Current Microbiology (2024) 81:236
https://doi.org/10.1007/s00284-024-03754-1

REVIEW ARTICLE

Orientia tsutsugamushi: An Unusual Intracellular Bacteria—Adaptation Strategies, Available Antibiotics, and Alternatives for Treatment

Prashansha Srivastava[1] · Aishwarya Shukla[1] · Rajeev Singh[1] · Rajni Kant[1] · Nalini Mishra[1] · Sthita P. Behera[1] · Gaurav R. Dwivedi[1] · Dharmendra K. Yadav[2]

Received: 16 November 2023 / Accepted: 28 May 2024 / Published online: 21 June 2024

Abstract

During evolution *Orientia tsutsugamushi* became a smarter obligate bacterium to establish as intracellular pathogens. *O. tsutsugamushi* is a human pathogenic bacterium responsible for 1 billion infections of scrub typhus. Several novel mechanisms make this bacterium unique (cell wall, genetic constitutions, secretion system, etc.). In 2007, *O. tsutsugamushi* Boryong was pioneer strain for whole-genome sequencing. But the fundamental biology of this bacterial cell is a mystery till date. The unusual biology makes this organism as model for host cell interaction. Only a few antibiotics are effective against this intracellular pathogen but emergence of less susceptibility toward antibiotics make the situation alarming. The review was captivated to highlight the unusual aspects of adaptation, antibiotics, and drugs beyond antibiotics.

Introduction

Over 50 years ago, Van Valen proposed 'Red Queen Hypothesis' in which the queen said to Alice, in 'Through the Looking Glass,'

"It takes all the running you can do, to keep in the same place" [1]

To maintain their own survival, one species can increase its fitness at the expense of the other species [2]. Charles Darwin published his book "The Origin of Species" in 1859; the fields of evolutionary biology have come a long way since then, this book emphasized that natural selection evolves the organism which is totally different from their ancestor. The natural selection lies in the center of shaping the adaptations (morphological, physiological, and behavioral) to find out the inside story of living organism across the generation [3]. Genetic material is immortal and transfer information from one generation to another generation. Mutation and genetic recombination are the key players of variation.

Natural selection is working on the concept of 'survival of the fittest' and that's why an evolutionary arm race, between host and pathogen, predator, and prey, continuously working in this dynamic environment [4, 5]. To make the environment dynamic, living, non-living, and different species of living things exist in a symbiotic environment. Symbiosis is a broad term which refers to 2 species existing together in an environment. The inter-specific correlation

Prashansha Srivastava and Aishwarya Shukla have equally contributed to this work.

✉ Gaurav R. Dwivedi
grd.rmrcb@gov.in; grdnikhil@gmail.com

Prashansha Srivastava
prashanshasrivastava1998@gmail.com

Aishwarya Shukla
aishwaraya.shukla@gmail.com

Rajeev Singh
rajeevbt22@gmail.com

Rajni Kant
rajnikant.srivastava@gmail.com

Nalini Mishra
nalini007mishra@gmail.com

Sthita P. Behera
sp.behera1@gmail.com

Dharmendra K. Yadav
dharmendra30oct@gmail.com

[1] ICMR-Regional Medical Research Centre, BRD Medical College Campus, Gorakhpur 273013, India

[2] College of Pharmacy, Gachon University of Medicine and Science, Hambakmoeiro 191, Yeonsu-Gu, Incheon 21924, Korea

among eukaryotic and prokaryotic species existed during the course of evolution [5]. In this hill climbing fight for survival, human and microbial world are in a persistent fight.

Human beings are shielded by their immune system in combating infections caused via virulent organisms, namely viruses, fungi, bacteria, etc. As the part of patrolling, immune cells target and kill microbes roaming around the circulatory system, still immunity alone was not sufficient to tackle the contagious organism. Several anti-viral, antibacterial, and anti-parasitic were invented and became crucial to counter the contagious agents [6]. When it comes to 'Minute forms of life,' understanding their adaptation strategies during evolutionary arm race, to get selected by nature and maintaining their population through generations, demands our special attention. For example, viruses, bacteria, fungi, and parasites started to develop resistance which became one of the dominant strategies for survival [7].

Pathogenic microbes may be categorized on their infection methods. First, the extracellular microbes do not enter inside the cells but prefer to grow in external environment (body fluids with different nutrients). Second, the intracellular bacterial pathogen adapted a different strategy to escape from getting caught by host immune system and replicate inside host cell to increase their number causing severity of disease [8]. After invasion, host cells provide a heavenly environment for pathogen replication. However, each host cell has a limited carrying capacity for intracellular bacteria. Therefore, after reaching the carrying capacity, they must leave the infected cell and infect other cells to replicate further [9]. To date, it is not clearly understood how these intracellular bacteria multiply and spread their infection without being caught by the host immune system.

Scrub typhus (ST) is a remerging neglected disease that represents an acute fever sickness caused by the bacteria *Orientia tsutsugamushi* and spread by the larvae (chiggers) of *Leptotrombidium* mites. This disease is very severe public health problem in Southeast, East Asia, and the Pacific Islands, called as the 'Tsutsugamushi Triangle' [10]. Now scrub typhus (ST) is not only reported from tsutsugamushi triangle but evidence indicated that scrub typhus started to expand outside the Triangle in eastern Africa, France, the Middle East, and South America [11]. It frightens one billion people worldwide and around one million people are affected each year [12]. Many Sero-epidemiological data in Asia have shown seroprevalence rates are 9.3 to 27.9% alongside a notable increase in disease incidence. The median fatality rate for untreated ST and treated ST varied as 6.0% versus 1.4%. Patient with central nervous system involvement experience a high fatality rate of 13.6%, while those with multi-organ dysfunction had a rate of 24.1%. Additionally, ST infection during pregnancy is associated with a high risk of miscarriage and poor neonatal outcomes [13].

For the treatment of *O. tsutsugamushi,* antibiotics are still a medical miracle. Chloramphenicol, tetracycline (doxycycline), macrolides (azithromycin), and rifampin are still the best option for treating the disease 'Scrub typhus' [14–17]. *O. tsutsugamushi* started to develop strategies to tolerate and replicate even in the presence of antibiotics. This adaptation strategy of *O. tsutsugamushi* started to alarm for the discovery of new drugs.

In view of unusual biology for host–pathogen interaction, less susceptibility of present antibiotics, and outbreak of the scrub typhus across the world, the present review is conceptualized to find out the adaptation strategy acquired by the deadly pathogen *O. tsutsugamushi.* This review is also conceptualized to focus on antibiotics, less susceptibility toward antibiotics, and alternatives of antibiotics.

History

The history of *O. tsutsugamushi* is too old, for the first time, it was reported in China by Hong Ge, in 313 AD, and this organism is still very less reported and falls in the category of neglected diseases to date [18]. It was strived to gather the historical evidence from 313 AD to till date (Fig. 1).

Taxonomy and Classification

The genus *Orientia* belongs to the family Rickettsiaceae, order Rickettsiales and phylum Proteobacteria [19]. There are different strains of the bacteria due to the presence of variable membrane protein which includes Shimokoshi, TA763, Gilliam, Kawasaki, TA716, Boryong, Saitama, Ikeda, Kato, Karp, TA686, and Kuroki. This gram-negative bacterium is rod in shape and shows pleomorphism [10]. *O. tsutsugamushi* is obligate intracellular rickettsia which is classified as a biosafety level-3 (BSL-3) pathogen [20]. It is the causative agent of an acute febrile illness named Scrub typhus. The most neglected disease, as claimed by World Health Organization (WHO), is scrub typhus which requires hospitalization.

Transmission

The name 'tsutsugamushi' was originated from the Japanese word 'tsutsuga' which means illness and 'mushi' means insect, thus tsutsugamushi refers an illness caused due to an arthropod [21]. *O. tsutsugamushi* is transmitted by an arthropod named trombiculid mites (Acarina: Trombiculidae) commonly called as chiggers, when their larval mites feeds on human [22]. The carrier and main reservoir of *Orientia* species is mites. The only stage which is ecto-parasitic and

Fig.1 History and discoveries in scrub typhus

feeds on serum exudates is the larvae of trombiculid mites, while all other active stages are free-living [22]. *O. tsutsugamushi* were transmitted and maintained in environment via vertical transmission, that is, infected adults to their larvae (Fig. S1); a study showed that horizontal transmission is also possible when an uninfected larva co-feeds with naturally infected larvae. This may explain the occurrence of many strains in individual larvae [23].

Life Cycle

Mites remain infected by *O. tsutsugamushi* in their different stages of life cycle (larva, nymph, adult, and egg) [24]. Through transovarial transmission female pass *O. tsutsugamushi* to their offspring via eggs and through transstadial transmission, the pathogen in the vector passes from one life stage to the next, i.e., from mite larva to nymph and nymph

to adult (Fig. S1) [25]. Larva mites are the only parasitic stage; humans are the accidental and are dead-end host [26]. The incubation period of *O. tsutsugamushi* is approximately 5 to 14 days.

Symptoms and Complications

Infected *Leptotrombidium* mites bite to human thereafter infection start to manifest. Initial symptoms of scrub typhus include development of eschar (dark dry scab) at the bite site followed by fever, headache, myalgia, cough, generalized lymphadenopathy (swollen lymph nodes), nausea, vomiting, and abdominal pain (Fig. S2) [24]. Maximum percentage of scrub typhus patients share a common symptoms of fever acute undifferentiated febrile illness (AUFI) and headache followed by several chronic complications such as multi-organ failure occur in some case which includes jaundice, acute renal failure, pneumonitis, acute respiratory distress syndrome (ARDS), myocarditis (inflammed heart), septic shock, meningoencephalitis (inflammed brain), coagulopathy, pericarditis (swollen pericardium), and disseminated intravascular coagulation (DIC) (Fig. S2) [12]

Prior to understanding the adaptation strategy of pathogen, it is crucial to understand the cell biology of *O. tsutsugamushi*.

Components of Bacteria and Its Unique Features

Cell Wall

The cell wall of this intracellular pathogen does not completely match with either gram-positive bacteria or gram-negative bacteria. A recent study done by Atwal et al. indicated that *O. tsutsugamushi* lack the set of genes which encode for Lipopolysaccharides (LPS), while this group was able to identify the set of genes which were expressing peptidoglycan-like structure (Fig. S3) [27]. This component is crucial for bacterial growth, host cell invasion, and cell integrity [28]. The cell wall of *Orientia* is dynamic and different from truly gram-positive bacteria which are sensitive to several antibiotics targeting the cell wall. The dynamism of cell wall also help the pathogen to escape and is helpful in adaptation as intracellular pathogen [28]. An unrelated group of bacteria named *chlamydiae* shows similarity in a conserved gene set (murA-G) responsible for peptidoglycan synthesis. Gene murA-G regulates the shape, elongation, division, and sporulation. Another protein Class B penicillin-binding proteins (PBPs) responsible for peptidoglycan transpeptidase activity are also conserved in both *Orientia* and *Chlamydiae* [9].

Membrane Proteins

As the part of dynamic adaptation and smart invasion inside the host cell is governed by membrane proteins. The outer and inner membrane have plenty of specific proteins like Type-specific antigen (TSA56, TSA22, TSA47), Surface cell antigen (ScaA-F), htrA, and secretion systems which modulate host cell immune system for successful establishment and spread of intracellular pathogen *O. tsutsugamushi* [29].

Genomic Feature

The whole-genome sequencing of *O. tsutsugamushi* has been completed in the past. The information of sequenced genome of different strains is given in Table 1. Even many strains of *O. tsutsugamushi* genome have been sequenced, but in this study we are taking Ikeda strain as a model system for biology and pathogenesis of *O. tsutsugamushi* [30]. The complete genome sequence of Ikeda strain was done by Nakayama et al. and major portion of the genome consists of Coding sequences (CDSs-1967) following Transposable elements (619), *O. tsutsugamushi* amplified genetic elements (OtAGEs-185), Short repeats (165), tRNA genes (34), r-RNA genes (3), and other RNA genes (1) (Fig. 2).

Ikeda strain has a single circular chromosome of 2,008,987 bp and having the coding sequence with number 1967 [31]. In this strain, no plasmid/prophage was reported, the protein-coding genes (1967ssCDSs) are higher compared to other member of Rickettsiales, and the second peculiar feature of its genome is repeated genes of 85 families (OtRG 1–85). In *O. tsutsugamushi,* several reports indicated that gene loss was at a high level, but enormous amplification of mobile elements induced in-depth genome shuffling. This resulted in origin of large number of repeated genes, and Nakayama et al. also reported 18 types of repeated sequence

Table 1 Sequencing and genome information of *O. tsutsugamushi* strains

Name of strain *O. tsutsugamushi*	Year	Genome length (bp)	CDSs	Assembly number
Ikeda	2008	2,008,987	1967	GCA_000010205.1
Boryong	2007	2,127,051	1638	GCA_000063545.1
Karp	2022	2,469,803	1951	GCA_022936085.1
Kato	2018	2,319,449	1809	GCA_900327265.1
Gilliam	2018	2,465,012	1886	GCA_900327245.1
TA686	2018	2,254,485	2546	GCA_900377405.1
TA763	2018	2,089,396	1750	GCA_900327225.1
FPW1038	2018	2,035,338	1667	GCA_900327215.1
UT76	2018	2,078,193	1665	GCA_900327255.1
UTI76	2018	1,932,116	1492	GCA_900327235.1
AFSC 4	2016	1,295,323	1038	GCA_001654795.1

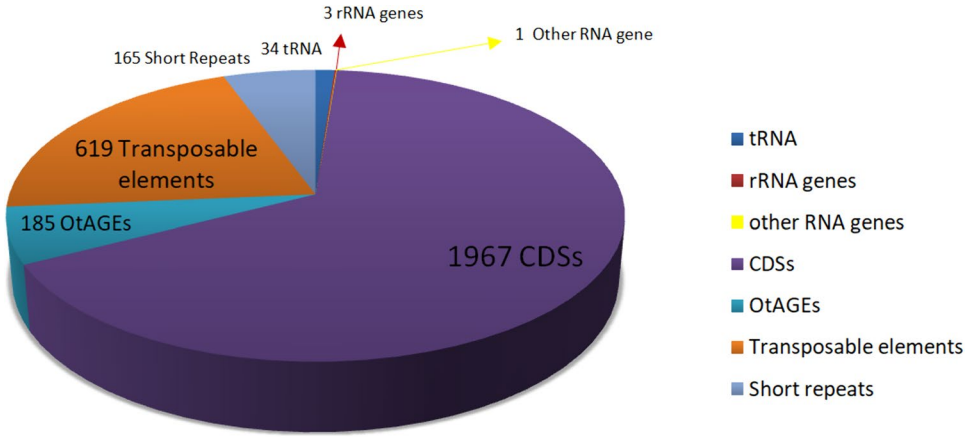

Fig. 2 Peculiar features of whole genome of Ikeda strain of *O. tsutsugamushi*

in *O. tsutsugamushi* genome which have exclusively amplified and scattered all around the genome; all these events allowed this bacterium a unique genome evolution. Poor colinearity of *O. tsutsugamushi* genome to any of the rickettsial genome is another peculiar feature. There are many genes reported which do not belong to any of the repeated gene families are referred as Singleton genes [30].

Secretion System

Many intracellular bacterial pathogens have a protein complex on cell membrane referred to as Secretion system. Bacteria translocate various micro- and macromolecules to designate sub-cellular locations via secretion system which modulate host cell processes; secretion systems are classified from Type I to Type VI in gram-negative bacteria, which deliver a particular group of proteins; as per available report, different strains of *O. tsutsugamushi* utilize Type I and Type IV secretion system frequently [29].

Type 1 Secretion System (T1SS)

Type I secretion systems (T1SS) reported to secrete various protein/enzymes (namely adhesins, proteins, lipases, proteases, or pore-forming toxins) in the unfolded state, T1SS translocate proteins in a single step due to its following components: (a) Plasma membrane harbors ABC transporter which recognizes and translocates substrate. (b) Membrane fusion protein (MFP) is the linker protein connecting outer membrane and inner membrane. (c) Outer membrane protein (OMP) forms a channel and opens after substrate recognition and transport from ABC transporter, a 33 residue ankyrin repeat is the substrate of T1SS, expressed during infection [29]. Ank repertoires containing protein (Anks) are reported as crucial virulent factor of *O. tsutsugamushi* [31]. Anks have binding diversity via two reasons: (i) variation of Ank repeats and (ii) high rate of degeneracy of amino acids. Most *O. tsutsugamushi* Anks also carry an F-box domain that is capable of interacting with SKP1 of the SCF1 ubiquitin ligase complex [32], which normally functions in eukaryotic cells to tag proteins with ubiquitin for degradation by the 26 s proteasome degradation pathway.

Ikeda strain of *O. tsutsugamushi* genome has 47 Ank genes (one of the highest number of *O. tsutsugamushi* strains) [33]. The Ank ORF is crucial for modulation of host cellular processes. To initiate the modulation, Anks translocated from pathogen to host cell.

Type 4 Secretion System (TFSS)

However, conjugation systems are rarely reported in intracellular bacteria, but *O. tsutsugamushi* genome is exceptional with 359 *tra* genes responsible for conjugative TFSS [34]. Conjugative TFSS mediate the transport of DNA among bacteria and transport several effector proteins into the host for successful initiation of infection [35]. TFSS *tra* genes are arranged into 24 fragmented repeat clusters. In Boryong strain of *O. tsutsugamushi*, there are reports of having 27 TPR (Tetratricopeptide repeats) and 50 ankyrin repeat proteins, among them several proteins reported to mediate DNA and protein interaction with host cell. Amid sequenced Rickettsiales, *O. tsutsugamushi* is the only sequenced species characterized by having full-length spotT/relA gene with both synthase and hydrolase catalytic residues [34].

Target Cells of *O. tsutsugamushi*

The target cells of *O. tsutsugamushi* can be classified as non-phagocytic and phagocytic cells, in which the former comprises endothelial and fibroblast cells and latter comprises phagocytic macrophages, polymorphonuclear leukocytes (PMNs), and dendritic cells in vitro and in vivo [36].

Adaptive Strategies of Host Cell by Generating Immune Response After ST Infection

After infection, Dendritic cells (DCs) play a key role for initializing antigen-specific immune response. DCs are one of the major antigen-presenting cells and play a crucial role to connect innate and adaptive immune response. Maturation of DCs is a multistep process, namely antigen uptake, migration, expression of co-stimulatory molecules on cell surface, and through the secretion of cytokine and chemokine [37].

Just after infection of *O. tsutsugamushi*, maturation of monocyte-derived DCs are induced which can be measured by enhanced expression of CD80, CD83, CD86, and MHC class I molecules; after induction, DCs show an increased level of IL-12p70, TNF-α, IL-6, and IL-8. Matured DCs interact with T cells and production of interferon (IFN γ) is stimulated. On one hand, the protective immunity against intracellular pathogen is provided by IFN γ [38]; on the other hand, IFN γ is also responsible to activate the production of reactive oxygen species in macrophage to kill the intracellular pathogen [39]. As the part of adaptive strategy of host to control the severity and antigen-specific immune response against *O. tsutsugamushi* several cytokines, for example, IL-6 and IL-8 activate lymphocytes and are responsible for neutrophil migration. IL-12 was reported to induce differentiation of T_H cells [40]. After phagocytosed by DCs, intracellular pathogen (*Shigella flexneri* [41] and *Listeria monocytogenes* [42]) are reported to enter in a degradation pathway (phagolysis). Just after phagolysis, major histocompatibility complex (MHC) presentation is done by antigenic short peptide which in turn activate T cells [43]. However, *O. tsutsugamushi* is reported to overcome the phagolysis mechanism which is different from *Shigella flexneri* and *Listeria monocytogenes* [38].

Infection Mechanism in Target Cell by *O. tsutsugamushi*

Attachment of O. tsutsugamushi to Host Cell

To establish a successful infection and growth of *O. tsutsugamushi* inside the cell requires efficient invasion of host cell. Intracellular invasion mechanism is controlled by signal transduction events which are complex and have not been clearly described. However, this review tries to compile up the molecular mechanism of invasion based on available literature till date. Cho et al. demonstrated that *O. tsutsugamushi* utilizes host integrin signaling pathways to mediate actin cytoskeleton rearrangement for entry into non-phagocytic host cell [18].

To initiate the attachment to the host cell *O. tsutsugamushi* utilizes TSA56-Fibronectin complex to interact with host cell integrin α5β1 (Fig. 3) [18]. The formation of

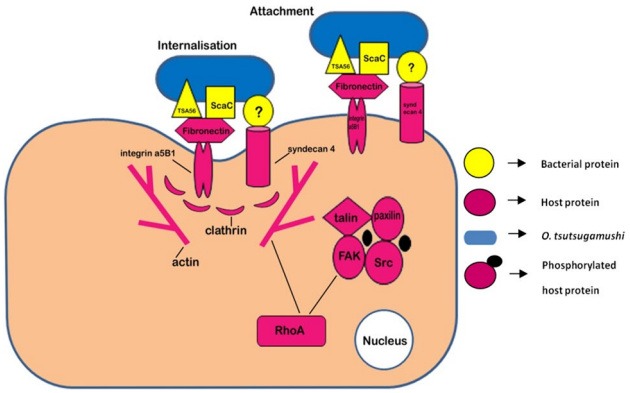

Fig. 3 Molecular mechanism of attachment and internalization involving bacterial protein TSA56, ScaC, and host protein Fibronectin-integrin complex and Clathrin protein

TSA56-Fibronectin and integrin complex induces activation of signaling molecules at the inner surface of cytoplasmic membrane [44]. The signaling molecule are non-receptor tyrosine kinase, namely FAK and Src family, which is responsible for the focal adhesion, FAK is a 125 kDa protein regarded as key player in integrin mediated signaling [45]. Just after FAK activation, RhoA GTPase becomes active and mediates rearrangement of actin cytoskeleton as well as promotes bacterial uptake [18]. *O. tsutsugamushi* invade into the host cell via a zipper-like mechanism which is mediated by Clathrin protein [9]

Evolutionary Strategy Adapted by *O. tsutsugamushi* to Actively Escape from Host Cell Autophagy

Autophagy, an evolutionarily conserved catabolic mechanism, is regulated intracellularly to degrade the cytosolic components, like misfolded protein aggregates and defaced organelles, in a lysosome-dependent manner [46]. ATGs, a highly conserved autophagy-related gene, regulate autophagy. Immune cells specifically adapted an autonomous effector mechanism of innate immunity for degradation of microorganisms invading intracellularly through autophagy. Several escape mechanisms were acquired by intracellular pathogens by blocking host autophagic defense mechanism or altering host autophagic response. This can be achieved by different mechanisms such as (a) antagonizing autophagy initiation or auto-phagosomal maturation, (b) escaping autophagic recognition, and (c) using host autophagy component for their own survival [47]. On the other way, host autophagy generates nutrient which is utilized by intracellular pathogen for survival and growth [47]. There are reports that endolysosomal pathway may be utilized as a protective intracellular niche by intracellular pathogen [29].

In the case of *O. tsutsugamushi*, several studies indicated that this bacterium activates cellular autophagy, but at the same time it evades cellular autophagic system without fusing the lysosome (Fig. 4) [48]. In *O. tsutsugamushi*-infected polymorphonuclear leukocyte (PMNs), more autophagosomes are found, within 1-h post-infection (hpi) [49]. Just after entering the host cell early endosome is formed. After some time, late endosome is formed and before fusion of the lysosome it escapes from autophagy mechanism of host cell (Fig. 4); this mechanism is not fully understood. When *O. tsutsugamushi* was cultured in L929 cells and a hemolysin gene, *tly*C, encodes phospholipase D protein [50]. This protein may disrupt the phagosomal membrane and allow the pathogen to discharge into the host cell's cytoplasm [51]. *O. tsutsugamushi* now moves toward perinuclear region via microtubule mediated trafficking [52]. At perinuclear region in polysaccharide matrix bacterial replication takes place [9].

Adapted Strategies of *O. tsutsugamushi* Inside the Host Cell (Hijacking the Host Cell 26 s-Proteasomal Degradation Machinery)

O. tsutsugamushi, an obligate auxotroph, relies on the host cell for amino acid (histidine and aromatic amino acid) [53]. During initial stage of invasion (24–48 h) minimal growth of *O. tsutsugamushi* is reported, after that it leads to log phase [54]. Requirement of amino acid was found less in initial phase (24 h). The demand of amino acids gets increased when the pathogen is ready for optimal growth (48 h). The pathogen reported to modulate host cellular processes to support its exponential growth [55].

An unfolded protein response (UPR) is evoked due to the accumulation of misfolded protein-guided stress of endoplasmic reticulum (ER) [55]. The UPR is regarded as a protective cellular pathway and reported as evolutionarily conserved. It relieves the stress of ER via inhibition of translation, enhancing protein folding capacity of ER and facilitates endoplasmic reticulum associated degradation (ERAD) (Fig. 5) [56]. ER recognize and transport misfolded newly synthesized protein to the 26 s-proteasome for degradation [57]. With the help of 26 s-proteasome unfolded peptides are degraded into amino acids via aminopeptidase enzyme [58]. As per available reports, viruses and some intracellular bacteria (*O. tsutsugamushi*) either induce or inhibit the UPR as per their requirement [56]. Optimum expression of Ank4 was reported during the 24 h for UPR induction [55]. *O. tsutsugamushi* effector Ank4 protein is linked to induce the UPR and inhibit ERAD during 24–48 h of infection. After expression Ank4 binds with ERAD chaperon Bat3 to inhibit

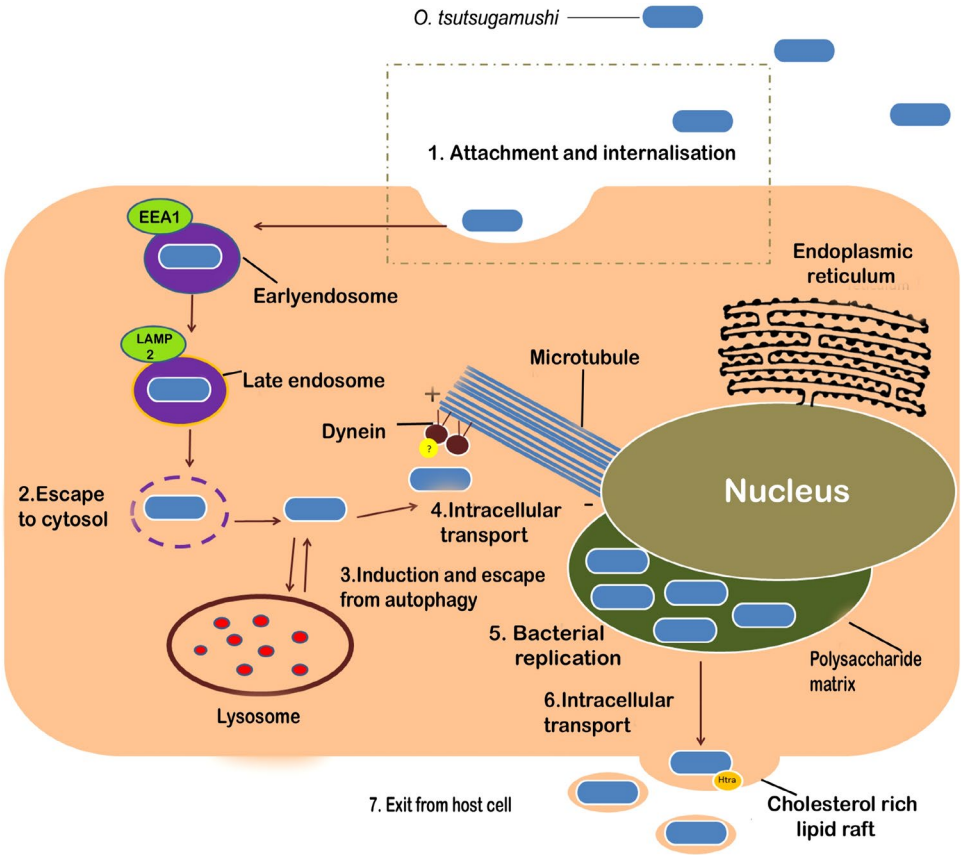

Fig. 4 Schematic overview of escaping and budding out mechanism of *O. tsutsugamushi* in host cell involves several steps. (1) Attachment and internalization, (2) Escape to cytosol, (3) Induction and escape from autophagy, (4) Intracellular transport, (5) Bacterial replication, (6) Intracellular transport toward the cell wall, (7) Exit from the host cell

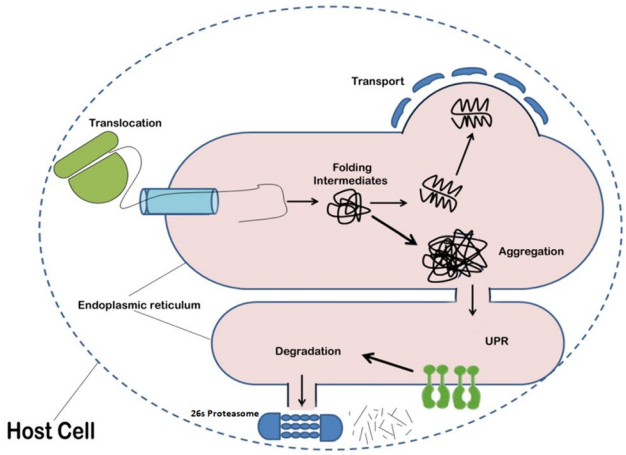

Fig. 5 ERAD pathway for degradation of misfolded ER proteins through 26 s-proteasomal machinery via **a** translocation, **b** aggregation, and **c** degradation

ERAD and accumulate the huge number of misfolded proteins until 72 h. After that, *O. tsutsugamushi* utilizes ERAD-derived amino acids to benefit its replication [55]

Camouflaging and Spreading of Infection

Every host cell has a carrying capacity of pathogens for its survival. After achieving the carrying capacity, every intracellular pathogen needs to exit from the infected cell. Intracellular pathogen has adapted several mechanisms to exit infected host cell as follows: (a) Lysis of host cell, e.g., Plasmodium falciparum and Chlamydia spp. (b) Efflux of vacuole (harboring bacteria) (Cryptococcus neoformans), and (c) Actin-mediated protuberance into adjacent cells (e.g., Shigella flexneri, Listeria monocytogenes, Rickettsia rickettsii) [52]. However, O. tsutsugamushi has adapted a unique mechanism to exit the host cell by utilizing an unusual budding out mechanism (bacteria encased via host cell membrane) [59]. This encasing enables infection of newly adjacent cells exempting extreme cellular environment and remain hidden from the host immune cells by camouflaging. The utilized host cell membrane for bacterial envelope are specialized membrane micro-domains referred as lipid raft [60]. Lipid rafts are rich in glycosylphosphatidylinositol (GPI)-linked molecules, cholesterol, and glycosphingolipids and contain a 22-kDa protein, caveolin-1 [61]. Several reports indicated that lipid raft bacteria interactions may be initial event for bacterial entry [62, 63], but in O. tsutsugamushi, disruption of lipid rafts has no significant effect on entry into host cells [60]. The encasing process depends on a bacterial surface antigen htrA, a 47-kDa protein, which binds some proteins of the lipid raft and this binding of htrA and lipid raft protein initiate the exit of bacterium via budding (Fig. 4) [60].

Antibiotics and Their Susceptibility for the Treatment of Scrub Typhus

The disease scrub typhus can vary from asymptomatic [64] to lethal but it mainly causes severe febrile illness. For the treatment of scrub typhus, the first effective treatment was introduced in the form of antibiotic chloramphenicol [65]. Later tetracycline group of antibiotics were introduced and found comparatively more effective than existing ones. Among tetracyclines, the antibiotic doxycycline was reported as drug of choice till date [66]. Antibiotics of class Tetracycline, principally doxycycline, is equally effective. Several other antibiotics were used for the treatment of scrub typhus. The list of antibiotics currently recommended to treat scrub typhus includes the following (Table 2).

Antibiotics: Resistance and Challenges in Developing New Antibiotics

As earlier it was said that only "survival of the fittest" evolved & evolved generation will dominate [67]. After the invention of antibiotics, the diseases caused by bacteria were regarded as diseases of the past. But the same time, bacteria also develop many mechanism to withstand and multiply in the presence of antibiotics [68]. Only a few groups of antibiotics are effective against *O. tsutsugamushi*, but bacteria started to show less susceptibility toward the present treatment options. The resistance in fluoroquinolones and less susceptibility reports toward doxycycline and chloramphenicol presented a pressure on macrolides group of antibiotics (azithromycin). In the current scenario, it is crucial to understand the reason behind the less susceptibility of antibiotics. There may be many reasons reported till date, among them one of the latest report [69] suggests that the

Table 2 Antibiotics used for treatment of scrub typhus

S.No	Name of the drugs	Class of the drugs	Mode of action	Status	References
1	Doxycycline	Tetracycline	Protein synthesis- 30 s subunit	In clinical use	[10, 66]
2	Azithromycin	Macrolides	Protein synthesis- 50 s subunit	In clinical use	[14, 16]
3	Chloramphenicol	Amphenicols	Protein synthesis- 50 s subunit	In clinical use	[14, 15]
4	Rifampicin	Ansamycin	RNA transcription- DNA-dependent RNA polymerase	In clinical use	[14, 17]

concentration required to kill the bacteria is not reaching to the pathogen. If the right drug will not be prescribed for the right time, then there is no life for the existing antibiotic and in this race, it is evident that bacteria adapted smartly than the human being.

From the golden age of antibiotics till date, several drug-resistant bacteria (Methicillin-resistant *Staphylococcus aureus* (MRSA), Multidrug-resistant *Mycobacterium tuberculosis* (MDRTB), *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species (ESKAPE), and DIRTY DOZEN) have been developed [70]. Initially, MRSA and MDRTB directed the discovery and development of antibiotics [70]. After development of resistance in ESKAPE pathogens, the direction of discovery of antibiotics retracted toward these pathogens [71]. In 2017, World health Organization (WHO) again emphasized the need of new antibiotics against dirty dozen [71].

In this time duration, the neglected pathogens *O. tsutsugamushi* started to develop resistance toward the available antibiotics [72]. This bacteria is not only neglected in terms of scientific discovery but also in the field of drug discovery [69]. Doxycycline, chloramphenicol, fluoroquinolone, and rifampicin were the drugs which became choice of treatment in due time [17]. The first less susceptibility was reported in 1995 against Doxycycline [16]. From 1995 to till date, *O. tsutsugamushi* acquired resistance/less susceptibility toward other available drugs except azithromycin (Table 3) [73].

As per report [74], there are unique and highly variable antibiotic resistance loci present in *O. tsutsugamushi* genomes in comparison with other rickettsial species. As earlier said, the genome of *O. tsutsugamushi* is highly repetitive. It possesses 2179 potential protein-coding loci. Among these, the number of presumed antibiotic-resistant loci is very low. Bacteria encode CRISPR-like elements that is more than 400 transposes, 60 phage integrases, and 70 reverse transcriptases in the major part of their protein-coding loci, explaining their capability to modify their genome under selection [34]. The *O. tsutsugamushi* was known to have high antigenic diversity. In India, for instance, Kato-like strains predominate (61.5%), followed by Karp-like strains (27.7%) and Gilliam and Ikeda strains [75]. In *O. tsutsugamushi*, genetic recombination occurs among diverse genotypes. The significant diversity and heterogeneity of putative antibiotic-resistant loci help to acquire antibiotic resistance under selection pressure and act as a challenge in developing new antibiotics.

Drug resistance in *O. tsutsugamushi* developed via horizontal gene transfer. *O. tsutsugamushi* genomes carry 359 *tra* genes which code integral components of conjugative type IV secretion system through which resistance genes are transferred horizontally. *abaF*, an efflux pump gene gets mutated due to antibiotic resistance and expressed actively. Another gene *gyrA* responsible for DNA gyrase enzyme gets mutated and becomes resistant to quinolones. [74]

New Approach for Effective Treatment and Drug in Pipeline

For treatment of scrub typhus, combination of antibiotics now became an attractive strategy to overcome the resistance. Combination therapy presents the best alternative to monotherapy due to the effectiveness and delay in development of resistance. A recent study by Varghese et al. reported the combination of doxycycline and azithromycin showed better results than giving alone [83]. The mode of actions of both the antibiotics are different, azithromycin binds the 23SrRNA of the 50S ribosomal subunit at the polypeptide exit tunnel, and doxycycline prevents aminoacyl-tRNA binding to the 30S ribosomal subunit, which results in complete blockage of protein synthesis with a consequently greater effect against O. tsutsugamushi [83].

A natural antibiotic Corallopyronin A isolated from an environmental soil bacteria named as Corallococcus coralloides is a new, highly effective agent for treating scrub typhus. Corallopyronin A (CorA) is a myxobacterial α-pyrone antibiotic which includes two side chains. Corallopyronin A was isolated from environmental bacteria and targets a bacterial enzyme called RNA polymerase. In preclinical trials, even low doses of the new antibiotic have

Table 3 Antibiotics showing less susceptibility or resistance

Year	Strain	Country/ region	Antibiotic resistance or low susceptibility	References
1995	AFSC-4	Thailand, Kanchanaburi	Doxycycline	[16]
1996	C3, C27	Thailand, Chiangrai	Chloramphenicol and Doxycycline	[76]
2003	NA	India, Tamil Nadu	Chloramphenicol, Doxycycline, and Ciprofloxacin	[77]
2009	AFSC-7	USA	NA	[78]
2010	Kato	Lao	Ciprofloxacin	[79]
2013	AFSC-4, AFSC-5	Korea	Doxycycline	[80]
2014	NA	Korea, Busan	Doxycycline	[81]
2016	NA	Thailand, Nakhon Ratchasima	Doxycycline, levofloxacin	[82]

proven to be very effective against *Orientia tsutsugamushi* [84].

Alternatives of Antibiotics

At present, antibiotics are the major weapon to treat the Scrub typhus, but development of less susceptibility/resistance against available antibiotics put our generation in dark [85]. This compelled us to think for the drugs beyond antibiotics and natural products became need of the hour for treating this neglected disease [86]. Nature and natural products always become the major supporter and guide for the humans being in the drug discovery [87]. Secondary metabolites of plants became the wonder drug antibiotics and became the major life-saving agent [14]. Plants secondary metabolites became one of the major source of new drugs or their molecules/compounds became the blueprint for the development of novel drugs [86]. As per report of WHO, maximum population depends on the natural products for their primary treatment [88]. Historically Indian and Chinese medicines are based on natural products. Several anti-parasitic and anti-cancerous drugs were developed from plants [89]. Several plants secondary metabolites like alkaloids, organosulfur compounds, terpenoids, and flavonoids reported to have antimicrobial activities for several bacterial pathogens [86]. In the era of drug resistance, these plants' secondary metabolites are being observed as promising alternatives of antibiotics (Table 4).

Saponins, allicin, apigenin, eugenol, curcumin, piperine, gallic acid, kaempferol, etc. are the plant secondary metabolites which are used in clinical trials for the discovery and development as anti-infectives, but these compounds need to be repurposed as antibacterials for the treatment of Scrub typhus [86, 91–97]. The potential challenges for drug development of these compounds are as follows:

1 In vitro antibacterial potential is found good but in preclinical and clinical stage maximum compounds do not qualify due to less efficacy and toxicity.
2 The high cost and long time for the drug development.
3 Secondary metabolite concentration varies from place to place and its industrial production affects secondary metabolite concentration.

Multiple secondary plant metabolites have been reported to be used for the treatment of MDR pathogens. But these

Table 4 Antibacterial agents from plants

Antibacterial products from natural sources	Origin	MDR pathogens	Possible mode of action	References
Laricresinol & berberine	*Zingiber officinale*	*Salmonella typhimurium*	Efflux pump inhibitor	[90]
Saponins & bromo-polyphenols	*Cassia fistula*	*E. coli & MTB*	RNA Polymerase inhibitor	[91]
Flavonoids & polyphenols + Phenylalanine β-naphthylamide	*Vernonia auriculifera*	*E. coli, E. aerogenes, K. pneumonia, P. aeruginosa, S. aureus*	Efflux pump inhibitor	[92]
Phytochemical + antibiotics	*Terminalia bellirica*	MRPA	Reduction of Quorum sensing regulated virulence factor & biofilm formation	[92]
Gallic Acid	*Phyllanthus emblica*	*E. coli*	Efflux pump inhibition/modulation	[93]
Allicin	*Allium spp.*	*P. aeruginosa*	DNA and protein synthesis inhibitor	[94]
Genistein	*Glycine max*	*S. aureus*	Efflux pump inhibition	[86]
Thymol	*Thymus capitatus, Thymus vulgaris*	*E. coli*	Cell membrane disturbance	[86]
Quercetin	*Capparis spinosa*	*E. coli*	Efflux pump inhibition	[86]
Kaempferol	*Moringa oleifera, Sambucus nigra, Aloe vera*	*S. aureus, MRSA*	Efflux pump inhibitor	[95]
Osthole	*Cnidium monnieri*	*Klebsiella pneumoniae*	DNA gyrase inhibitor	[96]
Piperine	*Piper nigrum*	*Enterococcus faecalis*	Efflux pump inhibitor	[97]
Apigenin	*Polymnia fruticosa*	*Helicobacter pylorii*	Type II Fatty acid biosynthesis inhibitor	[86]
Eugenol	*Syzygium aromaticum*	*Helicobacter pylorii*	Cell membrane disturbance	[86]
Piperine	*Piper nigrum*	*Mycobacterium tuberculosis*	Efflux pump inhibition	[86]
Allicin	*Allium sativum*	*Streptococcus pneumoniae*	DNA and protein synthesis inhibitor	[94]

researches are in infancy stage; similarly, Ayurveda recommends different plants (Veratrum, Belladonna, Gelsemium, Hyoscyamus, *Dostemia contrayerba*, *Melissa officinalis*) for the treatment of Scrub typhus [98]. These plants are reported to cure high-grade fever, sepsis, & complications of respiratory & nervous system.

Vaccine Development and Challenges Associated with it

Over the past 80 years, despite of many attempts to develop vaccine against *O. tsutsugamushi*, none of the attempts resulted in effective vaccine. To develop vaccines, many approaches were used, namely (1) cotton rat's lungs infected with *O. tsutsugamushi* that were formalin-fixed homogenized, (2) formalin-killed *O. tsutsugamushi*, (3) live strain of *O. tsutsugamushi* with low virulence and inoculation of live virulent strain followed by antibiotic treatment, and (4) live irradiated *O. tsutsugamushi* and its recombinant fusion of 56-kDa and 47-kDa proteins [99]

The above vaccines have their own short comings either it may provide strong protection against homologous challenge but weak protection against heterologous challenge or it may provide active immunity by sustaining antibodies for only a short duration which diminishes through time [100]. In humans, homologous immunity lasts up to 3.5 years but heterologous immunity lasts only a few months and humans can get infected by multiple strains simultaneously [99]. The lack of knowledge for a common antigen for most of *O. tsutsugamushi* strains which can stimulate both cell-mediated and humoral immunity is the main issue in developing an effective clinical vaccine.

Future

In view of the novel cell biology of *O. tsutsugamushi*, there is need of further studies for deeper understanding of role of intracellular events, such as autophagy in scrub typhus pathogenesis. Further, investigations are needed to understand the role of biphasic metabolic differences between intracellular and extracellular bacterial stages in Orientia infection. *O. tsutsugamushi* has been reported to cause chronic latent infection, but the mechanism needs to be investigated in detail. However, there is limited knowledge of immune invasion strategies utilized by Orientia. Hence, a combined approach will be needed to understand the molecular, cellular, host–cell interaction, pathogenesis, genomic diversity, etc. Moreover, there are mixed reports of antibiotic susceptibility/resistance to available antibiotics, this needs to address precisely. There are limited antibiotics available to treat scrub typhus and there are also reports of antibiotic susceptibility/resistance to available antibiotics in Orientia. Thus, this is the need of hour to identify new antibiotics, repurposing of available drugs, and identification of novel drug targets to treat scrub typhus.

Summary

The earliest life forms (prokaryotes) are omnipresent and flourish in any type of environment, while evolutionarily evolved eukaryotes would die. This indicates that during the time of evolution robust environmental conditions make prokaryotes more resilient. *O. tsutsugamushi* adapted several mechanisms to become a model intracellular organism. Its unique cellular components make it a vibrant pathogen to evade the host cell machinery for chronic infection. At present, *O. tsutsugamushi* is not limited to the *tsutsugamushi* triangle but started to emerge different parts of the world. Less susceptibility toward available antibiotics makes it important to bring *O. tsutsugamushi* outside the neglected disease category. The discovery and development of novel multiplex subunit vaccines and broad-spectrum antibiotics will be the future research priority to overcome the acute and chronic infections caused by *O. tsutsugamushi*.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00284-024-03754-1.

Acknowledgements The authors are thankful to Director, ICMR-RMRC, Gorakhpur for providing conducive work environment.

Author Contributions G.R.D. conceived the concept and design the manuscript. P.S., A.S., R.S., R.K., N.M., and S.P.B. wrote the manuscript. G.R.D., P.S., and D.K.Y. critically analyzed and finalized the manuscript.

Funding P.S. is grateful to UGC for JRF fellowship. Authors are also grateful to DHR, New Delhi for funding VRDL team (File No.R.15012/39/2021-HR-VRDL).

References

1. Brockhurst MA, Chapman T, King KC, Mank JE, Paterson S, Hurst GDD (2014) Running with the red queen: the role of biotic conflicts in evolution. Proc R Soc B 281:20141382. https://doi.org/10.1098/rspb.2014.1382
2. Medeiros LP, Garcia G, Thompson JN, Guimarães PR (2018) The geographic mosaic of coevolution in mutualistic networks. Proc Natl Acad Sci USA 115:12017–12022. https://doi.org/10.1073/pnas.1809088115
3. Lenski RE (2017) What is adaptation by natural selection? Perspectives of an experimental microbiologist. PLoS Genet 13:e1006668. https://doi.org/10.1371/journal.pgen.1006668
4. Sistrunk JR, Nickerson KP, Chanin RB, Rasko DA, Faherty CS (2016) Survival of the fittest: how bacterial pathogens utilize bile to enhance infection. Clin Microbiol Rev 29:819–836. https://doi.org/10.1128/CMR.00031-16
5. Chodasewicz K (2014) Evolution, reproduction and definition of life. Theory Biosci 133:39–45. https://doi.org/10.1007/s12064-013-0184-5

6. Burman LG (1980) Influence of antimicrobial agents on host-parasite interactions. Scand J Infect Dis Suppl. https://doi.org/10.3109/inf.1980.12.suppl-24.01
7. Penesyan A, Gillings M, Paulsen I (2015) Antibiotic discovery: combatting bacterial resistance in cells and in biofilm communities. Molecules 20:5286–5298. https://doi.org/10.3390/molecules20045286
8. Bourdonnay E, Henry T (2016) Catch me if you can. Elife 5:e14721. https://doi.org/10.7554/eLife.14721
9. Salje J (2017) *Orientia tsutsugamushi*: a neglected but fascinating obligate intracellular bacterial pathogen. PLoS Pathog 13:e1006657. https://doi.org/10.1371/journal.ppat.1006657
10. Wongsantichon J, Jaiyen Y, Dittrich S, Salje J (2020) *Orientia tsutsugamushi*. Trends Microbiol 28:780–781. https://doi.org/10.1016/j.tim.2020.02.014
11. Jiang J, Richards A (2018) Scrub typhus: no longer restricted to the *Tsutsugamushi* triangle. TropicalMed 3:11. https://doi.org/10.3390/tropicalmed3010011
12. Kelly DJ, Fuerst PA, Ching W, Richards AL (2009) Scrub typhus: the geographic distribution of phenotypic and genotypic variants of *Orientia tsutsugamushi*. Clin Infect Dis 48:S203–S230. https://doi.org/10.1086/596576
13. Zaman K (2023) Scrub typhus, a salient threat: needs attention. PLoS Negl Trop Dis 17:e0011427. https://doi.org/10.1371/journal.pntd.0011427
14. Hutchings MI, Truman AW, Wilkinson B (2019) Antibiotics: past, present and future. Curr Opin Microbiol 51:72–80. https://doi.org/10.1016/j.mib.2019.10.008
15. Lee M, Kim J, Jo DS (2017) Effects of clarithromycin treatment in scrub typhus in children: comparison with chloramphenicol and azithromycin. Korean J Pediatr 60:124. https://doi.org/10.3345/kjp.2017.60.4.124
16. Strickman D, Sheer T, Salata K, Hershey J, Dasch G, Kelly D, Kuschner R (1995) In vitro effectiveness of azithromycin against doxycycline-resistant and—susceptible strains of *Rickettsia tsutsugamushi*, etiologic agent of scrub typhus. Antimicrob Agents Chemother 39:2406–2410. https://doi.org/10.1128/AAC.39.11.2406
17. Watt G, Kantipong P, Jongsakul K, Watcharapichat P, Phulsuksombati D, Strickman D (2000) Doxycycline and rifampicin for mild scrub-typhus infections in Northern Thailand: a randomised trial. Lancet 356:1057–1061. https://doi.org/10.1016/S0140-6736(00)02728-8
18. Cho B-A, Cho N-H, Seong S-Y, Choi M-S, Kim I-S (2010) Intracellular invasion by *Orientia tsutsugamushi* is mediated by integrin signaling and actin cytoskeleton rearrangements. Infect Immun 78:1915–1923. https://doi.org/10.1128/IAI.01316-09
19. Elliott I, Thangnimitchok N, De Cesare M, Linsuwanon P, Paris DH, Day NPJ, Newton PN, Bowden R, Batty EM (2021) Targeted capture and sequencing of *Orientia tsutsugamushi* Genomes from chiggers and humans. Infect Genet Evol 91:104818. https://doi.org/10.1016/j.meegid.2021.104818
20. Ogawa M, Ando S, Saijo M (2020) Evaluation of recombinant type-specific antigens of *Orientia tsutsugamushi* expressed by a baculovirus-insect cell system as antigens for indirect immunofluorescence assay in the serological diagnosis of scrub typhus. Jpn J Infect Dis 73:330–335. https://doi.org/10.7883/yoken.JJID.2019.334
21. Banerjee A, Kulkarni S (2021) *Orientia tsutsugamushi*: the dangerous yet neglected foe from the east. Int J Med Microbiol 311:151467. https://doi.org/10.1016/j.ijmm.2020.151467
22. Frances SP, Watcharapichat P, Phulsuksombati D, Tanskul P (2000) Transmission of *Orientia tsutsugamushi*, the aetiological agent for scrub typhus, to co-feeding mites. Parasitology 120:601–607. https://doi.org/10.1017/S0031182099005909
23. Traub R, Wisseman CL (1974) Review article*: the ecology of chigger-borne rickettsiosis (scrub typhus)1, 2. J Med Entomol 11:237–303. https://doi.org/10.1093/jmedent/11.3.237
24. Jeong YJ, Kim S, Wook YD, Lee JW, Kim K-I, Lee SH (2007) Scrub typhus: clinical, pathologic, and imaging findings. Radiographics 27:161–172. https://doi.org/10.1148/rg.271065074
25. Phasomkusolsil S, Tanskul P, Ratanatham S, Watcharapichat P, Phulsuksombati D, Frances SP, Lerdthusnee K, Linthicum KJ (2009) Transstadial and transovarial transmission of *Orientia Tsutsugamushi* in L*eptotrombidium Imphalum* and L*eptotrombidium Chiangraiensis* (acari: trombiculidae). J Med Entomol 46:1442–1445. https://doi.org/10.1603/033.046.0628
26. Lerdthusnee K, Khuntirat B, Leepitakrat W, Tanskul P, Monkanna T, Khlaimanee N, Inlao I, Kengluecha A, Mungviriya S, Chandranoi K et al (2003) Scrub typhus: vector competence of leptotrombidium chiangraiensis chiggers and transmission efficacy and isolation of *Orientia tsutsugamushi*. Ann NY Acad Sci 990:25–35. https://doi.org/10.1111/j.1749-6632.2003.tb07333.x
27. Atwal S, Giengkam S, Chaemchuen S, Dorling J, Kosaisawe N, VanNieuwenhze M, Sampattavanich S, Schumann P, Salje J (2017) Evidence for a peptidoglycan-like structure in *Orientia tsutsugamushi*. Mol Microbiol 105:440–452. https://doi.org/10.1111/mmi.13709
28. Vollmer W, Bertsche U (2008) Murein (peptidoglycan) structure, architecture and biosynthesis in *Escherichia coli*. Bi chimica et Biophysica Acta BBA—Biomembranes 1778:1714–1734. https://doi.org/10.1016/j.bbamem.2007.06.007
29. VieBrock L, Evans SM, Beyer AR, Larson CL, Beare PA, Ge H, Singh S, Rodino KG, Heinzen RA, Richards AL et al (2015) *Orientia tsutsugamushi* Ankyrin repeat-containing protein family members are type 1 secretion system substrates that traffic to the host cell endoplasmic reticulum. Front Cell Infect Microbiol. https://doi.org/10.3389/fcimb.2014.00186
30. Nakayama K, Yamashita A, Kurokawa K, Morimoto T, Ogawa M, Fukuhara M, Urakami H, Ohnishi M, Uchiyama I, Ogura Y et al (2008) The whole-genome sequencing of the obligate intracellular bacterium *Orientia tsutsugamushi* revealed massive gene amplification during reductive genome evolution. DNA Res 15:185–199. https://doi.org/10.1093/dnares/dsn011
31. Al-Khodor S, Price CT, Kalia A, Abu Kwaik Y (2010) Functional diversity of ankyrin repeats in microbial proteins. Trends Microbiol 18:132–139. https://doi.org/10.1016/j.tim.2009.11.004
32. Beyer AR, VieBrock L, Rodino KG, Miller DP, Tegels BK, Marconi RT, Carlyon JA (2015) *Orientia tsutsugamushi* strain ikeda ankyrin repeat-containing proteins recruit SCF1 ubiquitin ligase machinery via poxvirus-like F-box motifs. J Bacteriol 197:3097–3109. https://doi.org/10.1128/JB.00276-15
33. Jernigan KK, Bordenstein SR (2014) Ankyrin domains across the tree of life. PeerJ 2:e264. https://doi.org/10.7717/peerj.264
34. Cho N-H, Kim H-R, Lee J-H, Kim S-Y, Kim J, Cha S, Kim S-Y, Darby AC, Fuxelius H-H, Yin J et al (2007) The *Orientia tsutsugamushi* genome reveals massive proliferation of conjugative type IV secretion system and host-cell interaction genes. Proc Natl Acad Sci USA 104:7981–7986. https://doi.org/10.1073/pnas.0611553104
35. Cascales E, Christie PJ (2003) The versatile bacterial type IV secretion systems. Nat Rev Microbiol 1:137–149. https://doi.org/10.1038/nrmicro753
36. Tango Y, Kano R, Maruyama H, Asano K, Tanaka S, Hasegawa A, Kamata H (2010) Detection of autoantibodies against survivin in sera from cancer dogs. J Vet Med Sci 72:917–920. https://doi.org/10.1292/jvms.09-0476
37. Ho L, Shaio M, Chang D, Liao C, Lai J (2004) Infection of human dendritic cells by dengue virus activates and primes T cells towards Th0-like phenotype producing both Th1 and Th2

cytokines. Immunol Invest 33:423–437. https://doi.org/10.1081/IMM-200038680
38. Chu H (2013) *Orientia tsutsugamushi* infection induces CD4+ T cell activation via human dendritic cell activity. J Microbiol Biotechnol 23:1159–1166. https://doi.org/10.4014/jmb.1303.03019
39. Metz G, Carlier Y, Vray B (1993) *Trypanosoma cruzi* upregulates nitric oxide release by IFN-γ-preactivated macrophages, limiting cell infection independently of the respiratory burst. Parasite Immunol 15:693–699. https://doi.org/10.1111/j.1365-3024.1993.tb00584.x
40. Harada A, Sekido N, Akahoshi T, Wada T, Mukaida N, Matsushima K (1994) Essential involvement of interleukin-8 (IL-8) in acute inflammation. J Leukoc Biol 56:559–564
41. Kim DW, Chu H, Joo DH, Jang MS, Choi JH, Park S-M, Choi Y-J, Han SH, Yun C-H (2008) OspF directly attenuates the activity of extracellular signal-regulated kinase during invasion by shigella flexneri in human dendritic cells. Mol Immunol 45:3295–3301. https://doi.org/10.1016/j.molimm.2008.02.013
42. Henry CJ, Ornelles DA, Mitchell LM, Brzoza-Lewis KL, Hiltbold EM (2008) IL-12 produced by dendritic cells augments CD8+ T cell activation through the production of the chemokines CCL1 and CCL17. J Immunol 181:8576–8584. https://doi.org/10.4049/jimmunol.181.12.8576
43. Shankar AH, Titus RG (1997) The influence of antigen-presenting cell type and interferon- on priming and cytokine secretion of *Leishmania* major-specific T cells. J Infect Dis 175:151–157. https://doi.org/10.1093/infdis/175.1.151
44. Rottner K, Stradal TEB, Wehland J (2005) Bacteria-host-cell interactions at the plasma membrane: stories on actin cytoskeleton subversion. Dev Cell 9:3–17. https://doi.org/10.1016/j.devcel.2005.06.002
45. Dupuy AG, Caron E (2008) Integrin-dependent phagocytosis—spreading from microadhesion to new concepts. J Cell Sci 121:1773–1783. https://doi.org/10.1242/jcs.018036
46. Mizushima N, Yoshimori T, Levine B (2010) Methods in mammalian autophagy research. Cell 140:313–326. https://doi.org/10.1016/j.cell.2010.01.028
47. Deretic V, Levine B (2009) Autophagy, immunity, and microbial adaptations. Cell Host Microbe 5:527–549. https://doi.org/10.1016/j.chom.2009.05.016
48. Choi J-H, Cheong T-C, Ha N-Y, Ko Y, Cho C-H, Jeon J-H, So I, Kim I-K, Choi M-S, Kim I-S et al (2013) *Orientia tsutsugamushi* subverts dendritic cell functions by escaping from autophagy and impairing their migration. PLoS Negl Trop Dis 7:e1981. https://doi.org/10.1371/journal.pntd.0001981
49. Ko Y, Choi J-H, Ha N-Y, Kim I-S, Cho N-H, Choi M-S (2013) Active escape of *Orientia tsutsugamushi* from cellular autophagy. Infect Immun 81:552–559. https://doi.org/10.1128/IAI.00861-12
50. Suttinont C, Losuwanaluk K, Niwatayakul K, Hoontrakul S, Intaranongpai W, Silpasakorn S, Suwancharoen D, Panlar P, Saisongkorh W, Rolain JM et al (2006) Causes of acute, undifferentiated, febrile illness in rural thailand: results of a prospective observational study. Ann Trop Med Parasitol 100:363–370. https://doi.org/10.1179/136485906X112158
51. Chattopadhyay S, Richards AL (2007) Scrub typhus vaccines: past history and recent developments. Hum Vaccin 3:73–80. https://doi.org/10.4161/hv.3.3.4009
52. Kim S-W, Ihn K-S, Han S-H, Seong S-Y, Kim I-S, Choi M-S (2001) Microtubule- and dynein-mediated movement of *Orientia tsutsugamushi* to the microtubule organizing center. Infect Immun 69:494–500. https://doi.org/10.1128/IAI.69.1.494-500.2001
53. Min C-K, Yang J-S, Kim S, Choi M-S, Kim I-S, Cho N-H (2008) Genome-based construction of the metabolic pathways of *Orientia tsutsugamushi* and comparative analysis within the rickettsiales order. Comp Funct Genomics 2008:1–14. https://doi.org/10.1155/2008/623145
54. Giengkam S, Blakes A, Utsahajit P, Chaemchuen S, Atwal S, Blacksell SD, Paris DH, Day NPJ, Salje J (2015) Improved quantification, propagation, purification and storage of the obligate intracellular human pathogen *Orientia tsutsugamushi*. PLoS Negl Trop Dis 9:e0004009. https://doi.org/10.1371/journal.pntd.0004009
55. Rodino KG, VieBrock L, Evans SM, Ge H, Richards AL, Carlyon JA (2018) *Orientia tsutsugamushi* modulates endoplasmic reticulum-associated degradation to benefit its growth. Infect Immun 86:e00596-e617. https://doi.org/10.1128/IAI.00596-17
56. Celli J, Tsolis RM (2015) Bacteria, the endoplasmic reticulum and the unfolded protein response: friends or foes? Nat Rev Microbiol 13:71–82. https://doi.org/10.1038/nrmicro3393
57. Kawahara H, Minami R, Yokota N (2013) BAG6/BAT3: emerging roles in quality control for nascent polypeptides. J Biochem 153:147–160. https://doi.org/10.1093/jb/mvs149
58. Saric T, Graef CI, Goldberg AL (2004) Pathway for degradation of peptides generated by proteasomes. J Biol Chem 279:46723–46732. https://doi.org/10.1074/jbc.M406537200
59. Rikihisa Y, Ito S (1980) Localization of electron-dense tracers during entry of *Rickettsia tsutsugamushi* into polymorphonuclear leukocytes. Infect Immun 30:231–243. https://doi.org/10.1128/iai.30.1.231-243.1980
60. Kim M-J, Kim M-K, Kang J-S (2013) Involvement of lipid rafts in the budding-like exit of *Orientia tsutsugamushi*. Microb Pathog 63:37–43. https://doi.org/10.1016/j.micpath.2013.06.002
61. Brown DA, London E (1998) Functions of lipid rafts in biological membranes. Annu Rev Cell Dev Biol 14:111–136. https://doi.org/10.1146/annurev.cellbio.14.1.111
62. Saito A, Kokubu E, Inagaki S, Imamura K, Kita D, Lamont RJ, Ishihara K (2012) Porphyromonas gingivalis entry into gingival epithelial cells modulated by fusobacterium nucleatum is dependent on lipid rafts. Microb Pathog 53:234–242. https://doi.org/10.1016/j.micpath.2012.08.005
63. Rohde M, Muller E, Chhatwal GS, Talay SR (2003) Host cell caveolae act as an entry-port for group a *Streptococci*. Cell Microbiol 5:323–342. https://doi.org/10.1046/j.1462-5822.2003.00279.x
64. Kawamura A, Murata M, Osono M, Nogami S, Shirasaka A, Tanaka H, Sudo K, Suzuki K, Miyairi T, Kijima H (1980) Studies on inapparent infection of tsutsugamuschi disease in izu shichito islands: seroepidemiology and demonstration of an avirulent rickettsia strain for mice. Jpn J Exp Med 50:91–105
65. Smadel JE, Jackson EB (1947) Chloromycetin, an antibiotic with chemotherapeutic activity in experimental rickettsial and viral infections. Science 106:418–419. https://doi.org/10.1126/science.106.2757.418
66. Richards AL, Jiang J (2020) Scrub typhus: historic perspective and current status of the worldwide presence of orientia species. TropicalMed 5:49. https://doi.org/10.3390/tropicalmed5020049
67. Rout UK, Sanket AS, Sisodia BS, Mohapatra PK, Pati S, Kant R, Dwivedi GR (2020) A comparative review on current and future drug targets against bacteria & malaria. CDT 21:736–775. https://doi.org/10.2174/1389450121666200129103618
68. Dwivedi GR, Rai R, Pratap R, Singh K, Pati S, Sahu SN, Kant R, Darokar MP, Yadav DK (2021) Drug resistance reversal potential of multifunctional thieno[3,2-c]pyran via potentiation of antibiotics in MDR P. Aeruginosa BioMed Pharmacother 142:112084. https://doi.org/10.1016/j.biopha.2021.112084
69. Wangrangsimakul T, Phuklia W, Newton PN, Richards AL, Day NPJ (2020) Scrub typhus and the misconception of doxycycline resistance. Clin Infect Dis 70:2444–2449. https://doi.org/10.1093/cid/ciz972

70. Espinosa-Pereiro J, Sánchez-Montalvá A, Aznar ML, Espiau M (2022) MDR tuberculosis treatment. Medicina 58:188. https://doi.org/10.3390/medicina58020188
71. Mohr KI (2016) History of antibiotics research. In: Stadler M, Dersch P (eds) How to overcome the antibiotic crisis: facts, challenges, technologies and future perspectives. Springer, Cham, pp 237–272
72. Watt G (2020) Drug-resistant scrub typhus. Clin Infect Dis 71:1580–1580. https://doi.org/10.1093/cid/ciz1190
73. Smadel JE, Woodward TE, Ley HL, Philip CB, Traub R, Lewthwaite B, Savoor SR (1948) Chloromycetin in the treatment of scrub typhus. Science 108:160–161. https://doi.org/10.1126/science.108.2798.160
74. Rao RSP, Ghate SD, Shastry RP, Kurthkoti K, Suravajhala P, Patil P, Shetty P (2022) Prevalence and heterogeneity of antibiotic-resistant genes in *Orientia tsutsugamushi* and other rickettsial genomes. Bioinformatics 13:423
75. Varghese GM, Janardhanan J, Mahajan SK, Tariang D, Trowbridge P, Prakash JAJ, David T, Sathendra S, Abraham OC (2015) Molecular epidemiology and genetic diversity of *Orientia tsutsugamushi* from patients with scrub typhus in 3 regions of India. Emerg Infect Dis 21:64–69. https://doi.org/10.3201/eid2101.140580
76. Watt G, Chouriyagune C, Ruangweerayud R, Watcharapichat P, Phulsuksombati D, Jongsakul K, Teja-Isavadharm P, Bhodhidatta D, Corcoran KD, Dasch GA et al (1996) Scrub typhus infections poorly responsive to antibiotics in Northern Thailand. Lancet 348:86–89. https://doi.org/10.1016/S0140-6736(96)02501-9
77. Mathai E, Rolain JM, Verghese GM, Abraham OC, Mathai D, Mathai M, Raoult D (2003) Outbreak of scrub typhus in Southern India during the cooler months. Ann NY Acad Sci 990:359–364. https://doi.org/10.1111/j.1749-6632.2003.tb07391.x
78. Chao C, Garland DL, Dasch GA, Ching W (2009) Comparative proteomic analysis of antibiotic-sensitive and insensitive isolates of *Orientia tsutsugamushi*. Ann NY Acad Sci 1166:27–37. https://doi.org/10.1111/j.1749-6632.2009.04525.x
79. Tantibhedhyangkul W, Angelakis E, Tongyoo N, Newton PN, Moore CE, Phetsouvanh R, Raoult D, Rolain J-M (2010) Intrinsic fluoroquinolone resistance in *Orientia tsutsugamushi*. Int J Antimicrob Agents 35:338–341. https://doi.org/10.1016/j.ijantimicag.2009.11.019
80. Kim MS, Baek JH, Lee J-S, Chung M-H, Lee SM, Kang J-S (2013) high in vitro infectivity of a doxycycline-insensitive strain of *Orientia tsutsugamushi*. Infect Chemother 45:431. https://doi.org/10.3947/ic.2013.45.4.431
81. Lee SH, Chung EJ, Kim EG, Sea JH (2014) A Case of doxycycline-resistant tsutsugamushi meningoencephalitis. Neurol Asia 19:205–206
82. Tantibhedhyangkul W, Wongsawat E, Suputtamongkol Y, Thipmontree W, Silpasakorn S, Waywa D (2016) Scrub typhus in northeastern Thailand: eschar distribution, abnormal electrocardiographic findings, and predictors of fatal outcome. Am J Trop Med Hyg 95:769–773. https://doi.org/10.4269/ajtmh.16-0088
83. Varghese GM, Dayanand D, Gunasekaran K, Kundu D, Wyawahare M, Sharma N, Chaudhry D, Mahajan SK, Saravu K, Aruldhas BW et al (2023) Intravenous doxycycline, azithromycin, or both for severe scrub typhus. N Engl J Med 388:792–803. https://doi.org/10.1056/NEJMoa2208449
84. Kock F, Hauptmann M, Osterloh A, Schäberle TF, Poppert S, Frickmann H, Menzel K-D, Peschel G, Pfarr K, Schiefer A et al (2018) *Orientia tsutsugamushi* is highly susceptible to the RNA polymerase switch region inhibitor corallopyronin a in vitro and in vivo. Antimicrob Agents Chemother 62:e01732-e1817. https://doi.org/10.1128/AAC.01732-17
85. Lu C-T, Wang L-S, Hsueh P-R (2021) Scrub typhus and antibiotic-resistant *Orientia tsutsugamushi*. Expert Rev Anti Infect Ther 19:1519–1527. https://doi.org/10.1080/14787210.2021.1941869
86. Gorlenko CL, Kiselev HYu, Budanova EV, Zamyatnin AA, Ikryannikova LN (2020) Plant secondary metabolites in the battle of drugs and drug-resistant bacteria: new heroes or worse clones of antibiotics? Antibiotics 9:170. https://doi.org/10.3390/antibiotics9040170
87. Li JW-H, Vederas JC (2009) Drug discovery and natural products: end of an era or an endless frontier? Science 325:161–165. https://doi.org/10.1126/science.1168243
88. Ekor M (2014) The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. https://doi.org/10.3389/fphar.2013.00177
89. Giordano A, Tommonaro G (2019) Curcumin and cancer. Nutrients 11(10):2376. https://doi.org/10.3390/nu11102376
90. Mehta J, Rolta R, Dev K (2022) Role of medicinal plants from north western himalayas as an efflux pump inhibitor against MDR AcrAB-TolC *Salmonella enterica Serovar typhimurium* in vitro and in silico studies. J Ethnopharmacol 282:114589. https://doi.org/10.1016/j.jep.2021.114589
91. Chakraborty AK, Saha S, Poria K, Samanta T, Gautam S, Mukhopadhyay J (2022) A saponin-polybromophenol antibiotic (CU1) from cassia fistula bark against multi-drug resistant bacteria targeting rna polymerase. Curr Res Pharmacol Drug Discov 3:100090. https://doi.org/10.1016/j.crphar.2022.100090
92. Jepkoech C, Omosa LK, Nchiozem-Ngnitedem V-A, Kenanda EO, Guefack M-GF, Mbaveng AT, Kuete V, Heydenreich M (2022) Antibacterial secondary metabolites from *Vernonia auriculifera* hiern (*Asteraceae*) against MDR phenotypes. Nat Prod Res 36:3203–3206. https://doi.org/10.1080/14786419.2021.1953024
93. Dharmaratne MPJ, Manoraj A, Thevanesam V, Ekanayake A, Kumar NS, Liyanapathirana V, Abeyratne E, Bandara BMR (2018) *Terminalia bellirica* fruit extracts: in-vitro antibacterial activity against selected multidrug-resistant bacteria, radical scavenging activity and cytotoxicity study on BHK-21 cells. BMC Complement Altern Med 18:325. https://doi.org/10.1186/s12906-018-2382-7
94. Reiter J, Levina N, Van Der Linden M, Gruhlke M, Martin C, Slusarenko A (2017) Diallylthiosulfinate (allicin), a volatile antimicrobial from garlic (*Allium sativum*), kills human lung pathogenic bacteria, including MDR strains, as a vapor. Molecules 22:1711. https://doi.org/10.3390/molecules22101711
95. Randhawa HK, Hundal KK, Ahirrao PN, Jachak SM, Nandanwar HS (2016) Efflux pump inhibitory activity of flavonoids isolated from *Alpinia calcarata* against methicillin-resistant *Staphylococcus aureus*. Biologia 71:484–493. https://doi.org/10.1515/biolog-2016-0073
96. Tan N, Yazıcı-Tütüniş S, Bilgin M, Tan E, Miski M (2017) Antibacterial activities of pyrenylated coumarins from the roots of prangos hulusii. Molecules 22:1098. https://doi.org/10.3390/molecules22071098
97. Khameneh B, Iranshahy M, Ghandadi M, Ghoochi Atashbeyk D, Fazly Bazzaz BS, Iranshahi M (2015) Investigation of the antibacterial activity and efflux pump inhibitory effect of co-loaded piperine and gentamicin nanoliposomes in methicillin-resistant *Staphylococcus aureus*. Drug Dev Ind Pharm 41:989–994. https://doi.org/10.3109/03639045.2014.920025
98. Latimer TS (1871) Report of five cases of typhus fever treated with belladonna. Ga Med Companion 1:350–352

99. Valbuena G, Walker DH (2012) Approaches to vaccines against *Orientia tsutsugamushi*. Front Cell Infect Microbiol 2:170. https://doi.org/10.3389/fcimb.2012.00170
100. Walker DH, Mendell NL (2023) A scrub typhus vaccine presents a challenging unmet need. npj Vaccin 8:11. https://doi.org/10.1038/s41541-023-00605-1