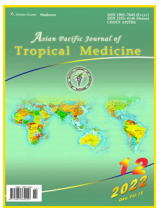

Review Article

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Neglected scrub typhus: An updated review with a focus on omics technologies

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ABSTRACT

Scrub typhus is a neglected disease and one of the most serious health problems in the Asia-Pacific region. The disease is caused by an obligate intracellular bacteria *Orientia tsutsugamushi*, which is transmitted by chigger bites or larval mite bites. Scrub typhus is a threat to billions of people worldwide causing different health complications and acute encephalitis in infants and growing children. The disease causes multiple organ failure and mortality rates may reach up to 70% due to a lack of appropriate healthcare. Currently available genome and proteome databases, and bioinformatics methods are valuable tools to develop novel therapeutics to curb the pathogen. This review discusses the state-of-the-art of information about *Orientia tsutsugamushi*-mediated scrub typhus and delineates the role of omics technologies to develop drugs against the pathogen. The role of proteome-wide *in silico* approaches for the identification of therapeutic targets is also highlighted.

KEYWORDS: *Orientia tsutsugamushi*; Scrub typhus; Therapeutic targets; Omics technologies; Drug discovery

1. Introduction

Scrub typhus is one of the neglected, underreported, and underdiagnosed febrile disease requiring comprehensive investigations to curb the infection[1,2]. Since the last decade, incidences of scrub typhus outbreaks have increased, which makes it a public health concern of significant importance[3,4].

Scrub typhus is the oldest Rickettsial vector-borne zoonotic disease caused by the intracellular pathogenic bacterium *Orientia* (*O.*) *tsutsugamushi* also known as "tsutsugamushi disease"[5]. *O. tsutsugamushi* has been infecting the humans for more than 200 years. The disease was first described in 1810 by Hashimoto and later in 1879 by Baelz and Kawakami as Japanese "flood fever"[6,7]. In India, the first case was reported in 1934 in Himachal Pradesh[6]. The human transmission of disease occurs by bite of trombiculid mite larva infected with *O. tsutsugamushi*[8,9]. Scrub typhus is also known as river/flood fever because infected mites are particularly found in dense scrub vegetation mainly during the wet season (June to November)[10]. The common symptoms of scrub typhus include fever, headache, dry cough, chills, myalgia, eschar, rashes, lymphadenopathy, and gastrointestinal disturbances[11,12]. Scrub typhus in some cases can lead to even more severe complications including multi-organ failure and death[12]. The disease has a greater impact on the rural population, especially in field or outdoor workers, though sporadic incidences from urban areas have also been reported. Impoverished people residing in unhygienic areas are more vulnerable to scrub typhus, *i.e.*, occupation and socioeconomic status are the potential factors for scrub typhus infection[13,14].

2. *Orientia tsutsugamushi*

O. tsutsugamushi, the etiological agent of scrub typhus is an obligate intracellular Gram-negative bacterium[8] that belongs to

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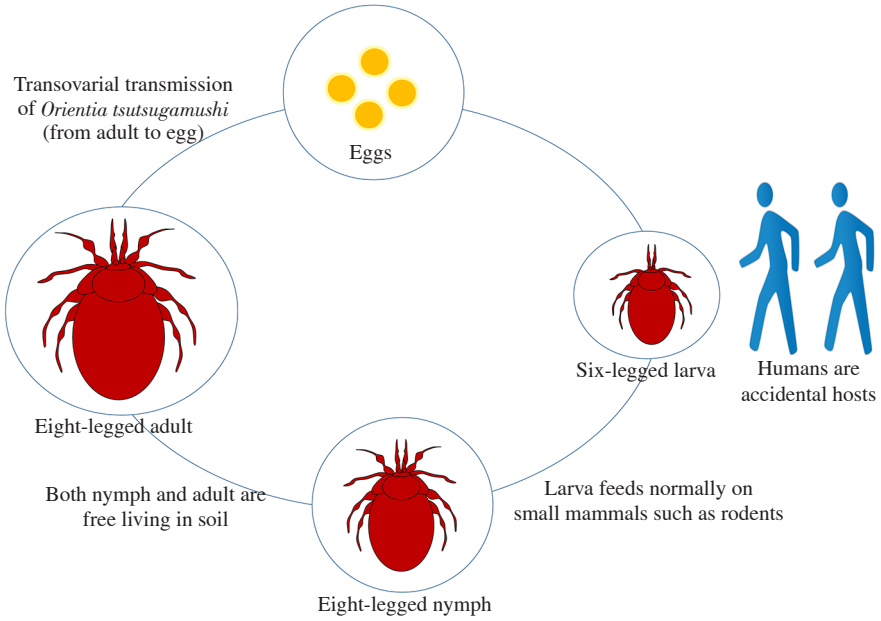

Figure 1. Life cycle of mite *Leptrotrombidium*. The larva (chigger) feeds normally on small mammals but humans are accidental hosts. The *Orientia tsutsugamushi* is transmitted through bite of chigger (larval stage of mite).

the α-subdivision of *Proteobacteria*, order Rickettsiales, and family Rickettsiaceae. Earlier, the bacterium was classified in *Rickettsia* genus, but at present, it is assigned to a new genus-*Orientia* in the family Rickettsiaceae, based on 16S-rRNA sequence, and biochemical and morphological differences[15]. The *Orientia* is linked with its initial reported cases in the 'Oriental' countries. The species name is acquired from the Japanese word 'tsutsuga', meaning "illness or fever" and 'mushi' meaning "insect or creature"[7,16]. Scrub typhus spreads in the body through both lymphatic and hematogenous routes[17]. The target site includes endothelial cells of different organs. The molecular pathology of *O. tsutsugamushi* is still poorly understood, and biological interactions between the host and the pathogen are not fully understood. It is primarily due to the obligatory intracellular nature of the bacterium, which needs to be cultured in mammalian cell lines and also has to be handled very carefully[18].

O. tsutsugamushi is transmitted by the bite of "chiggers", which are the tick larvae (family Trombiculidae, genus *Leptotrombidium*). The potential reservoir of *O. tsutsugamushi* is the mite which maintains the pathogen through transstadial and transovarial transmission[1,19]. *O. tsutsugamushi* establishes in the cell cytosol of several organs as well as oocytes of the mite, hence the mode of transmission is primarily transovarial[20,21]. *Leptotrombidium* chigger generally feeds on small rodents, but occasionally bites large mammals and humans, therefore, humans serve as accidental hosts (Figure 1).

O. tsutsugamushi is harbored in the salivary glands of infected mites and is transmitted to the human when chiggers bite and suck human blood[22]. The chiggers bite leaves a black dead skin tissue known as eschar whose appearance varies among patients depending on the area and genotype of the pathogen[14,23]. Fever arises around day 4 of the chiggers bite, and eschar develops after one week[24]. The presence of eschar is a characteristic supportive evidence for the clinical diagnosis of scrub typhus[25]. Although the transmission of *O. tsutsugamushi* is primarily through the chiggers, unconfirmed reports indicated that scrub typhus may also be spread by leech bites[26]. Sando *et al*. have reported *Rickettsia japonica* infection from a land leech (*Haemadipsa zeylanica* japonica) bite, a potential carrier of *Rickettsia japonica*[27].

3. Scrub typhus epidemiology

The febrile scrub typhus is endemic to the "Tsutsugamushi triangle" within the Asia Pacific region which cover about 8 million km^2 area from Japan in the east, to Pakistan in the west, Russian Far East in the north and Australia in the south (Figure 2)[5,14,28]. Scrub typhus has been a nationally notifiable disease in countries and regions such as China, Japan, South Korea, Thailand, Bhutan, and Taiwan[14]. Cases of scrub typhus infection from India, Nepal, Russia, Malaysia, Philippines, Indonesia, Sri Lanka, Afghanistan, Maldives, and northern Australia have also been documented[1,14,29]. It has been estimated that about one billion people are threatened by the disease globally and illness occurs in nearly one million people every year[14,30]. Scrub typhus is prevalent in the "Tsutsugamushi triangle", but few cases have been reported in the Middle East and South America[19,31]. Serological and molecular evidence of *Orientia*

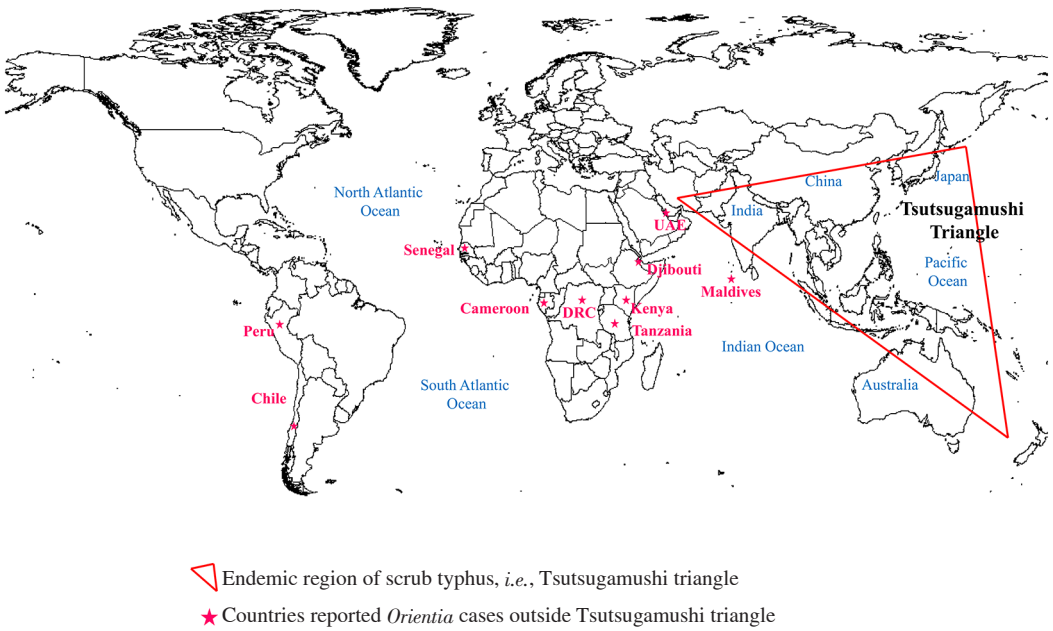

Figure 2. *Orientia* species distribution within endemic regions.

species from Africa and Europe were also seen earlier[32]. These reports indicated the broader geographical distribution of the genus *Orientia*, and also stated that scrub typhus infection is now no longer restricted to the "Tsutsugamushi triangle"[32]. Further, scrub typhus is a remarkably neglected disease, but it has an ever-widening impact worldwide[8].

Scrub typhus was a major cause of illness in military personnel during World War][over the India-Burma border and during the Indo-Pak conflict 1965[14]. The outbreak of scrub typhus was reported in various Indian provinces including Himachal Pradesh, Jammu & Kashmir, Uttarakhand, Rajasthan, Pondicherry, Maharashtra, Tamil Nadu, Kerala, and the North-Eastern region with different intensity and complications[7,28,33]. The primary and foremost vector of *O. tsutsugamushi* reported in India is *Leptotrombidium* deliense[29]. Precise data on the incidence of scrub typhus in India is currently not available due to underreported cases of the disease, however, outbreaks of disease are on a rise[34].

4. Health complications associated with scrub typhus

The associated health complications of scrub typhus include pneumonitis, acute respiratory distress syndrome, jaundice, acute kidney failure, meningoencephalitis, myocarditis, pericarditis, septic shock, and disseminated intravascular coagulation[34–37]. The main target organ of intracellular pathogen "*Orientia*" is the lungs, therefore, leads to numerous pulmonary complications such as interstitial pneumonia, acute respiratory failure, cardiogenic pulmonary edema, and death[39].

O. tsutsugamushi is responsible for central nervous system infections across endemic areas[40]. There are various reports of meningitis, acute encephalitis syndrome (AES), and other neurological complications due to scrub typhus[7,33]. Scrub typhus has been recognized as one of the primary causes of AES in Indian children. A recent report from the Uttar Pradesh State of India revealed that children of 2-15 year's age are at risk of acquiring scrub typhus, which is associated with outbreaks of AES[41]. The children visiting or playing in fields and handling fodder of cattle were at higher risk for scrub typhus[41]. Studies have shown that scrub typhus starts mimicking Parkinson's disease, which is of grave concern[42]. It was also proven pathologically that scrub typhus could lead to GI complications including small bowel bleeding[43]. According to Department of Health Research-Indian Council of Medical Research guidelines, the use of rapid tests for the diagnosis and management of rickettsial diseases could be helpful in countries like India[44].

5. Antibiotic resistance in *Orientia tsutsugamushi*

Natural immunity to scrub typhus is weak, and according to the "Centre for Disease Control and Prevention", the vaccine is not available yet. The cost-effective and broad-spectrum antibiotic "doxycycline" which is a derivative of oxytetracycline, is an empirical treatment and prophylaxis for scrub typhus. Doxycycline is given either orally or intravenously[45,46]. Doxycycline is more effective than other antibiotics if given in an earlier stage of

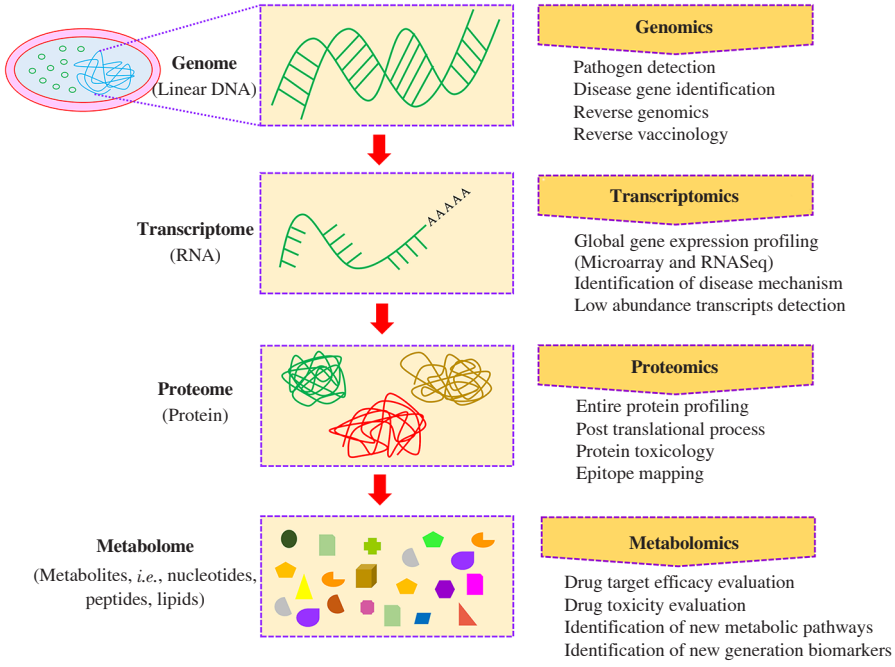

Figure 3. Description of omics approaches. The omics technologies are primarily aimed to screen genes (genomics), transcripts (transcriptomics), proterins (proteomics) and metabolites (metabolomics) in particular biological sample. The left layer of the figure indicates biological data and right layer denotes the corresponding omics techniques used to screen that particular sample.

infection[46]. Other recommended antibiotics include tetracycline, chloramphenicol, macrolides (azithromycin), quinolones, and rifampicin[46]. Tetracycline, chloramphenicol, and quinolones can cause complications in children below age 8 years and pregnant women[45]. Rifampicin was also found to be useful when pathogens become resistant to doxycycline[47]. The commonly recommended first-line antibiotics like penicillin and cephalosporin used for the treatment of a general febrile illness are not effective against scrub typhus[26]. Fluoroquinolones like ciprofloxacin could be used but are not good drugs for the treatment as *in vitro* study listed that *O. tsutsugamushi* was intrinsically resistant to these antibiotics[48,49]. Watt *et al*. reported that *O. tsutsugamushi* strains from Northern Thailand resistant to doxycycline and chloramphenicol[50]. Earlier doxycycline resistance has also been reported from Southern India and South Korea[51,52]. In Southeast Asia and Australia, an opinion about doxycycline resistance has also been made on the failure of prophylaxis in military personnel[53,54]. Wangrangsimakul *et al*. have concluded that doxycycline resistance against scrub typhus is a misconception and the variations in the treatment are due to other bacteria, host, and pharmaceutical factors[55]. The reports on the existence and importance of antibiotic resistant scrub typhus are still uncertain and need further studies to examine whether antibiotic resistance is present or how to cope with it if it has evolved[26,55].

6. Drug discovery and development

Drug discovery and development is a complex process that involves chemistry, pharmaceutical, and clinical sciences to design and discover drugs. The conventional pharmacological approach for drug discovery and development includes several phases such as target identification, target validation, lead identification, lead optimization, preclinical trial, clinical trial, food and drug administration approval, and new drug product launch to the public[56]. It needs 12-15 years and billions of dollars to launch a new drug, *i.e.*, the discovery and development of drugs using experimental approaches are arduous, costly, complicated, and time-consuming[56,57]. Most new drugs met with failure during clinical trials after enormous investment. Therefore, it is good to use omics and computational *in silico* approaches in screening large datasets in the initial stage to raise the efficiency of the drug discovery process.

The past decade has witnessed unprecedented advances in the development and use of alternative therapeutic strategies such as engineered microorganisms[58,59], phage therapy[60,61], antibody-antibiotics conjugates[62], and antibiotics to prevent microbial infections[63,64]. In addition, it is logical to consider the One Health approach while addressing the menace of microbial infections and drug resistance[65,66], and epidemiology[67].

7. Trends of omics technologies in drug discovery

The high throughput omics technologies are principally aimed to screen genes (genomics), transcripts (transcript omics), proteins (proteomics), and metabolites (metabolomics) in a particular biological sample (Figure 3). These techniques allow quantitative measurements of a biological sample and can generate gigantic scientific data exponentially on daily basis. The decrease in cost and time to generate enormous scientific data has created exciting opportunities and challenges for biologists and computational biologists[68,69].

Omics data in their original formats are useless, therefore, difficult to fuse systematically with already available scientific literature. There are various information, rich databases and repositories which provide omics-driven scientific data for further evaluation[70,71]. To accelerate the process of collection, processing, and interpreting omics data, various data mining algorithms, tools, databases, and software have been designed by computational biologists. Omics technologies have transformed biological research as these provide the researchers to get a deeper insight into molecular mechanisms of various biological processes, including complex diseases. Omics approaches are progressively being utilized in the drug discovery and development process, *i.e.*, identification of drug targets, uncovering molecular mechanisms of drugs and estimating efficacy of drugs[69–71].

7.1. Genomics

Genomics is the comprehensive investigation of the whole genome of an organism. The genome of an organism represents a complete set of genes including coding and non-coding regions. Conventionally, genes are analyzed independently, but the arrival of high throughput sequencing technologies has changed the whole scenario. DNA sequence analysis now has become fundamental for research in molecular biology and biotechnology. Traditional DNA sequencing methods can only analyze a limited number of DNA fragments. The advent of DNA microarray and next generation sequencing technologies to DNA sequencing or whole genome sequencing revolutionized the field of genomics[72,73].

DNA microarrays are widely used for genome wide association studies which help to identify genetic variants. Knowledge of these variations further aids in understanding molecular mechanisms of risk factors of the diseases and comparative genomics[74]. The whole genome sequencing or sequencing of a specific region can be achieved by next generation sequencing, which allows the detection of rare coding and non-coding variants and the identification of new mutations[75]. The repositories of genome wide associated data are extensively employed for various *in silico* interpretations to discover potential drug targets and to access drug efficacy and safety. Recently, the computational analysis of pathogens genomes and extracting information from them is one of the fast and time-saving strategies in the fields of disease identification and drug discovery[76].

The complete genomes of various strains of *O. tsutsugamushi* have been sequenced and available in public domain databases[11,21,77]. The genome of *O. tsutsugamushi* has a single circular chromosome which varies in size from strain to strain, ranging from approximately 2.0-2.1 Mb[11,21,77]. In order of Rickettsiales, *O. tsutsugamushi* has the largest genome size. The universally conserved genes are less in the genome of *O. tsutsugamushi*, and the expansion, complexity, and diversity in the genome are due to gene duplication and repeat amplification[11,77].

The whole genome sequencing of *O. tsutsugamushi* showed that it contains an immense number of repetitive sequences, most of them are pseudogenes and the repeat density is about 200-fold higher than its closely related species *Rickettsia prowazekii*[21,77]. The extensive gene loss of housekeeping genes was noticed among the core genes set of the family Rickettsiaceae. Although, housekeeping gene loss is higher in *O. tsutsugamushi* than *Rickettsia* but genome of *O. tsutsugamushi* has acquired the huge number of foreign genes. Therefore, the genome of *O. tsutsugamushi* is one of the bacterial genomes having high plasticity[21]. The repetitive sequences of *O. tsutsugamushi* were classified into three categories as amplified genetic elements, transposable elements, and others (short repeats of unknown origin). The *O. tsutsugamushi* amplified genetic element is a member of the integrative and conjugative element which consists of the integrase gene, conjugative transfer gene, type Ⅳ secretory system, and some probable effector protein, *i.e.*, ankyrin repeats-containing proteins, tetratricopeptide repeats-containing proteins, and histidine kinase[11,21]. Also, the *O. tsutsugamushi* genome has undergone reshuffling, hence there is evidence of horizontal gene transfer from *Parachlamydia* and *Legionella* spp.[78].

7.2. Transcriptomics

The transcriptome represents a set of all RNA transcripts in an organism. The primary aim of transcriptomics is to study all RNAs either coding mRNA (the transient intermediary molecule in the information network) or non-coding RNAs[79]. The genome wide transcriptional profiling measures the expression levels of many genes at a single time which provides a global view of cellular and biological processes under different conditions[71]. Microarray and RNA-Seq are the two main techniques sused in the field of transcriptomics[79]. The microarray is used to quantify predetermined sequences, whereas the emergence of high throughput sequencing technologies has provided a new method, *i.e.*, RNA-Seq

for transcriptome mapping and quantification[79,80]. RNA-Seq has numerous advantages over previous approaches and has consistently revolutionized the transcriptomics field[80].

Additionally, RNA-Seq may deliver information on unannotated exons, allele-specific expression, alternative splicing, and novel transcripts (non-coding RNA). The technology efficiently has been applied in the drug discovery process and assisted into recognize of drug-related genes, fusion proteins, long non-coding RNAs, and microRNAs[81]. Recently it is reported that long non-coding RNAs play critical roles in the regulation of host cell response and also provide valuable information about the disease[82,83]. MicroRNAs participate in bacterial infection by altering inflammatory responses, tissue remodeling, cell penetration, and host immune responses[84]. Therefore, distinctively expressed coding and non-coding RNAs identified by the RNA-Seq approach may provide novel insight into the pathogenesis and can help to improve the diagnosis, prevention, and therapy of the infectious disease.

The global transcriptomic analysis of *O. tsutsugamushi*-infected human monocytes presented evidence that the expression of thousand genes has altered[85]. The genes encoding cytokines, genes of type- Ⅰ interferon pathway, the gene linked with M1 (pro-inflammatory phenotype) polarization of macrophages, and apoptosis associated genes were highly upregulated which indicate their role in inflammatory complications of scrub typhus and also serve as new clues for the diagnosis of scrub typhus[85]. Recently, Chao *et al*. analyzed the mRNA expression profile of *O. tsutsugamushi* infected mice and stated that several metabolic pathways altered dynamically at different time points[86]. To survive inside the host in the initial stage of the infection, the *O. tsutsugamushi* affects the genes of the host involved in cell growth control, secretion of cytokines, cell adhesion, invasions, and cell development. Once the infection has established the genes of cellular immune pathways got stimulated to defend the host cell to destroy the pathogen[86].

7.3. Proteomics

The total protein content in an organism at a particular time represents its proteome. The proteome alters from cell to cell and from time to time. Fluctuations also occur in the proteome in response to external stimuli. The complex regulatory systems that are used to control protein expressions make the proteome dynamic in nature. Therefore, moving from genomics to proteomics, there is a massive increase in potential complexity[87].

The first comparative proteomics analysis by Chao *et al*. on antibiotic sensitive and insensitive isolates of *O. tsutsugamushi* using 2D gel electrophoresis and LC-MS-MS presented 14 differentially expressed genes which signified that antibiotic insensitivity in *O. tsutsugamushi* might arise due to multiple mechanisms[88]. The shotgun proteomics analysis of *O. tsutsugamushi* revealed that although the protein profile of *O. tsutsugamushi* is similar to other intracellular bacteria, yet it also has specific potentially virulent and immunogenic protein factors which play critical roles in virulence or host-pathogen interactions[89]. A combined study on mRNA profiling and proteomic analysis of *O. tsutsugamushi* in eukaryotic fibroblasts and macrophages cells showed that genes involved in translation, processing, and secretion are significantly downregulated in macrophages as compared to fibroblasts which are correlated with the decline in bacterial translation and growth rate potentially due to killing of bacteria in macrophages[90].

Studies on clinical blood serum samples from scrub typhus patients revealed that the expression of proteins linked with immune responses particularly the complement system was significantly up-regulated on infection of *O. tsutsugamushi* and subsequently down regulated on antibiotic treatment[91]. All these studies provide valuable clues to explore further pathophysiological mechanisms, potential diagnostic markers, and therapeutic agents of scrub typhus. Based on bioinformatics prediction of 321 meal-binding proteins in *O. tsutsugamushi*, 60 of the proteins exhibited the ability to interact with drug or drug-like molecules, indicating that these proteins can be used as broad-spectrum antibiotics to curtail scrub typhus[37]. Earlier pan-proteome profiling and hypothetical proteome mining of *O. tsutsugamushi* was carried out, which showed the involvement of proteins in various biological processes, antibiotic resistance, and virulence. These shortlisted proteins are envisioned as potential therapeutic candidates for drug discovery and development against *O. tsutsugamushi*[92,93].

7.4. Metabolomics

Another emerging field in omics is "metabolomics" which is defined as a comprehensive analysis of global metabolite profiles in a cell, tissue, or organism under a given set of conditions. Metabolites are regulators, intermediates, and output products of various biochemical reactions[94]. The global metabolite profiling of *O. tsutsugamushi*-infected mice (organs, tissue, and serum) showed altered metabolic patterns in the liver and spleen such as a decline in energy production, severe deficiency of remethylation and glutathione, abnormality in protein oxidation, fatty acid biosynthesis pathways, and enlargement of spleen[95]. Recently, a comprehensive metabolomics study of chigger borne *O. tsutsugamushi* infection on host serum revealed that a significant alteration had been found in numerous metabolites linked to immune response and energy production pathways[96]. Additionally, small alterations in biochemical profiles of lipid, carbohydrate, and nucleotide metabolism, and bile acid homeostasis have also been observed[96]. These findings provide an impressive platform to further get insight into mechanisms by which host response to *O. tsutsugamushi* infection.

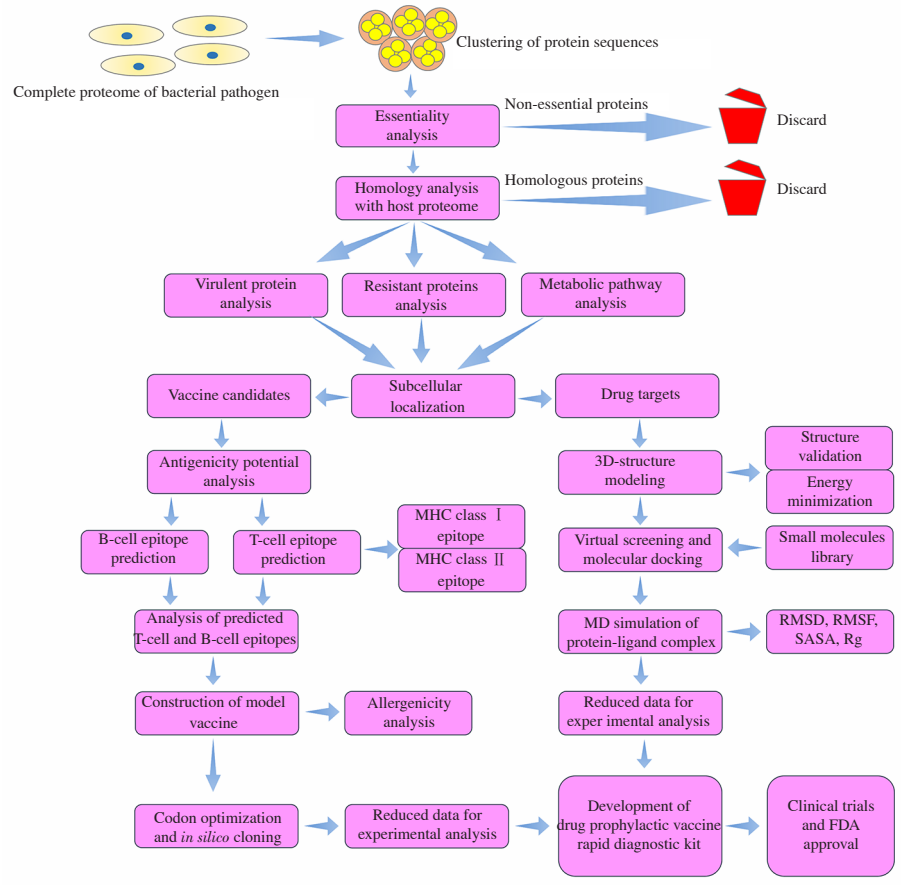

Figure 4. Subtractive proteome-wide *in silico* approach in drug discovery and development. The complete proteome of the bacterial pathogen can be exploited for the identification of potential therapeutic targets by means of *in silico* approaches, which are faster, more efficient, and cost-effective.

8. Proteome–wide *in silico* approaches for identification of probable drug targets

Over the last two decades, the computer has become a core part of biology and reshaped biological sciences[97]. Hallam Stevens writes in his book *Life Out of Sequence*, "Biology adapted itself to the computers, not the computer to biology"[98]. Computational biology and bioinformatics have emerged as a promising and effective approaches for retrieving information about genes and proteins of an organism and evolving industrial and therapeutic strategies[99,100]. The large datasets provided by proteomics technologies can be accessed by numerous bioinformatics tools to predict 3D structures, functional motifs and domains, evolutionary relationships, protein-protein interactions, and protein translational modifications[99–101]. Such analysis addressed the role of proteomics strategies, along with bioinformatics applications in various health and clinical sciences like detection of biomarkers for disease, knowing mechanisms of pathogenicity, analysis of expression patterns alteration in response to different signals, and exploration of metabolic pathways proteins in different diseases[71,104].

The preeminent mission in the biomedical field scientifically, economically, and socially is to discover new drug targets from massive datasets provided by omics technologies using *in silico* approaches[105]. The complete proteome of the bacterial pathogen provided by omics techniques can be exploited for the prediction of potential therapeutic candidates using *in silico* approaches which are efficient, cost-effective, and much faster[104–108]. Nowadays, subtractive, comparative, and functional proteomics are the best optimized proteome-wide *in silico* approaches used to identify the most excellent probable drug targets[111,112]. The workflow of proteome-wide *in silico* approaches in drug or vaccine or diagnostic kit development was shown in Figure 4. The two critical parameters used by these *in silico* approaches to find out probable drug targets are essentiality and selectivity/specificity, *i.e.*, the target must be critical for the growth, survival, and pathogenicity or not well-conserved in the host for avoiding issues of cytotoxicity[111,112]. Therefore, the use of a choice of bioinformatics tools and techniques symbolizes an attractive source of alternative strategies to identify probable drug targets. These putative drug targets are further used, consecutively, for the optimization and new lead detection using molecular modelling, multiple docking strategies, free energy calculations, molecular dynamics simulations, and drug-likeness determination to launch novel antimicrobial[100,113].

9. Conclusions

Scrub typhus is one of the most neglected febrile illness in India, which has re-emerged with some antibiotic resistance cases and has recently been associated with AES among children. Currently, the vaccine is not available for the disease, and studies on antibiotic resistance have also been uncertain. Therefore, the need is to understand the pathological behavior of *O. tsutsugamushi* to construct novel therapeutic targets.

The data generated by omics technologies probably opens up new horizons to accelerate the development of precise drugs or next generation vaccines against scrub typhus and other bacterial diseases. Furthermore, computational screening of drug targets before laboratory, preclinical, and clinical trials probably trims down the economic burden related to materials, manpower, and instrumentation involved in the initial phase of drug discovery. Therefore, using *in silico* computational approaches for screening novel therapeutic targets is a convenient strategy to develop novel therapeutics against infectious diseases.

Conflict of interest statement

The authors declare no conflict of interest.

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Authors' contribution

D.S. and A.S. conceptualize the work. D.S. and S.K.V. performed the formal analysis and validation of the data. S.K. and B.S. performed data curation, writing-review and editing. D.S. contributed in writing-original draft. A.S., S.K., S.K.V. and B.S. contributed to final version of the manuscript.

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