieved with the former to the latter, particularly regarding immunity. Also, meanwhile the liver stage of rodent Plasmodium species develops fully within 3 days and the human Plasmodium parasite undergo 10 days of liver stage development before exiting the liver to contaminate the erythrocytes. In addition, none of the rodent parasites form persistent liver stages that could model those found in *P. vivax* infection, affirming that PE biology in the rodent is not an ideal model for human malaria infection.

Human clinical trials have identified robust induction of both humoral and cellular immune responses after whole sporozoite immunization yet unequivocal identification of correlates of protection from these studies has proven to be challenging. To resolve differing observations, functional in vitro assays have been developed like, the examination of immune sera and its inhibitory activity on infection of cultured hepatoma cell lines with sporozoites [23–25]. Yet, in vitro cultured cells do not precisely demonstrate the complex design of the liver tissue, rendering them only partially physiologically relevant. In addition, tissue-resident memory T cells, which proved to be critical in controlling the liver stages in rodent malaria infection, do not undergo recirculation into the blood stream [26], hampering the examination of their contribution to immunity after *P. falciparum* immunization of humans. There were presence of peripheral T cells that could have corelated with rodent Plasmodium species. However no ex-vivo models were identified to give a clear and robust quantification of CD8 T cell killing of the functional liver cells or increasing the humoral and cellular immune responses. Thus, there was an urgency in developing the next model.

2.3.2 Humanized Mice to Model PE Immunity

The quantification clarity was lacking in the previously developed model, the robustness in ex vivo and in vitro assays requires an animal model that could better mimic or be the better mirrors of Plasmodium infection. Moreover, the immune response in non-human primate is similar to humans and tissue-resident immune populations can be sampled to query their importance to vaccine-engendered protection [27]. However, NHP (non-human primate) systems are still not an ideal model for human Plasmodium infection and in supplement to ethical and financial barriers and logistical concerns regarding artificial housing requirements for NHPs have curbed their use in malaria research. Thus, the advancement of immunodeficient mice that have been imbedded with functional human immune cells and therefore the human tissues will enable the assessment of infection in the human cells and tissues in the framework of human immune responses in vivo. Human liver-chimeric mice were believed to play an important and cheaper alternative to NHP models in the final selection of antibody-based vaccine antigens. The mice were utilized to demonstrate the passive transfer of human monoclonal antibodies from humans, those are immunized with whole *P. falciparum* sporozoites and hence block the liver infection [28–30]. To scrutinise intrinsic human immune responses, human immune system (HIS) mice have been developed, which enable the direct analysis of the HIS responses in vivo after infection with human pathogens or after vaccination. HIS mice are obtained by the transplantation of human CD34+ cells. To produce tamed mice with a competent human humoral response, immunocompromised mice were transduced with recombinant Adeno-associated virus (AAV) vectors [31].

Huang and colleagues also generated a humanized CD8 T cell mice referred to as HIS-CD8 [32] by transducing mice with AAV vectors and a cocktail of human cytokines. Inoculation of HIS-CD8 mice with AAV vectors bearing *P. falciparum* CSP resulted in an investiture of restricted human CD8 T cells. These immunized HIS-CD8 mice greatly reduced parasite burden in the liver after challenge with transgenic *P. berghei* parasites encoding full *P. falciparum* CSP [33]. Importantly, in vivo attenuation of human CD8 T cells completely exterminated the decline in liver burden in immunized HIS-CD8 mice [34]. The HIS studies, however, support the challenge with transgenic rodent malaria parasites voicing selected *P. falciparum* proteins. Thus, there is a necessity to combine humanized liver-chimeric mice with the models of HIS to generate dual-chimeric mice (HIS huHep) receptive to human Plasmodium liver infection. However, such a model will not yet fully imitate human immunity, although liver chimeric mice can be repopulated with high levels of huHeps, their liver sinusoidal endothelial cells, Kupffer cells, hepatic stellate cells and cells of the myeloid lineage all stay on of mouse origin. In rodent malaria systems, CD8+ dendritic cells have shown to be critical for the generation of an effective immune response after whole sporozoite immunization [35, 36]. The significance of liver-resident memory CD8 T cells in PE immunity [26, 37, 38], will be crucial to examine whether HIS-CD8 mice reiterate the critical roles of tissue resident cells observed in rodent malaria studies.

2.3.3 Humanized Mice to Model Malaria BS (Blood Smear) Infections

Although *P. falciparum* could have been cultured in vitro, a miniature animal model of human BS malaria would extend greater advantages, as it would allow the preclinical testing of drugs and vaccine candidates in an in vivo setting against the human pathogen. The liver-chimeric mice maintain liver infection and liver stage-to-BS transition after injection of target human RBCs [39, 40]. The presence of huRBCs (human red blood corpuscles) on the day of exo-erythrocytic merozoite emergence from the liver leads to a rapid period of low parasitaemia and these parasites can then be removed and maintained in huRBC culture. However, BS infection cannot be preserved in the mice as huRBCs are promptly cleared. Luckily, different immune-modulation etiquettes collective with daily injections of huRBCs can support high-engraftment levels and promote a continuous *P. falciparum* BS infection in NSG mice [41]. These mice show repossession of the parasite in bone marrow and spleen, suggesting it might resemble the behaviour of the parasite in humans. One downside is that in this study the mice were directly infected with BS parasites, as they are not human liver chimeric. Moreover, as *P. falciparum* gametocytes take [10, 11, 14–16] days to mature, this is the time period the infected RBC has to be maintained to make available themselves for transmission back to the mosquito. If this is achieved, the model might facilitate the study of transmission in an in vivo setting and allow the transmission of the blocking drugs and vaccines. A vigorous protocol was embedded to maintain huRBCs in human liver-chimeric mice to better evaluate the efficacy of transmission of blocking small molecules, antibodies and vaccines [42].

Another encouraging use for combining huHep/huRBC mice was the preclinical evaluation of attenuated *P. falciparum* whole sporozoite vaccine candidates, allowing for impeccable sensitivity in detecting potential breakthrough infection into the blood before testing of new attenuated strains in human trials [43]. The genetic crosses have been carried out in splenectomised chimpanzees. The recovery can be carried out using recombinant progeny. The opportunity to execute genetic crosses in a small animal model provides a vigorous avenue for genetics research and a new avenue to determine the underlying traits of *P. falciparum* drug resistance and other phenotypes of clinical importance. The in-vitro cultures prepared can especially benefit a small animal infection model. To institute a humanized mouse model that will transmit *P. vivax* BS infection, mice will have to be imbedded with these rare cells. Reticulocytes can be found in a very small fraction in umbilical cord, reticulocytes and invasion assay. The repeated injection of huRBCs, combined with different immunomodulatory protocols, is a burdensome process, needing an experienced researcher and often prominent to losses of mice. A sophisticated substitute to continual huRBC reorganization would be a mouse that inherently sustains human erythropoiesis after HSC transplantation. Human erythropoiesis is severely impaired noticed in the humanized mouse models. A mouse in which human CD34+ HSC transplantation leads to robust human erythropoiesis combined with an HIS, which in addition harbours a human-chimeric liver, would ultimately enable the production of reproducible data on the developmental life cycle of human malaria parasites. Very little is known about the essential molecular mechanisms of these

observations, as we lack a simulation system to probe them. Currently, the questions of drug–drug interactions or vaccine wellbeing in coinfected individuals can solely be forwarded in clinical trials, as carried out previously for the malaria vaccine candidate [44]. However, the sophisticated mouse model with a human-chimeric liver, human erythropoiesis and HIS could potentially fill this gap.

2.3.4 BS Malaria Pathology in Humanized Mice

The matter of whether a humanized mouse model can expedite the study of *P. falciparum* malaria associated pathophysiology remains largely unmapped. The pathology of severe malaria is mainly dogged by linkage interactions between infected erythrocytes and human endothelial cells. These adhesion interactions lead to the confiscation of infected erythrocytes in the microvasculature, which assists the parasite by avoiding clearance in the spleen. Unfortunately, this sequestration also leads to vascular occlusion and inflammation, which are important contributors to severe malaria pathology. Three forms of cemented interactions have been labelled: the cytoadherence of infected erythrocytes to endothelial cells, formation of rosettes with uninfected erythrocytes and platelet-mediated clumping of infected cells [45]. These connections are mediated by the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family, variant antigens expressed on the surface of infected RBCs that interact with multiple host receptors [46]. Depending on which gene is expressed, the parasite modifies the antigenic properties of infected erythrocytes, which allows it to evade the host immune system but also changes the binding specificity for host receptors. Var gene switching is currently under pervasive investigation and a small animal model allowing the regulated in vivo estimation of this phenomenon would be of great value to this important field of research. The most lethal complication of a *P. falciparum* infection is cerebral malaria. It has been conjectured that the targeting of different receptors via the representation of different PfEMP1 variants leads to tissue-specific sequestration of the parasites. To date, any knowledge of whether infected RBCs sequester in the brain of *P. falciparum*-infected humanized mice are observed or not are in vague. It is thus highly relevant to investigate whether the illustrated pathologies of human malaria are encapsulated in the humanized mouse models. If this is not the case, a mouse model must be developed in which human receptors are communicated on mouse endothelial cells. If this could be accomplished, it would wide open new possibilities for investigating the capability of anti-adhesion drugs or antibodies as novel malaria therapies.

2.3.5 BS Malaria Immunity in Humanized Mice

Animal models of malaria persists to deliver important perceptions in the immune response to *Plasmodium* BS infection. However, the development of novel immunotherapeutic strategies necessitates a thorough investigation of the human immune response, particularly to *P. falciparum* BS infection. [47–49]. Altogether with the

rodent Plasmodium studies, two immunological processes necessary for the control of BS malaria infection have been identified, namely, the inflammatory reaction from innate immune sensing of Plasmodium infections. Plasmodium pathogen-associated molecular patterns retain pattern recognition receptors on innate immune cells which activate an inflammatory response significant for early parasite control [50, 51]. However, this inflammatory response can also be pathogenic to the host. *P. berghei*-infected mice [52], which promotes the production of the immunosuppressives [53]. Antibodies have been able to play a central role in BS malaria immunity [54]. The parasite proteins that persuade protective antibody responses, the mechanism of Plasmodium humoral protection and why protective antibodies develop after years of repeated exposure, are finally maturing. Mounting evidence in the rodent models and correlative data in humans from endemic regions have established that Plasmodium evades humoral immunity through dysregulation of CD4+ T cell [55] and B cell dysfunction [56–61]. Although the mechanisms behind the roles of inflammation on dendritic cell function and B cell dysfunction have been well studied in rodent models of malaria, a mouse model with a humanized immune system will be life-threatening to confirm whether the mechanisms delineated in the rodent malaria models pertains to infection with the human *P. falciparum* parasite. However, in preceding sections, the existing humanized mouse models will not accurately mimic a complete HIS as they still contain murine antigen presenting cells or other murine myeloid cells. Conventional mouse model infections with rodent malaria parasites have critically contributed to our understanding of malarial parasite biology, pathogenesis, immunology, and have been important in malaria vaccine and drug discovery. However, differences between the genomes of the human infective and rodent infective Plasmodium species as well as significant divergence between the mouse and the human biology might preclude facile application of knowledge gleaned from traditional rodent systems to the design of effective interventions in the humans.

Humanized mouse models have emerged as a critical link between traditional rodent models and humans. The mice will enable better examination of the factors critical for hepatocyte infection and liver stage development and the understanding of liver stage directed, infection-preventing interventions. The mice are prepared to greatly accelerate our perception of the immune response to human Plasmodium parasites and vaccine candidates as the data gleaned from these studies will more closely represent the immune response in humans. Next generation HIS mice for malaria research will likely require humanization of the liver, bone marrow, lymphoid compartments and human erythrocytes. BLT (bone marrow, liver and thymus) mice, where human foetal thymus and liver tissues are engrafted into the same mouse represent the most extensive humanized mouse system to date [62]. Combining this with huHep mice would generate the triple-humanized mouse sorely needed for the study of human malarial parasite infection and immunology. However, the high costs and technical demands will likely preclude it from being widely employed. Ultimately, future humanized mouse models for Plasmodium research will utilize the transplantation of cells to develop robust human immune and erythropoietic compartments and hepatocyte transplantation to ensure human liver chimerism (Table 1).

Table 1 Models to study the disease prevalence [12, 13]

Sl. No.	Models
1.	IN-VITRO:
A.	Isolation of cord blood mononuclear cells
B.	Cultivation and differentiation of HSCs derived from CBMNs
C.	Cultivation of parasites and antigen preparations
D.	Stimulation of HSC-derived MNCs with malaria antigens
E.	Phenotypic characterization and expression of death receptor
2.	CELL LINE: (For *P. falciparum* and *P. vivax*) Stem cell derived and HSPC derived cells:
A.	Study of erythrocytes receptors
B.	Study of erythroid permissiveness
C.	Study of erythropoiesis impairment
D.	Study the influence on erythroid transcriptomics
E.	Study of reticulocyte permissiveness
F.	Establishment of in-vitro culture
3.	ANIMAL MODELS:
A.	Anti-PE Plasmodium immunity and vaccine development: from traditional mouse models to human clinical trial
B.	Humanized mice to model PE immunity
C.	Humanized mice to model malaria BS (blood smear) infections
D.	BS malaria pathology in humanized mice
E.	BS malaria immunity in humanized mice

3 Future Perspective Need of Malarial Research [12, 13]

It is very clear that animal models allow for more detailed examination of multiple and specific pathophysiologic processes caused by malarial infection that is not possible for clinical studies through the level, scope of experimental observation and intervention. Using in vivo imaging in conjunction in mice expressing human receptors creates a screening system for testing inhibitors that block *P. falciparum* cytoadherence. Rather using an ex-vivo two-dimensional models, the graft models showed the detailed examination of the interaction between parasites and the host in a three-dimensional model. In dissimilarity to the mouse model, the incidence of cerebral microvascular sequestration through cyto-adhesion in cerebral blood vessels is proven for these two macaque monkeys and they consequently bid an alternative for examining the association of parasite-specific adhesive events in the brain with disease and underlying pathogenic mechanisms. These models offer the possibility to use more sophisticated approaches for neuroimaging. Severe diseases in the

P. knowlesi rhesus model can imitate some clinical syndromes of *P. falciparum* severe disease in humans. Although it is unclear if *P. knowlesi* in rhesus monkeys is an acceptable model for HCM, from initial reports it is particular to be a model for human severe disease caused by this parasite. There are, however, limitations to the primate model since symptoms of cerebral vascular sequestration in monkeys infected with *P. falciparum*. Additionally, evaluation of the inflammatory responses and parasite sequestration in the placenta and foetus may provide insights into the role of proinflammatory cytokines in malarial placental pathology. Major constraints of many mouse models are the lack of a natural chronic infection. Several recent studies indicate that *P. berghei* in mice can be models to investigate malaria-associated lung pathology. For these models, it was felt that detailed comparisons between lung pathology in the rodent models and lung pathology in humans are required to validate their use for developing therapies and interventions. Models for anaemia were also considered to be important to support human clinical studies. However, the lack of chronic infection and the rapid possession of immunity impede the use of rodent models to study anaemia in chronic infection, which would be more reflective of severe malaria in children. However, mechanisms of disruption of bone marrow haematopoiesis and dyserythropoeisis can be readily investigated. Another important point of experimental model study was the direct injection of infected erythrocytes to initiate infections in the study of immune mechanisms and immunopathology. However, this does not reproduce the sequence of the natural infection where infection starts with a mosquito bite and inoculation of sporozoites. To simulate host infections using variables, 1–5 days were sufficient to represent the characteristic change. These theories can be categorized as host, vector, parasite, environment and intervention factors like hypothetical decision tree approach to modelling mosquito agents. At each step, the mosquitoes will check their status, determining their subsequent actions with probability of success. Human behaviours regarding intervention and treatment decisions have been simulated for both patients and carers. One model simulated the impact of anti-malarial use on HIV-positive pregnant women, including disease severity and improvements in birth weight.

The most simulated disease aspects were gametocyte density, including fever simulations such as the EMOD framework modelled the egg population as a cohort for their progression to adulthood, and two models simulated eggs as individual agents. When both hosts and vectors were simulated, models focused on mosquito life cycles, population dynamics, physical environments and interventions for vector control. Studies investigated the impacts of infection not only on hosts, but also on vector behaviours, such as altered biting rates. The choice of parasite was often based on the dominant species in the target location. The HYDREMATS model details about the epidemiology. They were allowed to interplay between the physical properties of the study area and the construction of the model. For example, the size of grid squares was related to parameters such as typical distance moved by mosquitoes in one time step or larval habitat size. Conversely, the models with a continuous landscape were used to allow variation in the location of houses, larval

habitats, sugar sources and resting sites and to model the impact of different physical environments. Each object had a specified location and mosquitoes could sense objects within a certain circular distance, as opposed to a particular grid square. The Malaria Atlas Project and the Markham Seasonality index have generated methods to predict by combining these tools with model outputs to generate maps of estimated disease burden. These models generally introduced a new modelling technique for assessing the hypothetical scenarios, as opposed to claiming accurate simulation of real-world situations. Calibration techniques included the use of calibration vectors, least squares, maximum likelihood functions and visual estimations. The validation was most performed by running a calibrated simulation and comparing model outputs to a dataset. Fruitful model validation was often used to justify extending a model framework to include interventions or to assess their potential impact in the location of interest. Several studies concluded that the model did not accurately fit the data used for validation. The greatest discrepancy was intervention efficacies predicted by models with in-built transmission heterogeneity, when compared to those with uniform transmission levels between individuals. The Open Malaria collection aggregated the outputs from model variants to investigate interventions, such as vaccination, seasonal malaria chemoprevention, mass test and treatment strategies and long-lasting insecticidal nets (LLINs). One study predicted changes to disease outcomes and transmission upon the removal of vector control interventions from elimination programmes. The aggregation of model outputs for each parameter set was performed to reduce the uncertainty arising from structural differences in models. Hence, the ensemble is considered as one of the models and its outputs interpreted as such. Despite the varied approaches, the consensus modelling largely drew consistent findings on the relationship between available malaria prevalence data and clinical incidence, on the impacts of vaccination and mass drug administration (MDA).

Mathematical modelling plays an important role in malaria elimination, and agent-based approaches make a major contribution to these efforts. The extension of compartmental models to their equivalents arose from the need to understand malaria transmission at the individual level. The result is a rich array of model families and simulation techniques, adapted to a range of key issues in transmission and control. Generally, there are three core themes which have emerged regarding justified usage. Firstly, the greater importance of stochasticity approaching elimination requires an alternative approach to traditional compartmental methods. Secondly, the attempts to eliminate local transmission require discrete population simulations to incorporate spatially explicit environments at increasingly fine resolutions. And finally, the heterogeneities in disease progression and severity on the individual patient level result in varying efficacy of the drug and vaccine interventions, which may be difficult to capture within a compartmental framework. These three arguments jointly inclusive of a compartmental structure, based on averaging over a population, have limitations when that average does not adequately represent the individuals. In addressing these issues, the benefits of agent-based techniques in this

space are evident. Many papers in this review explicitly aimed to fill the knowledge gap regarding intervention use in low-transmission environments. Most projects provided outputs robust at multiple transmission intensities, highlighting the flexibility. The HYDREMATS framework was used in multiple locations, incorporating environmental factors such as temperature and rainfall at different times. The Open Malaria models progressed from assessing the force of infection of malaria transmission to estimating cost-effectiveness of a vaccination programme. Given the similarities of the compartmental models, the depth and flexibility of agent-based methods are allowing new insights into malaria transmission and prevention. Instead of suggesting a "gold standard" approach, it may be preferable to ensure the model style used is appropriate for the question at hand. Open Malaria's early modelling of gametocyte densities did not use vector agents, but successfully provided insights into risks of fever, morbidity and mortality of patients. The EMOD models initially described host–vector interactions without spatial consideration but added this capability when required to assess interventions. Therefore, while not every model incorporated every aspect of malaria epidemiology, each was tailored to the research question at hand. Conversely, if modelling groups are considering extending their model frameworks, particularly to influence policy, there is potential to draw from the features of one another.

HYDREMATS currently includes human and mosquito agents, while the characteristics of human infection are more specific in the Open Malaria simulations. Therefore, the time variability of individual gametocyte density, probabilities of fever, morbidity, mortality and the infectivity of hosts to vectors used in the Open Malaria framework could be adapted into HYDREMATS to more realistically replicate disease transmission. However, in neither of these simulations, does it include human behavioural reactions to biting rates and the probabilities of successful blood meals. To some extent, combining the model structures across the research teams can be considered an extension of the use of submodules already undertaken by larger modelling frameworks. The HYDREMATS have successfully integrated detailed larval habitat and entomologic models and Open Malaria includes seven modules of human disease states and interventions. Modular projects such as HYDREMATS, EMOD and Open Malaria have provided insights into transmission dynamics, vector populations, disease severity and the contributors to these factors. Given the importance of comprehensive modelling to guide policy decisions, the potential for combining the strengths of validated models to enhance decision-making capabilities could be explored. These insights include the distances between larval habitats and houses to effectively reduce malaria transmission and the impact on systematic versus random location on mosquito abundance. Alternatively, techniques such as Markov chain Monte Carlo (MCMC) can search the entire parameter space. MCMC has already been used in malaria ensemble modelling. Approaches such as MCMC and approximate Bayesian computation are increasing in popularity as including uncertainty in model parameters (Table 2).

Table 2 Techniques applied [12, 13]

Sl. No.	Name of the techniques:
1	Graft models showed the detailed examination of the interaction between parasites and the host in a three-dimensional model
2	Cerebral microvascular sequestration through cyto-adhesion in cerebral blood vessels
3	Evaluation of the inflammatory responses and parasite sequestration in the placenta and foetus
4	Malaria-associated lung pathology
5	Models for anaemia
6	Mechanisms of disruption of bone marrow haematopoiesis and dyserythropoeisis
7	Direct injection of infected erythrocytes to initiate infections in the study of immune mechanisms and immunopathology
8	Hypothetical decision tree approach to modelling mosquito agents
9	Gametocyte density, fever Simulations such as the EMOD framework
10	HYDREMATS model
11	Models with a continuous landscape
12	Malaria Atlas Project and the Markham Seasonality Index
13	Calibration vectors, least squares, maximum likelihood functions and visual estimations
14	Open Malaria collection, vaccination, seasonal malaria chemoprevention, mass test and treatment strategies and long-lasting insecticidal nets
15	Mathematical modelling
16	EMOD models
17	Markov chain Monte Carlo
18	MCMC and approximate Bayesian computation

4 Conclusion

A protocol exists for the description of all the mathematical models. Beyond individual models, ensemble modelling utilized as a mathematical tool and the gap analysis performed, which has originated from the animal study and later mathematical models, serves as an important tool for generating robust conclusions about the malaria transmission.

Glossary

AAV Adeno-associated virus
BLT Bone marrow, liver and thymus
CSA Chondroitin sulphate A
EMOD Epidemiological modelling software
HCM Human cerebral malaria

HIS Human immune system
huRBCs Human red blood corpuscles
LLINs Long-lasting insecticidal nets
MCMC Markov chain Monte Carlo
MDA Mass drug administration
NHP Non-human primate
PfEMP1 *P. falciparum* erythrocyte membrane protein 1
WHO World Health Organization

References

1. (Malaria – WHO). https://www.who.int/news-room/fact-sheets/detail/malaria.
2. Craig AG, Grau GE, Janse C, Kazura JW, Milner D, Barnwell JW, et al. The role of animal models for research on severe malaria. PLoS Pathog. 2012;8(2):e1002401.
3. Langhorne J, Buffet P, Galinski M, Good M, Harty J, et al. The relevance of non-human primate and rodent malaria models for humans. Malar J. 2011;10:23.
4. WHO Severe falciparum malaria. World Health Organization, communicable diseases cluster. Trans R Soc Trop Med Hyg. 2000;94 Suppl 1:S1–90.
5. Fried M, Domingo GJ, Gowda CD, Mutabingwa TK, Duffy PE. *Plasmodium falciparum*: chondroitin sulfate A is the major receptor for adhesion of parasitized erythrocytes in the placenta. Exp Parasitol. 2006;113:36–42.
6. Mens PF, Bojtor EC, Schallig HD. Molecular interactions in the placenta during malaria infection. Eur J Obstet Gynecol Reprod Biol. 2010;152:126–32.
7. White VA, Lewallen S, Beare N, Kayira K, Carr RA, et al. Correlation of retinal haemorrhages with brain haemorrhages in children dying of cerebral malaria in Malawi. Trans R Soc Trop Med Hyg. 2001;95:618–21.
8. White VA, Lewallen S, Beare NA, Molyneux ME, Taylor TE. Retinal pathology of pediatric cerebral malaria in Malawi. PLoS One. 2009;4:e4317.
9. Beare NA, Lewis DK, Kublin JG, Harding SP, Zijlstra EE, et al. Retinal changes in adults with cerebral malaria. Ann Trop Med Parasitol. 2003;97:313–5.
10. Saggu R, Faille D, Grau G, Cozzone PJ, Viola A. In the eye of experimental cerebral malaria. Am J Pathol. 2011;179:1104.
11. Dondorp AM, Ince C, Charunwatthana P, Hanson J, van Kuijen A, et al. Direct in vivo assessment of microcirculatory dysfunction in severe falciparum malaria. J Infect Dis. 2008;197:79–84.
12. Noulin F. Malaria modeling: in vitrostem cellsvs in vivomodels. World J Stem Cells. 2016;8(3):88). Baishideng Publishing Group Inc. https://doi.org/10.4252/wjsc.v8.i3.88.
13. Chitsanoor S, Somsri S, Panburana P, Mungthin M, Ubalee R, Emyeam M, Jongwutiwes S, Sattabongkot J, Udomsangpetch R. A novel in vitro model reveals distinctive modulatory roles of *Plasmodium falciparum* and *Plasmodium vivax* on naïve cell-mediated immunity. Malar J. 2017;16(1). Springer Science and Business Media LLC. https://doi.org/10.1186/s12936-017-1781-4.
14. Nussenzweig RS, Vanderberg J, Most H, Orton C. Protective immunity produced by the injection of x-irradiated sporozoites of *Plasmodium berghei*. Nature. 1967;216:160–2.
15. Mueller AK, Camargo N, Kaiser K, Andorfer C, Frevert U, Matuschewski K, et al. Plasmodium liver stage developmental arrest by depletion of a protein at the parasite-host interface. Proc Natl Acad Sci U S A. 2005;102:3022–7.
16. Doll KL, Butler NS, Harty JT. CD8 T cell independent immunity after single dose infection-treatment-vaccination (ITV) against *Plasmodium yoelii*. Vaccine. 2014;32:483–91.

17. Beaudoin RL. Should cultivated exoerythrocytic parasites be considered as a source of antigen for a malaria vaccine? Bull World Health Organ. 1977;55:373–6.
18. Steel RW, Kappe SH, Sack BK. An expanding toolkit for preclinical preerythrocytic malaria vaccine development: bridging traditional mouse malaria models and human trials. Future Microbiol. 2016;11:1563–79.
19. Liehl P, Zuzarte-Luis V, Chan J, Zillinger T, Baptista F, Carapau D, et al. Host-cell sensors for Plasmodium activate innate immunity against liver-stage infection. Nat Med. 2014;20:47–53.
20. Liehl P, Meireles P, Albuquerque IS, Pinkevych M, Baptista F, Mota MM, et al. Innate immunity induced by Plasmodium liver infection inhibits malaria reinfections. Infect Immun. 2015;83:1172–80.
21. Miller JL, Sack BK, Baldwin M, Vaughan AM, Kappe SH. Interferon-mediated innate immune responses against malaria parasite liver stages. Cell Rep. 2014;7:436–47.
22. Zheng H, Tan Z, Zhou T, Zhu F, Ding Y, Liu T, et al. The TLR2 is activated by sporozoites and suppresses intrahepatic rodent malaria parasite development. Sci Rep. 2015;5:18239.
23. Kaushansky A, Rezakhani N, Mann H, Kappe SH. Development of a quantitative flow cytometry-based assay to assess infection by *Plasmodium falciparum* sporozoites. Mol Biochem Parasitol. 2012;183:100–3.
24. Finney OC, Keitany GJ, Smithers H, Kaushansky A, Kappe S, Wang R. Immunization with genetically attenuated P. falciparum parasites induces long-lived antibodies that efficiently block hepatocyte invasion by sporozoites. Vaccine. 2014;32:2135–8.
25. Seder RA, Chang LJ, Enama ME, Zephir KL, Sarwar UN, Gordon IJ, et al. Protection against malaria by intravenous immunization with a nonrepli-cating sporozoite vaccine. Science. 2013;341:1359–65.
26. Fernandez-Ruiz D, Ng WY, Holz LE, Ma JZ, Zaid A, Wong YC, et al. Liver-resident memory CD8(+) T cells form a front-line defense against malaria liver-stage infection. Immunity. 2016;45:889–902.
27. Joyner C, Barnwell JW, Galinski MR. No more monkeying around: primate malaria model systems are key to understanding *Plasmodium vivax* liver-stage biology, hypnozoites, and relapses. Front Microbiol. 2015;6:145.
28. Foquet L, Hermsen CC, van Gemert GJ, Van Braeckel E, Weening KE, Sauerwein R, et al. Vaccine-induced monoclonal antibodies targeting circumsporozoite protein prevent *Plasmodium falciparum* infection. J Clin Invest. 2014;124:140–4.
29. Behet MC, Foquet L, van Gemert GJ, Bijker EM, Meuleman P, Leroux-Roels G, et al. Sporozoite immunization of human volunteers under chemoprophylaxis induces functional antibodies against pre-erythrocytic stages of *Plasmodium falciparum*. Malar J. 2014;13:136.
30. Sack BK, Miller JL, Vaughan AM, Douglass A, Kaushansky A, Mikolajczak S, et al. Model for in vivo assessment of humoral protection against malaria sporozoite challenge by passive transfer of monoclonal antibodies and immune serum. Infect Immun. 2014;82:808–17.
31. Huang J, Li X, Coelho-dos-Reis JG, Zhang M, Mitchell R, Nogueira RT, et al. Human immune system mice immunized with *Plasmodium falciparum* circumsporozoite protein induce protective human humoral immunity against malaria. J Immunol Methods. 2015;427:42–50.
32. Huang J, Li X, Coelho-dos-Reis JG, Wilson JM, Tsuji M. An AAV vector-mediated gene delivery approach facilitates reconstitution of functional human CD8+ T cells in mice. PLoS One. 2014;9:e88205.
33. Li X, Huang J, Zhang M, Funakoshi R, Sheetij D, Spaccapelo R, et al. Human CD8+ T cells mediate protective immunity induced by a human malaria vaccine in human immune system mice. Vaccine. 2016;34:4501–6.
34. Li X, Huang J, Kaneko I, Zhang M, Iwanaga S, Yuda M, et al. A potent adjuvant effect of a CD1d-binding NKT cell ligand in human immune system mice. Expert Rev Vaccines. 2017;16:73–80.
35. Shortman K, Heath WR. The CD8+ dendritic cell subset. Immunol Rev. 2010;234:18–31.
36. Cockburn IA, Zavala F. Dendritic cell function and antigen presentation in malaria. Curr Opin Immunol. 2016;40:1–6.

37. Tse SW, Radtke AJ, Espinosa DA, Cockburn IA, Zavala F. The chemokine receptor CXCR6 is required for the maintenance of liver memory CD8(+) T cells specific for infectious pathogens. J Infect Dis. 2014;210:1508–16.
38. Tse SW, Cockburn IA, Zhang H, Scott AL, Zavala F. Unique transcriptional profile of liver-resident memory CD8+ T cells induced by immunization with malaria sporozoites. Genes Immun. 2013;14:302–9.
39. Mikolajczak SA, Vaughan AM, Kangwanrangsan N, Roobsoong W, Fishbaugher M, Yimamnuaychok N, et al. *Plasmodium vivax* liver stage development and hypnozoite persistence in human liver-chimeric mice. Cell Host Microbe. 2015;17:526–35.
40. Vaughan AM, Kappe SH, Ploss A, Mikolajczak SA. Development of humanized mouse models to study human malaria parasite infection. Future Microbiol. 2012;7:657–65.
41. Duffier Y, Lorthiois A, Cistero P, Dupuy F, Jouvion G, Fiette L, et al. A humanized mouse model for sequestration of *Plasmodium falciparum* sexual stages and in vivo evaluation of gametocytidal drugs. Sci Rep. 2016;6:35025.
42. Foquet L, Schafer C, Minkah NK, Alanine DGW, Flannery E, Steel RWJ, et al. *Plasmodium falciparum* liver stage infection and transition to stable blood stage infection in liver-humanized and blood-humanized FRGN KO mice enables testing of blood stage inhibitory antibodies (reticulocyte-binding protein homolog 5) in vivo. Front Immunol. 2018;9:524.
43. Mikolajczak SA, Lakshmanan V, Fishbaugher M, Camargo N, Harupa A, Kaushansky A, et al. A next-generation genetically attenuated *Plasmodium falciparum* parasite created by triple gene deletion. Mol Ther. 2014;22:1707–15.
44. Otieno L, Oneko M, Otieno W, Abuodha J, Owino E, Odero C, et al. Safety and immunogenicity of RTS,S/AS01 malaria vaccine in infants and children with WHO stage 1 or 2 HIV disease: a randomised, double-blind, controlled trial. Lancet Infect Dis. 2016;16:1134–44.
45. Dams Y, Kuhnrae P, Higgins MK, Ghumra A, Rowe JA. Rosetting *Plasmodium falciparum*-infected erythrocytes bind to human brain microvascular endothelial cells in vitro, demonstrating a dual adhesion phenotype mediated by distinct P. falciparum erythrocyte membrane protein 1 domains. Infect Immun. 2014;82:949–59.
46. Rowe JA, Claessens A, Corrigan RA, Arman M. Adhesion of *Plasmodium falciparum*-infected erythrocytes to human cells: molecular mechanisms and therapeutic implications. Expert Rev Mol Med. 2009;11:16.
47. Sauerwein RW, Roestenberg M, Moorthy VS. Experimental human challenge infections can accelerate clinical malaria vaccine development. Nat Rev Immunol. 2011;11:57–64.
48. Engwerda CR, Minigo G, Amante FH, McCarthy JS. Experimentally induced blood stage malaria infection as a tool for clinical research. Trends Parasitol. 2012;28:515–21.
49. Tran TM, Samal B, Kirkness E, Crompton PD. Systems immunology of human malaria. Trends Parasitol. 2012;28:248–57.
50. Stevenson MM, Riley EM. Innate immunity to malaria. Nat Rev Immunol. 2004;4:169–80.
51. Gazzinelli RT, Kalantari P, Fitzgerald KA, Golenbock DT. Innate sensing of malaria parasites. Nat Rev Immunol. 2014;14:744–57.
52. Haque A, Best SE, de Oca M, James KR, Ammerdorffer A, Edwards CL, et al. Type I IFN signaling in CD8- DCs impairs Th1-dependent malaria immunity. J Clin Invest. 2014;124:2483–96.
53. Montes de Oca M, Kumar R, Rivera FL, Amante FH, Sheel M, et al. Type I interferons regulate immune responses in humans with blood-stage *Plasmodium falciparum* infection. Cell Rep. 2016;17:399–412.
54. Cohen S, Butcher GA, Mitchell GH. Mechanisms of immunity to malaria. Bull World Health Organ. 1974;50:251–7.
55. Butler NS, Moebius J, Pewe LL, Traore B, Doumbo OK, Tygrett LT, et al. Therapeutic blockade of PD-L1 and LAG-3 rapidly clears established blood-stage *Plasmodium infection*. Nat Immunol. 2011;13:188–95.
56. Ryg-Cornejo V, Ioannidis LJ, Ly A, Chiu CY, Tellier J, Hill DL, et al. Severe malaria infections impair germinal center responses by inhibiting T follicular helper cell differentiation. Cell Rep. 2016;14:68–81.

57. Kurup SP, Obeng-Adjei N, Anthony SM, Traore B, Doumbo OK, Butler NS, et al. Regulatory T cells impede acute and long-term immunity to blood-stage malaria through CTLA-4. Nat Med. 2017;23:1220–5.
58. Hansen DS, Obeng-Adjei N, Ly A, Ioannidis LJ, Crompton PD. Emerging concepts in T follicular helper cell responses to malaria. Int J Parasitol. 2017;47:105–10.
59. Krishnamurty AT, Thouvenel CD, Portugal S, Keitany GJ, Kim KS, Holder A, et al. Somatically hypermutated Plasmodium-specific IgM(+) memory B cells are rapid, plastic, early responders upon malaria rechallenge. Immunity. 2016;45:402–14.
60. Zander RA, Obeng-Adjei N, Guthmiller JJ, Kulu DI, Li J, Ongoiba A, et al. PD-1 Co-inhibitory and OX40 Co-stimulatory crosstalk regulates helper T cell differentiation and anti-Plasmodium humoral immunity. Cell Host Microbe. 2015;17:628–41.
61. Obeng-Adjei N, Portugal S, Holla P, Li S, Sohn H, Ambegaonkar A, et al. Malaria-induced interferon-gamma drives the expansion of Tbethi atypical memory B cells. PLoS Pathog. 2017;13:e1006576.
62. Wege AK, Melkus MW, Denton PW, Estes JD, Garcia JV. Functional and phenotypic characterization of the humanized BLT mouse model. Curr Top Microbiol Immunol. 2008;324:149–65.

Disease Models in Tuberculosis Research

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Abstract Animal models in general and TB animal models in particular have been utilized in a variety of ways to enhance diagnosis and foster preventative medicine and therapy. They have played a significant role in both translational and personalized medicine through enhancement of multidrug-resistant TB, extrapulmonary TB, and hindering recurrence of the disease through effective drug and vaccine regiments. Animal models are in no way identical, their diversity brings with it positive features and discouraging drawbacks. Thus, diversification of the animal models will enrich the research experience, reduces bio-safety risks, provides consistent, precise, and more timely experimental cycles, and ultimately broaden the appeal of the findings.

In spite of the advancement in the medical management systems and implementation of global policies in population health, tuberculosis (TB) remains a major social and health threat to mankind . The detectable increase in the number of TB patients worldwide and associated fatalities necessitated the use of effective tools for the prevention, diagnosis, and treatment. Vaccination, medical therapy, community organization, and involvement of social and health workers are key for prevention and treatment of this disease. Development of biomarkers and the tools to investigate the pathogenesis of the etiologic infectious agents, and translational medical research require utilization of novel research animal models, and assessment of the clinical and epidemiological data.

Keywords Tuberculosis · Animal model · *Mycobacterium TB* · MDR · Latent TB Infection (LTBI) · Inoculation · Pathogenesis · Caseous necrosis · Granuloma · Vaccine

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1 Introduction

Tuberculosis is a devastating contagious disease that poses major health peril to the world population. Prevention of the spread of tuberculosis (TB) requires a viable public health effort with broad implementation of anti-TB measures including medical therapy and vaccination. In order to achieve the desired result in eradicating this disease, diagnostic biomarkers, medications, and vaccines need to be developed through the use of animal models. As we address this issue, the prevalence and the speed with which tuberculosis has been propagating, and the rising resistance to TB treatment must not be ignored.

In his regard, a brief discussion of various forms of tuberculosis will be helpful. Classification of TB is based on location of the lesions, and the developmental phases that the infection progresses. These categories can identify the course of the disease, clinical manifestations, diagnosis, and treatment as well as the animal model most suitable for TB research. This encompasses pulmonary, extra-pulmonary, latent TB infection (LTBI), and other active TB forms. Accordingly, investigation of pathogenesis, assessment of therapy methodologies and vaccine preparation for a target TB type must utilize animal models to establish a balanced and individualized approach [1]. Genetically engineered, and cross-collaborative (CC) mice can serve as means in TB's patient-centric research. Monkey TB model mimics that of human beings with some inter-group variations, a fact that makes monkey models suitable for investigation of patient-centric medicine [2].

Although animal model for TB research can be altered and further enhanced to enable typical TB characteristics, the tissue of a typical TB animal model must steadily manifest symptoms comparable to human TB symptoms and signs, show identical pathological lesions, and equivalent *Mycobacterium TB* loads, as well as immunological, radiographic, and hematologic alterations [3]. The role of animal model in sciences basic to medicine and in the biological and translational research of TB has been well documented.

An in-depth understanding of the mechanism of infection, immune response associated with the host–bacteria inter-relationships, and the limitations posed by TB experimental research must be established in order to move forward in the development of unique therapeutic methodologies and vaccines. Experiments that imitate human pathophysiology have been the focus of recent TB research that acquire lung-on-chip cultures and ends up creating lung organoids from healthy or patient donors. We will attempt to discuss the animal models of TB at cellular levels, investigate the potential application of pulmonary organoids, the drawbacks of experimental research, document the pros and cons of the currently utilized models, and the use of in vitro 3D models.

The importance of animal models in TB research, particularly in translational and basic medicine as well as in the study of biology of TB are visible in the considerable number of research publications and recorded data on the subject matter [4].

Several animal models have been proposed and used and their characteristics were verified. Because of the variations in inoculation methodologies of *Mycobacterium* TB and differences in the morphologic, histologic, and immunologic aspects of these models, a clear understanding of the comparative anatomy and structural organization of animal model must be achieved. In addition the variability that exists among animal models within a group has undoubtedly added a degree of complexity to uniform assessment of research data. Significant differences among animals at the cellular and molecular levels combined with the above challenges necessitated a standardized methodology to induce infection, use of immunologic reagents and selection of animal model that exhibits human-like clinical symptoms. Through these standard activities, TB-based research become precise, accurate, and produced applicable data that can be used to develop treatment regimens and TB vaccines. Data indicate that no single research tool or model will address the emerging challenges of TB research. We are hereby attempting to underscore the pros and cons of the current animal and cell-based culture and the potential role of lung organoids in TB research.

Tuberculosis (TB) is the leading cause of death, afflicting annually millions of people worldwide with an estimated one-third of world population living with the latent phase of this disease. In addition to human casualties, TB's economic impact remains over 0.5% of the total global gross national product [5]. In view of these facts, World Health Organization (WHO) considered an urgent path which targets a 90% reduction in the incidence of TB by the next decade [6]. To achieve this dramatic outcome, a massive effort is required to lower the annual TB incidence from one-fifth to fractions [7, 8].

The areas considered critical in this effort encompass enhanced diagnostic means, effective therapy alternatives, and development of innovative vaccines. For this, three areas in TB research are generally accepted as critical: development of innovative vaccines, improved diagnostic tools, and effective treatment alternative [8, 9]. To succeed in these tactics, innovative approaches, notably the establishment of well-suited experimental models for TB research study are required [10]. To that end an evaluation of various experimental approaches implemented in TB research including in vivo models and human cell culture. The focus will be on the most recent advancement recorded in vitro 3D models and the possible role of lung organoids in the investigation of host–pathogen interactions and immune reagents during *Mycobacterium tuberculosis* infection [11, 12].

2 Experimental Models in Tuberculosis Research

Various animal models are utilized in TB research ranging from nonhuman primates (NHPs) to zebrafish [13, 14]. A review of the number of publications compiled in relation to type of animal model in TB research, mouse appears to represent the

predominant model followed by guinea pig and to a lesser extent by rabbit model. While zebrafish and non-human primates shared the least research data.

As you will see below a number of animal species have been put into use as a model for TB infection, including rhesus monkey, guinea pigs, rabbits, mice, zebrafish, and guinea pigs. Not all models exhibit identical features, as individual models may or may not mimic one or more features of human TB, including clinical symptoms and signs, level of *Mycobacterium TB* load in the affected organ, immunologic loads, pathological changes, disease development and course as well as immunological constraints [15]. Rhesus monkey model mimics the clinical signs of a TB-infected human and the associated granulomatous lesions, and thus serves as an excellent model for TB pathogenesis research. Anti-TB vaccine research is well suited for guinea pig model because of the robust immune response elicited to TB infection. Similarly, mouse model serves well in the research of anti-TB drug's efficacy of active TB infection because of the uniformity of bacterial load and pathogenesis.

TB can assume active and latent forms of infection. An active TB is further classified into primary, blood disseminated, and secondary tuberculosis. To replicate human TB, all characteristics of TB must be reproduced in various animal TB models and meet specific research criteria. This may not always be possible in view of the fact that a TB animal model cannot manifest all types of TB [16, 17].

As discussed earlier, currently used mouse models fail to mimic human immune reaction to TB. To overcome this major disadvantage, humanized mice are used in studies through the reorganization of immunocompromised mice with human hematopoietic cells [18]. Humanized mice result from transplanting Human Leukocyte Antigen (HLA)-restricted cells into the animal model. Characteristics of human TB pathology, such as granulomatous lesions, lung cavitation, bronchial obstruction, and caseous necrosis are mimicked by infection of humanized mice with *M. tuberculosis* [19]. Aside from exhibiting typical human pathology listed above, T-cell exhibited abnormal responses combined with compromised control of bacterial infection [20].

Corollary to this, humanized mice demonstrated innate and adaptive immune responses, producing antigen-specific T-cell responses to mycobacterial infection.

Humanized immune response through the mouse model of TB infection can also be achieved by infecting transgenic mice expressing human cluster designation 1(CD1). Reviewing published data indicate that humanized mice are a good tool to study HIV/TB infection [21]. Further work must be planned in order to optimize the efficacy of mice model in TB research.

Certain strain of mice used in the evaluation of anti-TB medications and vaccines can determine the role of genes in TB infection and investigation of the mechanisms of TB immune response. The tail vein of H37RV used to inoculate active TB infection via multidrug-resistant *Mycobacterium TB* (MDR).

Research data support the fact that mice are preferred model animals for several reasons, such as access to immunologically based tools for mice, existence of

genetically engineered mice strains, the small size and the cost-effectiveness of maintaining mice in a small space within laboratory settings [22]. Several other advantages were reported including suitability for HIV and TB co-infection [21]. While other investigators affirmed that genetically modified mice are suitable for the investigation of the role of proteins and genes in TB infection. Cost-effectiveness, convenient operability, unusually small size and abundance of immune reagent with clear genetic background are additional pros of this model.

While many positive immune system-related factors that favor the use of this animal strain exist, there are also equally important issues that hamper the use of this model of infection and limit research of TB pathogenesis. Unlike a human patient, the mouse does not serve as a natural host for *M. tuberculosis* and fails to present lung cavitation, an important feature for disease transmission in humans [23], which is absent in commonly used mouse strains. The impact of genetic variation is evident in the tissue necrosis caused by *Mycobacterium TB infection in mouse strains*. The genetic variation in mouse strains appears in the susceptibility to TB infection and the efficacy of vaccination by Bacillus Calmette-Guerin (BCG) [24]. Research data appear to correlate with the mouse strains and diverse experimental end points. Variable findings are attributed to the dose, method of inoculation of *M. tuberculosis,* and the mouse genetic makeup [25].

Zhan et al. reported the use of certain strains for spontaneous latent TB infection model. Nuermberger et al. [26] reported a "modified latent TB Infection" model following medication therapy and vaccination. Zhang et al. [27] stated that through the use of this model mild granulomas are produced in the lungs, spleen, and liver, and the *Mycobacterium TB* maintained a low level during the latent phase, but showed dramatic surge when the infection relapsed. However, absence of infection recurrence predictors combined with an unusually high level of bacterial loads at latency stage, and the distinct variation observed within a group regarding level of relapse and length of latency have limited the efficacy of this model. In view of the above findings, the role of latent and modified latent TB infection model helped in the development of anti-TB drugs and opened the door for further research to limit the incubation period of *Mycobacterium TB*.

Guinea pig and rabbit are used to overcome the limitations posed by the use of mice model, particularly as it relates to humanized granulomas, a typical lung lesion in *M. tuberculosis* infection. But a clear difficulty still exists in maintaining these species in the laboratory environment and explore the immune system in these models [28].

Hartz guinea pig has been used as a model for active TB infection by subcutaneously inoculating H37Rv 10^3–10^5 CFU for assessing the safety and efficacy of vaccines, primary immunity, anti-TB drugs, pathological response of host, *Mycobacterium TB* coevolution in vivo. Researchers were able to determine that this model is more vulnerable to TB and capable of developing miliary caseous necrotic lesions in the lung, spleen, and liver, which are remarkably similar to that of humans. Yet, this model does not exhibit clinical manifestations of TB nor can

spontaneously produce latent infection and more importantly remains devoid of immunologic reagents essential for gene sequencing.

TB infection of *New Zealand rabbit* via spinal punching has also been used, since it presents liquefactive necrotic granulomatous lesions with cavitation in the meninges, vertebral column, skin, and pulmonary system and serves as the best model for TB transmission, bone TB treatment, and in the diagnosis of skin and meningeal TB, which are rare.

Rabbit's susceptibility to TB is clearly less than guinea pig and higher than mice, but absence of clinical manifestations and related immune reagent is a major drawback. Yet, it remains a model of choice for research on diagnosis and treatment of TB of the joints, spine, and cavities. Macaque/Rhesus monkey use as a model enables TB infection to be inoculated via bronchoscopy and presents a disease course and progression of latent and active TB Infection that mimic characteristics of human TB [29]. Among these characteristics are the Langerhans giant cells that are similar to that of human granulomatous lesions. A unique feature of this model is the clinical manifestations that are identical to the human version of the disease including, fever, dyspnea, cough, exhaustion, weakness, and depression. It presents TB lesions in the pulmonary and extrapulmonary structures, such as the liver, spleen, CNS, osseous structures, and kidney. This model appears to serve well in the establishment of treatment strategies, evaluation of specific medications and vaccine TB and in determining the immunological and pathological response of the animal model and in vivo *Mycobacterium TB* coevolution. Utilization of this model has certain limitations including difficulty obtaining transgenic monkeys, scarcity of immune reagents for specific TB genes, and the need for a large number of animals with associated cost and space requirement. Similarly, research of the efficacy of anti TB medications and related vaccine affected by the variability encountered within a group of animal models.

Genetic variation among monkey TB models is similar to human population, accounting for the differences observed among individual models. Similarity to human is a positive finding, but at the same time limits the ability to present a consistent response to *Mycobacterium TB* strain, a characteristic seen in multidrug-resistant TB (MDR TB). To acquire an optimum active TB model with consistent course, inoculation dose of *Mycobacterium TB* must be increased. Unlike latent infection of guinea pig or mouse where moderate doses or administration of immunosuppressant drugs can achieve similar results. Timely intervention and enhancement of *Mycobacterium TB* inoculation dose are additional measures that optimize the use of monkey as a research model, which provides stability and lowers recurrence. Cluver et al. recommends that lower doses of less-virulent strains are inoculated to reproduce TB symptoms and lesions in monkeys [30].

For TB-induced pleural effusion and active TB, Chinese tree shrew has been used as a model and *Mycobacterium TB* is introduced through caudal and inguinal veins. This model is suitable for the study of the mechanism of TB infection through

the application of transcriptomics and proteomics. Infected animal model shows grossly visible TB lesions and granulomas in the pleural, peritoneum, cerebellum, lung, kidneys, skin, and the vertebral column. Despite the steady and consistent infection methodologies, granulomas lack caseous necrosis and Langerhans cells. Infected animal exhibits hypokinesia, low grade fever, and weight loss, but without distinct clinical symptoms. This model allows investigation of the treatment methodologies of the cutaneous, pulmonary, and pleural TB as well as *Mycobacterium TB* pathogenesis, but hindered by the inability to identify immunologic reagents that annotate the genomic map.

A strain of *Wistar rat model* is utilized to induce latent (LTBI) and active tuberculosis infections through tracheostomy [31]. It allows the investigation of the characteristics of dormant phase of the *Mycobacterium tuberculosis*. Infection of this model induces the formation of granulomatous pulmonary lesions that harbor polymorphonuclear neutrophils and macrophages. This animal strain is cost-effective, but more resistant to infection than mice models. The makeup of tuberculosis-related genes, the mechanism of action, metabolism, toxic effects, and effectiveness of anti-tuberculosis drugs can be studied through this animal model. Despite the positive attributes, granulomatous lesions do not mimic that of human version of tuberculosis, exhibiting much higher level of bacterial load particularly in latent infection.

Another model used for bovine TB infection is the castrated Friesian-cross calf. In 2007, investigators reported lesions in the lower respiratory tract, mediastinal lymph nodes, and lung lobes with positive *Mycobacterium TB* culture in this infected model. Plattner et al. also indicated that fetal bovine model is ideal for immune research and that the difference in host response to anti-TB medication is attributed to the variation in the Gamma delta T (γδT0) cells in early granuloma. It has been proven as an ideal model for determining TB susceptible genes, immune reaction, the protective role of vaccines, and in determining immune response mechanism of TB.

The structural organization of granulomas in this model, whether intact or ruptured, shows major differences from that of human. Examination of intact granulomatous lesions reveal Langerhans giant cells and epithelial cells encircling a central

necrotic cavitation that contains the granuloma. On the other hand, ruptured granulomas exhibit structural characteristics in the early phase that are different from that of that of the late stage of infection. While lymphocytes, plasma cells, and macrophages make up the early phase of loose granuloma; the majority of epithelial cell-surrounded scattered granulomas are WC1 (Gamma delta or T19) cells. Gamma cells are concentrated around necrotic or mineral deposit containing center. Adult zebrafish and larvae utilized as a model for latent *Mycobacterium TB marinum* infection and associated granulomatous lesions receive inoculation via the caudal vein [32, 33].

As a fast-breeding model, zebrafish provides additional advantages including easy accessibility, small size, and limited space requirement. It can serve as a model

for research on TB innate immunity in larvae and for both innate and acquired immunity in the adult zebrafish. It is a suitable model for bacterial virulence and susceptibility research as well as for the assessment of toxic effects and efficacy of anti-TB medications. This model, which is used for the study of the dynamics that govern the development of granuloma, shows a transparent body and offers additional advantage in the ability to examine the *Mycobacterium marinum* interaction with the host.

Both adult and larval zebrafish models are used for the study of the mechanisms of the disease. Certain genetic mutations within this model produce different phenotypes in juvenile when compared with adult zebrafish. Adjusting the dosage of bacterial infection can serve in the development of various types of infection including active, chronic, and latent TB infection models. Because of the shorter cycle of replication and research intervals, biological safety risk of *Mycobacterium marinum* does not exist. Another advantage of this model is the exceedingly early development of post-infection of granuloma.

The International Conference on Harmonization (ICH) developed guidelines that help researchers in experimental research studies on animal models. This guidance was developed within a specialized working group of the International Conference on Harmonization (ICH) to establish the technical requirements for registration of pharmaceuticals for human utilization, safety pharmacology, exploratory clinical trials, reproductive toxicity as well as combination drug toxicity testing.

The complex nature, the diverse issues addressed, and broader scope of the elements within this guidance have necessitated revisions and periodic modifications. The guidance was subjected to rigorous approval processes at various phases (Step 1-Step 4) and has finally received endorsement by the ICH Steering Committee and the final draft was recommended for adoption by the regulatory bodies of the European Union, Japan, and the United States.

ICH guidance mirrors the Food and Drug Administration's (FDA's) current viewpoint on the use of animal models to develop drugs for human use. However, it does not grant specific rights nor establishes binding commitment of the FDA or the public and do not establish legally enforceable responsibilities. It should be viewed only as proposals and not to be construed as mandatory requirements, unless specific regulatory or statutory requirements are quoted.

FDA's compliance program provides directions for the assessment of nonclinical laboratories engaged in specific studies that define the animal model in which the efficacy of an investigational drug or biological product will be evaluated. It also addresses controlled animal efficacy studies designed to provide indication on the effectiveness to support the pharmacokinetic and/or pharmacodynamic experiments in animals used to determine the dose and regimen in humans. Inspections of these studies were conducted to authenticate, to the extent possible, the value and

integrity of the data contained in the final reports of the specific research studies submitted to FDA. Further the FDA in collaboration with the Clinical Data Interchange Standards Consortium was able to develop electronic data standards to assess the studies conducted on animals.

Research on immune cells and molecules remains a challenging task due to the fact that specific antibodies and immunological reagents are missing in the zebrafish model. As a result, sequencing and use of transcriptome remain the sole method for immune response research. Although infection with *Mycobacterium marinum* strain remains effective, use of standard strain H37Rv for the same purpose may not viable. Inability to present clinical symptoms that mimic human TB, as seen in other models, is a clear disadvantage in the use of this model. Despite the similarity of amino acid homologue of intracellular survival mechanisms of *Mycobacterium TB* and *Mycobacterium marinum,* reciprocal replacement of the intracellular survival mechanisms is not possible. This an additional drawback of this animal model. The zebrafish model is considered a favorite research tool because of the common cellular and molecular characteristics associated with *M. marinum* and *Mycobacterium TB* infections although some differences persist between these two types of infections [34].

The study of the adult zebrafish vs. larvae serves different, but parallel, purposes in TB research. Optical transparency of the zebrafish embryos permits the use of advanced imaging techniques. Similarly, the ease with which genetic manipulation of zebrafish occurs would facilitate exploration of the molecular features of TB infection. Further zebrafish larva infected with *Mycobacterium marinum* shows some resemblances to *Mycobacterium TB*. When it comes to the late stages of TB infection, adult zebrafish will be the model of choice for TB research. Although the physiological gap between zebrafish and humans is enormous, which inevitably imposes some limitations to the use of this model.

3 Characteristics and Area of Utilization of Tuberculosis (TB) Animal Models

Animal model strains	Strain selection	Infection method	Animal model type	Advantages	Disadvantages	Applications
C57BL/6 BALB/c C3HeB/FeJ Mice	H37Rv Multidrug-resistant *Mycobacterium tuberculosis* (MDR) 10^5–10^7 CFU (Colony Forming Unit)	Tail vein, aerosol	Active TB	Humanized mouse TB model could be a candidate model of HIV & TB co-infection Cost-effectiveness, small size, easy operability. Genetically engineered mice allows investigation of the role of certain genes or proteins in TB mechanism	No obvious clinical manifestations of TB infection. TB granuloma is different from that of humans, without Langerhans giant cells and class epithelioid cells in peripheral granulomas, do not form necrotic lesions in granuloma except in C3HeBJ mice. No disseminated disease throughout the whole body and no cavitation. Inter-individual variation in infection outcome. Both pathological lesions and bacterial loads in organs were non-uniform	Role of specific gene in TB. Rapid evaluation of anti-TB drugs and vaccines. Research on mechanism of TB immunological response
C57BL/6 C3HeB/FeJ Mice	H37Rv 10^2–10^3 CFU	Tail vein, aerosol	LTB (latent TB infection)	Spontaneous LTBI model could be obtained. Modified LTBI model could also be obtained after drug or vaccine intervention. Mild granulomatous lesions appeared in lung, spleen, and liver. Bacterial load kept low level throughout the latency phase, the relapsed with aggravated lesion and higher bacterial load levels	The latency length and relapse level of TB show great variation within group, and the latent-relapse period exceptionally long. Tissue bacterial loads were at high level latency phase. Lack of predictors for recurrence	Research on mechanisms of latency and relapse. Study the prevention and control of the incubation period, including development of drugs and vaccines. Vaccine safety and efficacy studies

Hartz Guinea pig	H37Rv 10^3–10^5 CFU	Aerosol subcutaneous	Active TB, vaccine evaluations	Tuberculous granuloma remarkably similar to that in humans, with caseous necrosis. Very susceptible to TB. Miliary nodules observed in lung, liver, and spleen; Anti-TB drugs and vaccination have a good response	Lack of specific immune reagents, so more difficult to research underlying mechanisms. Lack of general clinical manifestations of TB. Necrosis & caseation occurs, but cavitation is sporadic. Cannot spontaneously develop latent infection	Drug evaluation. Evaluation of the safety and efficacy of vaccines or immunity strategies, such as primary immunity, prime-boost, and therapeutic vaccines. Pathological response of host, *Mycobacterium TB* coevolution in vivo
The New Zealand rabbit	H37Rv 10^8 CFU	Spinal punching aerosol	Pulmonary, bone, meningeal and cutaneous TB	Model for rarer forms of TB (cutaneous and meningeal). Rabbit spinal TB was the best model to research treatment of bone TB. TB granulomas with necrosis and liquefaction, and easy to form cavitation	Less susceptible to TB than guinea pigs, although slightly higher than mice. Clinical symptoms of TB are not obvious. Lack of relevant immune reagent	Good model for TB transmission research. Preferred model for research on diagnosis and treatment of cavitary, spinal, and joint TB. Could be used for diagnosis and research of meningeal cutaneous TB

(continued)

Animal model strains	Strain selection	Infection method	Animal model type	Advantages	Disadvantages	Applications
Cynomolgus macaque/ Rhesus monkey	H37Rv, Erdman, MDR 100-500 CFU	Via bronchoscope	LTBI & active TB	Can develop pulmonary TB as well as extra-pulmonary in the liver, spleen, kidney, mediastinum, and occasionally the cerebellum and bone. Granuloma is similar to humans, with classic Langerhans giant cells. Can mimic a variety of clinical manifestations, such as low-grade fever, emaciation, cough, depression, and dyspnea. Can mimic LTBI and various forms of active TB progression	Cost and space requirements are high and limit the number of animals that can be used. High variation within groups, making it difficult to evaluate the effectiveness of drugs and vaccines. Transgenic monkeys difficult to obtain, and limited availability of immune reagent, restricting the study of the specific genes in TB	Study of the personalized mechanisms of disease, including pathological and immunological response of host, *Mycobacterium TB* coevolution in vivo (precision medicine). Assessment of individualized anti-tuberculosis drugs and vaccines, therapy strategy
Chinese tree shrew	H37Rv 10^3–10^6 CFU	Caudal and inguinal veins	Active TB, TB-induced pleural effusion	Weight loss, low grade fever, reduced mobility. Visible TB nodules in peritoneum, lung, kidneys, region along spine, and intercostal space. Cutaneous lesions and pleural effusion common; cerebellar TB can be established. Proteomics and transcriptions can be used to study the mechanism of TB	Clinical manifestations not obvious. Granuloma structure differs from humans as it lacks Langerhans cells and caseous necrosis. The same infection leads to various degrees of pathological changes. The whole genome has been sequenced but not annotated. Lack of immunological reagents	Research on TB treatment against pulmonary, pleural effusion, cutaneous tuberculosis models. Research on pathogenic mechanisms of *Mycobacterium TB*

Wistar rat	HN878/W4500 CFU	Tracheotomy	LTBI & active TB	TB model could be obtained in genetic engineered rat. Lower cost compared to large animals. Continuous latent infections facilitate study of biological characteristics of TB bacterium from early to late dormant phase. Formation of pulmonary granuloma containing lymphocytes, macrophages, and PMN	Rat are more resistant to *Mycobacterium TB* infection than mice. High pulmonary bacterial load in latent infection. Granuloma structure dissimilar to that of human TB	TB-related gene and protein would be investigated in genetic engineered rat. Research on Anti-TB drug absorption, distribution, metabolism, toxicology, and efficacy of drug

(continued)

Animal model strains	Strain selection	Infection method	Animal model type	Advantages	Disadvantages	Applications
Castrated male Friesian-cross calf	*Mycobacterium bovis* 10^4 CFU, *Mycobacterium avium* Chester	Aerosol	Bovine TB	Good model for research on TB susceptible genes. Fetal bovine TB model good for immune response research. Pathological lesions observed in the lower respiratory tract, the upper, middle, and lower lobes of lung, and mediastinal lymph nodes. *Mycobacterium TB* culture also positive. Difference in Gamma Delta T cells in early granuloma formation led to the different anti-tuberculosis response in host	In early phase, macrophages, lymphocytes, and a small amount of plasma cells make up the loose granuloma; in late phase, the scattered granulomas were encircled with epithelial cells but not fibroepithelial cells, and with necrosis or mineral deposits in the center; the majority of cells were WC1. Have both intact and interrupted structural granulomas, both of which are different from that of human. The ruptured granulomas are different from that of humans. The intact structural granulomas consisted of epithelial cells around granulomas and necrosis or cavitation in the center. Langerhans giant cells scattered in local lesions	Investigate the protective effects of vaccines. Research immune response mechanism if TB. Good model for research on TB susceptibility genes

Zebrafish larvae and adult	*Mycobacterium marinum*	Local & caudal vein injections	LTB (latent TB infection	Small size requires less space; fast breeding makes it easy to access; transparent body makes it easy to observe the interaction between bacteria and host. Larva can be particularly good mode; for TB innate immunity, while adults can be used for research on both innate and acquired immunity. *Mycobacterium marinum* has no biological safety risk for researchers, with shorter replication cycles (4 h) and shorter research periods. Research on the dynamics of granuloma formation; granuloma could form early at the first week post-infection. Adult and larval zebrafish models complement one another in studying disease mechanisms, certain genetic mutations lead to opposite phenotypes in juvenile and adult zebrafish	Could not be infected with standard strain H37Rv, but with *Mycobacterium marinum*. Although the amino acid homology of *Mycobacterium tuberculosis* and *M. marinum* is close to % 85, the intracellular survival mechanisms are different and could not be reciprocally replaced. Lack of zebrafish immunological reagents and specific antibodies; so mechanistic research on immune molecules and cells is difficult to conduct. Transcriptome and sequencing is the only method to study the immune response. Lack of clinical symptoms and manifestations of TB; therefore, unable to mimic most forms of human TB	Best model for bacterial virulence studies. Evaluation of efficacy and toxicity of anti-tuberculosis compounds. Good model for host susceptibility studies. Research on dynamics of granuloma formation. Larvae can be a particularly good model for TB innate immunity, while adults can be used for research on both innate and acquired immunity

4 Discussion

A number of challenges have risen in TB research that rendered diagnostic, therapeutic, and preventative measures of limited effect, but at the same time opened the horizon for further research utilizing live animal models. There is a long list of barriers, which seem to impede TB research including complexity of TB pathogenesis, lack of effective vaccine, brief therapy interval, and lack of diagnostic biomarkers combined with diagnostic failures. Additionally, multidrug resistance (MDR), latent-relapse, extra-pulmonary tuberculosis, and necessity of individualized treatment methodologies challenge the progress of the research in this field.

In order to detect TB, experimental animal model must be in an active-latent-relapse stage so that the developed biomarkers, such as immune cells, cytokines, and chemokines are detected in urine and blood for both *Mycobacterium TB* and animal model. The importance of early diagnosis of TB plays a key role in limiting TB spread. The traditional methods of TB diagnosis rely on locating the *Mycobacterium tuberculosis* in sputum culture obtained from necrotic tissue of the respiratory tract of affected patient, and on the findings of X-ray/Computed Tomography (CT), tuberculin skin test, along with patient's symptoms. Because of the delay experienced in obtaining the results from sputum test, and the fact that not all active TB patients present positive sputum test renders the use of this diagnostic tool of limited value. For instance, pediatric patients, diabetics, patients with combined HIV-TB infection or extra-pulmonary TB, are some examples where sputum cannot be used for the diagnosis *of TB*. Thus, it is not a universally applicable test.

Several additional diagnostic approaches and specimens are used, such as ELISA (Enzyme-Linked Immuno Sorbent Assay, cytokines such as IP10\VEGF\HO-1 in serum, ELISPOT (Enzyme-Linked Immunospot Assay), *Mycobacterium TB* DNA, Ag85 complex, LAM (lipoarabinomannan) a cell wall component of *Mycobacterium TB* in blood or urine; transcriptomic or metabolomic signatures; and phenotypes of PBMC detected by FACS (Fluorescence Activated Cell Sorting). However, the value of these tests remains questionable because of the low sensitivity, inconstant performance results, complexity, and prohibitive cost. While the results of the specific laboratory tests on blood and urine Ag85 showed remarkable variation in performance, urine LAM detection maintained reduced sensitivity. Prohibitive cost and complexity of transcriptional profiles make the routine use of this diagnostic test, in epidemic areas of TB an unfeasible task. Thus, a cost-effective operation, and extremely sensitive application tools with rapid detection results are needed in TB epidemic areas where opportunities to conduct experiments is not always possible [35].

Comparison of latent and active TB infection in the mouse and monkey enables identification of biomarkers. However, granulomas become visible by the third week in the monkey following infection using positron emission tomography. Similarly detectable changes in erythrocyte sedimentation rate (ESR) occur over a long period of time after infection. Findings from sputum or fluid culture obtained from bronchial alveolar lavage fail to show concrete evidence of latent and active

TB infection. In mouse, weeks have to pass after infection to uncover immunologic response. Therefore, when developing vaccines, biomarkers can predict TB relapse, and aid in establishing immune response that enhance vaccine research and its translation from an animal model to human. In view of the fact that Bacillus Calmette-Guérin vaccine (BCG) vaccine is primarily effective in preventing pediatric tuberculous meningitis, development of a new and universally effective vaccine is needed.

Newly developed vaccines are introduced using animal models with initial success. A prominent candidate in this group was MVA85A, which appeared to address the fundamentals issues associated with BCG vaccine. However, further testing and findings of Phase III clinical study proved to be tenuous [36]. Early diagnosis and vaccine efficacy necessitate tools that assess biomarkers and immune cells in various stages of TB infection.

Treatment methodologies of TB can range from few months to 2 years. This variation emanates from the type of TB, such as acute phase, chronic, or latent TB, and whether the patient is subjected to pretreatment. Furthermore, the use of conventional anti TB drugs or immunotherapy plays a role in the duration of treatment.

Documented data show successful outcome from efforts directed at shortening the treatment plan to few months. Efficient and shorter treatment duration with negligible drawbacks can be achieved through the use of animal models.

Investigators report success in the use of chronic or latent TB infection models in the evaluation of immunotherapy that targets the affected animal model. Corollary to this, active TB in the acute phase can be utilized to assess chemotherapeutic agents. The administration of Vitamin D or ganoderma lucidum (an enzyme secreted by white fungi) prior to standard therapy proved to be effective in latent TB models but not with active acute stage TB. The type of drug to be assessed determines the animal models used.

The serious economic damage and the danger to public health associated with the protracted treatment of Multidrug resistant TB and the low recovery rate require innovative approaches to establish fast and cost-effective diagnostic results that enhance good prognosis. Here the role of MDR TB animal models becomes significant in developing methods and regimens that optimize the process, but this would not be an easy task given the lack of active or latent infection MDR TB animal model [37]. The use of latent-relapse TB in the monkey, mouse, rat, and rabbit models seems to present the answer to the challenges associated with early diagnosis and relapse prevention of latent TB in human.

Although mouse is the most commonly utilized animal model among the above group and the LTB infection in the mouse can be determined at the time of inoculation, the high bacterial load during the latent phase, fluctuating latent period, level of relapse, and variable starting time-points limit the efficacy of this experimental model.

Unlike mouse strain, the monkey LTBI model is best utilized during relapse phase. However, the unusual length of the latent phase and the variation observed within groups rendered the utilization of this model in research of questionable value [38]. Extrapulmonary Tuberculosis (EPTB) as the name implies produces

lesions outside the respiratory tract including the lymph nodes, meninges, and osseous structures. Development of EPTB and transformation from latent TB to active form are closely related to stress factors including administration of immune therapy, aging, and exposure to other infectious agents, such as HIV, and associated with poor prognosis and high morbidity. A distinguishing feature of EPTB is that sputum culture is not attainable, but samples can be obtained from lesions and body fluids would help in reaching a diagnosis. Since the duration of EPTB treatment is longer and less effective than comparable treatment regimens, surgery combined with chemotherapy present a stronger alternative methodology for treatment, particularly with bone TB.

The fact that EPTB tissue examination is regulalrly performed in humans with biomarkers of *Mycobacterium TB*, allows possible use of animal models, for the same purpopse, an important factor to consider in the development of efficient, accurate, and cost-effective diagnostic methods. Because of the extensive structural involvement in EPTB and the vague nature of the clinical presentations, diagnosis and treatment of EPTB remain challenging and will continue to present major hazard to community health.

Several fundamental criteria have been established in the assessment of animal TB research model for drug and vaccine development. Among these standards are the pathological lesions in the pulmonary and extrapulmonary structures, bacterial load in affected organs, assessment of the clinical signs and symptoms, and determination of immune response to infection as well as radiographic findings. In view of biosafety challenges, it may take a minimum of 6–8 weeks to conduct research assessing drug therapy and vaccine efficacy using experimental animal models, a factor that impedes drug and vaccine research. However, the process of assessment of the infected models can be dramatically shortened by the administration of higher doses of inoculant and by the use of more receptive strains of mice models, such as TNF-α or IFN-γ knockout mice [39]. With these modifications the speed of the process could exceed that of the mice strain C57BL/6 or BALB/c. In order to maintain optimum utilization, achieve high sensitivity and safety margins in detection, the application of tracer techniques on laboratory animals may be necessary. These approaches enhance the bacterial load and distribution, enabling proper monitoring of lesions in affected organs. Establishment of a latency infection and relapse through the administration of immunotherapy. may elicit variable responses among models within the same group.

Among the challenges that face the establishment of models that address latency-recurrence infection are lack of consistency in replication of infection and the time needed to accomplish process. Further, amplification of latency period, difficulty sustaining a stable progress of the disease, and the lack of predictive TB biomarkers regarding long-term recurrence of the disease are equally valid disadvantages. Thus, lessening the latency period and keeping consistent progress toward latency and recurrence can overcome some of the challenges and improve the outcome of the infected model.

Using certain mice strains prone to infection with TB, such as such as C3HeB/FeJ, and TNF-α or IFN-γ-knockout after immunization or treatment with anti-TB

drugs or collaborative cross (CC) mice strain have shown success in shortening the cycle and achieving consistent and stable latency-relapse phase.

Administration of specific immune system suppressants such as TNF-α antibodies, gamma irradiation or specific hormone can hasten a consistent relapse with reasonable level of *Mycobacterium TB* organ load and provides a viable statistical data with minimal variation in group. Imaging techniques also play an important role in the early detection of the recurrence phase and in this manner shorten the monitoring latency course of action.

In order to study the drug-resistant (DR) animal models and rapidly assess the efficacy of individual drugs or drug combinations, animal models must be inoculated with a high-virulent drug-resistant *Mycobacterium TB* and drug-resistant mutations with common resistant phenotype and genotype. Less-virulent *Mycobacterium TB* strains best suit the research on pathogenesis and immune mechanism [40]. Reported screening of inoculated animal model with less-virulent drug-resistant strains indicates failure to evoke drug-resistant latency, active TB models or latency or latency-relapse TB mode.

Currently steady assessment of large samples of infected lesions obtained from multiple lesions of TB animal models at different time intervals continues, which necessitates innovative approaches to rectify the evaluation process of animal models. Variations among groups of models and presence of bio-safety risks make this process cumbersome, lengthy, and logistically unfeasible. It has been suggested that in vivo monitoring methodologies are particularly useful in ascertaining the site and level of organ load of TB pathogen.

18F-fluorodeoxyglucose (FDG) PET/CT, a common method used for the diagnosis, staging, and therapy of cancer appears to be also invaluable in determining early stages of infectious diseases including TB. The outcome of this application in TB infection is correlated with robustness of the immune system of the animal model, as a weakened system produces a blurred image that curtails the monitoring process, early detection, and bacterial organ load determination.

The utility of Green Fluorescence Protein (GFP) in in-vivo detection of the lesion sites and level of organ bacterial load used in the diagnosis of latent infection remain uncertain, and may require structural modification of GFP. As the search for new biomarkers continues, FDG-PET/CT and fluorescent-labeling is employed to boost accuracy of detection of TB infection and provide a workable, quick assessment of drug efficacy in EPTB models.

5 Conclusions

For the purpose of maximizing accuracy and reliability in detection innovative approaches should be employed when animal models for TB are considered. These measures must also focus on reducing systematic errors through regular monitoring, minimize uncoordinated and untimely testing of animal models, and reduce biosafety risk for researchers and staff. Among the most positive developments in the

diagnostic path is the employment of PET-CT for imaging lesions and the associated bacterial population. We should not ignore the role of gene-manipulation technologies that can modify or delete genes through CRISPR-Cas9 or other advanced approaches in the mice, rats, and monkeys in exploring the effects of genes and proteins on the mechanisms of therapy and vaccine plans. Depending on the specific purposes, such as prime immunization, prime-boost immunization, and therapeutic vaccine evaluation, multiple TB animal models need to be utilized. For a systematic study of the diagnosis and mechanism of therapy of TB recurrence, a monkey model may be utilized, and in vivo monitoring methodologies applied to achieve personalized medical research.

A gene-knockout mouse TB model with virulent and/or attenuated strains can be used for fast evaluation of drug efficacy and assessment of immune agents, respectively. For drug and vaccine assessment, attention must be directed toward models with rapidly advancing TB, using IFN-γ or TNF-α knockout mice with active TB than wild-type infected TB mice. Assessment of drugs and vaccines of a latency-recurrence TB model might benefit from utilization of TNF-α KO mice. Similarly latent-relapse monkey model can be developed with the administration of TNF-α antibody.

References

1. Peng X, Knouse JA, Hernon KM. Rabbit models for studying human infectious diseases. Comp Med. 2015;65:499–507.
2. Lin PL, Rodgers M, Smith L, Bigbee M, Myers A, Bigbee C, et al. Quantitative comparison of active and latent tuberculosis in the cynomolgus macaque model. Infect Immun. 2009;77:4631–42.
3. Kramnik I, Beamer G. Mouse models of human TB pathology: roles in the analysis of necrosis and the development of host-directed therapies. Semin Immunopathol. 2016;38:221–37.
4. Moskovic E, MacSweeney E, Law M, Price A. Survival, patterns of spread and prognostic factors in uterine sarcoma: a study of 76 patients. Br J Radiol. 1993;66:1009–15.
5. Diel R, Rutz S, Castell S, Schaberg T. Tuberculosis: cost of illness in Germany. Eur Respir J. 2012;40(1):143–51.
6. Uplekar M, Weil D, Lonnroth K, Jaramillo E, Lienhardt C, Dias HM, et al. WHO's new end TB strategy. Lancet. 2015;385(9979):1799–801.
7. Dye C, Glaziou P, Floyd K, Raviglione M. Prospects for tuberculosis elimination. Annu Rev Public Health. 2013;34:271–86.
8. Lienhardt C, Lonnroth K, Menzies D, Balasegaram M, Chakaya J, Cobelens F, et al. Translational research for tuberculosis elimination: priorities, challenges, and actions. PLoS Med. 2016;13(3):e1001965.
9. Pai M, Behr MA, Dowdy D, Dheda K, Divangahi M, Boehme CC, et al. Tuberculosis. Nat Rev Dis Primers. 2016;2:16076.
10. Karp CL, Wilson CB, Stuart LM. Tuberculosis vaccines: barriers and prospects on the quest for a transformative tool. Immunol Rev. 2015;264(1):363–81. pmid:25703572. Tuberculosis. Nat Rev Dis Primers. 2016;2:16076.
11. O'Garra A, Redford PS, McNab FW, Bloom CI, Wilkinson RJ, Berry MP. The immune response in tuberculosis. Annu Rev Immunol. 2013;31:475–527.

12. Orme IM, Robinson RT, Cooper AM. The balance between protective and pathogenic immune responses in the TB-infected lung. Nat Immunol. 2015;16(1):57–63.
13. McMurray DN. Disease model: pulmonary tuberculosis. Trends Mol Med. 2001;7(3):135–7.
14. Myllymaki H, Niskanen M, Oksanen KE, Ramet M. Animal models in tuberculosis research—where is the beef? Expert Opin Drug Discov. 2015;10(8):1–13.
15. Rodgers JD, Connery NL, McNair J, Welsh MD, Skuce RA, Bryson DG, et al. Tuberculosis (Edinb). 87(5):405–414.
16. Swanson RV, Ammerman NC, Ngcobo B, Adamson J, Moodley C, Dorasamy A, et al. Clofazimine contributes sustained antimicrobial activity after treatment cessation in a mouse model of tuberculosis chemotherapy. Antimicrob Agents Chemother. 2016;60:2864–9.
17. Kashino SS, Napolitano DR, Skobe Z, Campos-Neto A. Guinea pig model of *mycobacterium tuberculosis* latent/dormant infection. Microbes Infect. 2008;10:1469–76.
18. Legrand N, Weijer K, Spits H. Experimental models to study development and function of the human immune system in vivo. J Immunol. 2006;176(4):2053–8.
19. Heuts F, Gavier-Widén D, Carow B, Juarez J, Wigzell H, Rottenberga ME. CD4+ cell-dependent granuloma formation in humanized mice infected with mycobacteria. PNAS. 2013;110(16):6482–7.
20. Lee J, Brehm MA, Greiner D, Shultz LD, Kornfeld H. Engrafted human cells generate adaptive immune responses to *Mycobacterium bovis* BCG infection in humanized mice. BMC Immunol. 2013;14:53.
21. Nusbaum RJ, Calderon VE, Huante MB, Sutjita P, Vijayakumar S, Lancaster KL, et al. Pulmonary tuberculosis in humanized mice infected with HIV-1. Sci Rep. 2016;6:21522.
22. Cooper AM. Mouse model of tuberculosis. Cold Spring Harb Perspect Med. 2015;5(2):a018556.
23. Vilaplana C, Cardona PJ. The lack of a big picture in tuberculosis: the clinical point of view, the problems of experimental modeling and immunomodulation. The factors we should consider when designing novel treatment strategies. Front Microbiol. 2014;5:55.
24. Smith CM, Proulx MK, Olive AJ, Laddy D, Mishra BB, Moss C, et al. Tuberculosis susceptibility and vaccine protection are independently controlled by host genotype. MBio. 2016;7:e01516.
25. Franco NH, Correia-Neves M, Olsson IA. Animal welfare in studies on murine tuberculosis: assessing progress over a 12-year period and the need for further improvement. PLoS One. 2012;7(10):e47723.
26. Nuermberger EL, Yoshimatsu T, Tyagi S, Bishai WR, Grosset JH. Paucibacillary tuberculosis in mice after prior aerosol immunization with *Mycobacterium bovis* BCG. Infect Immun. 2004;72:1065.
27. Zhan L, Ding H, Lin S, Tang J, Deng W, Xu Y, et al. Experimental *Mycobacterium tuberculosis* infection in the Chinese tree shrew. FEMS Microbiol Lett. 2014;360:23.
28. Clark S, Hall Y, Williams A. Animal models of tuberculosis: guinea pigs. Cold Spring Harb Perspect Med. 2014;5(5):a018572.
29. Izzo AA, Leung-Theung-Long S, Gouanvic M, Coupet C-A, Ray A, Tupin E, et al. A novel MVA-based multiphasic vaccine for prevention or treatment of tuberculosis induces broad and multifunctional cell-mediated immunity in mice and primates. PLoS One. 2015;10:e0143552. https://doi.org/10.1371/journal.pone.0143552.
30. Cluver L, Orkin M, Moshabela M, Kuo C, Boyes M. The hidden harm of home-based care: pulmonary tuberculosis symptoms among children providing home medical care to HIV/AIDS-affected adults in South Africa. AIDS Care. 2013;25:748–55.
31. Singhal A, Mathys V, Kiass M, Creusy C, Delaire B, Aliouat El M, et al. BCG induces protection against *Mycobacterium tuberculosis* infection in the Wistar rat model. PLoS One. 2011b;6:e28082. https://doi.org/10.1371/journal.pone.0028082.
32. Berg RD, Ramakrishnan L. Insights into tuberculosis from the zebrafish model. Trends Mol Med. 2012;18(12):689–90. (119).

33. Myllymaki H, Bauerlein CA, Ramet M. The zebrafish breathes new life into the study of tuberculosis. Front Immunol. 2016;7:196. (120).
34. Tobin DM, Vary JC Jr, Ray JP, Walsh GS, Dunstan SJ, Bang ND, et al. The lta4h locus modulates susceptibility to mycobacterial infection in zebrafish and humans. Cell. 2010;140(5):717–30. 24643219
35. Goletti D, Petruccioli E, Joosten SA, Ottenhoff TH. Tuberculosis biomarkers: from diagnosis to protection. Infect Dis Rep. 2016;8:6568.
36. Ottenhoff TH, Kaufmann SH. Vaccines against tuberculosis: where are we and where do we need to go? PLoS Pathog. 2012;8:e1002607.
37. Harris RC, Khan MS, Martin LJ, Allen V, Moore DA, Fielding K, et al. The effect of surgery on the outcome of treatment for multidrug-resistant tuberculosis: a systematic review and meta-analysis. BMC Infect Dis. 2016;16:262. https://doi.org/10.1186/s12879-016-1585-0.
38. Lin PL, Maiello P, Gideon HP, Coleman MT. PET CT identifies reactivation risk in cynomolgus macaques with latent *M. tuberculosis*. PLoS Pathog. 2016;12:e1005739. https://doi.org/10.1371/journal.ppat.1005739.
39. Henao-Tamayo M, Obregon-Henao A, Creissen E, Shanley C, Orme I, Ordway DJ. Differential *Mycobacterium bovis* BCG vaccine-derived efficacy in C3Heb/FeJ and C3H/HeOuJ mice exposed to a clinical strain of *Mycobacterium tuberculosis*. Clin Vaccine Immunol. 2015;22:91–8.
40. D'Ambrosio L, Centis R, Sotgiu G, Pontali E, Spanevello A, Migliori GB. New anti-tuberculosis drugs and regimens: 2015 update. ERJ Open Res. 2015;1:00010–2015.

Disease Models in Viral Research

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Abstract Viral diseases have become a constant threat to human population as the rise of exposure to viruses like Dengue, Measles, Chikungunya, Influenza, SARS-CoV-2, Zika, and Ebola increased. To tackle this emerging problem, it is important to investigate the pathogenesis and immune responses by implementing various animal models. Animal models are also utilized in identifying newer drug targets, evaluating efficacy and potential toxicity of new drug and vaccine candidates. Furthermore, no single animal model can mimic all clinical manifestations of the human disease; understanding the lacunas of available models helps in choosing the model for a particular experiment. In this chapter, we cover the animal models, which include nonhuman primates, rodents used for various viral diseases and shows the areas where there is gap in knowledge.

Keywords Animal models · Viral · Virus · Pathogenesis · Infection

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1 Introduction

Animal models are necessary to understand disease progression, pathogenesis, and immunologic responses to viral infections in humans. A good animal model of viral disease should mimic the host defense mechanism and disease progression. As per the protocol mentioned by "FDA animal efficacy rule" the models should have the same receptors as those present in humans and should develop the same symptoms observed in humans. For the evaluation of efficacy and potential risks of new antiviral drugs and vaccines, small animal models are widely used in preclinical phase. Large animal models like nonhuman primate (NHP) models are used for more detailed characterization of disease pathogenesis. All laboratory animal models, from mice and hamsters to ferrets and NHPs, are important for understanding the disease mechanisms. In this manuscript, we summarize the animal models used to study the different types of viral pathogenesis along with their advantages and disadvantages.

2 Animal Models

Developing experimental animal models of dengue virus (DV) infection has been a struggle for the researchers since nonhuman species are unaffected with DV epidemic. There exist various animal models, which have its own merits and demerits and are distinctively used for studies pertaining to pathogenesis of dengue, immunopathogenesis, and/or preclinical studies of different drug candidates and vaccines to combat DV infection, which will be discussed in the upcoming sections.

2.1 Nonhuman Primate Models

The physiological, genetic, and immunological characteristics of NHPs are comparable to those of humans, making it a good experimental model for dengue virus (DENV) infection. The very first study on dengue pathogenesis was done on Rhesus macaque (Macaca mulatta) by administering defibrinated blood from dengue patients through subcutaneous or intravenous route [50]. Furthermore, other NHPs such as the squirrel monkeys (*Saimiri sciureus*), white face monkeys (*Cebus capucinus*), Aotus monkeys, black spider monkeys (*Ateles fusciceps*), howler monkeys (*Alouatta palliata*), cotton-top marmosets (*Marikini geoffroyi*), Saimiri monkeys, red spider monkeys (*Ateles geoffroyi*), and marmosets (*Callithrix jacchus*) has been extensively used as models in pathogenesis and immunopathogenesis studies of dengue [72]. Used for pathogenesis studies.

Animals infected with 10^4–10^6 PFU of DENV are thought to mimic the inoculum found in a mosquito bite. NHPs can maintain viral replication after subcutaneous

(s.c.) inoculation with 10^5 PFU of DENV even so, lower replication of virus is observed compared to humans, only affects the lymphatic abundant tissues and characterized by lymphadenopathy, lymphocytosis, and leukopenia [61]. Surprisingly, cyclophosphamide, an alkylating agent and immunosuppressive medication can be used that predominantly affects proliferative cells along with lymphocytopenia, allowed DENV infiltration of monocytes in Rhesus monkeys to be maintained for longer time interval [62]. After s.c. DENV infection, several Rhesus macaques had decreased platelet counts, however other significant clinical symptoms were absent [37]. Dengue haemorrhage was observed in *Rhesus macaques* treated intravenously with a high dosage of dengue virus [77]. Moreover, human dengue symptoms such as leukopenia, thrombocytopenia, and liver damage have been observed in marmosets [76]. However, there are still several other experimental constraints towards using NHPs, including the significant expense, trouble acquiring huge quantity of animals, and lack of consistency in clinical symptoms observed among patients. All these investigations might pave the way for more pathophysiologic research and treatment approaches for dengue.

2.1.1 Used for Immunopathogenesis Studies

NHPs may not show classic symptoms of infection, but they often generate an antibody (Ab) immunological response that is similar to those observed in individuals affected with DENV infection [61]. Each one of the four DENV serogroups causes a wide cross-reacting Ab and T cell response internally; however, the majority of the response is directed towards the pathogenic serogroup [2, 7]. Recurrent exposures caused a cross-reactive response in NHPs, having the highest reported Ab titers aimed towards the predominant pathogenic serogroup [46]. In NHPs, viremia rises after a subsequent DENV infection, implying that Ab-dependent enhancement (ADE) may boost viral burden via cross-reactive Abs. In Rhesus macaques, viremia rose from 3- to 100-fold after passively transferring anti-DENV monoclonal Ab 1A5 preceding DENV infection, despite no indications of sickness [32]. In NHP existing models, T cell responses are not so well defined. Another study found that DENV infection stimulates the production of proinflammatory cytokines, including interleukin-6 (IL-6) and interleukin-8 (IL-8). Additionally, DENV infection induces the production of two other proinflammatory cytokines, tumor necrosis factor (TNF) and monocyte chemotactic protein-1 (MCP-1). These cytokines can lead to inflammation and tissue damage in the affected area, which can contribute to the development of DHF and DSS. Moreover, an increased level of these cytokines has been associated with severe dengue cases, suggesting that they may play a role in the pathogenesis of DHF/DSS [70, 95]. In conclusion, whereas NHP models exhibit high viremia, experimental animals do not exhibit plasma leakage or symptoms similar to DSS or DHF, which limits their usefulness in immunopathogenesis and pathogenesis studies.

2.2 Rodent Models

Many attempts were undertaken in the past years to develop rodent models which matches with the clinical DENV infection to solve the shortcomings that the virus cannot multiply efficiently within the cells of rodents. Inbred mouse models reduce experimental heterogeneity, while genetically modified mouse models allow some features of dengue clinical symptoms to be replicated. The innate resistance of mice to DENV could be countered by using mouse-adapted strains and/or various route of administration techniques, making rodents an appealing model for dengue study.

2.2.1 Suckling Mice Model

Suckling mice model are the most preliminary animal models to be explored in the dengue research. DENV-induced encephalitis and paralysis were observed in this model of intracranial inoculation with the full-length dengue-2 viral RNA transcript, a type of illness that affects the central nervous system which is uncommon in case of infection in humans [53]. Several researchers used the ICR (Institute of Cancer Research strain) suckling mouse model to assess antiviral and vaccination efficiency based on the clinical severity and survivability scores, along with viral load decline in brain tissue [52]. However, because of DENV's modified tropism to preferentially infiltrate brain due to the presence of blood brain barrier along with rare route of administration of the virus, the biological and physiological significance of suckling mice to mimic DENV infection must be regarded with caution.

2.2.2 Immunocompetent Mice Model

The responses of cellular and innate immunity are observed in various stages of infection with DENV and are correlated with extensiveness of disease and its pathogenesis [36], so immunocompetent mice are best suited for studying the intrinsic role of host immune responses to immunopathogenesis.

Although DENV is inherently resistant in immunocompetent mice, infection can be produced with high viral inoculum ($10^6 – 10^8$ PFU). The viral load in the serum, spleen, liver, and brain of C57BL/6 mice after intravenous (i.v.) infection with 10^8 PFU DENV2 can be observed, indicating widespread virus spread and multiplication in the different tissues. The researchers also found a link between T cell infiltration and activation in liver as well as hepatic injury, a frequent condition seen in dengue patients. [16]. Interestingly, isolated hemorrhages (an essential sign in DSS and DHF) were observed inside the tissues of subcutaneous region when immunocompetent mice were infected with DENV2. This was also accompanied with low platelet count severity and viral load that can be detected in liver, brain, skin, spleen, and serum [15]. Since TNF-α deletion in mice reduced the incidence of hemorrhages, it was proposed that DENV-induced macrophage TNF-α production closely

corresponds with hemorrhage, highlighting the possible innate immunity role played in severe disease immunopathology [15].

Another research depicted a significant plasma leakage which is supported by the hematocrit levels rise, peaking 24–48 h after exposure when C57/BL6 mice are infected with this DENV clinical isolate. The scientists also showed that induction with DENV activated mast cells and release mediators that play a role in causing plasma leakage in mice model. As a result, DHF and DSS characteristics are mimicked by immunocompetent mice models that can give insights into the mechanisms, and the preventive and/or harmful function of generated immune response from DENV infection can also be unraveled by deletion of immune effectors, like mast cells, in the animals [106]. Further, in another study it was found that inoculating wild-type mice with infected K562 cells results in artificially produced temporary viremia, suggesting that this technique could be beneficial for evaluating vaccine candidates [116]. Mice models which are immunocompetent could be imagined as a decent substitute of NHP models for measuring efficacy of vaccines by enhancing this artificial viremia system from transitory to long-lasting. Altogether, these mice models offer a potential platform for exploring host immune response involvement in disease and can also be used to test preclinical vaccines.

2.2.3 Immunocompromised Mice Model

Clinical DENV infection usually incorporates a variety of strategies for bypassing cellular antiviral defenses that aren't possible with DENV homologs in mice, resulting in DENV's failure to reproduce efficiently in WT (wild type) immunocompetent mice [34]. Different immune-deficient mouse strains have shown varied vulnerabilities to DENV infection. Upon intra-peritoneal (i.p.) administration with the mouse-brain-adapted DENV1 strain, around 40–60% of BALB/c athymic nu/nu mice and littermates which were heterozygous died [40]. The C5-deficient mouse (A/J strain) succumbed to DENV infection with indications of paralysis and demonstrated certain clinical manifestations such as thrombocytopenia and an increased hematocrit [41]. RAG1−/− mice, where B cells and T cells are mostly absent, are prone to be infected by PL046, a clinical DENV2 strain, showing a 31% death rate [101]. Since the IFN system is so crucial in the host defense system against the virus, on the 129/Sv background (AG129) the mice which exhibited presence of IFN-α/β and IFN-γ had been investigated for vulnerability to DENV infection. 100% of AG129 mice exhibited paralysis when infected with DENV2 strain New Guinea C (NGC), adapted from mouse-brain, intraperitoneally [45]. Likewise, all AG129 mice succumbed after being infected with PL046 by intravenous (i.v.) injection. Serum, spleen, and liver post-infection showed presence of virus in these experiments, but titers in the brain escalated and the mice acquired paralysis [101]. Furthermore, one of the primary drawbacks of these immunocompromised mouse models has been that paralysis is not really a significant trait. To circumvent the problem of clinical strains of DENV being neuroinvasive in the mice, a novel strain of DENV2 was created by passing subsequently PL046 between C6/36 cells of mosquito and

AG129 mice, denoted as D2S10. Contrary to the paralysis seen among PL046-infected mice, D2S10-infected AG129 animals resulted in death soon after infection and also exhibited higher TNF-α levels and permeability in vasculature [102]. Recently, using reverse genetics technique performed in vivo, the viral coefficient was found to influence virus clearance and, as a result, was linked to a severe illness phenotype in AG129 mice [88]. Modified D2S10 virus by plaque purification produced the more virulent mouse-adapted strain S221, which may also kill AG129 mice completely in 4–6 days without causing neurologic illness [118]. Another study showed that AG129 mice were susceptible to a DENV2 clinical isolate after a single intraperitoneal injection. The mice exhibited viral load in blood and a serological analysis (NS1 secretion, Ab response and inflammation) that was highly comparable to that of human acute Dengue fever (DF) patients [99]. Another study found that a non-mouse-adapted DENV2 strain, D2Y98P, causes deadly systemic infection in AG129 mice, with indications such as leukopenia, liver damage, and vascular leakage, serving as a basis for more appropriate clinical DENV pathogenesis research [108]. When inoculated with S221 virus, STAT1 homozygous knockout mice having deficit in STAT2 (STAT1δ/STAT2 δ) or Type I IFNR (STAT1δ/AR δ) resulted in early death from DENV associated with increased viral load in blood and levels of TNF-α, in addition to AG129 mice. As a result, these models demonstrate that the molecular role of IFN signaling in DENV pathogenesis or prevention may be deconstructed [86]. Single-deficient animals lacking IFN-α/β receptors, on the other hand, may provide an alternate platform for revealing the probable immunoprotective function of T cell responses after DENV infection. S221 inoculation of IFN-α/βR δ mice (on a C57BL/6 background) boosted virulence and elicited a significant response of $CD8^+$ T cells, which was hypothesized to limit clearance of DENV, as reduction of $CD8^+$ T cells dramatically elevated viral load in the brain, serum, and spleen [118]. Recent investigation of DENV infection in IFN-α/βR−/− mice revealed the possibility of producing systemic infection prior to death [80]. Mice harbouring selective knockouts of Type I FNR in different immune cells have recently proved to deliver a superior response of immune system compared to mice which are immunocompromised [122]. Overall, immunocompromised mouse models do have the capacity to be more useful as models for dengue pathogenesis, antiviral testing, and vaccine development.

2.2.4 Humanized Mice Models

Mouse–human chimaeras have been used as an alternate approach over studying DENV infection in immunocompromised mice. In the beginning, researchers employed SCID mice implanted with human peripheral blood lymphocytes, which maintained lower levels of DENV-1 viremia [115]. DENV-2 infection was detected in the blood, liver, and brain of SCID mice grafted with human HepG2 hepatocarcinoma cells [3]. Human CD34+ hematopoietic stem cells when transplanted in non-obese diabetic/SCID (NOD/SCID) mice and infected with DENV developed thrombocytopenia as well as a rash comparable to human Dengue. The capacity to

Table 1 Different animal models used in Dengue

Models	Benefits	Limitations	References
Nonhuman primate	Viral load in blood sustained for a measurable period of time Immunogenicity (neutralizing antibody response) Schedules of immunization more inclined towards humans Plays important role in vaccine development studies	Expensive Clinical symptoms of the disease are lacking	[7, 37, 77]
Wild-type mice	Less expensive, easy reproduction Immune response of mouse can be induced	Clinical symptoms of the disease are lacking Route of infection cannot be mimicked Lacks sustainability of infection	[117]
AG129 mice	Less expensive, easy reproduction Plays important role in antiviral studies Sustains viral load in blood	Poor immune response due to lack of competent IFN pathway Only few strains of virus can be used Clinical symptoms are few	[88, 99]
IFNAR δ mice (C57BL/6)	Less expensive, easy reproduction Sustains viral load in blood Response of $CD8^+$ T cells	IFN type 1 pathway is lacking Only few strains of virus can be used Few clinical symptoms	[80, 118, 122]
Humanized mice	Proper clinical symptoms Cytokines/chemokines and cellular immune responses are induced Human neutralizing Anti-DENV-IgM are induced	No/poor induction of human anti-DENV IgG Time consuming and expensive	[3, 115]

examine human immune reactions to DENV infection and tropism in human leukocytes is one benefit of utilizing humanized mice. Despite this, humanized mice are not broadly utilized in the DENV field since: (a) they are expensive and complex to produce, limiting throughput; (b) there are varying levels of human cell transplant, resulting in animal-to-animal variability; and (c) infection in these animals somehow doesn't result in the serious DENV symptoms observed in humans (Table 1).

3 Animal Models in Measles

Measles is induced in humans and nonhuman primates (NHPs) by measles virus (MV) belonging to the Morbillivirus genus and family *Paramyxoviridae* [92]. Mostly because mice are not normally responsive to MV infection, transgenic

mouse models have been developed to investigate measles pathophysiology. Interestingly, the majority of these mice are receptor knock-in mice that lack signaling of Type I IFN, making entry of wild-type MV and multiplication easier. As an effective alternative, infections with strongly linked animal morbilliviruses can be further examined in their host species, such as canine distemper virus (CDV), which is a helpful proxy for studying the pathogenesis of MV infections. CDV affects a wide variety of animals, including dogs, raccoons, and ferrets [74, 110]. Ferrets (*Mustela putorius furo*) are one of such hosts that can be handled in a controlled laboratory environment and their respiratory tract is comparable to that of human. In ferret models, recombinant CDV (rCDV) strains harbouring a fluorescent reporter protein allowed for sensitive recognition of infected tissues and cells, as well as characterization of morbilliviral tropism in its host organism [110]. Finally, ferrets have been used as an animal model for several other viral infections, and reagents and instruments are available, albeit in limited quantities [28]. Even with these benefits, CDV wild-type infection in ferret models is not a perfect replica of MV infection in primates, because infection with CDV in ferrets usually leads to rapid progression of the disease, neurological dysfunction, and increased mortality, whereas MV infection in primates seldom leads to neurological abnormalities or fatality.

Significant research in NHPs, which are usually vulnerable to MV infection, have thus contributed significantly to our modern understanding of the pathogenesis of measles. They are an excellent animal model for understanding the pathogenesis of measles. New World monkeys can become severely ill or possibly die as a result of MV infection, making them inappropriate as animal models [1]. Furthermore, squirrel monkeys (*Saimiri sciureus*), a New World species, effectively replicated acute signs of measles in humans in a recent research [22]. However, the absence of cross-reactive reagents still restricts this concept. Rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) macaques have disease manifestations and virological, immunological, and pathological results that are identical to those seen in people, and have thus been extensively employed as animal models for measles [27]. Despite their resemblance, the clinical manifestations of the two macaque species differ. Rhesus macaques have a more noticeable skin rash and conjunctivitis over cynomolgus macaques, according to studies [65]. The utilization of NHPs in animal studies involves ethical implications as well, and it is becoming increasingly restricted. Despite this, macaques are a suitable animal for in vivo measles studies owing to the accessibility of cross-reactive reagents. Moreover, using recombinant MVs (rMVs) established on wild-type strains that produce a fluorescent reporter protein leads to more accurate analysis of viral tropism, disease dynamics, and pathogenesis.

To fill the gap between tissue culture and macaque investigations, a newer small animal model came to light. The cotton rat (*Sigmodon hispidus*) model has proven to be a good resource for exploring the MV pathogenesis. MV replicates in the respiratory tract and lymphoid organs of cotton rats (varying on virus strain). These have been used to research viruses with genetic alterations and have been inoculated with vaccine, wild-type, and recombinant measles viruses. The cotton rat has also

proven to be a useful animal method for analyzing immune suppression and vaccine suppression by maternal antibodies induced from MV infection. Furthermore, cotton rats model appears as a promising model used in studying polymicrobial infections (interaction between secondary pathogens and MV) [75].

Overall, an effective measles animal model demands the proper viral selection, host sensitivity, and the accessibility of tools and procedures to track the disease's progression.

4 Animal Models of Chikungunya Virus Infection

Chikungunya virus (CHIKV), a positive-sense RNA virus belonging to the genus Alphavirus and family *Togaviridae*. In humans, infection with CHIKV causes excruciating arthralgia, which causes acute swelling and pain in the peripheral joints, as well as a high fever and occasionally a rash. Given its expanding global distribution, several aspects of chikungunya pathogenesis remain unknown. As a result, there is indeed a significant focus in using animal models to study CHIKV infection.

4.1 Mouse Models

Chikungunya pathogenesis can be studied using mouse models, which can also be used as early prototype platforms for CHIKV vaccines and treatments. Numerous characteristics have made mice alluring model systems for analyzing CHIKV, which include (1) their minimal expense; (2) less space consumed due to its small size; (3) greater availability of reagents specific to mouse, like immune reagents, used in quantifying host response against CHIKV infection; (4) genetically similar inbred animals are available which is utilized to examine the reactions to various treatments in the same line or to assess phenotypic uniformity; (5) genetically modified animals or knockout mice are further helping in deciphering the role of specific genes and pathways involved in CHIKV infection. While rodent models are a valuable tool, they are limited in their potential to represent every element of CHIKV infection in humans.

4.1.1 Acute Infection Models

Neonatal mice are particularly prone to CHIKV infection and, previous to the advancement of new cell culture techniques, have been used to isolate and amplify viruses like CHIKV [94]. Furthermore, these mice exhibit deadly encephalitis, which is utilized in mimicking illness of human neonates, also offer the platform for host variable and viral research that can be correlated to neonatal illness severity

[19]. Neonatal mice offer efficient ways for evaluating the potential of polyclonal and monoclonal antibodies specific to CHIKV as well as other therapies due to their great susceptibility to CHIKV infection, in addition to being valuable as pathogenesis models. Moreover, neonatal mice cannot be utilized to precisely test vaccination efficacy due to their underdeveloped immune systems; however, they can also be used to examine the toxicity of live attenuated vaccines [55].

Animals without an effective type I IFN receptor or other type I IFN pathway components are highly vulnerable to systemic CHIKV (for example, IRF3/IRF7) [19, 98]. As a result, as demonstrated by the research, immunocompromised mice are effective instruments for exploring the role of type 1 IFN system in pathogenesis of CHIKV and are extremely sensitive threat models for evaluating the potential of anti- CHIKV Abs or the efficacy and safety of vaccines used for CHIKV [84]. Unfortunately, type I IFN receptor-deficient animals die quickly after contracting CHIKV, limiting their effectiveness.

Immunocompetent mouse models which involve subcutaneous infection in the footpads of C57BL/6 mice result in CHIKV-induced arthritis with symptoms like swelling, tendonitis peaking at 3–7 days after the infection [31].

4.1.2 Chronic Models

The use of mice models to study the persistence of CHIKV infection and its link to chronic condition has emerged. At early time periods after infection, C57/BL6 mice implanted with a clinical sample of the virus to the footpad exhibited viral RNA that could be detected in a range of tissues [38]. However no infectious virus has been isolated from these tissues during the chronic stage, data indicates that CHIKV survives in these tissues through low-level sustained reproduction [38]. Immunological processes that assist towards the regulation of CHIKV infection and how these may be modified during persistence are also being studied using mouse models. Multiple studies have identified essential functions for the adaptive immune response, notably the B-cell response, in CHIKV infection management [58, 100]. Improved models for exploring the influence of aging on CHIKV disease are also needed, in addition to models that better depict the various CHIKV disease outcomes seen in patients.

4.2 Nonhuman Primate Models

NHP CHIKV infection models have lately been utilized to investigate chikungunya pathogenesis as well as in preclinical studies to assess vaccination and immunotherapeutic efficacy. Novel NHP models involving cynomolgus macaques (*Macaca fascicularis*) have been developed, as well as additional evolution of the rhesus macaque model to mimic CHIKV exposure in elderly populations [68].

4.2.1 Cynomolgus Macaque Model

A novel CHIKV infection model using immunocompetent adult cynomolgus macaques replicated the viral, clinical, and pathological characteristics shown in human infection. Long- term CHIKV infection was detected in the joints, muscles, lymphoid organs, and liver of macaques, which may reflect the long-term CHIKV illness characteristics seen in people using high dose inoculation, about 10^8 PFU. Furthermore, during the late phases of CHIKV infection in vivo, macrophages were identified as the major cellular repositories. This model of CHIKV physiopathology may enable possible treatment and/or preventive approaches to be developed [49].

4.2.2 Rhesus Macaque Model

When rhesus macaques were infected with CHIKV, maximal viremia levels occurred 1–2 days after the onset and sustained for an average of 6 days along with high fever and rashes [68]. Studies in rhesus macaques employing CHIKV-specific mAbs yielded intriguing findings into CHIKV transmission paths [85]. The rhesus macaque model of CHIKV infection can be utilized to address fundamental concerns regarding chikungunya pathogenesis and as a preclinical subject for evaluating potential anti-CHIKV therapies, according to this study.

5 Influenza

5.1 Epidemiology and Pathogenesis

Influenza is one of the worst infectious disease resulting in millions of deaths. After black death, influenza pandemic ranks in the second position in terms of death toll. Annually, more than 40,000 deaths occur in the United States due to influenza virus [25]. Spanish flu pandemic in 1918 caused more than 50–100 million people deaths. Not only 1918, 1957, 1968, and 2009 influenza pandemic, world has faced another 10 pandemics due to this virus [59]. Influenza virus belongs to the family Orthomyxoviridae, has eight single stranded, negative-sense, RNA molecules that encode 11 or 12 viral proteins [44]. Out of the three types of influenza viruses influenza A, B, and C, most virulent type in humans is influenza A. Influenza A viruses are classified depending upon the antigenic properties of their haemagglutinin (HA) and neuraminidase (NA) glycoproteins. HA antigens are classified into 16 subtypes (H1 to H16), whereas NA antigens into nine subtypes (N1 to N9). Subtypes H1N1, H1N2, and H3N2 can cause infection in humans [89]. HA binds to α-2,6-linked or α-2,3-linked sialic acid residues on glycoproteins of the respiratory epithelial cell surface and this linkage is destroyed by NA, required for the release of progeny virions from the host cell surface [35]. Influenza virus transmission from another animal species like wild birds to humans

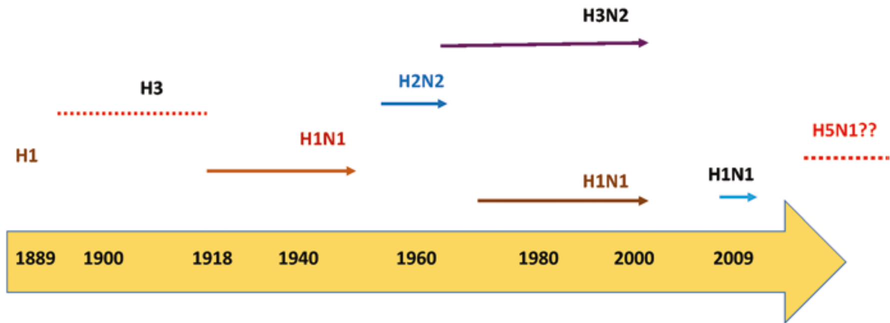

Fig. 1 The various types of human influenza viruses in respective years

occurs through air and from infected person through coughing or sneezing [113]. Clinical evidence shows that children with first time exposure and immunocompromised humans are good transmitters of influenza virus [114].

Common symptoms caused in human after the infection of influenza are fever, sore throat, muscle pains, severe headache, coughing, weakness and fatigue [26] and severe infection can cause pneumonia.

In near future, H5N1 subtype may produce another pandemic. Previously consistent evidence suggests that pandemic due to influenza virus occurs in every 10–40 years. For understanding, the underlying mechanisms of transmission and immune responses to influenza virus, to study the efficacy of vaccine and antiviral drug, selection of the appropriate laboratory animal model is very important (Fig. 1).

5.2 *Mice* (Mus musculus)

The use of these mouse has led to a better understanding of the role of different immune components during the course of influenza virus infection. For example, the use of C57BL/6 and BALB/C mice has provided insight into the role of antibodies in protection against infection. Similarly, the use of DBA.2 mice has shown that the type I interferon response plays a major role in controlling influenza virus infection. C57BL/6, BALB/C and, more recently, DBA.2 are the most common mice strains for the study of influenza virus [9]. Immune response depends on the different strain; therefore, the selection of appropriate strain is a concern. Although human influenza viruses generally cause disease in mice only if they are first adapted to the species and necessary to use murine-adapted viruses is required [64].

5.3 *Ferrets*

Ferrets are a requisite model for the study of pathogenesis of influenza viruses because infected ferrets mimic the symptoms of influenza virus infection in humans and they display many of the clinical signs which are nor seen in the other animal

models [6]. During the epidemic in England in early 1933, Wilson Smith and his team first isolated the virus causing human influenza [104]. Unlike mice models, ferrets are not required prior adaptation to the species and susceptible to a wide variety of human influenza virus. Airborne transmissibility among ferrets has generally correlated with transmissibility among humans. Limitations of ferret studies are that they are more expensive, require complex husbandry, and ferret genome is not fully annotated [90].

Ferret models are used to test the efficacy of antiviral agents. Approved drugs for influenza virus like oseltamivir, zanamivir, and peramivir have shown positive result on ferrets, responded decrease in inflammatory markers and a reduction of influenza virus shedding in the nares [119].

5.4 Guinea Pig (Cavia porcellus)

Influenza virus pathogenesis is not frequently studied in guinea pigs [87]. Guinea pig models offer an alternative for studying the progression of disease in humans as compare to mice and ferrets models. In 2006, Peter Palese first developed the guinea pig model of influenza virus transmission [57]. Anice C. Lowen et al. reported that guinea pig can transmit influenza virus in between themselves [56]. The models can be used to identify the key factors that lead to virus transmission. The main limitation with guinea pig model is that unlike ferrets, guinea pigs show minimal clinical evidence of influenza virus infection [109].

6 COVID-19

Wild animals are the major hosts for the emergence of the majority of infectious diseases. In 2002–03, the severe acute respiratory syndrome (SARS) outbreak caused by a novel coronavirus originated in China and spread to 37 countries. In 2012, the Middle East respiratory syndrome (MERS) outbreak was caused by another novel coronavirus originating in Saudi Arabia and spread to 27 countries. The total death count of 774 was reported due to severe acute respiratory syndrome coronavirus (SARSCoV) outbreak in November 2002 in China, where the total death rate was 9.6% [39, 78]. A higher death rate of 35% was reported due to another corona virus middle east respiratory syndrome coronavirus (MERS-CoV) in 2012 [79]. Very recently, a novel coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) broke out in December, 2019, which was first detected in Hubei province, China [120]. More than 950,000 deaths and 30 million people were infected by this novel coronavirus as of 18 September 2020. On the 11th of February, 2020, WHO termed this novel coronavirus as “COVID-19.”

SARS-CoV-2 made up with four structural proteins, namely spike protein, envelope protein, nucleocapsid protein, and membrane protein. The spike protein binds to target cells via the angiotensin-converting enzyme 2(ACE2) receptor and is

activated by the host human transmembrane protease serine 2 (hTMPRSS2) protease and coreceptor [24]. COVID-19 causes a wide range of disease symptoms in humans from asymptomatic to severe pneumonia. Mild symptoms like fever, muscle pain, sore throat, cough, and dyspnoea are common, depending upon the replication capacity of SARS-CoV-2 in the upper respiratory tract of the host. Animal models of COVID-19 are essential for understanding disease pathogenesis and for evaluating the safetyand efficacy of vaccine. Coronaviruses are pleomorphic, enveloped, single-stranded, positive-sense RNA viruses belonging to the family *Coronaviridae.* Based on genomic structures and phylogenetic relationships, this family is further divided into four genera, namely, alpha, beta, gamma, and delta, based on phylogenetic relationships and genomic structures. Out of this, alpha and beta genera can infect only mammals. On the other hand, gamma and delta coronaviruses infect birds, but can infect mammals to some extent [12].

6.1 Mouse Models

Mouse models are a valuable tool for studying the pathogenesis of SARS-CoV-2. They are used to understand how the virus replicates and how it causes disease in humans. K18-hACE2 transgenic mouse is one of the best models for SARS-CoV. Mice expressing hACE2 receptor and transgenic hACE2 under the control of an epithelial cell-specific promoter K18 (K18-hACE2) observed similar disease progression and infection pattern with humans [5]. Transgenic BABL/c mice produced neutralizing antibodies against SARSCoV-2 after the administration of DNA vaccine [103]. For understanding the pathology of COVID-19, knock out mouse model has been developed, ACE−/− knockout mice is one of them [43]. One of the major limitation with mice models is that mice needs to be made transgenic in most of the cases for causing infection.

6.2 Ferret Models

The use of ferrets in studying the pathogenesis of respiratory viruses has several benefits. They have been used to study the pathogenesis of human respiratory syncytial virus (hRSV), influenza virus (hIV) and adenovirus (AdV) [69]. Like mice models, administration of SARS-CoV at a high dose in ferrets shown infection in the respiratory system [63]. The infected ferrets were observed increased body temperature, coughing and upregulation of various cytokines and chemokines, indicates significant inflammatory response [8]. Broncho-alveolar inflammation observed in euthanized ferrets by the help of microscopic findings [91]. Significant reduced viral load was observed in the upper respiratory tract of ferrets by the administration of ribonucleoside analog inhibitor MK-4482/EIDD-2801 [20]. Furthermore, ferrets immune system and the expression of ACE2 protein in lungs share similarities with the human making them a possible animal models for studying the pathogenesis of SARS-CoV and testing of therapeutic drug molecule and lifesaving vaccines.

6.3 Hamster Model

Golden Syrian hamster (*Mesocricetus auratus*) is a good model for studying respiratory infection caused by viruses. Significant body weight loss and increased inflammatory cells in hamsters were observed after the transmission of SARS-CoV-2. Considering the gender factor, a more severe disease was observed in male hamsters than female hamsters [81]. Compared to other animal models hamsters exhibit a more consistent lung disease phenotype during COVID-19 infection [12]. These animal models can be used to study mild SARS-CoV-2 infections in humans and host defense response to the virus [13].

6.4 Nonhuman Primates

COVID-19 like disease was produced in *Cynomolgus macaques* by the inoculation of a SARS-CoV-2 strain through intratracheal and intranasal routes [93]. High levels of viral replication for 7–14 days in respiratory tract was observed by several researchers which is a pathological feature of viral pneumonia [14]. Scientists are undoubtedly interested to use nonhuman primates for testing suitable vaccine candidates. Recently inactivated SARS-CoV-2 vaccine candidate, PiCoVacc, was tested in rhesus macaques and showed a good immunogenic response [30] (Table 2).

Table 2 Animal models of SARS-CoV-2

SN	Animal models	Strains	Route used	Clinical signs	Advantages/ limitations
1	Mice [66, 103]	K18-hACE2 Transgenic, C57BL/6 J, BALB/c	Intranasal	Lung damage, bodyweight loss	Needs to be made transgenic in most of the cases for causing infection.
2	Ferret [8]	*Mustela putorius furo*	Intratracheal/ Intranasal	Nasal discharge, weight loss and elevated body temperature	For studying disease transmission and for the development of new vaccine Ferrets do not mimic all the clinical signs
3	Hamsters [13, 17]	*Mesocricetus auratus*, Golden Syrian Hamsters	Intranasal	Laboured breathing, weight loss and decreased activity	Hamsters do not mimic all the clinical signs, Lack of mortality
4	Nonhuman Primate [5]	*Rhesus macaques*	Intratracheal/ Intranasal	Upregulation of inflammatory cytokine and weight loss	Permissive for SARS-CoV-2 infection and develop pneumonia. Good model for evaluating vaccines. NHPs do not develop the acute lung injury.

7 Zika Virus

7.1 Epidemiology and Pathogenesis

Zika virus (ZIKV) is a member of *Flaviviridae* family. It was first isolated from the blood of a sentinel rhesus monkey in Ziika forest of Uganda in 1947, from this point it is known as Zika [23]. At the end of 2015, it became a serious public health concern after the outbreak in Brazil [96]. In 2016, WHO declared ZIKV to be a "Public Health Emergency of International Concern" [18]. ZIKV is a positive-stranded RNA enveloped viruses, same genus with dengue virus, Japanese encephalitis virus, yellow fever virus [60]. Daytime-active Aedes mosquitoes transmit this virus. It is also transmitted from human to human through sexual and vertical routes [21, 97]. Infection during pregnancy causes microcephaly in the infants [11]. Symptoms are almost same with dengue virus. There is no such vaccine or therapeutic treatment against this disease until now, so need of well-established animal models is necessary.

7.2 Mice Models

Mice models have been very powerful tools to understand ZIKV mechanism of pathogenesis, as well as for drug screening. Matthew J. Gorman et al. reported a mouse-adapted ZIKV (Dak-MA) with enhanced brain and NSC infectivity [33]. Helen M. Lazear and his team developed a knock out mice with lacking the interferon receptor (Ifnar1^{-}/$^{-}$). This mice has observed sustain high viral burden in brain, spinal cord and testes consistent with evidence that ZIKV causes neurodevelopmental defects in human foetuses [51]. For understanding the disease pathogenesis, this ZIKV model will be gold standard model (Fig. 2).

7.3 Guinea Pigs

Guinea pigs have been extensively used in viral research since from 1967, the year when MARV was discovered. Reproductive physiology and estrous cycle of guinea pigs are similar with humans. Mukesh Kumar et al., reported that infection with ZIKV produced potent cytokines, chemokines and growth factors in the serum of infected guinea pigs [48]. Long gestation period and pups are born with a mature CNS, selects this models for the studies of in utero transfer of ZIKV and neurological manifestations in infants [83].

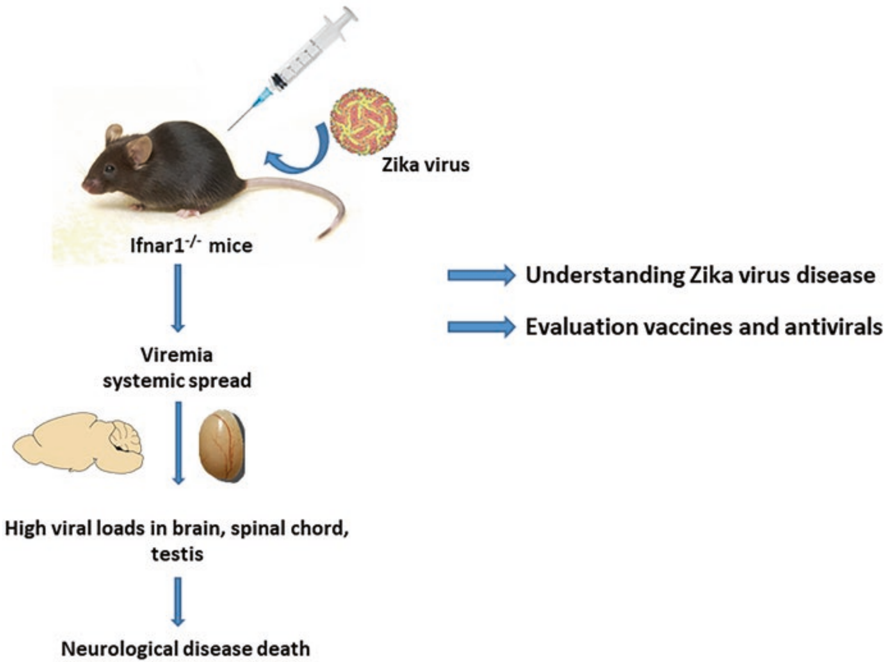

Fig. 2 Mouse model of zika virus pathogenesis. (Adapted from Helen M. Lazear et al. [51])

7.4 Ferrets

The ferret is an interesting and potentially translational animal model to understand the pathogenesis of ZIKV. It is the smallest animal model with a gyrencephalic cerebral cortex and possesses several key features and an immune system that make it appropriate for the study of neurological effect caused by ZIKV [42].

7.5 Nonhuman Primate (NHP) Models

Similar fetal development and gestation of macaque monkeys with human allows more faithfully enumerate human disease [112]. Osuna et al. reported that both rhesus and cynomolgus macaques have shown to be highly susceptible to ZIKV infection following subcutaneous (sc) inoculation [82]. Rhesus macaques are used to study the protective effect of ZIKV vaccine and for the evaluation of protective efficacy of active ZIKV immunization [71]. Limitations are the cost is high while the throughput is low and the presence of limited number of colonies for the study of antiviral or vaccine efficacy.

Table 3 Subtypes of Ebola and Marburgvirus

Genus	Species	Virus	Disease in humans
Ebola	*Zaire ebolavirus*	Ebola virus (EBOV)	✓
	Sudan ebolavirus	Sudan virus (SUDV)	✓
	Taï forest ebolavirus	Taï Forest virus (TAFV)	✓
	Reston ebolavirus	Reston virus (RESTV)	✕
	Bundibugyo ebolavirus	Bundibugyo virus (BDBV)	✓
Marburgvirus	*Marburg marburgvirus*	Marburg virus (MARV)	✓
		Ravn virus (RAVV)	✓

8 Ebola and Marburgvirus

Ebolavirus and Marburgvirus come under the family *Filoviridae*. Ebolavirus genus is divided into five distinct species: Zaire ebolavirus, Sudan ebolavirus, Tai Forest ebolavirus, Bundibugyo ebolavirus, and Reston ebolavirus; out of this five species Reston ebolavirus does not cause lethal disease in humans. The genus Marburgvirus has two subspecies: Marburgvirus and Ravnvirus [73]. In 1976, Sudan and Zaire species caused an outbreak in the Democratic Republic of Congo (DRC). The whole family belongs to the biosafety level 4 (BSL-4) agents, because they can cause high mortality in both humans and nonhuman primates (NHP) [47]. The transmission of filoviruses to the mammals is not well understood till now, but it confirmed that outbreaks are initiated from the dead NHPs [105]. Common clinical symptoms of filoviruses are fever, nausea, vomiting, headaches, sore throat, diarrhoea and abdominal pain [67] (Table 3).

8.1 Mice Models

Evidence showed that NHP are considered as the most appropriate model for the study of filovirus [105], but limitations like ethical and space issues convinced to use various small animals. Immunocompetent mice are unable to cause infections with wild type EBOV (WT-EBOV) [4]. However, wild-type inbred mice are susceptible to filovirus that has been mouse adapted (MA) by serial passage in mice [10]. Overall, mouse models of EBOV infection demonstrate rapid onset of viremia and high viral burden in the spleen, liver, and multiple organ tissues.

*8.2 Guinea Pigs (***Cavia porcellus***)*

Guinea pig models have been developed by using guinea pig adapted EBOV (GP-EBOV) and MARV (GP-MARV) through aerosol routes [54]. After 2 days of the infection, neutrophilia and lymphopenia are observed [107]. Advantages of

guinea pigs models are low cost, produce fever, which is one of the main symptom, larger animals to study disease progression, and easy to take blood samples, while the main limitations are transgenic and knockout models are not available and lack of immunological tools and reagents to evaluate cell-mediated responses to vaccines.

8.3 Syrian Hamsters

Hamsters models are an alternative to guinea pigs to compare differences in immune responses to EBOV infection. Because of their short life cycle they are easily available for experiment purpose. WT EBOV does not cause clinical signs in hamsters model [111], while MA-EBOV causes significant organ damage. Infected hamsters produce an upregulation of different cytokines levels in liver, spleen, and blood, which indicates potential immune response. Limitations are that mouse-adapted variants are needed and lack of commercially available reagents.

8.4 NHPs

NHPs are the gold standard model for the evaluation of filovirus infections because they can produce infection through various routes in humans [29]. This are the preferred models for studying of countermeasures for the resemble disease progression with humans. It is important to note that the severity and combination of symptoms may vary depending on the pathogen involved and the overall health of the NHP. The most common signs and symptoms reported are fever, lymphopenia, and alteration of various liver enzymes [121].

9 Conclusion

Animal models are inescapable for greater understanding of disease pathogenesis and testing of new therapeutic approaches in viral research. Several animal species have been used in viral research, each with advantages and disadvantages. The ideal animal model for human viral disease should mimic the symptoms of virus infection in humans. Smaller rodents are readily accessible to the researchers for their low cost and requirements compared to the NHPs, which can limit some research need. In addition, a single animal model cannot fulfill all the aspect of human disease accurately, because most disease processes involve multiple cells, tissues, and/or organs. In this review, we have discussed a number of animal models to study dengue, measles, chikungunya, influenza, COVID-19, zika, ebola, and marburg viruses. Their use is important as they offer the opportunity to study different parameters of virus infections in a well-controlled manner that cannot be performed safely in humans. Moreover, current models could be improved significantly with

recently evolved technological tools to comprehend clinical findings, allowing for better translation of antiviral and vaccine research.

Pls add elaborative conclusion on disease spread, limitations on animal models, advances in methodology, regulatory etc.

References

1. Albrecht P, Lorenz D, Klutch MJ, et al. Fatal measles infection in marmosets pathogenesis and prophylaxis. Infect Immun. 1980;27:969–78. https://doi.org/10.1128/IAI.27.3.969-978.1980.
2. de Alwis R, Beltramello M, Messer WB, et al. In-depth analysis of the antibody response of individuals exposed to primary dengue virus infection. PLoS Negl Trop Dis. 2011;5:e1188. https://doi.org/10.1371/JOURNAL.PNTD.0001188.
3. An J, Kimura-Kuroda J, Hirabayashi Y, Yasui K. Development of a novel mouse model for dengue virus infection. Virology. 1999;263:70–7. https://doi.org/10.1006/viro.1999.9887.
4. Banadyga L, Dolan MA, Ebihara H. Rodent-adapted filoviruses and the molecular basis of pathogenesis. J Mol Biol. 2016;428:3449–66.
5. Bao L, Deng W, Huang B, et al. The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice. Nature. 2020;583:830–3.
6. Belser JA, Katz JM, Tumpey TM. The ferret as a model organism to study influenza a virus infection. Dis Model Mech. 2011;4:575–9.
7. Beltramello M, Williams KL, Simmons CP, et al. The human immune response to dengue virus is dominated by highly cross-reactive antibodies endowed with neutralizing and enhancing activity. Cell Host Microbe. 2010;8:271–83. https://doi.org/10.1016/j.chom.2010.08.007.
8. Blanco-Melo D, Nilsson-Payant BE, Liu W-C, et al. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. Cell. 2020;181:1036–45.
9. Bouvier NM, Lowen AC. Animal models for influenza virus pathogenesis and transmission. Viruses. 2010;2:1530–63.
10. Bray M, Hatfill S, Hensley L, Huggins JW. Haematological, biochemical and coagulation changes in mice, Guinea-pigs and monkeys infected with a mouse-adapted variant of Ebola Zaire virus. J Comp Pathol. 2001;125:243–53.
11. Caine EA, Jagger BW, Diamond MS. Animal models of Zika virus infection during pregnancy. Viruses. 2018;10:598. https://doi.org/10.3390/v10110598.
12. Casel MAB, Rollon RG, Choi YK. Experimental animal models of coronavirus infections: strengths and limitations. Immune Netw. 2021;21:1–17. https://doi.org/10.4110/in.2021.21.e12.
13. Chan JF-W, Zhang AJ, Yuan S, et al. Simulation of the clinical and pathological manifestations of coronavirus disease 2019 (COVID-19) in a golden Syrian hamster model: implications for disease pathogenesis and transmissibility. Clin Infect Dis. 2020;71:2428–46.
14. Chandrashekar A, Liu J, Martinot AJ, et al. SARS-CoV-2 infection protects against rechallenge in rhesus macaques. Science. 2020;80(369):812–7.
15. Chen H-C, Hofman FM, Kung JT, et al. Both virus and tumor necrosis factor alpha are critical for endothelium damage in a mouse model of dengue virus-induced hemorrhage. J Virol. 2007;81:5518–26. https://doi.org/10.1128/jvi.02575-06.
16. Chen HC, Lai SY, Sung JM, et al. Lymphocyte activation and hepatic cellular infiltration in immunocompetent mice infected by dengue virus. J Med Virol. 2004;73:419–31. https://doi.org/10.1002/jmv.20108.
17. Cleary SJ, Pitchford SC, Amison RT, et al. Animal models of mechanisms of SARS-CoV-2 infection and COVID-19 pathology. Br J Pharmacol. 2020;177:4851–65.
18. Complications P. Zika Situation Report. 2016:1–6.

19. Couderc T, Chrétien F, Schilte C, et al. A mouse model for chikungunya: young age and inefficient type-I interferon signaling are risk factors for severe disease. PLoS Pathog. 2008;4:e29. https://doi.org/10.1371/journal.ppat.0040029.
20. Cox RM, Wolf JD, Plemper RK. Therapeutically administered ribonucleoside analogue MK-4482/EIDD-2801 blocks SARS-CoV-2 transmission in ferrets. Nat Microbiol. 2021;6:11–8.
21. Cunha MS, Esposito DLA, Rocco IM, et al. First complete genome sequence of Zika virus (Flaviviridae, Flavivirus) from an autochthonous transmission in Brazil. Genome Announc. 2016;4:e00032–16.
22. Delpeut S, Sawatsky B, Wong X-X, et al. Nectin-4 interactions govern measles virus virulence in a new model of pathogenesis, the squirrel monkey (Saimiri sciureus). J Virol. 2017;91:e02490–16. https://doi.org/10.1128/jvi.02490-16.
23. Dick GWA, Kitchen SF, Haddow AJ. Zika virus (I). Isolations and serological specificity. Trans R Soc Trop Med Hyg. 1952;46:509–20.
24. Djomkam ALZ, Ochieng'Olwal C, Sala TB, Paemka L. Commentary: SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Front Oncologia. 2020;10:1448.
25. Dushoff J, Plotkin JB, Viboud C, et al. Mortality due to influenza in the United States—an annualized regression approach using multiple-cause mortality data. Am J Epidemiol. 2006;163:181–7.
26. Eccles R. Understanding the symptoms of the common cold and influenza. Lancet Infect Dis. 2005;5:718–25.
27. El Mubarak HS, Yüksel S, van Amerongen G, et al. Infection of cynomolgus macaques (Macaca fascicularis) and rhesus macaques (Macaca mulatta) with different wild-type measles viruses. J Gen Virol. 2007;88:2028–34. https://doi.org/10.1099/vir.0.82804-0.
28. Enkirch T, von Messling V. Ferret models of viral pathogenesis. Virology. 2015;479–480:259–70. https://doi.org/10.1016/j.virol.2015.03.017.
29. Feldmann H, Jones S, Klenk H-D, Schnittler H-J. Ebola virus: from discovery to vaccine. Nat Rev Immunol. 2003;3:677–85.
30. Gao Q, Bao L, Mao H, et al. Development of an inactivated vaccine candidate for SARS-CoV-2. Science. 2020;80(369):77–81.
31. Gardner J, Anraku I, Le TT, et al. Chikungunya virus arthritis in adult wild-type mice. J Virol. 2010;84:8021–32. https://doi.org/10.1128/jvi.02603-09.
32. Goncalvez AP, Engle RE, St. Claire M, et al. Monoclonal antibody-mediated enhancement of dengue virus infection in vitro and in vivo and strategies for prevention. Proc Natl Acad Sci U S A. 2007;104:9422–7. https://doi.org/10.1073/pnas.0703498104.
33. Gorman MJ, Caine EA, Zaitsev K, et al. An immunocompetent mouse model of Zika virus infection. Cell Host Microbe. 2018;23:672–85. https://doi.org/10.1016/j.chom.2018.04.003.
34. Green AM, Beatty PR, Hadjilaou A, Harris E. Innate immunity to dengue virus infection and subversion of antiviral responses. J Mol Biol. 2014;426:1148–60. https://doi.org/10.1016/j.jmb.2013.11.023.
35. Gubareva LV, Kaiser L, Hayden FG. Influenza virus neuraminidase inhibitors. Lancet. 2000;355:827–35.
36. Guzman MG, Harris E. Dengue. Lancet. 2015;385:453–65. https://doi.org/10.1016/S0140-6736(14)60572-9.
37. Halstead SB, Shotwell H, Casals J. Studies on the pathogenesis of dengue infection in monkeys. I. Clinical laboratory responses to primary infection. J Infect Dis. 1973;128:7–14. https://doi.org/10.1093/infdis/128.1.7.
38. Hawman DW, Stoermer KA, Montgomery SA, et al. Chronic joint disease caused by persistent chikungunya virus infection is controlled by the adaptive immune response. J Virol. 2013;87:13878–88. https://doi.org/10.1128/jvi.02666-13.
39. Holmes KV. SARS-Associated coronavirus. N Engl J Med. 2009;348:1948–51. https://doi.org/101056/NEJMp030078

40. Hotta H, Murakami I, Miyasaki K, et al. Inoculation of dengue virus into nude mice. J Gen Virol. 1981;52:71–6. https://doi.org/10.1099/0022-1317-52-1-71.
41. Huang KJ, Li SYJ, Chen SC, et al. Manifestation of thrombocytopenia in dengue-2-virus-infected mice. J Gen Virol. 2000;81:2177–82. https://doi.org/10.1099/0022-1317-81-9-2177.
42. Hutchinson EB, Chatterjee M, Reyes L, Djankpa FT, Valiant WG, Dardzinski B, Mattapallil JJ, Pierpaoli C, Juliano SL. The effect of Zika virus infection in the ferret. J Comp Neurol. 2019;527(10):1706–1719. https://doi.org/10.1002/cne.24640. Epub 2019 Feb 15. PMID: 30680733; PMCID: PMC6593673.
43. Imai Y, Kuba K, Rao S, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature. 2005;436:112–6.
44. Jagger BW, Wise HM, Kash JC, et al. An overlapping protein-coding region in influenza A virus segment 3 modulates the host response. Science. 2012;337:199–204.
45. Johnson AJ, Roehrig JT. New mouse model for dengue virus vaccine testing. J Virol. 1999;73:783–6. https://doi.org/10.1128/jvi.73.1.783-786.1999.
46. Koraka P, Benton S, van Amerongen G, et al. Characterization of humoral and cellular immune responses in cynomolgus macaques upon primary and subsequent heterologous infections with dengue viruses. Microbes Infect. 2007;9:940–6. https://doi.org/10.1016/j.micinf.2007.03.012.
47. Kuhn JH, Bao Y, Bavari S, et al. Virus nomenclature below the species level: a standardized nomenclature for laboratory animal-adapted strains and variants of viruses assigned to the family Filoviridae. Arch Virol. 2013;158:1425–32.
48. Kumar M, Krause KK, Azouz F, et al. A guinea pig model of Zika virus infection. J Virol. 2017;14:75. https://doi.org/10.1186/s12985-017-0750-4
49. Labadie K, Larcher T, Joubert C, et al. Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages. J Clin Invest. 2010;120:894–906. https://doi.org/10.1172/JCI40104.
50. Lavinder CH, Francis E. The etiology of dengue. An attempt to produce the disease in the rhesus monkey by the inoculation of defibrinated blood. J Infect Dis. 1914;15:341–6. https://doi.org/10.1093/INFDIS/15.2.341.
51. Lazear HM, Govero J, Smith AM, et al. Resource a mouse model of Zika virus pathogenesis. Cell Host Microbe. 2016;19:720–30. https://doi.org/10.1016/j.chom.2016.03.010.
52. Lee JC, Tseng CK, Wu YH, et al. Characterization of the activity of 2′-C-methylcytidine against dengue virus replication. Antivir Res. 2015;116:1–9. https://doi.org/10.1016/j.antiviral.2015.01.002.
53. Lee YR, Huang KJ, Lei HY, et al. Suckling mice were used to detect infectious dengue-2 viruses by intracerebral injection of the full-length RNA transcript. Intervirology. 2005;48:161–6. https://doi.org/10.1159/000081744.
54. Leffel EK, Reed DS. Marburg and Ebola viruses as aerosol threats. Biosecur Bioterror. 2004;2:186–91.
55. Levitt NH, Ramsburg HH, Hasty SE, et al. Development of an attenuated strain of chikungunya virus for use in vaccine production. Vaccine. 1986;4:157–62. https://doi.org/10.1016/0264-410X(86)90003-4.
56. Lowen AC, Mubareka S, Tumpey TM, et al. The Guinea pig as a transmission model for human influenza viruses. Proc Natl Acad Sci U S A. 2006a;103:9988–92. https://doi.org/10.1073/pnas.0604157103.
57. Lowen AC, Mubareka S, Tumpey TM, et al. The Guinea pig as a transmission model for human influenza viruses. Proc Natl Acad Sci. 2006b;103:9988–92.
58. Lum F-M, Teo T-H, Lee WWL, et al. An essential role of antibodies in the control of chikungunya virus infection. J Immunol. 2013;190:6295–302. https://doi.org/10.4049/jimmunol.1300304.
59. Lycett SJ, Duchatel F, Digard P. A brief history of bird flu. Philos Trans R Soc B. 2019;374:20180257.

60. Marano G, Pupella S, Vaglio S, et al. Zika virus and the never-ending story of emerging pathogens and transfusion medicine. Blood Transfus. 2016;14:95.
61. Marchette NJ, Halstead SB, Falkler WA, et al. Studies on the pathogenesis of dengue infection in monkeys. III. Sequential distribution of virus in primary and heterologous infections. J Infect Dis. 1973;128:23–30. https://doi.org/10.1093/infdis/128.1.23.
62. Marchette NJ, O'Rourke T, Halstead SB. Studies on dengue 2 virus infection in cyclophosphamide-treated rhesus monkeys. Med Microbiol Immunol. 1980;168:35–47. https://doi.org/10.1007/BF02121650.
63. Martina BEE, Haagmans BL, Kuiken T, et al. SARS virus infection of cats and ferrets. Nature. 2003;425:915.
64. Matsuoka Y, Lamirande EW, Subbarao K. The mouse model for influenza. Curr Protoc Microbiol. 2009;13:1–30. https://doi.org/10.1002/9780471729259.mc15g03s13.
65. McChesney MB, Miller CJ, Rota PA, et al. Experimental measles: I. pathogenesis in the normal and the immunized host. Virology. 1997;233:74–84. https://doi.org/10.1006/viro.1997.8576.
66. McCray PB Jr, Pewe L, Wohlford-Lenane C, et al. Lethal infection of K18-hACE2 mice infected with severe acute respiratory syndrome coronavirus. J Virol. 2007;81:813–21.
67. Mehedi M, Groseth A, Feldmann H, Ebihara H. Clinical aspects of Marburg hemorrhagic fever. Future Virol. 2011;6:1091–106.
68. Messaoudi I, Vomaske J, Totonchy T, et al. Chikungunya virus infection results in higher and persistent viral replication in aged rhesus macaques due to defects in anti-viral immunity. PLoS Negl Trop Dis. 2013;7:e2343. https://doi.org/10.1371/JOURNAL.PNTD.0002343.
69. Miller ML, Andringa A, Rafales L, Vinegar A. Effect of exposure to 500 ppm sulfur dioxide on the lungs of the ferret. Respiration. 1985;48:346–54. https://doi.org/10.1159/000194849.
70. Mongkolsapaya J, Dejnirattisai W, Xu XN, et al. Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever. Nat Med. 2003;9:921–7. https://doi.org/10.1038/nm887.
71. Morrison TE, Diamond MS. Crossm animal models of Zika virus infection. 2015.
72. Muhammad Azami NA, Takasaki T, Kurane I, Moi ML. Non-human primate models of dengue virus infection: a comparison of viremia levels and antibody responses during primary and secondary infection among old world and new world monkeys. Pathog (Basel, Switzerland). 2020;9:247. https://doi.org/10.3390/pathogens9040247.
73. Nakayama E, Saijo M. Animal models for Ebola and Marburg virus infections. Front Microbiol. 2013;4:1–20. https://doi.org/10.3389/fmicb.2013.00267.
74. Nambulli S, Sharp CR, Acciardo AS, et al. Mapping the evolutionary trajectories of morbilliviruses: what, where and whither. Curr Opin Virol. 2016;16:95–105. https://doi.org/10.1016/j.coviro.2016.01.019.
75. Niewiesk S. Current animal models: Cotton rat animal model – Cotton rat. Curr Top Microbiol Immunol. 2009;330:89–110. https://doi.org/10.1007/978-3-540-70617-5_5.
76. Omatsu T, Moi ML, Hirayama T, et al. Common marmoset (Callithrix jacchus) as a primate model of dengue virus infection: development of high levels of viraemia and demonstration of protective immunity. J Gen Virol. 2011;92:2272–80. https://doi.org/10.1099/VIR.0.031229-0.
77. Onlamoon N, Noisakran S, Hsiao HM, et al. Dengue virus – induced hemorrhage in a nonhuman primate model. Blood. 2010;115:1823–34. https://doi.org/10.1182/blood-2009-09-242990.
78. Organization WH. Summary table of SARS cases by country, 1 November 2002-7 August 2003. Wkly Epidemiol Rec Relev épidémiologique Hebd. 2003;78:310–1.
79. Organization WH. Coronavirus disease 2019 (COVID-19): situation report. 2020;73.
80. Orozco S, Schmid MA, Parameswaran P, et al. Characterization of a model of lethal dengue virus 2 infection in C57BL/6 mice deficient in the alpha/beta interferon receptor. J Gen Virol. 2012;93:2152–7. https://doi.org/10.1099/vir.0.045088-0.
81. Osterrieder N, Bertzbach LD, Dietert K, et al. Age-dependent progression of SARS-CoV-2 infection in Syrian hamsters. Viruses. 2020;12:779.

82. Osuna CE, Lim S-Y, Deleage C, et al. Zika viral dynamics and shedding in rhesus and cynomolgus macaques. Nat Med. 2016;22:1448.
83. Padilla-Carlin DJ, McMurray DN, Hickey AJ. The guinea pig asa model of infectious diseases. Comp Med. 2008;58(4):324–40. PMID: 18724774; PMCID: PMC2706043.
84. Pal P, Dowd KA, Brien JD, et al. Development of a highly protective combination monoclonal antibody therapy against chikungunya virus. PLoS Pathog. 2013;9:e1003312. https://doi.org/10.1371/JOURNAL.PPAT.1003312.
85. Pal P, Fox JM, Hawman DW, et al. Chikungunya viruses that escape monoclonal antibody therapy are clinically attenuated, stable, and not purified in mosquitoes. J Virol. 2014;88:8213–26. https://doi.org/10.1128/jvi.01032-14.
86. Perry ST, Buck MD, Lada SM, et al. STAT2 mediates innate immunity to dengue virus in the absence of STAT1 via the type I interferon receptor. PLoS Pathog. 2011;7:e1001297. https://doi.org/10.1371/journal.ppat.1001297.
87. Phair JP, Kauffman CA, Jennings R, Potter CW. Influenza virus infection of the Guinea pig: immune response and resistance. Med Microbiol Immunol. 1979;165:241–54.
88. Prestwood TR, Prigozhin DM, Sharar KL, et al. A mouse-passaged dengue virus strain with reduced affinity for heparan sulfate causes severe disease in mice by establishing increased systemic viral loads. J Virol. 2008;82:8411–21. https://doi.org/10.1128/jvi.00611-08.
89. Prevention C for DC and Key facts about avian influenza (bird flu) and avian influenza A (H5N1) virus. 2005. Internet site www.cdc.gov/flu/avian/gen-info/facts.htm (Accessed May 15, 2006).
90. Radigan KA, Misharin AV, Chi M, Budinger GRS. Modeling human influenza infection in the laboratory. Infect Drug Resist. 2015;8:311–20. https://doi.org/10.2147/IDR.S58551.
91. Richard M, Kok A, de Meulder D, et al. SARS-CoV-2 is transmitted via contact and via the air between ferrets. Nat Commun. 2020;11:1–6.
92. Rima B, Balkema-Buschmann A, Dundon WG, et al. ICTV virus taxonomy profile: paramyxoviridae. J Gen Virol. 2019;100:1593–4. https://doi.org/10.1099/jgv.0.001328.
93. Rockx B, Kuiken T, Herfst S, et al. Comparative pathogenesis of COVID-19, MERS, and SARS in a nonhuman primate model. Science. 2020;368:1012–5.
94. Ross RW. The newala epidemic: III. The virus: isolation, pathogenic properties and relationship to the epidemic. J Hyg (Lond). 1956;54:177–91. https://doi.org/10.1017/S0022172400044442.
95. Rothman AL. Immunity to dengue virus: a tale of original antigenic sin and tropical cytokine storms. Nat Rev Immunol. 2011;11:532–43. https://doi.org/10.1038/nri3014.
96. Ruiz SI, Zumbrun EE, Nalca A. Animal models of human viral diseases. 2020.
97. Russell K, Hills SL, Oster AM, et al. Male-to-female sexual transmission of Zika virus—United States, January–April 2016. Clin Infect Dis. 2017;64:211–3.
98. Schilte C, Buckwalter MR, Laird ME, et al. Cutting edge: independent roles for IRF-3 and IRF-7 in hematopoietic and nonhematopoietic cells during host response to chikungunya infection. J Immunol. 2012;188:2967–71. https://doi.org/10.4049/jimmunol.1103185.
99. Schul W, Liu W, Xu HY, et al. A dengue fever viremia model in mice shows reduction in viral replication and suppression of the inflammatory response after treatment with antiviral drugs. J Infect Dis. 2007;195:665–74. https://doi.org/10.1086/511310.
100. Seymour RL, Adams AP, Leal G, et al. A rodent model of chikungunya virus infection in RAG1 −/− mice, with features of persistence, for vaccine safety evaluation. PLoS Negl Trop Dis. 2015;9:e0003800. https://doi.org/10.1371/journal.pntd.0003800.
101. Shresta S, Kyle JL, Snider HM, et al. Interferon-dependent immunity is essential for resistance to primary dengue virus infection in mice, whereas T- and B-cell-dependent immunity are less critical. J Virol. 2004;78:2701–10. https://doi.org/10.1128/jvi.78.6.2701-2710.2004.
102. Shresta S, Sharar KL, Prigozhin DM, et al. Murine model for dengue virus-induced lethal disease with increased vascular permeability. J Virol. 2006;80:10208–17. https://doi.org/10.1128/jvi.00062-06.
103. Smith TRF, Patel A, Ramos S, et al. Immunogenicity of a DNA vaccine candidate for COVID-19. Nat Commun. 2020;11:1–13.

104. Smith W, Andrewes CH, Laidlaw PP. A virus obtained from influenza patients. Lancet. 1933:66–8.
105. St Claire MC, Ragland DR, Bollinger L, Jahrling PB. Animal models of ebolavirus infection. Comp Med. 2017;67:253–62.
106. St John AL, Rathore APS, Raghavan B, et al. Contributions of mast cells and vasoactive products, leukotrienes and chymase, to dengue virus-induced vascular leakage. elife. 2013;2:e00481. https://doi.org/10.7554/eLife.00481.
107. Subbotina E, Dadaeva A, Kachko A, Chepurnov A. Genetic factors of Ebola virus virulence in Guinea pigs. Virus Res. 2010;153:121–33.
108. Tan GK, Ng JKW, Trasti SL, et al. A non mouse-adapted dengue virus strain as a new model of severe dengue infection in AG129 mice. PLoS Negl Trop Dis. 2010;4:e672. https://doi.org/10.1371/journal.pntd.0000672.
109. Van Hoeven N, Belser JA, Szretter KJ, et al. Pathogenesis of 1918 pandemic and H5N1 influenza virus infections in a Guinea pig model: antiviral potential of exogenous alpha interferon to reduce virus shedding. J Virol. 2009;83:2851–61.
110. von Messling V, Springfeld C, Devaux P, Cattaneo R. A ferret model of canine distemper virus virulence and immunosuppression. J Virol. 2003;77:12579–91. https://doi.org/10.1128/jvi.77.23.12579-12591.2003.
111. Wahl-Jensen V, Bollinger L, Safronetz D, et al. Use of the Syrian hamster as a new model of ebola virus disease and other viral hemorrhagic fevers. Viruses. 2012;4:3754–84.
112. Waldorf KMA, Stencel-Baerenwald JE, Kapur RP, et al. Fetal brain lesions after subcutaneous inoculation of Zika virus in a pregnant nonhuman primate. Nat Med. 2016;22:1256–9.
113. Weinstein RA, Bridges CB, Kuehnert MJ, Hall CB. Transmission of influenza: implications for control in health care settings. Clin Infect Dis. 2003;37:1094–101.
114. Weinstock DM, Gubareva LV, Zuccotti G. Prolonged shedding of multidrug-resistant influenza A virus in an immunocompromised patient. N Engl J Med. 2003;348:867–8.
115. Wu SJL, Hayes CG, Dubois DR, et al. Evaluation of the severe combined immunodeficient (SCID) mouse as an animal model for dengue viral infection. Am J Trop Med Hyg. 1995;52:468–76. https://doi.org/10.4269/ajtmh.1995.52.468.
116. Yamanaka A, Konishi E. A simple method for evaluating dengue vaccine effectiveness in mice based on levels of viremia caused by intraperitoneal injection of infected culture cells. Vaccine. 2009;27:3735–43. https://doi.org/10.1016/J.VACCINE.2009.03.083.
117. Yauch LE, Shresta S. Mouse models of dengue virus infection and disease. Antivir Res. 2008;80:87. https://doi.org/10.1016/J.ANTIVIRAL.2008.06.010.
118. Yauch LE, Zellweger RM, Kotturi MF, et al. A protective role for dengue virus-specific CD8 + T cells. J Immunol. 2009;182:4865–73. https://doi.org/10.4049/jimmunol.0801974.
119. Yun NE, Linde NS, Zacks MA, et al. Injectable peramivir mitigates disease and promotes survival in ferrets and mice infected with the highly virulent influenza virus, A/Vietnam/1203/04 (H5N1). Virology. 2008;374:198–209.
120. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382:727–33. https://doi.org/10.1056/NEJMOA2001017.
121. Zumbrun EE, Bloomfield HA, Dye JM, et al. A characterization of aerosolized Sudan virus infection in African green monkeys, cynomolgus macaques, and rhesus macaques. Viruses. 2012;4:2115–36.
122. Zust R, Toh Y-X, Valdes I, et al. Type I interferon signals in macrophages and dendritic cells control dengue virus infection: implications for a new mouse model to test dengue vaccines. J Virol. 2014;88:7276–85. https://doi.org/10.1128/jvi.03827-13.

Viral Diseases: Cellular Understanding of Disease: Vaccine Development

Rohit C. Ghan and Amish Patel

Abstract The past 2 years have arguably been the most challenging since last few decades. The COVID-19 pandemic has changed the world in many ways—good and bad. With more than six million deaths worldwide (and counting), this pandemic has been one of the worst, second only, to the Spanish Flu pandemic of 1918–19. The saving grace, if it can be called that, is that the vaccine(s) for COVID-19 were one of the fastest developed and marketed compared to any other vaccine, for this scale of a pandemic, in history. This has, in a way, renewed the focus and emphasis on, not just technological advancements enabling important diagnostic tools, but also put forth newer mechanisms for countering different modalities of viruses with corresponding vaccines. This chapter attempts to view viruses and their pathogenicity at a cellular level and purview various mechanism of their virulence. It also briefly presents an overview of various diagnostic tools available and their advancements along with different approaches to developing novel vaccines.

Keywords Vaccine · Virus · Pathogenesis · Virulence · Pandemic · Vector · Immunogenicity · Attenuated vaccines · Toxoid

Abbreviations

BCG	Bacillus Calmette-Guerin
Cas	CRISPR associated protein
CD4	Co-receptor protein found on helper T cells that binds to class II MHC molecules outside the antigen-binding site.

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CISPR	Clustered regularly interspaced short palindromic repeats
COV	Corona virus
COVID	Corona virus disease
COVID-19	Corona virus disease (2019 pandemic)
DETECTR	DNA endonuclease targeted CRIPSR trans reporter
DNA	Deoxyribonucleic acid
EVD	Ebola virus disease
FDA	Food and Drug Administration
GI	Gastrointestinal
GWAS	Genome-wide association studies
HIV	Human immunodeficiency virus
MERS	Middle East respiratory syndrome
mRNA	messenger Ribonucleic acid
RNA	Ribonucleic acid
SARS	Severe acute respiratory syndrome
SHERLOCK	Specific high-sensitivity enzymatic reporter un-LOCK
TMV	Tobacco mosaic virus
VLP	Virus-like particles
WHO	World Health Organization

1 Introduction

If there exists an entity which teeters on the border of living and non-living organisms, one might contend that viruses are the closest match to that description [1]. While composed of complex biochemicals, viruses don't exhibit basic metabolic activity required for "proof-of-life." However, when it associates with cell(s) of other living organisms, the replication mechanism encoded within its genetic code is activated and it starts to reproduce. This association, invariably parasitic, leads to infection, and ultimately death of the host cell. And that is why this, almost alienic organism, is of great interest to human species.

From discovery of the first Tobacco Mosaic Virus (TMV) in 1892/98 [2] to the more recent decoding of the SARS-COVID-19 virus [3], the more we learn about this organism, the more we think there is to know. A brief history of time (Fig. 1) indicates how viruses have wreaked havoc through spread of infectious diseases taking the form of pandemics .

Coined by Prof. M.W. Beijerinck [8], the term "virus" has come to represent this "agent of infection." Certain specific viral proteins that viruses exhibit while infecting other cellular organisms point towards the ancient origins of these parasitic organisms. Since the sole mechanism of replication for viruses is via infection of host cells, in the process of which the host cell ceases to exist, it therefore begets us to understand the mechanics of pathogenesis at a cellular level, delve into the diagnostics aspects for detection of virus infections, and discuss the potential pathways of vaccine development.

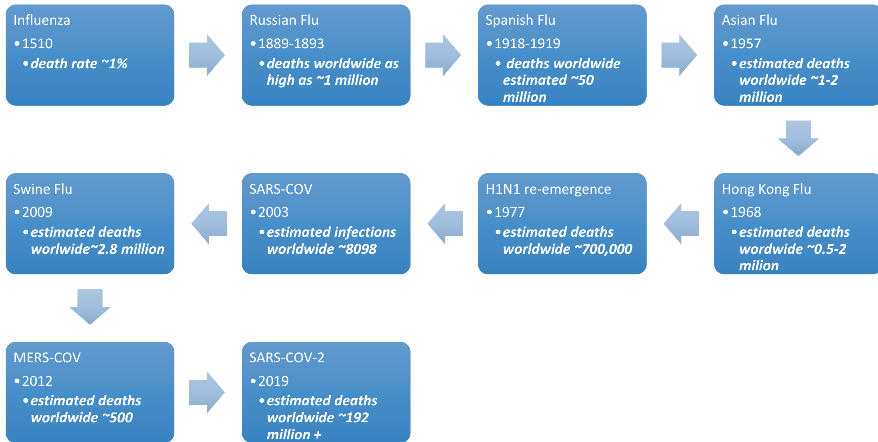

Fig. 1 Brief history of time of Virus Infections/Pandemics/Epidemics [4–7]

2 Understanding of Viral Interactions at a Cellular Level

2.1 Inception of Viruses

As old as life itself, viruses are believed to have existed almost 3.5 billion years before human life evolved on earth. The most probable cause of emergence of this simple life form from the inanimate via chemical process, known as abiogenesis, is thought to be the impetus for its origins [9–11].

Several different hypotheses have been proposed to stipulate the timeline and mechanism of origin of viruses. Some of the more prevalent and rational theories are virus-first [12], regression theory [13], and escaped-gene theory [14]. The "virus-first" theory hypothesizes that after planet-formation, as the earth cooled down, the fundamental molecules (RNA), which were able to replicate became the building blocks of viruses. This, in turn, led to evolution of single-celled and multi-cellular organisms which acted as hosts for these RNA-based organisms.

Alternatively, the "regression-theory" proposes that the cellular organisms regressed in their evolutionary pathway losing their ability to replicate. This led to conversion (regression) of self-sustaining cellular lifeforms to parasitic viruses. Two strains of Tupanviruses isolated from amoebae and whose genomes are coding for over 1400 proteins, were discovered by Abrahão, J and team [13]. This discovery supports the hypothesis that these viruses had evolved regressively from predecessors possessing a full set of genes.

A third theory, "escaped-gene" theory, argues that viruses evolved from genes that "escaped" cellular organisms at different stages of early evolution. In essence, this theory opposes the "virus-first" hypothesis. It goes on to propose that these "escaped" genes, somehow, enabled the viruses to replicate via parasitism.

2.2 "Turning Evil": Pathogenesis

Irrespective of the hypothesis of origins of viruses, one undeniable fact that precipitates is that viruses are parasitic organisms. They rely on "host" cells for replication. This process of infection, or "association" with host cells leading to disease is termed pathogenesis [15]. The virus first implants itself onto host cell via different routes of entry, namely respiratory tract, gastrointestinal (GI) tract, through the skin, and via genital organs; basically, any organ where exposure to tissue is possible. It then releases its genome and replicates the same while using the host's ribosomes to manufacture the proteins required for replication. The resulting virions spread to other target organs. Ultimately these virions reach "shedding" sites to spread the virus back to the environment to look for fresh hosts [16].

Thus, in an evil twist, the virus diverts the host cell's energy and shuts off its macromolecular synthesis mechanism. It competes with the mRNA for cellular ribosomes and inhibits the interferon defense mechanisms. Eventually this causes cell damage and ultimately death of the host cell.

2.3 Mechanisms of Virulence

The association of a pathogen with a host cell is generally described as virulence. Several advancements [17] have been made towards understanding the mechanism of virulence. Simplistically, virulence can be evaluated based on different factors including the pathogen, host, and environmental factors. However, the understanding of how a pathogen jumps across host species and the evolution of virulence in a virus is still a subject of debate. Genome-wide association studies (GWAS) [18] offer a promising avenue for understanding the impact of evolution of viral infections on the host's genome and thereby can provide a deeper insight into the interaction between the pathogen and the host. And this aspect is one of the most critical aspects of understanding virulence. Virulence is accentuated when pathogens shift to a new species. Ebola virus, prevalent in bats, is mostly asymptomatic; however, when it shifts to humans and other primates, it turns fatal [19]. Studies have suggested that while prediction of pathogenicity may not be assured in a novel host, if a pathogen exhibits high levels of virulence in one host species, it may result in similar levels of virulence in closely related hosts [19]. One other key factor of virulence of a virus is the proteins it chooses from the host cell for the synthesis of the virulence traits. Studies have also shown that amongst different strains of the same virus (pathogen), the strain found to be most virulent were the ones with higher non-structural protein content [20].

2.4 "Trojan Attack": Biology of Virus Infected Cells [21]

Unlike bacteria, viruses are intracellular pathogens, which means that they infect their host cells by entering them. This is achieved by binding of surface protein of a virus with a particular receptor on the surface of the host cell. Viruses, in a Trojan-like manner, employ a primary receptor and a secondary co-receptor in order to effectively attach to and gain and entry into host cells. The HIV virus uses CD4 as its primary receptor, which is a protein (which provides immune recognition) and is present on the surface of mainly T-cells and macrophages.

Once the pathogen recognizes and has attached to the host cell, its next order of business is to gain entry into the host cell and release its nucleic-acid genome contained within its protein coat/lipid envelope. Typically, the released nucleic acid stays complexed with viral proteins. Viruses with an envelope can enter the host cell by fusing with the plasma membrane. Alternatively, they can also enter via the endosomal membrane via endocytosis (Fig. 2I, II). Viruses regulate fusion such that virus particles will only fuse with the correct host-cell membrane, and also prevent self-fusing with other virus particles.

HIV viruses effect fusion at neutral pH at the plasma membrane. They bind to receptors or co-receptors. This causes a structural change in the protein of the virus envelope exposing a covered fusion peptide.

When polio virus binds to its receptors, receptor-mediated endocytosis as well as a structural change in the virus itself is triggered. This structural change uncovers a

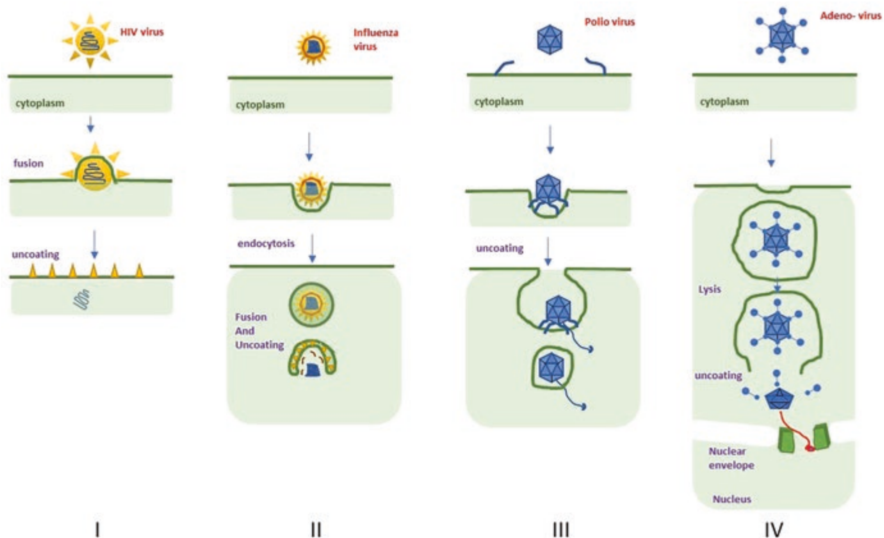

Fig. 2 Mechanisms of "uncoating" employed by (I) viruses with an envelope, (II) cell-surface binding first followed by fusion and uncoating, (III) non-enveloped viruses such as polio virus, and (IV) another unenveloped virus (adeno-virus) employing a more complex "uncoating strategy"

hydrophobic appendage on one of the capsid proteins. This appendage then inserts into the endosomal membrane and forms an opening. The viral genome enters the cytoplasm through the opening, leaving behind the capsid either in the endosome or on the surface of the cell, or both.

Adenovirus employs a more complex strategy. Receptor-mediated endocytosis takes up the virus into the cell. As the endosome turns more acidic, the adenovirus performs multiple uncoating steps. This causes the structural proteins to be removed from the capsid. One of the proteins released owing to lysis of the capsid releases the remainder of the virus into the cytoplasm. This adenovirus then attaches into the nuclear-pore complex and releases the genome into the nucleus.

3 Development of Treatments for Viral Infections

3.1 Diagnosis of Viral Infections

Owing to the unique nature of pathogenesis of viruses, especially across species, it is extremely challenging (as it is important) to diagnose viral infections and their potential fatality to humans. Diagnostic virology is therefore becoming increasingly important and interesting. Traditional diagnosis methods such as cultures, albeit effective, were slow and expensive and hence with the advent of modern-day viruses with the potential of turning into endemic or even pandemics required more robust and faster. Advantages and disadvantages of some contemporary diagnostic methods for virology are compared [22] (Table 1).

Broadly, the detection of virus-based infections can be classified into three categories (a) direct virus detection (performed about 1–3 days after the infection, (b) antibody-determination – quantitation of antibodies against some virus-specific proteins, and (c) detection of virus-induced disease (upon tissue-specific changes in the host-organism). Some of the latest cutting-edge diagnostic tools employed include CRISPR-Cas system. Specific High-sensitivity Enzymatic Reporter unLOCK (SHERLOCK) and DNA Endonuclease Targeted CRIPSR Trans Reporter (DETECTR) are cutting edge tools that are employed to detect specific RNA or DNA at lower concentrations [23]. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a natural tool for editing genomes. It is a part of the prokaryotic immune system employed to combat viruses and immunize the pathogen [24]. Cas (CRISPR-associated protein) within the CRISPR system is driven by RNA to the target location. Cas-proteins are enzymes like endonucleases or ribonucleases. These enzymes act to cleave DNA or RNA [25]. Nucleic acid-based amplification assays are fast becoming the new the standard-of-use methodologies for detection of viruses.

Table 1 Advantages and disadvantages of various diagnostic tests for virology

Diagnostic methods	Advantages	Disadvantages
Isolation of the virus	Highly sensitive	Slow, time-intensive, convoluted, expensive
	Additive in nature – produces more material for further evaluation if required	Specific to cell-type, ineffective for non-viable virus, or non-cultivatable agents
Detection of viral genome via PCR	Rapid and sensitive	May lead to detection of non-relevant co-infections
	Applicable to non-cultivatable agents as well	Risk of contamination of DNA
	Can be multiplexed	Target-specific
IgM serology	Rapid	Possibility of false positives
		Target-specific
Antibody seroconversion	Effective in case of absence of direct detection samples	Slow, late (retrospective)
		Interpretation is difficult
		Target-specific
Visual observation via electron microscopy	Rapid	Not sensitive owing to low sample-load
	Detects non-cultivatable and non-viable viruses	Limited to few viral infections
Serological identification of virus	Rapid and sensitive	Not applicable to all viruses
	Provides information on serotypes	Interpretation maybe difficult
	Readily available in the form of kits	Target-specific

3.2 Vaccine Development

One cannot speak about vaccine development in the present day and age without referring to COVID-19. Typically, vaccine development takes several years and a deep understanding of the virus, its virology, pathogenesis, interactions with the host, shedding pathway, and mechanism. Previously (prior to 2020), the fastest vaccine developed was the mumps vaccine in the 1960s, which was developed in about 4 years. The COVID-19 vaccine was developed and commercialized (on an emergency-use basis) within 12 months [26]. One of the main reasons for this record-breaking speed of development was the fact that the groundwork for these vaccines started decades ago with the SARS-COV and MERS-COV outbreaks in 2003 and 2012 respectively (Fig. 1).

Traditional approaches to vaccine development such as live attenuated vaccines or inactivated vaccines or vaccines based on protein subunits all came with their pros and cons [27–29]. Pertinent probability of reversion to a pathogenic form with attenuated vaccines can render them questionable forms of reliable treatments. At the same time, inactivated vaccines may not provide adequate immunogenicity.

Technology developed several decades ago, which involves delivering an antigen using a non-pathogenic viral backbone as the vehicle for the antigen was used to provide immunity to chimpanzees exposed to hepatitis B [30]. Viral-vectors have been employed, since, with success, in quite a few animal species [31, 32]. However, only one viral-vector has been approved by the FDA in 2019 for prevention of Ebola virus disease (EVD) [33].

Another alternative to traditional vaccines are nanoparticle-based vaccines [34]. They offer significant advantages such as the ability to control the size, tailored surface-modifications, ability to control release dynamics, increase payloads, and better stability [35]. Improvement in immunogenicity and stability of subunit vaccines in enabled by these nanoparticle vaccines as well as virus-like particles (VLPs). Expression of proteins that are antigenic in either a eukaryotic or prokaryotic system results in production of VLP vaccines. This also yields self-assembling antigenic proteins in particulate form. Thus,VLP vaccines can be said to produce particulates that are similar to the structure of the actual viruses [36, 37].

Genetic vaccines comprising DNA (in the form of plasmids) and RNA (in the form of mRNA) have gained a lot of attention in the recent years following data indicating that plasmid-DNA expresses an immune response in presence of antigen that is coated with plasmid [38]. Researchers reported viability of mRNA for the purpose of in vivo gene transfer in the 1990s [39]. However, further development of mRNA vaccines was delayed owing to issues with their stability. Encapsulation of RNA was found to increase the stability as well as augment the immunogenicity of RNA vaccines [40]. With certain factors taken into consideration such as cellular pathways for triggering immunity as a response to foreign RNA, RNA vaccines may exhibit superior performance as compared to vaccines with respect to immune response [41]. Table 2 shows the different types of vaccines, i.e., inactivated vaccines, live attenuated vaccines, mRNA, and toxoid vaccines approved, and license for use in the USA in recent years. This list gives an idea on the applicability of different vaccine platforms being employed for commercializing vaccines for various infections.

The SARS-CoV-2 virus and two SARS-like coronaviruses (bat-SL-CoVZXC21 and bat-SL-CoVZC45) are almost 88% similar. They also employ the same human angiotensin-converting enzyme-2 receptor for cell-entry during infection [43]. According to WHO (World Health Organization), production of a safe and effective vaccine ready for human use can easily take upwards of 18 months. Developing a vaccine in the middle of a pandemic outbreaks is extremely slow and can risk precious lives. Predicting the virus mutations which can trigger reemergence of highly pathogenic virulent strains (cross-species) may be a way to stay ready for new emerging pandemic strains in the future. In light of the SARS-COV outbreak in 2003, scientists had predicted that there could be a potential bat coronavirus that would likely cross species and infect humans [44]. However, this information did not receive proper attention. Had that not been the case, a predictive vaccine could have been developed for the first time in history and in time to possibly prevent the COVID-19 pandemic. Nonetheless, utilizing the mRNA (Pfizer and Moderna) and human monoclonal antibodies' platforms (Eli Lily, Regeneron) [45], effective vaccines were developed in record time.

Table 2 Different types of vaccines approved by FDA and licensed for use in the United States [42]

Vaccine type	Treatment	Commercial name	Initial Approval
Inactivated vaccines	Hepatitis A, Flu, Rabies, Polio	Havrix® - Hepatitis A	2005
	Diphtheria & Tetanus Toxoids & Acellular Pertussis Vaccine Adsorbed, Hepatitis B (recombinant) and Inactivated Poliovirus Vaccine Combined	Pediarix®	2002
	Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed and Inactivated Poliovirus Vaccine	Kinrix®	2008
	Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed and Inactivated Poliovirus Vaccine	Quadracel®	2015
	Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactivated Poliovirus, Haemophilus b Conjugate [Meningococcal Protein Conjugate] and Hepatitis B [Recombinant] Vaccine	Vaxelis®	2018
	Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactivated Poliovirus and Haemophilus b Conjugate (Tetanus Toxoid Conjugate) Vaccine	Pentacel®	2008
	Hepatitis A Vaccine, Inactivated	VAQTA®	1996
	Hepatitis A Inactivated and Hepatitis B (Recombinant) Vaccine	Twinrix®	2001
	Japanese Encephalitis Virus Vaccine, Inactivated, Adsorbed	Ixiaro®	2009
	Poliovirus Vaccine Inactivated (Monkey Kidney Cell)	IPOL®	1989
Live attenuated vaccines	Adenovirus Type 4 and Type 7 Vaccine, Live, Oral	No trade name	2011
	Cholera Vaccine Live	Vaxchora®	2016
	Dengue Tetravalent Vaccine, Live	Dengvaxia®	2019
	Ebola Zaire Vaccine	Ervebo®	2019
	Influenza Vaccine, Live, Intranasal	Flumist®	2003
	(Trivalent, Types A and B)	Flumist Quadrivalent®	2003
	Measles, Mumps, and Rubella Vaccine, Live	Priorix®	2022
		M-M-R-II	1978
	Measles, Mumps, Rubella, and Varicella Virus Vaccine Live	Proquad®	2005
	Rotavirus Vaccine, Live, Oral	Rotarix®	2008
	Rotavirus Vaccine, Live, Oral, Pentavalent	RotaTeq®	2006
	Smallpox and Monkeypox Vaccine, Live, Non-replicating	Jynneos®	2019

(continued)

Table 2 (continued)

Vaccine type	Treatment	Commercial name	Initial Approval
	Smallpox (Vaccinia) Vaccine, Live	ACAM2000®	2007
	Typhoid Vaccine Live Oral Ty21a	Vivotif®	1989
	Varicella Virus Vaccine Live	Varivax®	1995
	Zoster Vaccine, Live, (Oka/Merck)	Zostavax®	2006
Messenger RNA (mRNA) vaccines	Covid-19 Vaccine, mRNA	Comirnaty®	2021
		Spikevax®	2022
		Janssen Covid-19 vaccine (***Emergency use authorization)***	2021
Toxoid vaccines	Diphtheria & Tetanus Toxoids Adsorbed	No trade name	1997
		Infanrix®	1997
	Diphtheria & Tetanus Toxoids & Acellular Pertussis Vaccine Adsorbed	Daptacel®	2002
	Diphtheria & Tetanus Toxoids & Acellular Pertussis Vaccine Adsorbed, Hepatitis B (recombinant) and Inactivated Poliovirus Vaccine Combined	Pediarix®	2002
	Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed and Inactivated Poliovirus Vaccine	Kinrix®	2008
	Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed and Inactivated Poliovirus Vaccine	Quadracel®	2015
	Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactivated Poliovirus, Haemophilus b Conjugate [Meningococcal Protein Conjugate], and Hepatitis B [Recombinant] Vaccine	Vaxelis®	2018
	Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactivated Poliovirus, and Haemophilus b Conjugate (Tetanus Toxoid Conjugate) Vaccine	Pentacel®	2008
	Haemophilus b Conjugate Vaccine (Tetanus Toxoid Conjugate)	ActHIB®	1993
		Hiberix®	2009
	Meningococcal (Groups A, C, Y and W-135) Polysaccharide Diphtheria Toxoid Conjugate Vaccine	Menactra®	2005
	Tetanus & Diphtheria Toxoids, Adsorbed	TDVAX®	1970
	Tetanus & Diphtheria Toxoids Adsorbed for Adult Use	Tenivac®	2003
	Tetanus Toxoid Adsorbed	No trade name	1997
	Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Adsorbed	Adacel®	2005
		Boostrix®	2005

4 Conclusion

In closing, with the vast encyclopedic data available on viruses, right from their potential origins to the detailed knowledge on the mechanisms of virulence, pathogenesis, and outbreaks, it is possible to develop critical diagnostic methods and tools to develop effective vaccines proactively. Implementation of cutting-edge techniques like CRIPSR combined with predictive tools (driven by AI (Artificial Intelligence)) should enable global collaboration towards a common goal of preventing future pandemics.

In the words of Martin Luther King Jr., “We must accept finite disappointment, but never lose infinite hope”.

References

1. Villareal L. Are viruses alive? Sci Am. 2004;291(6):100–5.
2. Lecoq H. Discovery of the first virus, the tobacco mosaic virus: 1892 or 1898? C R Acad Sci III. 2001;10:929–33.
3. Ellis P, Somogyvári F, Virok DP, Noseda M, McLean GR. Decoding Covid-19 with the SARS-CoV-2 genome. Curr Genet Med Rep. 2021;9(1):1–12.
4. Lofgren E, Fefferman NH, Naumov YN, Gorski J, Naumova EN. Influenza seasonality: underlying causes and modeling theories. J Virol. 2007;81:5429–36.
5. Ma W, Lager KM, Vincent AL, Janke BH, Gramer MR, Richt JA. The role of swine in the generation of novel influenza viruses. Zoonoses Public Health. 2009;56:326–37.
6. Kawaoka Y, Krauss S, Webster RG. Avian-to-human transmission of the PB1 gene of influenza A viruses in the 1957 and 1968 pandemics. J Virol. 1989;63:4603–8.
7. Guan Y, Zheng BJ, He YQ, Liu XL, Zhuang ZX, Cheung CL, Luo SW, Li PH, Zhang LJ, Guan YJ, Butt KM, Wong KL, Chan KW, Lim W, Shortridge KF, Yuen KY, Peiris JS, Poon LL. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003;302:276–8.
8. Tang C. Biography of professor Martinus Willem Beijerinck. J Appl Virol. 2013;2(3):4.
9. Pross A. What is life? How chemistry becomes biology? Oxford, UK: Oxford University Press; 2012.
10. Pross A. Toward a general theory of evolution: extending Darwinian theory to inanimate matter. J Syst Chem. 2011;2(1):1759–2208.
11. Pascal R. Suitable energetic conditions for dynamic chemical complexity and the living state. J Syst Chem. 2012;3(3):1759–2208.
12. Bandea C. The origin and evolution of viruses as molecular organisms. Nature Precedings. 2009.
13. Abrahão J. Tailed giant Tupanvirus possesses the most complete translational apparatus of the known virosphere. Nat Commun. 2018;9:749.
14. Krupovic M, Koonin EV. Multiple origins of viral capsid proteins from cellular ancestors. Proc Natl Acad Sci U S A. 2017;10:1073.
15. Baron S, Fons M, Albrecht T. Viral pathogenesis. In: Medical microbiology. Galveston. https://www.ncbi.nlm.nih.gov/books/NBK8149/.; 1996.
16. Louten J. Virus replication. Essent Human Virol. 2016;May 6:49–70.
17. Milton Zaitlin PP. Advances in understanding plant viruses and virus diseases. Annu Rev Phytopathol. 2000;38:117–43.

18. Geoghegan JL, Holmes EC. The phylogenomics of evolving virus virulence. Nat Rev Genet. 2018;19:756–69.
19. Longdon B, Hadfield JD, Day JP, Smith SC, McGonigle JE, Cogni R, Cao C, Jiggins FM. The causes and consequences of changes in virulence following pathogen host shifts. PLoS Pathog. 2015;11(3):e1004728.
20. Zou C, et al. Virulence difference of five type I dengue viruses and the intrinsic molecular mechanism. PLoS Negl Trop Dis. 2019;13(3):1–16.
21. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Molecular biology of the cell. 4th ed. New York: Garland Science; 2002.
22. Burrell CJ, Howard CR, Murphy FA. Laboratory Diagnosis of Virus Diseases. Fenner and White's Medical Virology. 2017:135–54. https://doi.org/10.1016/B978-0-12-375156-0.00010-2. Epub 2016 Nov 11. PMCID: PMC7149825.
23. Gronowski AM. Who or what is SHERLOCK? Electron J Int Fed Clin Chem. 2018;29:201–4.
24. Sasnauskas G, Siksnys V. CRISPR adaptation from a structural perspective. Curr Opin Struct Biol. 2020;65:17–25.
25. Gasiunas G, Sinkunas T, Siksnys V. Molecular mechanisms of CRISPRmediated. Cell Mol Life Sci. 2014;71(3):449–65.
26. Cohen S. The fastest vaccine in history. UCLA Health. 2020.
27. Liu M. Immunologic basis of vaccine vectors. Immunity. 2010;33(October 29):504–15.
28. Plotkin S. History of vaccination. Proc Natl Acad Sci U S A. 2014;111:12283–7.
29. Rauch S, Jasny E, Schmidt KE, Petsch B. New vaccine technologies to combat outbreak situations. Front Immunol. 2018;9:1–24.
30. Smith GL, Mackett M, Moss B. Infectious vaccinia virus recombinants that express hepatitis B virus surface antigen. Nature. 1983;302:490–5.
31. Pastoret PP, Brochier B. The development and use of a vaccinia-rabies recombinant oral vaccine for the control of wildlife rabies; A link between Jenner and Pasteur. Epidemiol Infect. 1996;116:235–40.
32. Esaki M, Godoy A, Rosenberger JK, Rosenberger SC, Gardin Y, Yasuda A. Protection and antibody response caused by Turkey herpesvirus vector newcastle disease vaccine. Avian Dis. 2013;57:750–5.
33. FDA: First FDA-approved vaccine for the prevention of Ebola virus disease, marking a critical milestone in public health preparedness and response, FDA, [Online]. Available: https://www.fda.gov/news-events/press-announcements/first-fda-approved-vaccine-prevention-ebola-virus-disease-marking-critical-milestone-public-health. Accessed 30 June 2022.
34. Poon C, Patel AA. Organic and inorganic nanoparticle vaccines for prevention of infectious diseases. Nano Express. 2020;1:1–11.
35. Zhao L, Seth A, Wibowo N, Zhao CX, Mitter N, Yu C, Middelberg AP. Nanoparticle vaccines. Vaccine. 2014;32:327–37.
36. Kushnir N, Streatfield SJ, Yusibov V. Virus-like particles as a highly efficient vaccine platform: diversity of targets and production systems and advances in clinical development. Vaccine. 2012;31:58–83.
37. Vang L, Morello CS, Mendy J, Thompson D, Manayani D, Guenther B, Julander J, Sanford D, Jain A, Patel A, Shabram P, Smith J, Alexander J. Zika virus-like particle vaccine protects AG219 mice and rhesus macaques against Zika virus. PLoS Negl Trop Dis. 2021;15(March 12):e0009195.
38. Wolff JA, Malone RW, Williams P, Chong W, Acsadi G, Jani A, Felgner PL. Direct gene transfer into mouse muscle in vivo. Science. 1990;247:1465–8.
39. Zhang C, Maruggi G, Shan H, Li J. Advances in mRNA vaccines for infectious diseases. Front Immunol. 2019;10:594.
40. Hood JL. Post isolation modification of exosomes for nanomedicine applications. Nanomedicine. 2016;11:1745–56.
41. van den Boorn JG, Hartmann G. Turning tumors into vaccines: co-opting the innate immune system. Immunity. 2013;39:27–37.

42. “Vaccibnes Blood Biologics,” FDA, [Online]. Available: https://www.fda.gov/vaccines-blood-biologics/vaccines/vaccines-licensed-use-united-states. Accessed 5 July 2022.
43. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng Y, Wang J, Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen W, Shi W, Tan W. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020;395:565–74.
44. Fan Y, Zhao K, Shi ZL, Zhou P. Bat coronaviruses in China. Viruses. 2019;11(3):210.
45. Cohen J. Monoclonal antibodies can prevent COVID-19—but successful vaccines complicate their future. Science. 2021. https://www.science.org/content/article/monoclonal-antibodies-can-prevent-covid-19-successful-vaccinescomplicate-their-future

Pneumocystis Carnii Pneumonia Infections: Disease, Diagnosis, and Treatment Options

Nayyer Islam, Muhammad Irfan, Ameer Fawad Zahoor, Haroon Khalid Syed, Shahid Shah, Muhammad Ajmal Shah, Muhammad Ali Syed, and Syed Atif Raza

Abstract By reading this chapter, readers will be able to understand what is *Pneumocystis carinii* pneumonia (PCP) infection, how it affects immunocompromised patients, and on prior treatment different diagnostic parameters are performed for sampling and afterwards treatment is recommended by a physican. Readers will be able to distinguish PCP patients infected with HIV/AIDS and PCP patients infected without HIV/AIDS. Also, by going through this chapter, readers will be able to distinguish between pneumonia, PCP fungal infection and the compatibility of COVID-19 with PCP fungal infection. Different treatment strategies are discussed in this chapter in order to treat PCP-infected patients and risk factors are also considered so that by keeping in view these risk factors preventive measures are adopted to treat PCP fungal-infected patients. Epidemiology of PCP infection is elaborated so that reader will be able to understand whether it is endemic or pandemic infectious disease. In addition, the pathophysiology of PCP is discussed so that reader should come to know its mode of transmission in lungs where this fungal infection starts replicating. Importantly, different case studies are discussed in this chapter for the purpose of understanding that more than one infection also affects

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PCP-infected patients and different antibiotic therapies are performed along with adjunctive corticosteroids therapy for better recovery of the patient. It is the expected responsibility of readers after studying this chapter to provide awareness of PCP infection among health care professionals and people.

Keywords *Pneumocystis carinii* pneumonia · Epidemiology · Diagnosis · Treatment · Case studies

1 Background

It is commonly called as *Pneumocystis carinii* pneumonia (PCP)/*Pneumocystis jirovecii* pneumonia (PJP). PCP is a fungal infection that can affect one or both of the lungs and its causative agent is *Pneumocystis jirovecii*, which is also called as *Pneumocystis carinii* [1]. It mostly affects individuals who are infected with HIV infections [2]. In 1980, this case was first reported in the USA and it occurred in an individual infected with HIV. In the beginning, scientists were confused about whether PCP is caused by protozoa or fungus so they classified it as protozoan but with the passage of time and advancement in medical knowledge they classified it as fungus [3]. Today, PJP is considered one of the several fungal infections which can cause severe life-threatening problems in individuals suffering with AIDS/HIV infections [4]. In the USA, the first decade of HIV epidemic cases associated with PJP were reported approximately to be 100,000, but today with current anti-retroviral therapy (ART), individuals with HIV/AIDS are less likely to be infected with PCP infection [5]. PCP is a fungal infection that affects the lungs of individuals with weakened immune system and it is usually caused by *Pneumocystis jirovecii*. There are several reasons for a weakened immune system such as cancer, HIV infection, AIDS, high dose corticosteroids, or medicine taken after having bone marrow or organ transplant. This fungal infection mostly attacks individuals suffering from HIV/AIDS infection but seems to rarely occur in healthy individuals who do not acquire AIDS/HIV infections [6]. Nearly, all the individuals suffering from PCP either have low oxygen levels in their blood (hypoxemia) at rest or an increase in their alveolar-arterial oxygen tension gradient, which causes difficulty for the individual to breathe [7].

2 Microbiology

PJP occurs in the respiratory tracts of humans and mammals and it belongs to unicellular fungi group. Distinct species flexibility is present between members of host-specific genus. In 1909, Chagas first introduced this organism and then few years later Dr. Carni isolated it from infected rats and proposed this organism's name as *Pneumocystis carinii* and some years later Dr. Otto Jirovec and his group members found that this organism was also present in humans, later, they isolated

this organism and renamed it as *Pneumocystis jirovecii*. Because of that reason, this organism has dual names i.e., PCP and PJP. There was a clash among the scientists whether *Pneumocystis jirovecii* pneumonia belongs to protozoa group or trypanosome group, further nucleic acid biochemical analysis suggest that *Pneumocystis* RNA and mitochondrial DNA considered the organism as a fungus which is unicellular rather than a protozoa [8]. Later on, scientists found that these organisms exist in three structural forms, namely, trophozoite, sporozoite, and cyst. The trophozoite is sometimes called as trophic form due to its existence in clusters arrangement. While sporozoite is also ranked as a precystic form in which it lays down the resting phase, and the cyst is a kind of form containing several spores which are sometimes called as intracystic bodies.

3 Epidemiology

PJP diagnosis is difficult because modern medical facilities are not present in underdeveloped regions of the world. In Africa, its frequency is found to occur at the rate of 80% in infants suffering with pneumonia and other HIV infections [9]. In sub-Saharan Africa, individuals suffering from tuberculosis, which is a serious lungs infection, also have a chance to be infected with PJP pneumonia [10]. Similarly in Pakistan, PCP pneumonia prevalence rate is 16% and different antibiotic therapies are performed to prevent form PCP infection [11]. According to latest research prevalence rate of PCP infection seems to be 32–38% in India [10]. In USA, 75% overall PCP cases were reported [5] and in China PCP prevalence rate is 40% according to latest research [12]. In Malaysia, PCP prevalence rate is 60% and in Europe PCP detection ratio is 18% [13]. In Mozambique, PCP occurs at the incidence of 6.8% and a specimen study was performed via nasopharyngeal aspirates through polymerase chain reaction (PCR) test to confirm the presence of infection.

In Malawi, 5% PCP detection ratio was observed and sample specimen was taken via lung aspiration through a PCR detection technique [5]. Similarly in Namibia, PCP detection ratio is 5%, but sample specimen was taken via sputum induction through Grocott's Methenamine Silver stain (GMS) and PCR detection ratios [14]. In France, PCP occurs at the incidence of 26.1% and in Brazil 20% PCP detection rate was observed [5, 15]. In Uganda, 4% PCP detection ratio was noticed and a specimen study was performed through BAL test, and a modified Giemsa detection method was used in this regard [16]. In Vietnam and Tanzania, the PCP detection rate were found to be 3% and 1.5%, respectively, when sample specimens were taken via oral route using a PCP detection technique [17]. In Poland, 21% PCP detection rate was observed, and in Malawi, PCP occurs at incidence rate of 9% and specimen study was performed via BAL test and detection methods used in this technique were IF and PCP [18]. These are the different regions of the world where different studies and techniques are performed for PCP detection. By covering the epidemiological factors of PCP fungal infection, it was found that PCP infectious disease is a pandemic disease because it is present in different regions of the world.

4 Etiology

The causative agent of PCP is the fungus *Pneumocystis jirovecii* pneumonia [19]. Individuals with healthy immune system do not get infected with this organism, but those individuals who have weakened immune system may easily be affected by this fungus organism named as PCP. The immune system may be weakened due to several reasons such as in the case of cancer therapy, organ transplant, and using medicines (steroids) that suppress your immune system [20]. If PCP is not treated in the appropriate manner the patient's condition may worsen. So, it is recommended to boost up your immune system in the case of PCP infection.

5 Pathophysiology

Pneumocystis pneumonia is a worldwide infectious fungal disease and it occurs mostly in children who are 3 to 4 years of age [21]. Furthermore, preclinical studies have suggested that PCP transmission is airborne and clinical trials on humans has also been reported due to depressed immunity in individuals [22]. PCP occurs due to defective humoral and cellular immunity. Once PCP spores are inhaled via alveoli, the host organism starts replicating and ultimately causes disease. The role of immunity in the case of PCP infection is attributed due to the reasons such as (1) defects in cellular or humoral immunity, (2) CD4+ production is low, (3) CD4+ T-cell count is >200 cells/μL, and (4) development of PCP infection [9].

6 Risk Factors

It is most likely to develop in those individuals in which HIV/AIDS infection has been reported. It also occurs in individuals who are immunodeficient. In this regard, patients receive long-term immunosuppressive therapy. It can also occur in individuals who are at risk of malnutrition [22]. Patients, whose organ transplantation is performed by providing corticosteroids therapy for suppressing immune system, the chances of PCP fungal infection began to increase in such cases. PCP fungal infection is more susceptible in those patients whose are hematologic and nonhematologic malignant including solid tumors and cancerous cells [23].

7 Pneumonia & *Pneumocystis Carinii* Pneumonia

Pneumonia is an infection of lungs affecting one or both lung parts [24]. In this infection, alveoli are filled with fluid and pus. Its causative agent is *Streptococcus pneumoniae*. Pneumonia affects mainly patients with 65 years of age or more and

children about 2 years of age [25]. The signs and symptoms of pneumonia in adults are clearly observed but in case of children signs and symptoms are monitored carefully. They may have fever, cough, or they may have difficulty in breathing and eating. Pneumonia classification is based according to infectious agent or place from where infection spread. Community acquired pneumonia is caused by viruses, bacteria, or fungi. Hospital acquired pneumonia is caused when a patient is admitted for another illness but after recovering from particular illness got infectious pneumonia disease. It is more severe because bacteria are more resistant to antibiotic therapies and patients who acquire this infection are already immunosuppressed. Hospital acquired pneumonia develops when people are visiting to attend the patient for longer times, it is also more resistant to antibiotics, and can also be caused by visiting out-patient clinics. Aspiration pneumonia is caused when patient inhaled food, drink, saliva, or vomit and disturbs body's normal reflux mechanism. This can also be spread by excessive intake of alcohol.

Pneumocystis carinii pneumonia is caused by fungal infection and can affect one or both lungs [26]. It is usually spread through individuals who are either immunocompromised or have already been infected with HIV/AIDS. Before adopting treatment strategy, its diagnosis is performed based on PCP patient infected with or without PCP. Factors that increase the risk of PCP fungal infections include smoking, alcohol consumption, dyspnea, and malnutrition. From above discussion, the difference of pneumonia and PCP fungal infection is clearly observed. Further basic details of pneumonia and PCP fungal infection are given below to summarize our findings and by observing these findings, it can easily diagnose whether patient is infected with pneumonia or PCP fungal infection (Table 1).

Table 1 Difference between pneumonia and PCP

Pneumonia	*Pneumocystis carinii* pneumonia
Inflammation of lungs in one or both parts.	PCP is a fungal infection that may affect one or both parts of lungs.
Alveoli are filled with fluid and pus.	Before opting treatment strategy, diagnosis is performed to rule out PCP.
Streptococcus pneumoniae is the most causative agent which causes inflammation in alveoli.	Its causative agent is *pneumocystis jirovecii*.
Pneumonia develops when body immune system is weak.	It lowers infected patient's immune system and CD4+ level, and different antibiotics along with adjunctive corticosteroids are given to treat PCP infection.
Factors that may lead to pneumonia are pre-existing lungs disease, recent influenza infections, smoking, and upper respiratory tract infections.	Factors that may lead to PCP are smoking, alcohol intake, and malnutrition. These conditions may trigger PCP infection.
Vaccines are available	No vaccines are currently available

8 Differential Diagnosis of COVID 19 and PCP Infection

Clinical conditions of coronavirus disease 2019 (COVID-19) and pneumocystis pneumonia (PCP) have similarities and are clinically indistinguishable at the early stages in HIV-positive patients [27]. Differential diagnosis should be performed to rule out similar clinical manifestations. Diagnostic parameters should be performed on priority basis to determine whether underlying disease is COVID-19 or PCP fungal infection. Similarities and differences based on COVID-19 and PCP fungal infection can be better described and explained with the help of case studies. Fewer such cases have been discussed in this context to understand the pathological condition and suspected diseased condition.

8.1 Case Study 1

During the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, a 25-year-old patient presented with significant hypoxemia despite using a non-rebreather mask. A massive right pneumothorax and severe interstitial illness were discovered on a chest X-ray. Despite the installation of a chest tube, hypoxemia persisted, prompting emergency intubation. A CT scan of the chest was performed, and the nasopharyngeal SARS-CoV-2 PCR was positive. His absolute CD4+ count was 32 cells/Mm^3 and his HIV serology was positive. Because of his severe acquired immunodeficiency, radiographic results suggested a life-threatening co-infection with *Pneumocystis jirovecii*, prompting therapy with trimethoprim–sulfamethoxazole, prednisolone, and remdesivir. Pneumocystis pneumonia (PCP) was confirmed 4 days later by bronchoscopic pneumocystis antigen. Clinically, the patient recovered and was discharged from hospital successfully 21 days later [28] (Fig. 1).

In both PCP and SARS-CoV-2 infection, widespread ground-glass nodules are the most common finding, making radiographic distinction challenging, especially in immunosuppressed patients. One-third of patients with serious PCP may develop cystic tumors [29, 30]. The identification of *Pneumocystis jirovecii* co-infection would have been difficult without these cystic radiographic features. As a result, in the present SARS-CoV-2 pandemic, knowledge of co-infections is vital in order to correctly diagnose and treat these co-infections, decreasing morbidity and mortality rates [31].

8.2 Case Study 2

With a three-week history of cough, myalgia, fever, and increasing dyspnea, a 54-year-old man was taken to a medical center. The BMI of patient was normal. However, patient had an 8-year history of hypertension and type 2 diabetes as well

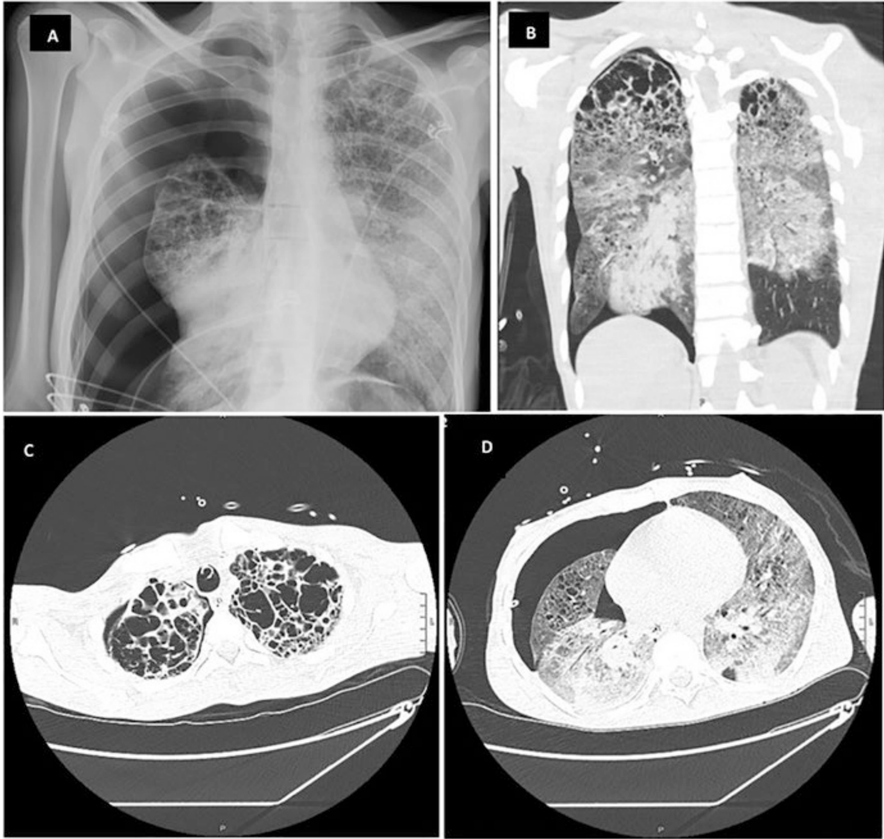

Fig. 1 Depicting a chest X-ray and a CT scan of the chest at the time of presentation, (**a**) X-ray of the chest reveals a massive right pneumothorax and severe interstitial illness, (**b**) Coronal CT chest picture demonstrating apical cystic alterations, diffuse ground-glass opacities, thick consolidation, and pneumothorax, (**c**) An axial image of the most prominent apical cystic alterations, and (**d**) Axial image with the chest tube revealing diffuse ground-glass opacities and a right pneumothorax, reproduced with permission [28]. (continue directly)

as electrocardiographic signs of left ventricular hypertrophy. The patient previously had two bouts of drug-sensitive pulmonary tuberculosis, both of which the subject had successfully treated. The patient was HIV positive at the time of admission, with a CD4+ count of 26 cells/L and a viral load of 2,447,646 copies/mL. His SARS-CoV-2 polymerase chain reaction (PCR) nasopharyngeal swab was likewise positive. He was moved to a field hospital for coronavirus illness (COVID), where the subject needed nasal prong oxygen to keep his oxygen saturation (SpO_2) at 96%. His oxygen needs increased within 24 h of transfer, therefore the patient was transferred to our intensive care unit (ICU) for high-flow nasal cannula (HFNC) oxygen (Figs. 2 and 3).

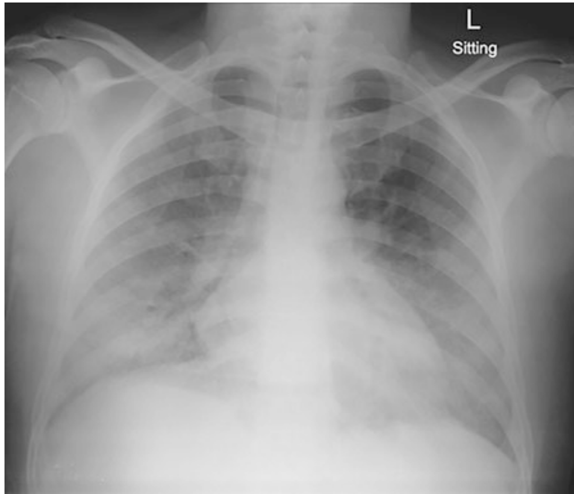

Fig. 2 The above figure shows that on arrival, the patient's chest radiograph revealed bilateral ground-glass opacifications, mostly in the lower zone. The patient was started on empiric dexamethasone and therapeutic co-trimoxazole after a working diagnosis of COVID-19 and/or PCP. On the third day of hospitalization, however, persistent fever, hemodynamic instability, increased oxygen needs with worsening pulmonary infiltrates on chest X-ray, and growing inflammatory markers led to a diagnosis of nosocomial pneumonia. In the absence of culture findings, empiric meropenem and fluconazole were started, reproduced with permission [28]

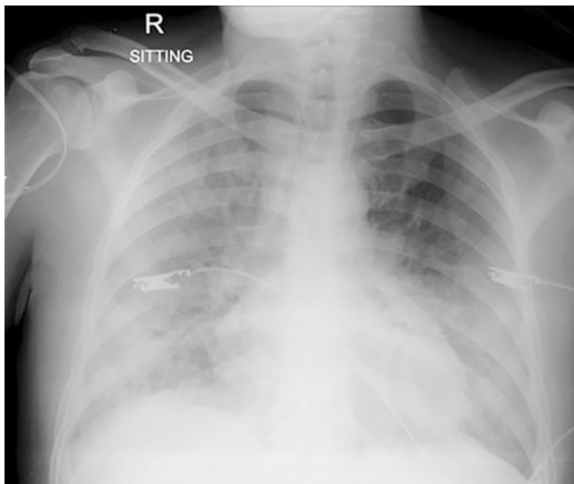

Fig. 3 The above figure shows the consolidation including all but the left upper zone shown on this chest radiograph, indicating a severe deterioration. Sputum DFAT confirmed PCP, which was backed up by a β-D-glucan level of >500 pg/mL (normal 60). *Acinetobacter baumannii* was also found in blood cultures, for which colistin was started pending sensitivity. Legionella species were not found in the urine. On the eighth day, after admission to the ICU, the patient died of increasing respiratory failure. (Image reproduced with permission from [28])

8.3 Case Study 3

A middle-aged man with shortness of breath, nocturnal sweats, and weight loss came to community care in England in March 2020. The subject was administered oral antibiotics after the chest X-ray, however, revealed nothing unusual. The subject was brought to emergency treatment a week later. In the absence of laboratory testing, the subject was discharged with a suspected mild COVID-19 diagnosis after a chest X-ray revealed bilateral apical ground glass alterations (Fig. 1a). The patient was clinically stable without severe hypoxia and was thus discharged with a suspected light COVID-19 diagnosis. Four days later with substantial hypoxia, increasing bilateral upper lobe airspace shadowing on chest X-ray, anemia (hemoglobin of 87 g/dL), lymphopenia (0.14 cells/Mm^3), and increased C-reactive protein (212 mg/L), as well as yeast infections were observed.

COVID-19 was initially regarded the most likely diagnosis in the emergency department, may be due to the relative frequency of COVID-19 during the peak of the epidemic and the resulting limitations in clinical reasoning. The diagnoses explored on the second presentation, 4 days later, were community acquired pneumonia (CAP) and moderate/severe COVID-19. Although the lymphopenia and bilateral chest X-ray alterations were consistent with COVID-19, the patient also had several unusual characteristics, such as oral thrush, weight loss, and a long clinical history. Following admission and a negative SARS-CoV-2 polymerase chain reaction test (PCR) result, *Pneumocystis jirovecii* pneumonia (PCP) was evaluated initially (Fig. 4).

The patient's condition worsened on day 5 of hospitalization, and was moved to the critical care unit for mechanical ventilation. SARS-CoV-2, influenza, parainfluenza, rhinovirus, adenovirus, respiratory syncytial virus, human metapneumovirus, and *Pneumocystis jirovecii* were all found to be negative by PCR, and acid-fast bacilli (AFB) staining was negative. Co-trimoxazole was switched to IV pentamidine on the ninth day of admission due to bone marrow suppression and hyperkalemia. A bronchial alveolar lavage on day 10 revealed *Pneumocystis jirovecii* confirming the diagnosis. CMV and herpes simplex type 1 (HSV-1) were also found in the lavage, but not SARS-CoV-2, respiratory viruses, or AFB. On day 15, a right tension pneumothorax occurred, as well as an intractable bronchopleural fistula. The patient died on the 17th day of his hospital stay, around 29 days after his first appearance in the community.

A lengthier clinical history previous to admission to emergency care is normal with COVID-19, oral thrush, and radiographic indications of apical alterations with basal sparing were among the clinical signals that may have prompted an earlier HIV test in the patient presented. Adding an HIV test to the order panel for all COVID-19 admissions, as well as educating and being attentive about potential differential illnesses like PCP, is one low-cost technique of enabling a differential diagnosis [33]. This will help determine how HIV-positive persons with and without COVID-19 should be treated, including cohort nursing and a focused therapy approach.

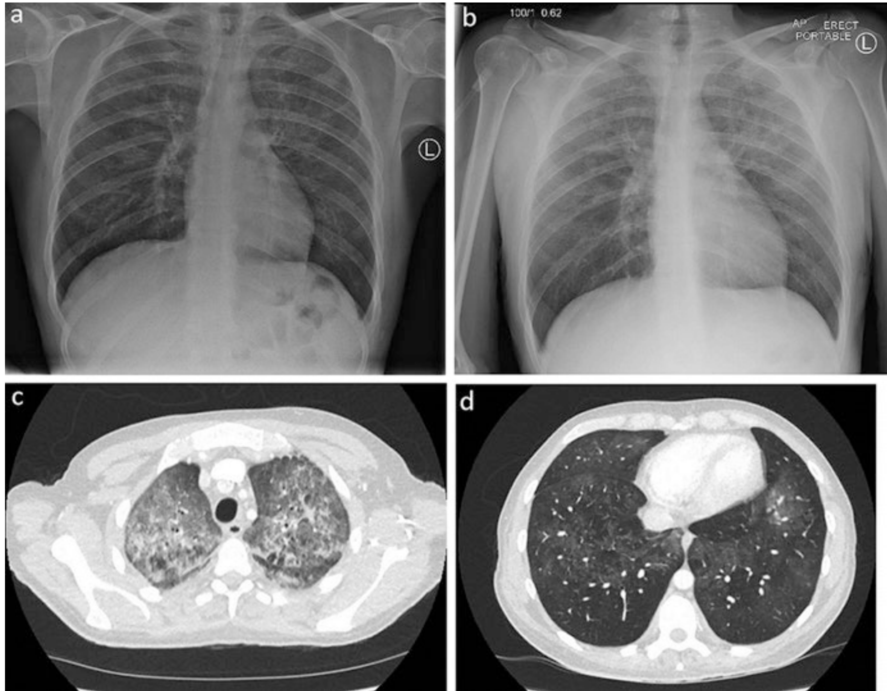

Fig. 4 (**a**) X-ray of the chest revealing bilateral apical ground glass alterations. (**b**) An X-ray of the chest reveals increasing bilateral upper lobe airspace shadowing, (**c** and **d**) chest computed CT demonstrating extensive ground glass alterations with basal sparing, reprinted with permission [32]

8.4 Case Study 4

During prednisolone therapy for autoimmune hepatitis, a 76-year-old lady got pneumocystis pneumonia (PCP) (AIH). A hematological test performed by patient's family physician revealed that the lady had excessive levels of hepatobiliary enzymes. The patient was sent to the hospital for further testing and was admitted to the hospital. The subject was diagnosed with autoimmune hepatitis (AIH) and began treatment on 40 mg of prednisolone per day. The transaminase levels of patient were improved due to which the dose of prednisolone was tailored downwards every 2 weeks. Fever of 38 °C was noted during the fourth week of medication. The chest X-ray revealed ground glass opacities, related to cough complaint. An induced sputum cytodiagnosis was conducted since the respiratory illness was suspected. The respiratory condition was followed after she was diagnosed with PCP.

Cotrimoxazole [trimethoprim/sulfamethoxazole] was begun after the patient's fever was managed symptomatically. The subject had acute exhaustion, hyponatremia, and a drop in platelets. ADRs were thought to be the cause of these signs and symptoms. The cough was suppressed once hyponatremia was cured. Eventually, the fever subsided, and the pneumonia began to recover. Prednisolone was not

lowered in dosage throughout this time and was kept at 15 mg/day for the whole 4-week period. Cotrimoxazole was added as a preventive measure, and AIH therapy was continued. As a result, multiple stomach ulcers and oral candidiasis was developed. The subject received treatment and was released on a daily dose of oral prednisolone of 10 mg. The IgG and transaminase values were both in the normal range when the patient was taking prednisolone 10 mg every other day at the time of the previous follow-up.

8.5 Case Study 5

On day 7 of the fifth chemotherapy session, a 69-year-old lady receiving biweekly pirarubicin hydrochloride, oncovin, cyclophosphamide, and prednisolone for mycosis fungoides developed a fever. The first chest CT pictures were unremarkable, and serum procalcitonin and -D-glucan levels were normal. Meropenem and amphotericin B were used to treat a bacterial or fungal illness, but was useless. A contrast-enhanced CT was performed around 10 days following the initial chest CT. Apart from two mass lesions in S6 and S10 in the right lower lobe, no source of fever was discovered (Fig. 5).

Compared to HIV-positive individuals, *Pneumocystis jirovecii* is particularly difficult to identify in specimens from HIV-negative patients, who have a higher immune response to pneumocystis but fewer pathogens. *Pneumocystis jirovecii* can be detected using a polymerase chain reaction based on bronchoalveolar lavage fluid. Because the tumors were confined, biopsy and Grocott's staining proved to be the most crucial measures for a correct diagnosis in this case. Because the chest CT results were abnormal and the patient's blood -D-glucan level was within normal ranges, PCP was excluded from the differential diagnosis in this case. In a retrospective investigation of patients with PCP identified via bronchoalveolar lavage, Tasaka et al. found that blood -D-glucan level was the most accurate PCP predictor among serum levels of lactate dehydrogenase, -D-glucan, Krebs von den Lungen-6

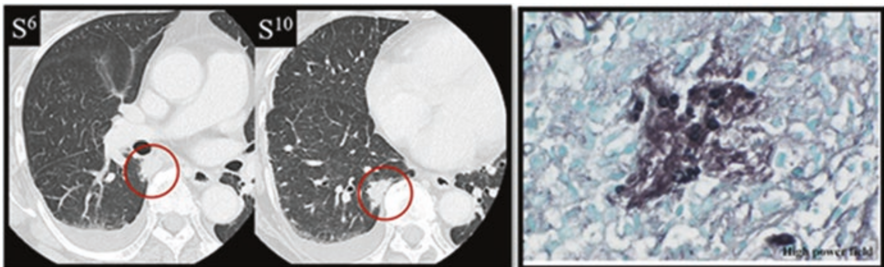

Fig. 5 In the right lung, pleural effusion can be observed, which might be impacted by inflammation. The SpO_2 level was within normal range, and was asymptomatic. Bronchoscopy was conducted, and bronchial biopsy specimens stained with Grocott's staining indicated cysts of *Pneumocystis jirovecii*, reproduced with permission [28]

(KL-6), and C-reactive protein. The threshold value for D-glucan concentration for PCP in the above-mentioned study was 31.1 pg/mL, with a sensitivity and specificity of 92.3% and 86.1% respectively. The reference range for -D-glucan, on the other hand, varies depending on the test technique used. Although the -D-glucan concentration was within normal ranges in this case, which might be due to the limited number of infections, there was insufficient data to evaluate if the D-glucan concentration represents the pneumocystis load in the lungs.

Depending on the patient's immunological state, PCP might appear with a variety of chest CT abnormalities. PCP is more frequent in immunocompromised persons and can be fatal. Even when chest CT images are abnormal, such as many lesions, PCP should be examined, and bronchoscopy should be attempted to diagnosis PCP if the patient's health allows. In suspected PCP cases, quick diagnosis and anti-PCP therapy are essential.

9 PCP Prognosis

The prognosis of PCP is worst due to its late diagnosis and it is a major cause of death in the US due to AIDS. It is most likely to occur in those patients who develop pneumothorax, and in this case patients receive mechanical ventilation. Currently, 20–50% cases have been reported due to large-scale studies.

10 Clinical Manifestations

These are basically signs and symptoms which distinguish and tell the medical expert whether infection is caused by *Streptococcus pneumoniae* and *Pneumocystis jirovecii*. By keeping in view these findings diagnostic parameters are performed prior to treatment. PCP signs & symptoms and different findings are discussed in below section for further observations and understanding (Table 2).

Table 2 Signs and symptoms for diagnosis of PCP

Symptoms	Signs	CXR Findings
Fever	Hypoxia	Diffuse, bilateral, hazy infiltrates
Dyspnea	Tachypnea, tachycardia	Pneumothorax
Dry cough	Inspiratory crackles	Pleural effusion, lobar infiltrate, nodules less common
Pleuritic chest pain	Elevated A-a gradient	CXR normal in 25%
Malaise	Chest exam normal in 50%	

Table 3 Differential diagnostic parameters of PCP infected patient

Acute respiratory distress syndrome (ARDS)	Cytomegalovirus	Lymphocytic interstitial pneumonia
Mycoplasma infections	Viral pneumonia	Pulmonary embolism
Legionellosis	Tuberculosis	Mycobacterium avium complex (MAC) infection

11 Differential Diagnosis of PCP

Patients who are PCP infected may also be infected with other pneumonia diseases [34]. This condition is not necessary in this regard that PCP patient is infected with fungal disease only. PCP patient may also be infected with other fungal disease, that's why differential diagnosis is performed to ensure whether patient is infected with PCP or multiple viral, bacterial, or fungal diseases are involved. Table 3 tells us about the differential diagnostic parameters of PCP-infected patient.

12 Laboratory Studies

A lactic dehydrogenase (LDH) test is performed to detect the degree of lung injury [35]. Individuals who are infected with HIV are at great risk of PCP and it is uplifted in 90% patients. There exists an alternative to invasive testing procedure names as sputum *P. jirovecii* PCR, which is a time-consuming method for sample collection and it is done in case of patient's respiratory failure. For PCP detection a sensitive test known as β-D-Glucan (BDG) is preferred which is comprised of Aspergillus, Candida, and Pneumocystis, but zygomycetes are excluded. The accuracy of this test is determined with Quantitative studies.

13 Chest Radiography

Those patients with defective immune system and other symptoms such as fever or respiratory signs are observed then in this regard chest radiography is performed. These results may be normal in patients with early mild disease. Common signs and symptoms include asymmetric infiltrates, pneumothorax, and pneumatoceles. In this overall process, a small amount of radiation is placed on the targeted organs such as lungs and then the image is detected in x-ray form.

14 Computed Tomography

For the detection and imaging of PJP infection high-resolution computed tomography (HRCT) is used rather than CT scan because it can easily detect PJP patients infected with HIV infection. This process is monitored by the radiologist and after obtaining HRCT of the patients with the help of specified computers, concluded results are sent to the physician for treatment purposes.

15 Other Noninvasive Tests

15.1 Pulmonary Function Tests

This test is performed on the basis of DLCO which stands for decreased diffusion capacity of carbon monoxide; patients whose DLCO value is normal are less likely to be susceptible with PCP infection. Decreased DLCO value indicates higher risk of PCP infection (89–100%). When this test is compared with HRCT, then it is used to distinguish whether the PCP patient is infected with HIV/AIDS or the patient is infected with PCP infection only. In such type of tests, patient's observation is made on regular basis.

15.2 Pulse Oximetry

It should be calculated in all the patients at room temperature [36]. It should be calculated at rest or after some activity in specified patients. If hypoxemia is detected in which oxygen saturation is less than 90%, then arterial blood gas level should be attained along with corticosteroids.

15.3 HIV Testing

In case of possible HIV testing in PCP patients, observations and results made before the test and after the test should be evaluated carefully [37].

15.4 Laboratory Testing

In laboratory testing, the blood sample of the affected patient is evaluated according to LDH level.

16 Sputum Induction

Another method of detecting the PCP infection is via sputum induction [38]. In this process, the patient is nebulized with 3% hypertonic saline and the specified patient is provided with a box for sputum collection. The nebulization sputum is sent to the laboratory to detect if the PCP infection is caused by HIV or it is caused by only pneumonia. The sensitivity of this test is usually based on the effective technique applied in laboratory and its specificity varies from 99% to 100%.

17 Bronchoalveolar Lavage (BAL) Test

BAL test is performed if the sputum induction is negative and this test has higher diagnostic sensitivity, this test is performed on the recommendation of pulmonologist when the patient mental status is altered and the patient is unable to give sample by sputum induction. This test gives more sensitivity to detect PCP infection than sputum induction [39].

18 Lungs Biopsy

This test is performed to detect higher sensitivity and specificity, in which the results obtained are 100%. In this test, tissue samples are obtained from infected PCP patient's lungs for diagnostic purposes [40].

19 Histologic Evaluation

P. jiroveci cannot grow in vitro, therefore, histologic findings are observed before patient diagnosis. Several staining techniques are applied for PCP detection. (1) Crystal violet, (2) Giemsa, (3) Diff-Quik, and (4) Wright stain [41]. For trophozoite and cyst form identification, mostly these staining methods are used.

20 Treatment of PCP

Although PCP is declared as fungal pneumonia, it does not respond to anti-fungal drugs. It is treated via TMP-SMX and other second line agents such as pentamidine and dapsone which are mostly along with pyrimethamine or atovaquone. A few successful case studies indicate that caspofungin can also treat PCP infection [42].

Treatment of PCP depends upon degree of illness and there are different parameters for tackling with PCP fungal infection which involves different drug combinations via oral or IV route depending upon the diagnosis and severity of the infection. There are two types of PCP patients, the first one are those who do not acquire HIV/AIDS infection and recovers within 4–5 days and the second one are those who acquire PCP infection and recovers within 21 days or longer [43].

20.1 Antibiotic Therapy (Table 4)

20.2 Adjunctive Corticosteroids Therapy

In severe cases, corticosteroids therapy should be administered to the PCP patient because of suppression of immune response. Latest research indicates that patients who are infected with PCP fungal infection should not keep in contact with other immunocompromised patients (Table 5).

Table 4 Antibiotic Therapy for PCP

Mild to moderate PCP (oral route)	
First choice	Trimethoprim-sulfamethoxazole
Second choice	Trimethoprim (Proloprim) and dapsone Or Clindamycin (Cleocin) and primaquine
Third choice	Atovaquone (Mepron)
Moderate to severe PCP (IV regimens)	
First choice	Trimethoprim-sulfamethoxazole
Second choice	Trimetrexate/leucovorin and oral dapsone Or Clindamycin (Cleocin phosphate) and oral primaquine
Third choice	Pentamidine

Table 5 Adjunctive Corticosteroids Therapy for PCP

Schedule	Dosage
Days 1–5	40 mg of prednisone twice daily
Days 6–10	40 mg of prednisone once daily
Days 11–21	20 mg of prednisone once daily

21 PCP Prevention

Smokers are at a greater risk of getting PCP infection, so it is advisable to quit smoking in order to prevent lungs from PCP infections. Currently, no vaccine available for PCP as a preventive measure. If an individual is infected with PCP, avoid direct contact with the person and practice safety precautions. If the patient is immunocompromised and CD4+ level is low, the physicians recommend to take medications that boost immune response thereby maintaining CD4+ level.

22 Conclusion

By reading this chapter, readers can easily get to know about PCP infection and can easily distinguish between PCP patients infected with HIV or PCP patients infected without HIV. Hospitals should have separate wards for dealing with PCP patients for better patient care services and different tests are performed before diagnosis based on patient disease response and conditions. Antibiotics and adjunctive steroids are given to PCP patients for better recovery and maintaining their immune system. Patients must be observed carefully for clinical outcomes and it must also be assured that no drug interactions and toxicity occurs during treatment. If the treatment is carried out by a general family physician, then the patient should be aware of this PCP fungal infection transmission and treatment protocols.

References

1. Sokulska M, Kicia M, Wesołowska M, Hendrich AB. Pneumocystis jirovecii—from a commensal to pathogen: clinical and diagnostic review. Parasitol Res. 2015;114(10):3577–85.
2. Lu J-J, Lee C-H. Pneumocystis pneumonia. J Formos Med Assoc. 2008;107(11):830–42.
3. Sritangratanakul S, Nuchprayoon S, Nuchprayoon I. Pneumocystis pneumonia: an update. J Med Assoc Thail. 2004;87(Suppl 2):S309–17.
4. Arichi N, Kishikawa H, Mitsui Y, Kato T, Nishimura K, Tachikawa R, et al. Cluster outbreak of pneumocystis pneumonia among kidney transplant patients within a single center. Paper presented at the Transplantation proceedings, vol. 41, 2009. p. 170.
5. Morris A, Lundgren JD, Masur H, Walzer PD, Hanson DL, Frederick T, et al. Current epidemiology of pneumocystis pneumonia. Emerg Infect Dis. 2004;10(10):1713.
6. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. Science Transl Med. 2012;4(165):165rv113.
7. White PL, Backx M, Barnes RA. Diagnosis and management of pneumocystis jirovecii infection. Expert Rev Anti-Infect Ther. 2017;15(5):435–47.
8. Bray PG, Barrett MP, Ward SA, de Koning HP. Pentamidine uptake and resistance in pathogenic protozoa: past, present and future. Trends Parasitol. 2003;19(5):232–9.
9. Gingerich AD, Norris KA, Mousa JJ. Pneumocystis pneumonia: immunity, vaccines and treatments. Pathogens. 2021;10(2):236.

10. Rodríguez YDA, Wissmann G, Müller A, Pederiva M, Brum M, Brackmann R, et al. Pneumocystis jirovecii pneumonia in developing countries. Parasite. 2011;18(3):219.
11. Zubairi ABS, Shahzad H, Zafar A. Clinical outcomes of pneumocystis pneumonia from a tertiary care centre in Pakistan. JPMA. 2016;66(11):1367.
12. Chen M, Xu Y, Hong N, Yang Y, Lei W, Du L, et al. Epidemiology of fungal infections in China. Front Med. 2018;12(1):58–75.
13. Velayuthan RD, Samudi C, Lakhbeer Singh HK, Ng KP, Shankar EM, Denning DW. Estimation of the burden of serious human fungal infections in Malaysia. J Fungi. 2018;4(1):38.
14. Nowaseb V, Gaeb E, Fraczek MG, Richardson MD, Denning DW. Frequency of pneumocystis jirovecii in sputum from HIV and TB patients in Namibia. J Infect Dev Ctries. 2014;8(03):349–57.
15. Dunbar A, Schauwvlieghe A, Algoe S, Van Hellemond JJ, Reynders M, Vandecasteele S, et al. Epidemiology of pneumocystis jirovecii pneumonia and (non-) use of prophylaxis. Front Cell Infect Microbiol. 2020;10:224.
16. Taylor SM, Meshnick SR, Worodria W, Andama A, Cattamanchi A, Davis JL, et al. Low prevalence of pneumocystis pneumonia (PCP) but high prevalence of pneumocystis dihydropteroate synthase (dhps) gene mutations in HIV-infected persons in Uganda. PLoS One. 2012;7(11):e49991.
17. Masoza TS, Rwezaula R, Msanga DR, Chami N, Kabirigi J, Ambrose E, et al. Prevalence and outcome of HIV infected children admitted in a tertiary hospital in Northern Tanzania. BMC Pediatr. 2022;22(1):1–9.
18. Gajewska M, Goryński P, Paradowska-Stankiewicz I, Lewtak K, Piotrowicz M, Urban E, et al. Monitoring of community-acquired pneumonia hospitalisations before the introduction of pneumococcal conjugate vaccine into Polish National Immunisation Programme (2009–2016): a nationwide retrospective database analysis. Vaccine. 2020;38(2):194–201.
19. Schmidt JJ, Lueck C, Ziesing S, Stoll M, Haller H, Gottlieb J, et al. Clinical course, treatment and outcome of pneumocystis pneumonia in immunocompromised adults: a retrospective analysis over 17 years. Crit Care. 2018;22(1):1–9.
20. Suzuki T, Shimoda Y, Teruya K, Gatanaga H, Kikuchi Y, Oka S, Watanabe K. Case report: new development of fibrosing interstitial lung disease triggered by HIV-related pneumocystis pneumonia. BMC Pulm Med. 2019;19(1):1–3.
21. García-Moreno J, Melendo-Pérez S, Martín-Gómez MT, Frick MA, Balcells-Ramírez J, Pujol-Jover M, et al. Pneumocystis jirovecii pneumonia in children. A retrospective study in a single center over three decades. Enferm Infecc Microbiol Clin (Engl Ed). 2020;38(3):111–8.
22. Chesnay A, Paget C, Heuzé-Vourc'h N, Baranek T, Desoubeaux G. Pneumocystis pneumonia: pitfalls and hindrances to establishing a reliable animal model. J Fungi. 2022;8(2):129.
23. Choo R, Naser NSH, Nadkarni NV, Anantham D. Utility of bronchoalveolar lavage in the management of immunocompromised patients presenting with lung infiltrates. BMC Pulm Med. 2019;19(1):1–12.
24. Andrews CP, Coalson JJ, Smith JD, Johanson WG. Diagnosis of nosocomial bacterial pneumonia in acute, diffuse lung injury. Chest. 1981;80(3):254–8.
25. Miller E, Andrews NJ, Waight PA, Slack MP, George RC. Herd immunity and serotype replacement 4 years after seven-valent pneumococcal conjugate vaccination in England and Wales: an observational cohort study. Lancet Infect Dis. 2011;11(10):760–8.
26. Sjögren P, Nilsson E, Forsell M, Johansson O, Hoogstraate J. A systematic review of the preventive effect of oral hygiene on pneumonia and respiratory tract infection in elderly people in hospitals and nursing homes: effect estimates and methodological quality of randomized controlled trials. J Am Geriatr Soc. 2008;56(11):2124–30.
27. Xu X, Jiang X, Ma C, Du P, Li X, Lv S, et al. A deep learning system to screen novel coronavirus disease 2019 pneumonia. Engineering. 2020;6(10):1122–9.
28. Amini B, Sharma R. Pulmonary Pneumocystis jiroveci infection. 2008. Retrieved from https://radiopaedia.org/articles/1901.
29. Crans CA, Boiselle PM. Imaging features of pneumocystis carinii pneumonia. Crit Rev Diagn Imaging. 1999;40(4):251–84.

30. Kanne JP, Yandow DR, Meyer CA. Pneumocystis jiroveci pneumonia: high-resolution CT findings in patients with and without HIV infection. AJR Am J Roentgenol. 2012;198(6):W555.
31. Zahar J, Robin M, Azoulay E, Fieux F, Nitenberg G, Schlemmer B. Pneumocystis carinii pneumonia in critically ill patients with malignancy: a descriptive study. Clin Infect Dis. 2002;35(8):929–34.
32. Amini B, Sharma R. Pulmonary Pneumocystis jiroveci infection. 2022. Retrieved from https://radiopaedia.org/articles/1901.
33. Stansell JD, Osmond DH, Charlebois E, Lavange L, Wallace JM, Alexander BV, et al. Predictors of pneumocystis carinii pneumonia in HIV-infected persons. Pulmonary complications of HIV infection study group. Am J Respir Crit Care Med. 1997;155(1):60–6.
34. Sepkowitz KA. Pneumocystis carinii pneumonia in patients without AIDS. Clin Infect Dis. 1993;17(Supplement_2):S416–22.
35. Mossman BT, Marsh JP, Sesko A, Hill S, Shatos MA, Doherty J, et al. Inhibition of lung injury, inflammation, and interstitial pulmonary fibrosis by polyethylene glycol-conjugated catalase in a rapid inhalation model of asbestosis. Am Rev Respir Dis. 1990;141(5 Pt 1):1266–71.
36. Selwyn PA, Pumerantz AS, Durante A, Alcabes PG, Gourevitch MN, Boiselle PG, Elmore JG. Clinical predictors of pneumocystis carinii pneumonia, bacterial pneumonia and tuberculosis in HIV-infected patients. AIDS. 1998;12(8):885–93.
37. Pulvirenti J, Herrera P, Venkataraman P, Ahmed N. Pneumocystis carinii pneumonia in HIV-infected patients in the HAART era. AIDS Patient Care STDs. 2003;17(6):261–5.
38. Leigh T, Hume C, Gazzard B, Parsons P, Husain O, Collins J. Sputum induction for diagnosis of pneumocystis carinii pneumonia. Lancet. 1989;334(8656):205–6.
39. Golden JA, Hollander H, Stulbarg MS, Gamsu G. Bronchoalveolar lavage as the exclusive diagnostic modality for pneumocystis carinii pneumonia: a prospective study among patients with acquired immunodeficiency syndrome. Chest. 1986;90(1):18–22.
40. Walzer PD, Perl DP, Krogstad DJ, RAWSON PG, SCHULTZ MG. Pneumocystis carinii pneumonia in the United States: epidemiologic, diagnostic, and clinical features. Ann Intern Med. 1974;80(1):83–93.
41. Dako F, Kako B, Nirag J, Simpson S. High-resolution CT, histopathologic, and clinical features of granulomatous pneumocystis jiroveci pneumonia. Radiol Case Rep. 2019;14(6):746–9.
42. Chen L, Zhang X, Peng X, Yang Y, Yu H. Baicalin tetrazole acts as anti-pneumocystis carinii pneumonia candidate in immunosuppressed rat model. Microb Pathog. 2019;132:59–65.
43. Dunphy L, Patel N, Palmer B, McKeown E. Missed opportunity to diagnose HIV with pneumocystis carinii pneumonia as its sequela. BMJ Case Reports CP. 2020;13(6):e235386.

Recent Developments in the Treatment of Bacterial Pneumonia

Grace Johnson, Michael Young, Jonah Gordon, and Charles Preuss

Abstract Bacterial pneumonia acquired from either the community, hospital, ventilators, aspiration, or tuberculous organisms represents a threat to an immunocompromised host as much as a previously healthy host. The treatment of bacterial pneumonia is largely based on organismal prevalence in a community or hospital, and the decision to treat in an outpatient versus inpatient setting is guided by severity-of-illness scoring systems. In the setting of mild community-acquired pneumonia (CAP), *Streptococcus pneumoniae* is the presumed perpetrator, and amoxicillin or doxycycline is administered as the mainstay treatment. Severe CAP leads to suspicions of methicillin-resistant *Staphylococcus aureus* (MRSA) in addition to *Streptococcus pneumoniae* or atypical organisms, and fluoroquinolones are added to the regimen with a macrolide, doxycycline, and a beta-lactam. Bacterial pneumonia acquired in the hospital setting bears great risk for resistant organisms such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *Klebsiella pneumoniae*. In such cases, anti-pseudomonal penicillins, cephalosporins, carbapenems, or fluoroquinolones are implemented accordingly, with vancomycin or linezolid being added when MRSA is suspected. Bacterial pneumonia acquired from mechanical ventilators is identified via culturing of bronchial secretions, and is most likely underlain by MRSA, *Acinetobacter baumanii,* or *Pseudomonas aeruginosa.* Antibiograms guide the treatment regimens for these cases. Recent advances in siderophore technology are facilitating targeted cephalosporin treatment of Gram-negative organisms causing these infections. For all types of bacterial pneumonia, resistance to these historically fool-proof empiric treatments is growing. Additionally, pneumonia in patients with chronic conditions such as cystic fibrosis warrants increasing ingenuity in targeting resistant *Pseudomonas aeruginosa* or *Burkholderia cepacia*, such as recent developments in bacteriophage therapy. For

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all types of bacterial pneumonia in all subsets of patients, pinpointing the causative organism through culturing or nucleic acid hybridization for resistance gene identification, as well as greater incorporation of targeted drug-delivery systems, will allow for deceleration of the growing resistance threats and economic costs in treating bacterial pneumonia.

Keywords Pneumonia · Bacterial pneumonia · Tuberculosis · Community-acquired pneumonia · Hospital-acquired pneumonia · Ventilator-associated pneumonia · Aspiration pneumonia · Drug resistance · Cystic fibrosis · Immunocompromise · Sickle cell · Inhaled phage therapy · Inflammasome inhibitor therapy

1 Introduction

Pneumonia is an expensive, prevalent, and relevant infection of the decade. In 2018, it ranked fourth among the 10 most frequent diagnoses among inpatients, accounted for 740,700 inpatient hospital stays, and comprised $7.7 billion dollars of healthcare costs in the United States [1]. Pneumonia accounted for 1,485,000 emergency department visits in 2018 [2]. In 2020, bacterial pneumonia bore a mortality rate of 14 deaths per 100,000 population [3]. Pneumonia is traditionally categorized as community-acquired or nosocomial (hospital-acquired or ventilator-associated), with empiric treatment aimed at the most likely causative organisms, as a pathogen is detected in only 38% of cases [4]. These organisms are described as either "typical" organisms such as *Streptococcus pneumoniae* or *Hemophilus influenzae* that can be cultured on standard media and Gram stained, and give a classic presentation of lobar pneumonia, or "atypical" organisms that are intracellular or gram-indeterminant, and thus cannot easily be cultured – such as *Mycoplasma pneumoniae* or *Chlamydia* species; these cause milder symptoms of an infiltrative pneumonia [5]. Decisions for therapy can be guided by severity-of-illness scoring systems, such as the CURB-65, which allots a point for confusion, uremia, respiratory rate >30/minute, blood pressure <90/60, and age >65, such that a patient with a score of 0–1 is a candidate for outpatient treatment, where a score of 2 or higher indicates a potential need for hospital admission [6]. The Pneumonia Severity Index is a composite of 20 factors and stratifies patients into mortality classes, such that patients in risk class IV or greater should be managed inpatient, while lower-risk classes be maintained as outpatient [7].

This chapter delineates the types of pneumonia, their causative pathogens, and appropriate treatments, as well as present recent advances in experimental therapies.

2 Community-Acquired Pneumonia

Community-acquired pneumonia (CAP) is defined as "the acute infection of lung tissue in a patient who has acquired it from the community or within 48 hours of the hospital admission" [5]. Presentation is typically composed of systemic symptoms such as chills fever, cough, pleuritic chest pain, fatigue, and anorexia. Physical exam findings are consistent with decreased breath sounds, increased tactile fremitus, and crackles upon auscultation [8].

2.1 Etiology and Diagnosis of CAP

Though institution of the conjugated pneumococcal vaccine for infants and older adults has decreased pneumonia-related hospitalizations [9], *Streptococcus pneumoniae (S. pneumoniae)* is the most common cause of CAP; other bacteria to consider are *Hemophilus influenzae, Staphylococcus aureus (S. aureus), Mycoplasma pneumoniae, Chlamydia pneumoniae, Legionella* sp., and less commonly *Pseudomonas aeruginosa* [4, 9]. Gram-staining and culture of respiratory secretions and blood culturing are not recommended as a part of outpatient management, as there is no evidence that this portends better outcomes when compared to not performing these techniques [10]. However, in the setting of severe CAP managed in the hospital, Gram-staining and respiratory specimen culturing is recommended when the patient is intubated, or has risk factors for methicillin-resistant *Staphylococcus aureus* (MRSA) or *Pseudomonas* sp. such as prior parenteral antibiotic therapy in the last 90 days or previous infection with these organisms. Blood cultures should be obtained on these inpatients if they have severe pneumonia or are being treated for MRSA or *Pseudomonas* sp. [10]. This distinction between severe, intensive-care unit (ICU)-requiring pneumonia and non-severe pneumonia can be judged based on a set of major and minor criteria indicated by a joint committee of the Infectious Diseases Society of America (IDSA) and the American Thoracic Society (ATS) [11]: major criteria consist of either respiratory failure necessitating ventilation, or septic shock requiring pressors, or meeting three minor criteria: leukopenia (WBC <4000/uL), thrombocytopenia (<100,000/uL), or hypothermia (core temperature <36 °C), as these have been shown to portend a poor prognosis [11].

2.2 Outpatient Treatment of CAP

Outpatient treatment for patients of no comorbidities or risk for drug-resistant *S. pneumoniae* is amoxicillin 1 g 3 times per day, or doxycycline 100 mg twice daily, or a macrolide if there is low regional prevalence of macrolide-resistant *S. pneumoniae* [10] (Fig. 1). For patients with co-morbidities such as heart disease,

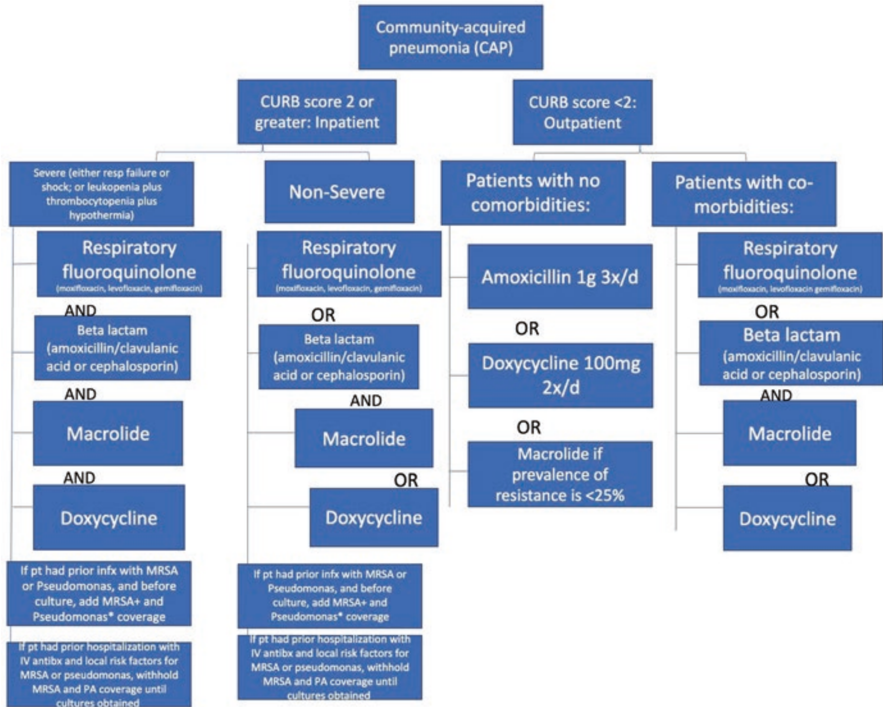

Fig. 1 Overview of treatment algorithm for community-acquired pneumonia. +Linezolid + Vancomycin for MRSA. *Piperacillin + Tazobactam, cefepime, ceftazidime, imipenem, meropenem, or azotrenam for Pseudomonas. *pt. = patient. (Adapted from Metlay et al. [10])

diabetes mellitus, obesity, liver, lung, renal disease, malignancy, or alcoholism, therapy should cover beta-lactamase-producing *Hemophilus influenzae,* resistant *S. pneumoniae, S. aureus,* enteric gram-negatives, and atypicals such as *Chlamydia* spp. Monotherapy with a respiratory fluoroquinolone such as moxifloxacin, levofloxacin, or gemifloxacin may be used, or – and equally effective – a beta-lactam such as amoxicillin/clavulanic acid or a cephalosporin, plus a macrolide or doxycycline [10, 12]. A benefit of quinolone monotherapy is fewer gastrointestinal side effects [12].

2.3 Inpatient Treatment of CAP

Community-acquired pneumonia requiring hospitalization is more common in elderly populations, and has an annual incidence of about 24.8 cases per 10,000 population [4]. Patients with CAP requiring hospitalization are most likely to have *Streptococcus pneumoniae*, and *H. influenzae,* followed by atypicals such as *Mycoplasma pneumoniae* and *Legionella* spp. [13, 14]. Inpatient treatment of adults

with non-severe pneumonia may suffice with quinolone monotherapy or a beta-lactam plus macrolide or doxycycline [10] (Fig. 1). A 2020 study that performed a secondary analysis on the Global Initiative for MRSA Pneumonia (GLIMP) study, which sought to identify the pathogens underlying CAP in hospitalized patients in 54 different countries, found that in the U.S., CAP necessitating ICU admission is likely to be underlain by infection by *Staphylococcus aureus* (MRSA and methicillin-sensitive *Staphylococcus aureus*), *Pseudomonas aeruginosa*, *S. pneumoniae, and Klebsiella* sp. [13]. A 2011 prospective study of 3523 adult patients treated for CAP at a center in Barcelona found the most common agent underlying CAP necessitating ICU admission was *S. pneumoniae* [14]. Adults hospitalized for severe CAP should combine a respiratory fluoroquinolone, macrolide, doxycycline, and beta-lactam [10] for coverage of these most prevalent organisms. As for treatment with corticosteroids, there is presently no evidence supporting their use for non-severe or severe CAP [10, 15].

2.4 Pseudomonas aeruginosa *CAP*

A 2018 point-prevalence study investigating rates of and risk factors for *Pseudomonas* sp. pulmonary infection across 54 countries found that though the rate is low, at 4.2% of all community-acquired pneumonia cases, most cases occur individuals who had prior infection with *Pseudomonas aeruginosa* and had a preexisting pulmonary comorbidity such as COPD, bronchiectasis, or tracheostomy [16]. In patients with *Pseudomonas* sp. CAP, resistances must be considered, as 2% of patients hospitalized with CAP had antibiotic-resistant *Pseudomonas* sp.. Piperacillin-tazobactam, ceftazidime, aztreonam, imipenem, or meropenem is recommended for the treatment of *Pseudomonas* sp. CAP [10].

2.5 Antibacterial Resistance in CAP

Methicillin-resistant *Staphylococcus aureus* must be considered in patients with risk features such as past MRSA infection, recent antibiotic use, and recent hospitalization [17]. A 2021 retrospective study identified a strong association between lung abscess and MRSA pneumonia, with a 4.24-times likelihood of having MRSA pneumonia in the presence of a lung abscess ($P < 0.0001$) [18]. Influenza co-infection, end-stage renal disease, and use of illicit substances were also strong independent risk factors for MRSA CAP. Patients with MRSA CAP should be treated with vancomycin or linezolid [10].

Drug-resistant *Streptococcus pneumoniae* is more likely in older patients >65 years, patients who have been treated with a beta-lactam, macrolide, or fluoroquinolone in the past 3–6 months, medical comorbidities, immunosuppression, or

alcoholism [10, 19]. Combining a beta-lactam or cephalosporin with either a macrolide or doxycycline should cover drug-resistant *S. pneumoniae.*

2.6 Future of CAP

A 2019 randomized controlled clinical trial comparing moxifloxacin to a newer fluoroquinolone, delafloxacin, found that delafloxacin was non-inferior to moxifloxacin in treating community-acquired bacterial pneumonia [20]. Delafloxacin has lower MICs than other fluoroquinolones against Gram-positive pathogens and anaerobes, and demonstrates similar potency as ciprofloxacin against Gram-negatives [21]. Because it is weakly acidic, it is able to penetrate intracellularly when in the acidic environment of infected tissue [22]. The non-inferiority of delafloxacin may be an auspicious finding as it lacks some of the adverse effects seen in other fluoroquinolones, such as QT prolongation, phototoxicity, and CNS effects [20, 23]. Lefamulin is a pleuromutilin antibiotic that inhibits protein synthesis, binding the 50S ribosomal subunit to interfere with peptidyl transferase activity [24]. It is effective against typical and atypical bacterial pneumonia pathogens such as *S. aureus* and MRSA*, S pneumoniae,* group A and B streptococci, *M. catarrhalis, H. influenzae, Legionella* sp.*, Mycoplasma* sp.*,* and *Chlamydia pneumoniae* [24]. A safety and efficacy analysis published in 2021 for phase 3 clinical trial data comparing moxifloxacin to lefamulin for CAP found non-inferiority and clinical efficacy, as well as tolerability in populations with co-morbidities [25]. Successes with these alternative drugs are promising in light of drug resistances and intolerability surrounding other typically used medications such as macrolides and fluoroquinolones.

3 *Mycobacterium tuberculosis* Pneumonia

Globally, mortality from *Mycobacterium tuberculosis* (TB) infection is number 13 of the top 20 most common causes of death worldwide [26]. According to the World Health Organization's 2021 Global Tuberculosis Report, TB traditionally accounted for the most deaths from a single microorganism, until the SARS-CoV-2 pandemic struck in 2019. Though 9.9 million individuals were estimated to have been assailed by TB infection, only 5.8 million cases were reported; this decline in diagnosis and reports of TB infection during 2020 has been attributed to healthcare disruptions imposed by the COVID-19 pandemic. However, deaths from TB are estimated to have increased to 1.3 million deaths among HIV-negative people worldwide, compared to 1.2 million in 2019. The deaths from HIV-positive individuals are also estimated to have slightly increased to 214,000 from 209,000 deaths in 2019 [26]. This is all despite a consistent decline in mortality seen up

until 2019, as well as a slight decrease in TB incidence even through 2020, demonstrating the impact of the COVID-19 pandemic on TB mortality and access to care [26]. Over 86% of incident cases in 2020 occurred in WHO regions of Southeast Asia, Africa, and the Western Pacific, with the greatest incidences found in these countries listed in descending incidence: India, China, Indonesia, the Philippines, Pakistan, Nigeria, Bangladesh, and South Africa [26]. TB burden is inversely associated with gross domestic product per capita, directly associated with prevalence of undernutrition, and is commonly seen as co-infection with human immunodeficiency virus (HIV); in South Africa, 50% of TB cases were co-infected with HIV [26]. According to the World Health Organization (WHO), the greatest risk factors for TB infection are alcohol use disorders, diabetes mellitus, HIV, smoking, and poor nutrition – all features that blunt the vigor of the immune system to extinguish such a subversive pathogen. While implementation of anti-retroviral therapy for HIV over the past couple of decades has helped decline the cases of TB expected in the HIV population [27], a greater proportion of deaths from TB burden those with HIV [28]. This is due to the synergy of HIV and TB infection that drive each other – active TB infection provokes HIV replication in mononuclear cells activated against the local TB infection site [29]. Conversely, the immunosuppression induced by HIV allows TB to thrive and escape effective cell-mediated responses. The concert of inflammatory signaling allows the mycobacteria to thrive at the expense of the host.

In a healthy host, exposure may not yield infection, as *Mycobacteria* sp. can be cleared by the innate immune system [30]. Otherwise, inhaled *Mycobacteria*-laden droplets are engulfed by alveolar macrophages, prevent phagolysosome acidification, and can reside quiescently in macrophages and surrounded in granulomas. This balance is maintained by macrophage TNF-alpha release, Th1 cell interferon-gamma signaling, as well as a tolerizing Th2 response that tempers inflammation [31, 32]. However, with an immune system perturbation – such as HIV infection, immunosuppressive therapy such as glucocorticoids or TNF-alpha inhibitors, malnutrition – the reciprocal latency is disrupted and TB is permitted to induce necrosis of granulomas and cause pulmonary TB disease, and disseminate to well-vascularized tissues like bone, liver, spleen, brain parenchyma, or meninges [31].

Mathematical modeling estimates that a quarter of the world's population harbors latent TB [33]. Reactivation of latent TB is estimated to occur in 5–10% of cases of latent TB [34, 35]. Thus, treatment of latent TB is essential. While latent TB is asymptomatic, lacking the features typical of active TB disease such as cough, hemoptysis, fevers, night sweats, and weight loss, it is strongly recommended for individuals at risk of latent TB to be tested using the tuberculin skin test, or interferon gamma release assay (IGRA), which provoke a memory T-cell response to current infection [36]. These individuals include those with HIV, exposure to TB, immunosuppressive therapy such as TNF-alpha inhibitors, silicosis, or pre-blood or marrow transplant [36].

3.1 Treatment of Latent TB

Traditionally, a 9-month regimen of daily isoniazid had been recommended for treatment of latent TB [36, 37]. However, given the difficulty in adherence to lengthy drug regimens that frustrates treatment success [38], and evidence showing similar efficacy yet improved adherence of once-weekly isoniazid plus rifapentine for 3 months compared to daily isoniazid over 9 months [39], the CDC elicited updated recommendations in 2020 for treatment of latent TB to prioritize shorter regimens: either weekly isoniazid plus rifapentine over 3 months, rifampin daily for 4 months, or rifampin plus isoniazid daily for 3 months [40]. Alternative courses include the 6- or 9-month isoniazid monotherapy [40]. The regimen consisting of 3-month weekly rifapentine plus isoniazid therapy is also seen to have less risk of hepatotoxicity compared to a prolonged 9-month course of isoniazid alone [41].

3.2 Treatment of Active TB

85% of individuals diagnosed with active TB can be cured with a drug regimen administered over 6 months [42]. Active TB is confirmed with chest X-ray, acid-fact bacilli smear, sputum culture, and nucleic acid amplification [43]. Rifampin-resistant (RR-TB) or multidrug (resistant to rifampin and isoniazid)-resistant (MDR-TB) strains of TB are determined by drug susceptibility assays, and have displayed a stable prevalence of at 3–4% of newly diagnosed TB cases and 18–21% of previously treated TB cases [42]. For treatment of active tuberculous disease, the 2016 American Thoracic Society/CDC/Infectious Disease Society of American advises 2 months (8 weeks) of daily isoniazid, rifampin, pyrazinamide, and ethambutol, followed by 4.5 months (18 weeks) of daily isoniazid and rifampin [44]. If a patient's TB strain is susceptible to isoniazid and rifampin, then ethambutol can be discontinued [44]. The continuation phase may be prolonged to 9 months if the patient had a cavitation on initial chest X ray, and had a positive sputum culture after the initial 2 months of therapy [44]. A recent clinical trial comparing the traditional 6-month quadruple therapy to a 4-month course of rifabutin plus moxifloxacin found no difference between the therapies in producing TB-free survival at 12 months [45], providing evidence for future recommendations to consider a shorter regimen.

3.3 Drug-Resistant Tuberculosis

The 2020 WHO update for treating drug-resistant tuberculosis recommends a four-drug regimen for treating isoniazid-resistant TB: rifampicin, ethambutol, pyrazinamide, and levofloxacin for 6 months [42]. For multidrug-resistant TB, an oral regimen for 9–12 months is recommended [42]; this entails seven drugs to be

Table 1 Drug combinations used for treatment of multidrug-resistant tuberculosis

Group	Drugs
Group A (highly effective, strongly recommended according to GRADE evidence)	Moxifloxacin or levofloxacin Bedaquiline Linezolid
Group B (second choice; conditionally recommended)	Clofazimine Cycloserine or terizidone
Group C (for completion of treatment regimen)	Ethionamide, ethambutol, delamanid, pyrazinamide, imipenem/cilastin, meropenem, amikacin or streptomycin, and p-Aminosalicylic acid

Adapted from WHO [42]

implemented for 4 months or until sputum conversion occurs, including bedaquiline, levofloxacin or moxifloxacin, ethionamide, ethambutol, high-dose isoniazid, pyrazinamide, and clofazimine. The subsequent 5 months of continuation consist of treatment with moxifloxacin or levofloxacin, clofazimine, ethambutol, and pyrazinamide [42]. If the strain is resistant to fluoroquinolones, a regimen composed of bedaquiline, pretomanid, and linezolid can be used [42, 46, 47]. Agents for treatment of MDR-TB are classified by the WHO according to Grading of Recommendations, Assessment, Evaluation (GRADE) evidence into groups A–C: group A including fluoroquinolones moxifloxacin and levofloxacin, bedaquiline, and linezolid; group B including clofazimine and cycloserine or terizidone, and group C including other drugs such as ethionamide, ethambutol, delamanid, pyrazinamide, imipenem/cilastatin, meropenem, amikacin or streptomycin, and p-aminosalicylic acid (Table 1). It is recommended for patients requiring longer regimens encompassing 18–20 months to be initiated on four drugs until sputum conversion – the three from group A, and at least one from group B – and then continue with three drugs for the continuation phase consisting of 15–17 months after sputum conversion [42].

4 Nosocomial Pneumonia

Nosocomial pneumonia can be separated into two distinct types: hospital-acquired (HAP) and ventilator-associated (VAP). HAP and VAP have the highest mortality rates, and are the most common of all nosocomial infections in the intensive-care unit (ICU) [48].

4.1 Ventilator-Associated Pneumonia

Intubation and mechanical ventilation are associated with risk of pneumonia known as ventilator-associated pneumonia (VAP). VAP is an infection of the lungs occurring after a minimum of 48 hours of mechanical ventilation (MV). An initial step in

the pathogenesis of VAP involves aspiration of oropharyngeal or gastric contents and subsequent lung colonization. With an average incidence of 20–25%, VAP most commonly occurs within the first 5 days of MV [49]. Burn victims, as well as patients with increased duration of MV and impaired consciousness, are associated with increased risk of acquiring VAP [50]. Further, the 30-day mortality due to VAP has been reported to be around 30% [51]. It has been noted that developing countries have higher rates of VAP due to ineffective or lack of prevention strategies [50].

4.2 Etiology of VAP

VAP is believed to exist on a spectrum with ventilator-associated tracheobronchitis (VAT), which has also been increasing in incidence [52]. Identification of the causative pathogen can be determined via bronchoalveolar lavage and subsequent culture of the aspirate. A 2018 study noted isolation of 2387 different pathogens for 1474 episodes of VAP over a 10-year period in the United States [53]. This same study reported increasing incidence of *S. aureus*, including MRSA, as the causative agent. This trend is believed to be due to increasing antibiotic resistance among pathogens. A 2012 Korean study determined the most common pathogens for VAP in the ICU-setting were *S. aureus* and *Acinetobacter baumanii (A. baumanii)*, a Gram-negative coccobacillus [54]. These two pathogens were responsible for 75% of all VAP infections within the study. A 2018 meta-analysis, including 27 years of publications across the Asian continent, reported *A. baumanii* as being the most common cause of VAP with *P. aeruginosa* a close second [55]. Resistance to carbapenems, a strong class of antibiotic covering Gram-negatives, was also noted to be very high at 57%. The aforementioned studies highlight the necessity of local antibiograms as different pathogens with differing resistance patterns are responsible for VAP in each geographic region.

4.3 Treatment of VAP

As highlighted, VAP is relatively common phenomenon in the ICU setting with significant mortality, thus necessitating robust prevention and treatment strategies. The most recent 2016 guidelines from both the Infectious Disease Society of America (IDSA) and American Thoracic Society (ATS) recommend use of local antibiograms to guide treatment regimens. Empiric treatment includes risk stratification for methicillin-sensitive or methicillin-resistant *S. aureus* (MSSA and MRSA, respectively) as well as coverage for Gram-negative rods, including *P. aeruginosa*. Vancomycin or linezolid are recommended for MRSA risk and piperacillin-tazobactam, cefepime, levofloxacin, the carbapenems meropenem and imipenem, or aminoglycosides are recommended for their respective antipseudomonal properties [56]. Further, many of the aforementioned antibiotics have inherent side effects

such as nephro- and ototoxicity (aminoglycosides) and tendon damage (fluoroquinolones). Combination therapy, including coverage for MRSA/MSSA and *P. aeruginosa*, is the current recommended treatment modality for VAP. Antibiotic choice should depend on both local antibiograms and patient comorbidities to minimize unwanted side effects.

4.4 Prevention of VAP

Intubation and MV are both obvious risk factors for VAP and minimizing the duration of MV can help mitigate risk. It has been established that, when mechanically ventilated, supine patients have a higher risk of pulmonary aspiration compared to patients in the 45° upright position [57]. As a result, many critical care units adopted this practice of elevating ventilated patients as lower aspiration risk should, in theory, minimize VAP risk. Despite this common practice, “head of bed” elevation has been shown to have no significant clinical benefit [58]. Many of the pathogens responsible for VAP colonize the oropharynx, thus making effective oral care a target for VAP prevention. Bedside nurses in the critical care setting focus on oral cavity assessment (validated scoring tools such as the Beck oral assessment scale), saliva maintenance, and suctioning of oral secretions [59]. Oral rinse of 2% chlorhexidine has been shown to be an effective prevention strategy for VAP and subsequent colonization of the oropharynx [60]. Use of probiotics in patients undergoing critical care has been shown to reduce length of ICU stay with no effect of VAP prevention [61].

4.5 Future of VAP

Although a viral infection, the onset of the COVID-19 pandemic has generated massive amounts of research related to infectious disease. A handful of studies have noted that patients with severe forms of COVID-19 pneumonia undergoing MV had a concomitant bacterial lung infection [62, 63], with *A. baumanii* being the responsible pathogen in one of these case series. Currently, much research surrounding VAP is focused on multidrug-resistant organisms, especially *A. baumanii.* This Gram-negative bacterium is able to survive in the hospital environment through strong biofilms [64]. Although carbapenems have long been noted to be the last resort for *A. baumanii* VAP treatment, it seems as though carbapenem-resistant *A. baumanii* (CRAB) levels are rising [65].

A new siderophore cephalosporin, known as cifderocol, has been recently developed with promising and potent anti-Gram-negative activity, including activity against CRAB [66]. The polymyxin colistin, a significant nephrotoxin when given IV, has been shown to be efficacious against CRAB in the nebulized form, event without additional intravenous dosing [67]. Trials have shown ceftazidime-avibactam

to be non-inferior to meropenem in the treatment of VAP [68]. Some trials have examined the use of tigecycline as a treatment of VAP with insignificant results. In 2010, the FDA issued a safety announcement noting increased mortality risk when IV tigecycline is used to treat patients with VAP. A black box warning was added to the medication in 2013 [69].

There have been recent developments in diagnostics for VAP as well. The BioFire FilmArray Blood Culture Identification (BCID) panel is a respiratory sample diagnostic test with 1-hour turnaround time and high sensitivity and negative predictive value [70]. The BCID panel, with rapid results and the ability to identify drug-resistant pathogens, is promising with quick turnaround for antibiotic choice, minimizing the overall duration of empiric treatment.

4.6 Hospital-Acquired Pneumonia

Hospital-acquired pneumonia (HAP) is a form of pneumonia that occurs at least 48 hours after admission without prior indication of underlying pulmonary infection and is not associated with use of mechanical ventilation nor intubation. A 2018 study noted the incidence of HAP of just under 2%, but with a higher mortality rate than VAP [48]. Although less common than VAP, HAP leads to significant healthcare costs, thus there is a strong a focus of both prevention and treatment strategies in the hospital setting.

4.7 Etiology of HAP

As both VAP and HAP commonly occur in the hospital critical care setting, similar causative pathogens are to be expected. A 2019 retrospective study in China noted HAP and VAP with similar causative pathogens, namely *A. baumannii*, *P. aeruginosa,* and *Klebsiella pneumoniae* (*K. pneumoniae*) [71]. A multicenter study of nine different Chinese cities reported the most common HAP pathogens to be, once again, *A. baumannii*, *P. aeruginosa, S. aureus,* and *K. pneumoniae* [72] *S. pneumoniae* has been noted to be cultured in ~5% of cases and is more common as the causative pathogen in early-onset HAP versus late-onset [73]. A 2017 Indian study reported the most common cause of HAP as *S. aureus* with high levels of resistance in antibiotics such as cefepime, amoxicillin, and ceftazidime [74]. Between 2008 and 2012, the incidence of MRSA-associated HAP increased despite an overall decrease in MRSA infections globally [75]. In general, etiology of HAP can vary depending on geographic location, with strains of drug-resistant pathogens on the rise.

4.8 Risk and Treatment of HAP

There are many potential risk factors that increase the likelihood of a HAP. Utilization of IV antibiotics within 90 days of HAP diagnosis is a risk factor for many drug-resistant strains of pathogens [76]. As noted prior, HAP secondary to MRSA colonization is increasing in incidence. In general, risk factors associated with increased likelihood of MRSA colonization include chronic skin breakdown [77] as well as hospital length of stay greater than 7 days, age greater than 65 years, previous history of MRSA colonization, and recent antibiotic use, including fluoroquinolones [78]. Once hospitalized, use of a ventilator after HAP diagnosis, as well as a diagnosis of septic shock, are two underlying risk factors that increase mortality rate in HAP patients [56]. Further, use of acid blockers such as proton-pump inhibitors and histamine blockers have been associated with a 30% increase in odds of developing HAP [79].

Patients diagnosed with HAP undergo risk stratification to determine if there is a high likelihood of MRSA infection, high mortality risk, or both [56]. The 2016 clinical practice guidelines from the IDSA and ATS utilize risk stratification when recommending treatment options. For patients with no increased mortality risk nor additional risk factors for MRSA colonization, one of the following antibiotics is recommended as empiric treatment: piperacillin-tazobactam, or cefepime. It is recommended that patients with increased risk of MRSA colonization initiate either vancomycin or linezolid for MRSA coverage as well as one of the following antibiotics: piperacillin-tazobactam, the fourth generation cephalosporins cefepime or ceftazidime, levofloxacin or ciprofloxacin, imipenem or meropenem, or aztreonam. For patients with recent IV antibiotic use or high mortality risk, either linezolid or vancomycin is recommended as well one of the following antibiotics: piperacillin-tazobactam, cefepime or ceftazidime, levofloxacin or ciprofloxacin, imipenem or meropenem, aztreonam, or one of the aminoglycosides amikacin, gentamicin, or tobramycin. Specific populations at risk for Gram negative infections, including those with underlying lung disease such as cystic fibrosis, are recommended to receive two anti-pseudomonal antibiotics (piperacillin-tazobactam, fourth generation cephalosporins, aminoglycosides, carbapenems). The aforementioned recommendations must be supplemented with the local antibiograms at each institution in order determine the most effective treatment for each individual.

4.9 Future of HAP

Given that a HAP diagnosis can be avoided, the prevention of HAP is the primary focus in critical care settings. Techniques for prevention are similar to those of VAP, including a strong focus on proper oral care to prevent transmission of oral flora to the lungs [80]. Despite a focus on prevention, adequate treatment of HAP needs to be optimized as many of the recommended empiric antibiotics have significant side

effects including ototoxicity, nephrotoxicity, myelosuppression, and thrombocytopenia, as well as seizures [81–84]. A new scoring tool, known as the CarbaSCORE, has been recently developed and validated to aid physicians in determining if the causative pathogen in HAP requires carbapenem treatment [85]. The goal of this validated tool is to shorten duration to initial antibiotic therapy as well as limit unnecessary empiric therapy to prevent both the development of multidrug-resistant organisms and toxic side effects to patients.

Many clinical trials have been performed in the past decade to determine the most effective treatment with minimal side effects. The 2019 ASPECT-NP trial determined that a high-dose combination of ceftolozane-tazobactam is non-inferior to meropenem in the treatment of HAP [85]. The REPROVE trial found ceftazidime-avibactam to be non-inferior to meropenem in the treatment of HAP as well [68]. These findings are significant as cephalosporins have much favorable side effect profiles when compared to carbapenems.

5 Bacterial Aspiration Pneumonia

5.1 Etiology and Risks of BAP

Upper airway, gastric, and oropharyngeal secretions are normally colonized by bacteria within their respective microbiome. When large amounts of these secretions or contents are aspirated into the lungs, bacterial aspiration pneumonia (BAP) can occur. The lung parenchyma has its own microbiome. It is hypothesized that, following aspiration of different organisms into the lungs, “dysbiosis” occurs, especially in patient populations with inability to cough or impaired consciousness, such as the elderly [87]. In a 2003 study including 95 elderly individuals with diagnosed BAP, 67 different causative pathogens were reported, with Gram-negative enteric rods (49% of infections) and anaerobes (16%) being the most common [88]. The study reported the most common causative anaerobes were *Prevotella* sp. and *Fusobacterium* sp., and no significant difference was found in mortality between patients with aerobic or anaerobic infections. More recent data suggest the common thought that BAP is primarily due to anaerobic pathogens is, in actuality, quite rare [89]. A 2010 Taiwanese study noted the most common pathogen associated with community-acquired lung abscesses, a sequela of untreated BAP, were the *Streptococcus* species [90].

Common risk factors for the development of BAP include altered mental status, poor oropharyngeal hygiene, recent stroke, recurrent vomiting, impaired gag reflex, dysphagia or swallowing impairment, and altered feeding mechanisms such as nasogastric tube feeds [89]. BAP can be further categorized by nosocomial, hospital-acquired BAP or community-acquired infection. Similar to other forms of bacterial pneumonia in the hospital setting, oral hygiene is a staple in hospital-acquired BAP prevention.

5.2 *Treatment of BAP*

Currently, no specific guidelines exist for the treatment for BAP. As a result, treatment typically consists of following the 2016 IDSA and ATS for either HAP- or VAP-based patient history, with the addition of anaerobic coverage if a high likelihood of aspiration is present. Current IDSA/AST guidelines for the treatment of HAP can been seen in Fig. 2. As with HAP/VAP, determining the causative pathogen can help narrow initial antibiotic coverage. A 2018 systematic review from Australia cites common BAP diagnostic methods as sputum culture, protected brush specimens, bronchoalveolar lavage, and blood culture [91]. Regarding additional anaerobic coverage, the most commonly used antibiotics include metronidazole and clindamycin. As noted prior, the thought that anaerobes are common causative pathogens in BAP is fading away. As a result, it seems as though the use of metronidazole or clindamycin for the treatment of BAP is mainly used when culture results indicate anaerobic infection, not as an empiric treatment. A 2020 meta-analysis noted, for severe bacterial infections in hospitalized patients (nonspecific to BAP), there was no significant difference in 90-day mortality when empiric metronidazole was prescribed [92]. Further, there are no large, double-blinded, randomized control trials for the treatment of BAP to date, thus the IDSA/AST guidelines for HAP and VAP are routinely used given that the causative pathogens for BAP, HAP, and VAP are similar.

5.3 *Future of BAP*

A handful of clinical trials are currently recruiting patients to elucidate further strategies for diagnosis, prevention, and treatment of chemical pneumonitis and BAP. The “Early Stop (Ruling) of the Antibiotic Treatment During Pneumopathies d’ Inhalation (APAPI)” in France is studying the use of PCR-based diagnostics. The “Early Antibiotics After Aspiration in ICU Patients” from UConn Health in the United States is studying the use of either ceftriaxone, amoxicillin-clavulanate, cefepime, vancomycin, or levofloxacin as empiric agents in ICU patients with clinical signs of aspiration. The “Nutrition Monitoring and Feeding Optimization With the smART+ System- Comparative Study” in Israel is investigating the use of the smART+ feeding tube nutrition system, which has the ability to prevent reflux episodes with automatic balloon inflation to prevent reflux of gastric contents into the oropharynx. This study has the potential to be extremely useful in ICU patients at risk for BAP as it may minimize aspiration events. The “Validation of Aspiration Markers in Intubated Patients” out of Massachusetts General Hospital is investigating the use of quinine as a biomarker for aspirated contents and endotracheal tube cuff leakage in the ICU. The results of these aforementioned clinical trials are highly anticipated as they have the potential to change the future of BAP management.

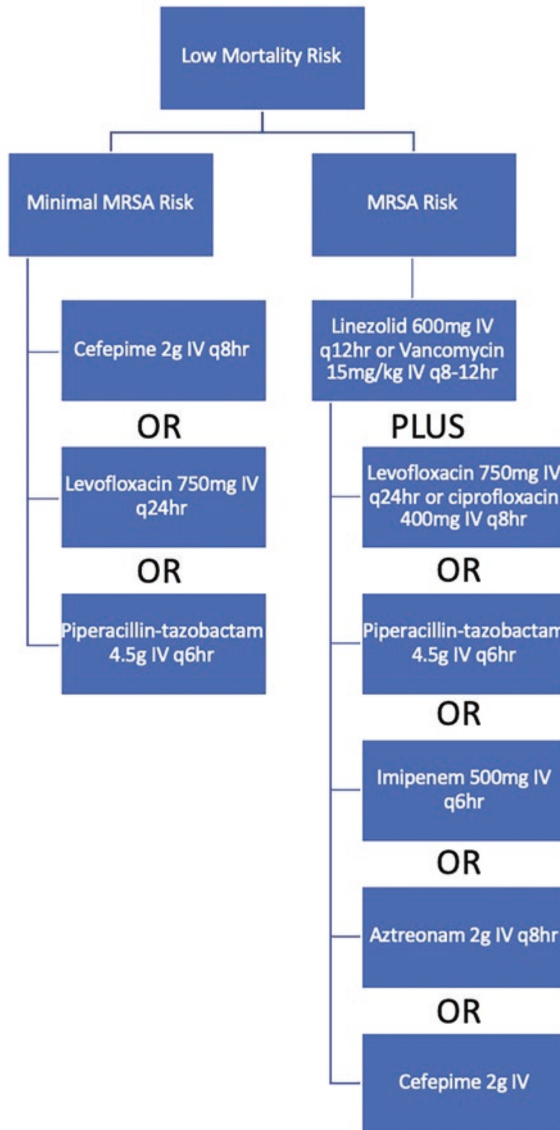

Fig. 2 Treatment of HAP in patients with low mortality risk. (Adapted from Kalil et al. [56])

6 Pneumonia in the Pediatric Population

Bacterial pneumonia is a frequent cause of hospitalization in the pediatric population. Although the focus of this chapter is on bacterial pneumonia, the presumed cause of most cases of pediatric CAP are of viral origin. This is supported by the fact that routine childhood vaccinations are given for respiratory bacteria such as *H. influenzae* and *S. pneumoniae.* More recent studies suggest that, upon

Table 2 Current 2011 IDSA guidelines for the treatment of bacterial pneumonia in the pediatric population

Etiology	Outpatient management	Inpatient management
S. pneumoniae	Amoxicillin	Ampicillin, penicillin, or ceftriaxone
S. aureus	Cephalexin	Cefazolin
MRSA	Oral clindamycin	Vancomycin, clindamycin, or linezolid
S. pyogenes	Amoxicillin or penicillin V	IV penicillin or ampicillin
H. influenza (typeable or nontypeable)	Amoxicillin with or without clavulanate	IV ampicillin, cefotaxime, ceftriaxone, ciprofloxacin, or levofloxacin
Mycoplasma sp.	Azithromycin or clarithromycin, doxycycline in adolescent population	IV azithromycin or erythromycin
C. pneumoniae	Azithromycin or clarithromycin	IV azithromycin or erythromycin

Adapted from Bradley et al. [94]

presentation at an outpatient clinic, children with a diagnosis of mild pneumonia do not need antibiotic treatment [93].

IDSA guidelines for the treatment of bacterial CAP in infants and children are currently undergoing updates, with the most recent published guidelines being released in 2011. In the outpatient setting these guidelines recommend use of amoxicillin, for *Streptococcus pneumoniae* coverage, in pediatric patients with suspected bacterial CAP as well as macrolides for the treatment of atypical causes of CAP such as *Mycoplasma pneumoniae* [94]. On the inpatient unit, third generation cephalosporins (such as ceftriaxone) are recommended for the treatment of a child or infant without full immunization status. Ampicillin is recommended for the treatment of hospitalized CAP where *S. pneumoniae* is suspected whereas an empiric combination of vancomycin or clindamycin plus a beta-lactam is recommended for the treatment of *Staphylococcus aureus* pneumonia. Finally, in a hospitalized pediatric patient in which atypical organisms (such as *Mycoplasma* sp. or *Chlamydia pneumoniae*) are suspected, a macrolide and beta-lactam are recommended [94]. Ultimate antibiotic choice must depend on both patient characteristics as well as current antibiograms of the practicing institution. Current recommendations from the IDSA can be further seen in Table 2.

7 Novel Developments in Pneumonia Therapy

7.1 Inhaled Phage Therapy

The constant evolution of antibiotic resistance among the etiologic agents of pneumonia has enhanced the need for novel therapeutic approaches. A promising approach to this growing concern is inhaled phage therapy, which utilizes lytic

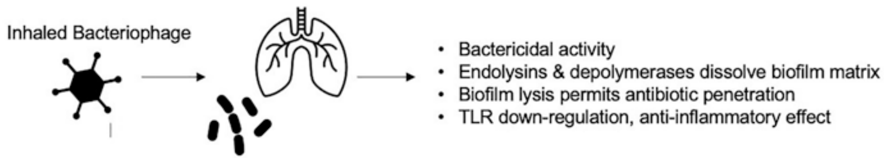

Fig. 3 Schema of phage therapy for chronic lung infection

bacteriophages to selectively target replicating bacterial cells within the respiratory system [95]. Lytic bacteriophages are an optimal choice as a novel therapeutic for a number of reasons, including bactericidal activity, low toxicity, and a high specificity which protects the natural microbiome of the lungs [95]. Following inhalation, lytic phages will bind to specific bacterial receptors to inject genetic material into a cell where replication machinery can then be redirected to produce bacteriophage progeny, which serves as an important mechanism of control for bacterial population size through phage titer build-up [96]. Further, whereas a hallmark of airway infections is the formation of biofilms with antimicrobial resistance, inhaled phage therapy has demonstrated anti-biofilm activity by preventing the initial formation of biofilms and disrupting those already formed [96] (Fig. 3). Biofilm disruption stems from the virion-associated depolymerases and endolysins associated with the phages that damage peptidoglycan and dissolve the outer extracellular polymeric substance (EPS) matrix, which increases the penetrative capability of both the phage and other classical antimicrobials to bacterial cells contained deep within the biofilm [96].

One etiologic agent of pneumonia of growing importance for alternative treatment approaches is *Pseudomonas aeruginosa*, which has demonstrated significant multidrug resistance [97]. While the use of phage therapy has received limited attention in the past, persistent lung infections by *Pseudomonas aeruginosa* within pediatric patients with cystic fibrosis have successfully been treated with this novel approach [98, 99]. Outside of these specific populations, animal models have shown promise for phage therapy in a broad-treatment approach to *Pseudomonas aeruginosa* infections, with findings indicating comparability to traditional antimicrobial treatment and increased survival compared to controls when phage administration was performed early during the course of infection [97]. Bacteriophages in this model were even found to have entered the bloodstream, which may play an important role in preventing sepsis associated with the initial pneumonia [97]. Earlier animal models have highlighted the efficacy of intranasally administered liposome-encapsulated phages in pneumonia caused by *Klebsiella pneumoniae* [100]. Nebulized phage therapy was demonstrated to be an effective approach in treating infections with *Burkholderia cenocepacia* and was compared to an intraperitoneal phage administration which had no impact on treating the respiratory infection [101].

While phage therapy is certainly a promising approach to treating bacterial pneumonia, a number of safety and efficacy concerns would need to be addressed before its adoption as a standard practice. Due to the biological nature of phage therapy,

there exists the potential for co-evolution amongst bacteria and the administered phages, thus creating difficulty in investigating therapeutic outcomes in such a dynamic environment [102]. Akin to antibiotic-resistance, phage-resistance would be a near-certainty in the widespread adoption of phage therapies through a number of extracellular and intracellular mechanisms ranging from receptor modifications to enhanced abortive infection phenomena [102]. Additionally, there is concern regarding the interactions of bacteriophages with the human immune system, as well as pharmacokinetic considerations related to poorly defined dosing and administration routes [102]. At this time, no bacteriophage therapies have been registered in the Western world, though there are a number of phase I and II trials ongoing, as well as instances of limited use under the FDA's Emergency Investigational New Drug scheme [103].

7.2 Inflammasome Inhibitor Therapy

A promising approach to managing and treating pneumonia are therapies that modulate the immune response to limit excessive activity of often damaging inflammatory mechanisms. Inflammasomes are a critical component of such immune activity that exist as a complex of proteins that processes a number of pro-inflammatory signals to stimulate a downstream response, and have been associated with a number of pulmonary pathologies including bacterial pneumonia [104]. Primarily, inflammasomes promote the synthesis and release of interleukin-1ß and interleukin-18 as the driving mechanism behind pulmonary inflammation [104]. The aim of inflammasome inhibitor therapy is to repress the chronic activation that often leads to adverse outcomes while balancing the importance of inflammation in targeting invasive bacteria [105].

The primary inflammasome involved in pneumococcal pneumonia is NLRP3, which cleaves and activates caspase-1 and has demonstrated significant activity in the presence of virulence factors such as pneumolysin [105]. Additionally, RNA from staphylococcal and group B streptococcal bacteria has been documented as activating NLRP3 in even small concentrations [106]. As has been stated, inflammasomes such as NLRP3 certainly possess a critical role in bacterial clearance; however, NLRP3 overactivation has been associated with the release of a number of damaging factors including reactive oxygen species, ATP, chemokines, and cytokines, all of which enhance disease burden associated with pneumonia [107].

Resveratrol is a plant-derived natural polyphenol with anti-inflammatory activity that acts by inhibition of NLRP3 in a number of pathologies induced by *Staphylococcus aureus* [108]. While a greater number of studies are required to identify the clear role and mechanism resveratrol plays in NLRP3 downregulation, early experimentation has demonstrated decreased mRNA and protein expression of NLRP3 and caspase-1 in mice models infected with *S. aureus* pneumonia, as well as a reduction in inflammatory cytokines including interleukin-1ß [108]. Further, while outside the realm of bacteriology, the importance of inflammasome inhibition

was demonstrated in studies of pneumonia associated with SARS-CoV-2, in which NLRP3-knockout mice models showed less signs of interstitial pneumonia and a decrease in inflammatory cell aggregation [109].

While certainly a novel therapeutic approach to bacterial pneumonia, research into inflammasome inhibitor therapy must address the risks of downregulating a natural immune response that has clear benefits for bacterial clearance. For instance, patients on inflammasome inhibitors for other conditions have been documented to be at greater risk of pneumococcal infections, a fact which both accentuates the critical role of the inflammasome in immune defense against pneumonia and emphasizes the risks involved in such therapy [105]. A greater library of data is essential to explore the role of the inflammasome, specifically NLRP3, in bacterial pneumonia so that its complex associations with genetics and immunity can be fully understood.

8 Pneumonia in Special Populations

8.1 Sickle Cell Disease

Individuals with a diagnosis of sickle cell disease are a particularly at-risk group for bacterial pneumonia, and thus warrant special attention in recognizing etiological and epidemiological trends as well as the current treatment modalities unique to this population.

A number of immunological deficits have been associated with sickle cell disease, including impaired splenic function, complement inactivity, and micronutrient deficiencies, each of which enhances the risk for acquiring a bacterial infection [110]. Specifically, sickle cell disease has been correlated with an upregulation of the platelet-activating factor receptor, which allows for increased binding of pneumococcal bacteria to activated cells and thus promotes bacterial invasion [111]. One study comparing rates of pneumococcal disease found that Black individuals with sickle cell or hemoglobin C trait had 77% and 42% increased rates of disease compared to Whites and Blacks with normal hemoglobin phenotypes [112]. Children with sickle cell disease are at a particularly high risk of developing pneumonia, with an odds ratio of 7.38 compared to controls [113]. Further highlighting the correlation between sickle cell disease and pneumonia was a large systematic review and meta-analysis among African populations that found that patients with pneumococcal disease had 36-times greater odds of having sickle cell disease compared to controls without sickle cell disease [114].

Prophylaxis is an important modality of treatment concerning sickle cell patients and bacterial pneumonia. The pneumococcal polysaccharide vaccine has shown to provide protection from 75% of pneumococcal serotypes through a T-cell independent response, therefore meaning that responses diminish quickly following vaccination [111]. The seven-valent pneumococcal conjugate vaccine is composed of the

polysaccharide capsule conjugated to a carrier protein such as diphtheria toxin mutant or tetanus toxoid, and has been shown to build strong immunologic memory in children under 2, thus contributing to an overall reduction in the incidence of bacterial pneumonia [111]. Prophylactic oral penicillin has been proven effective in reducing infection and mortality rates related to pneumonia; however, it does not cover all causes of community-acquired pneumonia and poor adherence to treatment regiments may inadvertently enhance the evolution of penicillin-resistant strains [115]. Of note, prophylactic oral penicillin significantly reduces the rates of nasopharyngeal carriage in sickle cell patients – an important risk factor for developing bacterial pneumonia [111].

Regarding treatment, a recent systematic review could not identify any randomized control trials that examined safety or efficacy in antibiotic usage for sickle cell patients presenting with community-acquired pneumonia [115]. Therefore, current recommendation for presenting cases is intravenous administration of broad-spectrum antibiotics following relevant cultures [111]. Given the critical correlations between sickle cell disease and bacterial pneumonia from both a pathophysiologic and epidemiologic standpoint, it is of utmost importance for future research to address clinical practices such as antibiotic administration in this high-risk population in order to optimize the quality of treatment.

8.2 Pneumonia in the Immunocompromised

Individuals with immunocompromised conditions such as those with AIDS, cancer/on myeloablative chemotherapy, transplant recipients, or others on immunosuppressive therapies are susceptible to a variety of bacterial pneumonia-causing organisms (Table 3). Those with depleted humoral immunity are susceptible to encapsulated pathogens such as *S. pneumoniae* and *H. influenzae*; those with impaired cell-mediated immunity are vulnerable to intracellular organisms and mycobacteria; others with neutropenia can easily contract Gram-negative rods or catalase-positive organisms such as *S. aureus* or MRSA, as well as fungi [116]. ESKAPE organisms (i.e., *Enterococcus faecium, S. aureus, Klebsiella* sp., *Acinetobacter* sp., *Pseudomonas* sp., *and Enterobacter* spp.) are most likely to cause pneumonia post-lung transplant, as well as *Norcardia* sp. [117]. *S. pneumoniae, S. aureus, Klebsiella* sp., and Gram-negative rods are most likely to cause pneumonia immediately following hematopoietic stem cell transplant [116]. Other than ensuring receipt of both the 13- and 23-valent pneumococcal vaccines, empiric antimicrobials in the case of pneumonia in an immunocompromised patient should include anti-pseudomonals (e.g., piperacillin-tazobactam, cefepime, carbapenems), beta-lactam with a macrolide or doxycycline; if inpatient, cefepime and vancomycin should also be administered to cover *Pseudomonas* sp. and MRSA [116, 118]. While pneumonia severity index scores are useful for guiding decisions for inpatient versus outpatient management in the immunocompetent, these should be used with caution in the immunocompromised as these individuals' conditions can precipitously deteriorate [118].

Table 3 Most common agents causing bacterial pneumonia in immunocompromised hosts

Immunocompromised condition	Common organisms
Humoral immunity (e.g., X-linked agammaglobulinemia, hyper-IgM)	Encapsulated: *S pneumoniae, H influenzae*
Cell-mediated immunity (e.g., AIDS, DiGeorge syndrome)	Intracellular organisms (*Legionella, Coxiella*), *Mycoplasma, Mycobacterium*
Neutropenia	*S aureus*, MRSA, Gram-negative rods
Post-lung transplant	ESKAPE pathogens
Post-hematopoietic stem cell transplant	*S pneumoniae, S aureus, Klebsiella*, other Gram-negative rods

8.3 Pneumonia and Cystic Fibrosis

In cystic fibrosis (CF), mutation of the CFTR gene precludes sufficient mucus production that normally facilitates clearance of inhaled pathogens along the mucociliary escalator. Thus, chronic bacterial colonization and subsequent inflammation contribute to pulmonary decline through the lifetime [119]. While *Staphylococcus aureus* colonization is most prevalent in the first few years of life, by the second decade, more than 50% of individuals with cystic fibrosis develop chronic *Pseudomonas* sp. infection. Treatment of chronic *Pseudomonas* sp. infection includes inhaled tobramycin powder or inhaled aztreonam [120], with intravenous aminoglycosides for an acute exacerbation [121]. Azithromycin is also recommended for ongoing therapy mainly due to its anti-inflammatory properties in the CF lung, showing to reduce pulmonary exacerbations by 44% compared to placebo in a 2018 randomized controlled clinical trial [122].

9 Conclusion

The goal of this chapter is to highlight the evolving nature of bacterial pneumonia in a variety of presentations, including CAP, HAP, VAP, and TB, with an emphasis on recent advancements in both treatment and diagnostics. Bacterial pneumonia is a disease that warrants serious attention from within the medical community due to its significant prevalence and great economic burden on healthcare systems. A number of novel therapeutic agents show promise in advancing the treatment of bacterial pneumonia through modalities designed for specific targeting of pathogenic bacteria or immunomodulation to limit the common, immune-mediated damage associated with the normal inflammatory response. However, as both traditional and novel approaches to treatment continue to evolve, a number of biological and ethical concerns will need to be addressed in order to minimize the risk of emerging treatment-resistant bacterial strains.

Multidrug-resistant bacteria are on the rise throughout healthcare, especially in the context of bacterial pneumonia [123]. Pneumonia is the leading cause of death

from nosocomial infection [124], and multidrug and extensively drug-resistant strains in nosocomial pneumonia bear mortality rates near 50% [125]. While this prompts increasing innovation in combinations of antibiotics to overcome resistance, this effort is also what imposes the selection pressure to breed those mechanisms conferred by either chromosomal mutation, plasmid exchange, or transposable elements – such as efflux pumps, alterations in penicillin-binding proteins, beta-lactamase production, DNA gyrase mutations, and lipo-oligosaccharide changes [123]. As a result, antibiotic stewardship and use of local antibiograms are key in helping curb the development of new multidrug-resistant organisms. However, diagnosis is limited by time, as culturing cannot be accelerated beyond the 48–72 hours it takes for this method of microbe identification. Thus, the future of bacterial pneumonia – particularly in hospitalized patients or those with high likelihoods of harboring resistant strains – must lie in molecular techniques to expedite diagnosis, and prompt implementation of targeted therapies. Real-time polymerase chain reaction (PCR) or nucleic acid hybridization to identify resistance genes can be performed in 2–6 hours [126] and facilitate efficient diagnosis. Rather than inundating these bacterial pathogens with antibiotics in hopes of extinguishing their resistances, it will be important to increasingly rely on therapies that directly target the invader, such as the aforementioned phage therapy for treating Gram-negative bacillus pneumonia. Where the creativity of microbial survival tactics is cunning, the innovation of novel therapies must prevail to curtail the growing burden of bacterial pneumonia in both the community and hospital.

References

1. McDermott KW (IBM Watson Health), Roemer M (AHRQ). Most frequent principal diagnoses for inpatient stays in U.S. hospitals, 2018. HCUP Statistical Brief #277. July 2021. Agency for Healthcare Research and Quality, Rockville, MD. https://www.hcup-us.ahrq.gov/reports/statbriefs/sb277-TopReasons-Hospital-Stays-2018.pdf.
2. Cairns C, Kang K, Santo L. National Hospital Ambulatory Medical Care Survey: 2018 emergency department summary tables. Available from: https://www.cdc.gov/nchs/data/nhamcs/web_tables/2018_ed_web_tables-508.pdf.
3. Centers for Disease Control and Prevention, National Center for Health Statistics. Underlying cause of death 1999–2020 on CDC WONDER online database, released in 2021. Data are from the Multiple Cause of Death Files, 1999–2020, as compiled from data provided by the 57 vital statistics jurisdictions through the Vital Statistics Cooperative Program. Accessed at http://wonder.cdc.gov/ucd-icd10.html on Mar 4, 2022.
4. Jain S, Self WH, Wunderink RG, et al. Community-acquired pneumonia requiring hospitalization among U.S. adults. N Engl J Med. 2015;373(5):415–27. https://doi.org/10.1056/NEJMoa1500245.
5. Sattar SBA, Sharma S. Bacterial pneumonia. [Updated 2021 Dec 28]. In: StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK513321/.
6. Chalmers JD, Singanayagam A, Akram AR, et al. Severity assessment tools for predicting mortality in hospitalised patients with community-acquired pneumonia. Systematic review and meta-analysis. Thorax. 2010;65(10):878–83. https://doi.org/10.1136/thx.2009.133280.

7. Fine MJ, Auble TE, Yealy DM, et al. A prediction rule to identify low-risk patients with community-acquired pneumonia. N Engl J Med. 1997;336(4):243–50. https://doi.org/10.1056/NEJM199701233360402.
8. Grief SN, Loza JK. Guidelines for the evaluation and treatment of pneumonia. Prim Care. 2018;45(3):485–503. https://doi.org/10.1016/j.pop.2018.04.001.
9. Griffin MR, Zhu Y, Moore MR, Whitney CG, Grijalva CG. U.S. hospitalizations for pneumonia after a decade of pneumococcal vaccination. N Engl J Med. 2013;369(2):155–63. https://doi.org/10.1056/NEJMoa1209165.
10. Metlay JP, Waterer GW, Long AC, Anzueto A, Brozek J, Crothers K, et al. Diagnosis and treatment of adults with community-acquired pneumonia. An official clinical practice guideline of the American Thoracic Society and Infectious Diseases Society of America. Am J Respir Crit Care Med. 2019;200:e45–67.
11. Mandell LA, Wunderink RG, Anzueto A, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis. 2007;44 Suppl 2(Suppl 2):S27–72. https://doi.org/10.1086/511159.
12. Raz-Pasteur A, Shasha D, Paul M. Fluoroquinolones or macrolides alone versus combined with β-lactams for adults with community-acquired pneumonia: systematic review and meta-analysis. Int J Antimicrob Agents. 2015;46:242–8.
13. Carugati M, Aliberti S, Sotgiu G, et al. Bacterial etiology of community-acquired pneumonia in immunocompetent hospitalized patients and appropriateness of empirical treatment recommendations: an international point-prevalence study [published correction appears in Eur J Clin Microbiol Infect Dis. 2021 Sep;40(9):2051]. Eur J Clin Microbiol Infect Dis. 2020;39(8):1513–25. https://doi.org/10.1007/s10096-020-03870-3.
14. Cillóniz C, Ewig S, Polverino E, et al. Microbial aetiology of community-acquired pneumonia and its relation to severity. Thorax. 2011;66:340–6.
15. Waterer G, Metersky ML. Corticosteroids for community-acquired pneumonia: overstated benefits and understated risks. Chest. 2019;156(6):1049–53. https://doi.org/10.1016/j.chest.2019.06.017.
16. Restrepo MI, Babu BL, Reyes LF, et al. Burden and risk factors for Pseudomonas aeruginosa community-acquired pneumonia: a multinational point prevalence study of hospitalised patients. Eur Respir J. 2018;52(2):1701190. Published 2018 Aug 9. https://doi.org/10.1183/13993003.01190-2017.
17. Metersky ML, Frei CR, Mortensen EM. Predictors of Pseudomonas and methicillin-resistant Staphylococcus aureus in hospitalized patients with healthcare-associated pneumonia. Respirology. 2016;21(1):157–63. https://doi.org/10.1111/resp.12651.
18. Lewis PO. Risk factor evaluation for methicillin-resistant Staphylococcus aureus and Pseudomonas aeruginosa in community-acquired pneumonia. Ann Pharmacother. 2021;55(1):36–43. https://doi.org/10.1177/1060028020935106.
19. Jenkins TC, Sakai J, Knepper BC, et al. Risk factors for drug-resistant Streptococcus pneumoniae and antibiotic prescribing practices in outpatient community-acquired pneumonia. Acad Emerg Med. 2012;19(6):703–6. https://doi.org/10.1111/j.1553-2712.2012.01365.x.
20. Horcajada JP, Salata RA, Álvarez-Sala R, Nitu FM, Lawrence L, Quintas M, Cheng C-Y, Cammarata S. A phase 3 study to compare delafloxacin with moxifloxacin for the treatment of adults with community-acquired bacterial pneumonia (DEFINE-CABP). Open Forum Infect Dis. 2020;7(1):ofz514. https://doi.org/10.1093/ofid/ofz514.
21. Van Bambeke F. Delafloxacin, a non-zwitterionic fluoroquinolone in Phase III of clinical development: evaluation of its pharmacology, pharmacokinetics, pharmacodynamics and clinical efficacy. Future Microbiol. 2015;10(7):1111–23. https://doi.org/10.2217/fmb.15.39.
22. Jorgensen SCJ, Mercuro NJ, Davis SL, Rybak MJ. Delafloxacin: place in therapy and review of microbiologic, clinical and pharmacologic properties. Infect Dis Ther. 2018;7(2):197–217. https://doi.org/10.1007/s40121-018-0198-x.

23. Lodise T, Corey R, Hooper D, Cammarata S. Safety of delafloxacin: focus on adverse events of special interest. Open Forum Infect Dis. 2018;5(10):ofy220. https://doi.org/10.1093/ofid/ofy220.
24. Veve MP, Wagner JL. Lefamulin: review of a promising novel pleuromutilin antibiotic. Pharmacotherapy. 2018;38(9):935–46. https://doi.org/10.1002/phar.2166.
25. File TM Jr, Alexander E, Goldberg L, et al. Lefamulin efficacy and safety in a pooled phase 3 clinical trial population with community-acquired bacterial pneumonia and common clinical comorbidities. BMC Pulm Med. 2021;21(1):154. Published 2021 May 8. https://doi.org/10.1186/s12890-021-01472-z.
26. World Health Organization. Global tuberculosis report 2021. 14 Oct 2021. ISBN: 9789240037021.
27. Dye C, Williams BG. Tuberculosis decline in populations affected by HIV: a retrospective study of 12 countries in the WHO African region. Bull World Health Organ. 2019;97(6):405–14. https://doi.org/10.2471/BLT.18.228577.
28. Panel on Guidelines for the Prevention and Treatment of Opportunistic Infections in Adults and Adolescents with HIV. Guidelines for the Prevention and Treatment of Opportunistic Infections in HIV-infected Adults and Adolescents: recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. Available at https://clinicalinfo.hiv.gov/en/guidelines/adult-and-adolescent-opportunistic-infection. Accessed 25 Apr 2022. Mycobacterium tuberculosis infection and disease, updated 17 Feb 2022.
29. Meng Q, Sayin I, Canaday DH, Mayanja-Kizza H, Baseke J, Toossi Z. Immune activation at sites of HIV/TB co-infection contributes to the pathogenesis of HIV-1 disease. PLoS One. 2016;11(11):e0166954. Published 2016 Nov 21. https://doi.org/10.1371/journal.pone.0166954.
30. Young DB, Gideon HP, Wilkinson RJ. Eliminating latent tuberculosis. Trends Microbiol. 2009;17(5):183–8. https://doi.org/10.1016/j.tim.2009.02.005.
31. Chai Q, Zhang Y, Liu CH. Mycobacterium tuberculosis: an adaptable pathogen associated with multiple human diseases. Front Cell Infect Microbiol. 2018:8.
32. Getahun H, Matteelli A, Chaisson RE, Raviglione M. Latent Mycobacterium tuberculosis infection. N Engl J Med. 2015;372:2127–35.
33. Houben RM, Dodd PJ. The global burden of latent tuberculosis infection: a re-estimation using mathematical modelling. PLoS Med. 2016;13(10):e1002152. Published 2016 Oct 25. https://doi.org/10.1371/journal.pmed.1002152.
34. Comstock GW, Livesay VT, Woolpert SF. The prognosis of a positive tuberculin reaction in childhood and adolescence. Am J Epidemiol. 1974;99(2):131–8. https://doi.org/10.1093/oxfordjournals.aje.a121593.
35. Kiazyk S, Ball TB. Latent tuberculosis infection: an overview. Can Commun Dis Rep. 2017;43(3–4):62–6. Published 2017 Mar 2. https://doi.org/10.14745/ccdr.v43i34a01.
36. Getahun H, Matteelli A, Abubakar I, Aziz MA, Baddeley A, Barreira D, et al. Management of latent Mycobacterium tuberculosis infection: WHO guidelines for low tuberculosis burden countries. Eur Respir J. 2015;46:1563.
37. Stagg HR, Zenner D, Harris RJ, Muñoz L, Lipman MC, Abubakar I. Treatment of latent tuberculosis infection. Ann Intern Med. 2014;161:419–28.
38. Horsburgh CR, Goldberg S, Bethel J, Chen S, Colson PW, Hirsch-Moverman Y, et al. Latent TB infection treatment acceptance and completion in the United States and Canada. Chest. 2010;137:401–9.
39. Sterling TR, Villarino ME, Borisov AS, Shang N, Gordin F, Bliven-Sizemore E, et al. Three months of Rifapentine and isoniazid for latent tuberculosis infection. N Engl J Med. 2011;365:2155–66.
40. Sterling TR, Njie G, Zenner D, et al. Guidelines for the treatment of latent tuberculosis infection: recommendations from the National Tuberculosis Controllers Association and

CDC, 2020. MMWR Recomm Rep. 2020;69(RR-1):1–11. https://doi.org/10.15585/mmwr.rr6901a1external. icon.
41. Tseng SY, Huang YS, Chang TE, Perng CL, Huang YH. Hepatotoxicity, efficacy and completion rate between 3 months of isoniazid plus rifapentine and 9 months of isoniazid in treating latent tuberculosis infection: a systematic review and meta-analysis. J Chin Med Assoc. 2021;84(11):993–1000. https://doi.org/10.1097/JCMA.0000000000000605.
42. WHO Consolidated Guidelines on Tuberculosis. Module 4: treatment – drug-resistant tuberculosis treatment; 2021. ISBN: 978 92 4 000704 8.
43. Jilani TN, Avula A, Zafar Gondal A, et al. Active tuberculosis. [Updated 2021 Oct 19]. In: StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK513246/.
44. Nahid P, Dorman SE, Alipanah N, Barry PM, Brozek JL, Cattamanchi A, et al. 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. 2016;63:e147–e95.
45. Dorman SE, Nahid P, Kurbatova EV, Phillips PPJ, Bryant K, Dooley KE, et al. Four-month Rifapentine regimens with or without moxifloxacin for tuberculosis. N Engl J Med. 2021;384:1705–18.
46. Nahid P, Mase SR, Migliori GB, et al. Treatment of drug-resistant tuberculosis. An official ATS/CDC/ERS/IDSA Clinical Practice Guideline [published correction appears in Am J Respir Crit Care Med. 2020 Feb 15;201(4):500–501]. Am J Respir Crit Care Med. 2019;200(10):e93–e142. https://doi.org/10.1164/rccm.201909-1874ST.
47. Mirzayev F, Viney K, Linh NN, Gonzalez-Angulo L, Gegia M, Jaramillo E, et al. World Health Organization recommendations on the treatment of drug-resistant tuberculosis, 2020 update. Eur Respir J. 2021;57:2003300.
48. Giuliano KK, Baker D, Quinn B. The epidemiology of nonventilator hospital-acquired pneumonia in the United States. Am J Infect Control. 2018;46(3):322–7. https://doi.org/10.1016/j.ajic.2017.09.005. Epub 2017 Oct 16. PMID: 29050905.
49. Bauer TT, Ferrer R, Angrill J, Schultze-Werninghaus G, Torres A. Ventilator-associated pneumonia: incidence, risk factors, and microbiology. Semin Respir Infect. 2000;15(4):272–9. https://doi.org/10.1053/srin.2000.20938. PMID: 11220409.
50. Wu D, Wu C, Zhang S, Zhong Y. Risk factors of ventilator-associated pneumonia in critically III patients. Front Pharmacol. 2019;10:482. Published 2019 May 9. https://doi.org/10.3389/fphar.2019.00482.
51. Martin-Loeches I, Rodriguez AH, Torres A. New guidelines for hospital-acquired pneumonia/ventilator-associated pneumonia: USA vs. Europe. Curr Opin Crit Care. 2018;24(5):347–52. https://doi.org/10.1097/MCC.0000000000000535. PMID: 30063491.
52. Zaragoza R, Vidal-Cortés P, Aguilar G, et al. Update of the treatment of nosocomial pneumonia in the ICU. Crit Care. 2020;24:383. https://doi.org/10.1186/s13054-020-03091-2.
53. Evans CR, Sharpe JP, Swanson JM, Wood GC, Fabian TC, Croce MA, Magnotti LJ. Keeping it simple: impact of a restrictive antibiotic policy for ventilator-associated pneumonia in trauma patients on incidence and sensitivities of causative pathogens. Surg Infect. 2018;19(7):672–8. https://doi.org/10.1089/sur.2018.087. Epub 2018 Sep 11. PMID: 30204541.
54. Chi SY, Kim TO, Park CW, et al. Bacterial pathogens of ventilator associated pneumonia in a tertiary referral hospital. Tuberc Respir Dis (Seoul). 2012;73(1):32–7. https://doi.org/10.4046/trd.2012.73.1.32.
55. Bonell A, Ryan Azarrafiy V, Huong TL, Le Viet T, Phu VD, Dat VQ, Wertheim H, van Doorn HR, Lewycka S, Nadjm B. A systematic review and meta-analysis of ventilator-associated pneumonia in adults in Asia: an analysis of National Income Level on Incidence and Etiology. Clin Infect Dis. 2019;68(3):511–8. https://doi.org/10.1093/cid/ciy543.
56. Kalil AC, Metersky ML, Klompas M, Muscedere J, Sweeney DA, Palmer LB, Napolitano LM, O'Grady NP, Bartlett JG, Carratalà J, El Solh AA, Ewig S, Fey PD, File TM Jr, Restrepo MI, Roberts JA, Waterer GW, Cruse P, Knight SL, Brozek JL. Management of adults with

hospital-acquired and ventilator-associated pneumonia: 2016 clinical practice guidelines by the Infectious Diseases Society of America and the American Thoracic Society. Clin Infect Dis. 2016;63(5):e61–e111. https://doi.org/10.1093/cid/ciw353. Epub 2016 Jul 14. Erratum in: Clin Infect Dis. 2017;64(9):1298. Erratum in: Clin Infect Dis. 2017;65(8):1435. Erratum in: Clin Infect Dis. 2017;65(12):2161. PMID: 27418577; PMCID: PMC4981759.

57. Torres A, Serra-Batlles J, Ros E, Piera C, Puig de la Bellacasa J, Cobos A, Lomeña F, Rodríguez-Roisin R. Pulmonary aspiration of gastric contents in patients receiving mechanical ventilation: the effect of body position. Ann Intern Med. 1992;116(7):540–3. https://doi.org/10.7326/0003-4819-116-7-540. PMID: 1543307.
58. Niël-Weise BS, Gastmeier P, Kola A, et al. An evidence-based recommendation on bed head elevation for mechanically ventilated patients. Crit Care. 2011;15(2):R111. https://doi.org/10.1186/cc10135.
59. Gupta A, Gupta A, Singh TK, Saxsena A. Role of oral care to prevent VAP in mechanically ventilated intensive care unit patients. Saudi J Anaesth. 2016;10(1):95–7. https://doi.org/10.4103/1658-354X.169484.
60. Zand F, Zahed L, Mansouri P, Dehghanrad F, Bahrani M, Ghorbani M. The effects of oral rinse with 0.2% and 2% chlorhexidine on oropharyngeal colonization and ventilator associated pneumonia in adults' intensive care units. J Crit Care. 2017;40:318–22. https://doi.org/10.1016/j.jcrc.2017.02.029. Epub 2017 Mar 1. PMID: 28320561.
61. Mahmoodpoor A, Hamishehkar H, Asghari R, Abri R, Shadvar K, Sanaie S. Effect of a probiotic preparation on ventilator-associated pneumonia in critically ill patients admitted to the intensive care unit: a prospective double-blind randomized controlled trial. Nutr Clin Pract. 2019;34(1):156–62. https://doi.org/10.1002/ncp.10191. Epub 2018 Aug 8. PMID: 30088841.
62. Lescure FX, Bouadma L, Nguyen D, Parisey M, Wicky PH, Behillil S, Gaymard A, Bouscambert-Duchamp M, Donati F, Le Hingrat Q, Enouf V, Houhou-Fidouh N, Valette M, Mailles A, Lucet JC, Mentre F, Duval X, Descamps D, Malvy D, Timsit JF, Lina B, van-der-Werf S, Yazdanpanah Y. Clinical and virological data of the first cases of COVID-19 in Europe: a case series. Lancet Infect Dis. 2020;20(6):697–706. https://doi.org/10.1016/S1473-3099(20)30200-0. Epub 2020 Mar 27. Erratum in: Lancet Infect Dis. 2020: Erratum in: Lancet Infect Dis. 2020;20(6):e116. PMID: 32224310; PMCID: PMC7156120.
63. Dong X, Cao YY, Lu XX, Zhang JJ, Du H, Yan YQ, Akdis CA, Gao YD. Eleven faces of coronavirus disease 2019. Allergy. 2020;75(7):1699–709. https://doi.org/10.1111/all.14289. Epub 2020 Apr 6. PMID: 32196678; PMCID: PMC7228397
64. Espinal P, Martí S, Vila J. Effect of biofilm formation on the survival of Acinetobacter baumannii on dry surfaces. J Hosp Infect. 2012;80(1):56–60. https://doi.org/10.1016/j.jhin.2011.08.013. Epub 2011 Oct 4. PMID: 21975219.
65. Isler B, Doi Y, Bonomo RA, Paterson DL. New treatment options against carbapenem-resistant *Acinetobacter baumannii* infections. Antimicrob Agents Chemother. 2018;63(1):e01110–8. https://doi.org/10.1128/AAC.01110-18. PMID: 30323035; PMCID: PMC6325237
66. Dobias J, Dénervaud-Tendon V, Poirel L, Nordmann P. Activity of the novel siderophore cephalosporin cefiderocol against multidrug-resistant Gram-negative pathogens. Eur J Clin Microbiol Infect Dis. 2017;36(12):2319–27. https://doi.org/10.1007/s10096-017-3063-z. Epub 2017 Jul 26. PMID: 28748397.
67. Kim YK, Lee JH, Lee HK, et al. Efficacy of nebulized colistin-based therapy without concurrent intravenous colistin for ventilator-associated pneumonia caused by carbapenem-resistant *Acinetobacter baumannii*. J Thorac Dis. 2017;9(3):555–67. https://doi.org/10.21037/jtd.2017.02.61.
68. Torres A, Zhong N, Pachl J, Timsit JF, Kollef M, Chen Z, Song J, Taylor D, Laud PJ, Stone GG, Chow JW. Ceftazidime-avibactam versus meropenem in nosocomial pneumonia, including ventilator-associated pneumonia (REPROVE): a randomised, double-blind, phase 3 non-inferiority trial. Lancet Infect Dis. 2018;18(3):285–95. https://doi.org/10.1016/S1473-3099(17)30747-8. Epub 2017 Dec 16. PMID: 29254862.

69. https://www.fda.gov/drugs/drug-safety-and-availability/fda-drug-safety-communication-fda-warns-increased-risk-death-iv-antibacterial-tygacil-tigecycline.
70. Pulido MR, Moreno-Martínez P, González-Galán V, Fernández Cuenca F, Pascual Á, Garnacho-Montero J, Antonelli M, Dimopoulos G, Lepe JA, MJ MC, Cisneros JM, MagicBullet Working Group. Application of BioFire FilmArray Blood Culture Identification panel for rapid identification of the causative agents of ventilator-associated pneumonia. Clin Microbiol Infect. 2018;24(11):1213.e1–4. https://doi.org/10.1016/j.cmi.2018.06.001. Epub 2018 Jun 12. PMID: 29906599.
71. Feng DY, Zhou YQ, Zou XL, et al. Differences in microbial etiology between hospital-acquired pneumonia and ventilator-associated pneumonia: a single-center retrospective study in Guang Zhou. Infect Drug Resist. 2019;12:993–1000. Published 2019 Apr 29. https://doi.org/10.2147/IDR.S204671.
72. Liu YN, Cao B, Wang H, Chen LA, She DY, Zhao TM, Liang ZX, Sun TY, Li YM, Tong ZH, Wang Z, He B, Yang W, Qu JM, Li XY, Chen RC, Ye F, Kang J, Li ER, Chen P, Zheng Y, Ma ZS, Ren J, Shi Y, Sun WK, Feng YL, Fan H, Jiang SJ, Zhang S, Xiong SD, Zuo P, Wang ZW. [Adult hospital acquired pneumonia: a multicenter study on microbiology and clinical characteristics of patients from 9 Chinese cities]. Zhonghua Jie He He Hu Xi Za Zhi. 2012;35(10):739–46. Chinese. PMID: 23289990.
73. Suaya JA, Fletcher MA, Georgalis L, Arguedas AG, McLaughlin JM, Ferreira G, Theilacker C, Gessner BD, Verstraeten T. Identification of Streptococcus pneumoniae in hospital-acquired pneumonia in adults. J Hosp Infect. 2021;108:146–57. https://doi.org/10.1016/j.jhin.2020.09.036. Epub 2020 Nov 8. PMID: 33176175.
74. Bhadade R, Harde M, deSouza R, More A, Bharmal R. Emerging trends of nosocomial pneumonia in intensive care unit of a tertiary care public teaching hospital in Western India. Ann Afr Med. 2017;16(3):107–13. https://doi.org/10.4103/aam.aam_7_17. PMID: 28671150; PMCID: PMC5579893
75. Lewis SS, Walker VJ, Lee MS, Chen L, Moehring RW, Cox CE, Sexton DJ, Anderson DJ. Epidemiology of methicillin-resistant Staphylococcus aureus pneumonia in community hospitals. Infect Control Hosp Epidemiol. 2014;35(12):1452–7. https://doi.org/10.1086/678594. Epub 2014 Oct 24. PMID: 25419766; PMCID: PMC4408547.
76. Shebl E, Gulick PG. Nosocomial pneumonia. [Updated 2021 Jul 21]. In: StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK535441/.
77. Rioux C, Armand-Lefevre L, Guerinot W, Andremont A, Lucet JC. Acquisition of methicillin-resistant Staphylococcus aureus in the acute care setting: incidence and risk factors. Infect Control Hosp Epidemiol. 2007;28(6):733–6. https://doi.org/10.1086/516664. Epub 2007 Apr 19. PMID: 17520551.
78. Loke HY, Kyaw WM, Chen MIC, Lim JW, Ang B, Chow A. Length of stay and odds of MRSA acquisition: a dose-response relationship? Epidemiol Infect. 2019;147:e223. https://doi.org/10.1017/S0950268819001110.
79. Herzig SJ, Howell MD, Ngo LH, Marcantonio ER. Acid-suppressive medication use and the risk for hospital-acquired pneumonia. JAMA. 2009;301(20):2120–8. https://doi.org/10.1001/jama.2009.722. PMID: 19470989.
80. Pássaro L, Harbarth S, Landelle C. Prevention of hospital-acquired pneumonia in non-ventilated adult patients: a narrative review. Antimicrob Resist Infect Control. 2016;5:43. Published 2016 Nov 14. https://doi.org/10.1186/s13756-016-0150-3.
81. Kros CJ, Steyger PS. Aminoglycoside- and cisplatin-induced ototoxicity: mechanisms and otoprotective strategies. Cold Spring Harb Perspect Med. 2019;9(11):a033548. https://doi.org/10.1101/cshperspect.a033548. PMID: 30559254; PMCID: PMC6579718.
82. Filippone EJ, Kraft WK, Farber JL. The nephrotoxicity of vancomycin. Clin Pharmacol Ther. 2017;102(3):459–69. https://doi.org/10.1002/cpt.726. Epub 2017 Jun 5. PMID: 28474732; PMCID: PMC5579760

83. Tsuji Y, Holford NHG, Kasai H, Ogami C, Heo YA, Higashi Y, Mizoguchi A, To H, Yamamoto Y. Population pharmacokinetics and pharmacodynamics of linezolid-induced thrombocytopenia in hospitalized patients. Br J Clin Pharmacol. 2017;83(8):1758–72. https://doi.org/10.1111/bcp.13262. Epub 2017 Mar 31. PMID: 28186644; PMCID: PMC5510085
84. Cannon JP, Lee TA, Clark NM, Setlak P, Grim SA. The risk of seizures among the carbapenems: a meta-analysis. J Antimicrob Chemother. 2014;69(8):2043–55. https://doi.org/10.1093/jac/dku111. Epub 2014 Apr 16. PMID: 24744302.
85. Teysseyre L, Ferdynus C, Miltgen G, Lair T, Aujoulat T, Lugagne N, Allou N, Allyn J. Derivation and validation of a simple score to predict the presence of bacteria requiring carbapenem treatment in ICU-acquired bloodstream infection and pneumonia: CarbaSCORE. Antimicrob Resist Infect Control. 2019;8:78. https://doi.org/10.1186/s13756-019-0529-z. PMID: 31139361; PMCID: PMC6528287.
86. Kollef MH, Nováček M, Kivistik Ü, Réa-Neto Á, Shime N, Martin-Loeches I, Timsit JF, Wunderink RG, Bruno CJ, Huntington JA, Lin G, Yu B, Butterton JR, Rhee EG. Ceftolozane-tazobactam versus meropenem for treatment of nosocomial pneumonia (ASPECT-NP): a randomised, controlled, double-blind, phase 3, non-inferiority trial. Lancet Infect Dis. 2019;19(12):1299–311. https://doi.org/10.1016/S1473-3099(19)30403-7. Epub 2019 Sep 25. PMID: 31563344.
87. Mandell LA, Niederman MS. Aspiration pneumonia. N Engl J Med. 2019;380(7):651–63. https://doi.org/10.1056/NEJMra1714562. PMID: 30763196.
88. El-Solh AA, Pietrantoni C, Bhat A, Aquilina AT, Okada M, Grover V, Gifford N. Microbiology of severe aspiration pneumonia in institutionalized elderly. Am J Respir Crit Care Med. 2003;167(12):1650–4. https://doi.org/10.1164/rccm.200212-1543OC. Epub 2003 Apr 10. PMID: 12689848.
89. DiBardino DM, Wunderink RG. Aspiration pneumonia: a review of modern trends. J Crit Care. 2015;30(1):40–8. https://doi.org/10.1016/j.jcrc.2014.07.011. Epub 2014 Jul 22. PMID: 25129577.
90. Takayanagi N, Kagiyama N, Ishiguro T, Tokunaga D, Sugita Y. Etiology and outcome of community-acquired lung abscess. Respiration. 2010;80(2):98–105. https://doi.org/10.1159/000312404. Epub 2010 Apr 10. PMID: 20389050.
91. Bowerman TJ, Zhang J, Waite LM. Antibacterial treatment of aspiration pneumonia in older people: a systematic review. Clin Interv Aging. 2018;13:2201–13. Published 2018 Oct 30. 10.2147/CIA.S183344
92. Petersen MW, Perner A, Bahador M, Sjövall F, Møller MH. Empirical metronidazole for patients with severe bacterial infection: protocol for a systematic review. Acta Anaesthesiol Scand. 2018;62(5):724–30. https://doi.org/10.1111/aas.13101. Epub 2018 Mar 8. PMID: 29520759.
93. Lipshaw MJ, Eckerle M, Florin TA, Crotty EJ, Lipscomb J, Jacobs J, Rattan MS, Ruddy RM, Shah SS, Ambroggio L. Antibiotic use and outcomes in children in the emergency department with suspected pneumonia. Pediatrics. 2020;145(4):e20193138. https://doi.org/10.1542/peds.2019-3138. Epub 2020 Mar 16. PMID: 32179662; PMCID: PMC7111492.
94. Bradley JS, Byington CL, Shah SS, Alverson B, Carter ER, Harrison C, Kaplan SL, Mace SE, McCracken GH Jr, Moore MR, St Peter SD, Stockwell JA, Swanson JT. The Management of Community-Acquired Pneumonia in Infants and Children Older Than 3 Months of Age: Clinical Practice Guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. Clin Infect Dis. 2011;53(7):e25–76. https://doi.org/10.1093/cid/cir531.
95. Bodier-Montagutelli E, Morello E, L'Hostis G, Guillon A, Dalloneau E, Respaud R, et al. Inhaled phage therapy: a promising and challenging approach to treat bacterial respiratory infections. Expert Opin Drug Deliv. 2017;14(8):959–72. https://doi.org/10.1080/17425247.2017.1252329.

96. Wang X, Xie Z, Zhao J, Zhu Z, Yang C, Liu Y. Prospects of inhaled phage therapy for combatting pulmonary infections. Front Cell Infect Microbiol. 2021;11:758392. https://doi.org/10.3389/fcimb.2021.758392.
97. Yang X, Haque A, Matsuzaki S, Matsumoto T, Nakamura S. The efficacy of phage therapy in a murine model of Pseudomonas aeruginosa pneumonia and sepsis. Front Microbiol. 2021;12:682255. https://doi.org/10.3389/fmicb.2021.682255.
98. Krylov V, Shaburova O, Pleteneva E, Bourkaltseva M, Krylov S, Kaplan A, et al. Modular approach to select bacteriophages targeting Pseudomonas aeruginosa for their application to children suffering with cystic fibrosis. Front Microbiol. 2016;7:1631. https://doi.org/10.3389/fmicb.2016.01631.
99. Parkins MD, Somayaji R, Waters VJ. Epidemiology, biology, and impact of clonal Pseudomonas aeruginosa infections in cystic fibrosis. Clin Microbiol Rev. 2018;31(4):e00019–8. https://doi.org/10.1128/CMR.00019-18.
100. Singla S, Harjai K, Katare OP, Chhibber S. Bacteriophage-loaded nanostructured lipid carrier: improved pharmacokinetics mediates effective resolution of Klebsiella pneumoniae-induced lobar pneumonia. J Infect Dis. 2015;212(2):325–34. https://doi.org/10.1093/infdis/jiv029.
101. Semler DD, Goudie AD, Finlay WH, Dennis JJ. Aerosol phage therapy efficacy in Burkholderia cepacia complex respiratory infections. Antimicrob Agents Chemother. 2014;58(7):4005–13. https://doi.org/10.1128/aac.02388-13.
102. Caflisch KM, Suh GA, Patel R. Biological challenges of phage therapy and proposed solutions: a literature review. Expert Rev Anti-Infect Ther. 2019;17(12):1011–41. https://doi.org/10.1080/14787210.2019.1694905.
103. Kwiatek M, Parasion S, Nakonieczna A. Therapeutic bacteriophages as a rescue treatment for drug-resistant infections – an in vivo studies overview. J Appl Microbiol. 2020;128(4):985–1002. https://doi.org/10.1111/jam.14535.
104. McVey MJ, Steinberg BE, Goldenberg NM. Inflammasome activation in acute lung injury. Am J Phys Lung Cell Mol Phys. 2021;320(2):L165–78. https://doi.org/10.1152/ajplung.00303.2020.
105. Surabhi S, Cuypers F, Hammerschmidt S, Siemens N. The role of NLRP3 inflammasome in pneumococcal infections. Front Immunol. 2020;11:614801. https://doi.org/10.3389/fimmu.2020.614801.
106. Sha W, Mitoma H, Hanabuchi S, Bao M, Weng L, Sugimoto N, et al. Human NLRP3 inflammasome senses multiple types of bacterial RNAs. Proc Natl Acad Sci U S A. 2014;111(45):16059–64. https://doi.org/10.1073/pnas.1412487111.
107. Yap JKY, Moriyama M, Iwasaki A. Inflammasomes and pyroptosis as therapeutic targets for COVID-19. J Immunol. 2020;205(2):307–12. https://doi.org/10.4049/jimmunol.2000513.
108. Wu S, Huang J. Resveratrol alleviates Staphylococcus aureus pneumonia by inhibition of the NLRP3 inflammasome. Exp Ther Med. 2017;14(6):6099–104. https://doi.org/10.3892/etm.2017.5337.
109. Zeng J, Xie X, Feng X-L, Xu L, Han J-B, Yu D, et al. Specific inhibition of the NLRP3 inflammasome suppresses immune overactivation and alleviates COVID-19 like pathology in mice. EBioMedicine. 2022;75:103803. https://doi.org/10.1016/j.ebiom.2021.103803.
110. Booth C, Inusa B, Obaro SK. Infection in sickle cell disease: a review. Int J Infect Dis. 2010;14(1):e2–e12. https://doi.org/10.1016/j.ijid.2009.03.010.
111. Battersby AJ, Knox-Macaulay HHM, Carrol ED. Susceptibility to invasive bacterial infections in children with sickle cell disease. Pediatr Blood Cancer. 2010;55(3):401–6. https://doi.org/10.1002/pbc.22461.
112. Poehling KA, Light LS, Rhodes M, Snively BM, Halasa NB, Mitchel E, et al. Sickle cell trait, hemoglobin C trait, and invasive pneumococcal disease. Epidemiology. 2010;21(3):340–6. https://doi.org/10.1097/EDE.0b013e3181d61af8.
113. Reeves SL, Jary HK, Gondhi JP, Kleyn M, Dombkowski KJ. Health outcomes and services in children with sickle cell trait, sickle cell anemia, and normal hemoglobin. Blood Adv. 2019;3(10):1574–80. https://doi.org/10.1182/bloodadvances.2018028043.

114. Ramakrishnan M, Moïsi JC, Klugman KP, Iglesias JMF, Grant LR, Mpoudi-Etame M, Levine OS. Increased risk of invasive bacterial infections in African people with sickle-cell disease: a systematic review and meta-analysis. Lancet Infect Dis. 2010;10(5):329–37. https://doi.org/10.1016/S1473-3099(10)70055-4.
115. Martí-Carvajal AJ, Conterno LO. Antibiotics for treating community-acquired pneumonia in people with sickle cell disease. Cochrane Database Syst Rev. 2016;11(11):CD005598-CD005598. https://doi.org/10.1002/14651858.CD005598.pub4.
116. Aleem MS, Sexton R, Akella J. Pneumonia in an immunocompromised patient. [Updated 2021 Jul 31]. In: StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557843/.
117. Murali S, Marks A, Heeger A, Dako F, Febbo J. Pneumonia in the immunocompromised host. Semin Roentgenol. 2022;57:90–104.
118. Ramirez JA, Musher DM, Evans SE, Dela Cruz C, Crothers KA, Hage CA, et al. Treatment of community-acquired pneumonia in immunocompromised adults: a consensus statement regarding initial strategies. Chest. 2020;158:1896–911.
119. Elborn JS, Flume PA, Devanter DRV, Procaccianti C. Management of chronic Pseudomonas aeruginosa infection with inhaled levofloxacin in people with cystic fibrosis. Future Microbiol. 2021;16:1087–104.
120. Castellani C, Duff AJA, Bell SC, Heijerman HGM, Munck A, Ratjen F, et al. ECFS best practice guidelines: the 2018 revision. J Cyst Fibros. 2018;17:153–78.
121. Flume PA, Peter J, Mogayzel J, Robinson KA, Goss CH, Rosenblatt RL, Kuhn RJ, et al. Cystic fibrosis pulmonary guidelines. Am J Respir Crit Care Med. 2009;180:802–8.
122. Mayer-Hamblett N, Retsch-Bogart G, Kloster M, Accurso F, Rosenfeld M, Albers G, et al. Azithromycin for early Pseudomonas infection in cystic fibrosis: the OPTIMIZE randomized trial. Am J Respir Crit Care Med. 2018;198:1177–87.
123. Horcajada JP, Montero M, Oliver A, Sorlí L, Luque S, Gómez-Zorrilla S, et al. Epidemiology and treatment of multidrug-resistant and extensively drug-resistant Pseudomonas aeruginosa infections. Clin Microbiol Rev. 2019;32:e00031–19.
124. Peleg AY, Hooper DC. Hospital-acquired infections due to gram-negative bacteria. N Engl J Med. 2010;362(19):1804–13. https://doi.org/10.1056/NEJMra0904124.
125. Cillóniz C, Dominedò C, Torres A. Multidrug resistant gram-negative bacteria in community-acquired pneumonia. Crit Care. 2019;23:79. https://doi.org/10.1186/s13054-019-2371-3.
126. Lung M, Codina G. Molecular diagnosis in HAP/VAP. Curr Opin Crit Care. 2012;18(5):487–94. https://doi.org/10.1097/MCC.0b013e3283577d37.

Lipid-Based Drug Delivery for Pneumonia Treatment

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Abstract Respiratory tract infections are a significant problem that severely impacts public health due to high morbidity and mortality rates. The causes of respiratory tract infections are bacteria, fungi, and viruses, thus requiring antibiotics, antifungal, and antivirus agents. In addition to treating the causes of bacterial, fungal, or viral pneumonia, alternative therapies are required to address the symptoms caused by infection. The use of the pulmonary route is increasingly common because it is possible to target drugs specifically on the respiratory tract locally and systemically. Undesirable systemic effects can be minimized because the drug acts with maximum pulmonary specificity and rapid onset of action. However, the drug's solubility and permeability highly determine its capability to reach target sites and produce therapeutic efficacy. Therefore, an innovative drug delivery system using a non-invasive and practical approach is required to enhance therapeutic efficacy and drug safety. Considering the pathophysiology of pneumonia, the physicochemical properties of delivery carriers significantly affect drug deposition. The use of lipids in liposomes, microspheres, and solid-lipid nanoparticles successfully improved drug solubility and enhanced its penetration for achieving high drug accumulation in lung tissue, providing future use for pneumonia treatment.

Keywords Public health · Pneumonia · Pulmonary route · Lipids · Liposomes · Microspheres · Solid-lipid nanoparticles

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1 Introduction

Respiratory tract infections are a significant problem that severely impacts public health due to high morbidity and mortality rates. According to the World Health Organization (WHO), respiratory tract infections rank highest in diseases causing death or disability. The Global Burden of Disease (GBD) study estimates that 2.74 million deaths have resulted from lower respiratory tract infections [1], which represent a leading cause of morbidity and mortality worldwide, particularly in the under-5 s and adults aged 70 and over. This infection affects the lower respiratory tract of the larynx, including the trachea and alveoli, causing acute bronchitis, bronchiolitis, pneumonia, tracheitis, and influenza. In general, patients with lower respiratory tract infections will present symptoms of pneumonia or the presence of certain infections, which can lead to a high risk of long healing and poorer outcomes.

The leading causes of respiratory tract infections are bacteria and viruses. The latter reportedly lead to the majority of lower respiratory tract infections, including bronchitis, bronchiolitis, and pneumonia. Viruses can infect and replicate in the epithelium, causing damage to the proximal airways, the alveoli, and parenchyma. Viral infections can present several clinical manifestations, one of them being pneumonia, an inflammation of the pulmonary parenchyma. In developing countries, viruses cause 40–90% of respiratory tract infections in children. Viruses that most commonly affect this group include respiratory syncytial virus (RSV), influenza A and B viruses (IAV and IBV), parainfluenza viruses (PIVs), adenoviruses (AdV), human rhinoviruses (hRV), and human enteroviruses (hEV) [2].

In addition to viruses, bacteria, fungi, and parasites are also known to cause respiratory tract infections. Bacterial infection due to *Streptococcus pneumoniae* is most commonly found in cases of pneumonia, while atypical pneumonia is an infection caused by *Mycoplasma pneumoniae*, *Chlamydia* spp., *Legionella*, and *Coxiella burnetti* [3]. The ability of bacteria to infect the respiratory tract is influenced by the nature of the respiratory tract epithelium. The delayed release of bacteria from the respiratory tract is caused by a viral infection, smoking, or the characteristics of the bacteria themselves, which can inhibit the clearance of respiratory mucous membranes. When the process is inhibited, bacteria can easily attach to the surface of epithelial cells, subsequently forming and increasing bacterial colonization in the respiratory tract. Meanwhile, fungi have been reported as causing respiratory tract infections, with *Aspergillus* sp. reputedly the most common cause. The fungus is frequently present in residential environments, both indoors and outdoors.

The use of the pulmonary route is increasingly common because it is possible to target drugs specifically on the respiratory tract, both locally and systemically. The benefits of administering inhalation therapy through oral suspension dosages during the treatment of pulmonary diseases have been recognized for many years. Moreover, inhalation has been recognized as a more effective form of inflammatory therapy than its conventional counterparts. Compared with oral or parenteral formulations, therapeutic doses of the drug are typically administered in a matter of minutes into the airways, where the active drug exerts its beneficial effect. Undesirable systemic

effects can be minimized because the drug acts with maximum pulmonary specificity together with a rapid onset of action. There are currently several forms of inhalation for local delivery to the lungs, including dry powder inhalers, metered-dose inhalers, and nebulizers.

Pulmonary drug delivery systems are not only intended to deliver drugs locally to the lungs but can also perform systemic delivery. However, the complexity of the respiratory system, comprising the mouth, nose, upper respiratory tract, and lungs, with their respective anatomies and histologies, renders this a considerable challenge. The flow and rate of respiration, neurological, and muscular responses are also affected. Furthermore, the asymmetrical branches of the lungs also provide horizontal heterogeneity between the pulmonary regions.

Innovative drug delivery using a non-invasive and practical approach is required to enhance therapeutic efficacy and drug safety. Several formulation strategies have been developed to maintain drug release in the lungs using nanocarriers as the most researched approaches. Nanocarriers allow controlled drug release at optimal therapeutic levels.

2 Pneumonia

Pneumonia is the severest inflammatory condition of the respiratory system and the most common infectious disease. The word “pneumonia,” meaning “lung disease,” is derived from the ancient Greek word *Pneumon* meaning “lungs.” Pneumonia is an acute respiratory infection that affects the alveoli and the distal bronchi of the lungs and is broadly divided into community-acquired pneumonia (CAP), hospital-acquired pneumonia (HAP), and ventilation-acquired pneumonia (VAP). The global incidence of CAP varies from 1.5 to 14 cases per 1000 people annually. At the same time, cases of HAP range from 6 to 8.6 per 1000 individuals. The highest incidence is reported in the ICU, where it occurs in 12–29% of patients, 90% being VAP cases with mortality between 33% and 50% [4].

Pneumonia occurs when the lower respiratory tract is invaded by infective microorganisms, bacteria, viruses, or fungi. In general, *Streptococcus pneumoniae* (pneumococcus) is a common pathogen causing CAP. It was reported that infections due to gram-negative bacteria were more common than those caused by gram-positive bacteria [3]. Certain viruses are also known to cause infections of the upper and lower respiratory tracts. The virus group includes influenza (IV), human respiratory syncytial virus (HRSV), human rhinovirus (HRV), human metapneumovirus (HMPV), parainfluenza, and severe acute respiratory syndrome coronavirus (SARS-CoV). Among the various pathogens that cause infections of the respiratory tract, fungi represent an extremely limited variety. *Aspergillus* is the leading cause of fungal infections of the lungs. Pathogens can reach the lungs through inhalation of droplets, aspiration of pathogens in the oropharynx, or dissemination through the bloodstream. Microscopic bacteria, less than 5 μm in size, can penetrate the lungs.

Clinical symptoms in patients with pneumonia can be influenced by the infective microorganisms and include fever, chills, malaise, cough, increased sputum production, tachypnea, decreased oxygen saturation, and shortness of breath. Coughing constitutes a defense mechanism of the lungs which can also be indicative of a severe illness. While it constitutes one of the common symptoms of pneumonia, coughing is not a feature of the form of the illness caused by viruses or mycoplasma. In bacterial pneumonia, the infection produces a fever which activates the body's defense system with alveolar macrophages eradicating the proliferating bacteria. However, when the invading bacteria exceed the capacity of the body to defend itself, they multiply. The alveolar macrophages then respond by releasing cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF), which can cause fever [5]. Pneumonia patients can also experience shortness of breath due to IL-8 and granulocyte colony-stimulating factor (G-CSF) inducing neutrophil maturation which, in turn, results in leukocytosis and purulent secretion. The released cytokines are responsible for leakage in the alveolar-capillary membrane causing shortness of breath [5], also referred to as hypoxemia, which indicates a lack of oxygen in the blood.

3 Pharmacological Therapy for Pneumonia

3.1 Antibiotics

Pneumonia is an infection of the respiratory tract requiring appropriate antibiotic therapy. In patients with CAP, macrolide antibiotics such as azithromycin or doxycycline can be administered. In this regard, fluoroquinolones and β-lactams can also be considered. Macrolides are generally used as the first-line treatment for CAP. These antibiotics impede bacterial growth by inhibiting binding to the bacterial 50S ribosomal subunit and interfering with protein synthesis [6]. These macrolides can reduce mortality due to severe sepsis through their immunomodulatory properties. Azithromycin is administered via the parenteral or oral route in single 500 mg doses for 7–10 days.

Azithromycin is effective against *Haemophilus influenzae* and Moraxella. Furthermore, this drug can be used to treat *Mycoplasma* and chlamydia, both common causes of pneumonia in adults. Azithromycin is widely distributed in the lower respiratory tract, and the drug levels can be maintained for a certain period [7], although one of the main problems associated with its use is that of extremely poor solubility. This drug belongs to the Biopharmaceutics Classification System (BCS) Class II in biological fluids, which demonstrate low bioavailability on oral administration.

Azithromycin induces certain side effects, including gastrointestinal disturbances, headaches, and dizziness. Hearing loss has also been reported by individuals using this drug, including those with respiratory disorders [8]. Therefore, an

azithromycin nanocarrier design, which can increase bioavailability and target lung tissue while reducing systemic side effects, needs to be developed.

In CAP patients who are not admitted to an ICU, fluoroquinolone monotherapy or the use of β-lactams with or without macrolides for empiric therapy may also be recommended. Fluoroquinolones are also drugs that can treat upper and lower respiratory tract infections. Drugs of this class include ciprofloxacin, moxifloxacin, gatifloxacin, and levofloxacin and trigger a broad spectrum of action on both gram-positive and gram-negative bacteria. Quinolones function by converting DNA gyrase and topoisomerase IV into toxic enzymes that break down bacterial chromosomes [9]. Drugs belonging to the fluoroquinolone therapeutic class promote increased oral absorption and systemic distribution. Thus, the clinical application of these compounds has been extended to the treatment of lower respiratory tract infections, skin and soft tissue infections, sexually transmitted diseases, and urinary tract infections.

The use of fluoroquinolones has side effects such as gastrointestinal disturbances, toxicity to the central nervous system, rashes, and QT prolongation, among others [10]. In addition, fluoroquinolone drugs such as norfloxacin and ciprofloxacin have low solubility and poor permeability. This characteristic can limit their bioavailability. Consequently, it is necessary to formulate drugs such as lipid nanocarriers that can enhance these properties in order to increase their therapeutic efficacies when targeting the lungs.

3.2 Antiviral Agents

In the case of viral pneumonia, it is necessary to use antiviral therapy, including drugs such as oseltamivir, zanamivir, or peramivir. A 2021 study conducted by Moreno et al. reported that the early use of oseltamivir in adults with influenza or viral pneumonia reduced fatalities in the ICU by as much as 33% [11].

Oseltamivir is an antiviral neuraminidase inhibitor administered orally in capsule form. This drug can competitively and selectively inhibit the influenza virus neuraminidase, an enzyme required for viral replication. Oseltamivir can impede the release of the influenza virus and prevent the spread of infection to new host cells [12]. Oseltamivir reportedly causes gastrointestinal disturbances such as nausea and vomiting, while other side effects include abdominal pain, diarrhea, headache, insomnia, and vertigo [13]. It belongs to a class of drugs with limited absorption characteristics. However, given the current lack of drug solubility data, oseltamivir cannot be classified in the BCS yet.

Lopinavir is used in conjunction with ritonavir to enhance its therapeutic effect on patients with viral pneumonia. Since lopinavir in isolation has a low bioavailability of 25%, combining it with ritonavir is necessary to increase its bioavailability. Lopinavir is an antiviral protease inhibitor previously used to treat HIV infections which now forms part of the treatment of COVID-19 patients. The use of lopinavir/ritonavir reportedly aids patients with pneumonia resulting from COVID-19

infection because the lopinavir mechanism binds to the SARS-CoV 3CL protease receptor [14].

In a previous study [15], the tissue distribution of lopinavir with isotope labels was observed in mice. The radioactivity level in the lungs was lower than that in their plasma, indicating that the pulmonary distribution of lopinavir was relatively low [15]. Therefore, it is necessary to formulate a drug delivery system capable of targeting lopinavir in lung tissue in order to increase the drug's effectiveness as an antiviral medication in the treatment of pneumonia.

3.3 Antifungal

In patients with fungal pneumonia, appropriate antifungal therapy is required. Conventional antifungals include amphotericin B, flucytosine, and several azole groups.

Polyenes are one antifungal group used to treat fungal pneumonia. Amphotericin B is one such drug prescribed to combat severe fungal infections. Polyenes bind to sterols in fungal cell membranes, forming transmembrane channels that lead to cell leakage and death. However, monitoring of serum creatinine parameters, blood urea nitrogen (BUN), and electrolytes (especially potassium and magnesium) present in the blood, in addition to liver function, is required due to the potential side effects of amphotericin B, which can cause renal toxicity [16].

Amphotericin B belongs to BCS class IV. The problems connected with its oral delivery include low water solubility, poor permeability, and poor drug absorption in the digestive tract. The formulation of drugs included in BCS class IV is necessary to increase their clinical application.

In addition to amphotericin B, fluconazole, an antifungal belonging to the azole group, can be used to treat pneumonia. Fluconazole is currently more effective in treating infection in lung tissues than other azole groups. Fluconazole works by inhibiting C-14 demethylase, which is required to synthesize ergosterol. C-14 demethylase is part of the fungal cytochrome P450 complex that causes subsequent disruption of fungal membranes. In human studies, it was reported that fluconazole was distributed rapidly in lung tissue, with concentrations twice as high as those in plasma [17]. Therefore, this drug is suitable for the treatment of lung infections.

Belonging to the BCS class 1 as a highly soluble and permeable drug, this drug dissolves rapidly, enabling its effective therapeutic applications.

3.4 Symptomatic Therapy: Anti-inflammation and Analgesic

In addition to treating the causes of bacterial, fungal, or viral pneumonia, alternative therapies are required to address the symptoms caused by infection. Other drugs widely prescribed to treat pneumonia patients include anti-inflammatory and

analgesic groups, such as non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs are a class of FDA-approved drugs that act as antipyretic, anti-inflammatory, and analgesic agents. They are commonly prescribed to treat localized inflammation in lungs infected due to injury to the parenchyma. NSAIDs represent a class of drugs that are highly effective at treating pain and inflammation. The inflammatory response involves cyclooxygenase (COX), which is necessary to convert arachidonic acid into thromboxane, prostaglandins, and prostacyclin, which are all mediators of inflammation. NSAIDs inhibit the cyclooxygenase (COX) enzyme, whose function is speed determining for prostaglandins and the synthesis of other prostanoids such as thromboxane.

In pneumonia patients, infection in the lungs can cause inflammation leading to the release of various cytokines and inflammatory mediators. As vital organs are involved in gas exchange, excessive pulmonary inflammation can be life-threatening since the lungs are constantly exposed to harmful pathogens. Therefore, immediate and intense defensive measures, especially inflammation, are required to eliminate the pathogens as rapidly as possible.

In general, this drug is administered orally in tablet form. Although NSAIDs are generally considered safe and, therefore, readily available without a prescription, numerous reports exist of risks and safety concerns associated with their use. Potential side effects of NSAIDs include kidney damage and gastrointestinal bleeding. Moreover, these drugs have been reported to have cardiovascular side effects and induce nephrotoxicity. Side effects affecting the gastrointestinal tract are caused by inhibition of COX-1, which prevents the formation of prostaglandins, whose function is to protect the gastric mucosa. Cardiovascular side effects reported as resulting from NSAID use, particularly COX-2 selective NSAIDs, include myocardial infarction, thromboembolism, and atrial fibrillation. Selective inhibition of COX-2 by specific NSAIDs may exacerbate the risk of cardiovascular events in patients with a medical history of cardiovascular disease. This prothrombotic risk results from thromboxane A2-mediated vasoconstriction and platelet aggregation, which is unbalanced and cannot be counteracted when prostacyclin activity is suppressed through COX-2 inhibition [18].

COX-1 and COX-2 have been acknowledged as facilitating the production of prostaglandins that play a role in renal hemodynamics. In patients with normal renal function, inhibition of prostaglandin synthesis does not pose a significant problem. However, in patients with renal dysfunction, these prostaglandins assume greater significance and can be a source of problems if the use of NSAIDs compromises their effective functioning. Potential complications include acute kidney function disorders, fluid and electrolyte disturbances, renal papillary necrosis, and nephrotic syndrome/interstitial nephritis. Side effects such as bleeding are also possible, especially with nonselective NSAIDs, due to their antiplatelet activity, which is usually only problematic if the patient has a history of GI ulcers, diseases that impair platelet activity (hemophilia, thrombocytopenia, von Willebrand, etc.), in certain cases, perioperatively.

The NSAID class of drugs belongs to the BCS class II weak acid groups whose members dissolve rapidly in the small intestine but demonstrate low solubility at

gastric pH. Fifteen of the 18 NSAIDs were classified as BCS class II. Because the majority of the drugs in this class are included in BCS class II, whose solubility is low, a formulation is required to increase the solubility and bioavailability of NSAID drugs.

3.5 Symptomatic Therapy: Corticosteroid

Corticosteroids are synthetic analogs of naturally occurring steroid hormones produced by the adrenal cortex. This synthetic compound has glucocorticoid and/or mineralocorticoid properties. Mineralocorticoids affect ion transport in renal tubular epithelial cells and are primarily involved in regulating electrolyte and water balance. Glucocorticoids are primarily involved in carbohydrate, fat, and protein metabolism and have anti-inflammatory, immunosuppressive, antiproliferative, and vasoconstrictive effects. Corticosteroid drugs such as prednisone, prednisolone, methylprednisolone, and dexamethasone have potent anti-inflammatory, immunomodulatory, and antineoplastic properties and are important to the treatment of various conditions.

The use of corticosteroids in patients with severe pneumonia has been reported to reduce lung inflammation. In addition, it has been asserted that this drug can play a role in preventing respiratory failure. Corticosteroids can inhibit the expression of numerous molecules involved in the inflammatory response caused by pneumonia. The molecular mechanism that occurs increases the transcription of anti-inflammatory molecular genes. In addition, corticosteroids are known to cause transrepression by decreasing gene transcription of various inflammatory cytokines, chemokines, or adhesion molecules [19]. This drug can be given orally or parenterally. High doses of glucocorticoids can be administered intravenously to patients who experience an emergency or those with respiratory tract disorders. On the other hand, oral administration can be an alternative applied to reduce patient discomfort and facilitate administration.

Dexamethasone is reported to be highly water-soluble. Consequently, in some of the literature, this drug is included in BCS class I. However, in other studies, it is classified as falling within BCS class III. In healthy individuals, the bioavailability of oral dexamethasone ranges between 70% and 78%. However, extrapolating the bioavailability assigned to healthy individuals to patients hospitalized with pneumonia may be inaccurate because of the potential for impaired absorption caused by delayed gastric emptying or changes in first-pass metabolism.

Several in vitro studies have shown that corticosteroids reduce cytokine expression in human cells and inhibit phagocytic cell migration. Corticosteroids can reduce the release of the cytokine Interleukin-6 (IL-6), C-reactive protein, and the number of neutrophils. Another potential benefit of using corticosteroids in pneumonia is to block a Jarisch-Herxheimer-like reaction to antibiotic initiation in patients with a high bacterial load. Jarisch-Herxheimer-like reactions are thought to be caused by high concentrations of cytokines immediately after initiation of

antibiotics, possibly through the release of endotoxins or other bacterial mediators in individuals with a high bacterial load [20]. Experimental studies have shown that corticosteroid administration reduces the inflammatory response in cases of severe CAP [21].

The use of corticosteroids can cause various side effects ranging from mild to severe. Factors influencing the occurrence of side effects due to the use of corticosteroids include the dose administered and duration of therapy. The most common side effects of corticosteroids include osteoporosis and fractures, hypothalamic-pituitary-adrenal suppression, cushingoid, hyperglycemia, myopathy, glaucoma, cataracts, psychiatric disorders, immunosuppression, cardiovascular disease, gastrointestinal, and skin disorders.

Corticosteroid drugs such as methylprednisolone and dexamethasone have been reported as accumulating at relatively low levels in the lungs [22].

3.6 Symptomatic Therapy: Beta-Agonist

β2-adrenergic receptor agonists, a class of drugs used to manage respiratory disorders, mimic the functioning of catecholamines such as epinephrine, norepinephrine, and dopamine in producing different autonomic responses in the body. In particular, the smooth muscle of the respiratory tract, uterus, intestines, and systemic blood vessels in the organ experience the most significant β-2 agonist effect.

β2-Agonists are effective bronchodilators primarily because of their ability to relax airway smooth muscle. The mechanism involves binding to the active site of β2-adrenergic receptors found in the respiratory tract. This can increase intracellular cyclic-AMP, which controls smooth muscle tone. Thus, activation of β2-adrenergic receptors results in direct bronchodilation.

Based on the onset of this action, this class of drugs is subdivided into two groups, namely, short-acting beta-agonists (SABA) and long-acting beta-agonists (LABAs). SABAs are characterized by rapid onset of action and immediate symptom relief. In contrast, LABAs have a long onset of action and provide prolonged and sustained treatment due to their increased half-life. SABA class drugs include salbutamol, terbutaline, levalbuterol, and pirbuterol, while salmeterol and formoterol are members of the LABA group.

The main side effects of salbutamol use are nervousness and tremors. The occurrence of the latter is caused by the activation of β2 receptors on motor nerve terminals which increases intracellular cAMP. Other side effects include nausea, sleep disturbances, fever, bronchospasm, vomiting, headache, and dry mouth. Moreover, salbutamol can also increase blood pressure and cause hypokalemia. Elevated blood sugar levels and prolongation of the QTc interval and ST-segment depression can also occur, although these side effects are rare.

Salbutamol belongs to the BCS class 1 drug group commonly used to treat respiratory disorders. The administering of salbutamol in combination with budesonide produced a therapeutic effect in cases of bacterial pneumonia in neonates. Its

administration can effectively alleviate symptoms such as shortness of breath and cough, reduce treatment time, and mitigate the inflammatory response in neonates.

The drug overview used for pneumonia therapy can be seen in Table 1.

Table 1 The overview of drugs used for pneumonia therapy

No	Pharmacological class therapy	Active drug	Approval date	Dosage form(s)
1	Antibiotics	Azithromycin (ZITHROMAX®)	24 May 2002	Azithromycin 250 mg tablet. Azithromycin 500 mg tablet. Azithromycin 300 mg suspension. Azithromycin 600 mg suspension. Azithromycin 900 mg suspension. Azithromycin 1200 mg suspension.
		Doxycycline (DORYX®)	6 May 2005	Doxycycline hyclate delayed-release tablets 75 mg. Doxycycline hyclate delayed-release tablets 100 mg.
		Ciprofloxacin (CIPRO®)	25 March 2004	Ciprofloxacin hydrochloride 100 mg tablet. Ciprofloxacin hydrochloride 250 mg tablet. Ciprofloxacin hydrochloride 500 mg tablet. Ciprofloxacin hydrochloride 750 mg tablet. Ciprofloxacin 5 g/100 mL oral suspension. Ciprofloxacin 10 g/100 mL oral suspension.
		Moxifloxacin (AVELOX®)	10 December 1999	Moxifloxacin hydrochloride 400 mg tablets. Moxifloxacin hydrochloride 400 mg in 250 mL sodium chloride injection flexibag.
		Levofloxacin (LEVAQUIN®)	17 December 1998	Levofloxacin 200 mg tablet. Levofloxacin 500 mg tablet.
2	Antiviral	Oseltamivir (TAMIFLU®)	14 December 2000	Oseltamivir phosphate 75 mg capsule. Oseltamivir phosphate 12 mg/mL powder for oral suspension.
		Lopinavir/ritonavir (KALETRA®)	15 September 2000	KALETRA capsule (133.3 mg lopinavir and 33.3 mg ritonavir). KALETRA oral solution (80 mg lopinavir and 20 mg ritonavir).

(continued)

Table 1 (continued)

No	Pharmacological class therapy	Active drug	Approval date	Dosage form(s)
3	Antifungal	Amphotericin B (Ambisome)	11 August 1997	Liposomal amphotericin B 50 mg/vial injection.
		Flucytosine (ANCOBON®)	26 November 1971	Flucytosine 250 mg capsule. Flucytosine 500 mg capsule.
		Fluconazole (DIFLUCAN®)	29 January 1990	Fluconazole 50 mg tablet. Fluconazole 100 mg tablet. Fluconazole 150 mg tablet. Fluconazole 200 mg tablet.
4	NSAIDs	Aspirin	25 June 1965	Oral 650 mg 8-h Bayer extended release tablet. Oral 650 mg Measurin extended release tablet.
		Piroxicam (FELDENE®)	06 April 1982	Piroxicam 10 mg capsule. Piroxicam 20 mg capsule.
		Diclofenac Sodium (VOLTAREN®)	28 July 1988	Diclofenac sodium 25 mg delayed-release tablet. Diclofenac sodium 50 mg delayed-release tablet. Diclofenac sodium 75 mg delayed-release tablet.
5	Corticosteroid	Prednisone	21 April 2012	Prednisone 5 mg tablet.
			26 July 2012	Prednisone 1 mg delayed-release tablet. Prednisone 2 mg delayed-release tablet. Prednisone 5 mg delayed-release tablet.
		Prednisolone (Orapred® ODT)	02 June 2006	Prednisolone sodium phosphate equivalent to 10 mg prednisolone base orally disintegrating tablet (ODT). Prednisolone sodium phosphate equivalent to 15 mg prednisolone base ODT. Prednisolone sodium phosphate equivalent to 30 mg prednisolone base ODT.
		Methylprednisolone	31 October 1997	Methylprednisolone 4 mg tablet. Methylprednisolone 8 mg tablet. Methylprednisolone 16 mg tablet. Methylprednisolone 32 mg tablet.
		Dexamethasone	15 December 1983	Dexamethasone 0.5 mg tablet. Dexamethasone 0.75 mg tablet. Dexamethasone 4 mg tablet. Dexamethasone 6 mg tablet.
			11 April 2003	Dexamethasone sodium phosphate 10 mg/mL injection.

(continued)

Table 1 (continued)

No	Pharmacological class therapy	Active drug	Approval date	Dosage form(s)
6	Beta-agonist	Salbutamol (VENTOLIN™)	10 July 1986	Albuterol sulfate equivalent to 2 mg albuterol tablet. Albuterol sulfate equivalent to 4 mg albuterol tablet.
		Terbutaline (BRETHINE®)	17 May 1976	Terbutaline sulfate 2.5 mg tablet. Terbutaline sulfate 5 mg tablet.
		Levalbuterol (XOPENEX®)	25 March 1999	Levalbuterol hydrochloride equivalent to 0.021% Levalbuterol solution for inhalation. Levalbuterol hydrochloride equivalent to 0.042% Levalbuterol solution for inhalation. Levalbuterol hydrochloride equivalent to 0.0103% Levalbuterol solution for inhalation. Levalbuterol hydrochloride equivalent 0.25% Levalbuterol solution for inhalation.
		Pirbuterol (MAXAIR®)	30 December 1986	Pirbuterol acetate equivalent to 0.2 mg pirbuterol aerosol metered inhalation.
		Salmeterol (ADVAiR® DISKUS)	24 August 2000	Combination of100/50 mcg fluticasone propionate/salmeterol xinafoate powder inhalation. Combination of 250/50 mcg fluticasone propionate/ Salmeterol xinafoate powder inhalation. Combination of 500/50 mcg luticasone propionate/ Salmeterol xinafoate powder inhalation.
		Formoterol (SYMBICORT®)	21 July 2006	Budesonide 80 μg and formoterol fumarate dihydrate 4.5 μg /inh aerosol metered inhalation. Budesonide 160 μg and formoterol fumarate dihydrate 4.5 μg /inh aerosol metered inhalation.

4 Challenges in Drug Delivery for Pneumonia

The pulmonary drug delivery system is a non-invasive route for local and systemic therapy, which increases patient comfort and compliance. The pulmonary route is an efficient method for absorbing macro- and micro-molecules through deep lung tissue into the bloodstream. This drug delivery system is superior to others with regard to certain diseases. This route offers many advantages over conventional oral

administration, such as a large surface area enabling rapid absorption due to high vascularity and avoiding the first-pass effect. The large absorption surface area of the alveoli, the skinny diffusion pathways from the air spaces into the blood, and the increased blood flow render the lungs the gateway to the circulation system. Drug molecules are absorbed more efficiently from the lungs than other non-invasive drug delivery routes. As a result, ever-increasing amounts of inhaled drugs are available to treat various diseases. Modifying the formulation in the drug delivery system may enable delayed release in the respiratory tract. These benefits include a more prolonged duration of action, reduced drug use, increased patient compliance, and fewer drug-related side effects.

4.1 Particle Size

Pulmonary drug delivery systems have numerous advantages, although these depend on optimal particle size. If the drug particle size is relatively large, deposition in the larynx can ensue, possibly proving to be an irritant. However, if comparatively small, drug particles can be directly exhaled from the lungs without being deposited.

Particles larger than 5 μm in size will be deposited in the upper respiratory tract, such as in the oropharyngeal area. In comparison, smaller particles in the range of 1–5 μm are deposited in the bronchiolus area through sedimentation. This is an effective measure of drug distribution throughout the lungs. Meanwhile, particles smaller than 1 μm are deposited in the deeper alveolar region through Brownian diffusion, while those less than 0.5 μm in size will be directly expelled during the exhalation process.

The disposition of drug particles in the lungs is highly dependent on the particle size at the time of formulation. The drug disposition reflects the ability of drug particles that are inhaled to be deposited in the respiratory tract. Drug disposition mechanisms based on particle size include impaction, sedimentation, and diffusion. Particles smaller than 0.1 μm in diameter are only deposited by diffusion. Particles 1 μm to 5 μm in size will be gradually deposited in the bronchioles over a relatively long period. Inertial transport becomes an effective transport mechanism for larger particles, while deposition due to impaction increases with particle size, particle density, and airflow rate. Aerosol particles pass through the oropharynx and upper respiratory tract at high velocity in the impaction deposition mechanism. Due to centrifugal force, the particles collide with the respiratory wall and are deposited in the oropharynx [23].

However, the effectiveness of pulmonary route drug delivery therapy is limited due to rapid lung clearance. Small particles delivered to the lungs will rapidly diffuse across the epithelial cells of the lungs and penetrate the bloodstream within a few minutes. On the other hand, although macromolecules can also be absorbed within the systemic circulation after a few hours, they are rapidly taken up by alveolar macrophages and excreted by mucociliary cells.

4.2 Drug Solubility

Active ingredients with poor solubility become a significant challenge in the development of drug dosage forms for clinical use. Drug absorption will be limited by the amount of active ingredient dissolved, which subsequently diffuses through the biological membrane. Poor drug solubility can lead to low bioavailability. Solubility enhancement remains one of the most challenging aspects of drug development.

Drug delivery through inhalation has considerable potential for the treatment of various pulmonary conditions such as tuberculosis, asthma, chronic obstructive pulmonary disease (COPD), and pulmonary infections. This delivery route can assist in the direct and efficient delivery of high-dose drugs to the target site while reducing systemic exposure and side effects and avoiding first-pass metabolism. However, its effectiveness is hampered by drug clearance at the target site, poor accumulation in the respiratory tract, and inefficient penetration of the lung mucosa and epithelium. This scenario necessitates more drug doses. Thus, a nanocarrier is necessary to encapsulate drugs that can maintain drug release, prolong residence time in the respiratory tract, and support drug penetration through the mucosa and epithelium.

4.3 Local Therapy and Mucoadhesive Property

The mucoadhesive delivery system is intended to prolong the contact between the drug and the mucosa. Particles with mucoadhesive systems have been extensively investigated for pulmonary drug delivery because of their benefit in increasing the residence time of particles in the lungs. However, retention of adhesively trapped particles in the mucus of the airways may be limited by physiological mucus clearance mechanisms [24].

The short duration of a drug's action and the requirement that it be administered several times a day represents a drawback of pulmonary route therapy. Therefore, drug formulation with a controlled drug release rate is required. Controlled release drugs such as antibiotics can maintain a relatively constant concentration in pulmonary tissue for an extended period to enhance their effect. In addition, the advantages of controlled-release formulations can protect the drug against rapid clearance in the respiratory tract, including mucociliary clearance and macrophage phagocytosis.

The nanocarrier drug delivery system can provide continuous release in the lungs to increase the therapeutic effect of inhaled drugs. Modification of the release system is intended to maintain the drug in the lungs for an extended period and sustainably release it locally at the desired therapeutic level. In addition to increasing the effectiveness of therapy, this can reduce the systemic side effects of drugs.

4.4 Low Dose

Drug delivery systems to the lungs are generally formulated at low doses. However, under certain conditions, higher doses are required, such as is the case with antibiotics directly targeted at the lungs. High doses are required to ensure sufficient drug concentrations in the lungs to provide optimal results. On the other hand, the administration of high drug doses with a larger number of carriers will result in an increase in powder volume, which is inconvenient for multidose inhalers and can result in certain side effects such as throat irritation and coughing [25].

The pulmonary alveoli possess a surface area of more than 100 m^2 with a wall thickness of less than 1 μm, allowing rapid absorption into the systemic circulation. This can produce a more rapid onset of action than other routes and reduce common systemic side effects. Administering drugs locally via the pulmonary route can also avoid first-pass metabolism. Consequently, these advantages support the use of lower doses when compared to those of other drug delivery routes.

4.5 Rapid Onset of Action

Lung physiology featuring a large surface area, high blood supply, and rapid onset of action with high drug bioavailability means that the drug delivery system has significant potential as a form of treatment for lung disorders such as pneumonia [26].

5 Nanocarriers for Pulmonary Drug Delivery

5.1 Liposomes

Liposomes are spherical vesicles with an aqueous core surrounded by phospholipids. Liposomes are nanocarriers that can trap various therapeutic agents, control the release of drugs trapped in liposomes, and improve the pharmacokinetic profile of drugs after in vivo administration. This delivery system can be used in drug delivery systems aimed at the lungs because it can trap drugs and target their effects on these organs for a protracted period. This can increase the benefits of drug therapy and reduce the potential for negative side effects.

Liposomes are produced using phospholipids with or without cholesterol, components that are very similar to the pulmonary biological membrane. This system has biocompatible and biodegradable characteristics, which render it suitable as a drug carrier to formulate inhaled drugs. Liposomal formulations with compositions similar to pulmonary surfactants can be potential drug carriers for drug delivery to the lungs. This is due to its low local irritation in the lung parenchyma, high biodegradability, and sustained release.

Several drugs have been developed into liposomes for the treatment of pneumonia. Liposomal azithromycin is a potential therapy to increase the efficacy of azithromycin in combatting lung infections, in addition to its safe use [27]. In the form of liposomes, oseltamivir is also reported as capable of enabling direct targeting of the influenza virus in the lungs. The delivery system provides an enhanced safety profile as well as rapid and effective delivery of antivirals within pneumonia therapy [25]. In addition, the formulation of amphotericin B, one of the treatments for pneumonia in the form of liposomes and lipid complexes, can reportedly reduce kidney toxicity in patients with renal impairment. The use of amphotericin B at a dose of 1 mg/kg/day is known to cause a high incidence of toxicity. The liposome formulation produced fewer side effects [28]. Therefore, the use of amphotericin B in the form of liposomes can be recommended for patients with kidney disorders to reduce renal toxicity [16]. There are other liposomal drugs that maintain their release in the lungs, including ciprofloxacin liposome [29], isoniazid liposome for the treatment of tuberculosis [30], and salbutamol sulfate liposome as part of asthma therapy [31].

5.1.1 Tedizolid Phosphate Liposome

Tedizolid forms part of the second generation of oxazolidinone antibiotics. It is a protein synthesis inhibitor that acts as an antibacterial by binding to the 50 s subunit of the bacterial ribosome in order to inhibit bacterial protein synthesis [32]. In the study conducted by Yang et al. (2017), it was reported that the tedizolid phosphate liposome contained spherical formations uniform in size, namely, 194.9 ± 2.93 nm with a zeta potential of 20.4 mV and encapsulation efficiency of 5.52% [32]. In the in vitro release study, it was found that the tedizolid liposome released approximately 80% of drug content within 10 h. Slow-release commenced after the first 2 h precipitating a sustained-release effect.

In vivo biodistribution studies have shown that the liposome can be distributed in the lungs. The use of stearylamine in liposome formulations produced a positive charge of liposomes, thereby increasing drug affinity in the lungs. The relative uptake of liposomes in the lungs is 1.527, which indicates that the drug can be delivered in a targeted manner.

5.1.2 Amikacin Liposome (Arikayce™)

In the amikacin liposome, which is composed of lipid dipalmitoylphosphatidylcholine (DPPC) and cholesterol, the average particle size of liposomes ranged from 248 to 282 nm [33]. This drug is given to patients by a nebulizer and produces droplets with a diameter of ~4.7 μm, which includes sizes that can be widely distributed in the lungs (<5 μm). Amikacin liposomes release about 30% of amikacin when initially produced during nebulization [34]. The development of the amikacin liposome for the treatment of respiratory tract infections via inhalation

showed promising results. In vitro studies reported that liposomes could diffuse through the patient's sputum and penetrate the biofilm of *Pseudomonas aeruginosa*, thereby enabling them to get closer to the target site. Liposomes can also increase drug levels locally and prolong the half-life of the drug [33].

In the in vivo study on mice, the use of liposomes in cases of infection was able to reduce and eradicate *P. aeruginosa* compared to conventional preparations [35]. In clinical trials in phase I and phase II, amikacin liposome was a safe drug therapy devoid of side effects. The use of amikacin in the form of liposomes also improved lung function in 14–28 days [36].

5.1.3 Tobramycin Liposome (LipoBiEDT-TOB ™)

Encapsulation of tobramycin in a liposome increases its in vitro efficacy against *P. aeruginosa* [37, 38]. The tobramycin liposome has a particle size of between 200 and 500 nm with a zeta potential of −22.3 to −0.5 mV [39]. Only 20% of the drug was released from the liposome in phosphate buffer media at 37 °C for 72 h, although this figure rose over time at increasing temperatures. These results indicate that tobramycin liposome is stable when releasing drugs in the body at physiological temperatures, increasing precise drug delivery to target sites such as inflamed lungs [40]. The in vivo study using liposomes produced drug levels 16 times higher than those of native drugs [41].

5.1.4 Ciprofloxacin Liposome

The ciprofloxacin liposome can be administered either through inhalation or intravenously. At the preclinical stage, ciprofloxacin liposomes can treat lung infections due to *P. aeruginosa*. In vivo studies have shown that the ciprofloxacin liposomes can maintain their concentration for 24 h with a half-life of 12 h [42, 43]. In clinical study phase II, this system was reported to be safe and capable of increasing the antimicrobial activity of Ciprofloxacin [44].

5.2 Microspheres

Microspheres are one of the new drug delivery systems used as an effective therapeutic alternative to conventional or immediate-release dosage forms. Microspheres are spherical solid particles with diameters ranging from 1 μm to 1000 μm, with the drug dispersed in a particular solution in a microcrystalline form.

Microspheres consist of natural and synthetic polymers that are biocompatible and biodegradable such as chitosan, poly (lactic) acid, poly (lactic-co-glycolic) acid, and poly (lactic-co-lysine graft lysine). Using polymeric microparticles as a carrier system for drug delivery to the lungs offers potential advantages such as drug

stability, high drug encapsulation efficiency, and targeting specific drugs to the site of action. This system is capable of enabling drug deposition in the lungs, delaying drug release, and protecting biomacromolecules such as proteins and peptides from hydrolysis by enzymes. Preparation of drug formulations in microspheres with aerodynamic diameters of 1–5 μm and suitable shape and porosity can be undertaken to meet pulmonary administration requirements. In addition, microspheres usually demonstrate high stability with robust moisture resistance capabilities. These characteristics have determined the broad application of microspheres in lung delivery.

Vishwa et al. (2021) studied the moxifloxacin microsphere [45]. An in vitro study of moxifloxacin microspheres as a treatment for tuberculosis showed them to be a potent antibiotic against *M. tuberculosis*. An in vivo study demonstrated that the moxifloxacin microsphere increases the duration of the drug's residence in the lungs. The microsphere is also capable of eradicating bacteria and reducing drug delivery. However, there is currently no available data regarding clinical studies of moxifloxacin microspheres.

5.2.1 Solid Lipid Nanoparticle (SLNs)

SLNs can potentially be used for pulmonary drug delivery. SLNs are nanoscale aqueous suspensions prepared from physiological lipids, mainly triglycerides, and phospholipids. Since the formulation is based on physiological components, this system is less toxic and, consequently, more acceptable for pulmonary drug delivery. Phospholipids based on surfactant proteins are found on the alveolar surface, where they are essential for maintaining optimal surface tension and reducing friction in lung tissue. SLNs are ideal for delivering drugs with lipophilic characteristics and low water solubility. Thus, drug formulations in the SLN can increase solubility and therapeutic effectiveness. In the study by Esmaeili et al. (2016), it was reported that the use of budesonide in SLN at a size of 170–200 nm produced an increase in system performance [46]. In addition, the high stability and slow drug release of SLN indicate its potential as an alternative to conventional formulations.

The rifabutin SLN is intended for antibiotic delivery to the lungs. This formulation prepared with glyceryl dibehenate, glyceryl tristearate lipids, and Tween produced uniform particle sizes ranging from 100 to 200 nm with a polydispersity index less than 0.2. The zeta potential value was obtained at −24 mV [47].

The release study of rifabutin from SLN in a 0.1% lung surfactant medium showed a release of 95.6% from SLN composed of glyceryl tristearate and 97.4% from SLN composed of glyceryl dibehenate in 24 h. During the first 30 min, there is a rapid release of around 65%, subsequently followed by a slow release lasting for 12 h [47].

In the in vitro study, the rifabutin SLN showed an intensification of the antimicrobial effect with a decrease in the viability of the bacteria. Meanwhile, in the in vivo studies, the rifabutin SLN system produced a reasonably high accumulation in the lungs compared to the conventional rifabutin formulation [48].

The SLN formulation of amikacin prepared with Tween 80 and cholesterol has a particle size of 150 nm with a zeta potential of +4 mV [49]. In the study undertaken by Ghaffari et al. (2011), it was reported that the release of amikacin SLN at 25 °C was 12% in 24 h, reaching 25% in 48 h, and 33% in 72 h. The drug release stood at 95% after 144 h of observation [49].

Based on the previous study [50], it was reported that the in vivo biodistribution of amikacin SLN for pulmonary delivery produced an increase in drug concentration compared to intravenous administration. Moreover, with regard to the nephrotoxic side effect of amikacin, the administration of the amikacin SLN via the pulmonary route causes lower drug concentrations in the kidney than intravenous administration. Therefore, it can reduce the side effects of nephrotoxic amikacin. This study indicates that the use of amikacin SLN via the pulmonary route can increase the effectiveness of the drug in cases of respiratory tract infection because the inhalation of nanoparticles can reduce the frequency of drug administration and increase its pharmacological index.

The summary of nanocarrier studies for delivering drugs in pneumonia treatment can be seen in Table 2.

Table 2 In vitro and in vivo activities of nanocarrier drug delivery for pneumonia

No	Types of nanocarrier	Active drug	In vitro and in vivo activities	Reference(s)
1	Liposome	Azithromycin	Azithromycin liposomes significantly reduced *P. aeruginosa* colonies, bacterial counts within biofilms, virulence factors, and motility. Azithromycin liposomes showed no toxicity in vitro.	[27]
		Tedizolid phosphate	Tedizolid liposomes provided a sustained-release effect, with approximately 80% of the drug released within 10 h. Tedizolid liposome increased the uptake of the drug into the lungs, indicating a targeted manner.	[32]
		Amikacin	Amikacin liposomes could diffuse through the patient's sputum and penetrate the biofilm of *Pseudomonas aeruginosa.* Liposomes increased amikacin levels in the lung and prolonged the half-life of amikacin. Amikacin liposomes successfully reduced and eradicated *P. aeruginosa* infection compared to the conventional preparation. Amikacin liposome provided a safe therapy for patients with improved lung functions within 14–28 days.	[33, 35, 36]

(continued)

Table 2 (continued)

No	Types of nanocarrier	Active drug	In vitro and in vivo activities	Reference(s)
		Tobramycin	Tobramycin liposomes increased in vitro efficacy against *P. aeruginosa*. Tobramycin liposome is stable with minimum drug leakage, thus increasing the precise drug to target the inflamed lungs. It produced drug levels 16 times higher than those of free drug.	[37, 38, 40, 41]
		Ciprofloxacin	The concentration of ciprofloxacin liposomes could be maintained for 24 h with a 12 h half-life time. Liposomes were reported to be safe and capable of increasing the antimicrobial activity of ciprofloxacin.	[42–44]
2	Microsphere	Moxifloxacin	Moxifloxacin microsphere produced potent antimicrobial activity against *M. tuberculosis*. Moxifloxacin microsphere increased the duration of the drug's residence in the lungs and was capable of eradicating the bacteria.	[45]
3	Solid lipid nanoparticles (SLNs)	Budesonide SLN	Budesonide SLN successfully increased drug stability and produced slow drug release.	[46]
		Rifabutin SLN	Rifabutin SLN showed an intensification of the antimicrobial effect with a decrease in the viability of the bacteria. Rifabutin SLN produced high drug accumulation in the lungs.	[48]
		Amikacin SLN	Amikacin SLN had slow drug release up to 144 h. SLN increased drug concentration in the lung. Amikacin SLN had a lower drug concentration in the kidney, thus reducing the nephrotoxicity.	[49, 50]

6 Conclusion

Pulmonary drug delivery systems represent attractive carriers for managing pneumonia. They offer various advantages over the conventional oral route. Nanoparticle-based drug carriers are of great interest in the delivery of drugs to the lungs due to their extensive surface area with rapid absorption due to their high drug vascularity,

thus having significant potential for successfully treating lung diseases. Various drugs prescribed in the treatment of pneumonia are subject to limitations on their clinical use, such as poor bioavailability. The use of nanoparticles such as liposomes, microspheres, and solid lipid nanoparticles successfully improves bioavailability and targets the drug to the lungs. Nanoparticles with specific particle sizes can be deposited at high levels in the lungs, thus improving effective drug concentration in the treatment of lung infections such as pneumonia. Moreover, encapsulating active drugs into nanoparticles also significantly reduces drug toxicity. The use of lipid nanocarriers can be promising carriers for pulmonary drug delivery for the effective therapy of pneumonia infections.

References

1. Troeger C, Forouzanfar M, Rao PC, Khalil I, Brown A, Swartz S, et al. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory tract infections in 195 countries: a systematic analysis for the global burden of disease study 2015. Lancet Infect Dis. 2017;17(11):1133–61.
2. Khomich OA, Kochetkov SN, Bartosch B, Ivanov AV. Redox biology of respiratory viral infections. Viruses. 2018;10(8):392.
3. Cilloniz C, Martin-Loeches I, Garcia-Vidal C, Jose AS, Torres A. Microbial etiology of pneumonia: epidemiology, diagnosis and resistance patterns. Int J Mol Sci. 2016;17(12):2120.
4. Howie SRC, Hamer DH, Graham SM. Pneumonia. In: International encyclopedia of public health (Second Edition). 2017. p. 500–8.
5. Sattar SBA, Sharma S. Bacterial pneumonia. In: StatPearls [internet]. Treasure Island: StatPearls Publishing; 2022 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK513321/.
6. Dinos GP. The macrolide antibiotic renaissance. Br J Pharmacol. 2017;174:2967–83.
7. Danesi R, Lupetti A, Barbara C, Ghelardi E, Chella A, Malizia T, et al. Comparative distribution of azithromycin in lung tissue of patients given oral daily doses of 500 and 1000 mg. J Antimicrob Chemother. 2003;51(4):939–45.
8. Zuckerman JM, Qamar F, Bono BR. Macrolides, ketolides, and glycylcyclines: azithromycin, clarithromycin, telithromycin, tigecycline. Infect Dis Clin N Am. 2009;23(4):997–1026.
9. Aldred KJ, Kerns RJ, Oshero N. Mechanism of quinolone action and resistance. Biochemistry. 2014;53(10):1565–74.
10. Cowling T, Farrah K. Fluoroquinolones for the treatment of respiratory tract infections: a review of clinical effectiveness, cost-effectiveness, and guidelines [internet]. Ottawa: Canadian Agency for Drugs and Technologies in Health; 2019. Available from: https://www.ncbi.nlm.nih.gov/books/NBK543514/.
11. Moreno G, Rodríguez A, Sole-Violán J, Martín-Loeches I, Díaz E, Bodí M, et al. Early oseltamivir treatment improves survival in critically ill patients with influenza pneumonia. ERJ Open Res. 2021;7(1):00888–2020.
12. Treanor JJ, Hayden FG, Vrooman PS, Barbarash R, Bettis R, Riff D, et al. Efficacy and safety of the oral neuraminidase inhibitor oseltamivir in treating acute influenza: a randomized controlled trial. J Am Med Assoc. 2000;283(8):1016–24.
13. Malosh RE, Martin ET, Heikkinen T, Brooks WA, Whitley RJ, Monto AS. Efficacy and safety of oseltamivir in children: systematic review and individual patient data meta-analysis of randomized controlled trials. Clin Infect Dis. 2018;66(10):1492–500.
14. Orbak Z, Laloglu F, Akat H. Effectiveness of lopinavir/ritonavir on COVID-19-related pneumonia in a child with COVID-19-associated Kawasaki disease. Cardiol Young. 2021;31(3):507–10.

15. Wang Y, Chen L. Lung tissue distribution of drugs as a key factor for COVID-19 treatment. Br J Pharmacol. 2020;177(21):4995–6.
16. Limper AH, Knox KS, Sarosi GA, Ampel NM, Bennett JE, Catanzaro A, et al. An official American Thoracic Society statement: treatment of fungal infections in adult pulmonary and critical care patients. Am J Respir Crit Care Med. 2011;183(1):96–128.
17. Felton T, Troke PF, Hope WW. Tissue penetration of antifungal agents. Clin Microbiol Rev. 2014;27(1):68–88.
18. Perry LA, Mosler C, Atkins A, Minehart M. Cardiovascular risk associated with NSAIDs and COX-2 inhibitors. US Pharm. 2014;39:35–8.
19. Rhen T, Cidlowski JA. Anti-inflammatory action of glucocorticoids — new mechanisms for old drugs. N Engl J Med. 2005;353(16):1711–23.
20. Wunderink RG. Corticosteroids for severe community-acquired pneumonia: not for everyone. JAMA. 2015;313(7):673–4.
21. Sibila O, Luna CM, Agustí C, Baquero S, Gando S, Patrón JR, et al. Effects of glucocorticoids in ventilated piglets with severe pneumonia. Eur Respir J. 2008;32(4):1037–46.
22. Ayyar VS, Song D, DuBois DC, Almon RR, Jusko WJ. Modeling corticosteroid pharmacokinetics and pharmacodynamics, part I: determination and prediction of dexamethasone and methylprednisolone tissue binding in the rats. J Pharmacol Exp Ther. 2019;370(2):318–26.
23. Heyder J. Deposition of inhaled particles in the human respiratory tract and consequences for regional targeting in respiratory drug delivery. Proc Am Thorac Soc. 2004;1(4):315–20.
24. Schneider CS, Xu Q, Boylan NJ, Chisholm J, Tang BC, Schuster BS, et al. Nanoparticles that do not adhere to mucus provide uniform and long-lasting drug delivery to airways following inhalation. Sci Adv. 2017;3(4):e1601556.
25. Aziz S, Scherlieβ R, Steckel H. Development of high dose oseltamivir phosphate dry powder for inhalation therapy in viral pneumonia. Pharmaceutics. 2020;12(12):1–21.
26. Ali M. Pulmonary drug delivery. In: Kulkarni VS, editor. Handbook of non-invasive drug delivery systems. USA: William Andrew Publishing; 2010. p. 209–46.
27. Solleti VS, Alhariri M, Halwani M, Omri A. Antimicrobial properties of liposomal azithromycin for pseudomonas infections in cystic fibrosis patients. J Antimicrob Chemother. 2015;70(3):784–96.
28. Akbarzadeh A, Rezaei-sadabady R, Davaran S, Joo SW, Zarghami N. Liposome: classification, preparation, and applications. Nanoscale Res Lett. 2013;8:1–9.
29. Finlay WH, Wong JP. Regional lung deposition of nebulized liposome-encapsulated ciprofloxacin. Int J Pharm. 1998;167(1–2):121–7.
30. Chimote G, Banerjee R. In vitro evaluation of inhalable isoniazid-loaded surfactant liposomes as an adjunct therapy in pulmonary tuberculosis. J Biomed Mater Res. 2010;94(1):1–10.
31. Huang WH, Yang ZJ, Wu H, Wong YF, Zhao ZZ, Liu L. Development of liposomal salbutamol sulfate dry powder inhaler formulation. Biol Pharm Bull. 2010;33(3):512–7.
32. Yang Z, Tian L, Liu J, Huang G. Construction and evaluation in vitro and in vivo of tedizolid phosphate loaded cationic liposomes. J Liposome Res. 2017;28(4):322.
33. Li Z, Zhang Y, Wurtz W, Lee JK, Malinin VS, Durwas-Krishnan S, et al. Characterization of nebulized liposomal amikacin (Arikace™) as a function of droplet size. J Aerosol Med Pulm Drug Deliv. 2008;21(3):245–53.
34. Zhang J, Leifer F, Rose S, Chun DY, Thaisz J, Herr T, et al. Amikacin liposome inhalation suspension (ALIS) penetrates non-tuberculous mycobacterial biofilms and enhances amikacin uptake into macrophages. Front Microbiol. 2018;9(May):1–12.
35. Meers P, Neville M, Malinin V, Scotto AW, Sardaryan G, Kurumunda R, et al. Biofilm penetration, triggered release and in vivo activity of inhaled liposomal amikacin in chronic Pseudomonas aeruginosa lung infections. J Antimicrob Chemother. 2008;61(4):859–68.
36. Waters V, Ratjen F. Inhaled liposomal amikacin. Expert Rev Respir Med. 2014;8(4):401–9.
37. Halwani M, Mugabe C, Azghani AO, Lafrenie RM, Kumar A, Omri A. Bactericidal efficacy of liposomal aminoglycosides against Burkholderia cenocepacia. J Antimicrob Chemother. 2007;60(4):760–9.

38. Marier JF, Brazier JL, Lavigne J, Ducharme MP. Liposomal tobramycin against pulmonary infections of Pseudomonas aeruginosa: a pharmacokinetic and efficacy study following single and multiple intratracheal administrations in rats. J Antimicrob Chemother. 2003;52(2):247–52.
39. Messiaen A, Forier K, Nelis H, Braeckmans K, Coenye T. Transport of nanoparticles and tobramycin-loaded liposomes in burkholderia cepacia complex biofilms. PLoS One. 2013;8(11):e79220.
40. Zhao Y, Dai X, Wei X, Yu Y, Chen X, Zhang X, et al. Near-infrared light-activated thermo-sensitive liposomes as efficient agents for photothermal and antibiotic synergistic therapy of bacterial biofilm. ACS Appl Mater Interfaces. 2018;10(17):14426–37.
41. Güngör N, Knaapen AM, Munnia A, Peluso M, Haenen GR, Chiu RK, et al. Genotoxic effects of neutrophils and hypochlorous acid. Mutagenesis. 2010;25(2):149–54.
42. Khatib I, Khanal D, Ruan J, Cipolla D, Dayton F, Blanchard JD, et al. Ciprofloxacin nanocrystals liposomal powders for controlled drug release via inhalation. Int J Pharm. 2019;566(May):641–51.
43. Bassetti M, Vena A, Russo A, Peghin M. Inhaled liposomal antimicrobial delivery in lung infections. Vol. 80, Drugs. Springer International Publishing; 2020. p. 1309–18.
44. Serisier DJ, Bilton D, De Soyza A, Thompson PJ, Kolbe J, Greville HW, et al. Inhaled, dual release liposomal ciprofloxacin in non-cystic fibrosis bronchiectasis (ORBIT-2): a randomised, double-blind, placebo-controlled trial. Thorax. 2013;68(9):812–7.
45. Vishwa B, Moin A, Gowda DV, Rizvi SMD, Hegazy WAH, Abu Lila AS, et al. Pulmonary targeting of inhalable moxifloxacin microspheres for effective management of tuberculosis. Pharmaceutics. 2021;13(1):1–17.
46. Esmaeili M, Aghajani M, Abbasalipourkabir R, Amani A. Budesonide-loaded solid lipid nanoparticles for pulmonary delivery: preparation, optimization, and aerodynamic behavior. Artif Cells Nanomed Biotechnol. 2016;44(8):1964–71.
47. Gaspar DP, Faria V, Goncalves LMD, Taboada P, Remunan-Lopez C, Almeida AJ. Rifabutin-loaded solid lipid nanoparticles for inhaled antitubercular therapy: physicochemical and in vitro studies. Int J Pharm. 2016;497(1–2):199–209.
48. Gaspar DP, Gaspar MM, Eleute CV, Grenha A, Blanco M, Taboada P, et al. Microencapsulated solid lipid nanoparticles as a hybrid platform for pulmonary antibiotic delivery. Mol Pharm. 2017;14(9):2977–90.
49. Ghaffari S, Varshosaz J, Saadat A, Atyabi F. Stability and antimicrobial effect of amikacin-loaded solid lipid nanoparticles. Int J Nanomedicine. 2011;6:35–43.
50. Varshosaz J, Ghaffari S, Mirshojaei SF, Jafarian A, Atyabi F, Kobarfard F, et al. Biodistribution of amikacin solid lipid nanoparticles after pulmonary delivery. Biomed Res Int. 2013;2013:136859.

Hepatitis C: Exploration of Diseases, Diagnosis, and Treatment Strategies

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Abstract By reading this chapter, readers will be able to understand what is hepatitis and how it affects different patients, and on prior treatment, different diagnostic parameters are performed. Also, by going through this chapter, readers will be able to distinguish between different types of hepatitis and their effect on different people. Different treatment strategies are discussed in this chapter in order to treat hepatitis-infected patients, and risk factors are also considered so that by keeping in view these risk factors, preventive measures are adopted to treat hepatitis. Epidemiology of hepatitis is elaborated so that reader will be able to understand whether it is endemic or pandemic infectious disease. In addition, the pathophysiology of hepatitis C is discussed so that the reader should come to know its mode of transmission in the liver where this viral infection starts replicating. Certain outbreaks of hepatitis C are also discussed in this chapter. Some generally used treatment strategies are elaborated in this chapter along with necessary preventive

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measures. It is the expected responsibility of readers after studying this chapter to provide awareness of hepatitis C infection among healthcare professionals and people. At the end of this chapter, there is a complete summary of all of the topics in the form of conclusion.

Keywords Hepatitis C · Epidemiology · Diagnosis · Treatment options

1 Introduction

The word "hepatitis" described inflammation of the liver. It is the vital organ that operates to process nutrition with filtration of blood as well as combats against infections [1]. Any wound or inflammation can damage the functions of the liver. Excessive alcohol intake, some medicines, pollution diseases, and injuries can all cause hepatitis. The causative agent of hepatitis is a virus [2]. Hepatitis A, B, and C are the most common types of viral hepatitis in the USA. Hepatitis A symptoms might last anywhere from a few weeks to several months. It is transferred when someone consumes solids, even in tiny amounts, or when they come into contact with objects, foods, or drinks that have been contaminated by an infectious individual. There is a vaccine of hepatitis A that works by inhibiting hepatitis A (HCA) virus. Infections continue to happen in the USA with hepatitis A. In most of the countries, particularly those where there is lack of a modern sewage disposal system suffer from hepatitis A more. Every year, approximately 24,900 new infections occur with hepatitis A [3]. The symptoms of hepatitis B can vary from a moderate, short-term sickness to serious chronic condition. Greater than 90% of unvaccinated newborns, who become infected, develop chronic hepatitis B; only 6.2–10.5% of adults and older children that were infected develop the same chronic condition [4]. It spreads from blood plasma, bio fluids or ejeculation conatining sperms, even in trace quantity, of a person infected with the virus enter the body of a healthy individual. Hepatitis B can sometimes be spread through delivery from a mother to child, sexual contact with an infected individual, and reusing needles, syringes, and even medical equipment contaminated with blood from an infected person, like glucose monitors. Personal items like toothbrushes and razors should not be shared that might spread hepatitis B. Infection outbreaks have occurred in healthcare settings due to a lack of infection control. Chronic liver disease, for example, liver failure, cirrhosis, or liver cancer, affects 15–25% of persistently infected patients [5]. There is an effective hepatitis B vaccine available. About two-thirds of those infected with hepatitis B are unaware they are afflicted, and Asians make up almost half of those infected in the USA. Hepatitis B is the most prevalent trigger of liver cancer [6]. Hepatitis B vaccination is an essential requirement for all newborns, children, and adolescents under the age of 19. Hepatitis B infection is a concern for people who have hepatitis B in their sex partners, homosexuals, and treatment. Persons who inject drugs, those who share a residence with a hepatitis B-infected person, residents and staff of developmentally handicapped facilities, and

healthcare and public safety professionals who make contact with blood-contaminated fluids are all at risk of contracting hepatitis B. The hepatitis C virus causes a liver infection called hepatitis C (HCV) [7]. Hepatitis C is transferred by coming into contact with the blood of an infected person. Sharing needles or other injecting equipment is the most common way to develop hepatitis C right now. Hepatitis C is a short-term condition for some people, but it is a long-term, chronic infection for more than half of those who contract it. Cirrhosis and liver cancer are two significant and potentially fatal chronic hepatitis C consequences [8]. As a significant liver disease progresses, symptoms are common. Hepatitis C is a disease that does not have a vaccine. The simplest approach to avoid contracting hepatitis C is to avoid risky habits like drug injections [9]. Hepatitis C analysis is crucial since most persons with the disease can be cured in 8–12 weeks if they receive the right therapy. When injected intravenously, hepatitis C is a flavivirus with an RNA genome that is the most common cause of non-A, non-B hepatitis (NANBH) [10]. To distinguish it from viral causes of nonalcoholic hepatitis, the virus was given the moniker NANBH before it was discovered. Chronic liver disease, which can lead to cirrhosis and hepatocellular carcinoma, affects the majority of HCV patients.

2 Epidemiology

Hepatitis C is a worldwide infection that affects millions of people. HCV infection affects more than 3% of the world's population [11]. HCV is thought to infect 170 million people around the world [12], out of which approximately 71 million (Fig. 1) are chronic patients according to the report of United Nations in 2017 [13]. It is the most common cause of parenteral NANBH in the globe. Due to the use of parenteral anti-schistosomal medication, prevalence rates in Egypt have been reported to be as high as 22% [14]. Healthy blood donors have a high prevalence rate as well. In central Europe, the Middle East, Spain, Italy, and Japan, hepatitis C is very common. The most common way for HCV to spread is through blood transfusion. The risk of HCV infection through blood transfusions is now estimated to be one case per 100,000 transfused units [15]. In 2018, the Centers for Disease Control and Prevention (CDC) received reports of 3621 cases of acute hepatitis C. In 2018, the estimated 50,300 acute hepatitis C infections were recorded after accounting for under-ascertainment and underreporting [16]. The CDC has further information about hepatitis C surveillance. Between 2013 and 2016, 2.4 million persons in the USA were predicted to contract hepatitis C [17]. Cirrhosis will develop in about 5–25 people infected with HCV in 10–20 years. Cirrhotic patients have a 1–4% annual risk of developing hepatocellular carcinoma and a 3–6% annual risk of hepatic decompensation, with a 15–20% risk of death the next year [18]. The prevalence of patients with HCV load worldwide is approximately 170 million, out of which almost 70 million patients are of chronic type [12]. Another study in 2005 reported that the prevalence of HCV was 2.8% with a total patient burden of approximately 185 million. The countries with the highest proportion of HCV patients

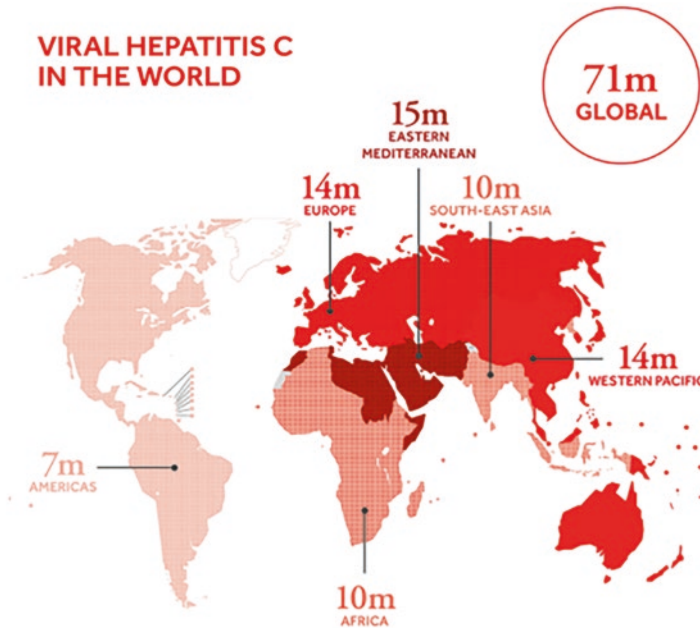

Fig. 1 Global prevalence of chronic hepatitis C-infected virus positive patients according to World Health Organization (WHO) Report 2015. (Reproduced with permission from [23])

mainly belong to lower middle income countries which correlates the association with poor socioeconomic conditions [19]. According to a report, the alarming prevalence of HCV patients was found in Egypt, and it contributes almost 15% [20]. Other countries with higher prevalence of HCV disease proceeded by Egypt are Cameron (13.8%), Gabon (11.2%), Uzbekistan (11.3%), Mongolia (10.8%), Nigeria (8.4%), and Pakistan (6.8%). However, in abovementioned countries, there were associated contributing factors. For instance, according to national literature in meta-analysis, it was found that exposure to medical procedures was one of the determinants of quick spread of HCV between 1989–2013 in Egypt [21]. These findings were supported as the cause of spread of disease in other countries like India, Gabon, and Nigeria as well [21]. The occurrence of hepatitis is even prevailing at lower pace in developed countries such as Austria, Cyprus, Italy, Israel, Germany, Denmark, France, and the UK between 0.4% and 1.5% [22].

In Pakistan, HCV has infected 12.9 million people, with 8.7 million of them chronically infected. The figure is greater than previous estimates of 6.7–7.8 million chronic infections, but it is comparable. HCV transmission is fostered by barbering, ear/nose piercing, and healthcare practices (blood transfusions, medical injections) as well as injecting drug usage in Pakistan which has the world's second-highest prevalence. According to a countrywide study conducted in 2007, 4.8% of the population or around eight million people have been exposed to HCV. In 2013, 17.0 million people in Pakistan were chronically infected with HCV, accounting for one

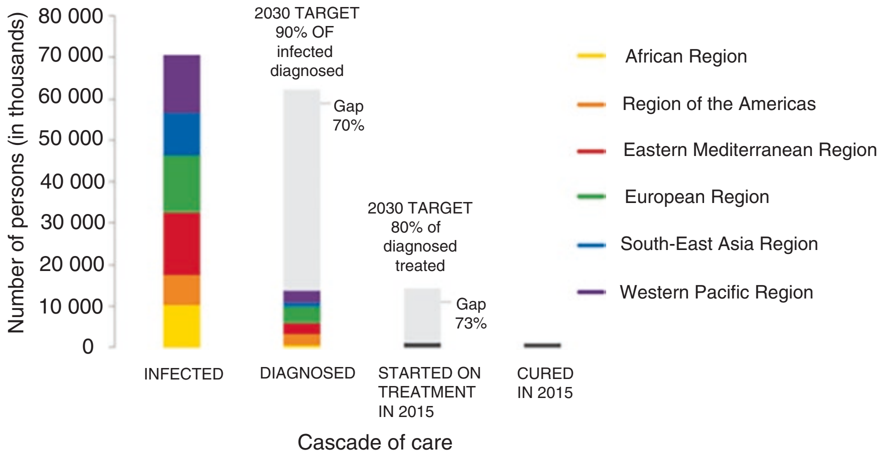

Fig. 2 Global cascade of care given by region. (Adopted with permission from [23])

tenth of the global HCV burden [24]. This emphasizes how critical it is to treat the HCV epidemic in Pakistan as part of any worldwide effort to eradicate the virus. Pakistan has had national and provincial hepatitis prevention and control programs including HCV screening and treatment in place since 2005. Local HCV prevention efforts have also focused on educational activities. The effectiveness of these treatments is unknown. Pakistan's population is expected to grow by a third to almost 250 million people between 2016 and 2030 with chronic HCV prevalence rising from 3.9% (95% confidence interval 3.7–4.1%) to 5.1% (4.6–5.5%) [24]. The number of prevalent chronic infections will increase from 7.5 (7.2–7.9) million to 12.6 (11.8–13.6) million as a result of these changes with yearly incident infections increasing from 700,000 (620,000–780,000) to 1.1 (1.0–1.3) million (from 3.7 to 4.8 per 1000 person-years) [24]. There will be significant increases in HCV-related disease during this decade, with 1.4 (1.0–2.0) million HCV-related deaths expected among those over the age of 20. According to the report of World Health Organization (WHO) in 2015, 20% of the patients were aware of their diagnosis based upon which treatment started (Fig. 2). The American regions had a maximum diagnosing rate compared with others, while the lowest diagnosis rate was associated with the African region which was 6% [23].

3 Pathogenesis

The ability of HCV to stay cell-associated and evade host cell death is the most important factor in viral pathogenicity, which results in chronic liver infection. One of the key determinants in the survival of HCV infection in the liver is the presence of a closely related but diverse population of virus genomes. Infections with the

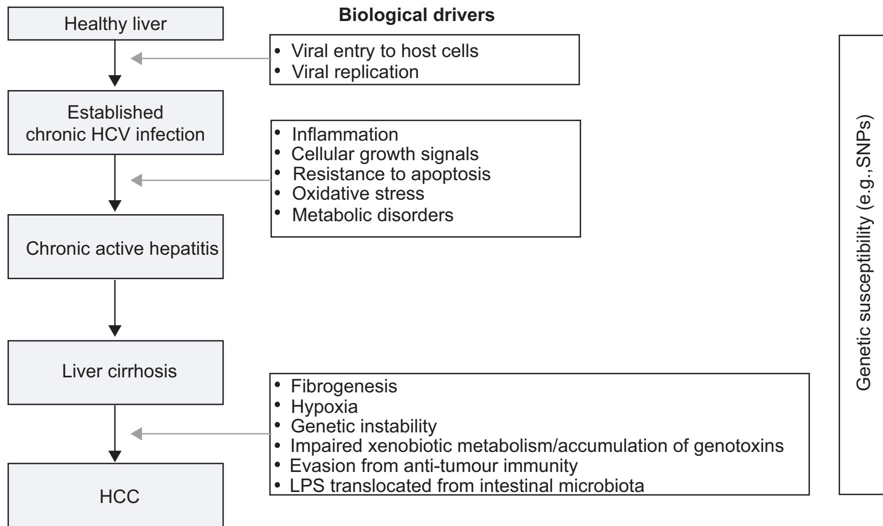

Fig. 3 Pathophysiology of hepatitis C infection. (Reprinted with permission from [29])

hepatitis C virus and their pathogenesis HCV's natural targets are hepatocytes and probably B lymphocytes [25]. According to current research, people with chronic hepatitis may have at least 50% of their hepatocytes infected with HCV. Viremia remains in the majority of infected patients and is associated with varying degrees of hepatic inflammation and fibrosis [26]. Lymphocytic infiltration in the portal tube or the liver lobule, as well as portal and periportal fibrosis, characterizes chronic hepatitis [27]. The primary histological markers of chronic hepatitis induced by HCV are portal inflammation, interphase hepatitis, and lobular necrosis. Immunity of host HCV immunity is not always permanent, and serum antibodies to the virus are typically protective [28]. Cell-mediated immunity, particularly cytotoxic T cells, plays a major role in liver inflammation and tissue necrosis (Fig. 3).

4 Clinical Syndromes

The majority of people with acute HCV infections do not show any symptoms and do not get jaundice. Acute HCV infection is often accompanied by mild symptoms that are comparable to HBV infection. Hepatomegaly affects a third of acutely infected patients in symptomatic settings, but jaundice affects less than a quarter. The vast majority of instances (80%) are asymptomatic and do not result in jaundice [30]. Hepatitis C is the most frequent cause of chronic hepatitis all over the world. In the absence of hepatic synthesis malfunction, most people with chronic hepatitis are asymptomatic; however, they may feel nonspecific symptoms like weariness or malaise. The hepatitis C virus is the most common cause of cirrhosis and hepatitis

today. Cirrhosis develops in roughly 20% of chronic hepatitis patients [31]. Globally, this process could take up to 20 years after the original infection. Secondary consequences include liver failure, portal hypertension, and other difficulties in patients with this illness. In 1–5% of patients with underlying cirrhosis, hepatocellular carcinoma is one of the most serious consequences [32]. Serological tests to detect antibodies to HCV can be used to confirm hepatitis C infection. Antibodies are directed against the core envelope as well as the NS3 and NS4 proteins and have a low titer. Acute HCV antibodies are frequently detected 6–8 weeks after the initial infection in acute infections. The antibodies that are created then last for the rest of your life if you have a persistent infection. Anti-HCV antibodies can be detected using ELISAs, including second- and third-generation ELISAs [33]. HCV RNA in the serum is detected using PCR and branched DNA tests. The most specific test for HCV infection is HCV RNA testing, which can be used to diagnose acute HCV infections before antibodies are formed. The most accurate way to determine the amount of HCV-related liver damage is to perform a liver biopsy. Before starting antiviral therapy, it is usually suggested for all patients.

5 Transmission and Symptoms

HCV is mostly transmitted through parenteral administration of infectious blood or bodily fluids. Two examples of probable exposures are using drugs and having a child with an HCV-infected mother. HCV can also be spread through unregulated tattooing, receiving donated blood and blood products, and needle stick injuries in healthcare settings, as well as through sexual contact with an HCV-infected person or exchanging personal items contaminated with infectious blood, such as razors or toothbrushes [34]. In the USA, there have been nationwide seroprevalence surveys aimed at people who inject drugs (PWID), and estimates based on smaller surveys in regional and urban areas vary substantially [35]. According to a 2018 study, the overall prevalence of hepatitis C among PWID in the USA is around 53%, ranging from 38.1% to 68.0% depending on the state. Although it is difficult to distinguish this risk from that linked with injectable drug use and intercourse with HCV-infected partners, limited epidemiologic evidence suggests that non-injection snorted or inhaled cocaine adds an additional risk. Because blood banks use more advanced hepatitis C screening tests, the risk of transmission to recipients of blood or blood products is extremely minimal, with about one case per two million units transfused. Blood transfusions were a major source of hepatitis C virus transmission prior to 1992 [36]. Medical and dental treatments done in the USA do not pose a danger of hepatitis C transmission as long as standard precautions and other infection control protocols are routinely followed. When injection equipment, such as syringes, is shared among patients, or when injectable drugs or intravenous solutions are mismanaged and contaminated with blood, hepatitis C can spread in healthcare settings. Standard precautions should be known and followed by healthcare staff, including maintaining injection safety procedures aimed at reducing

blood-borne pathogen dangers for patients and healthcare employees. Cases of suspected HCV infection caused by a healthcare professional should be reported to state and local public health officials as soon as possible for investigation and treatment. If standard precautions and other infection-control practices are not followed on a regular basis, hepatitis C can spread in healthcare settings. In the USA, poor infection-prevention measures in inpatient, outpatient, and hemodialysis settings have been associated to hepatitis C transmission. Syringe reuse and other aseptic technique failures, contamination of multidose vials, and poor equipment cleaning were among the infection control difficulties [37]. Controlled substance diversion for malicious purposes has also been linked to outbreaks. Physicians who uncover novel diseases in people who have no risk factors and then report them to public health authorities are frequently the source of healthcare-related outbreaks. People who have recently contracted HCV are often asymptomatic or have just minor symptoms that do not necessitate medical attention. Some of the symptoms include fever, lethargy, dark urine, clay-colored feces, abdominal pain, loss of appetite, nausea, vomiting, joint pain, and jaundice. The majority of people who have chronic HCV infection are asymptomatic or have nonspecific symptoms such as chronic fatigue and depression. Many people develop chronic liver disease, which can range in severity from mild to severe and can lead to cirrhosis and cancer of the liver. HCV-infected people's chronic liver disease is mostly quiet, progressing over decades without presenting any signs or symptoms. HCV infection is typically missed until asymptomatic patients are discovered to be HCV-positive via blood donation screens or when high alanine aminotransferase (ALT) levels are discovered during routine checkups [38]. Hepatitis C can cause medical issues that are not restricted to the liver in some patients who have a prolonged HCV infection. Only a few examples are diabetes, glomerulonephritis, essential mixed cryoglobulinemia, porphyria, and non-lymphoma Hodgkin's lymphoma.

6 Testing and Diagnosis

The patients were chosen from the hospitals' outpatient, inpatient, and blood bank departments. The outpatients were people who had been instructed by their doctor that an anti-HCV ELISA test was required. Inpatients were people who had previously tested positive for anti-HCV antibodies, having evidence of infection in their files, and were recruited at the blood bank. To confirm that all possible volunteers met the eligibility standards, they were further questioned. Patients who have been eligible for the study were invited to participate, with the goal to learn more about HCV infection. They were assured that any information obtained during the interview would be kept absolutely confidential. Outpatients, inpatients, and blood bank controls were also chosen. We chose outpatients who had been instructed by their attending physician to have an ELISA done and had tested negative for anti-HCV. Patients with a negative anti-HCV test before surgery were chosen as

inpatients in allied surgical wards (ENT, Eye) or general surgery patients other than hepato-biliary surgery [39]. Among blood donors, those who tested negative by ELISA were considered possible controls. Injecting drugs and sharing needles, syringes, or other medical items, as well as the individuals with certain medical disorders, should undergo routine periodic testing while risk factors exist. Regardless of setting prevalence, testing of those at risk should be done. If no hepatitis C prevalence data are available in a hospital or patient catchment area, physicians and program administrators should begin screening all adults including pregnant women for HCV infection during each pregnancy. Some of the blood tests that can detect HCV infection include enzyme immunoassay (EIA), enhanced chemiluminescence immunoassay (CLIA), chemiluminescence microparticle immunoassay (CMIA), microparticle immunoassay (MEIA), electrochemiluminescence immunoassay (ECLIA), immunochromatographic assay, and immunochromatographic assay (rapid test) [40]. Other methods to determine the presence and quantities of HCV RNA include qualitative and quantitative nucleic acid testing. Anti-HCV seroconversion takes 8–11 weeks on average following HCV exposure; however, it can take longer in immunocompromised patients, such as those with HIV. HCV RNA levels can be detected as early as 1–2 weeks following exposure to the virus in people who have recently developed an acute infection. HCV antibody testing (anti-HCV) is insufficient to diagnose active HCV infection [41]. Anti-HCV testing simply detects whether or not you have ever been exposed to HCV. Testing is only used to rule out HCV infection if a patient tests negative, indicating that the patient has never been exposed to the virus. After a positive anti-HCV test, an HCV RNA test must be conducted to complete hepatitis C testing. In patients with chronic hepatitis C, liver enzyme levels associated with hepatic inflammation (Fig. 4) vary often and then recover to normal or near normal on a regular basis. Despite chronic liver illness, liver enzyme values can stay normal for over a year.

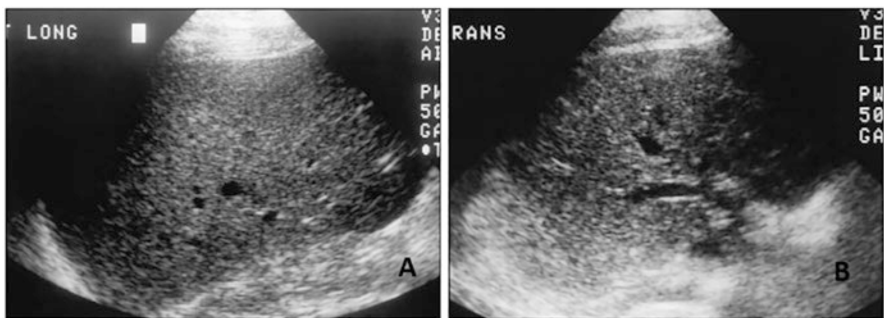

Fig. 4 Ultrasonogram showing (**a**) fibrotic liver with regions of pinhead echoes, moderate inflammation, mild steatosis, and fibrosis. (**b**) mild-severe swelling, absence of fibrosis, and steatosis with presence of nodular pattern. (Reprinted with permissions from [42])

7 Management and Treatment

People with hepatitis C should be evaluated by a primary-care clinician or a specialist in hepatology, gastroenterology, or infectious disease for chronic liver disease, including treatment and monitoring, hepatitis A and B vaccination, alcohol screening and brief intervention, and HIV risk assessment and testing, as per experts [43]. Liver inflammation can be caused by HCV, which can lead to a variety of issues, including cancer. People with chronic hepatitis C require medicine to manage their condition. These medications can assist to alleviate symptoms. Even if an HCV infection has not yet manifested symptoms, it is critical to treat it. This is because medications can reduce the risk of hepatitis C consequences like serious liver disorders. HCV has a number of genetic variants (genotypes) [44]. The genotype of the patient determines the treatment for hepatitis C. Genotype 1 is the most common in the USA. The drugs available to treat hepatitis C are listed below, along with some helpful information on what to expect throughout therapy. PEGylated interferon and the antiviral medication ribavirin are presently used to treat patients with chronic infections. Protease inhibitors, ribozymes, and viral vaccinations are some of the other therapeutic alternatives. There are many treatment plans adopted in Pakistan. Hepatitis C can now be eradicated as a public health issue to the development of directly acting antiviral (DAA) medicines [45]. DAA treatment is highly tolerated and more effective than prior interferon-based treatments; with cures attainable in as little as 8 weeks because such therapies are so simple, they may now be administered through more simplified care models, such as primary care, which has been demonstrated to enhance adoption and be more cost-effective than hospital-based therapy. For hepatitis C to be eradicated, maximum treatment uptake at the lowest possible cost will be necessary. Employing reversible addition-fragmentation chain transfer, an antiviral prodrug containing tenofovir alafenamide (TAF) was synthesized by first conjugating it to glycerol monomethacrylate and then polymerizing it to make a diblock copolymer (pTAF) (RAFT) (RAFT) [46]. Using mix electrospinning with poly (caprolactone) (PCL) as the carrier polymer, this copolymer was formed into an electrospun nanofiber (ESF) scaffold to provide an efficient drug delivery method. Ribavirin is a medicine that is used to treat hepatitis C. It works by preventing viruses from multiplying and spreading; however, it might cause birth abnormalities if a child is conceived while the father is taking it. Protease inhibitors (NS5A inhibitors) have an unclear mechanism of action in treatment of hepatitis C. They may act by preventing the virus from replicating. They may also aid in the prevention of medication resistance (when a drug no longer works to treat a condition). All HCV genotypes are treated with these medicines. They can be taken by themselves or in combination with other drugs. Ledipasvir, a component of the combination medicine Harvoni, and elbasvir, a component of the combination treatment Zepatier, are two examples of directed inhibitors. To patients diagnosed with viral hepatitis, the importance of avoiding alcohol because it hastens cirrhosis and end-stage liver disease; the importance of eating a healthy diet and staying

physically active, especially for overweight patients; the importance of consulting a health professional before starting any new prescription pills; and the importance of consulting a health professional before starting any over-the-counter medications should all be emphasized. People should avoid or stop donating blood, tissue, or sperm, as well as the small but real risk of transmission to sex partners and when sharing personal items containing blood, such as toothbrushes, dental equipment, razors, nail clippers, glucose monitors, and lancet devices, to reduce the prevalence of hepatitis C. Hepatitis C therapy has been simplified, and a wide range of experts, including internal medicine and family practice physicians, nurse practitioners, physician assistants, and pharmacists, can now effectively manage HCV-infected patients. Children with hepatitis C and patients with severe HCV-related sequelae or advanced disease, such as those who require a liver transplant, may be best served by specialists such as infectious-disease physicians, gastroenterologists, pediatricians, and hepatologists. Genotyping is no longer required before starting hepatitis C therapy due to the availability of medications that are effective against a wide range of genotypes.

Pretreatment genotyping is still suggested for patients with cirrhosis and/or a history of failed hepatitis C treatment, since this information can help modify treatment regimens and enhance patient outcomes. With 8–12 weeks of oral medication, about 90% of persons infected with hepatitis C virus (HCV) can be cured of their infection, independent of HCV genotype. In collaboration with the International Antiviral Society–USA (IAS–USA), the Infectious Diseases Society of America (IDSA), and the American Association for the Study of Liver Diseases (AASLD) developed evidence-based recommendations for hepatitis C management to provide healthcare professionals with timely guidance as new therapies become available and integrated into hepatitis C treatment regimens and HBV reactivation was recently documented [47]. As a result, people starting HCV DAA treatment should be tested for HBV using HBsAg, anti-HBs, and anti-HBc antibodies. Also during HCV treatment, people who test positive for HBsAg and/or anti-HBc should be closely watched. Although there are many treatment plans of hepatitis C implicated in Pakistan, its prevalence rate is still very high due to use of injection drugs. It is transmitted by sexual activity with an infected person or the sharing of things such as razors or toothbrushes, unregulated tattooing, receiving donated blood and blood products, and needle stick injuries in healthcare settings. Hepatitis B and C infect around five and ten million persons in Pakistan, respectively [48]. Due to a lack of prevention, diagnostic, and treatment resources, as well as inadequately screened blood transfusions, improperly sterilized intrusive medical equipment, and risky injections, tens of thousands of additional patients are added each year. In order to lessen the large disease burden, the government has set a goal of eradicating hepatitis infections in the country by 2030. The program's goal is to set preventive measures and treatment plan with the provision of leadership and coordination. To avoid liver damage, people with hepatitis should maintain a healthy lifestyle. Begin by paying closer attention to your diet to safeguard your liver. Hepatitis does not

require a particular diet; instead, those with the disease should eat a healthy, well-balanced diet. Patients with hepatitis C should consume a diet high in fruits and vegetables. Whole grains such as oats, brown rice, barley, and quinoa must be included in the diet of HCV patients [49]. Lean protein can be found in fish, skinless chicken, egg whites, and lentils. Nuts, avocados, and olive oil, as well as low-fat or non-fat dairy products, are all good sources of healthy fats. Liver damage can be exacerbated by an unhealthy diet. You will gain weight if you eat too much high-calorie greasy, fatty, or sugary food, and fat will begin to build up in your liver. Cirrhosis, or scarring of the liver, may be accelerated by a "fatty liver" [50]. Hepatitis virus-targeting drugs may be hampered by fat in the liver. Butter, sour cream, and other high-fat dairy foods, as well as fatty cuts of meat and fried dishes, should be avoided. Sugar is abundant in cookies, cake, soda, and pre-packaged baked goods. The liver is harmed by salty foods and alcoholic beverages. Hepatitis patients should avoid eating raw or undercooked shellfish, according to some doctors, because they can carry viruses and bacteria.

8 Hepatitis C Prevention Methods

The most effective strategy to limit the spread of blood-borne infections among healthcare workers is to avoid occupational exposure to blood. Health-care workers should seek expert advice on infectious disease control to ensure blood safety. Potential for harm has decreased in recent decades due to better preventative tactics, but they still happen, exposing healthcare personnel to blood-borne illnesses. Those who are exposed to HCV-antibody-positive blood through needle-stick or sharps injuries have a 0.2% chance of becoming infected, according to a recent review of multiple studies [51]. Although there have been a few accounts of the hepatitis C virus being transmitted through blood splashed into the eye, the chances of this occurring are exceedingly remote. Between 2002 and 2015, no HCV transmission was observed among 458 healthcare workers who were exposed to mucosal membranes in the USA [52]. However, it was unclear whether the HCV-antibody-positive patients were infected at the time of exposure.

9 Hepatitis C and Pregnancy

All pregnant women should be checked for anti-HCV throughout their pregnancy unless they live in an area where the prevalence of (HCV RNA positive) is less than 0.1% [53]. Pregnant women with known risk factors should be checked throughout their pregnancy, regardless of the setting predominance. Anti-HCV-positive pregnant women should get a PCR test for HCV RNA to see if they are still infected. The total risk of transmitting HCV to an unborn child for an infected mother is between

4% and 8% every pregnancy [54]. During pregnancy or labor, transmission occurs, and there is no prophylactic treatment available to protect the newborn at this time. If the mother's HCV viral load is high and she also has HIV, she should be tested, which has a transfer rate of 8% to 15%; the danger is considerably increased [55]. The majority of infants who are born with this viral disease have no indications or symptoms. Hepatitis C is not transferred through nursing, according to study. According to standards, children should be tested for HCV antibodies no sooner than the age of 18 months, because anti-HCV from the mother may persist until then [56]. If a diagnosis is desired before the kid reaches the age of 18, testing for HCV RNA can be done at or after the infant's first well-child check at the age of 1–2 months. The testing should be repeated at a subsequent visit, regardless of the first test result.

10 Hepatitis C (HCV) Outbreaks

Hepatitis C virus (HCV) was first discovered in 1989 and has since grown to become the world's most pervasive health epidemic, infecting over 185 million people globally, becoming the leading cause of death and illness all over the world [57]. This infection is usually asymptomatic, and 50–80% of persons infected are unaware of their illness. One-third of those infected develop end-stage liver disease, cirrhosis, and hepatocellular carcinoma (HCC), with an estimated half-million individuals dying each year from these complications. Pakistan has an estimated eight million infected people, making it the world's second-largest burden behind China [58]. More than 3000 people were notified for outpatient primary care screening in 2019. There are eight infections linked to the outbreak. The suspected mode of transmission is non-sterile IV (intravenous) infusions of vitamins, antibiotics, steroids, and other drugs. In 2018, 425 people working in outpatient clinics were suspected of using dangerous injection techniques that could lead to hepatitis C. Non-sterile glassware and tubing were used to create IV (intravenous) infusions, which were not adequately reprocessed between patients also cause hepatitis C in 2017 in 584 cases according to research. The main causes of HCV transmission in undeveloped nations like Pakistan are a lack of suitable blood transfusion protocols, reuse of syringes and needles for tattooing and ear piercing, and insufficient cleaning of possibly contaminated surgical and dental equipment. According to a countrywide survey conducted in 2007, roughly 4.8% of the population was infected with HCV. Reusing syringes to access multidose ketamine vials that were likely used for more than one patient, accessed in the immediate patient treatment area with medication vials, and the space where they are prepared are not disinfected were the causes of hepatitis C in 121 cases, according to a 2016 study. In skilled nursing facilities, a 2013 epidemiological study discovered a link between HCV infection and podiatry, phlebotomy, and nail care [59] (Table 1).

Table 1 Hepatitis C (HCV) outbreaks as reported to CDC during 2008–2019 [59]

Setting	Year	State	Persons notified for screening	Outbreak-associated infections	Known or suspected mode of transmission	Comments
Outpatient primary care practice	2019	NY	>3000	8	IV (intravenous) infusions of vitamins, antibiotics, steroids, and other medications were prepared/administered using non-sterile technique. Scope of practice issues were identified: medical assistant prepared and administered injections and IV infusions	Investigation ongoing
Outpatient clinic	2018	CA	425	6	Suspected unsafe injection safety practices	
Alternative medicine practice	2017	NY	584	5	IV (intravenous) infusions were prepared using non-sterile glassware and tubing, which was not properly reprocessed between patients. Scope of practice issues were also identified with a phlebotomist preparing and administering injections and IV infusions	In addition to the five cases determined to be transmission-linked with HCV genetic sequencing, three clinic patients with resolved HCV may have had outbreak-associated infection
Vascular access clinic	2016	PA	121	2	Reuse of syringes to access multidose vials of ketamine that were possibly used for >1 patient; multidose vials accessed in the immediate patient treatment area; lack of disinfection of medication vials and medication preparation area	

(continued)

Table 1 (continued)

Setting	Year	State	Persons notified for screening	Outbreak-associated infections	Known or suspected mode of transmission	Comments
Skilled nursing	2013	ND	>500	46	Epidemiologic analysis suggested podiatry care, phlebotomy, and nail care performed at the skilled nursing facility were associated with HCV infection	
Hospital emergency room	2018	WA	2762	12	Narcotics tampering by nurse	
Hospital	2015	UT	7217	7	Drug diversion by nurse	

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11 Conclusion

By reading this chapter, readers can easily get access about HCV infection and can easily distinguish between different types of hepatitis. Hospitals should have separate wards for dealing with Hepatitis C patients for better patient care services, and different tests are performed before diagnosis based on patient disease response and conditions. Antivirals are given to HCV patients for better recovery and maintaining their immune system. Patients must be observed carefully for clinical outcomes, and it must also be assured that no drug interactions and toxicity occur during treatment. If the treatment is carried out by a general family physician, then he should be aware of detailed history of HCV patients.

References

1. Rossetto A, De Re V, Steffan A, Ravaioli M, Miolo G, Leone P, et al. Carcinogenesis and metastasis in liver: cell physiological basis. Cancers. 2019;11(11):1731.
2. Petruzziello A. Suppl-1, M3: epidemiology of hepatitis B virus (HBV) and hepatitis C virus (HCV) related hepatocellular carcinoma. Open Virol J. 2018;12:26.
3. Mehta P, Reddivari AKR. Hepatitis. StatPearls [Internet]. 2021.
4. Gomes C, Wong RJ, Gish RG. Global perspective on hepatitis B virus infections in the era of effective vaccines. Clin Liver Dis. 2019;23(3):383–99.
5. Li T-Y, Yang Y, Zhou G, Tu Z-K. Immune suppression in chronic hepatitis B infection associated liver disease: a review. World J Gastroenterol. 2019;25(27):3527.
6. Maucort-Boulch D, de Martel C, Franceschi S, Plummer M. Fraction and incidence of liver cancer attributable to hepatitis B and C viruses worldwide. Int J Cancer. 2018;142(12):2471–7.

7. Alavi M, Law MG, Valerio H, Grebely J, Amin J, Hajarizadeh B, et al. Declining hepatitis C virus-related liver disease burden in the direct-acting antiviral therapy era in New South Wales, Australia. J Hepatol. 2019;71(2):281–8.
8. Negro F. Natural history of hepatic and extrahepatic hepatitis C virus diseases and impact of interferon-free HCV therapy. Cold Spring Harb Perspect Med. 2020;10(4):a036921.
9. Lafferty L, Rance J, Treloar C. Who goes first? Understanding hepatitis C risk among injecting networks in the prison setting. Drug Alcohol Depend. 2018;183:96–101.
10. Houghton M. The discovery of the hepatitis C virus. HCV: The Journey from Discovery to a Cure. Springer; 2019. p. 19–27.
11. Zając M, Muszalska I, Sobczak A, Dadej A, Tomczak S, Jelińska A. Hepatitis C–New drugs and treatment prospects. Eur J Med Chem. 2019;165:225–49.
12. Elbaz T, Abdo M, Omar H, Hassan EA, Zaghloul AM, Abdel-Samiee M, et al. Efficacy and safety of sofosbuvir and daclatasvir with or without ribavirin in elderly patients with chronic hepatitis C virus infection. J Med Virol. 2019;91(2):272–7.
13. Li H-C, Lo S-Y. Hepatitis C virus: virology, diagnosis and treatment. World J Hepatol. 2015;7(10):1377.
14. El-Ghitany EM, Farghaly AG. Geospatial epidemiology of hepatitis C infection in Egypt 2017 by governorate. Heliyon. 2019;5(8):e02249.
15. Yooda AP, Sawadogo S, Soubeiga ST, Obiri-Yeboah D, Nebie K, Ouattara AK, et al. Residual risk of HIV, HCV, and HBV transmission by blood transfusion between 2015 and 2017 at the Regional Blood Transfusion Center of Ouagadougou, Burkina Faso. J Blood Med. 2019;10:53.
16. Edunjobi A, Aina F, Lewis K. The implication of exces-sive illicit drug and alcohol use on the liver and a possible link to the rising incidence of HCV infection in Farmington, New Mexico. Am J Hepato Gastroenterol. 2022;1(1):1003.
17. Sulkowski M, Cheng W-H, Marx S, Sanchez Gonzalez Y, Strezewski J, Reau N. Estimating the year each state in the United States will achieve the World Health Organization's elimination targets for Hepatitis C. Adv Ther. 2021;38(1):423–40.
18. Ioannou GN, Tang W, Beste LA, Tincopa MA, Su GL, Van T, et al. Assessment of a deep learning model to predict hepatocellular carcinoma in patients with hepatitis C cirrhosis. JAMA Netw Open. 2020;3(9):e2015626.
19. Butt AS. Epidemiology of viral hepatitis and liver diseases in Pakistan. Euroasian J Hepatogastroenterol. 2015;5(1):43.
20. El-Zanaty F. Ministry of Health and Population [Egypt], El-Zanaty and Associates [Egypt], ICF International, Egypt health issues survey 2015. Rockville: Ministry of Health and Population, ICF International Cairo; 2015.
21. Lanini S, Easterbrook PJ, Zumla A, Ippolito G. Hepatitis C: global epidemiology and strategies for control. Clin Microbiol Infect. 2016;22(10):833–8.
22. Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology. 2013;57(4):1333–42.
23. Organization WH. Global hepatitis report 2017. World Health Organization; 2017.
24. Lim AG, Qureshi H, Mahmood H, Hamid S, Davies CF, Trickey A, et al. Curbing the hepatitis C virus epidemic in Pakistan: the impact of scaling up treatment and prevention for achieving elimination. Int J Epidemiol. 2018;47(2):550–60.
25. Kishta S, Tabll A, Omanovic Kolaric T, Smolic R, Smolic M. Risk factors contributing to the occurrence and recurrence of hepatocellular carcinoma in hepatitis C virus patients treated with direct-acting antivirals. Biomedicine. 2020;8(6):175.
26. Hsu Y-C, Chen C-C, Lee W-H, Chang C-Y, Lee F-J, Tseng C-H, et al. Compositions of gut microbiota before and shortly after hepatitis C viral eradication by direct antiviral agents. Sci Rep. 2022;12(1):1–10.
27. Ali FAZ, Abdel-Maksoud FM, Abd Elaziz HO, Al-Brakati A, Elmahallawy EK. Descriptive histopathological and ultrastructural study of hepatocellular alterations induced by aflatoxin B1 in rats. Animals. 2021;11(2):509.

28. Tomlinson JE, Wolfisberg R, Fahnøe U, Patel RS, Trivedi S, Kumar A, et al. Pathogenesis, microRNA-122 gene-regulation, and protective immune responses after acute equine hepacivirus infection. Hepatology. 2021;74(3):1148.
29. Hoshida Y, Fuchs BC, Bardeesy N, Baumert TF, Chung RT. Pathogenesis and prevention of hepatitis C virus-induced hepatocellular carcinoma. J Hepatol. 2014;61(1):S79–90.
30. Murphy M, Gibney B, Gillespie C, Hynes J, Bolster F. Gallstones top to toe: what the radiologist needs to know. Insights Imaging. 2020;11(1):1–14.
31. Arafa M, Besheer T, El-Eraky A, Abo El-khair S, Elsamanoudy A. Genetic variants of XRCC1 and risk of hepatocellular carcinoma in chronic hepatitis C patients. Br J Biomed Sci. 2019;76(2):64–9.
32. Bertot LC, Adams LA. Trends in hepatocellular carcinoma due to non-alcoholic fatty liver disease. Expert Rev Gastroenterol Hepatol. 2019;13(2):179–87.
33. Kheirabad AK, Nategh R, Shokri F, Pashabeyg KR, Norouzi M. Evaluation of a novel enzyme-linked immuno assay model to detect E2 antigen and antibodies against core, NS3, NS4, and NS5 antigens of hepatitis C virus. Hepat Mon. 2020;20(9).
34. Abdel-Raheem EM, Mosallem FA, Raheem MMA, Mohamed A, Malek MG. Screening for hepatitis C virus infection among Minia city school students. Egypt Pediatr Assoc Gaz. 2018;66(3):66–70.
35. Paquette CE, Pollini RA. Injection drug use, HIV/HCV, and related services in nonurban areas of the United States: a systematic review. Drug Alcohol Depend. 2018;188:239–50.
36. Boudova S, Mark K, El-Kamary SS, editors. Risk-based hepatitis C screening in pregnancy is less reliable than universal screening: a retrospective chart review. Open forum infectious diseases. Oxford University Press US; 2018.
37. Charboneau RL. Engineering a prophylactic cap for multi-dose vial disinfection. Rowan University; 2021.
38. Cottone C, Bhamidimarri KR, editors. Evaluating CKD/ESRD patient with hepatitis C infection: how to interpret diagnostic testing and assess liver injury. Seminars in dialysis. Wiley Online Library; 2019.
39. Opoku B, Effah K, Yawson A, Antwi-Boasiako C, Djagbletey R, Abindau E, et al. Postgrad Med J Ghana.
40. Tiwari AK, Upadhyay AP, Arora D, Wadhwa T, Aggarwal G, Pabbi S, et al. Head-to-head comparison of Enzyme Linked Immunosorbent Assay (ELISA) and Enhanced Chemiluminescence Immunoassay (ECLIA) for the detection of Transfusion Transmitted Disease (TTD) Markers; HIV, HCV and HBV in blood donors, in India. J Virol Methods. 2020;285:113962.
41. Wong XZ, Gan CC, Mohamed R, Yahya R, Ganapathy S, Tan SS, et al. Hepatitis C core antigen testing to diagnose active hepatitis C infection among haemodialysis patients. BMC Nephrol. 2020;21(1):1–6.
42. Kutcher R, Smith GS, Sen F, Gelman SF, Mitsudo S, Thung SN, et al. Comparison of sonograms and liver histologic findings in patients with chronic hepatitis C virus infection. J Ultrasound Med. 1998;17(5):321–5.
43. Guss D, Sherigar J, Rosen P, Mohanty SR. Diagnosis and management of hepatitis C infection in primary care settings. J Gen Intern Med. 2018;33(4):551–7.
44. O'Brien TR, Yang H-I, Groover S, Jeng W-J. Genetic factors that affect spontaneous clearance of hepatitis C or B virus, response to treatment, and disease progression. Gastroenterology. 2019;156(2):400–17.
45. Lazarus JV, Roel E, Elsharkawy AM. Hepatitis C virus epidemiology and the impact of interferon-free hepatitis C virus therapy. Cold Spring Harb Perspect Med. 2020;10(3):a036913.
46. Dart A, Roy D, Vlaskin V, Limqueco E, Lowe NM, Srinivasan S, et al. A nanofiber based antiviral (TAF) prodrug delivery system. Mater Sci Eng C. 2022;133:112626.
47. Irvin R, Ward K, Agee T, Nelson NP, Vellozzi C, Thomas DL, et al. Comparison of hepatitis C virus testing recommendations in high-income countries. World J Hepatol. 2018;10(10):743.
48. Maqsood S, Iqbal S, Zakar R, Zakar MZ, Fischer F. Determinants of overall knowledge and health behaviours in relation to hepatitis B and C among ever-married women in

Pakistan: evidence based on Demographic and Health Survey 2017–18. BMC Public Health. 2021;21(1):1–13.
49. Sawicka B, Skiba D, Umachandran K, Dickson A. Alternative and new plants. Preparation of phytopharmaceuticals for the management of disorders. Elsevier; 2021. p. 491–537.
50. Eslam M, Sarin SK, Wong VW-S, Fan J-G, Kawaguchi T, Ahn SH, et al. The Asian Pacific Association for the Study of the Liver clinical practice guidelines for the diagnosis and management of metabolic associated fatty liver disease. Hepatol Int. 2020;14(6):889–919.
51. Pournaghi-Azar F, Mohseni M, Ghojazadeh M, Derakhshani N, Azami-Aghdash S. Prevalence and causes of needlestick injuries among dental care providers: a systematic review and meta-analysis. 2019.
52. Nijmeijer BM, Koopsen J, Schinkel J, Prins M, Geijtenbeek TB. Sexually transmitted hepatitis C virus infections: current trends, and recent advances in understanding the spread in men who have sex with men. J Int AIDS Soc. 2019;22:e25348.
53. Ahrens KA, Rossen LM, Burgess AR, Palmsten KK, Ziller EC. Rural–urban residence and maternal hepatitis C infection, US: 2010–2018. Am J Prev Med. 2021;60(6):820–30.
54. García-Romero CS, Guzman C, Cervantes A, Cerbón M. Liver disease in pregnancy: Medical aspects and their implications for mother and child. Ann Hepatol. 2019;18(4):553–62.
55. Tajiri H, Bessho K, Nakayama Y, Abukawa D, Iitsuka Y, Ito Y, et al. Clinical practice guidelines for the management of children with mother-to-child transmitted hepatitis C virus infection. Pediatr Int. 2022;64(1):e14962.
56. Schillie S, Wester C, Osborne M, Wesolowski L, Ryerson AB. CDC recommendations for hepatitis C screening among adults—United States, 2020. MMWR Recomm Rep. 2020;69(2):1.
57. El-Ghitany EM. Hepatitis C virus infection in Egypt: current situation and future perspective. J High Inst Public Health. 2019;49(1):1–9.
58. Jafri W, Siddiqui B, Awan S. HCV-discovery to elimination, "myth or reality". Hepatoma Res. 2018;4:54.
59. Khan A. Rapid point-of-care testing for hepatitis C–assessment of feasibility, knowledge of participants and outcomes. University of Otago; 2021.
60. Prevention CfDCa. Health care-associated hepatitis B and C outbreaks (≥2 cases) reported to the CDC 2008–2019 United States 11-May-2020. Available from: https://www.cdc.gov/hepatitis/outbreaks/healthcarehepoutbreaktable.htm.

Recent Developments in the Treatment of Bacterial Meningitis

Dao Le, Deborah Liaw, Nicole Naterelli, Brian Longbottom, and Charles Preuss

Abstract There are 1.2 million cases of bacterial meningitis each year, with about one fifth of these cases resulting in significant morbidity and mortality, usually in lower-resource countries. Early antibiotic treatment has been demonstrated to improve the morbidity and mortality of this disease. Because many organisms can cause bacterial meningitis, empiric antibiotic treatment must remain broad based on the likelihood of the causative organism, which can be estimated by the age of the patient. The most common organisms that cause bacterial meningitis include *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Listeria monocytogenes*, and *Haemophilus influenzae*. Most of the disease burden is concentrated in vulnerable populations, such as the neonatal and geriatric populations as well as immunocompromised individuals. Though there are currently many effective antibiotic therapies for bacterial meningitis, with the increasing issue of antibiotic resistance and remaining significant morbidity and mortality from bacterial meningitis, non-antibiotic therapies are currently being trialed to propel this area forward. In addition to antibiotic therapy, immunotherapy and vaccination show great promise in lessening the disease burden of bacterial meningitis worldwide.

Keywords Bacterial meningitis · Antibiotic therapy · Immunotherapy · Vaccination · *Streptococcus pneumoniae* · *Neisseria meningitidis* · *Listeria monocytogenes* · *Haemophilus influenzae* · Lyme meningitis

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1 Bacterial Meningitis

Bacterial meningitis is a bacterial infection of the meninges, the three-layer protective covering of the brain and spinal cord. The resulting meningeal inflammation and swelling can press on the brain and spinal cord, with a multitude of potential complications. As meningitis is a serious and life-threatening condition, prompt diagnosis and treatment are necessary to reduce associated morbidity and mortality [1].

Although meningitis-causing agents can be viral, bacterial, parasitic, or even non-infectious (arising from another condition, such as lupus), bacterial meningitis constitutes the most severe and serious form. Leading causes of bacterial meningitis in the United States include *Streptococcus pneumoniae*, Group B *Streptococcus*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Listeria monocytogenes*, and *Escherichia coli* [2]. The proportion of cases caused by each bacterial serogroup varies by age group; similarly, standard antibiotic treatment differs according to age. Clinical presentation and associated symptoms vary greatly depending on age and host immune status, though hallmark symptoms include fever, nuchal rigidity, photophobia, and/or purpuric rash. Non-specific symptoms can include headaches, dizziness, confusion, delirium, irritability, and nausea/vomiting [3].

1.1 Disease Epidemiology

The morbidity and mortality of bacterial meningitis is most pronounced in lower-resource countries, with a total of approximately 1.2 million cases worldwide per year. The prevalence, morbidity, and mortality of bacterial meningitis is dependent on region, causative organism, and age group. For example, *Neisseria meningitidis* meningitis is endemic to a region in sub-Saharan Africa that encompasses Senegal to Ethiopia. This region has approximately 350 million people at risk of *Neisseria meningitidis* meningitis per year, with relatively less access to antibiotics and vaccination as well [4].

The epidemiology of bacterial meningitis has undergone drastic changes resulting from widespread use of vaccines and antimicrobial treatment of pregnant women [5]. The incidence of bacterial meningitis in Western countries has declined by 3–4% per year to 0.7–0.9 per 100,000 per year since the 1990s [6], notably due to the introduction of four vaccines targeting meningitis-causing bacterial agents: a conjugate *Haemophilus influenzae* type b vaccine (1990), a 7-valent *S. pneumoniae* conjugate vaccine (2000), a 4-valent meningococcal vaccine (2005), and a 13-valent *pneumococcal* vaccine (2010). The decreasing proportion of disease caused by *Haemophilus influenzae* type b, *Neisseria meningitidis*, and *Streptococcus pneumoniae* serotypes reflects the positive impact of these vaccination efforts. Furthermore, as *Haemophilus influenzae* type b primarily affects infants aged

6–12 months, the 1990 implementation of its associated conjugate vaccine shifted disease burden from children under 5 years old to adults [7].

Prior to the implementation of the *H. influenzae* type b conjugate vaccine, an epidemiologic study including 13,974 US cases of bacterial meningitis from 1978 to 1981 described an overall incidence of 3.0 cases per 100,000. Incidence varied drastically with age; 76.7 cases per 100,000 were described for children under 1 year [8]. The three most common bacterial agents, accounting for over 80% of cases, included *H. influenzae*, *N. meningitidis*, and *S. pneumoniae*.

In 1986, a similar surveillance study for all cases of bacterial meningitis was conducted in five US states: Missouri, New Jersey, Oklahoma, Tennessee, and Washington, in addition to Los Angeles County [9]. The overall incidence described was greater than the prior study, likely owing to an improved system for searching active cases [5]. The three most common bacterial agents remained *H. influenzae* (45%), *N. meningitidis* (14%), and *S. pneumoniae* (18%). In children under 5 years old, 95% of meningitis was caused by type b strains of *H. influenzae*, suggesting a significant need for an effective immunization initiative [9]. In addition, the 19% case fatality rate described from *S. pneumoniae*-induced meningitis, compared to 3% from *H. influenzae*, highlighted the importance of addressing both bacterial agents.

In the early 1990s, following the introduction of the *H. influenzae* type b vaccine (Hib) for infants, the rate of bacterial meningitis declined by 55% [7]. More specifically, a 94% reduction in *H. Influenzae*-induced meningitis was described based on 1995 surveillance data including 22 counties of 4 states. As anticipated, this was accompanied by an increase in the median age of affected individuals from 15 months (1986) to 25 years (1995) [10]. These drastic shifts in epidemiologic patterns depict the success of this infant vaccination initiative.

However, the 1995 surveillance data demonstrated that *N. meningitidis* was the predominant meningitis-causing bacterial agent among children aged 2 to 18 [10]. Yet, in 2005, Menactra® was approved in the United States as the first 4-valent conjugate vaccine for immunization against meningococcal disease, lending way for further epidemiological shift. A population-based observational study in the United States described the decreasing incidence of *Neisseria meningitidis* infection from 0.721 per 100,000 people in 1997 to 0.123 per 100,000 people in 2010 [11].

The 1995 data also depicted a relatively high case fatality rate attributed to pneumococcal meningitis (21%), highlighting the need for an effective pneumococcal vaccine [10]. A 7-valent pneumococcal conjugate vaccine was ultimately introduced in 2000; the vaccine is termed "7-valent," as it consists of a solution of capsular antigen saccharides of *Streptococcus* serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F conjugated to diphtheria CRM 197 protein [12].

A baseline study on incidence, mortality, and morbidity of pneumococcal meningitis prior to the introduction of the 7-valent conjugant pneumococcal vaccine was conducted in England between 1998 and 2005. Of note, this data is considered baseline information as the 7-valent conjugated pneumococcal vaccine was implemented in the routine childhood immunization program in 2006. The researchers found the highest incidence in children aged 2–11 months; furthermore, using the

serotype distribution of isolates obtained in 2005, they described a 72% serotype coverage with the 7-valent conjugate vaccine for patients less than 2 years of age [13]. In line with this observation, a CDC surveillance study of bacterial meningitis in the United States from 1998 to 2003, following the 2000 7-valent vaccine implementation in the United States, depicted a significant reduction in the incidence of pneumococcal meningitis in patients less than 2 years of age [5].

In 2010, the 13-valent pneumococcal vaccine (PCV13) replaced the 7-valent conjugate vaccine for pediatric populations, and it was subsequently recommended for immunocompromised adults greater than or equal to 19 years old [14]. Implementation of these conjugate vaccines not only protected vaccinated individuals but led to a decline in disease incidence among unvaccinated children and adults via prevention of carriage and transmission of covered pneumococcal serotypes [15]. Although PCV13 was recommended for all adults ≥65 years in 2014, the recognition that pneumococcal disease incidence had been drastically reduced among adults through indirect effects from PCV13 use in populations led the Advisory Committee on Immunization Practices to revise this recommendation. PCV13 is now recommended for adults aged ≥65 years who do not have an immunocompromising condition, CSF leak, or cochlear implants, while the remaining recommended for immunocompromised adults ≥19 years [15].

Ultimately, the success of these vaccination initiatives is reflected by the declining incidence of *H. influenzae*, *S. pneumoniae*, *N. meningitidis*, and associated meningitis following the introduction of the conjugate *H. influenzae* type b vaccine, 7-valent *S. pneumoniae* conjugate vaccine, the 4-valent meningococcal vaccine, and the 13-valent *pneumococcal* vaccine. Epidemiologic patterns have subsequently changed, as peak incidence of bacterial meningitis has shifted from children under 5 years old to adults [7]. *S. pneumoniae* and *N. meningitidis* now represent the most common bacterial agents of community-acquired meningitis, rather than *H. influenzae*, the most common cause of bacterial meningitis prior to the introduction of the Hib conjugate vaccine; furthermore, whereas *N. meningitidis* was the most common meningitis-causing bacterial agent among children aged 2 to 18 based on the US 1995 surveillance data, *S. pneumoniae* is now the leading cause of bacterial meningitis in children and young adults. Despite the implementation of *pneumococcal* vaccines, serotype replacement contributes to its relatively higher incidence [6]. Different organisms continue to preferentially affect different individuals based on age and immunocompetency, and unvaccinated children are primarily affected.

1.2 Bacterial Agents of Meningitis

Although the proportion of meningitis-causing bacterial agents differs according to age group, the most common agents of bacterial meningitis include *Streptococcus pneumoniae*, Group B *Streptococcus*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Listeria monocytogenes*, and *Escherichia coli* [3]. As newborns often get meningitis following exposure to a multitude of pathogens during the vaginal delivery

[16], the meningitis-causing bugs are unique from those of other age groups. In addition, *Listeria monocytogenes* affects those with poor cell-mediated immunity, thereby affecting primarily newborns and the elderly [17].

In order of decreasing prevalence, newborns (0–6 months) are most affected by Group B *Streptococcus*, *E. coli*, and *Listeria* sp. [17]. The leading cause of bacterial meningitis in the remaining age groups is *Streptococcus pneumoniae*. This bacterial agent is encapsulated, thus leaving asplenic patients or sickle cells patients, who may be *functionally* asplenic, particularly susceptible to infection [18]. The second leading cause of bacterial meningitis is *N. meningitidis* in children and young adults and Gram-negative bacilli in the elderly. The polysaccharide capsule, lipooligosaccharide, and type IV pili contribute to the virulence of *N. meningitidis* [19]. Meningococcal infection can lead to life-threatening adrenal hemorrhage and subsequent hypotension due to insufficient production of cortisol, termed Waterhouse-Friedrichsen Syndrome [20]. Lastly, although the prevalence of *H. influenzae*-induced meningitis has decreased significantly following the implementation of the Hib vaccine, it remains the third most common meningitis-causing bacterial agent of children aged 6 months to 6 years. *Listeria* sp. is the third most common agent in newborns and elderly due to reduced cell-mediated immunity. The meningitis-causing bacterial agents and their associated age groups are summarized in Table 1.

1.3 Pathophysiology

The brain and spinal cord are protected by three meningeal layers: the dura mater, the arachnoid mater, and the pia mater. The dura mater is a tough, outer layer composed of fibrous connective tissue; the pia mater is the thin, innermost layer lying directly upon the surface of the brain; and the arachnoid mater is the middle layer lying between. The arachnoid and pia mater are collectively known as the leptomeninges, linked to one another via arachnoid trabeculae spanning the cerebrospinal fluid (CSF)-filled subarachnoid space [21]. The meninges act as a physical barrier to protect the central nervous system (CNS) and provide stability and anchorage, preventing side-to-side movement [21]. Furthermore, the cerebrospinal fluid within the subarachnoid space allows the meninges to act as a "shock absorber" in the event of rapid acceleration or deacceleration.

Table 1 Age-specific agents of bacterial meningitis

Age group	Newborn 0–6 months	Children 6 months to 6 years	Young adults 6–60 Years	Elderly 60+ Years
Bacterial agent	1. Group B *Streptococcus* 2. *Escherichia coli* 3. *Listeria monocytogenes*	1. *Streptococcus pneumoniae* 2. *Neisseria meningitidis* 3. *Haemophilus influenzae* type b	1.*Streptococcus pneumoniae* 2.*Neisseria meningitidis*	1. *Streptococcus pneumoniae* 2. Gram-negative bacilli 3. *Listeria monocytogenes*

The cerebrospinal fluid is normally sterile, with an absence of bacteria, viruses, or fungi and less than five white blood cells (WBC) per μL. Bacterial meningitis, however, is characterized by the entry of bacteria into the cerebrospinal fluid, resulting in inflammation of the leptomeninges [22]. Although some bacterial agents are spread from mother to infant upon delivery or via unprepared food (Group B *streptococcus* and *E. coli*, respectively), meningitis-causing bacteria typically colonize the nose and throat and are thus spread via respiratory secretions [2]. Respiratory transmission can occur via coughing and sneezing, sharing drinks and utensils, kissing, and living in close quarters with other individuals.

Bacteria first colonize mucosal linings, invade the bloodstream, and multiply. The resulting bacteremia allows bacteria to cross the blood-brain barrier (BBB) and invade the leptomeninges. In addition, bacteria can alter the permeability of the BBB, increasing the number of cells entering the cerebrospinal fluid. For example, *N. meningitidis* increases paracellular translocation by exploiting brain endothelial cell signaling [23]. In turn, cerebrospinal fluid WBC counts increase to between 100 and 10,000 cells/mm^3, with 80–95% being neutrophils [24]. Infiltrating WBCs release proinflammatory cytokines, leading to neuronal injury. Ultimately, bacterial colonization of the cerebrospinal fluid and resulting inflammation of the meninges may increase intracranial pressure. Furthermore, the breakdown of the blood-brain barrier and leakage of serum fluid, proteins, and cells into the subarachnoid space can cause interstitial and parenchymal edema [22].

Meningitis classically presents with altered mental status, high fever, and nuchal rigidity [25]. Photophobia is commonly described, which is thought to be caused by irritation of the basal meninges surrounding the diaphragma sellae [26]. Furthermore, meningococcal meningitis often presents with petechiae or purpura, commonly known as the “meningitis rash.” This complication of *Neisseria meningitidis* is associated with a high mortality rate, beginning with dermal microvascular thrombosis that rapidly becomes hemorrhagic skin necrosis [27]. Signs and symptoms can be non-specific and potentially differ by age, including headaches, dizziness, confusion, delirium, irritability, and nausea/vomiting [3]. For example, an infant presenting with unexplained irritability and failure to thrive may require a spinal tap to rule out meningitis.

1.4 Complications

Although morbidity and mortality of bacterial meningitis have decreased due to the implementation of effective antibiotics, meningitis-induced mortality remains a significant problem globally. The mortality for bacterial meningitis is estimated to be 10–15% of US cases, with delayed administration of antibiotics associated with increased mortality [1]. This is precisely why antibiotics are administered prior to receiving the diagnostic CSF bacterial culture to determine the bug, as this can take multiple days. Delayed antibiotic treatment can increase intracranial pressure,

potentially resulting in decreased cerebral perfusion, loss of consciousness, and/or death [1].

In addition, communicating hydrocephalus, in which the rate of CSF production in the choroid plexus is greater than the rate of CSF absorption, can result from meningeal scarring and fibrosis. CSF is normally produced in the choroid plexuses of the ventricles, absorbed by the arachnoid granulations extending into the venous sinuses, and ultimately drained into the venous system. Fibrosis, scarring, and inflammation of the arachnoid villi secondary to bacterial meningitis can impede CSF reabsorption, leading to excess cerebrospinal fluid in the ventricular system (communicating hydrocephalus), enlarged ventricles, increased intracranial pressure, and compression of the brain. A prospective nationwide cohort study from Dutch hospitals (2006–2009) found hydrocephalus to be the primary factor of mortality; a multivariate analysis found hydrocephalus to be an independent predictor of death, with an odds ratio of 7.81 (95% confidence interval 2.91–20.8) [28].

Meningeal fibrosis and cochlear ossification can also result in profound hearing loss; meningitis is responsible for more than half of acquired cases of deafness [29]. A 2006 retrospective review of 171 children with bacterial meningitis found that of 134 patients undergoing audiologic testing, 30.6% were found to have at least a unilateral mild sensorineural hearing loss [30]. Furthermore, the incidence of hearing loss was greater for patients infected with *S. pneumoniae* meningitis than patients with *N. meningitidis* meningitis.

Lastly, bacterial toxins in the cerebrospinal fluid and resulting swelling and pressure in the brain can disrupt function and result in seizures, brain damage, paralysis, or stroke. The probability of developing unprovoked seizures or epilepsy appears greater for patients with *Streptococcus pneumoniae*-induced meningitis. The resulting seizures tend to occur within 5 years of the infection and are often recurrent [31].

2 Treatment Options for Bacterial Meningitis

In order to reduce the likelihood of meningitis-induced complications, prompt antibiotic treatment is necessary. Diagnosis of meningitis requires a lumbar puncture (spinal tap), in which a healthcare professional inserts a needle between two lumbar vertebrae to retrieve a sample of cerebrospinal fluid. Spinal fluid testing reveals the cellular, protein, and glucose content of the CSF, in addition to the infectious agent.

Organism identification may take up to 48 hours, as a CSF culture must be performed to detect the growth of the specific meningitis-causing microorganism, whether it be bacterial, fungal, or viral [32]. Prior to receiving this test result, however, the CSF cellular, protein, and glucose content can be analyzed to seek patterns reflective of bacterial meningitis. The turbid-appearing cerebrospinal fluid of a bacterial meningitis patient is characterized by elevated white blood cells predominantly composed of neutrophils [24], an elevated protein concentration, and a decreased glucose concentration. Viral meningitis, in contrast, typically depicts clear cerebrospinal fluid with a normal glucose concentration.

Once the suspected diagnosis is bacterial meningitis, prompt administration of antibiotics is essential, as delays of just 3 to 6 hours are associated with increased mortality [1]. Thus, a regimen of antibiotic treatment is often initiated prior to the identification of the bacterial microorganism. Age and host immunocompetency are the primary factors that dictate the specific antibiotics administered. As children and young adults share similarities in the common meningitis-causing bacterial agents, both age groups should be treated with ceftriaxone and vancomycin [1]. Ampicillin should also be administered to patients who are immunocompromised or older than 50 in the event the bacterial agent is *Listeria monocytogenes*. Furthermore, given the unique bugs characteristic of neonatal meningitis, infants should receive a unique regimen of ampicillin and gentamicin or cefotaxime [16]. Lastly, individuals presenting with bacterial meningitis following head trauma or post-neurosurgical procedure should receive vancomycin and ceftazidime or cefepime to cover methicillin-resistant *Staphylococcus aureus* [1]. The age-specific antibiotic regimens are summarized in Table 2.

Dexamethasone has been shown to increase survival and decrease neurologic complications in patients with meningitis caused by *S. pneumoniae* [1]. Although there is no evidence that glucocorticoids improve outcomes for meningitis caused by other bacteria, it is recommended that dexamethasone be intravenously administered prior or at antibiotic initiation when the causative organism has not yet been elucidated. Dexamethasone should then be continued if the CSF culture depicts *S. pneumoniae*.

In addition to initiating a regimen of antibiotics for those suspected of having bacterial meningitis, close contacts should be treated prophylactically with ciprofloxacin, rifampin, or ceftriaxone [1]. Rifampin can result in orange discoloration of body fluids including sweat, saliva, urine, feces, and tears, which can stain contact lenses [33]. Furthermore, both rifampin and ciprofloxacin are contraindicated in pregnant women due to adverse effects in laboratory animals [34].

Table 2 Age-specific antibiotic treatment of bacterial meningitis

Age group	Newborn 0–6 months	Children 6 months to 6 years	Young adults 6–50 years	Elderly 50+ years
Antibiotic regimen	Ampicillin + gentamicin or cefotaxime	Ceftriaxone + vancomycin	Ceftriaxone + vancomycin	Ceftriaxone + vancomycin + ampicillin
Duration of treatment and respective dose	14–21 days Ampicillin – 300 mg/kg Gentamicin – 5 mg/kg Cefotaxime – 50 mg/kg	10–14 days Ceftriaxone – 100 mg Vancomycin – 60 mg	10–14 days Ceftriaxone – 2 g Vancomycin – 750–1000 mg	14–21 days Ceftriaxone – 2 g Vancomycin – 15–20 mg/kg Ampicillin – 2 g

2.1 Antibiotic Delivery

The blood-brain barrier and blood-CSF barrier impede the penetration of antibiotics into the cerebrospinal fluid; specifically, intact tight junctions between vascular endothelial cells limit antibiotic penetration [35]. This poses a therapeutic challenge as the concentration of an antibiotic in the cerebrospinal fluid depends on the balance of drug entry and elimination through the blood-brain barrier [36]. However, the separation of endothelial tight junctions and increased vesicular transport resulting from meningeal inflammation increases the permeability of the BBB, allowing greater CSF penetration.

Whereas the penetration of lipophilic antibiotics, such as chloramphenicol, rifampin, and trimethoprim, may reach 30–40% of serum concentration, the degree of CSF penetration of other antibiotics, such as β-lactams, may only increase to 5–10% of serum concentration [35]. As penetration remains relatively low, antibiotic doses are often increased for CNS infections compared to other bacterial infections. Table 3 depicts a non-exhaustive list of recommended intravenous antibiotic doses for infants, children, and adults with bacterial meningitis [37]. Over the course of antibiotic treatment, reduced meningeal inflammation, and thus restoration of BBB integrity, is expected; high dose antibiotic delivery should be maintained to achieve sufficient CSF concentrations.

Antibiotics for the treatment of bacterial meningitis should be administered intravenously to achieve adequate concentrations despite difficulty in penetration. Intravenous-to-Oral Switch Therapy, in which patients begin intravenous antibiotics and later switch to oral antibiotics, is not recommended [38]. Instead, prolonged intravenous antibiotic use is indicated, with the duration dependent on the causative agent.

Table 3 Recommended antibiotic doses in infants, children, and adults with normal renal and hepatic function, non-exhaustive list [36]

Antibiotic	Infants and children	Adults
Ampicillin	300–400 mg/kg [6]	12 g [4]
Cefotaxime	300 mg/kg [6–8]	8–12 g [4–6]
Ceftriaxone	100 mg/kg [12–23]	4 g [12]
Gentamicin[a]	7.5 mg/kg [8]	5 mg/kg [8]
Rifampin	20 mg/kg [23][b]	600 mg [23]
Trimethoprim-sulfamethoxazole[c]	10–20 mg/kg [6–12]	10–20 mg/kg [6–12]
Vancomycin[d]	60 mg/kg [6]	30–60 mg/kg [8–12]

Dosages reported as total daily dose (dosing interval in hours)

[a]Peak and trough serum concentration monitoring required

[b]Maximum dosage of 600 mg

[c]Dose based on trimethoprim component

[d]Serum trough concentration must be maintained at 15–20 μg/mL in adult patients receiving intermittent bolus administration

3 Pneumococcal Meningitis

Streptococcus pneumoniae is an encapsulated, Gram-positive, lancet shaped, facultative anaerobic organism that can cause meningitis, an inflammation of the membranes that surround the brain and spinal cord [39]. The most common agents of community-acquired bacterial meningitis are *Streptococcus pneumoniae* and *Neisseria Meningitidis* with *Streptococcus pneumoniae* responsible for two-thirds of the cases in Europe and United States. Even with advances in medicine, mortality is still high ranging between 16% and 37% with neurological sequelae occurring in between 30% and 52% of patients who have survived [40].

Streptococcus pneumoniae is the leading cause of bacterial meningitis in children 1–24 months and is the second most common cause in children older than 24 months [41]. Patients at risk are at opposite sides of the age spectrum, with greater risk in the population less than 2 years old and greater than 50 years old. Furthermore, individuals with certain underlying conditions are at higher risk, such as those with a previous splenectomy, sickle cell, multiple myeloma, hypogammaglobulinemia, alcoholism, chronic liver or kidney disease, malignancy, Wiskott-Aldrich syndrome, thalassemia major, and diabetes mellitus and in children with cochlear implants.

Pneumococcal conjugate vaccine includes many virulent serotypes, so invasive pneumococcal disease incidence has decreased due to herd immunity [42]. However, management of pneumococcal meningitis is more complicated than it was 20 years ago when penicillin was the main line of treatment, due to antibiotic resistance [43]. Penicillin became less effective around the 1970s when the previously recommended doses were not sufficient to reliably kill the pneumococcal isolates. Chloramphenicol was also advised against, especially for penicillin-resistant pneumococcus because the recommended dose was not achieving high enough bactericidal activity. More recently, starting in the 1980s, cefotaxime or ceftriaxone became the most recommended empiric treatment for children with meningitis. However, about a decade later, many patients started displaying signs of resistance even to extended spectrum cephalosporins [43], in which the new addition of vancomycin was found to be synergistic and helpful in eliminating pneumococcal meningitis [44].

If the combination of vancomycin and ceftriaxone is not effective, shown through further deterioration of the patient's condition or no decrease in bacterial colonies in cerebrospinal fluid (CSF), rifampin can be added. Lastly, meropenem is an approved antibiotic even though studies have not shown well-established activity against resistant pneumococcal isolates [45]. Despite expanding antibiotic resistance since the 1970s, the mortality of pneumococcal meningitis cases in US adults and children has not been affected [46]. The main line of treatment used by physicians is ceftriaxone with vancomycin even if the strain demonstrates susceptibility to ceftriaxone [43].

Novel adjunctive therapies targeting inflammation, coagulation, and complement are currently being studied to improve the prognosis of meningitis. In adult

patients, dexamethasone was introduced to reduce inflammation since outcome of meningitis was shown to be related to the severity of inflammation in the subarachnoid space. Even though dexamethasone's mechanism of action has not been fully elucidated, studies have shown widespread decreases in inflammatory cytokines from an array of leukocytes [47, 48] and increases in the production of anti-inflammatory cytokines such as IL-10, decreases in reactive oxygen species, and leukocyte adhesion [49]. Dexamethasone usage is currently controversial with many mixed results because the efficacy is unclear and using ceftriaxone with dexamethasone reduces ceftriaxone's penetration into the central nervous system (CNS) decreasing ceftriaxone's efficacy [50].

Other adjunctive treatments being studied are fluoroquinolones such as gatifloxacin and moxifloxacin because they have demonstrated similar efficacy against cephalosporin resistant pneumococcal isolates with ceftriaxone and vancomycin [51, 52]. Daptomycin, rifampin, and clindamycin have been introduced as potential targets for adjunctive therapy as they have been shown to decrease inflammation and cortical damage compared to ceftriaxone alone [53, 54]. Glycerol, an essential component of cell membranes, has traditionally been used in neurosurgery, neurology, and ophthalmology to decrease tissue pressures. It is thought to increase plasma osmolality, resulting in increased cerebral blood flow and oxygenation, and some preliminary studies with glycerol administration have exhibited a reduction in incidence of neurological sequelae [55]. However, these studies also yield inconclusive results due to differences in reduction of various neurological sequelae in addition to some harmful effects seen in some of the clinical trials [56].

3.1 Inhibition of Complement

Complement is important for the initial phases of host defenses against *S. pneumoniae*, of preventing bacterial colonization from becoming invasive. Part of the complement cascade is cleavage of complement components, ultimately creating MAC attack which is a membrane pore that causes cell lysis. However, in the transition, C3a, C4a, and C5a are also created during cleavage which are potent anaphylatoxins. Therefore, a C1 inhibitor, a mediator of the first step of classical pathway, was tested in a mouse model, resulting in decreases in cytokine levels, bacterial growth, and increased survival of the mice [57].

3.2 Inhibition of Proinflammatory Cytokines

Inflammatory cytokines such as TNF-α, interleukin (IL) 1, and 6 are produced by the body as a necessary response to bacterial infection but, at high levels, can produce complications. Thalidomide, a TNF-α inhibitor used traditionally to treat multiple myeloma, has shown to decrease TNF-α and pleocytosis in

CSF. TNF-α-converting enzyme (TACE) inhibitors have also been shown to increase survivability of pneumococcal mouse models [58]. Blocking IL 6 and 10 produced similar results to thalidomide and TACE inhibitor with the addition of decreasing CSF protein [59].

3.3 Inhibition of Pattern Recognition Receptors

Pattern recognition receptors are important for host cells to discern specific molecules on pneumococcus that are essential for their survival. Once TLR2, TLR4, TLR9, and nucleotide oligomerization domain 1 (nod 1) are activated on antigen-presenting cells (APCs), they release cytokines, inducing an inflammatory cascade. To prevent this subsequent step, TLR inhibitors such as tyrphostin AG126, were tested in mouse models. Tryphostin AG126 inhibits phosphorylation of extracellular signal-regulated kinase ½ (ERK1/2) in microglial cells, and when administered into pneumococcal mouse models, TNF- α, pleocytosis, and edema were decreased [60].

3.4 Inhibition of Caspases

NOD-like receptors (NLR) are another group of pattern recognition receptors that, when activated, lead to cleavage and activation of caspase-1. This leads to subsequent cleavage of inflammatory cytokines and programmed neuronal death within the hippocampal dentate gyrus [61]. Mouse models with knocked out caspase-1 showed lower levels of IL-1B, cytokines, and sequelae. Inhibition of caspase-3 has shown protective effects against neuronal damage. Caspase inhibitor z-VAD-fmk shows reduced hippocampal apoptosis, and pan-caspase inhibitor bocaspartyl (OMe)-flouromethylketone exhibited less cognitive decline [62].

3.5 Antioxidant Agents

Antioxidant therapies are also being studied in animal models, such as iNOS inhibitors, uric acid, and N-acetyl-L-cysteine (NAC). They have shown ambiguous results with many beneficial effects such as reduced inflammatory cytokines, cortical damage [63], CSF pleocytosis, and incidence of hearing loss [63], while some unfavorable effects seen were impaired bactericidal activity, increased neuronal death, and diminished learning function [63].

4 Meningococcal Meningitis

Meningococcal meningitis is caused by *Neisseria meningitidis*, an encapsulated aerobic or facultative anerobic, Gram-negative diplococci that is an important cause of community-acquired bacterial meningitis. It is a common cause of bacterial meningitis in children, especially under 2 years old [64], and two-thirds of patients suffering from meningococcal meningitis in the first year of life are under 6 months old.

They have many virulence factors, some examples being the polysaccharide capsule, surface adhesion proteins, lipooligosaccharide (LOS), and iron sequestration mechanisms [65]. Early meningococcal vaccines were made through killed/inactivated whole bacteria, and that was not successful because of very high reactogenicity of LOS. Antibiotics became the mainstay treatment until antibiotic resistance started to develop and vaccine research picked up, focusing more instead on targeting the polysaccharide capsule and components of the cell wall [66]. The majority of meningococcal disease is caused by serotypes A, B, C, Y, and W135, of which each serotype is prevalent in different geographical areas. Serotypes C and Y account for more than two-thirds of the cases in the United States [67]. It has been shown in many early studies that the main component deciding whether individuals are infected with *N. meningitidis* is whether they have antibodies [68]. Patients are also more susceptible to invasive *N. meningitidis* if they are deficient in complement, especially C5, C6, C7, and C8.

Polysaccharide vaccines (PS) are not very immunogenic in infants or children except for the serotype A polysaccharide vaccine. However, children and infants respond well to vaccines that have polysaccharides conjugated to proteins; this stimulates T-cell-dependent reactions, resulting in production of memory B cells and IgG antibodies. Therefore, in the context of outbreaks, the PS-conjugate vaccine is recommended for children under the age of 10, whereas the PS vaccine is already 85–90% effective in adults [69].

Early recognition and treatment are imperative because patients can deteriorate rapidly with a significant possibility of death. Meningococcal meningitis can be difficult to diagnose because there are many nonspecific symptoms such as headache, nausea, vomiting, headache, myalgia, rash, sore throat, and upper respiratory symptoms, many of which overlap with the influenza virus, and both occur concurrently in the winter. Furthermore, the classic triad of fever, altered mental status, and nuchal rigidity only occur in about 44% of people. There is high mortality of approximately 10–14% even in patients that receive treatment and as high as 50% of patients that are untreated [69].

Sequelae are also common, occurring in 11–19% of patients, including but not limited to chronic pain; skin scarring; limb amputation; neurological impairment such as hearing, visual, and motor function impairments; arthritis; and post-infection inflammatory syndrome [70]. Management of meningococcal meningitis includes antibiotics, isolation and contact precautions, infectious disease consultation, and management of coagulopathies.

Empirical treatment with ceftriaxone or cefotaxime should be given to patients, while physicians are waiting on cultures to come back since bacterial meningitis caused by *Neisseria* sp. looks similar to meningitis caused by other organisms. They are preferable to other antibiotics due to easy dosing and high efficacy. If the isolate is susceptible to penicillin, penicillin G can be administered instead and chloramphenicol if the patient is allergic to penicillin or beta lactams. The duration of penicillin should be 5–7 days, but can go up to 21 days depending on how sensitive the strain of *Neisseria* sp. is to antibiotics [71].

The dosing for ceftriaxone is 2 g intravenously every 12 hours, and cefotaxime dosing is 2 g every 6 hours. Penicillin G should be given at 300,000 units/kg per day intravenously (IV) or intramuscularly (IM) with a maximum dose of 24 million units per day. Penicillin G is usually given as four million units every 4 hours IV in adults and patients over 1 month old. Chloramphenicol is given at 100 mg/kg per day IV every 6 hours with maximum dose of 4 g per day and should be monitored for toxicity. The recommended therapeutic levels include trough of 5–10 mcg/mL and peak of 10–20 mcg/mL. Dexamethasone dosing is 0.15 mg/kg with a maximum dose of 10 mg every 6 hours but should be discontinued if the culture confirms meningococcal meningitis due to absent therapeutic effect [70].

If the patient has sepsis, he/she will need supportive care such as intravenous fluid resuscitation and vasopressors such as norepinephrine. If the patient has disseminated intravascular coagulation (DIC), aggressive hydration, blood transfusions, platelets, and coagulation factor replacement should be administered to the patient.

Prophylaxis is very effective and should be considered when close contacts become infected. They will show symptoms within 10 days of infection. Close contacts are generally others that are within 3 feet of the patient for more than 8 hours and have been exposed to his/her respiratory oral secretions. Treatments can include ceftriaxone, rifampin, or ciprofloxacin. Ceftriaxone can be given 250 mg intramuscularly as a one-time dose, whereas in patients that are less than 15 years old, then 125 mg. Rifampin can be given in 600 mg doses orally or IV twice daily for 2 days, whereas in patients less than 1 month, give 5 mg/kg and 10 mg/kg in patients older than 1 month. For ciprofloxacin, give 500 mg orally to adult patients and 20 mg/kg to pediatric patients older than 1 month as a one-time dose. Lastly, azithromycin can be given as a 500 mg orally as a one-time dose, whereas in pediatric patients over 1 month, give 10 mg/kg [72].

5 Treatment of *Listeria monocytogenes* Meningitis

Listeria monocytogenes is a Gram-positive facultative intracellular anaerobe that is predominantly transmitted via contaminated food such as unpasteurized dairy products and deli meats. Though it may cause only mild gastrointestinal symptoms in immunocompetent individuals, it can penetrate the blood-brain barrier and cause meningitis in immunocompromised populations such as infants and the elderly. In

fact, *Listeria monocytogenes* is the third most common meningitis etiology in elderly individuals only after *Streptococcus pneumoniae* and *Neisseria meningitidis* [73]. This section will explore the current antibiotic and anti-inflammatory treatments available for *Listeria monocytogenes* meningitis and the special considerations required for specific populations.

5.1 *Antibiotic Treatment of* **Listeria monocytogenes** *Meningitis*

Listeria monocytogenes meningitis can be fatal with inadequate antibiotic therapy. A retrospective cohort study performed in Denmark showed that the most prominent risk factors for mortality in 30 days after *Listeria monocytogenes* meningitis were sepsis and hemodynamic instability, altered mental status, and insufficient antibiotic therapy that does not cover this species in high-risk populations [74]. Therefore, it is imperative to ensure proper empiric antibiotic coverage, especially in immunocompromised individuals. *Listeria monocytogenes* is notably unresponsive to antibiotic therapy with cephalosporins, which are typically a popular choice once meningitis is suspected. Antibiotics that are considered proper definitive treatment for *Listeria monocytogenes* include benzylpenicillins (such as penicillin G), aminopenicillins (such as ampicillin), meropenem, sulfamethoxazole with trimethoprim, and piperacillin with tazobactam. A study has shown that treatment of *Listeria monocytogenes* meningitis with benzylpenicillins or aminopenicillins may result in lower 30-day mortality risks in comparison to treatment with meropenem [74].

Though ampicillin is currently the drug of choice for *Listeria monocytogenes* meningitis, other antibiotics and adjunctive therapies also deserve some discussion. Amoxicillin has been shown to have increased bactericidal activity against *Listeria monocytogenes* in in vitro studies in comparison to ampicillin, which requires at least 9 g per day for 21 days when treating *Listeria monocytogenes* meningitis in an adult. In in vitro studies, a dose of only 8 g per day is required for effective bactericidal action of amoxicillin against *Listeria monocytogenes* [73]. Trimethoprim with sulfamethoxazole may be considered as an alternative treatment in those with severe penicillin anaphylactic reactions due to its excellent penetration of the brain and nervous system. However, it does have reduced efficacy in comparison to the aminopenicillins and even fluoroquinolones in the treatment of *Listeria monocytogenes* meningitis in experimental models [73]. Fluoroquinolones, such as levofloxacin, can be extremely effective agents in treating *Listeria monocytogenes* meningitis due to their excellent ability to penetrate tissues and cells. The downside to empiric treatment of meningitis with fluoroquinolones, however, is that fluoroquinolone administration can cause increased teichoic acid release if *Streptococcus pneumoniae*, a far more common species in meningitis, is the causative agent. Such release can contribute to more brain damage in patients during their treatment

course [73]. Therefore, fluoroquinolones can be considered for more definitive treatment of *Listeria monocytogenes* meningitis once this causative agent is identified with little response to ampicillin. Another agent that can be used to treat *Listeria monocytogenes* meningitis is linezolid, which has excellent penetration of the cerebrospinal fluid (CSF) and does not have the same teichoic acid release effects in *Streptococcus pneumoniae*. However, it has only shown efficacy against *Listeria monocytogenes* in a few in vitro studies and has not been corroborated by any clinical trials [73].

Aminopenicillin combination with another antibiotic, specifically an aminoglycoside like gentamicin, has been shown to reduce the likelihood of mortality in practice [75]. In vitro studies have also demonstrated the best killing rates of *Listeria monocytogenes* in ampicillin and gentamicin combinations despite gentamicin's relative ineffectiveness as regards to killing intracellular bacteria [73]. Another potential effective treatment regimen for *Listeria monocytogenes* meningitis is ampicillin with trimethoprim plus sulfamethoxazole, but the small sample size of the study regarding this treatment regimen is unable to fully support this regimen in clinical practice [73]. The different antibiotic regimens for *Listeria monocytogenes* meningitis can be found in Table 4.

5.2 *Anti-inflammatory Treatment of* **Listeria monocytogenes** *Meningitis*

Though many other types of meningitis demonstrate reduced mortality with anti-inflammatory therapy such as dexamethasone, *Listeria monocytogenes* meningitis is associated with worse outcomes when steroid therapy is administered [75]. This may be due to the worsened immunity associated with steroid use, and due to the facultatively intracellular nature of *Listeria monocytogenes*, strong cell-mediated immunity is essential to fighting off this infection [73]. Therefore, if *Listeria monocytogenes* is determined to be the causative agent of meningitis, it is recommended to terminate dexamethasone or any other steroid administration [73].

Table 4 Antibiotics used for the treatment of *Listeria monocytogenes* meningitis along with their total daily doses

Antibiotic	Daily dosage	Form/frequency
Penicillin G	24 million units	i.v./every 4 hours
Ampicillin	9–12 g	i.v./every 4 hours
Amoxicillin	8 g	i.v./every 4 hours
Meropenem	6 g	i.v./every 8 hours
Gentamicin	5 mg/kg	i.v./every 8 hours
Trimethoprim/sulfamethoxazole	10–20 mg/kg	i.v./every 8 hours
Levofloxacin	1000 mg	i.v./every 24 hours
Linezolid	1200 mg	i.v./every 12 hours

5.3 *Treatment of* Listeria monocytogenes *Meningitis in Special Populations*

As previously stated, *Listeria monocytogenes* meningitis does not typically manifest in healthy, immunocompetent individuals. There should, however, be an index of suspicion in any young patient presenting with meningitis symptoms since *Listeria monocytogenes* meningitis has also been shown to occur in immunocompetent and healthy children. Even in completely immunocompetent children, *Listeria monocytogenes* meningitis can have severe sequelae, such as acute hydrocephalus and rapid progression to death. Therefore, treatment for *Listeria monocytogenes* meningitis should be actively considered in instances where a third-generation cephalosporin does not result in clinical improvement within a few days in healthy children with a meningitis picture. A treatment regimen consisting of ampicillin (200 mg/kg/day in 4 doses) and gentamicin (5 mg/kg/day) for 21 days has been demonstrated to reduce morbidity and mortality in healthy children [17].

In addition to children, there are other populations that deserve attention when it comes to *Listeria monocytogenes* meningitis risk as well. A multisite study conducted in Korea showed that the most predictive risk factor of *Listeria monocytogenes* meningitis was immunosuppressive therapy, while the second most predictive risk factor was chronic liver disease [76]. Increased mortality was also associated with delayed antibiotic therapy beyond 6 hours of symptoms and presentation, suggesting that empiric antibiotic therapy for *Listeria monocytogenes* meningitis should be initiated as rapidly as possible in these specific populations when presenting with a meningitis picture [76].

Some other groups may require early empiric antibiotic treatment covering *Listeria monocytogenes* as well when showing meningeal signs. The largest group at increased risk for *Listeria monocytogenes* meningitis consists of the elderly population above the age of 65 [77]. Old age, however, is not considered an independent risk factor for *Listeria monocytogenes* meningitis; rather, its association with decreased cell-mediated immunity might contribute to older populations being more adversely affected [78]. Listeriosis in pregnant women may result in only a mild febrile illness in mothers, but a greater risk of neonatal meningitis and sepsis. Therefore, *Listeria monocytogenes* should always be considered as a cause of fever in pregnant women, and ampicillin or amoxicillin can be initiated along with a bodily fluid culture to confirm infection. Ampicillin and amoxicillin are first line for these cases and can be administered 6–12 g per day IV and 100 mg/kg/day orally respectively for 14 days [79].

6 Neonatal Meningitis

6.1 *Empirical Antibiotic Therapy for Neonatal Meningitis*

Group B *Streptococcus* (GBS) is the most common cause of bacterial meningitis in neonates, whereas *Escherichia coli* is the most common cause of mortality. *Listeria monocytogenes* is also an important contributor to neonatal bacterial meningitis.

The CDC developed guidelines for treating GBS. For GBS and *E. coli*, ampicillin plus gentamicin are recommended as empirical antibiotic therapy [80, 81]. More community extended-spectrum beta-lactamase (ESBL) producers have emerged as causes of neonatal meningitis, which has been associated with nosocomial *E. coli* and *Klebsiella pneumoniae* infections [81–84]. ESBLs confer resistance to beta-lactam antibiotics, such as penicillins, cephalosporins, and the monobactam aztreonam [80, 84, 85].

Cefotaxime can be used empirically, as well. Ceftriaxone is not recommended in neonates due to acute bilirubin encephalopathy [80, 86].

Ampicillin and cefotaxime may be used in combination as an alternative, but there is a greater risk of necrotizing enterocolitis, more resistant Gram-negative organisms and invasive candidiasis [80, 87]. Therefore, ampicillin and gentamicin remain the mainstay treatment.

6.2 *Group B* Streptococcus *Treatment*

Penicillin G can be used to treat GBS in neonates. If empiric antibiotic therapy with ampicillin and gentamicin is administered, then gentamicin can be discontinued once GBS meningitis is confirmed by culture, and ampicillin can be continued alone [80]. Treatment should last for 14 days for uncomplicated GBS meningitis; some studies recommend 21 days at least [80]. Ampicillin and gentamicin can be continued until clearance of CNS and bacteremia are documented by lumbar puncture [80, 88].

6.3 Escherichia coli *(*E. coli*) Meningitis Treatment*

E. coli meningitis should be treated for 21 days minimum [80, 89]. Complications, such as ventriculitis, subdural effusions, and brain abscesses, necessitate increased duration of treatment [79, 89]. Ampicillin-resistant *E. coli* can be treated with cefotaxime. Despite the increase in resistant strains to ampicillin and gentamicin, these are still used in combination to empirically treat neonatal sepsis. Meropenem has been shown to be effective in treating community-acquired ESBL??? Strains; these strains will have more resistance against the penicillins, cephalosporins, and aminoglycosides [80, 82, 83, 90–93]. The beta lactam ring in penicillins and cephalosporins will be degraded by the beta-lactamases.

6.4 Listeria monocytogenes *Treatment*

Listeria monocytogenes meningitis is rare in previously healthy, immunocompetent children. It can be severe and carries a high rate of mortality if not diagnosed early in this group. It tends to infect pregnant women, neonates, the elderly, and the

immunocompromised. Only 5 cases had positive blood cultures out of 21 cases of previously healthy, immunocompetent children [17].

In patients that do not improve with first-line antibiotics (extended-spectrum cephalosporins), then *Listeria* sp. should be on the differential. Gram-staining may appear negative, whereas real-time polymerase chain reaction (RT-PCR) is more sensitive. First-line empiric treatment may include third-generation cephalosporins and vancomycin [17, 94]. As soon as *Listeria* sp. is confirmed via CSF or RT-PCR, treatment for *Listeria* sp. meningitis should be ampicillin and an aminoglycoside like gentamicin or amikacin. Vancomycin will not penetrate the CSF well and has not been successful in in vitro studies [17, 95, 96]. In refractory CNS listeriosis, trimethoprim-sulfamethoxazole (TPM/SMX) has been used as an alternative [17, 97]. TPM/SMX can cross the blood-brain barrier well and has a bactericidal effect on intracellular *Listeria monocytogenes* [97]. Carbapenems, alone or in combination with an aminoglycoside, have also been used as an alternative [17, 98–100]. Treatment lasted 10 days to 8 weeks [17].

6.5 Anti-inflammatory Therapy

The alpha7 nicotinic receptor (α7 nAChR) impairs the immune response against neonatal sepsis and meningitis (NSM). Memantine has been shown to have an effect on α7 nAChR and has been shown to prevent *E. coli*-induced NSM in a mouse model. Memantine also exhibited synergy with ampicillin in *E. coli* K1 (E44)-infected HBMEC and neonatal mice with bacteremia and meningitis from E44. Based on RNA sequencing data, use of memantine compared to no treatment decreased inflammatory factors and increased anti-inflammatory factors in BMEC cells. The NF-κB pathway can be affected by these factors. Circulating BMEC (cBMEC) could be biomarkers for NSM, and memantine reduces cBMEC that is increased in E44-infected mice [101].

6.6 Other Adjunctive Therapies

For GBS meningitis, one study by Zhu et al. demonstrated that penetration of the blood-brain barrier (BBB) happened first in the meningeal and cortex capillaries and involved host cell signaling factors $S1P_2$, EGFR, and CysLT1. GBS' utilization of these factors to penetrate the BBB was demonstrated by the pharmacologic inhibition, gene knockout, knockdown cells, gene knockout animals, and interrogation upstream and downstream of $S1P_2$, EGFR, and CysLT1; in animals with GBS meningitis, inhibiting these factors showed improved outcome when used as adjunctive therapy in addition to the standard antibiotic therapy. Therefore, BBB penetration by GBS is a promising, new potential therapeutic target to treat GBS meningitis [102].

In the MONALISA study, neurolisteriosis presented with meningoencephalitis in 212 (84%) of 252 patients, and mortality was increased in patients with neurolisteriosis on dexamethasone as adjunctive therapy [75].

6.7 Other Considerations

Serratia marcescens also has been shown to cause neonatal meningitis in the immunocompromised in neonatal intensive care units. The main risk factor is hospitalization, and infection control measures, such as rigorous hand hygiene and contact precautions, are most important to prevent the nosocomial infection [103].

6.8 Other Advances

In bacterial meningitis in infants, one study demonstrated the use of IL-23, alone or combined with IL-18 and sRAGE, is useful in identification of the infection. These biomarkers could be used in the diagnosis and determining the length of antibiotic therapy [104].

7 Haemophilus Influenzae

7.1 Encapsulated Versus Non-encapsulated Types

Haemophilus influenzae has both encapsulated and non-encapsulated types. It is a pleomorphic Gram-negative coccobacillus. The encapsulated type is typeable, whereas the unencapsulated is not. Serotypes "a" through "f" are assigned due to the capsular polysaccharides. Standard slide agglutination serotyping (SAST) is the method used for serologic capsule typing and distinguishes encapsulated from non-encapsulated types. This test is not a perfect tool for typing, as there may be user error in the protocol or interpretation of slide agglutination results. Furthermore, previously serotypeable strains that now exhibit a complete deletion of the capsule locus or strains with a partial deletion of capsule constituents such as bexA will be incorrectly classified [105]. The encapsulated type uses protein H and *Haemophilus* surface fibrils (Hsf). Protein H plays a major role in Hib and Hif's ability to bind to factor H, the main regulator of the alternative pathway of the complement system's activation [114]. The capsule helps evade the phagocytosis and recognition by the immune system; without anti-capsular antibodies, this allows for further survival and growth [108, 114]. The non-encapsulated strains directly attach to surface epithelial cells and gain access to the extracellular matrix. This binding allows

adhesion, colonization, and host cell entry. These can also develop biofilm-like colonies and evade the complement and immune system via similar interactions [108].

7.2 Hib Conjugate Vaccine: Epidemiology

The Hib conjugate vaccine targets the capsular polysaccharide type "b" [108]. Before discovery of the Hib conjugate vaccine, *H. influenzae* type b was the leading cause of bacterial meningitis in children. It also caused pneumonia, epiglottitis, and septic arthritis. Now, most cases of *H. influenzae*-invasive disease are due to non-typeable *H. influenzae* (NTHi) across all age groups in the US non-immunized children, and infants still completing the vaccination series are still at risk for Hib infection [108, 111]. On the global scale, *H. influenzae* is still causing significant illness in children. After the Hib conjugate vaccine, the amount of respiratory and invasive cases of non-typeable *H. influenzae* decreased in children and adults. Antibiotic resistance is variable on the area, but remains a growing concern [105].

7.3 Symptoms

Infants, children less than 5 years old, and adults 65 or older run a greater risk of infection by *H. influenzae*. American Indians and Alaska natives also are affected more. Patients with sickle cell disease, human immunodeficiency virus (HIV), asplenia, antibody and complement deficiency states, cancer and chemotherapy, radiotherapy, or post bone marrow transplant are also affected more due to an immunocompromised state [108].

Infection of the meninges commonly exhibits as fever, headache, altered consciousness, photophobia, nausea, vomiting, and nuchal rigidity [108]. The symptoms of *H. influenzae* acute bacterial meningitis are nonspecific in neonates and infants. These include hypotonia, lethargy, general weakness, fussiness, sleepiness, jitteriness, anorexia, apnea, jaundice, and diarrhea. Fever or hypothermia may or not also be present. In 15–34% of infants, seizures may happen [106]. Infants may demonstrate irritability, drowsiness, poor feeding, and vomiting [108]. There may be a history of upper respiratory tract infection. On the physical exam, meningismus is detected. According to Gorga et al., on physical exam, there may be stridor (81%), respiratory distress (78%), sore throat (65%), fever (57%), drooling (42%), painful swallowing (49%), tender neck (65%), altered voice (33%), cervical adenopathy (39%), and cough at times [112]. Children who did not receive the Hib conjugate vaccine may also exhibit epiglottitis. The tripod position is assumed to open the airway more, and the child will look ill [108].

7.4 Potential Complications

Subdural effusion is a complication associated with bacterial meningitis. Hib *S. pneumoniae* and *N. meningitidis* do not have a significant difference in the subdural effusion. 20–39% of cases of bacterial meningitis in children result in this complication, and it is more common in infants under 1 year of age.

Focal neurological deficits are another complication occurring in 3–14% of cases of bacterial meningitis. This deficit will exhibit specific signs and symptoms based on the location of the lesion in the central nervous system. The incidence is great in infants under 1 year of age. These deficits usually do not persist after acute bacterial meningitis in children [107].

Other potential complications of *H. influenzae* bacterial meningitis are empyema, seizures, cerebral abscess, brain edema, syndrome of inappropriate antidiuretic hormone, hydrocephalus, cerebral herniation, and hearing loss. If not treated promptly within a few hours, hemiparesis, coma, and death may result [108].

7.5 Antibiotic Treatments

Initially, a third-generation cephalosporin is administered, while culture and sensitivity results are pending. Antibiotic resistance is an emerging concern, which makes monitoring response to treatment very important. For suspected Hib meningitis, ceftriaxone, ceftazidime, cefotaxime, ampicillin-sulbactam, fluoroquinolones, or azithromycin is administered parenterally for a week [108].

From a prospective cohort study in the Netherlands of bacterial meningitis by *H. influenzae* from 2006 to 2018, 24 of 81 patients (30%) received amoxicillin alone; 49 of 81 patients received a third-generation cephalosporin (60%), and 7 of 81 received a combination of the two (9%). The median duration of antibiotic treatment was 10 days (IQR 8–14). Sixty-eight of 80 patients (85%) also received adjunctive dexamethasone therapy [109].

7.6 Differentials

H. influenzae bacterial meningitis must receive appropriate workup including a sputum specimen, Gram stain, and culture. It cannot be differentiated from other types of bacterial meningitis [108]. Therefore, on the differential for neonate would be the main causes of bacterial meningitis, such as Group B *Streptococcus*, *Listeria monocytogenes*, and *Escherichia coli*. If the child is unvaccinated or does not mount an adequate response to vaccination, Hib-caused meningitis should be considered, too. NTHi causes most of the cases of *H. influenzae* invasive disease and should be considered at all ages. According to the Centers for Disease Control and Prevention's

webpage about bacterial meningitis, in babies and young children, *S. pneumoniae*, *N. meningitidis*, group B *Streptococcus*, and *M. tuberculosis* should also be considered. In teens and young adults, *N. meningitidis* and *S. pneumoniae* should also be considered. In older adults, *S. pneumoniae*, *N. meningitidis*, group B *Streptococcus*, and *L. monocytogenes s*hould also be considered. *H. influenzae* maybe seen more in babies, young children, and older adults [113].

For diagnosing *H. influenzae* meningitis, Oxford Nanopore Technologies real-time sequencing has been shown to work in detecting *H. influenzae* in the CSF in 6 hours. BLAST analysis also helped identify a sequence for a protein of a non-b serotype, non-typeable strain of *H. influenzae* of lineage 22.1–21 [110].

8 Treatment of Lyme Meningitis

Lyme disease is a sequela caused by the bacteria *Borrelia burgdorferi* through tick-borne transmission. Meningitis and neurological manifestations are typically more severe symptoms of Lyme disease, which is more commonly associated with a bullseye rash, fever, general fatigue, and arthritis [115]. Lyme disease that is not treated early or in an immunocompromised host may lead to meningitis, which may warrant more aggressive treatment than non-disseminated Lyme disease [116]. This section will cover available antibiotic and anti-inflammatory treatments for Lyme meningitis and any special considerations when it comes to treatment.

8.1 Antibiotic Treatment of Lyme Meningitis

Many different antibiotic regimens are effective against Lyme meningitis, and different professional societies may have different recommendations. The American Academy of Neurology recommends intravenous penicillin, ceftriaxone, or cefotaxime for both children and adults with Lyme meningitis [117]. Many European societies, however, advocate for the use of oral doxycycline in adults with Lyme meningitis, saving regimens for those with no response to oral regimens or brain tissue penetration beyond the CSF [118]. Doxycycline, however, is not recommended for use children younger than 8 years old or pregnant or breastfeeding women due to its teeth discoloration effects in young children.

The specific regimens employed for Lyme meningitis include intravenous penicillin 20 million units daily, intravenous ceftriaxone 2–4 g daily, intravenous cefotaxime 2 g every 8 hours, or oral doxycycline 200 mg daily for 2 days and then 100 mg daily for 8 days. These antibiotic courses have been shown to be successful after 10–14 days of use, but 28 days of use is typically recommended with central nervous system involvement [117].

It is generally not recommended to treat Lyme meningitis with an antibiotic course longer than 30 days, even in the scenario of ongoing neurological sequelae

Table 5 Antibiotics used for the treatment of Lyme meningitis along with their total daily doses

Antibiotic	Daily dosage for adults
Penicillin G	20 million units i.v.
Ceftriaxone	2–4 g i.v.
Cefotaxime	6 g i.v.
Doxycycline	100–200 mg[a] oral tablet or capsule

[a]The doxycycline regimen involves 200 mg daily for 2 days and then 100 mg for 8 days total

and other symptoms. This phenomenon is called posttreatment Lyme disease syndrome (PTLDS) and includes long-term symptoms such as cognitive dysfunction, fatigue, chronic pain, and depression that can persist for over 6 months to a few years. Antibiotic therapy, however, provides no benefit to patients with this syndrome [117]. The different antibiotic regimens for Lyme meningitis can be found in Table 5.

8.2 Anti-inflammatory Treatment of Lyme Meningitis

Like *Listeria monocytogenes* meningitis, Lyme meningitis can worsen with corticosteroid use as well. Because its manifestations are relatively distinct from other types of meningitis, dexamethasone should not be initiated in patients with a Lyme meningitis clinical picture [117]. Lyme meningitis presents usually with predominant headache symptoms and a lack of meningeal signs or fever. Cranial nerve palsies may also be a telltale sign of Lyme disease dissemination to the central nervous system as well as other signs of Lyme, such as the bullseye rash, arthralgias, and potential heart block [117]. These symptoms warrant aggressive antibiotic treatment with no adjunctive steroid use.

Non-steroidal inflammatory (NSAID) drugs, such as aspirin, ibuprofen, naproxen, and indomethacin, however, have shown some benefit in the treatment course of Lyme meningitis [116]. Patients can greatly vary in how they respond to NSAIDs, however, and it is best for the treating physician to prescribe an NSAID that a patient has previously responded well to when treating Lyme meningitis. NSAIDs do not necessarily have mortality benefit in treating Lyme meningitis, but they can increase comfort and decrease the amount of time needed for full recovery in this disease process [116].

8.3 Treatment of Lyme Meningitis in Special Populations

In the pediatric population, Lyme meningitis is typically treated with one of the parenteral antibiotic regimens previously stated, while adults usually receive an oral course of doxycycline. A recent retrospective cohort study, however, has shown that

32 children aged 6–14 with Lyme meningitis treated exclusively with oral doxycycline and amoxicillin for a median of 25 days demonstrated complete resolution of their symptoms. Only two of these children suffered from increased intracranial pressure, a potential adverse effect of doxycycline or possibly a disease manifestation of Lyme meningitis. Acetazolamide administered concurrently with doxycycline was able to resolve these symptoms as well as allow for full resolution of Lyme meningitis in this subset of the pediatric population with only oral antibiotics [119].

The pediatric population is also more susceptible to stroke and vasculitis as a sequelae of Lyme meningitis. In these cases, prompt antibiotic therapy is the best treatment. There has been little evidence that antiplatelet or corticosteroid therapy improves clinical course in these cases [120, 121].

9 New Advancements

In addition to the current recommended treatments for bacterial meningitis, with the increasing issue of antibiotic resistance, some institutions have taken initiative to develop non-antibiotic treatments to decrease the morbidity and mortality of bacterial meningitis worldwide. Specifically at the University of Copenhagen and Lund University, researchers were able to use a form of immunotherapy in rats to kill bacterial meningitis without causing central nervous system swelling [122]. In the typical pathogenesis of bacterial meningitis, neutrophils form a net-like structure in the meninges to fight off infection; however, this mechanism of immune cell mobilization leaves the host more susceptible to cerebral edema. The researchers at the University of Copenhagen and Lund University were able to use DNAse to dissolve this structure in rats, allowing other immune cells to fight off the infection without causing increased central nervous system pressure and swelling. At this point, they have plans to conduct an international clinical trial to utilize DNAse in patients with bacterial meningitis, hoping that this treatment can treat this fatal disease without increasing antibiotic resistance and preventing significant morbidity and mortality from the brain swelling that ensues meningeal infection [122]. Other institutions, however, have opted to focus on meningitis prevention rather than treatment. The multinational healthcare company, Sanofi, has developed a meningococcal vaccination that covers groups A, C, Y, and W, which was approved by the Food and Drug Administration (FDA) in 2020. Previously, vaccination had only been offered for group B serotype meningococcal meningitis. The vaccination formulation developed by Sanofi utilizes tetanus toxoid as a protein carrier to ensure greater immune response in individuals of all ages [123]. These new efforts in both the treatment and prevention of bacterial meningitis will hopefully alleviate the international burden of this disease. Though there is still much progress to be made, these new developments show much promise.

10 Conclusion

Overall, there have been many advancements in the treatment of bacterial meningitis that have turned this almost certainly fatal disease in the past into a manageable disease course in the present. There is, however, still much to be discovered in this realm with increasing antibiotic resistance and the ineffectiveness of anti-inflammatory therapies in resource-poor areas posing as challenges in the treatment of bacterial meningitis moving into the future. There are still many advancements to be made in the realm of vaccination and prophylactic therapy for bacterial meningitis besides these already existing therapies for meningococcal and pneumococcal meningitis. Though the treatment of bacterial meningitis has come a long way, there is still much progress to be made, particularly internationally where the disease burden and mortality are still extremely high due to lack of resources.

References

1. Runde TJ, Anjum F, Hafner JW. Bacterial meningitis. In: StatPearls. StatPearls Publishing Copyright © 2022. StatPearls Publishing LLC; 2022.
2. Bacterial Meningitis Centers for Disease Control and Prevention Updated July 15, 2021. https://www.cdc.gov/meningitis/bacterial.html.
3. Hersi K, Gonzalez FJ, Kondamudi NP. Meningitis. In: StatPearls. StatPearls Publishing Copyright © 2022. StatPearls Publishing LLC; 2022.
4. Rosenstein NE, Perkins BA, Stephens DS, Popovic T, Hughes JM. Meningococcal disease. N Engl J Med. 2001;344:1378–88.
5. Brouwer MC, Tunkel AR, van de Beek D. Epidemiology, diagnosis, and antimicrobial treatment of acute bacterial meningitis. Clin Microbiol Rev. 2010;23(3):467–92. https://doi.org/10.1128/CMR.00070-09.
6. Brouwer MC, van de Beek D. Epidemiology of community-acquired bacterial meningitis. Curr Opin Infect Dis. 2018;31(1):78.
7. Thigpen MC, Whitney CG, Messonnier NE, et al. Bacterial meningitis in the United States, 1998-2007. N Engl J Med. 2011;364(21):2016–25. https://doi.org/10.1056/NEJMoa1005384.
8. Schlech WF 3rd, Ward JI, Band JD, Hightower A, Fraser DW, Broome CV. Bacterial meningitis in the United States, 1978 through 1981. The National Bacterial Meningitis Surveillance Study. JAMA. 1985;253(12):1749–54.
9. Wenger JD, Hightower AW, Facklam RR, Gaventa S, Broome CV, The Bacterial Meningitis Study G. Bacterial meningitis in the United States, 1986: report of a multistate surveillance study. J Infect Dis. 1990;162(6):1316–23.
10. Schuchat A, Robinson K, Wenger JD, et al. Bacterial meningitis in the United States in 1995. Active surveillance team. N Engl J Med. 1997;337(14):970–6. https://doi.org/10.1056/nejm199710023371404.
11. Castelblanco RL, Lee M, Hasbun R. Epidemiology of bacterial meningitis in the USA from 1997 to 2010: a population-based observational study. Lancet Infect Dis. 2014;14(9):813–9. https://doi.org/10.1016/s1473-3099(14)70805-9.
12. NCI Drug Dictionary National Cancer Institute. https://www.cancer.gov/publications/dictionaries/cancer-drug/def/pneumococcal-7-valent-conjugate-vaccine.
13. Johnson AP, Waight P, Andrews N, Pebody R, George RC, Miller E. Morbidity and mortality of pneumococcal meningitis and serotypes of causative strains prior to introduction of the

7-valent conjugant pneumococcal vaccine in England. J Infect. 2007;55(5):394–9. https://doi.org/10.1016/j.jinf.2007.07.009.
14. Ahmed SS, Pondo T, Xing W, et al. Early impact of 13-valent pneumococcal conjugate vaccine use on invasive pneumococcal disease among adults with and without underlying medical conditions—United States. Clin Infect Dis. 2019;70(12):2484–92. https://doi.org/10.1093/cid/ciz739.
15. Matanock ALG, Gierke R, Kobayashi M, Leidner A, Pilishvili T. Use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine among adults aged ≥65 years: updated recommendations of the Advisory Committee on Immunization Practices. MMWR Morb Mortal Wkly Rep. 2019;68:1069–75.
16. Bundy LM, Noor A. Neonatal Meningitis. In: StatPearls. StatPearls Publishing. Copyright © 2022. StatPearls Publishing LLC; 2022.
17. Castellazzi ML, Marchisio P, Bosis S. Listeria monocytogenes meningitis in immunocompetent and healthy children: a case report and a review of the literature. Ital J Pediatr. 2018;44(1):152. https://doi.org/10.1186/s13052-018-0595-5. PMID: 30594251; PMCID: PMC6311039. Ben-Joseph. EP e. Meninigits KidsHealth https://kidshealth.org/en/parents/meningitis.html#:~:text=Many%20different%20types%20of%20bacteria,meningococcus)%20are%20often%20the%20causes.
18. Ashorobi D, Fernandez R. Asplenia. In: StatPearls. StatPearls Publishing. Copyright © 2022. StatPearls Publishing LLC; 2022.
19. Rouphael NG, Stephens DS. Neisseria meningitidis: biology, microbiology, and epidemiology. Methods Mol Biol. 2012;799:1–20. https://doi.org/10.1007/978-1-61779-346-2_1.
20. Karki BR, Sedhai YR, Bokhari SRA. Waterhouse-Friderichsen syndrome. In: StatPearls. StatPearls Publishing. Copyright © 2022. StatPearls Publishing LLC; 2022.
21. Decimo I, Fumagalli G, Berton V, Krampera M, Bifari F. Meninges: from protective membrane to stem cell niche. Am J Stem Cells. 2012;1(2):92–105.
22. Rohde S. Inflammatory diseases of the meninges. In: Inflammatory diseases of the brain; 2012. p. 127–37. https://doi.org/10.1007/174_2012_633.
23. van Sorge NM, Doran KS. Defense at the border: the blood-brain barrier versus bacterial foreigners. Future Microbiol. 2012;7(3):383–94. https://doi.org/10.2217/fmb.12.1.
24. Guarner J, Liu L, Bhatnagar J, et al. Neutrophilic bacterial meningitis: pathology and etiologic diagnosis of fatal cases. Mod Pathol. 2013;26(8):1076–85. https://doi.org/10.1038/modpathol.2013.30.
25. van de Beek D, de Gans J, Spanjaard L, Weisfelt M, Reitsma JB, Vermeulen M. Clinical features and prognostic factors in adults with bacterial meningitis. N Engl J Med. 2004;351(18):1849–59. https://doi.org/10.1056/NEJMoa040845.
26. Digre KB, Brennan KC. Shedding light on photophobia. J Neuroophthalmol. 2012;32(1):68–81. https://doi.org/10.1097/WNO.0b013e3182474548.
27. Lécuyer H, Borgel D, Nassif X, Coureuil M. Pathogenesis of meningococcal purpura fulminans. Pathog Dis. 2017;75(3). https://doi.org/10.1093/femspd/ftx027.
28. Kasanmoentalib ES, Brouwer MC, van der Ende A, van de Beek D. Hydrocephalus in adults with community-acquired bacterial meningitis. Neurology. 2010;75(10):918–23. https://doi.org/10.1212/WNL.0b013e3181f11e10.
29. Zeeshan F, Bari A, Dugal MN, Saeed F. Hearing impairment after acute bacterial meningitis in children. Pak J Med Sci. 2018;34(3):655–9. https://doi.org/10.12669/pjms.343.14373.
30. Kutz JW, Simon LM, Chennupati SK, Giannoni CM, Manolidis S. Clinical predictors for hearing loss in children with bacterial meningitis. Arch Otolaryngol Head Neck Surg. 2006;132(9):941–5. https://doi.org/10.1001/archotol.132.9.941.
31. Murthy JM, Prabhakar S. Bacterial meningitis and epilepsy. Epilepsia. 2008;49 Suppl 6:8–12. https://doi.org/10.1111/j.1528-1167.2008.01750.x.
32. Benninger F, Steiner I. Chapter 12 - CSF in acute and chronic infectious diseases. In: Deisenhammer F, Teunissen CE, Tumani H, editors. Handbook of clinical neurology. Elsevier; 2018. p. 187–206.

33. Beloor Suresh A, Rosani A, Wadhwa R. Rifampin. In: StatPearls. StatPearls Publishing Copyright © 2022. StatPearls Publishing LLC; 2022.
34. Control and Prevention of Meningococcal Disease: Recommendations of the Advisory Committee on Immunization Practices (ACIP). Centers for Disease Control and Prevention. https://www.cdc.gov/mmwr/preview/mmwrhtml/00046263.htm.
35. Wood AJJMD, Quagliarello VJMD, Scheld WMMD. Treatment of bacterial meningitis. N Engl J Med. 1997;336(10):708–16. 1997 Mar 06 2017-10-31.
36. Lutsar I, McCracken GH Jr, Friedland IR. Antibiotic pharmacodynamics in cerebrospinal fluid. Clin Infect Dis. 1998;27(5):1117–27, quiz 1128–9. https://doi.org/10.1086/515003.
37. Tunkel AR, Hasbun R, Bhimraj A, et al. 2017 Infectious Diseases Society of America's clinical practice guidelines for healthcare-associated ventriculitis and meningitis*. Clin Infect Dis. 2017;64(6):e34–65. https://doi.org/10.1093/cid/ciw861.
38. McMullan BJ, Andresen D, Blyth CC, et al. Antibiotic duration and timing of the switch from intravenous to oral route for bacterial infections in children: systematic review and guidelines. Lancet Infect Dis. 2016;16(8):e139–52. https://doi.org/10.1016/S1473-3099(16)30024-X.
39. Mańdziuk J, Kuchar EP. Streptococcal meningitis. [Updated 2021 Apr 19]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554448/.
40. Mook-Kanamori BB, Geldhoff M, van der Poll T, van de Beek D. Pathogenesis and pathophysiology of pneumococcal meningitis. Clin Microbiol Rev. 2011;24(3):557–91. https://doi.org/10.1128/CMR.00008-11.
41. Schuchat A, Robinson K, Wenger JD, Harrison LH, Farley M, Reingold AL, Lefkowitz L, Perkins BA. Bacterial meningitis in the United States in 1995. Active surveillance team. N Engl J Med. 1997;337(14):970–6. https://doi.org/10.1056/NEJM199710023371404. PMID: 9395430.
42. Fine P, Eames K, Heymann DL. "Herd immunity": a rough guide. Clin Infect Dis. 2011;52(7):911–6. https://doi.org/10.1093/cid/cir007. PMID: 21427399.
43. Kaplan SL. Management of pneumococcal meningitis. Pediatr Infect Dis J. 2002;21(6):589–91; discussion 613–4. https://doi.org/10.1097/00006454-200206000-00034. PMID: 12182395.
44. Tan TQ, Schutze GE, Mason EO Jr, Kaplan SL. Antibiotic therapy and acute outcome of meningitis due to Streptococcus pneumoniae considered intermediately susceptible to broad-spectrum cephalosporins. Antimicrob Agents Chemother. 1994;38:918–23.
45. Klugman KP, Dagan R, the Meropenem Meningitis Study Group. Randomized comparison of meropenem with cefotaxime for treatment of bacterial meningitis. Antimicrob Agents Chemother. 1995;39:1140–6.
46. Arditi M, Mason EO Jr, Bradley JS, et al. Three-year surveillance of pneumococcal meningitis in children: clinical characteristics, and outcome related to penicillin susceptibility and dexamethasone use. Pediatrics. 1998;102:1087–97.
47. Dandona P, et al. Effect of dexamethasone on reactive oxygen species generation by leukocytes and plasma interleukin-10 concentrations: a pharmacodynamic study. Clin Pharmacol Ther. 1999;66:58–65.
48. Freyer D, et al. Pneumococcal cell wall components induce nitric oxide synthase and TNF-alpha in astroglial-enriched cultures. Glia. 1996;16:1–6.
49. Lorenzl S, Koedel U, Dirnagl U, Ruckdeschel G, Pfister HW. Imaging of leukocyte-endothelium interaction using in vivo confocal laser scanning microscopy during the early phase of experimental pneumococcal meningitis. J Infect Dis. 1993;168:927–33.
50. Viladrich PF, Gudiol F, Liñares R, et al. Evaluation of vancomycin therapy of adult pneumococcal meningitis. Antimicrob Agents Chemother. 1991;35:2467–72.
51. Lutsar I, Friedland IR, Wubbel L, et al. Pharmacodynamics of gatifloxacin in cerebrospinal fluid in experimental cephalosporin-resistant pneumococcal meningitis. Antimicrob Agents Chemother. 1998;42:2650–5.

52. Stuertz K, et al. Lower lipoteichoic and teichoic acid CSF concentrations during treatment of pneumococcal meningitis with non-bacteriolytic antibiotics than with ceftriaxone. Scand J Infect Dis. 1999;31:367–70.
53. Grandgirard D, Oberson K, Buhlmann A, Gaumann R, Leib SL. Attenuation of cerebrospinal fluid inflammation by the nonbacteriolytic antibiotic daptomycin versus that by ceftriaxone in experimental pneumococcal meningitis. Antimicrob Agents Chemother. 2010;54:1323–6.
54. Bottcher T, et al. Rifampin reduces production of reactive oxygen species of cerebrospinal fluid phagocytes and hippocampal neuronal apoptosis in experimental Streptococcus pneumoniae meningitis. J Infect Dis. 2000;181:2095–8.
55. Singhi S, Jarvinen A, Peltola H. Increase in serum osmolality is possible mechanism for the beneficial effects of glycerol in childhood bacterial meningitis. Pediatr Infect Dis J. 2008;27:892–6.
56. Brouwer MC, van de Beek D. Glycerol in bacterial meningitis: one strike and out? Lancet Infect Dis. 2011;11:257–8.
57. Zwijnenburg PJ, et al. C1 inhibitor treatment improves host defense in pneumococcal meningitis in rats and mice. J Infect Dis. 2007;196:115–23.
58. Echchannaoui H, Leib SL, Neumann U, Landmann RM. Adjuvant TACE inhibitor treatment improves the outcome of TLR2−/− mice with experimental pneumococcal meningitis. BMC Infect Dis. 2007;7:25.
59. Marby D, Lockhart GR, Raymond R, Linakis JG. Anti-interleukin-6 antibodies attenuate inflammation in a rat meningitis model. Acad Emerg Med. 2001;8:946–9.
60. Hanisch UK, et al. The protein tyrosine kinase inhibitor AG126 prevents the massive microglial cytokine induction by pneumococcal cell walls. Eur J Immunol. 2001;31:2104–15.
61. Bermpohl D, et al. Bacterial programmed cell death of cerebral endothelial cells involves dual death pathways. J Clin Invest. 2005;115:1607–15.
62. Irazuzta J, Pretzlaff RK, Zingarelli B. Caspases inhibition decreases neurological sequelae in meningitis. Crit Care Med. 2008;36:1603–6.
63. Loeffler JM, Ringer R, Hablutzel M, Tauber MG, Leib SL. The free radical scavenger alpha-phenyl-tert-butyl nitrone aggravates hippocampal apoptosis and learning deficits in experimental pneumococcal meningitis. J Infect Dis. 2001;183:247–52.
64. Rosenstein NE, Perkins BA, Stephens DS, et al. The changing epidemiology of meningococcal disease in the United States, 1992–1996. J Infect Dis. 1999;180:1894–901.
65. Stephens DS. Biology and pathogenesis of the evolutionarily successful, obligate human bacterium Neisseria meningitidis. Vaccine. 2009;27(Suppl 2):B71–7.
66. Christodoulides M, Heckels J. Novel approaches to Neisseria meningitidis vaccine design. Pathogens and Disease. 2017;75(3):ftx033. https://doi.org/10.1093/femspd/ftx033.
67. Frasch CE, Bash MC. Neisseria meningitidis Vaccines. In: Madame Curie Bioscience Database [Internet]. Austin: Landes Bioscience; 2000–2013. Available from: https://www.ncbi.nlm.nih.gov/books/NBK6030/.
68. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. II. Development of natural immunity. J Exp Med. 1969;129:1327–48.
69. Maslanka SE, Tappero JW, Plikaytis BD, et al. Age-dependent Neisseria meningitidis serogroup C class-specific antibody concentrations and bactericidal titers in sera from young children from Montana immunized with a licensed polysaccharide vaccine. Infect Immun. 1998;66:2453–9.
70. Nadel S. Treatment of meningococcal disease. J Adolesc Health. 2016;59(2 Suppl):S21–8.
71. McGill F, Heyderman RS, Michael BD, Defres S, Beeching NJ, Borrow R, Glennie L, Gaillemin O, Wyncoll D, Kaczmarski E, Nadel S, Thwaites G, Cohen J, Davies NW, Miller A, Rhodes A, Read RC, Solomon T. The UK joint specialist societies guideline on the diagnosis and management of acute meningitis and meningococcal sepsis in immunocompetent adults. J Infect. 2016;72(4):405–38.

72. Nguyen N, Ashong D. Neisseria Meningitidis. [Updated 2021 Oct 12]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK549849/.
73. Pagliano P, Ascione T, Boccia G, De Caro F, Esposito S. Listeria monocytogenes meningitis in the elderly: epidemiological, clinical and therapeutic findings. Infez Med. 2016;24(2):105–11. PMID: 27367319.
74. Thønnings S, Knudsen JD, Schønheyder HC, Søgaard M, Arpi M, Gradel KO, Østergaard C, Danish Collaborative Bacteraemia Network (DACOBAN). Antibiotic treatment and mortality in patients with Listeria monocytogenes meningitis or bacteraemia. Clin Microbiol Infect. 2016;22(8):725–30. https://doi.org/10.1016/j.cmi.2016.06.006. Epub 2016 Jun 23. PMID: 27345176.
75. Charlier C, Perrodeau É, Leclercq A, Cazenave B, Pilmis B, Henry B, Lopes A, Maury MM, Moura A, Goffinet F, Dieye HB, Thouvenot P, Ungeheuer MN, Tourdjman M, Goulet V, de Valk H, Lortholary O, Ravaud P, Lecuit M, MONALISA study group. Clinical features and prognostic factors of listeriosis: the MONALISA national prospective cohort study. Lancet Infect Dis. 2017;17(5):510–9. https://doi.org/10.1016/S1473-3099(16)30521-7. Epub 2017 Jan 28. Erratum in: Lancet Infect Dis. 2017 Sep;17(9):897. PMID: 28139432.
76. Lim S, Chung DR, Kim YS, Sohn KM, Kang SJ, Jung SI, Kim SW, Chang HH, Lee SS, Bae IG, Moon C, Rhee JY, Lee JS, Ki HK, Kim HA, Ryu SY, Yeom JS, Son JS, Moon SY, Kwon KT, Lee H, Heo ST, Kang CI, Peck KR, Song JH. Predictive risk factors for listeria monocytogenes meningitis compared to pneumococcal meningitis: a multicenter case-control study. Infection. 2017;45(1):67–74. https://doi.org/10.1007/s15010-016-0939-2. Epub 2016 Aug 19. PMID: 27541039.
77. Laguna-Del Estal P, Lledó-Ibáñez GM, Ríos-Garcés R, Pintos-Pascual I. Meningitis por listeria monocytogenes en adultos [Meningitis due to Listeria monocytogenes in adults]. Rev Neurol. 2013;56(1):13–8. Spanish. PMID: 23250677.
78. Paciorek M, Bienkowski C, Bednarska A, Kowalczyk M, Krogulec D, Makowiecki M, Bursa D, Pula J, Raczynska J, Porowski D, Skrzat-Klapaczynska A, Zielenkiewicz M, Radkowski M, Laskus T, Horban A. The clinical course and outcome of listeria monocytogenes meningitis: a retrospective single center study. Neuro Endocrinol Lett. 2019;40(2):79–84. PMID: 31785214.
79. Madjunkov M, Chaudhry S, Ito S. Listeriosis during pregnancy. Arch Gynecol Obstet. 2017;296(2):143–52. https://doi.org/10.1007/s00404-017-4401-1. Epub 2017 May 23. PMID: 28536811.
80. Simonsen KA, Anderson-Berry AL, Delair SF, Davies HD. Early-onset neonatal sepsis. Clin Microbiol Rev. 2014;27(1):21–47. https://doi.org/10.1128/CMR.00031-13.
81. Stoll BJ, Hansen NI, Sanchez PJ, Faix RG, Poindexter BB, Van Meurs KP, Bizzarro MJ, Goldberg RN, Frantz ID III, Hale EC, Shankaran S, Kennedy K, Carlo WA, Watterberg KL, Bell EF, Walsh MC, Schibler K, Laptook AR, Shane AL, Schrag SJ, Das A, Higgins RD, Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Early onset neonatal sepsis: the burden of group B streptococcal and E. coli disease continues. Pediatrics. 2011;127:817–26. https://doi.org/10.1542/peds.2010-2217.
82. Chandel DS, Johnson JA, Chaudhry R, Sharma N, Shinkre N, Parida S, Misra PR, Panigrahi P. Extended-spectrum beta-lactamaseproducing Gram-negative bacteria causing neonatal sepsis in India in rural and urban settings. J Med Microbiol. 2011;60:500–7.
83. Dubois V, De Barbeyrac B, Rogues AM, Arpin C, Coulange L, Andre C, M'zali F, Megraud F, Quentin C. CTX-M-producing Escherichia coli in a maternity ward: a likely community importation and evidence of mother-to-neonate transmission. J Antimicrob Chemother. 2010;65:1368–71. https://doi.org/10.1093/jac/dkq153.
84. Bradford PA. Extended-spectrum -lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev. 2001;14:933–51. https://doi.org/10.1128/CMR.14.4.933-951.2001.

85. Pitout JD, Laupland KB. Extended-spectrum beta-lactamaseproducing Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis. 2008;8:159–66.
86. Fink S, Karp W, Robertson A. Ceftriaxone effect on bilirubinalbumin binding. Pediatrics. 1987;80:873–5.
87. Polin RA, Committee on Fetus and Newborn. Management of neonates with suspected or proven early-onset bacterial sepsis. Pediatrics. 2012;129:1006–15. https://doi.org/10.1542/peds.2012-0541.
88. Edwards MS, Baker CJ. Streptococcus agalactiae (group B streptococcus). In: Long SS, Pickering LK, Prober CG, editors. Principles and practice of pediatric infectious diseases. 4th ed. Edinburgh: Elsevier; 2012. p. 707–12.
89. Cordero L, Sananes M, Ayers LW. Bloodstream infections in a neonatal intensive-care unit: 12 years' experience with an antibiotic control program. Infect Control Hosp Epidemiol. 1999;20:242–6. https://doi.org/10.1086/501619.
90. Villegas MV, Kattan JN, Quinteros MG, Casellas JM. Prevalence of extended-spectrum beta-lactamases in South America. Clin Microbiol Infect. 2008;14(Suppl 1):154–8. https://doi.org/10.1111/j.1469-0691.2007.01869.x.
91. American Academy of Pediatrics. Escherichia coli and other gram-negative bacilli (septicemia and meningitis in neonates). In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. Red book: 2012 report of the Committee on Infectious Diseases. Elk Grove Village: American Academy of Pediatrics; 2012. p. 321–4.
92. Arrieta A. Use of meropenem in the treatment of serious infections in children: review of the current literature. Clin Infect Dis. 1997;24(Suppl2):S207–12.
93. van den Anker JN, Pokorna P, Kinzig-Schippers M, Martinkova J, de Groot R, Drusano GL, Sorgel F. Meropenem pharmacokinetics in the newborn. Antimicrob Agents Chemother. 2009;53:3871–9. https://doi.org/10.1128/AAC.00351-09.
94. Tunkel AR, Hartman BJ, Kaplan SL, Kaufman BA, Roos KL, Scheld WM, Whitley RJ. Practice guidelines for the management of bacterial meningitis. Clin Infect Dis. 2004;39:1267. https://doi.org/10.1086/425368.
95. Lee JE, Cho WK, Nam CH, Jung MH, Kang JH, Suh BK. A case of meningoencephalitis caused by Listeria monocytogenes in a healthy child. Korean J Pediatr. 2010;53:653. https://doi.org/10.3345/kjp.2010.53.5.653.
96. Weston VC, Punt J, Vloebeghs M, Watson AR, Ispahani P. Listeria monocytogenes meningitis in a penicillin-allergic paediatric renal transplant patient. J Inf Secur. 1998;37(1):77–8.
97. Brisca G, La Valle A, Campanello C, et al. Listeria meningitis complicated by hydrocephalus in an immunocompetent child: case report and review of the literature. Ital J Pediatr. 2020;46(1):111. Published 2020 Aug 2. https://doi.org/10.1186/s13052-020-00873-w.
98. Ulloa-Gutierrez R, Avila-Agüero ML, Huertas E. Fulminant Listeria monocytogenes meningitis complicated with acute hydrocephalus in healthy children beyond the newborn period. Pediatr Emerg Care. 2004;20:233. https://doi.org/10.1097/01.pec.0000121243.99242.a9.
99. Hitomi S, Ohto T, Okamoto M, Nishimura Y, Iwasaki N, Matsui A. A case of listerial meningitis treated with a regimen containing panipenembetamipron. J Infect Chemother. 2004;10:242. https://doi.org/10.1007/s10156-004-0323-5.
100. Dilber E, Aksoy A, Cakir M, Bahat E, Kamaşak T, Dilber B. Listeria monocytogenes meningitis in two immunocompetent children. Ann Trop Paediatr. 2009;29:225. https://doi.org/10.1179/027249309X12467994694058.
101. Yu JY, Zhang B, Peng L, et al. Repositioning of memantine as a potential novel therapeutic agent against Meningitic E. coli-induced pathogenicities through disease-associated Alpha7 cholinergic pathway and RNA sequencing-based transcriptome analysis of host inflammatory responses. PLoS One. 2015;10(5):e0121911. Published 2015 May 19. https://doi.org/10.1371/journal.pone.0121911.
102. Zhu N, Zhang C, Prakash A, et al. Therapeutic development of group B Streptococcus meningitis by targeting a host cell signaling network involving EGFR. EMBO Mol Med. 2021;13(3):e12651. https://doi.org/10.15252/emmm.202012651.

103. Cristina ML, Sartini M, Spagnolo AM. *Serratia marcescens* infections in neonatal intensive care units (NICUs). Int J Environ Res Public Health. 2019;16(4):610. Published 2019 Feb 20. https://doi.org/10.3390/ijerph16040610.
104. Srinivasan L, Kilpatrick L, Shah SS, Abbasi S, Harris MC. Elevations of novel cytokines in bacterial meningitis in infants. PLoS One. 2018;13(2):e0181449. Published 2018 Feb 2. https://doi.org/10.1371/journal.pone.0181449.
105. Wen S, Feng D, Chen D, Yang L, Xu Z. Molecular epidemiology and evolution of Haemophilus influenzae. Infect Genet Evol. 2020;80:104205. https://doi.org/10.1016/j.meegid.2020.104205.
106. Davis LE. Acute bacterial meningitis. CONTINUUM: lifelong learning. Neurology. 2018;24:1264–83. https://doi.org/10.1212/con.0000000000000660.
107. Zainel A, Mitchell H, Sadarangani M. Bacterial meningitis in children: neurological complications, associated risk factors, and prevention. Microorganisms. 2021;9. https://doi.org/10.3390/microorganisms9030535.
108. Khattak ZE, Anjum F. Haemophilus Influenzae. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2021.
109. Chekrouni N, Koelman DLH, Brouwer MC, van der Ende A, van de Beek D. Community-acquired Haemophilus influenzae meningitis in adults. J Infect. 2021;82(5):145–50. https://doi.org/10.1016/j.jinf.2021.03.016.
110. Morsli M, Kerharo Q, Delerce J, Roche PH, Troude L, Drancourt M. *Haemophilus influenzae* meningitis direct diagnosis by metagenomic next-generation sequencing: a case report. Pathogens. 2021;10(4):461. Published 2021 Apr 12. https://doi.org/10.3390/pathogens10040461.
111. Shooraj F, Mirzaei B, Mousavi SF, Hosseini F. Clonal diversity of Haemophilus influenzae carriage isolated from under the age of 6 years children. BMC Res Notes. 2019;12(1):565. Published 2019 Sep 11. https://doi.org/10.1186/s13104-019-4603-7.
112. Gorga SM, Gilsdorf JR, Mychaliska KP. Haemophilus influenzae serotype f epiglottitis: a case report and review. Hosp Pediatr. 2017;7(1):54–6. [PubMed]
113. Bacterial meningitis. Centers for Disease Control and Prevention. https://www.cdc.gov/meningitis/bacterial.html. Published July 15, 2021. Accessed 17 May 2022.
114. Fleury C, Su YC, Hallström T, Sandblad L, Zipfel PF, Riesbeck K. Identification of a Haemophilus influenzae factor H-Binding lipoprotein involved in serum resistance. J Immunol. 2014;192(12):5913–23. https://doi.org/10.4049/jimmunol.1303449.
115. Chomel B. Lyme disease. Rev Sci Tech. 2015;34(2):569–76. https://doi.org/10.20506/rst.34.2.2380. PMID: 26601457.
116. Pachner AR. Early disseminated Lyme disease: Lyme meningitis. Am J Med. 1995;98(4A):30S–7S; discussion 37S-43S. PMID: 7726190. https://doi.org/10.1016/s0002-9343(99)80042-4.
117. Garcia-Monco JC, Benach JL. Lyme neuroborreliosis: clinical outcomes, controversy, pathogenesis, and polymicrobial infections. Ann Neurol. 2019;85(1):21–31. https://doi.org/10.1002/ana.25389.
118. Halperin JJ, Shapiro ED, Logigian E, et al. Practice parameter: treatment of nervous system Lyme disease (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology. 2007;69:91–102.
119. Lopez SMC, Campfield BT, Nowalk AJ. Oral Management for Pediatric Lyme Meningitis. J Pediatric Infect Dis Soc. 2019;8(3):272–5. https://doi.org/10.1093/jpids/piy072. PMID: 30169816.
120. Monteventi O, Steinlin M, Regényi M, Roulet-Perez E, Weber P, Fluss J. Pediatric stroke related to Lyme neuroborreliosis: data from the Swiss NeuroPaediatric Stroke Registry and literature review. Eur J Paediatr Neurol. 2018;22(1):113–21. https://doi.org/10.1016/j.ejpn.2017.10.010. Epub 2017 Nov 24. PMID: 29208342.

121. Carod Artal FJ. Clinical management of infectious cerebral vasculitides. Expert Rev Neurother. 2016;16(2):205–21. https://doi.org/10.1586/14737175.2015.1134321. Epub 2016 Jan 19. PMID: 26689107.
122. Pavan C, Xavier ALR, Ramos M, Fisher J, Kritsilis M, Linder A, Bentzer P, Nedergaard M, Lundgaard I. DNase treatment prevents cerebrospinal fluid block in early experimental pneumococcal meningitis. Ann Neurol. 2021;90(4):653. https://doi.org/10.1002/ana.26186.
123. Chang LJ, Hedrick J, Christensen S, Pan J, Jordanov E, Dhingra MS. A phase II, randomized, immunogenicity and safety study of a quadrivalent meningococcal conjugate vaccine, MenACYW-TT, in healthy adolescents in the United States. 2020. https://doi.org/10.1016/j.vaccine.2020.03.017.

Recent Developments in the Treatment of Bacterial Urinary Tract Infections

Caroline Kelmis, Katarina Stephan, Ajay Varadhan, Jeffrey B. Brown, and Charles Preuss

Abstract Urinary tract infections (UTIs) are one of the most common infectious diseases among humans and account for over one million hospitalizations and $1.6 billion in medical costs per year in the United States. Bacterial etiologies are the most common cause of UTI, with uropathogenic *Escherichia coli* (UPEC) accounting for over 80% of all infections. The development of UTIs can be influenced by urinary tract anatomy, congenital anomalies, and bacterial pathogenic proteins.

Cystitis, urethritis, and pyelonephritis represent common UTIs that remain prevalent globally with symptoms including dysuria, frequency, urgency, suprapubic or flank pain, hematuria, and fever. Events that must occur between the host and the microbes for bacteria to colonize include adhesion to host cells, colonization of tissues, cellular invasion, and intracellular multiplication and dissemination. Current antibiotic guidelines to treat these infections depend on various comorbidities or infection etiology.

Treatment of UTIs caused by *E. coli* with standard antibiotics is becoming increasingly difficult due to antibiotic resistance. Alternative therapies in the treatment of UTIs caused by UPEC include anti-adhesion agents to disrupt biofilms, phage therapy, phytochemicals, nanomedicine, and natural remedies such as cranberry juice and probiotics. Creation of a vaccine may have the largest potential for protection, and there are several in development and in clinical trials around the world. The abundance of antigens as targets for vaccines makes the hope for an effective vaccine in the future seem possible.

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1 Introduction

A urinary tract infection (UTI) is defined as an infection of the urinary system, ranging from the urethra to the kidney. UTIs are one of the most common infectious diseases among humans and are the most cause of nosocomial infections in the developed world [1]. UTIs account for over one million hospitalizations and $1.6 billion in medical costs per year in the United States, alone, and an estimated 40–50% of females and 5% of males will get at least one UTI in their lifetimes [1].

Symptoms of UTIs vary depending on the pathogen and location. Most infections are caused by ascending bacteria from the urethral orifice, and the urethra and bladder are the most commonly involved locations of infection. These infections are confined to the lower urinary tract and are referred to as urethritis and cystitis, respectively. Common symptoms of these infections include pain or burning with urination, increased frequency of urination, blood in the urine, and pressure in the groin [2]. More severe infections can occur in the kidney, termed pyelonephritis or an upper urinary tract infection. Pyelonephritis can have additional symptoms of fever, chills, nausea, vomiting, and low back pain [2].

Risk factors for UTI include actions that introduce bacteria into the urethral area, anatomic factors that allow for easier access to the bladder, or factors that reduce the flow of urine. Women are at higher risk of UTI than men due to their shorter urethral lengths as bacteria have a shorter distance to travel to cause infection. Similarly, young children have short urethras (as well as poor urogenital hygiene), predisposing to UTIs. The shorter length of travel is also why lower UTIs are more common than upper UTIs. Other UTI risk factors introduce bacteria to the urethral orifice and include poor urogenital hygiene, which displaces bacteria from the anus, and sexual intercourse, which displaces bacteria from the vagina. Lastly, factors that decrease urinary flow, including urinary catheters, benign prostatic hyperplasia, and neurogenic bladder, increase UTI risk due to absent or decreased urine flow, decreasing bacterial flushing from the urinary tract (Fig. 1).

Diagnosis for UTI is based on symptoms, physical exam findings, and urine tests, and treatment generally involves a short course of oral antibiotics [2]. Common risk factors for UTIs include female sex (shorter length of urethra), introduction of bacteria to the urinary tract (e.g., sexual activity, poor genital hygiene), or stasis of the urinary tract [e.g., pregnancy, structural abnormalities, dehydration] [2]. Prevention of UTIs tries to counteract these factors via hydration, post-intercourse urination, and proper genital hygiene (e.g., wiping from front to back).

Common pathogens of community acquired UTIs are bacteria of the skin, gastrointestinal tract, or genital tract [2]. Nosocomial pathogens are frequently introduced to the urinary tract via catheters and tend to be more drug resistant [3]. The

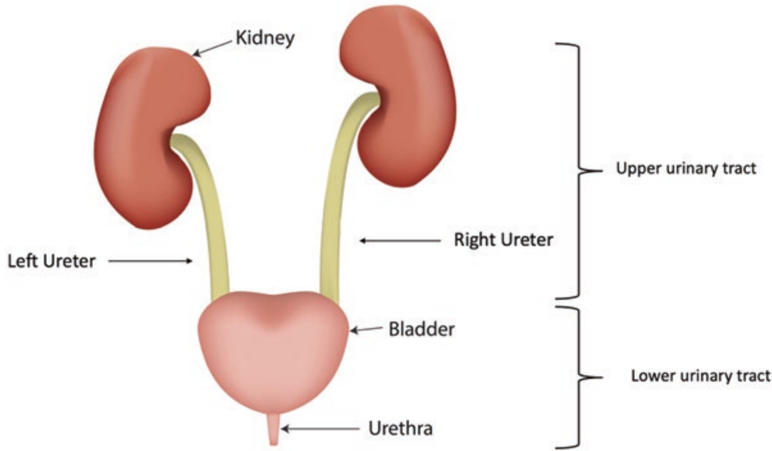

Fig. 1 Urinary Tract Anatomy

most common cause of UTI, particularly those that are community acquired, is uropathogenic *Escherichia coli* (UPEC) [2].

1.1 Anatomy of Urinary Tract

Before discussing pathogenicity and pharmacology of UTIs, it is appropriate to first discuss the relevant anatomy of the urinary tract. Urine production occurs in the kidneys, with the goal of removing extra fluid and metabolism waste products from the body [4]. Urine exits the kidneys via the renal pelvis and travels to the ureters. The ureters feed into the bladder, which stores urine before urination via the urethra with the male urethra traveling through the prostate [4]. As previously mentioned, UTIs most commonly occur from ascending bacterial infection. Thus, infections of the lower urinary tract (urethra, bladder, and prostate) occur much more frequently than those of the upper urinary tract (ureters and kidneys).

1.2 Uropathogenic *Escherichia coli*

UPEC accounts for an estimated 80% of all UTIs and has affinity for the bladder and kidneys, causing cystitis and acute pyelonephritis [5]. UPEC is normal flora of the gastrointestinal system, primarily utilizes peptides and amino acids for energy, and has evolved to acquire machinery that allows it to ascend through the urethra to its target tissue [5]. To cause infection, UPEC must overcome host defense

mechanisms, such as regular urine flow and shedding of urinary tract cells, as well as be able to survive in the urinary tract environment [6].

Being part of the *Escherichia coli (E. coli)* family, UPEC is described as a Gram-negative bacillus. Similar to other *E. coli* strains, UPEC has a polysaccharide capsule and contains flagella for motility [5]. Multiple virulence factors of UPEC aid in entry and adhesion, infection, motility, and immune evasion.

UPEC is introduced into the urinary tract via the urethral orifice and targets urothelial bladder cells. UPEC utilizes pili, or small arm-like appendages, for adhesion to urothelial cells [5]. UPEC fimbrial adhesin H (FimH) binds uroplakin IIIa on urothelial cells, causing intracellular signal cascades leading to cellular invasion and apoptosis [7]. UPEC also enters cells via its Type 1 pili, which bind urothelial alpha-3 and beta-1 integrins. This connection leads to urothelial microtubules destabilization, kinase signaling, and GTPase activation which all support bacterial internalization [8, 9]. Once intracellular, UPEC replicates and forms biofilm-like aggregates termed intracellular bacterial communities [IBCs] [10]. IBC formations are protective to the bacteria and serve as areas for further replication. From IBCs, UPEC can travel to adjacent urothelial cells for continued infection [6]. Some UPEC populations possess hemolysin A (HlyA) toxin, which aids in forming primary infections and establishing latent infections. HlyA toxin has been shown to inhibit urothelial kinase signaling, causing apoptosis and exfoliation [11]. Shedding of the top layer of urothelial cells allows for bacterial invasion of the deeper cells and can lead to the formation of quiescent intracellular reservoirs [QIRs] [11]. Following primary infection, UPEC within QIRs can reactivate and cause subsequent and recurrent infections [11].

Bacterial movement within the urinary tract is primarily accomplished by flagella. These flagella first play an important role in ascension through the urethra. Further, flagella aid with bacterial movement within and between the cells of the urinary tract [6].

Some populations of UPEC contain additional virulence factors allowing for colonization and infection of the kidney. UPEC strains causing pyelonephritis generally have been shown with more flagella and less Type 1 pili [12]. The expression of P fimbriae also aids in establishment of UPEC pyelonephritis. *P fimbriae* helps the bacteria adhere to renal surface epithelial cells by binding galactose-alpha-(1-4)-galactose-beta on globoseries glycolipids [13].

Host response to UPEC UTI is similar to those of other bacterial infection, primarily involving polymorphonuclear cells (PMNs). Toll-like receptors on host PMNs and macrophages recognize lipopolysaccharide (LPS) on the UPEC outer membrane, triggering a proinflammatory response and cytokine release [6]. While this inflammatory response leads to bacterial killing, it also causes local tissue damage including apoptosis of urothelial cells, which can lead to further UPEC infection and colonization.

LPS on the UPEC outer membrane elicits a strong host immune response, and thus, UPEC must be able to survive this response to cause infection. Host PMNs primarily kill the bacteria via phagocytosis and intracellular killing. The UPEC

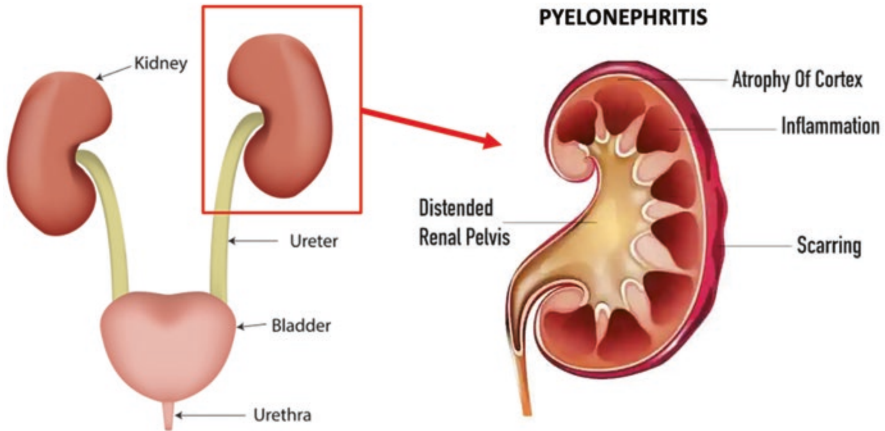

Fig. 2 Anatomy of pyelonephritis

polysaccharide capsule helps avoid this phagocytosis. Urothelial cells also play a part in host innate immunity by endocytosing UPEC into vesicles. Then, urothelial cells utilize actin fibers to trap the bacteria within the vesicles and restrict their replication [8, 14]. UPEC can counter this host response by disrupting the actin filaments. Once they escape the host vesicles, UPEC are free to start forming IBCs or exit the host urothelial cell.

2 Pyelonephritis (Fig. 2)

2.1 Pathophysiology

Pyelonephritis is the most severe manifestation of UTI. It occurs from bacteria ascending from the lower to the upper parts of the genitourinary system. UPEC is the bacterial pathogen most frequently responsible for pyelonephritis in roughly 80% of women and constitutes a major cause of morbidity and mortality in humans [15]. The invading bacteria cause a deviation from the native-tissue physiology, leading to dramatic changes in the micro-environment of the genitourinary system. The kidney is typically considered mostly resistant to infection due to it consisting of approximately one million nephrons continuously filtering blood from waste products. This anatomy causes dramatic changes to the local microenvironments, presenting several challenges to invading bacteria in the urinary tract. Several integrated events must occur between the host and the microbes for bacteria to colonize the dynamically changing microenvironment [16]. These integrated events include adhesion to host cells, colonization of tissues, and, in some contexts, cellular invasion; then intracellular multiplication, dissemination to other tissues, or persistence occurs [16].

The renal tubule epithelia cells expressing TLRs have demonstrated to play a key role in the inflammatory disorders and in initiating host defenses caused by UPEC. The primary response to UPEC infection in the kidneys is through the TLR4 signaling cascade. UPEC has TL4R receptors on the surface of renal epithelial cells and other immune cells in the interstitial compartment, including macrophages and dendritic cells [17, 18]. Following UPEC binding to the renal tubule, the pro-inflammatory pathways are activated, leading to the release of IL-8 [19]. In addition to TLR4 receptors, other toll-like receptors, such as the toll-like receptor 4, play a role in the response to UPEC infection [20]. The kidneys also have macrophages, T cells, and dendritic cells that assist in the detection of UPEC infection, modulate immune response through the activation of neutrophils, and induce inflammation [21].

Bacteria adhesion is an essential feature for its successful colonization, withstanding the hydrodynamic pressures of the renal tubule by adhering to the microtubule epithelium. Attachment organelles for UPEC include P, type 1, F1C, S fimbriae, and Afa/Dr. adhesin [16]. In approximately 80% of the UPEC strains, the *P fimbriae* is the adhesion factor expressed [22]. The *P fimbriae* plays an important role in the pathogenesis of pyelonephritis, binding to the glycosphingolipids and mannosylated glycoproteins expressed on the surface of renal epithelia [23]. *P fimbriae* have demonstrated to play an important role for early bacterial colonization and often act synergistically to facilitate colonization in the face of challenges, specifically from the hydrodynamic pressure of the renal filtrate.

Upon UPEC adhering to the renal tubule, early-stage tissue changes include vascular coagulation, epithelial breakdown, vascular leakage, immune cell recruitment, and general tissue destruction [24]. Bacterial clearance usually occurs within 22 hours of invasion and leaves behind local tissue destruction and vascular shutdown [24]. As the invasion progresses, coagulation in local peritubular capillaries, subsequent vascular shut-down and increasing hypoxic conditions promote a host response to the local infection site. Injury, such as ischemia, can initiate molecular crosstalk between the kidney and distant organs [25]. Boekel et al. studied early (8-hr post-infection) tissue responses in pyelonephritis and found approximately 80 upregulated genes in Gram-negative infection and inflammation [26]. This suggests that the local infection sites communicate in rapid succession with distant sites, although the mechanism governing this specific communication is unknown. Specific abnormalities in both the kidney's structure and function can increase the host susceptibility to infection [27]. Besides genetic susceptibilities linked to pyelonephritis, voiding dysfunctions and vesicoureteral reflux are identified as risk factors in children, whereas behavioral factors tend to affect adults [28].

3 Cystitis (Fig. 3)

3.1 Pathophysiology

Common cystitis, a bacterial infection of the bladder, is the most common type of UTI. The clinical presentation of cystitis can be dysuria, frequency, urgency, suprapubic pain, and hematuria and is often associated with symptoms perceived to

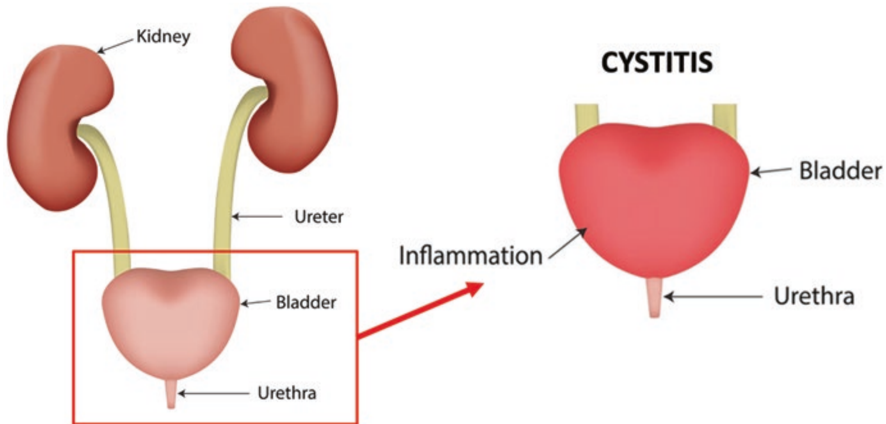

Fig. 3 Anatomy of cystitis

originate from the bladder [29]. These include the strong and sudden urge to void, increased frequency in urination, and nocturia [30]. Risk factors that should be considered for the development of bladder disorders such as cystitis revolve around chronic stress [31, 32]. The pathophysiology surrounding these stress-induced changes in the sympathetic division of the autonomic nervous system has been reported in literature. The enhanced severity and duration of pain symptoms for patients with cystitis may be related to the descending inhibition of the nervous system, termed stress-induced hyperalgesia [33]. Furthermore, epithelia alterations have also been suggested to be associated with cystitis. Specifically, changes in the bladder urothelium may lead to disruption of the mucosal barrier and allow water, urea, and toxins to move into the underlying tissues at the relevant site. This may produce symptoms of frequency, pain, and urgency seen in cystitis [34]. Similarly, it has been shown that in the presences of UPEC, this mechanism of epithelial cell and receptor expression may be disrupted and subsequently may impair normal function [35].

3.2 Current Treatments for Acute Pyelonephritis

In 2010, the Infectious Diseases Society of America (IDSA) updated its guidelines for the treatment of acute, uncomplicated cystitis and pyelonephritis in women [36]. The medical guidelines inform clinicians today through recommendations for antimicrobial regimens in patients with acute pyelonephritis. The guidelines address young, otherwise healthy women who are not pregnant, but the best clinical management for men, elderly induvial, and patients with comorbidities remains unclear. Despite publication of the guidelines, studies demonstrate a wide variation in prescribing practices regarding the selection of antimicrobial agents and duration of therapy [37, 38]. It encourages physicians to consider the effectiveness, risk of adverse side effects, and resistance rates in the local community when selecting an antibiotic for the treatment of acute pyelonephritis. The early initiation of therapy is

effective at preventing pyelonephritis; however, it is especially important to understand the risk of antibiotic resistance in antibiotic treatment. Regardless of the antibiotic selected for the initial therapy, the regimen should be revised as needed after urine culture susceptibility results become available to the physician. The selection of the most appropriate, narrowest-spectrum antibiotic is encouraged over a short duration to prevent further development of antimicrobial resistance.

With increasing antimicrobial resistance, efforts are made to use the shortest duration of antibiotics that are recommended. Oral fluoroquinolones have the shortest recommended length of treatment and remain the preferred antimicrobial class as a first-line outpatient therapy [36]. If the resistance rate of fluoroquinolones does not exceed 10%, patients requiring outpatient therapy can be treated with a once-daily oral fluoroquinolone, such as ciprofloxacin [Cipro; 1000 mg, extended-release, for 7 days] or levofloxacin [Levaquin; 750 mg for 5 days] or oral ciprofloxacin [Cipro; 500 mg ×2/day for 7 days] [36]. If the prevalence of resistance among the community exceeds 10%, an initial intravenous dose of ceftriaxone or gentamicin should be given, followed by the oral fluoroquinolone regimen [36]. Trimethoprim/sulfamethoxazole (Bactrim, Septra), a previous front-line treatment, is no longer recommended as an empiric treatment option for uncomplicated pyelonephritis in several geographical locations due to a high resistance rate to UPEC. Due to the high prevalence of resistance to oral beta-lactam antibiotics and trimethoprim/sulfamethoxazole, these drugs are typically reserved for acute cases where susceptibility results are known. However, in certain contexts, such as allergy history, drug availability, and drug-drug interactions, the IDSA recommends the use of oral beta-lactam antibiotics or trimethoprim/sulfamethoxazole if susceptibilities are unknown. When this occurs, the IDSA recommends the administration of a long-lasting, broad-spectrum drug such as ceftriaxone (Rocephin; 1 g) or gentamicin (5 mg/kg) concurrently to ensure that possible resistance was addressed [36].

Initial intravenous antibiotic therapy is recommended in patients with acute pyelonephritis who require in-patient treatment. The current IDSA guidelines recommend initiating a therapy that is appropriate based on local resistance patterns and patient factors. These include fluoroquinolones, extended-spectrum penicillin, extended-spectrum cephalosporins, carbapenems, and aminoglycosides as appropriate choices for empiric therapy [36]. There are no reported guidelines on when to transition from intravenous to oral therapy.

Following appropriate guidelines, antibiotics should produce improvements within 48–72 hours. Specifically, there should be an observable reduction or resolution of the local and systemic signs and symptoms that led to the diagnosis. Complications of acute pyelonephritis or an alternative diagnosis should be suspected if these changes are not observed.

3.3 Cystitis Treatment

With respect to the current treatment landscape, many antibiotic regimens exist for the management of cystitis. The 2011 IDSA guidelines address cystitis management and provide insight into the risks of broad-spectrum antibiotics [39]. These

guidelines indicate that no single agent is classified as the most effective treatment in acute, uncomplicated cystitis. First-line treatments is suggested to include 100 mg of nitrofurantoin twice a day for 5 days or a 3-day course of double-strength trimethoprim-sulfamethoxazole. A single dose of 3 grams of Fosfomycin may be utilized in the case of resistance, allergy, or kidney disfunction as well [36]. Second-tier antibiotics include fluroquinolones as indicated by the IDSA guidelines. It is suggested that fluoroquinolones are effective given their propensity for collateral damage and may be highly considered for serious infections. This becomes more relevant given the high resistance rates of urinary *E. coli* which may exceed 20% in some large urban locations [40].

4 Antimicrobial Resistance

Regional clustering of resistance among UPEC within the Unites States contribute to a more challenging treatment regimen in acute pyelonephritis and cystitis [41, 42]. The possible driving force for this observable trend may be due to variation in prescribing practices among physicians in the United States [20]. It has previously been established that there is a linear relationship between regions with more extensive antimicrobial use may have greater prevalence of antimicrobial resistance [43]. The statistically significant west-to-east descending gradient of antimicrobial resistance prevalence observed for ampicillin, trimethoprim, and trimethoprim/sulfamethoxazole, but not sulfisoxazole, and the north-south gradient observed for trimethoprim and trimethoprim/sulfamethoxazole but not ampicillin or sulfisoxazole, suggest that the various resistance phenotypes exhibited distinctive patterns of geographical distribution, which suggest a geographic segregation of composite resistance profiles [42]. Understanding these trends is relevant to both clinical practice and in understanding the basis for the current antimicrobial resistance of UPEC epidemic in the context of cystitis and pyelonephritis.

Increased resistance of UPEC to multiple antibiotics has been reported worldwide. In the United States, rates of *E. coli* from UTI increased from 7.8% in 2010 to 18.3% in 2014 [44]. In a longitudinal survey focused on South American, Seifert and colleagues reported that ceftriaxone resistance among *E. coli* isolates increased from 9.6% in 2004 to 44.1% in 2016 [45]. In Asian countries, the rates of *E. coli* increased from 25% in 2010 to 41.7% in 2013 in Hong Kong, 18.4% in 2010 to 28.1% in 2013 in Singapore, and 15.8% in 2010 to 34% in the Philippines [46]. Kwon and colleagues discuss how increased antimicrobial resistance of *E. coli* might have resulted in the increase in the total duration of antibiotic treatment and hospitalization days [47]. For example, the total duration of antibiotic therapy for the treatment of acute pyelonephritis has increased significantly (16.55 ± 9.68 vs. 19.12 ± 9.90, $p < 0.001$), and more alarming, the duration of carbapenem usage has also increased [0.59 ± 2.87 days in 2010–2011 to 1.79 ± 4.89 days in 2017–2018, $p < 0.001$] [47]. Moreover, an increase in antimicrobial resistance rate to commonly prescribed antibiotic classes may have led to a more frequent mismatch between initial antimicrobial regimen and pathogens [47]. It is important to address this

phenomenon because increased consumption of antimicrobial agents causes the emergence of other antimicrobial-resistant pathogens [48]. Recent analysis of the pharmaceutical industry has revealed that development of new antimicrobial agents is not on the priority list of many major companies [35]. Therefore, as the levels of resistance are rising, there are very few new clinical discoveries to address the threat. Judicious use of antibiotics is an urgent need and must be enforced at all levels of healthcare with respect to cystitis and pyelonephritis treatment.

4.1 Antibiotic Resistance on the Rise

There is a growing rate of antibiotic resistance among *E. coli* strains even leading to multidrug resistance (MDR). Risk factors for MDR include using antibiotics too early, hospitalization for long periods of time, use of urinary catheters and other medical devices, and having underlying diseases or old age [49, 50]. Awareness of regional susceptibility data is necessary when choosing an empirical antibiotic to treat *E. coli* [51]. The Infectious Diseases Society of America (IDSA) recommended in 2011 that trimethoprim-sulfamethoxazole, fosfomycin, nitrofurantoin, or pivmecillinam be prescribed for treatment if local resistance rates that do not exceed 20%; alternatives include fluoroquinolones or cephalosporins [36].

Trimethoprim-sulfamethoxazole is a commonly recommended antibiotic with resistance rates on the rise. Looking at resistance rates of *E. coli* strains to trimethoprim-sulfamethoxazole in the past decade, Japan has reported a 10–15% resistance rate [52], China and South Korea have reported about 30% [53, 54], and Europe and the Mediterranean region have reported about 15% resistance [55, 56]. The resistance rates of *E. coli* strains to fluoroquinolones were reported as about 10% for Japan and Australia [55], 12–30% in the United States [57], 16–26% in China [54], 20% in middle and north European countries [56], and up to 40% in other European and some Mediterranean regions [55, 58]. Both trimethoprim-sulfamethoxazole and fluroquinolone did show some evidence that escape from exposure to these antibiotics has the potential to increase susceptibility of antibiotics when treating UTIs caused by *E. coli* [51].

There has also been an increase in the prevalence of extended spectrum beta lactamase (ESBL)-producing *E. coli*. Before 2010, most countries only had about a 5–10% prevalence of ESBL-producing *E. coli.* After 2010, several countries have a greater than 10% prevalence such as Spain (8.9–23.6%), Turkey (24%), South Asia (33.2%), and Latin America (7.1–12.5%) [51].

4.2 New Antibiotic in Multidrug-Resistant Bacteria

Cefiderocol is a recent antibiotic proved to be effective against ESBL-producing Enterobacteriaceae, nonfermenting Gram-negative bacilli including MDR *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*,

Klebsiella pneumoniae, and carbapenem-resistant *Enterobacteriaceae* [59]. It is the first siderophore antibiotic to be approved by the FDA that has a catechol-type siderophore and cephalosporin core allowing it enhanced stability against extended spectrum β-lactamases and carbapenemases [60, 61]. Cefiderocol has been approved by the FDA for treatment of complicated UTIs. While the side effects are mostly mild including diarrhea, nausea, vomiting, constipation, and upper abdominal pain, a phase III clinical trial found cefiderocol to have a higher all-cause mortality [34]. Use of cefiderocol is thus limited to complicated UTIs caused by Gram-negative bacteria in patients who have no alternative treatment options [62].

4.3 Mechanisms of Resistance to Antibiotics in UPEC Strains

There are several mechanisms that contribute to resistance to antibiotics in UPEC strains via acquisition of resistance genes. Acquisition of these resistance genes can occur via plasmids, transposons, gene cassettes, or changes in the multiple antibiotic resistance locus on the chromosome of *E. coli* [63].

ESBL-producing *E. coli* are resistant to β-lactam antibiotics such as cephalosporins, ampicillin, and amoxicillin. ESBLs are transferred by plasmids after mutations in enzymes such as blaTEM-1, blaTEM-2, and blaSHV-1 as well as blaCTX-M newly identified in the early 2000s [64–66]. ESBL-producing *E. coli* pose a challenge in treatment of UTIs due to their increased rates of resistance to several antibiotics such as β-lactams, quinolones, and aminoglycosides. The most effective antibiotic against ESBL-producing *E. coli* are carbapenems; however, there are still mechanisms of resistance to these antibiotics either through overexpression of efflux pumps, decreased permeability, or secretion of carbapenemases which are carbapenem-hydrolyzing enzymes [67, 68].

Resistance to quinolones and fluoroquinolones has also developed among UPEC strains due to mutations in DNA topoisomerases II and IV, overexpression of efflux pumps, decreased uptake of antibiotics, and plasmid-mediated quinolone resistance (PMQR) genes [69, 70]. Of the PMQR genes, the most important are *Qnr* genes in creating antibiotic resistance. *Qnr* are located on different integrons and inhibit binding of quinolones to DNA gyrase and topoisomerases [71].

Another characteristic of *E. coli* that makes it prone to resistance to antibiotics is the ability to form biofilms. Biofilms secrete substances such as exopolysaccharides (EPS) that are protective and enhance their ability to survive [72]. Bacteria in the biofilm communicate with each other and secrete autoinducer substances (AI) which help the cells secrete virulence factors, accrue genetic changes, and modulate the host immune response [73]. Penetration by antibiotics is greatly hindered by biofilms, and alternative therapeutic agents need to be explored to treat *E. coli* biofilm formation [72].

Acquisition of resistance to different antibiotics poses a threat to treatment of all UTIs especially by biofilm-producing bacteria such as *E. coli*. Continual monitoring of resistance in different countries and communities is needed to find the best therapy against infection.

4.4 Alternative Therapies in the Treatment of UTIs Caused by UPEC

To specifically target biofilms formed by *E. coli*, antiadhesion agents can disrupt curli and pili formation which normally maintain cell–cell and cell–surface interactions in the formation of a biofilm. Lo et al. showed that in vivo and in vitro type 1 pili were disrupted and biofilm formation suppressed by *N*-(4-chloro-phenyl)-2-{5-[4-(pyrrolidine-1-sulfonyl)-phenyl]-[1,3,4]oxadiazol-2-yl sulfanyl}-acetamide (AL1) [74]. FimH antagonists, a mannoside, are another antiadhesion therapy option. They are designed to fit the binding pocket of FimH mannose, a receptor present on the urinary bladder that type 1 pili of *E. coli* normally binds to trigger infection. FimH antagonists have been more potent than FimH inhibitors against *E. coli*. These inhibitors can reduce catheter-associated UTI caused by UPEC [75, 76]. Mannosides also have synergistic effects when used with trimethoprim-sulfamethoxazole [77]. Alternatively, curlicides such as BibC6 and FN075 inhibit curli formation in UPEC by inhibiting the major curli subunit protein CsgA [72].

Phage therapy is another option to eradicate biofilms of *E. coli* or mixed bacteria biofilms. Using multiple types of bacteriophages at once have been shown to have greater efficacy than a single bacteriophage [72]. Phages that produce depolymerase can degrade the EPS matrix of the biofilm facilitating penetration of the phage into the biofilm and causing lysis of bacterial cells. T7 is an engineered phage that expresses recombinant dispersin B (DsbB) and lactonase to degrade EPS and AHL respectively and is even active against mixed biofilms of *Pseudomonas aeruginosa* and *E. coli* [78, 79]. Using a combination of phage therapy and antibiotics has also been shown to decrease antibiotic resistance in *E. coli* [80].

There are several phytochemicals being studied as potential therapies against *E. coli* due to their ability to kill the bacteria without developing resistance. Examples include 7-hydroxycoumarin (7-HC), salicylic acid, saponin, and indole-3-carbinol (I3C) which can inhibit activity against a planktonic culture of *E. coli* and *Staphylococcus aureus* while restricting the growth of the biofilm [81]. When phenolic-rich maple syrup (PMRSE) was tested on *E. coli*, it was found that multiple aspects of *E. coli* were suppressed such as multiple drug resistance genes, motility genes, as well as biofilm adhesion and formation [82].

Nanomedicine is another area of advancement in the treatment of UTIs. Nanotechnology particles can attach and penetrate bacterial cells, damage the membrane, and interact with DNA [83]. There has been research showing nanotechnology's ability to target biofilm production to reduce infections caused by catheter use. Successful inhibition of biofilm activity has been shown from coating catheters with nitrofurazone, silver hydrogel [84], furacilinum, and furanone [85]. While effective in inhibiting many pathogenic microorganisms, there was recurrence of UTIs due to an inability to provide long-term inhibition of biofilm production. Lellouche et al. have coated catheters with magnesium fluoride and yttrium fluoride nanoparticles that both proved inhibition of biofilm formation and a decline in bacterial colonization over 7 days [83, 85]. Nanotechnology antimicrobial spray has

been found to decrease the occurrence of catheter-associated UTIs caused by UPEC and can be incorporated into medical devices and wound dressings due to its small size, high stability, and high bioavailability [72]. Wei He et al. tested the use of JUC, a nanotechnology antimicrobial spray, on urinary catheters and found a significant difference in the incidence of bacteriuria (4.52% for therapy group vs 13.04% in control group, $p < 0.001$) [86].

Nanotechnology also has the potential to change how we deliver drugs and antibiotics in the treatment of UTIs. Nanodrug delivery vehicles advanced in the past decade can enhance specificity to target cells, increase solubility and bioavailability of the drug, allow a controlled release of the drugs, and make it possible for two drugs to be delivered simultaneously [87, 88]. These advantages are very attractive in the context of treating UTIs and specifically targeting the bladder. Chang et al. used poly(ethyl-2-cyanoacrylate (PECA) nanoparticles with epirubicin targeted to bladder cells and had higher penetration and specificity compared to epirubicin in a powdered form [89].

Nanoantibiotics, nanoparticles that have antimicrobial properties or enhance antibiotic efficacy, may also be an important advancement in the treatment of UTIs. Nanoantibiotics destroy pathogens with several different mechanisms and are more useful for the eradication of intracellular infections which is a large advantage over traditional antibiotics. Nanoantibiotics may help clear infection including quiescent intracellular reservoirs thus hindering recurrence of UTIs as well [90].

Cranberry juice has long been a natural remedy to protect against UTIs and may be increasingly important in the age of antibiotic resistance. Cranberries contain anthocyanidin/proanthocyanidin antiadhesive biocompounds that work by inhibiting type I and P-fimbria-preventing attachment of uroepithelial cells by UPEC [91]. While their antiadhesive properties are one factor in how cranberries mitigate UTIs, there are many other compounds, such as flavonoids and phenolic acids, in cranberries that may also play a role with several hypotheses that are not fully understood [92]. There have also been mixed results in the efficacy of using cranberry juice in UTI prophylaxis. Recent studies have suggested that there may be a large interindividual variability in the effectiveness of cranberries; a subpopulation analysis suggested that cranberries can prevent UTI recurrence but is less effective in populations at an increased risk for UTIs [93].

Finally, probiotics are another alternative strategy in preventing recurrent UTIs. Maintaining the normal ecology of the vagina, urethra, and bladder is important in diminishing the risk of UTIs. Lactobacilli are the predominate bacteria in the urogenital flora of healthy reproductive-aged women, but this flora can be disturbed in many scenarios such as temporarily after menstruation, after antibiotic administration especially long-term, and post-menopausal women [91]. The supplementation of different strains of lactobacilli can maintain or return the normal urogenital flora to protect against UTIs. *Lactobacillus rhamnosus GR-1* and *Lactobacillus fermentum RC-14* are two strains that have been shown to be the most effective in lowering the risk of urogenital infections [94]. Looking specifically at effectiveness against UPEC, several strains of lactic acid bacteria (*L. salivarius*, *L. paracasei*, two *L. plantarum* strains, two *Pediococcus pentosaceus* strains, and *L. crispatus*)

exhibited clear zones of inhibition when plated with UPEC [95]. Probiotics are a safe and natural way to lower rates of UTIs, but like cranberry juice, there still needs to be more studies to prove the effectiveness and standardize dosage and concentration of various compounds before recommendations can be made.

There is potential of combining multiple alternative therapies in the prevention of UTIs. One study looked at the potential benefit of cranberries, probiotics, and vitamin C supplementation in women with a history of recurrent UTIs [96]. There is not conclusive evidence on these supplements, but they are safe alternatives with the potential to decrease the occurrence of UTIs.

4.5 Vaccines Against Urinary Tract Infections

Due to how common UTIs are and the increasing rate of antibiotic resistance, the development of a vaccine could provide immense benefit for prevention of UTIs. So far vaccines developed can be classified in two groups: (1) cell-based vaccines and (2) antigen-based vaccines [63]. The following table describes three main cell-based vaccines that have been developed and are going through clinical trials (Table 1).

In a systematic review on the effectiveness of Uromune, two retrospective comparative studies were found that significantly decreased the incidence of UTIs in women. The therapy group treated with Uromune for 3 months reported UTI-free rates of 35–90%, while the control group treated with antibiotic prophylaxis for 6 months had a 0% UTI-free rate after 15 months ($p < 0.001$). Three prospective, uncontrolled studies were also assessed, and UTI-free rates in the therapy groups ranged from 33% to 78% for a period of 9–24 months [97].

Table 1 Description of cell-based vaccines and their availability

Name	Description	Route of administration	Availability
Uro-Vaxom	Composed of membrane proteins of 18 strains of UPEC	Oral capsule	Developed and manufactured in Switzerland and used in 40 countries
Uromune	Composed of killed bacteria in equal amounts of *E. coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, and *Enterococcus faecalis*	Sublingual	Developed and manufactured in Spain and available in special access programs in the UK, Netherlands, Sweden, Norway, Spain, Australia, and New Zealand, and a clinical trial in Canada
Urovac	Composed of killed bacteria (6 strains of UPEC, 1 strain of *Proteus mirabilis*, *Morganella morganii*, *Klebsiella pneumoniae*, and *Enterococcus faecalis*)	Intramuscular, oral capsule, or sublingual	Manufactured in Switzerland

Another systematic review of 10 randomized control trials looked at Uro-Vaxom and Urovac. UTI recurrence was found to be reduced compared to placebo for both Uro-Vaxom and Urovac. Uro-Vaxom showed the greatest effect at 3 months with a risk ratio of 0.67 (95% confidence interval (CI) 0.57–0.78) compared to 6 months with a risk ratio of 0.78 (95% CI 0.69–0.88). The overall Urovac risk ratio was found to be 0.75 [95% CI 0.63–0.89] [98]. There is evidence that these vaccines are efficacious in preventing UTIs; however, the trials evaluated were determined to be low quality of evidence [98].

Antigen-based vaccines come with other challenges, and several types are described in the following table. These vaccines utilize proteins and peptides, small molecules, or a combination to elicit an immune response. Many of these vaccines are in the early stage of research to determine the immunogenicity of these targets (Table 2).

A phase II double-blind study of ExPEC4V found that a robust immune response against all serotypes was observed, and there were no vaccine safety findings [101]. However, a systemic review found that ExPEC4V did not significantly reduce UTI recurrence compared to placebo [Risk Ratio 0.82, 95% CI 0.62–1.10] [98].

A phase I clinical study of the FimH vaccine showed that individuals immunized with the vaccine produced FimH-binding antibodies in women with a history of recurrent UTIs and women with no history of recurrent UTIs [102]. There are several other variations using the FimH antigen that elicited an immune response in mouse models such as a FimH.FliC mixed with Cholera toxin [63] and fusion of FimH and MrpH adhesins of UPEC and *Proteus mirabilis* with monophosphoryl lipid A adjuvant [100]. Other adhesin molecules being investigated are autotransporters such as UpaG that provided immune protection in a mouse model after both active and passive immunization [103, 104].

Table 2 Description of antigen-based vaccines

Type	Notes on antigen	Vaccine description
LPS-based vaccine	Difficult to cover all UPEC serotypes; low immunogenicity [63]	ExPEC4V consists of four bioconjugates containing O-antigens of three *E. coli* serotypes [O1A, O2, O6A, and O25B] [98]
FimH adhesin vaccine	High immunogenicity, antigenic structure is conserved across different strains of UPEC, high expression at site of infection, important role in pathogenesis [63]	FimH vaccine uses an antigen FimH with a TLR-4 agonist adjuvant
Iron scavenger receptor-based vaccines	Expressed on the surface of bacteria, high prevalence in different UPEC strains, and is expressed during an infection [99, 100]	Iron absorption receptors such as IutA, Iha, IroN, ChuA, FyuA, and IreA have been under investigation as potential vaccine targets [63]
Siderophore-based vaccines	Small iron-chelating molecules that are an important virulence factor for UPEC [101]	Siderophores (Ybt and Aer) conjugated to an immunogenic protein [101]

While the iron scavenger receptor-based vaccines have the potential to produce a robust immune response, the evidence is still in preliminary stages. Intranasal immunization of mice targeting these iron receptors has been shown to elicit a systemic, mucosal, and cellular immune response with lutA, Hma, and Ire providing significant protection in the mouse model [105]. Multi-epitope subunit vaccines have shown promise in protection in UPEC colonization in mice [106].

Iron acquisition is essential for bacterial growth, and UPEC strains code several siderophore genes to secure iron and evade host immune system. Ybt and Aer were found to be more prevalent among strains of *E. coli* causing UTIs compared to commensal bacteria [107]. Mike et al. conjugated Ybt and Aer to an immunogenic protein, cantonized BSA (cBSA), and immunized mice intranasally. These conjugates protected against UTIs by showing a significant decrease in bacterial burden in the urine and kidneys [101].

Overall, there is a lot of potential for a vaccine to protect against UTIs. Cell-based vaccines have shown a lot of promise and are available in many countries or are currently going through clinical trials. However, there is still no FDA-approved vaccine in the United States or Canada. There is an abundance of antigens as potential targets for vaccines that are still under investigation with the hope of an effective vaccine available in the future.

5 Future Perspectives

Acute cystitis and pyelonephritis, in particular, has a high rate of reoccurrence, with women having up to a 10% risk and men having up to a 6% risk of reoccurrence in the year following a first acute episode [108, 109]. Due to the increased use of fluoroquinolones for infections, the growing rates of resistance to fluoroquinolones among the most common uropathogens have challenged the widespread use of this drug class and have led researchers to search for alternatives.

Ceftriaxone is a widely used third-generation cephalosporin antibiotic that has a broad spectrum of bactericidal activity against aerobic Gram-positive and Gram-negative bacteria [110, 111]. It has previously been demonstrated that similar clinical outcomes are achieved between ceftriaxone and fluoroquinolone-treatment groups in acute pyelonephritis [57, 112]. Ceftriaxone has also been shown to achieve similar clinical outcomes in acute pyelonephritis when compared to ertapenem, a carbapenem [113]. Drawing upon this available data, ceftriaxone seems to be an effective therapy for some patients and offers a promising alternative to fluoroquinolones.

Another therapy, piperacillin-tazobactam, has been explored as an alternative for the management of acute pyelonephritis and cystitis in light the emergence of carbapenem-resistant organisms. Sharara et al. performed a multicenter observational study comparing clinical outcomes of hospitalizations with acute pyelonephritis who received piperacillin-tazobactam versus carbapenems [114]. These researchers found no difference between the two groups in the proportion of patients

(20% vs 25%) with recurrent cystitis or pyelonephritis with the same bacteria producing organisms within 30 days (OR, 0.75; 95% CI, 0.31–1.81; $p = 0.52$) [114]. This study demonstrates that piperacillin-tazobactam offers a reasonable alternative to the treatment of pyelonephritis and may mitigate the risks that carbapenems pose.

Amdinocillin, also known as mecillinam, has also been used successfully as the first choice for cystitis in Scandinavia, with guidelines recommending oral mecillinam for acute uncomplicated pyelonephritis [115]. Across 12 clinical studies, clinical success was seen in 38/51 (75%) patients treated with mecillinam as monotherapy and in 152/164 (93%) patients treated with mecillinam and one other beta-lactam [115]. Furthermore, a Danish retrospective study found a favorable 30-day mortality outcome of mecillinam (23%) compared to other antibiotics (43%) for *Klebsiella pneumoniae* bacteremia (OR, 0.4; 95% CI, 0.2–0.9; p = not reported) [116]. While mecillinam is an older antimicrobial drug, its unique properties still make it a relevant and important drug today. Synergism with mecillinam and other beta-lactams occurs because mecillinam has more selective affinity to penicillin-binding protein 2, and it is an amidinopenicillin [117]. Although most studies are outdated, mecillinam could be considered an alternative to acute pyelonephritis, especially in patients with a high predicted probability of resistance to fluoroquinolone and other first-line agents.

Ceftolozane-tazobactam, a novel antibacterial with Gram-negative activity, was developed to address the rising rates of antimicrobial resistance [118]. In the Assessment of the Safety Profile and Efficacy of Ceftolozane-Tazobactam in Complicated Urinary Tract Infections (ASPECT-cUTI) clinical program, the safety profiles and efficacy of ceftolozane-tazobactam were compared with those of levofloxacin [119]. ASPECT-cUTI reported a clinical cure in 90 (90.0%) of 100 patients in the ceftolozane-tazobactam group versus 86 (76.8%) of 112 in the levofloxacin group with levofloxacin-resistant uropathogens (95% CI: 3.1–22·9) and in 55 (90.2%) of 61 versus 42 (73.7%) of 57 for patients with ESBL-producing uropathogens (95% CI 2.6–30.2) [119]. Ceftolozane-tazobactam demonstrated its efficacy for the treatment of acute pyelonephritis and in infections caused by difficult-to-treat uropathogens. This drug might add a therapeutic option for patients with potentially life-threatening infections in the fact of increasing prevalence of bacteria resistant to fluoroquinolones.

Preventative therapies are being explored, although it is important to note that additional studies are needed before routinely recommending these methods. Low estrogen levels are hypothesized to be an important contributor to high incidence of UTIs in postmenopausal women. Perrotta et al. found that vaginal estrogen significantly decreased the number of UTIs in postmenopausal women [120]. Other preventative therapies include probiotics by helping restore the normal vaginal microbiome; cranberries which contain proanthocyanidins that inhibit *E. coli* from adhering to the bladder walls; hyaluronic acid and chondroitin sulfate which play a role in preventing the adherence of bacteria to the urothelium; and *D*-Mannose which intervenes with protein binding along the urothelium [121]. The integrated pathophysiology of pyelonephritis and cystitis constitutes several integrated events where the host and the microbes mutually influence each other. Full understanding

of these events is required to identify new potential angles for disease intervention amid increasing antibiotic resistance. In the future, there will be both non-antimicrobial and antimicrobial regimens, allowing for a new focus placed on monitoring and mimicking host-pathogen interaction in the dynamic micro-ecology significant in the live host.

References

1. Ulett GC, Totsika M, Schaale K, Carey AJ, Sweet MJ, Schembri MA. Uropathogenic Escherichia coli virulence and innate immune responses during urinary tract infection. Curr Opin Microbiol. 2013;16(1):100–7.
2. CDC. Suffering from a urinary tract infection? [Internet]. Centers for Disease Control and Prevention. 2022 [cited 2022 May 28]. Available from: https://www.cdc.gov/antibiotic-use/uti.html
3. Iacovelli V, Gaziev G, Topazio L, Bove P, Vespasiani G, Finazzi AE. Nosocomial urinary tract infections: a review. Urologia. 2014;81(4):222–7.
4. Anatomy of the Urinary System | Johns Hopkins Medicine [Internet]. [cited 2022 May 28]. Available from: https://www.hopkinsmedicine.org/health/wellness-and-prevention/anatomy-of-the-urinary-system
5. Croxen MA, Finlay BB. Molecular mechanisms of Escherichia coli pathogenicity. Nat Rev Microbiol. 2010;8(1):26–38.
6. Lüthje P, Brauner A. Virulence factors of uropathogenic E. coli and their interaction with the host. Adv Microb Physiol. 2014;65:337–72.
7. Thumbikat P, Berry RE, Zhou G, Billips BK, Yaggie RE, Zaichuk T, et al. Bacteria-induced uroplakin signaling mediates bladder response to infection. PLoS Pathog. 2009;5(5):e1000415.
8. Eto DS, Jones TA, Sundsbak JL, Mulvey MA. Integrin-mediated host cell invasion by type 1–piliated uropathogenic Escherichia coli. PLoS Pathog. 2007;3(7):e100.
9. Dhakal BK, Mulvey MA. Uropathogenic Escherichia coli invades host cells via an HDAC6-modulated microtubule-dependent pathway. J Biol Chem. 2009;284(1):446–54.
10. Anderson GG, Palermo JJ, Schilling JD, Roth R, Heuser J, Hultgren SJ. Intracellular bacterial biofilm-like pods in urinary tract infections. Science. 2003;301(5629):105–7.
11. Wiles TJ, Dhakal BK, Eto DS, Mulvey MA. Inactivation of host Akt/protein kinase B signaling by bacterial pore-forming toxins. Mol Biol Cell. 2008;19(4):1427–38.
12. Lane MC, Simms AN, Mobley HLT. Complex interplay between type 1 fimbrial expression and flagellum-mediated motility of uropathogenic Escherichia coli. J Bacteriol. 2007;189(15):5523–33.
13. Kaper JB, Nataro JP, Mobley HLT. Pathogenic Escherichia coli. Nat Rev Microbiol. 2004;2(2):123–40.
14. Mulvey MA, Schilling JD, Hultgren SJ. Establishment of a persistent Escherichia coli reservoir during the acute phase of a bladder infection. Infect Immun. 2001;69(7):4572–9.
15. Foxman B, Brown P. Epidemiology of urinary tract infections: transmission and risk factors, incidence, and costs. Infect Dis Clin N Am. 2003;17(2):227–41.
16. Bien J, Sokolova O, Bozko P. Role of uropathogenic Escherichia coli virulence factors in development of urinary tract infection and kidney damage. Int J Nephrol. 2012;2012:681473.
17. Tittel AP, Heuser C, Ohliger C, Knolle PA, Engel DR, Kurts C. Kidney dendritic cells induce innate immunity against bacterial pyelonephritis. J Am Soc Nephrol. 2011;22(8):1435–41.
18. Chassin C, Goujon JM, Darche S, du Merle L, Bens M, Cluzeaud F, et al. Renal collecting duct epithelial cells react to pyelonephritis-associated Escherichia coli by activating distinct TLR4-dependent and -independent inflammatory pathways. J Immunol. 2006;177(7):4773–84.

19. Ingersoll MA, Kline KA, Nielsen HV, Hultgren SJ. G-CSF induction early in uropathogenic Escherichia coli infection of the urinary tract modulates host immunity. Cell Microbiol. 2008;10(12):2568–78.
20. Samuelsson P, Hang L, Wullt B, Irjala H, Svanborg C. Toll-like receptor 4 expression and cytokine responses in the human urinary tract mucosa. Infect Immun. 2004;72(6):3179–86.
21. Weisheit CK, Engel DR, Kurts C. Dendritic cells and macrophages: sentinels in the kidney. Clin J Am Soc Nephrol CJASN. 2015;10(10):1841–51.
22. Mobley HL, Island MD, Massad G. Virulence determinants of uropathogenic Escherichia coli and Proteus mirabilis. Kidney Int Suppl. 1994;47:S129–36.
23. Uropathogenic Escherichia Coli – an overview | ScienceDirect Topics [Internet]. [cited 2022 May 26]. Available from: https://www.sciencedirect.com/topics/medicine-and-dentistry/uropathogenic-escherichia-coli.
24. Choong FX, Antypas H, Richter-Dahlfors A. Integrated pathophysiology of pyelonephritis. Mulvey MA, Stapleton AE, Klumpp DJ, editors. Microbiol Spectr. 2015;3(5):3.5.09.
25. Li X, Hassoun HT, Santora R, Rabb H. Organ crosstalk: the role of the kidney. Curr Opin Crit Care. 2009;15(6):481–7.
26. Boekel J, Källskog O, Rydén-Aulin M, Rhen M, Richter-Dahlfors A. Comparative tissue transcriptomics reveal prompt inter-organ communication in response to local bacterial kidney infection. BMC Genomics. 2011;12:123.
27. Jackson GG. Pathogenesis of renal infection. Arch Intern Med. 1957;100(5):692.
28. Finer G, Landau D. Pathogenesis of urinary tract infections with normal female anatomy. Lancet Infect Dis. 2004;4(10):631–5.
29. Bent S, Nallamothu BK, Simel DL, Fihn SD, Saint S. Does this woman have an acute uncomplicated urinary tract infection? JAMA. 2002;287(20):2701–10.
30. Fall M, Baranowski AP, Elneil S, Engeler D, Hughes J, Messelink EJ, et al. EAU guidelines on chronic pelvic pain. Eur Urol. 2010;57(1):35–48.
31. Lovick TA. Central control of visceral pain and urinary tract function. Auton Neurosci Basic Clin. 2016;200:35–42.
32. Kanter G, Komesu YM, Qaedan F, Jeppson PC, Dunivan GC, Cichowski SB, et al. Mindfulness-based stress reduction as a novel treatment for interstitial cystitis/bladder pain syndrome: a randomized controlled trial. Int Urogynecol J. 2016;27(11):1705–11.
33. Charrua A, Pinto R, Taylor A, Canelas A, Ribeiro-da-Silva A, Cruz CD, et al. Can the adrenergic system be implicated in the pathophysiology of bladder pain syndrome/interstitial cystitis? A clinical and experimental study. Neurourol Urodyn. 2015;34(5):489–96.
34. Birder L, Andersson KE. Urothelial signaling. Physiol Rev. 2013;93(2):653–80.
35. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. P T Peer-Rev J Formul Manag. 2015;40(4):277–83.
36. Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, et al. International Clinical Practice Guidelines for the Treatment of Acute Uncomplicated Cystitis and Pyelonephritis in Women: A 2010 Update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis. 2011;52(5):e103–20.
37. Huang ES, Stafford RS. National patterns in the treatment of urinary tract infections in women by ambulatory care physicians. Arch Intern Med. 2002;162(1):41–7.
38. Stamm W. Evaluating guidelines. Clin Infect Dis. 2007;44:775–6.
39. Uncomplicated Cystitis and Pyelonephritis (UTI) [Internet]. [cited 2022 May 28]. Available from: https://www.idsociety.org/practice-guideline/uncomplicated-cystitis-and-pyelonephritis-uti/.
40. Colgan R, Williams M. Diagnosis and treatment of acute uncomplicated cystitis. Am Fam Physician. 2011;84(7):771–6.
41. den Heijer CDJ, Donker GA, Maes J, Stobberingh EE. Antibiotic susceptibility of unselected uropathogenic Escherichia coli from female Dutch general practice patients: a comparison of two surveys with a 5 year interval. J Antimicrob Chemother. 2010;65(10):2128–33.

42. Sannes MR, Kuskowski MA, Johnson JR. Geographical distribution of antimicrobial resistance among Escherichia coli causing acute uncomplicated pyelonephritis in the United States. FEMS Immunol Med Microbiol. 2004;42(2):213–8.
43. Harbarth S, Albrich W, Brun-Buisson C. Outpatient antibiotic use and prevalence of antibiotic-resistant pneumococci in France and Germany: a sociocultural perspective. Emerg Infect Dis. 2002;8(12):1460–7.
44. Lob SH, Nicolle LE, Hoban DJ, Kazmierczak KM, Badal RE, Sahm DF. Susceptibility patterns and ESBL rates of Escherichia coli from urinary tract infections in Canada and the United States, SMART 2010–2014. Diagn Microbiol Infect Dis. 2016;85(4):459–65.
45. Seifert H, Blondeau J, Dowzicky MJ. In vitro activity of tigecycline and comparators (2014–2016) among key WHO "priority pathogens" and longitudinal assessment (2004–2016) of antimicrobial resistance: a report from the T.E.S.T. study. Int J Antimicrob Agents. 2018;52(4):474–84.
46. Jean SS, Coombs G, Ling T, Balaji V, Rodrigues C, Mikamo H, et al. Epidemiology and antimicrobial susceptibility profiles of pathogens causing urinary tract infections in the Asia-Pacific region: results from the Study for Monitoring Antimicrobial Resistance Trends (SMART), 2010–2013. Int J Antimicrob Agents. 2016;47(4):328–34.
47. Kwon KT, Kim B, Yeol RS, Wie SH, Kim J, Uk JH, et al. Changes in clinical characteristics of community-acquired acute pyelonephritis and antimicrobial resistance of uropathogenic Escherichia coli in South Korea in the past decade. Antibiotics. 2020;9(9):617.
48. Spellberg B, Guidos R, Gilbert D, Bradley J, Boucher HW, Scheld WM, et al. The epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. Clin Infect Dis. 2008;46(2):155–64.
49. Ejrnæs K. Bacterial characteristics of importance for recurrent urinary tract infections caused by Escherichia coli. Dan Med Bull. 2011;58(4):B4187.
50. Walker E, Lyman A, Gupta K, Mahoney MV, Snyder GM, Hirsch EB. Clinical management of an increasing threat: outpatient urinary tract infections due to multidrug-resistant uropathogens. Clin Infect Dis. 2016;63(7):960–5.
51. Lee DS, Lee SJ, Choe HS. Community-acquired urinary tract infection by *Escherichia coli* in the era of antibiotic resistance. Biomed Res Int. 2018;(2018):1–14.
52. Hayami H, Takahashi S, Ishikawa K, Yasuda M, Yamamoto S, Uehara S, et al. Nationwide surveillance of bacterial pathogens from patients with acute uncomplicated cystitis conducted by the Japanese surveillance committee during 2009 and 2010: antimicrobial susceptibility of Escherichia coli and Staphylococcus saprophyticus. J Infect Chemother. 2013;19(3):393–403.
53. Seo MR, Kim SJ, Kim Y, Kim J, Choi TY, Kang JO, et al. Susceptibility of *Escherichia coli* from community-acquired urinary tract infection to fosfomycin, nitrofurantoin, and temocillin in Korea. J Korean Med Sci. 2014;29(8):1178.
54. Yang B, Yang F, Wang S, Wang Q, Liu Z, Feng W, et al. Analysis of the spectrum and antibiotic resistance of uropathogens in outpatients at a tertiary hospital. J Chemother. 2018;30(3):145–9.
55. Treviño M, Losada I, Fernández-Pérez B, Coira A, Peña-Rodríguez MF, Hervada X, et al. [Surveillance of antimicrobial susceptibility of Escherichia coli producing urinary tract infections in Galicia (Spain)]. Rev Espanola Quimioter Publicacion Of Soc Espanola Quimioter. 2016;29(2):86–90.
56. Chervet D, Lortholary O, Zahar JR, Dufougeray A, Pilmis B, Partouche H. Antimicrobial resistance in community-acquired urinary tract infections in Paris in 2015. Médecine Mal Infect. 2018;48(3):188–92.
57. Sanchez M. Short-term effectiveness of ceftriaxone single dose in the initial treatment of acute uncomplicated pyelonephritis in women. A randomised controlled trial. Emerg Med J. 2002;19(1):19–22.
58. Yilmaz N, Agus N, Bayram A, Samlioglu P, Sirin MC, Karaca Derici Y, et al. Antimicrobial susceptibilities of Escherichia coli isolates as agents of community-acquired urinary tract infection (2008–2014). Türk Ürol Dergisi (Turkish J Urol). 2016;42(1):32–6.

59. Lee YR, Yeo S. Cefiderocol, a new siderophore cephalosporin for the treatment of complicated urinary tract infections caused by multidrug-resistant pathogens: preclinical and clinical pharmacokinetics, pharmacodynamics, efficacy and safety. Clin Drug Investig. 2020;40(10):901–13.
60. El-Lababidi RM, Rizk JG. Cefiderocol: a siderophore cephalosporin. Ann Pharmacother. 2020;54(12):1215–31.
61. Wu JY, Srinivas P, Pogue JM. Cefiderocol: a novel agent for the management of multidrug-resistant gram-negative organisms. Infect Dis Ther. 2020;9(1):17–40.
62. Birder LA. Pathophysiology of interstitial cystitis. Int J Urol. 2019;26(S1):12–5.
63. Asadi Karam MR, Habibi M, Bouzari S. Urinary tract infection: pathogenicity, antibiotic resistance and development of effective vaccines against uropathogenic Escherichia coli. Mol Immunol. 2019;(108):56–67.
64. Dashti AA, West P, Paton R, Amyes SGB. Characterization of extended-spectrum β-lactamase (ESBL)-producing Kuwait and UK strains identified by the Vitek system, and subsequent comparison of the Vitek system with other commercial ESBL-testing systems using these strains. J Med Microbiol. 2006;55(4):417–21.
65. Hawkey PM, Munday CJ. Multiple resistance in Gram-negative bacteria. Rev Res Med Microbiol. 2004;15(2):51–61.
66. Barguigua A, El Otmani F, Talmi M, Bourjilat F, Haouzane F, Zerouali K, et al. Characterization of extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolates from the community in Morocco. J Med Microbiol. 2011;60(9):1344–52.
67. Fusté E, López-Jiménez L, Segura C, Gainza E, Vinuesa T, Viñas M. Carbapenem-resistance mechanisms of multidrug-resistant Pseudomonas aeruginosa. J Med Microbiol. 2013;62(9):1317–25.
68. Patel G, Bonomo R. "Stormy waters ahead": global emergence of carbapenemases. Front Microbiol [Internet]. 2013 [cited 2022 May 28];4. Available from: https://www.frontiersin.org/article/10.3389/fmicb.2013.00048
69. Strahilevitz J. Plasmid-mediated quinolone resistance: a multifaceted threat [Internet]. [cited 2022 May 28]. Available from: https://journals.asm.org/doi/epub/10.1128/CMR.00016-09
70. Zurfluh K, Abgottspon H, Hächler H, Nüesch-Inderbinen M, Stephan R. Quinolone resistance mechanisms among Extended-Spectrum Beta-Lactamase (ESBL) producing Escherichia coli isolated from rivers and lakes in Switzerland. Khan AU, editor. PLoS ONE. 2014;9(4):e95864.
71. Robicsek A, Jacoby GA, Hooper DC. The worldwide emergence of plasmid-mediated quinolone resistance. Lancet Infect Dis. 2006;6(10):629–40.
72. Sharma G, Sharma S, Sharma P, Chandola D, Dang S, Gupta S, et al. Escherichia coli biofilm: development and therapeutic strategies. J Appl Microbiol. 2016;121(2):309–19.
73. Sturbelle RT, da C de Avila LF, Roos TB, Borchardt JL, de Cássia dos Santos da Conceição R, Dellagostin OA, et al. The role of quorum sensing in Escherichia coli (ETEC) virulence factors. Vet Microbiol. 2015;180(3):245–52.
74. Lo AWH, Van de Water K, Gane PJ, Chan AWE, Steadman D, Stevens K, et al. Suppression of type 1 pilus assembly in uropathogenic Escherichia coli by chemical inhibition of subunit polymerization. J Antimicrob Chemother. 2014;69(4):1017–26.
75. Han Z, Pinkner JS, Ford B, Obermann R, Nolan W, Wildman SA, et al. Structure-based drug design and optimization of mannoside bacterial FimH antagonists. J Med Chem. 2010;53(12):4779–92.
76. Kostakioti M, Hadjifrangiskou M, Hultgren SJ. Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. Cold Spring Harb Perspect Med. 2013;3(4):a010306.
77. Guiton PS, Cusumano CK, Kline KA, Dodson KW, Han Z, Janetka JW, et al. Combinatorial small-molecule therapy prevents uropathogenic Escherichia coli catheter-associated urinary tract infections in mice. Antimicrob Agents Chemother. 2012;56(9):4738–45.

78. Pei R, Lamas-Samanamud GR. Inhibition of biofilm formation by T7 bacteriophages producing quorum-quenching enzymes. Appl Environ Microbiol. 2014;80(17):5340–8.
79. Chibeu A, Lingohr EJ, Masson L, Manges A, Harel J, Ackermann HW, et al. Bacteriophages with the ability to degrade uropathogenic Escherichia Coli biofilms. Viruses. 2012;4(4):471–87.
80. Coulter LB, McLean RJC, Rohde RE, Aron GM. Effect of bacteriophage infection in combination with tobramycin on the emergence of resistance in Escherichia coli and Pseudomonas aeruginosa biofilms. Viruses. 2014;6(10):3778–86.
81. Monte J, Abreu AC, Borges A, Simões LC, Simões M. Antimicrobial activity of selected phytochemicals against Escherichia coli and Staphylococcus aureus and their biofilms. Pathogens. 2014;3(2):473–98.
82. Maisuria VB, Hosseinidoust Z, Tufenkji N. Polyphenolic extract from maple syrup potentiates antibiotic susceptibility and reduces biofilm formation of pathogenic bacteria. Appl Environ Microbiol. 2015;81(11):3782–92.
83. Lellouche J, Kahana E, Elias S, Gedanken A, Banin E. Antibiofilm activity of nanosized magnesium fluoride. Biomaterials. 2009;30(30):5969–78.
84. Johnson JR, Delavari P, Azar M. Activities of a nitrofurazone-containing urinary catheter and a silver hydrogel catheter against multidrug-resistant bacteria characteristic of catheter-associated urinary tract infection. Antimicrob Agents Chemother. 1999;43(12):2990–5.
85. Lellouche J, Friedman A, Gedanken A, Banin E. Antibacterial and antibiofilm properties of yttrium fluoride nanoparticles. Int J Nanomedicine. 2012;8(7):5611–24.
86. He W, Wang D, Ye Z, Qian W, Tao Y, Shi X, et al. Application of a nanotechnology antimicrobial spray to prevent lower urinary tract infection: a multicenter urology trial. J Transl Med. 2012;10(1):S14.
87. Emerich DF, Thanos CG. The pinpoint promise of nanoparticle-based drug delivery and molecular diagnosis. Biomol Eng. 2006;23(4):171–84.
88. Allen TM, Cullis PR. Drug delivery systems: entering the mainstream. Science. 2004;303(5665):1818–22.
89. Chang LC, Wu SC, Tsai JW, Yu TJ, Tsai TR. Optimization of epirubicin nanoparticles using experimental design for enhanced intravesical drug delivery. Int J Pharm. 2009;376(1):195–203.
90. Abed N, Couvreur P. Nanocarriers for antibiotics: a promising solution to treat intracellular bacterial infections. Int J Antimicrob Agents. 2014;43(6):485–96.
91. Das S. Natural therapeutics for urinary tract infections—a review. Future J Pharm Sci. 2020;6(1):64.
92. González de Llano D, Moreno-Arribas MV, Bartolomé B. Cranberry polyphenols and prevention against urinary tract infections: relevant considerations. Molecules. 2020;25(15):3523.
93. Mantzorou M. Cranberry consumption against urinary tract infections: clinical state of- the-art and future perspectives | Bentham Science. 2018 [cited 2022 May 28];19(13). Available from: http://www.eurekaselect.com/article/95063
94. Reid G, Bruce AW, Fraser N, Heinemann C, Owen J, Henning B. Oral probiotics can resolve urogenital infections. FEMS Immunol Med Microbiol. 2001;30(1):49–52.
95. Inhibitory Effect of Lactic Acid Bacteria on Uropathogenic Escherichia coli-Induced Urinary Tract Infections [Internet]. [cited 2022 May 28]. Available from: https://www.longdom.org/open-access/inhibitory-effect-of-lactic-acid-bacteria-on-uropathogenic-escherichia-coliinduced-urinary-tract-infections-35463.html
96. Montorsi F, Gandaglia G, Salonia A, Briganti A, Mirone V. Effectiveness of a combination of Cranberries, Lactobacillus rhamnosus, and vitamin C for the management of recurrent urinary tract infections in women: results of a pilot study. Eur Urol. 2016;70(6):912–5.
97. Nickel JC, Saz-Leal P, Doiron RC. Could sublingual vaccination be a viable option for the prevention of recurrent urinary tract infection in Canada? A systematic review of the current literature and plans for the future. Can Urol Assoc J. 2020;14(8):281–7.

98. Aziminia N, Hadjipavlou M, Philippou Y, Pandian SS, Malde S, Hammadeh MY. Vaccines for the prevention of recurrent urinary tract infections: a systematic review. BJU Int. 2019;123(5):753–68.
99. Garcia EC, Brumbaugh AR, Mobley HLT. Redundancy and specificity of *Escherichia coli* iron acquisition systems during urinary tract infection. Payne SM, editor. Infect Immun. 2011;79(3):1225–35.
100. Habibi M, Asadi Karam MR, Bouzari S. Evaluation of prevalence, immunogenicity and efficacy of FyuA iron receptor in uropathogenic Escherichia coli isolates as a vaccine target against urinary tract infection. Microb Pathog. 2017;110:477–83.
101. Mike LA, Smith SN, Sumner CA, Eaton KA, Mobley HLT. Siderophore vaccine conjugates protect against uropathogenic Escherichia coli urinary tract infection. Proc Natl Acad Sci. 2016;113(47):13468–73.
102. Starks CM, Miller MM, Broglie PM, Cubbison J, Martin SM, Eldridge GR. Optimization and qualification of an assay that demonstrates that a FimH vaccine induces functional antibody responses in women with histories of urinary tract infections. Hum Vaccines Immunother. 2021;17(1):283–92.
103. Durant L, Metais A, Soulama-Mouze C, Genevard JM, Nassif X, Escaich S. Identification of candidates for a subunit vaccine against extraintestinal pathogenic Escherichia coli. Infect Immun. 2007;75(4):1916–25.
104. Valle J, Mabbett AN, Ulett GC, Toledo-Arana A, Wecker K, Totsika M, et al. UpaG, a new member of the trimeric autotransporter family of adhesins in uropathogenic Escherichia coli. J Bacteriol. 2008;190(12):4147–61.
105. Alteri CJ, Hagan EC, Sivick KE, Smith SN, Mobley HLT. Mucosal immunization with iron receptor antigens protects against urinary tract infection. PLoS Pathog. 2009;5(9):e1000586.
106. Wieser A, Romann E, Magistro G, Hoffmann C, Nörenberg D, Weinert K, et al. A multiepitope subunit vaccine conveys protection against extraintestinal pathogenic Escherichia coli in mice. Infect Immun. 2010;78(8):3432–42.
107. Searle LJ, Méric G, Porcelli I, Sheppard SK, Lucchini S. Variation in siderophore biosynthetic gene distribution and production across environmental and faecal populations of Escherichia coli. PLoS One. 2015;10(3):e0117906.
108. Shields J, Maxwell AP. Acute pyelonephritis can have serious complications. Practitioner. 2010;254(1728):19. 21, 23–4, 2
109. Ki M. The epidemiology of acute pyelonephritis in South Korea, 1997–1999. Am J Epidemiol. 2004;160(10):985–93.
110. Brogden RN, Ward A. Ceftriaxone. A reappraisal of its antibacterial activity and pharmacokinetic properties, and an update on its therapeutic use with particular reference to once-daily administration. Drugs. 1988;35(6):604–45.
111. Rodman DP, McKnight JT, Anderson RL. A critical review of the new oral cephalosporins. Considerations and place in therapy. Arch Fam Med. 1994;3(11):975–80.
112. Hong MT, Seifert CF. Clinical impact of discordant prescribing of fluoroquinolones and alternative treatments in Escherichia coli pyelonephritis. J Pharm Pract. 2016;29(5):467–71.
113. Wells WG, Woods GL, Jiang Q, Gesser RM. Treatment of complicated urinary tract infection in adults: combined analysis of two randomized, double-blind, multicentre trials comparing ertapenem and ceftriaxone followed by appropriate oral therapy. J Antimicrob Chemother. 2004;53 Suppl 2:ii67–74.
114. Sharara SL, Amoah J, Pana ZD, Simner PJ, Cosgrove SE, Tamma PD. Is piperacillin-tazobactam effective for the treatment of pyelonephritis caused by extended-spectrum β-lactamase–producing organisms? Clin Infect Dis. 2020;71(8):e331–7.
115. Jansåker F, Frimodt-Møller N, Benfield TL, Knudsen JD. Mecillinam for the treatment of acute pyelonephritis and bacteremia caused by Enterobacteriaceae: a literature review. Infect Drug Resist. 2018;(11):761–71.

116. Pedersen G, Schønheyder HC, Sørensen HT. Antibiotic therapy and outcome of monomicrobial gram-negative bacteraemia: a 3-year population-based study. Scand J Infect Dis. 1997;29(6):601–6.
117. Neu HC. Synergy of mecillinam, a beta-amidinopenicillanic acid derivative, combined with beta-lactam antibiotics. Antimicrob Agents Chemother. 1976;10(3):535–42.
118. Zhanel GG, Chung P, Adam H, Zelenitsky S, Denisuik A, Schweizer F, et al. Ceftolozane/tazobactam: a novel cephalosporin/β-lactamase inhibitor combination with activity against multidrug-resistant gram-negative bacilli. Drugs. 2014;74(1):31–51.
119. Wagenlehner FM, Umeh O, Steenbergen J, Yuan G, Darouiche RO. Ceftolozane-tazobactam compared with levofloxacin in the treatment of complicated urinary-tract infections, including pyelonephritis: a randomised, double-blind, phase 3 trial (ASPECT-cUTI). Lancet. 2015;385(9981):1949–56.
120. Perrotta C, Aznar M, Mejia R, Albert X, Ng CW. Oestrogens for preventing recurrent urinary tract infection in postmenopausal women. Cochrane Database Syst Rev [Internet] 2008 [cited 2022 May 26];(2). Available from: https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD005131.pub2/abstract
121. Kolman KB. Cystitis and pyelonephritis. Prim Care Clin. 2019;46(2):191–202.

Drug Delivery to Diseases Caused by *E. coli* Infection

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Abstract The *Escherichia coli* are one of the most common facultative anaerobe in the intestinal tract of humans and many other endothermic species. Mostly, strains remained non-pathogenic which co-exist in harmony with the host. The relation may be symbiotic. Some of the strains are pathogenic which are capable of causing wide variety of illnesses. *E. coli*, a gram-negative bacterium, mainly targets gastro-intestinal tract, meninges, and kidneys leading to conditions like sepsis, diarrhea, dysentery, renal failure, pyelonephritis, hemolytic-uremic syndrome, and death. Treatment/management is determined by both the strain and the disease type. Symptomatic care is the first step in treating a patient with *E. coli*-related intestinal illness. The pace at which new diseases, newer pathways of already known diseases, and increasing understanding of the drug-resistant mechanisms are being uncovered is accelerating. This pace is not met by the discovery of new and effective medicines. To meet this gap, increasing attention to develop effective delivery strategies would improve risk benefit profiles and switch routes of administration. Multiple novel formulation concepts have been put forth in scientific arena for improving existing molecules for the treatment.

The covid19 pandemic has emphasized to the world once again importance of vaccine, which may be true for *E. coli* as well.

Keywords *E. coli* · Pathogenic · Non-pathogenic · Drug delivery · Nanoparticles

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1 Introduction

Theodore Escherich (1857–1911) identified 19 bacterial species in neonatal and infant fecal flora and designated it as *Bacterium coli commune* (the common colon bacterium) [1]. One of these species, *Escherichia coli* (*E.coli*), is the most prevalent facultative anaerobe in the intestinal tract of humans and numerous other endothermic animals [2]. Within hours of birth, *E. coli* colonizes neonates, and it was found to be serologically identical with the *E. coli* detected in the mother. It was thought to get transferred at the time of birth and permanently colonized in the human body [3].

Mostly, strains remained non-pathogenic which co-exist in harmony with the host. The relation may be symbiotic. The bacteria get benefitted from the host, whereas it synthesizes cofactors and contribute to resistance against pathogens in host [4]. It was also observed that some of the strains are pathogenic which can cause wide variety of illnesses. *E. coli*, a gram-negative bacterium, mainly targets gastrointestinal tract, meninges, and kidneys leading to conditions like sepsis, diarrhea, dysentery, renal failure, pyelonephritis, hemolytic-uremic syndrome, and death [5].

The pathogenicity of various *E. coli* species is attributed to their specific genes or gene combinations with virulence factor and capacity for genetic exchange. Only 20% of average genome size is retained in these species. Genes are continually acquired and exchanged through various mechanisms like plasmid transfer and bacteriophages and also by some unknown mechanism [6].

Our understanding of the variety of bacteria has been improved as a result of techniques like multi-locus sequence typing (MLST) [7] and multi-locus sequence analysis (MLSA) [8]. These revealed considerably large genetic variation in the genus *Escherichia*. Besides that, it is revealed to be five “cryptic clades” of *Escherichia* exist, which are phenotypically indistinguishable from *E. coli*. Majorly, the species *E. coli* can be classified in two broad classes, those causing intestinal infection (causing diarrheal disease) and those causing extraintestinal infection [8, 9] (Table 1).

A typical *E. coli* genome consists of about 4700 genes, among which only 2000 genes are common to all *E. coli* strains. It exhibit considerable genetic substructure

Table 1 Broad classification of *E. coli* depending on location of infection [9]

Causing intestinal infection	Causing extraintestinal infection	Non-infectious/or symbiotic
Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Shiga-toxin producing *E. coli* (STEC), Enteroaggregative *E. coli* (EAEC), Shigella	Extraintestinal pathogenic *E. coli* (ExPEC)	*E. coli* strain MG-1655 *E. coli* strain EcN (Nissle 1917)

denoted as phylo-group A, B1, B2, C, D, E, and F. Among these, phylo-groups A and B1 appear predominantly in humans from developing countries, while in developed countries, strains other than B1 appear frequently [10].

E. coli is found in plants as well as animals. It is isolated from ectothermic as well as homeothermic animals. The body size of host significantly affects the likelihood of isolation of *E. coli*. The probability of presence of *E. coli* increases with increase in weight (may be because of existence of relationship between body size and gut transit time). Pregnancy coupled with excessive weight gain or starvation in children may influence *E. coli* cell densities. Gut morphology and gut dynamics along with diet also play important role. Various antibiotics also depicted significant increase in *E. coli* cell densities. Along with this, background levels of contamination and climate or environmental conditions can also affect the presence of *E. coli* in host [11].

2 Pathovars of *E. coli*

2.1 Uropathogenic E. coli (UPEC)

UPEC, a prototype of extraintestinal pathogenic *E. coli* (ExPEC), is harmless in the gastrointestinal tract, but becomes pathogenic in the urinary tract. It accounts for substantial medical costs and morbidity worldwide by producing community-acquired UTIs as well as nosocomial UTIs. It leads to recurrent or relapsing UTIs [12, 13].

UPEC is an opportunistic intracellular pathogen colonizing the urinary tract and able to get transmitted sexually. It causes cystitis by colonizing in the bladder, leads to pyelonephritis by ascending through ureters in to the kidneys, and triggers host inflammatory responses in urinary tract. It colonizes the urinary tract and persists there by evading the innate immune responses like cytokine production, neutrophil influx, and generation of reactive nitrogen and oxygen species [12–14].

2.2 Shiga-Toxin Producing E. coli/Enterohemorrhagic E. coli (STEC/EHEC)

STEC releases shiga-toxin which is responsible for morbidity and mortality associated with STEC infection. It causes severe foodborne diseases which lead to abdominal cramps and diarrhea which progress to hemorrhagic colitis. Importantly, it is associated with life-threatening disease like hemolytic uremic syndrome (HUS) in young children leading to neurological complications (like seizure, stroke, coma, etc.) and chronic renal sequelae [15, 16].

The shiga-toxin composed of two subunits, subunit A and subunit B. The A subunit inhibits host protein synthesis and induces apoptosis. Subunit B is released by STEC/EHEC in large intestine where it gets bind to globotriaosylceramide-3 (Gb3) of endothelial cells, get absorbed, and disseminate to other organs. In the kidney, shiga-toxin binds with renal endothelial Gb3 and leads to acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia (symptoms of HUS) [15, 16].

2.3 Enteropathogenic E. coli (EPEC)

EPEC is a diarrheagenic *E. coli* pathotype leading to infection in infants and children worldwide. It is the prominent cause of persistent diarrhea which produces attaching and effacing (A/E) lesions. In this lesion, the bacteria disrupt the cell surface by attaching tightly to the cell membrane, leading to effacement (erasing or elimination) of the microvilli, F-actin rearrangement, and outgrowth of cuplike pedestals beneath the adherence site [17].

The essential virulence determinants of this tight attachment are T3SS, Intimin, and Tir. The insertion of Tir into the plasma membrane is governed by T3SS. Tir then serves as a receptor for bacterial binding through Tir-Intimin interaction. This interaction triggers signaling cascades, which binds cytoskeletal proteins beneath the adhering bacteria and phosphorylate the host phospholipase. Map (mitochondrial-associated protein) also involved in the process. Along with the host cell mitochondria, it also disrupts the function of the epithelial barrier, which has been linked to diarrheal illness. Microvilli effacement requires Map, EspF, Tir, and Intimin which retains the detached microvillar material [18].

2.4 Enterotoxigenic E. coli (ETEC)

ETEC is a diverse group of *E. coli* that ferments lactose and produces heat-labile (LET) and heat-stable (SET) enterotoxins. There is much similarity in LET and cholera toxin along with same mode of action. There are two subunits of LET toxin, active A subunit (LET-A) surrounded by binding B subunit (LET-B). Once ETEC colonize the small intestine, LET-B binds reversibly to GM1 ganglioside, while subunit A activates adenylate cyclase. This stimulates production of cyclic AMP; thereby, the crypt cells' ability to secrete chloride increases, which in turn prevents neutral sodium chloride from building up at the terminals of villus fibers. When this action surpasses the bowel's capacity for absorption, watery diarrhea develops [19, 20].

SET is a non-antigenic peptide with two variants, i.e., SETp (discovered in pigs) and SETh (discovered in humans), which shows similar mechanism of action. It increases levels of cGMP in small intestine by reversibly binding to guanylate

cyclase, leading to diarrhea by increasing the chloride secretion through crypt cells and inhibition of neutral sodium chloride absorption [19, 20].

2.5 Enteroaggregative E. coli (EAEC)

EAEC is a diarrheagenic *E. coli* pathotype characterized by aggregative adherence like "stacked bricks" when cultured with HEp-2 cells. It causes persistent diarrhea in children and HIV define-infected patients leading to malnutrition. It also causes traveler's diarrhea and acute diarrheal illness in children and adults [21, 22].

The EAEC virulence is prominently regulated by *AggR*, by controlling the expression of adherence factors, a dispersin protein, and other cluster of genes. The pathogenesis involved three steps: (1) aggregative adherence fimbriae (AAF) and adherence factors adhere to the intestinal mucosa, (2) EAEC encrusts on the surface of enterocytes by increased production of mucus, and (3) releases the toxins and elicits the inflammatory response, mucosal toxicity, and intestinal secretion [21, 22].

2.6 Diffusely Adherent E. coli (DAEC)

DAEC is a heterogeneous pathotype of *E. coli* causing diarrhea in children, UTI, and pregnancy complications in adults. It is also found in the intestine of adults and children as commensal bacterium. Adults are asymptomatic carriers, while younger children aged 1.5–5 years. exhibits persistent watery diarrhea. In adults, DAEC infection though not developing diarrhea can contribute to IBD, Crohn's disease, and coeliac [14, 23].

DAEC colonizes in the small bowel through afimbrial adhesion (Afa)/mannose-resistant adhesin (Dr adhesion) family. F1845 (a member of Afa/Dr. adhesin family) and Dr. adhesins bind to apical surface of urinary and intestinal epithelial cells through decay-accelerating factor (DAF). This interaction induces cytoskeleton rearrangement and destroys microvilli. Across the epithelial cells, increased levels of DAF expression contribute to inflammation in patients of Crohn's disease. MICA, the expression of which is also induced by DAEC strains, mediates IBD [14, 23].

Afa/Dr. adhesins can bind to carcinoembryonic antigen-related cell adhesion molecule (CEACAM) on epithelial cell surface, activates CDC42, aggregates CEACAM at bacterial adhesion site and effacement of brush border microvilli, and mediates internalization of the bacterial pathogen leading to infection of intestinal epithelial cells [14].

2.7 Adherent Invasive *E. coli* (AIEC)

AIEC is as such commensal bacterium which can be one of the causative organisms for IBD and CD. Its pathogenesis involves three steps, i.e., adhesion, invasion, and multiplication in the host epithelial cells. AIEC using CAECAM6 gets to adhere the epithelial cells in the ileum. CAECAM6 levels increased in CD patient due to stimulation of TNF-α, leading to intestinal inflammation through AIEC colonization. IAEC strain invades, infects, and replicates in the macrophage through several virulence factors, such as outer membrane vesicles, outer membrane proteins, and long polar fimbriae [14, 24, 25].

3 Treatment and Management of *E. coli* Infection

The gram-negative bacillus *E. coli* is a member of the normal intestinal flora but can potentially cause intestinal and extraintestinal disease in people. Hundreds of *E. coli* strains have been found, causing symptoms ranging from mild, self-limited gastroenteritis to renal failure and septic shock. *E. coli* is a causative organism of many diarrheal illnesses, including traveler's diarrhea and dysentery. *E. coli* is the most common pathogen leading to uncomplicated cystitis and also results in other extraintestinal illnesses, including pneumonia, bacteremia, and abdominal infections such as spontaneous bacterial peritonitis. *E. coli's* virulence allows it to bypass host defenses and acquire resistance to commonly used antibiotics [26].

Treatment/management is determined by both the strain and the disease. Symptomatic care is the first step in treating a patient with *E. coli*-related intestinal illness [26, 27]. Diarrhea may be a very upsetting experience for individuals. Fluid replacement and antidiarrheals are the pillars of management for mild illness, according to experts. When tolerated, oral rehydration is suggested as the first-line therapy for all patients with diarrheal disease, and it is equally effective as intravenous hydration (IV). When patients are unable to accept oral hydration, IV hydration is indicated. Antimotility drugs like bismuth-subsalicylate and loperamide are used to relieve distressing symptoms.

Because of the negative side effects and link to antibiotic resistance, antibiotics are not indicated as a first-line therapy for *E. coli*-related diarrhea in the majority of patients. Antibiotics may be appropriate for patients who have severe illness (more than six stools per day, fever, dehydration requiring hospitalization, diarrhea lasting more than 7 days, or bloody diarrhea). The Infectious Diseases Society of America (IDSA) and the International Society of Travel Medicine (ISTM) presently prescribe rifaximin, azithromycin, and ciprofloxacin for the treatment of *E. coli* diarrhea. Antibiotics are not indicated for individuals suspected of having EHEC/STEC because they raise the risk of hemolytic uremic syndrome, especially in children and the elderly [27].

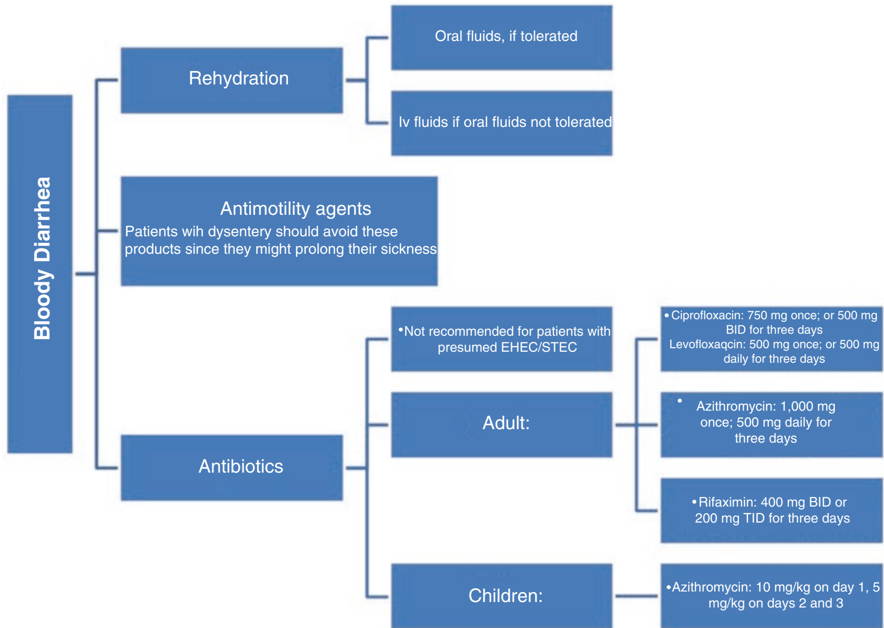

Fig. 1 Treatment algorithm for watery diarrhea

3.1 *Intestinal Infections*

The following treatment algorithms were recommended for *E. coli* intestinal infections (Figs. 1 and 2) [28].

3.2 *Extraintestinal Illness*

Local antibiograms exhibiting sensitivity and resistance patterns should be used to guide *E. coli* antimicrobial treatment (Tables 2 and 3).

4 Drug Delivery Technologies

Drug delivery systems are used to transport therapeutic drugs in the body as needed to safely achieve the desired therapeutic effect. Such systems are usually designed as follows:

1. Improve aqueous solubility and chemical stability of active agents.
2. Increase pharmacological activity.
3. Reduce side effects [30].

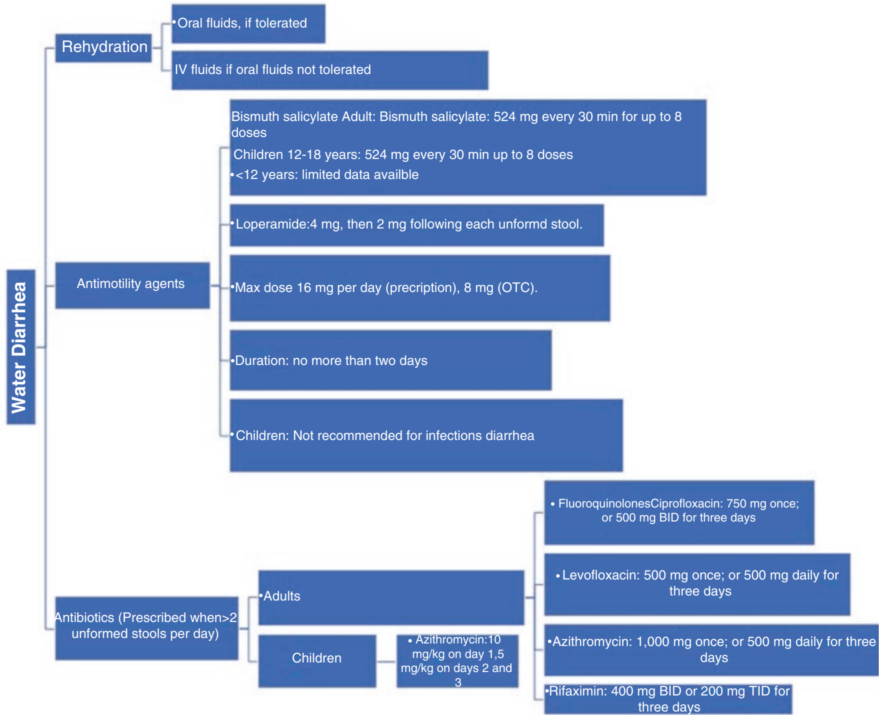

Fig. 2 Treatment algorithm for bloody diarrhea

Table 2 List of antibiotics prescribed in extra intestinal infections caused by *E. coli*

Antibiotics suitable for *E. coli* infections	ESBL-producing *E. coli*	Carbapenemase-producing *E. coli*
Beta-lactam antibiotics (Cephalosporins, Carbapenems, Monobactams) Nitrofurantoin Trimethoprim-sulfamethoxazole Fluoroquinolones (Ciprofloxacin, Levofloxacin)	Cefepime Ceftazidime Imipenem Ertapenem Meropenem	Ceftazidime-avibactam Colistin Polymyxin B

Biomedical research has advanced our understanding of diseases – their causes and remedies. Specifically, the remedies include approaches to prevent, manage, or treat a particular disease using a drug (e.g., chemical and biological molecules) or a drug-like (e.g., supplements) compound. The pace at which new diseases, newer pathways of already known diseases, and increasing understanding of the drug-resistant mechanisms are being uncovered is accelerating. This pace is not met by the discovery of new and effective medicines. To meet this gap, increasing attention to develop effective delivery strategies would improve risk-benefit profiles and switch routes of administration. Each mode of delivery such as oral, nasal, injection, sublingual, rectal, vaginal, ocular, optic, or nasal has its respective advantages and

Table 3 Antibiotic therapy for urinary tract infections in adults [29]

Indications	Antibiotic	Dosage regimen
Lower tract infections	Trimethoprim-sulfamethoxazole	1 DS tablet; Twice a day; 3 days
Uncomplicated		
	Ciprofloxacin	250 mg; Twice a day; 3 days
	Levofloxacin	250 mg; Once a day; 3 days
	Amoxicillin	500 mg; Twice a day; 5–7 days
	Amoxicillin-clavulanate	500 mg; Every 8 hours; 5–7 days
	Trimethoprim	100 mg; Twice a day; 3–5 days
	Nitrofurantoin macrocrystal	100 mg; Every 6 hours; 7 days
	Nitrofurantoin monohydrate	100 mg; Twice a day;5 days
Complicated	Trimethoprim-sulfamethoxazole	1 DS tablet; Twice a day; 7–10 days
	Ciprofloxacin	250–500 mg; Twice a day; 7–10 days
	Levofloxacin	250 mg; Once a day; 10 days
		750 mg; Once a day; 5 days
	Amoxicillin-clavulanate	500 mg; Every 8 hours; 7–10 days
Recurrent infections	Nitrofurantoin	50 mg; Once a day; 6 months
	Trimethoprim-sulfamethoxazole	1/2 SS tablet; Once a day; 6 months
Acute urethral syndrome	Trimethoprim-sulfamethoxazole	1 DS tablet; Twice a day; 3 days
Failure of trimethoprim-sulfamethoxazole	Azithromycin	1 g; Single dose
	Doxycycline	100 mg; Twice a day; 7 days
Acute pyelonephritis	Trimethoprim-sulfamethoxazole	1 DS tablet; Twice a day; 14 days
	Ciprofloxacin	500 mg; Twice a day; 14 days
	Levofloxacin	250 mg; Once a day; 10 days
		750 mg; Once a day; 5 days
	Amoxicillin-clavulanate	500 mg; Every 8 hours; 14 days

disadvantages. Nevertheless, oral drug delivery (pills, powders, suspensions, and solutions) is the singular, superior method of administration due to its convenience and safety as compared to other methods. The advantages of oral drug administration over other methods include ease of use, being painless, lesser cost of care, lesser patient supervision, and high patient compliance.

However, the oral route of drug administration has some disadvantages when it comes to the drug molecules exhibiting low solubility, lesser permeability, and degradation rates. Moreover, the uptake of certain biomolecules/drugs in oral route is largely affected by physiological barriers such as pH change in gastrointestinal tract (GIT): acidic pH in the stomach followed by basic pH in the intestine and enzymatic degradation. Almost 60% of drugs degrade in the harsh gastric environments of the stomach. Many have theorized making salts out of the drugs, thereby increasing its solubility and bioavailability [31].

Modified release (MR) drug delivery systems are developed to modulate the apparent absorption and/or alter the site of release of drugs to achieve specific clinical objectives that cannot be attained with the conventional immediate release (IR) dosage forms. Possible therapeutic benefits of a properly designed MR dosage form include improved efficacy and reduced adverse events, increased convenience and patient compliance, optimized clinical performance, a greater selectivity of activity, or new indications [32].

4.1 Challenges and Opportunities for Treatment of E. coli *Infections*

Due to *E. coli*'s thick cell walls, which exhibit poor drug absorption, conventional antibiotic therapy used to treat *E. coli* infection has significant obstacles. Antimicrobial resistance emerged as a result of ineffective therapy. Low bioavailability, GI tract drug degradation, and short GI retention times are problems with treatment-related medications.

Modified drug release and cutting-edge drug delivery methods can aid in the fight against *E. coli* infection.

Nanoparticles can increase the cell wall's permeability. Chitosan-based nanoparticles have antimicrobial activity by attaching to DNA and preventing DNA replication after adhering to the negatively charged bacterial cell wall and altering permeability and destabilizing the cell envelope. Additionally, the medicine can be exposed to microorganisms for a longer period of time, thanks to its positive ionic interactions with the negative charges of cell surface membranes. By increasing the concentration of the antibiotic in the microorganism without increasing the dose of antibiotic administered, nanoparticulate drug delivery methods may enhance therapeutic efficacy [33].

Antibiotic resistance is caused by a variety of processes used by microbes, including drug target modification, enzyme inactivation, efflux transport inhibition, and the development of alternative metabolic pathways for growth. Ineffective drug delivery and a lack of effective dose availability are other factors contributing to treatment resistance [34].

Newer technologies like nanoparticles, liposomes, hydrogels, modified release, floating, and bio-adhesive can be employed to enhance medicine delivery at the infection site.

Ciprofloxacin, along with other fluroquinolones, is a commonly used medicine as an antibiotic therapy for *E. coli* infection. It has gastrointestinal side effects, such as nausea and diarrhea, and a 70% bioavailability rate. The most frequent reason for stopping ciprofloxacin therapy remains adverse effects, which are increased with frequent dose [35, 36].

Fluroquinolone dosage forms that are antimicrobial in nature have enhanced residence durations in the stomach due to floating, bio-adhesive, swelling, and low-density devices. This improved contact time with the stomach mucosa and improved drug absorption and bioavailability [37].

Drugs with modified release mechanisms, such as sustained release or prolonged release, require fewer doses and retain effective concentration for longer periods of time, eradicating bacterial flora and reducing the likelihood of drug resistance. Modified release dosage forms also reduce adverse events like GI distress and nausea [38].

To treat GI tract infections, antimicrobial nanoparticles can be used orally. In order to prolong medication release and boost half-life and bioavailability, the nanocarriers shield antibiotics from GI fluid breakdown. The nano-sized nature is advantageous for efficient accumulation in infectious nidus by extravasating through the endothelium in the inflammatory site [39]. The use of nanotechnology in medicine to enhance antibiotic delivery for the killing of germs suggests a decrease in adverse effects and drug resistance [40].

4.2 Modified Release Dosage Forms

A major challenge with the majority of the conventional immediate release preparations is that they require administration multiple times per day to achieve and maintain the drug levels within the therapeutic range. In turn, this causes a somewhat undesirable "seesaw" effect of drug level fluctuations in the body critically; the advent of modified release drug delivery systems provides a host of benefits compared with conventional immediate release preparations, including the following:

- Reduced frequency of dosing: Plasma concentration can be maintained over an extended period of time, extremely valuable for improving medication adherence for patients with chronic conditions.
- Reduction or avoidance of side effects due to high plasma drug concentrations or "dose dumping."
- Improved control of therapeutic drug concentration, particularly important for patients that suffer from breakthrough symptoms.
- More cost-effective manufacturing due to a reduced number of dosage forms required per patient compared with its immediate release counterpart.

To date, modified release dosage forms refer to three main types of oral drug product release pathway:

1. Extended-release: In general, these oral dosage forms should exhibit at least a twofold reduction in dosage frequency as compared to the conventional immediate-release drug product. Examples of extended-release dosage forms include sustained-release, controlled-release, and long-acting drug products
2. Delayed-release: A dosage form that releases a discrete portion or portions of drug at a specific time following administration. Enteric-coated dosage forms are common delayed-release products (e.g., enteric-coated aspirin and other steroidal products).
3. Targeted-release: A dosage form that releases drug at or near the intended physiologic site of action or drug absorption. Targeted-release dosage forms may have either immediate- or extended-release characteristics. Examples include gastroretentive devices and colonic drug delivery systems [37].

New approaches for extending the retention time of drugs in the stomach comprise of various techniques, such as utilizing bioadhesive devices, swellable drug delivery systems (including low density devices, floating, high density systems, magnetic systems, unfoldable and expandable systems, and magnetic systems), and superporous, biodegradable hydrogel. For medications with small absorption windows, stability issues in the intestinal or colonic environments, locally acting in the stomach, and poor solubility in the intestine, extended-release dosage forms with prolonged residence durations in the stomach are very desirable [38].

Ciprofloxacin has 70% bioavailability and is absorbed mostly from the upper gastrointestinal tract, up to the jejunum. By increasing the gastric residence period of the dose form, the medicine can be given to the stomach, the so-called absorption window. GI side effects, including as nausea and diarrhea, continue to be the most common reason for ciprofloxacin medication cessation [36].

Polymers such as polyacrylic acid, polymethacrylic acid, sodium carboxymethyl cellulose, and hydroxypropyl methylcellulose were used to make bioadhesive extended-release tablets by Varshosaz et al. Because these polymers are sufficiently hydrated, they are able to cling to mucosal membranes. These polymers create gel-forming matrices and, when in contact with stomach fluid, have enough structure to form a gel layer with a specific gravity lower than gastric fluid. To create carbon dioxide and increase the buoyancy of the tablets, citric acid and sodium bicarbonate were utilized as an effervescent base.

An attempt reported with above preamble that the tablets with the shortest lag time of buoyancy were made with 10% effervescent base, although modifying the polymer type of the mixture ratio had no effect on the duration of buoyancy. Tablets containing 20% HPMC and 80% CMC or 80% PAA and 20% PMA were the best in terms of bioadhesion and prolonged drug release rate [41].

4.3 Nanoparticle-Based Drug Delivery

Antibiotics are used to treat infectious diseases by killing or disrupting microorganisms' development cycles in the traditional antimicrobial strategy. Antibiotics work on bacteria by inhibiting cell wall synthesis (−lactams, vancomycin, and bacitracin), protein synthesis (chloramphenicol, tetracyclines, aminoglycosides, macrolids), nucleic acid synthesis (fluoroquinolones, rifampicin), and membrane integrity (polymixinB) [42].

The narrow spectrum of efficacy, safety, and acceptability of these antibiotics are all problems. When taken orally or topically, traditional drug delivery systems (tablets, capsules, etc.) have a major drawback: poor drug delivery. Drugs are distributed in a non-specific manner in traditional dose forms, resulting in systemic adverse effects, inadequate absorption, and drug degradation. Another major difficulty with antimicrobial therapy is the development of bacterial resistance as a result of ineffective treatment and delivery.

In the realm of nanotechnology and its application in drug delivery, there are numerous success stories. Nanotechnology has provided new tools for fighting a variety of ailments [43].

Ultrafine particles featuring a size distribution in the nanometer range are described as nanoparticles (NPs). This nanosize gives a large surface area, which increases drug molecule reactivity [44].

These NPs have numerous advantages as drug delivery carriers over conventional approaches. Nanosize carriers reduce drug-induced adverse effects by overcoming solubility and stability difficulties. Nanotechnology allows two to three medicinal molecules to be delivered on the same substrate. Drug molecules delivered via NPs have a higher efficiency at the location, better circulation, and a better therapeutic index, which leads to a reduction in bacterial resistance. Improved bioavailability is the result of greater control over medication release and degradation profiles. Drug molecules and drug carriers with altered surface characteristics deliver drugs to specific locations and increase the number of administrative paths available [34].

4.3.1 Types of Nanoparticles

Nanoparticles are grouped into organic and inorganic nanoparticles.

Inorganic nanoparticles like as titanium oxide, zinc oxide, silver oxides, and magnesium oxides enjoy popularity in the field of nanoparticles because of their ability to withstand processing condition and optical and physical stability.

Organic nanoparticles obtained from organic materials like carbon nanotubes, lipids, and polymers have the advantage of electronic, metallic, and structural properties [45].

4.3.2 Methods of Preparation of Nanoparticles

- Inorganic nanoparticles prepared by two approaches

  A. Top-down Approach – Mechanical milling, laser ablation, and sputtering
  B. Bottom-up Approach – Solid-state method, liquid state, gas phase, and from biological tools [46]

- Organic nanoparticles prepared by two approaches

  A. Two step procedure based on emulsification

    (a) Methods of emulsification – Low energy and high energy emulsification
    (b) Generation of nanoparticles from emulsion – Solvent evaporation, solvent diffusion, salting out, gelation, polymerization

  B. One-step procedure – nanoprecipitation, dialysis, desolvation, gelation, spray drying [47, 48]

4.4 Organic Nanoparticles

Natural and synthetic polymers can be used to make polymeric nanoparticles. Because of their stability and ease of surface modification, they are commonly used. Biopolymeric nanoparticles have additional benefits such as biodegradability, biocompatibility, and nontoxicity, as well as availability from marine (chitin and chitosan) or agricultural (cellulose, starch, and pectin) resources. Biodegradable polymers like chitosan are primarily investigated as delivery systems for active ingredients, as well as the stabilization of biological molecules such as proteins, peptides, and genetic material [49].

Chitosan also has a high antibacterial activity, as well as other desirable characteristics including non-toxicity, biodegradability, low cost, high biocompatibility, and non-antigenicity. The theorized mechanism for its antimicrobial effect is that it binds to the negatively charged bacterial cell wall, causing instability of the cell envelope and altered permeability, and then attaches to DNA, preventing it from replicating. Furthermore, the medication can be exposed to microorganisms for longer periods of time due to its positive ionic interactions with the negative charges of cell surface membranes. Furthermore, chitosan and its derivatives have been demonstrated to have antibacterial properties against both gram-negative and gram-positive bacteria.

Because of its unique properties, chitosan nanoparticles have a stronger antibacterial activity than chitosan. The polycation's target site is the negatively charged surface of the bacterial cell. As a result, the polycationic chitosan nanoparticles with higher surface charge density interact with bacteria more than chitosan alone. Due

to the larger surface area of the chitosan nanoparticles, which could be tightly adsorbed onto the surface of the bacteria cells to disrupt the membrane, which would lead to the leakage of intracellular components, thus killing the bacteria cells, chitosan nanoparticles have a higher affinity with bacteria cells for a quantum-size effect [50, 51].

Chitosan nanoparticles have been used as medication delivery vehicles due to their numerous advantages. To begin with, because chitosan is biocompatible and biodegradable, it is a safe material. Second, because water-soluble polymers are ideal for drug delivery carriers, they can be made with simple and straightforward techniques. Chitosan nanoparticles can thus be utilized to manufacture a wide range of medicines, including macromolecules and labile chemicals. Third, chitosan is available in a wide range of molecular weights and can be chemically altered by coupling with ligands, providing for more formulation flexibility. Finally, chitosan promotes absorption by extending the time between the substrate and the cell membrane.

Furthermore, their nanosize makes medication uptake via the cell membrane easier. The ability to boost drug bioavailability was demonstrated when the absorption enhancing action and nanosized particles were combined. Fifth, chitosan nanoparticles have a variety of delivery routes, including non-invasive routes such as the oral, nasal, and ocular mucosa, which are preferred. Furthermore, chitosan nanoparticles have been shown to be an effective vaccination adjuvant [52].

Ionic gelation was used to make chitosan nanoparticles by Sobhani and colleagues. Dropwise addition of tripolyphosphate aqueous solution to chitosan aqueous solution (in 1% v/v acetic acid) under stirring at room temperature until faint turbidity was achieved. Ciprofloxacin-loaded nanoparticles were also created in the same way. The antibacterial activity of ciprofloxacin-loaded nanoparticles formulation was tested against *E. coli*, a gram-negative strain, and *S. aureus*, a gram-positive strain, and it was discovered that the potency of ciprofloxacin HCl improved by 50%. Because of the reduced particle size and improved penetration through the cell wall, the medication has a higher efficacy in this formulation [33].

Ibrahim and colleagues employed the ionic gelation process to create chitosan nanoparticles that were used as polydrug delivery systems for antibiotics such as ciprofloxacin HCl, chlortetracycline hydrochloride, and gentamycin sulphate. Antimicrobial activity studies demonstrated that these antibiotics showed enhanced potency against gram-positive and gram-negative bacteria compared with free medicines [53].

Kumar and his colleagues used the ionic gelation process to make ciprofloxacin-loaded genipin cross-linked chitosan/heparin nanoparticles (CIPRO-GP-CS/Hep NPs). The produced particles have a spherical form, a z-average of 250 nm, and a positive surface charge, indicating that they have high antibacterial activity. In simulated gastrointestinal (GI) pH circumstances, genipin cross-linking enhanced the stability of the nanoparticles, according to the drug delivery studies. As a result, this combination can be effectively used for medication administration [54].

Another approach for antimicrobial nanoparticles is administration by oral route to treat GI tract infection. The nanocarriers protect the antibiotics from degradation

in GI fluids. Some nanoparticles have the bioadhesive feature to prolong the retention in GI tract for enhanced oral bioavailability. Oral mesoporous silica nanoparticles (MSN) are ideal candidates to protect the drugs from enzymolysis in GI. Zhao et al. prepared antimicrobial peptide defensin-loaded MSNs for targeting the intestine. Defensin is easily degraded in the stomach. In order to achieve the intention of intestinal targeting, succinylated casein, which can be degraded by intestinal protease, was coated onto the surface of MSNs. Casein decoration decreased defensin release in acidic environment, whereas a controlled release fashion was found in the presence of trypsin. Multidrug-resistant *E. coli* was administered by oral gavage to evoke intestinal infection. The nanoparticles were orally administered every day for 5 days. The casein-coated nanomedicine significantly lowered bacteria colonization compared with free ciprofloxacin as the positive control. The proinflammatory mediator TNF-α in intestine was decreased by 1.5- and 2.2-fold after casein-coated nanoparticle application, compared to non-coated MSNs and free peptide, respectively [40, 55].

4.5 Inorganic Nanoparticles

Silver Nanoparticles (Ag NPs)

Silver nanoparticles (Ag NPs) have sparked a lot of attention [56]. They have a substantially stronger biocidal activity than their bulk counterparts, as well [57]. In recent years, the growth of multidrug-resistant bacteria has heightened interest in Ag NPs as an alternative to conventional antibiotics. More importantly, silver nanoparticles have demonstrated broad spectrum antibacterial action, killing a wide range of microorganisms found in everyday life, including antibiotic-resistant bacteria.

It has also been established that the bactericidal impact of Ag NPs is size dependent and increases as the dimensions decrease [58]. However, Ag NPs with a small size (less than 30 nm) tend to agglomerate, reducing their antibacterial effectiveness. Ag NPs can be included into appropriate organic or inorganic matrices to reduce the aggregation problem. This will maintain Ag NP's high surface-to-mass ratio, which is essential for effective antibacterial action.

Nanocomposites containing Ag NPs and one magnetic component, on the other hand, have recently been widely investigated for the ease of separation and recycling purposes [59]. A composite made up of Ag NPs and a magnetic component can provide two distinct advantages. First, it could be easily manipulated with a magnetic field, and second, Ag NPs could be delivered to a specific target, which is an important factor in drug delivery system design. In this situation, Ag NP availability in specific positions will be raised, but their concentration in the bulk solution will be reduced.

With its unique and intriguing features, graphene oxide (GO) could be a potential support for Ag NPs. Supporting Ag NPs on GO pre-coated with a magnetic

substance, on the other hand, will result in a magnetic antibacterial composite, which will widen the frontiers of using GO and Ag NPs in water treatment as well as in medicine for drug delivery [60, 61].

The antibacterial activity of graphene oxide, cobalt ferrite, and silver nanoparticles ($GO@CoFe_2O_4@Ag$) was significantly increased when combined with the antibiotic ciprofloxacin, resulting in a substantial synergistic impact in the system. The combination of ciprofloxacin with the $GO@CoFe_2O_4@Ag$ composite led to an increase in its antibacterial activity, attributed to the antibiotic's interaction with bacterial genetic material. Whether free in solution or on the composite's surface, ciprofloxacin can interact with cell walls through its functional groups, enhancing the composite's penetration containing Ag NPs into the bacteria. The impregnated composite with ciprofloxacin can then interact with the cells' DNA, stopping supercoiling, and ultimately causing significant harm to the bacteria, leading to the observed synergistic effect [62].

Copper Sulfide (CuS) Nanoparticles

The co-precipitation approach was used to make chitosan-g-poly(acrylamide)/copper sulfide (CPA/CuS) nanocomposite in a microwave reactor. After preparation, the product (CPA/CuS) was ground into powder.

For 24 hours, 750 mg of CPA/CS nanocomposite sample in pellet form was immersed in ofloxacin solution. The ofloxacin loading efficiency in nanocomposite samples was measured spectrophotometrically. The drug release of ofloxacin in vitro was investigated utilizing a CPA/CS nanocomposite at various pH levels. In an acidic media, the highest drug release was recorded. Initially, there was a higher and faster drug release, but this decreased over time.

It has been discovered that the CPA/CS nanocomposite suppresses *E. coli* bacterium growth by attaching to the outer membrane of the bacteria. At high concentrations, the antimicrobial action was more prominent. The suppression of dehydrogenase enzyme, periplasmic enzyme activity, and active transport may also contribute to the antibacterial impact of nanocomposite against *E. coli* bacteria [63].

4.6 Nanofibers

Electrospun nanofibers have a high surface area-to-volume ratio, which is beneficial for drug administration since it increases total drug release over time, which improves drug efficacy. However, the use of such sustained release technology for oral medication administration has been limited due to gastrointestinal transit time, which limits the real period for effective drug release.

Gastroretentive/mucoadhesive drug delivery systems are advantageous because they extend the gastric residence time of oral dosages and increase the contact time between medications and the gastric mucosa, hence improving drug bioavailability and absorption in the upper GIT.

Ofloxacin has certain drawbacks, such as a short half-life and rapid breakdown in the stomach media, necessitating repeated administration.

Priya Vashisth and colleagues put ofloxacin into electrospungellan/polyvinyl alcohol (PVA) nanofibers. Gellan is a microbial mucoadhesive polymer that can extend drug residence time by providing a simple and practical method of achieving stomach retention. Due to its hydrophilicity, gellan is a biocompatible thermostable polymer with restricted applications. PVA, on the other hand, is a thermostable hydrophilic polymer that is unstable in aqueous conditions. In comparison to non-crosslinked nanofibers, heat-treated gellan/PVA nanofibers had good mechanical strength, swelling properties, and biocompatibility, so heat crosslinking at 150 °C for 15 minutes was used to stabilize gellan/PVA and ofloxacin-loaded gellan/PVA nanofibers in aqueous media and body fluids.

In contrast to pure drug, the gellan/PVA nanofibers demonstrated an initial burst release followed by sustained release of ofloxacin for up to 24 hours. Furthermore, the nanofibers displayed improved antibacterial activity in vitro against test pathogens, as well as mucoadhesion and gastro-retention capabilities [64].

4.7 Metal Organic Framework (MOFs)

Metal organic frameworks are porous compounds containing metal ions and organic ligands that are employed as drug carriers. MOFs are made with organic ligands such as 2-methylimidazole (2-melm) and terephthalic acid and metal structures such as Zr^{2+}, Zn^{2+}, Fe^{2+}, and Cu^{2+}. These MOFs are effective in drug delivery because they have qualities including high porosity, flexible sizes, and high loading capacity. Drug release is accomplished via a variety of mechanisms, including temperature, pressure, and pH, and is dependent on the structure of MOFs. MOFs are made up of paramagnetic metal ions like iron, which allow for easier magnetic imaging and targeted magnetic transfer, improving the therapeutic index

MOF was made by reacting $FeCl_3.6H_2O$ and polyacrylic acid in ethylene glycol and then allowing $Zn(NO_3)2.6H_2O$ and 2-Melm to react with the produced complex from the first stage to produce ZIF-8 functionalized Fe_3O_4@Polyacrylic acid (PAA) in the second phase. By dissolving both MOF and ciprofloxacin in deionized water, ciprofloxacin was loaded into the framework. MOF containing iron oxide, polyacrylic acid, and zeolithicimidazole to carry ciprofloxacin (Fe_3O_4@PAA@ZIF-8@Ciprofloxacin) was tested for antimicrobial activity against two reference strains, *E. coli* and *S. aureus*. The MOF structure degrades over time, and the bacteria's cell wall is killed when ciprofloxacin is released. For *E. coli* and S bacteria, Fe_3O_4@PAA@ZIF-8@Ciprofloxacin provided outstanding results. The findings reveal that the framework boosts the drug's antibacterial efficacy significantly. It can be inferred that using Fe_3O_4@PAA@ZIF-8 as a carrier can increase the antibacterial characteristics of the medicine in addition to slow release in physiological settings [65].

In another instance, Nasrabadi et al. synthesized UiO-66 (Universitetet i Oslo) typical MOF built of $[Zr_6O_4(OH)_4]$ octahedron clusters and 1,4-benzene dicarboxylic acid (BDC) ligands. UiO-66 MOF shows thermal, chemical, and mechanical stability. Non-toxicity and ease of forming nanoparticles make it suitable for drug

delivery. The UiO-66 framework is pH sensitive, indicating that it is stable under physiological pH, but it is degraded under acidic conditions. Based on this feature, UiO-66 framework is used as a drug carrier to release the ciprofloxacin from the framework by changing the pH.

The ciprofloxacin drug has a particle size of about 500 μm. On the other hand, CIP-UiO-66 has a smaller particle size, which is about 80 nm. The role of particle size in determining antibacterial activity is very important. Thus, smaller nanoparticles have more antibacterial activity than larger particles because they pass through the cell membranes and cell walls easily. As a result, ciprofloxacin -UiO-66 antibacterial activity against both bacteria is considered a special surprise, by the gradual degradation of the UiO-66 structure, and the release of metal ions (Zr^{4+}) and drug (ciprofloxacin), leading to the physical destruction of the bacterial cell wall and death.

The ciprofloxacin release of UiO-66 is controlled at pH 7.4, which is considerably slower than acidic pH (pH 5.0). Surprisingly, a significant antibacterial activity was observed in the ciprofloxacin UiO-66 synthesized material, based on the inhibition zone of 24 mm against *E. coli* and 22 mm related to *Staphylococcus aureus*. Such results are obtained when the ciprofloxacin of an inhibitory behavior is shown against *E. coli* with an inhibition zone of 14 mm [66].

4.8 Nanoshells

Nanoshells are obtained by removing the cores of these particles or by over coating the inert core with a thin coat. Core shell nanoparticles consist of concentric particles, in which particles of one material are coated with a thin layer of another material using specialized procedures.

Nanoshells made of oxides such as silica and titania find application in the field of drug delivery. The outer surface of these shells can be used for attaching antibodies so that the silica shell-antibody complex can be used for targeted drug delivery in biological systems.

Silica-gold core nanoshell in drug delivery system may offer plenty of advantages over conventional dosage forms, which improves efficacy, reduced toxicity, enhanced biodistribution, and reduces patient's compliance. The particles have the ability to conjugate the molecules without affecting the core and also can be used to encapsulate the drugs [67].

Rosemary and co-workers synthesized silica gold nanoshells containing ciprofloxacin by combining gold solution with propanolic ciprofloxacin. Antibiotic action is determined by two factors: first, the drug's capacity to traverse the cell envelope and, second, its effective binding at the target site, in this case DNA gyrase. For fluoroquinolone penetration through the cell envelope in gram-negative organisms, three different routes have been proposed:

(i) The hydrophilic pathway through porin channels
(ii) The hydrophobic pathway through the membrane bilayer matrix
(iii) The uptake pathway that is self-promoted

The pharmacological features, such as hydrophobicity, size, and structure, would influence the first two entrance paths. Self-promoted uptake is based on the displacement of divalent cations from outer membrane lipopolysaccharides. It is likely that the medicine will permeate through a different pathway than a free fluoroquinolone because it is contained in a silica shell. In terms of absolute intensities, *E. coli* uptake is higher. The amount of medicines accumulated by an organism is influenced by the pH of the outer media as well as the surface charge on the cell membrane. Fluoroquinolones with a net charge are shown to not pass through the cell membrane. Passive diffusion is more likely to be mediated by uncharged species. Because the silica surface is hydrated and has no net charge, silica encapsulation has the potential to increase medication entry rates into the periplasmic region. It is inferred that improved accumulation is achievable in *E. coli* because ciprofloxacin encapsulated silica nanoshells (cip@SiO_2) can pass through both hydrophilic and hydrophobic channels, as indicated by the partition experiments. In partition experiment, the value obtained for cip@SiO_2 is 0.913 and for free ciprofloxacin was 0.505 indicating that cip@SiO_2 is distributed almost equally in the hydrophobic and hydrophilic phases. Thus, the difference between a free drug and cip@SiO_2 is that, after encapsulation, the hydrophobicity of the material changes, which affects the penetration and results in a decrease of the MIC for *E. coli*. Due to higher drug penetration within bacterial cells, a silica-shell-based drug delivery method can improve the antibacterial effect of loaded fluoroquinolone medicines [68].

4.9 Hydrogels

Hydrogels are cross-linked polymeric networks that absorb and hold enormous volumes of water and physiological fluids while being insoluble in aqueous solutions. At equilibrium, these hydrogels contain 60–90% fluid and only 10–30% polymer. Hydrogels have been used in a variety of biological, medical, pharmacological, and environmental applications due to their unique qualities such as the capacity to swell in water, high water content, and elastic nature akin to genuine tissue, biocompatibility, and lack of toxicity.

Hydrogels are polymers with a high hydrophilicity and water insolubility. In water, hydrogels swell to an equilibrium volume while maintaining their shape. The presence of water solubilizing groups such as –OH, –COOH, and –CONH accounts for the hydrophilicity. The existence of 3D network structures in the hydrogel is essential for the shape's insolubility and stability. Hydrogels are comprised of hydrophilic polymer molecules that are joined together by chemical bonds or other cohesive factors like ionic connection, hydrogen bonding, or hydrophobic interaction.

Hydrogels are widely employed as drug delivery systems in medicine and pharmacy, with one of the most prominent uses being controlled release systems and medication targeting to specific parts of the body. When the hydrogels make contact with the target site, the rate and duration of drug release is determined by their swelling behavior [69].

Chitosan and its derivative hydrogels have been shown to be a viable carrier for a variety of medicinal molecules of various sizes and types. However, one of the fundamental constraints of employing chitosan in hydrogels is its insolubility at pH > 6.5, which limits its biomedical applications, especially in circumstances when drug administration inside the body is required, particularly at physiological pH.

Chemically modified chitosan was employed in drug delivery devices to address this issue. In comparison to chitosan, N-trimethyl chitosan (TMC), a partly quaternized derivative of chitosan, has strong water solubility across a wide pH range. It has a high capacity for transporting hydrophilic macromolecules across mucosal epithelia, even at neutral and basic pH values, and has thus been employed in a variety of biotechnological and medicinal applications.

Ciprofloxacin-loaded hydrogel was created by Hanna and Saad. Ciprofloxacin was successfully integrated and released from the produced gel without causing any structural or functional changes. The produced hydrogel's entrapment efficiency was observed to rise as the drug concentration was raised, reaching 93.82.1% with 250 g/mL of ciprofloxacin, and it was shown that the drug entrapped within the gel without substantial interaction, as validated by FTIR spectra and DSC analyses. When compared to reference antibiotics, the release of ciprofloxacin from the ciprofloxacin-loaded hydrogel was found to be structurally intact as well as effective in inhibiting gram-positive and gram-negative bacterial strains with a larger width of inhibition zone (Gentamicin and Ampicilin) [70].

Another way to improve targeted antimicrobial drug delivery is a bioadhesive nanoparticle-hydrogel hybrid (NP-gel) system was created, especially under high shear force conditions. Antibiotics were put into polymeric nanoparticles, which were then embedded in a tissue-adhesive hydrogel in the design. On biological surfaces such as a bacterial film, a mammalian cell monolayer, and mouse skin tissue, the hybrid system demonstrated improved adhesion and antibiotic retention under high shear stress. The NP-gel efficiently reduced the production of *E. coli* bacterial film in a flow environment in vitro [71].

4.10 Prodrug Method to Deliver Drug

A prodrug is a drug substance that is inactive in its intended pharmacological activities and must be turned into a pharmacologically active agent through metabolic or physicochemical change. Prodrugs can be found in nature, such as various phytochemicals/botanical compounds and endogenous substances, or they can be created through synthetic or semisynthetic methods, either purposefully or inadvertently during drug development [72].

Because ciprofloxacin has a half-life of 4–5 hours, dosing takes a large amount of medication to sustain therapeutic levels. Due to the high clearance value, this could result in toxicity, side effects, or low bioavailability. Thus, a biopolymer

matrix including chitosan and ciprofloxacin was produced and tested for antibacterial efficacy in order to optimize the pharmacokinetic profile.

The film was created using the solution casting approach, and chitosan/ciprofloxacin prodrug conjugates were successfully manufactured using isopropyl alcohol/water and glacial acetic acid mixture. Chitosan increases the flexibility of the films and allows the film wall to widen its surface for swelling to compensate for osmotic variations between the interior and exterior of the films.

Ciprofloxacin appears to be released through both diffusion and film erosion. The films disintegrated in a pH-dependent manner. Without releasing significant amounts of the loaded drug, these biocompatible film devices can bypass the acidity of gastric fluid. The antimicrobial activity of ciprofloxacin and chitosan alone is effective against gram-positive and gram-negative bacteria, and the complexation of ciprofloxacin with chitosan increased the antimicrobial activity [73].

4.11 Liquid Crystals

Liquid crystals are a new type of drug delivery system composed of polar lipids that can spontaneously reorganize into three-dimensional structures (liquid crystalline phases) when exposed to water. They feature a flexible structure that can be modified to allow for both ease of administration and long-term drug release, as defined by local physicochemical parameters.

Optical and electrical anisotropy, as well as flow characteristics and molecule mobility, are all features of liquid crystals. While liquid crystals do not directly transition from a liquid to a solid state in practice, they do display molecularly organized intermediary phases (mesophases) with liquid- and solid-state properties under particular conditions. Lyotropic liquid crystals are based on lipids that spontaneously self-assemble in an aqueous environment and form mesophases in suitable solvents. They have nanostructured hydrophilic and hydrophobic domains separated by lipid bilayers, and they form in a polar (aqueous) environment.

Furthermore, the disclosed liquid crystal platform has a slow-release characteristic, enabling for longer treatments without the need for multiple dosage regimens. The platform is also non-invasive and can be used locally, eliminating the requirement for oral or intravenous delivery. While the liquid crystal was in the lamellar phase, an emulsion was created. Ciprofloxacin encapsulation increased bacterial cell death considerably, in addition to reducing toxicity. The lamellar phase liquid crystal treatment was five times more effective than free-ciprofloxacin at killing *E. coli*, suggesting the formulation's considerable potential for improving antibiotic efficacy and sustained administration [74] (Tables 4 and 5).

Table 4 List of drug delivery system and drugs encapsulated for treatment of *E. coli* infections

Sr. No.	Drug delivery system	Drugs encapsulated
1	Hydrogel	Ciprofloxacin [71], Ofloxacin [75], Amoxicillin [76], Cotrimoxazole [77]
2	Dendrimers	Ciprofloxacin [78], Amoxicillin [79]
3	Sustained Release Tablets	Ofloxacin [80], Levofloxacin [81], Amoxicillin [82]
4	Extended-Release	Nitrofurantoin [83], Ciprofloxacin [84]
5	Floating and Bio adhesive	Ciprofloxacin [41], Ofloxacin [85], Amoxicillin [86], Levofloxacin [87]
6	Metal Organic Frame work	Ciprofloxacin [65]
7	Nanoparticles	Ciprofloxacin [54], Ofloxacin [88], Amoxicillin [89], Levofloxacin [90], Cotrimoxazole [91], Azithromycin [92], Nitrofurantoin [93]
8	Nano shells	Ciprofloxacin [68], Amoxicillin [94]
9	Prodrug	Ciprofloxacin [73], Levofloxacin [95]
10	Thermosensitive Gel	Levofloxacin [96]
11	Liposomes	Ciprofloxacin [97], Amoxicillin [98]
12	Microspheres	Amoxicillin [99]

Table 5 List of observational and interventional clinical trials related to *E. coli* [100]

Sr. No.	Title	Type of trial
1	Multi-Drug Resistant Organism (MDRO): Study of Highly Resistant Escherichia Coli	Observational
2	Clinical and Microbiologic Characteristics of Cephalosporin-Resistant E. Coli-PITT Protocol	Observational
3	Evaluation of a Candidate Vaccine Against Uropathogenic *Escherichia Coli* in Women With a Clinical History of Recurrent Urinary Tract *Infection*	Interventional
4	Prospective Epidemiological Study to Estimate the O-serotype Distribution of Extraintestinal Pathogenic *Escherichia Coli* (ExPEC) Isolated From Hospitalized Patients Aged 60 Years or Older With Invasive ExPEC Disease (IED) and to Evaluate the Clinical Case Definition and Risk Factors.	Observational
5	Effects of Nutritional Fat on the Growth of Intestinal *E. Coli*	Interventional
6	Dose Escalating Study of Two Enterotoxigenic *Escherichia Coli* Prototype Adhesin-based Vaccines With or Without Modified Heat-labile Enterotoxin by Intradermal or Transcutaneous Immunization	Interventional
7	Randomized Double-Blinded Pilot Study Confirming A Human Challenge Model Using LSN03-016011/A Expressing LT And CS17 And Investigating The Safety Of VLA1701 (An Investigational Oral Cholera And ETEC (Enterotoxigenic *E Coli*) (Vaccine)	Interventional
8	A Multi-Center Randomized, Double-Blind Study to Assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of LBP-EC01 in Patients With Lower Urinary Tract Colonization Caused by *E. Coli*	Interventional

(continued)

Table 5 (continued)

Sr. No.	Title	Type of trial
9	Effect of Gastric Acid and H. Pylori *Infection* on *Infection* With Enteropathogenic *E. Coli*	Interventional
10	Hyperhydration to Improve Kidney Outcomes in Children With Shiga Toxin-Producing *E. Coli Infection*: A Multinational Embedded Cluster Crossover Randomized Trial	Interventional
11	Role of the NLRP3 Inflammasome in *Escherichia Coli* and Staphylococcus Aureus Bacteria	Observational
12	A Randomized Controlled Trial of Sitafloxacin and Ertapenem Treatment for Acute Pyelonephritis Caused by Extended-Spectrum ß-lactamase-producing *Escherichia Coli*	Interventional
13	Randomized, Controlled Multicentric, Open-label Clinical Trial to Prove Non-Inferiority of Fosfomycin vs Meropenem or Ceftriaxone in the Treatment of Bacteriemic Urinary *Infection* Due to Multidrug Resistance in *E.Coli*	Interventional
14	Preventing Urinary Tract *Infections* in Infants and Young Children With Probiotic *E. Coli* Nissle	Interventional
15	Granzyme A in Patients With *E. Coli* Bacteremic Urinary Tract *Infections*	Observational
16	A Study to Assess the Immunogenicity Consistency of Three Consecutive Batches of Commercial-scale of Recombinant Human Papillomavirus Bivalent (Types 16, 18) Vaccine (*Escherichia Coli*) in Healthy Female Subjects Aged 9–14 Years After Two-Dose Regimens Immunization Procedure	Interventional

5 Conclusion

Escherichia coli's propensity to colonize the guts of humans and animals, allowing for fecal-oral transfer, as well as its ability to transmit and uptake antibiotic resistance genes via plasmids to and from other bacteria, has made it a crucial target in the fight against antimicrobial resistance. Antibiotic resistance has been found to be multifaceted, yet all factors point to one primary issue: antibiotic overuse in both human and veterinary medicine. Existing drug molecules problems could be resolved by using novel drug delivery approaches to increase the lifecycle of it. Till date, various organic and inorganic nanoparticles have been studied for effective delivery of existing molecules or completely novel approach for delivering drugs. Additionally, emphasis on novel vaccine development can serve the purpose in the future.

References

1. Shulman ST, Friedmann HC, Sims RH. Theodor Escherich: the first pediatric infectious diseases physician? Clin Infect Dis. 2007;45(8):1025–9.
2. Ramos S, Silva V, Dapkevicius MLE, Caniça M, Tejedor-Junco MT, Igrejas G, et al. Escherichia coli as commensal and pathogenic bacteria among food-producing animals: health implications of extended spectrum β-lactamase (ESBL) production. Animals. 2020;10(12):2239.

3. Bettelheim K, Lennox-King S. The acquisition of Escherichia coli by new-born babies. Infection. 1976;4(3):174–9.
4. Paletta AC, Castro VS, Conte-Junior CA. Shiga toxin-producing and enteroaggregative Escherichia coli in animal, foods, and humans: pathogenicity mechanisms, detection methods, and epidemiology. Curr Microbiol. 2020;77(4):612–20.
5. Denamur E, Clermont O, Bonacorsi S, Gordon D. The population genetics of pathogenic Escherichia coli. Nat Rev Microbiol. 2021;19(1):37–54.
6. Sarowska J, Futoma-Koloch B, Jama-Kmiecik A, Frej-Madrzak M, Ksiazczyk M, Bugla-Ploskonska G, et al. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic Escherichia coli isolated from different sources: recent reports. Gut Pathog. 2019;11(1):1–16.
7. Urwin R, Maiden MC. Multi-locus sequence typing: a tool for global epidemiology. Trends Microbiol. 2003;11(10):479–87.
8. Johnson JR, Owens KL, Clabots CR, Weissman SJ, Cannon SB. Phylogenetic relationships among clonal groups of extraintestinal pathogenic Escherichia coli as assessed by multi-locus sequence analysis. Microbes Infect. 2006;8(7):1702–13.
9. Riley LW. Distinguishing pathovars from nonpathovars: Escherichia coli. Microbiol Spectrum. 2020;8(4):1.
10. Gordon DM. Strain typing and the ecological structure of Escherichia coli. J AOAC Int. 2010;93(3):974–84.
11. Gordon DM. The ecology of Escherichia coli. Elsevier; 2013. p. 3–20.
12. Karam MRA, Habibi M, Bouzari S. Urinary tract infection: pathogenicity, antibiotic resistance and development of effective vaccines against uropathogenic Escherichia coli. Mol Immunol. 2019;108:56–67.
13. Shah C, Baral R, Bartaula B, Shrestha LB. Virulence factors of uropathogenic Escherichia coli (UPEC) and correlation with antimicrobial resistance. BMC Microbiol. 2019;19(1):1–6.
14. Pakbin B, Brück WM, Rossen JW. Virulence factors of enteric pathogenic Escherichia coli: a review. Int J Mol Sci. 2021;22(18):9922.
15. Kim J-S, Lee M-S, Kim JH. Recent updates on outbreaks of Shiga toxin-producing Escherichia coli and its potential reservoirs. Front Cell Infect Microbiol. 2020;10:273.
16. Mühlen S, Dersch P. Treatment strategies for infections with Shiga toxin-producing Escherichia coli. Front Cell Infect Microbiol. 2020;10:169.
17. Mare AD, Ciurea CN, Man A, Tudor B, Moldovan V, Decean L, et al. Enteropathogenic Escherichia coli—a summary of the literature. Gastroenterol Insights. 2021;12(1):28–40.
18. Ochoa TJ, Contreras CA. Enteropathogenic E. coli (EPEC) infection in children. Curr Opin Infect Dis. 2011;24(5):478.
19. Fleckenstein JM, Kuhlmann FM. Enterotoxigenic Escherichia coli infections. Curr Infect Dis Rep. 2019;21(3):1–9.
20. Mirhoseini A, Amani J, Nazarian S. Review on pathogenicity mechanism of enterotoxigenic Escherichia coli and vaccines against it. Microb Pathog. 2018;117:162–9.
21. Jenkins C. Enteroaggregative escherichia coli. Curr Top Microbiol Immunol. 2018:27–50.
22. Lima AA, Medeiros PH, Havt A. Enteroaggregative Escherichia coli subclinical and clinical infections. Curr Opin Infect Dis. 2018;31(5):433–9.
23. Walczuk U, Sobieszczańska B, Turniak M, Rzeszutko M, Duda-Madej A, Iwańczak B. The prevalence of mucosa-associated diffusely adherent Escherichia coli in children with inflammatory bowel disease. Adv Clin Exp Med. 2019;28(7):919–25.
24. Chervy M, Barnich N, Denizot J. Adherent-invasive E. coli: update on the lifestyle of a troublemaker in Crohn's disease. Int J Mol Sci. 2020;21(10):3734.
25. Palmela C, Chevarin C, Xu Z, Torres J, Sevrin G, Hirten R, et al. Adherent-invasive Escherichia coli in inflammatory bowel disease. Gut. 2018;67(3):574–87.
26. Shane AL, Mody RK, Crump JA, Tarr PI, Steiner TS, Kotloff K, et al. 2017 Infectious Diseases Society of America clinical practice guidelines for the diagnosis and management of infectious diarrhea. Clin Infect Dis. 2017;65(12):e45–80.

27. Riddle MS, Connor BA, Beeching NJ, DuPont HL, Hamer DH, Kozarsky P, et al. Guidelines for the prevention and treatment of travelers' diarrhea: a graded expert panel report. J Travel Med. 2017;24(suppl_1):S63–80.
28. Mueller M, Tainter CR. Escherichia Coli. In: StatPearls. StatPearls Publishing, Treasure Island (FL); 2022.
29. Dipiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey LM. Pharmacotherapy: a pathophysiologic approach, ed. Connecticut: Appleton and Lange; 2014;4:141–2.
30. Li C, Wang J, Wang Y, Gao H, Wei G, Huang Y, et al. Recent progress in drug delivery. Acta Pharm Sin B. 2019;9(6):1145–62.
31. Kaur G, Arora M, Kumar MR. Oral drug delivery technologies—a decade of developments. J Pharmacol Exp Ther. 2019;370(3):529–43.
32. Qiu Y, Lee P. Rational design of oral modified-release drug delivery systems. Developing solid oral dosage forms. Elsevier; 2017. p. 519–54.
33. Sobhani Z, Samani SM, Montaseri H, Khezri E. Nanoparticles of chitosan loaded ciprofloxacin: fabrication and antimicrobial activity. Adv Pharm Bull. 2017;7(3):427.
34. Ranghar S, Sirohi P, Verma P, Agarwal V. Nanoparticle-based drug delivery systems: promising approaches against infections. Braz Arch Biol Technol. 2014;57:209–22.
35. Somasundaram S, Manivannan K. An overview of fluoroquinolones. Ann Res Rev Biol. 2013:296–313.
36. Fourcroy JL, Berner B, Chiang Y-K, Cramer M, Rowe L, Shore N. Efficacy and safety of a novel once-daily extended-release ciprofloxacin tablet formulation for treatment of uncomplicated urinary tract infection in women. Antimicrob Agents Chemother. 2005;49(10):4137–43.
37. Trenfield SJ, Basit AW. Modified drug release: current strategies and novel technologies for oral drug delivery. Elsevier; 2020. p. 177–97.
38. Streubel A, Siepmann J, Bodmeier R. Floating matrix tablets based on low density foam powder: effects of formulation and processing parameters on drug release. Eur J Pharm Sci. 2003;18(1):37–45.
39. Walvekar P, Gannimani R, and Govender T. Combination drug therapy via nanocarriers against infectious diseases. Eur J Pharm Sci 2019;27:121–41.
40. Yeh Y-C, Huang T-H, Yang S-C, Chen C-C, Fang J-Y. Nano-based drug delivery or targeting to eradicate bacteria for infection mitigation: a review of recent advances. Front Chem. 2020;8:286.
41. Varshosaz J, Tavakoli N, Roozbahani F. Formulation and in vitro characterization of ciprofloxacin floating and bioadhesive extended-release tablets. Drug Deliv. 2006;13(4):277–85.
42. Walker CB. Selected antimicrobial agents: mechanisms of action, side effects and drug interactions. Periodontol. 1996;10(1):12–28.
43. Ferrari M. Cancer nanotechnology: opportunities and challenges. Nat Rev Cancer. 2005;5(3):161–71.
44. Niemeyer CM. Nanoparticles, proteins, and nucleic acids: biotechnology meets materials science. Angew Chem Int Ed. 2001;40(22):4128–58.
45. Divya K, Jisha M. Chitosan nanoparticles preparation and applications. Environ Chem Lett. 2018;16(1):101–12.
46. Jamkhande PG, Ghule NW, Bamer AH, Kalaskar MG. Metal nanoparticles synthesis: an overview on methods of preparation, advantages and disadvantages, and applications. J Drug Deliv Sci Technol. 2019;53:101174.
47. Allouche J. Synthesis of organic and bioorganic nanoparticles: an overview of the preparation methods; 2013. p. 27–74.
48. Grenha A. Chitosan nanoparticles: a survey of preparation methods. J Drug Target. 2012;20(4):291–300.
49. Ghormade V, Pathan E, Deshpande M. Can fungi compete with marine sources for chitosan production? Int J Biol Macromol. 2017;104:1415–21.

50. Avadi M, Sadeghi A, Tahzibi A, Bayati K, Pouladzadeh M, Zohuriaan-Mehr M, et al. Diethylmethyl chitosan as an antimicrobial agent: synthesis, characterization and antibacterial effects. Eur Polym J. 2004;40(7):1355–61.
51. Abdel-Fattah WI, Sallam ASM, Atwa NA, Salama E, Maghraby AM, Ali GW. Functionality, antibacterial efficiency and biocompatibility of nanosilver/chitosan/silk/phosphate scaffolds 1. Synthesis and optimization of nanosilver/chitosan matrices through gamma rays irradiation and their antibacterial activity. Mater Res Express. 2014;1(3):035024.
52. Kim TH, Park IK, Nah JW, Choi YJ, Cho CS. Galactosylated chitosan/DNA nanoparticles prepared using water-soluble chitosan as a gene carrier. Biomaterials. 2004;25(17):3783–92.
53. Ibrahim HM, El-Bisi MK, Taha GM, El-Alfy EA. Chitosan nanoparticles loaded antibiotics as drug delivery biomaterial. J Appl Pharm Sci. 2015;5(10):085–90.
54. Kumar GV, Su C-H, Velusamy P. Ciprofloxacin loaded genipin cross-linked chitosan/heparin nanoparticles for drug delivery application. Mater Lett. 2016;180:119–22.
55. Zhao G, Chen Y, He Y, Chen F, Gong Y, Chen S, et al. Succinylated casein-coated peptide-mesoporous silica nanoparticles as an antibiotic against intestinal bacterial infection. Biomater Sci. 2019;7(6):2440–51.
56. Ghodake G, Lim S-R, Lee DS. Casein hydrolytic peptides mediated green synthesis of antibacterial silver nanoparticles. Colloids Surf B: Biointerfaces. 2013;108:147–51.
57. Choi O, Hu Z. Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. Environ Sci Technol. 2008;42(12):4583–8.
58. Sadeghi B, Garmaroudi FS, Hashemi M, Nezhad H, Nasrollahi A, Ardalan S, et al. Comparison of the anti-bacterial activity on the nanosilver shapes: nanoparticles, nanorods and nanoplates. Adv Powder Technol. 2012;23(1):22–6.
59. Kooti M, Kharazi P, Motamedi H. Preparation, characterization, and antibacterial activity of CoFe2O4/polyaniline/Ag nanocomposite. J Taiwan Inst Chem Eng. 2014;45(5):2698–704.
60. Wang G, Ma Y, Wei Z, Qi M. Development of multifunctional cobalt ferrite/graphene oxide nanocomposites for magnetic resonance imaging and controlled drug delivery. Chem Eng J. 2016;289:150–60.
61. Wang G, Ma Y, Zhang L, Mu J, Zhang Z, Zhang X, et al. Facile synthesis of manganese ferrite/graphene oxide nanocomposites for controlled targeted drug delivery. J Magn Magn Mater. 2016;401:647–50.
62. Kooti M, Sedeh AN, Motamedi H, Rezatofighi SE. Magnetic graphene oxide inlaid with silver nanoparticles as antibacterial and drug delivery composite. Appl Microbiol Biotechnol. 2018;102(8):3607–21.
63. Pathania D, Gupta D, Agarwal S, Asif M, Gupta VK. Fabrication of chitosan-g-poly (acrylamide)/CuS nanocomposite for controlled drug delivery and antibacterial activity. Mater Sci Eng C. 2016;64:428–35.
64. Vashisth P, Raghuwanshi N, Srivastava AK, Singh H, Nagar H, Pruthi V. Ofloxacin loaded gellan/PVA nanofibers-synthesis, characterization and evaluation of their gastroretentive/mucoadhesive drug delivery potential. Mater Sci Eng C. 2017;71:611–9.
65. Esfahanian M, Ghasemzadeh MA, Razavian SMH. Synthesis, identification and application of the novel metal-organic framework Fe3O4@ PAA@ ZIF-8 for the drug delivery of ciprofloxacin and investigation of antibacterial activity. Artif Cells Nanomed Biotechnol. 2019;47(1):2024–30.
66. Nasrabadi M, Ghasemzadeh MA, Monfared MRZ. The preparation and characterization of UiO-66 metal–organic frameworks for the delivery of the drug ciprofloxacin and an evaluation of their antibacterial activities. New J Chem. 2019;43(40):16033–40.
67. Amirthalingam T, Kalirajan J, Chockalingam A. Use of silica-gold core shell structured nanoparticles for targeted drug delivery system. J Nanomed Nanotechnol. 2011;2(6)
68. Rosemary M, MacLaren I, Pradeep T. Investigations of the antibacterial properties of ciprofloxacin@ SiO2. Langmuir. 2006;22(24):10125–9.
69. Maziad NA, El-Hamouly S, Zied E, Kelani EL, TA, Nasef NR. Radiation preparation of smart hydrogel has antimicrobial properties for controlled release of ciprofloxacin in drug delivery systems. Asian J Pharm Clin Res. 2015;14:15.

70. Hanna DH, Saad GR. Encapsulation of ciprofloxacin within modified xanthan gum-chitosan based hydrogel for drug delivery. Bioorg Chem. 2019;84:115–24.
71. Zhang Y, Zhang J, Chen M, Gong H, Thamphiwatana S, Eckmann L, et al. A bioadhesive nanoparticle–hydrogel hybrid system for localized antimicrobial drug delivery. ACS Appl Mater Interfaces. 2016;8(28):18367–74.
72. Wu K-M. A new classification of prodrugs: regulatory perspectives. Pharmaceuticals. 2009;2(3):77–81.
73. Semwal A, Singh BK, Archana D, Verma A, Dutta P. Macromolecular chitosan/ciprofloxacin pro-drugs: synthesis, physico-chemical and biological assessment for drug delivery systems. J Polym Mater. 2012;29(1):1.
74. Pisano S, Giustiniani M, Francis L, Gonzalez D, Margarit L, Sheldon IM, et al. Liquid crystal delivery of ciprofloxacin to treat infections of the female reproductive tract. Biomed Microdevices. 2019;21(2):1–12.
75. Pathania D, Gupta D, Kothiyal N, Eldesoky G, Naushad M. Preparation of a novel chitosan-g-poly (acrylamide)/Zn nanocomposite hydrogel and its applications for controlled drug delivery of ofloxacin. Int J Biol Macromol. 2016;84:340–8.
76. Liang Y, Zhao X, Ma PX, Guo B, Du Y, Han X. pH-responsive injectable hydrogels with mucosal adhesiveness based on chitosan-grafted-dihydrocaffeic acid and oxidized pullulan for localized drug delivery. J Colloid Interface Sci. 2019;536:224–34.
77. Kumar P, Narang RK, Swamy S. Development and evaluation of nanoparticle-loaded hydrogel of co-trimoxazole. Int J Pharm Sci Nanotechnol. 2016;9(1):3131–41.
78. Wong PT, Tang S, Mukherjee J, Tang K, Gam K, Isham D, et al. Light-controlled active release of photocaged ciprofloxacin for lipopolysaccharide-targeted drug delivery using dendrimer conjugates. Chem Commun. 2016;52(68):10357–60.
79. Mintzer MA, Dane EL, O'Toole GA, Grinstaff MW. Exploiting dendrimer multivalency to combat emerging and re-emerging infectious diseases. Mol Pharm. 2012;9(3):342–54.
80. Chavanpatil MD, Jain P, Chaudhari S, Shear R, Vavia PR. Novel sustained release, swellable and bioadhesive gastroretentive drug delivery system for ofloxacin. Int J Pharm. 2006;316(1–2):86–92.
81. Wilson B, Sitarambhai PH, Sajeev M, Vinothapooshan G. Design and evaluation of sustained release matrix tablets of levofloxacin for effective treatment of microbial infections. Int J Drug Deliv. 2011;3(2):305.
82. Beg S, Nayak AK, Kohli K, Swain S, Hasnain M. Antimicrobial activity assessment of time-dependent release bilayer tablets of amoxicillin trihydrate. Braz J Pharm Sci. 2012;48:265–72.
83. Spencer R, Moseley D, Greensmith M. Nitrofurantoin modified release versus trimetfaoprim or co-trimoxazole in the treatment of uncomplicated urinary tract infection in general practice. J Antimicrob Chemother. 1994;33(suppl_A):121–9.
84. Talan DA, Naber KG, Palou J, Elkharrat D. Extended-release ciprofloxacin (Cipro XR) for treatment of urinary tract infections. Int J Antimicrob Agents. 2004;23:54–66.
85. Qi X, Chen H, Rui Y, Yang F, Ma N, Wu Z. Floating tablets for controlled release of ofloxacin via compression coating of hydroxypropyl cellulose combined with effervescent agent. Int J Pharm. 2015;489(1–2):210–7.
86. Arianto A, Bangun H, Yohana A, Silalahi J. Floating gastroretentive of amoxicillin using hard alginate capsules and its antibacterial activities. Asian J Pharm Clin Res. 2017;10(5):413–9.
87. Pandey NK, Ghatuary SK, Dubey A, Jain PK. Formulation and in vitro evaluation of sustained release floating matrix tablet of levofloxacin by direct compression method. J Drug Deliv Therap. 2019;9(4-s):398–403.
88. Nuti S, Fernández-Lodeiro J, Del Secco B, Rampazzo E, Rodríguez-González B, Capelo JL, et al. Engineered nanostructured materials for ofloxacin delivery. Front Chem. 2018;6:554.
89. Demurtas M, Perry CC. Facile one-pot synthesis of amoxicillin-coated gold nanoparticles and their antimicrobial activity. Gold Bull. 2014;47(1):103–7.
90. López-López M, Fernández-Delgado A, Moyá ML, Blanco-Arévalo D, Carrera C, de la Haba RR, et al. Optimized preparation of levofloxacin loaded polymeric nanoparticles. Pharmaceutics. 2019;11(2):57.

91. Shahverdi AR, Fakhimi A, Shahverdi HR, Minaian S. Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against Staphylococcus aureus and Escherichia coli. Nanomedicine. 2007;3(2):168–71.
92. Saddik MS, Elsayed MM, El-Mokhtar MA, Sedky H, Abdel-Aleem JA, Abu-Dief AM, et al. Tailoring of novel azithromycin-loaded zinc oxide nanoparticles for wound healing. Pharmaceutics. 2022;14(1):111.
93. Cortesi R, Esposito E. Pharmaceutical and biotechnological applications of multiple emulsions. Colloids Drug Deliv. 2010;20100581:203–20.
94. Vafayi L, Gharibe S. Investigation of in vitro drug release from porous hollow silica nanospheres prepared of ZnS@ SiO2 core-shell. Bioinorg Chem Appl. 2013;2013:1.
95. Pal S, Ramu V, Taye N, Mogare DG, Yeware AM, Sarkar D, et al. GSH induced controlled release of levofloxacin from a purpose-built prodrug: luminescence response for probing the drug release in Escherichia coli and Staphylococcus aureus. Bioconjug Chem. 2016;27(9):2062–70.
96. Alves DA, Machado D, Melo A, Pereira RFC, Severino P, Hollanda LM, et al. Preparation of thermosensitive gel for controlled release of levofloxacin and their application in the treatment of multidrug-resistant bacteria. Biomed Res Int. 2016;2016:1.
97. Li T, Mudie S, Cipolla D, Rades T, Boyd BJ. Solid state characterization of ciprofloxacin liposome nanocrystals. Mol Pharm. 2018;16(1):184–94.
98. Trucillo P, Ferrari P, Campardelli R, Reverchon E, Perego P. A supercritical assisted process for the production of amoxicillin-loaded liposomes for antimicrobial applications. J Supercrit Fluids. 2020;163:104842.
99. Pooresmaeil M, Asl EA, Namazi H. Simple fabrication of biocompatible chitosan/graphene oxide microspheres for pH-controlled amoxicillin delivery. Eur Polym J. 2021;159:110706.
100. Clinical Trials – Escherichia Coli Infections. [23/06/2022]. Available from: https://clinicaltrials.gov/ct2/results?cond=Escherichia+Coli+Infections&term=&cntry=&state=&city=&dist=.

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