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State-of-the-art strategies to prioritize *Mycobacterium tuberculosis* drug targets for drug discovery using a subtractive genomics approach

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Tuberculosis remains one of the causes of death from a single infectious bacterium. The inappropriate use of antibiotics and patients' non-compliance among other factors drive the emergence of drug-resistant tuberculosis. Multidrug-resistant and extensively drug-resistant strains of tuberculosis pose significant challenges to current treatment regimens, as their reduced efficacy against these strains limits successful patient outcomes. Furthermore, the limited effectiveness and associated toxicity of second-line drugs further compound the issue. Moreover, the scarcity of novel pharmacological targets and the subsequent decline in the number of anti-TB compounds in the drug development pipeline has further hindered the emergence of new therapies. As a result, researchers need to develop innovative approaches to identify potential new anti-TB drugs. The evolution of technology and the breakthrough in omics data allow the use of computational biology approaches, for example, metabolomic analysis to uncover pharmacological targets for structured-based drug design. The role of metabolism in pathogen development, growth, survival, and infection has been established. Therefore, this review focuses on the *M. tb* metabolic network as a hub for novel target identification and highlights a step-by-step subtractive genomics approach for target prioritization.

KEYWORDS

***Mycobacterium tuberculosis*, drug discovery, metabolic pathway, subtractive genomic analysis, multi-drug resistance tuberculosis, choke point enzymes, essential genes, protein-protein interaction**

1 Introduction

1.1 Tuberculosis epidemiology and physiology

Tuberculosis (TB) is still one of the most common infectious diseases that threaten the public health system. In 2020, the WHO estimated 5.8 million new cases of tuberculosis and 1.5 million deaths, including people co-infected with HIV (WHO, 2020). Adults made up 88 percent of TB patients, while children made up 12 percent. South-East Asia, Africa, and the Western Pacific were the WHO regions with the highest number of TB patients.Two-thirds of the global TB cases were found in India (26 percent), Indonesia (8.5 percent), China (8.4 percent), the Philippines (6.0 percent), Pakistan (5.7 percent), Nigeria (4.4 percent),

Bangladesh (3.6 percent), and South Africa (3.6 percent) (Chakaya et al., 2021). TB has been responsible for more than a billion deaths since its discovery and this number has doubled the number of death due to malaria, smallpox, influenza, cholera, plague, and human immunodeficiency virus (HIV) put together (Heemskerk et al., 2015).

Mycobacterium tuberculosis (*M. tb*), the causative agent of tuberculosis (WHO, 2020), is an opportunistic intracellular pathogen that has adapted to live and thrive within the human host. *M. tb* mainly attacks the lungs, causing significant tissue damage and symptoms such as chronic cough, night sweats, weight loss, and doldrums (Macalino et al., 2020). In addition to the lungs, the bacteria can travel to other organs like the brain (causing TB meningitis), liver, spleen, and bones (Peters et al., 2020). The bacterium is spread via coughing whereby an infectious droplet containing the bacteria is inhaled by a healthy person. *M. tb* is taken up by alveolar macrophages, which might result in one of three outcomes: in an acidic environment, *M. tb*-containing phagosomes merge with lysosomes to destroy the bacteria, the bacteria remain latent in macrophages, or infection develops (Shim et al., 2020). Although the bacteria has infected about a quarter of the world's population, approximately 5–15 percent of those infected develop active tuberculosis throughout their lives, with the rest having latent tuberculosis and only developing active TB when their immune system is compromised (Sterling et al., 2020).

1.2 Current tuberculosis treatment

In the mid-twentieth century, compounds generated from soil-derived actinomycetes were the gold standard for antibiotic development. The agar-based diffusion and cross-steak techniques were used to screen compounds for antimicrobial effects against bacteria. Streptomycin (SM), the first anti-TB medicine, and other antibiotics were discovered as a result of these methods (Lewis, 2013). The combination of streptomycin (SM) and p-aminosalicylic (PAS) acid are the first drugs used to reduce the risk of antibiotic resistance. Isoniazid (INH), a prodrug activated by catalase-peroxidase (KatG) was discovered in 1912 but its antitubercular efficacy was not recognized until 1951 when Herbert Hyman Fox and Harry L. Yale conducted *in-vivo* screenings in mice infected with *M. tb*. INH is a thiosemicarbazone synthesis intermediate that has higher antitubercular action in lab animals than isonicotinyl-aldehyde thiosemicarbazone, the final product (Verma and Kalra, 2012). The inclusion of INH to PAS and SM ("triple therapy") led to a decrease in drug resistance tuberculosis cases even more and improved the treatment outcomes, although the treatment duration lasted for up to 24 months (Iseman, 2002).

Rifampicin (RIF) is the most widely used rifamycin derivative in TB treatment because of its bactericidal effect (Xu et al., 2021). An Italian research group led by Piero Sensi and Maria Teresa Timbal (1925–1969) identified RIF from a soil sample. RIF is a breakthrough in TB treatment since it reduces the treatment time from 18 to 9 months (Verma and Kalra, 2012). Pyrazinamide is a sterilizing medication and the most potent pyrazine derivative of nicotinamide for treating tuberculosis in mice. PZA was first synthesized by Dalmer and Walter in 1936 and rediscovered in 1972 (Zhang et al., 2014). Because of its capacity to lower TB relapse rates and shorten medication therapy by 3 months, PZA was added to the SM, INH, and RIF regimens. It is currently regarded as an essential component of routine first-line short-course treatment for drug-susceptible TB (Lamont et al., 2020). Ethambutol (EMB) was developed in the early 1960s and quickly replaced SM (Lodha and Bedi, 2020).

Current TB treatment includes a two-month intensive phase of four medications (INH, RIF, EMB, and PZA) followed by a four-month continuation phase of INH and RIF, this combination is to date successful for drug-sensitive TB treatment (Chakraborty and Rhee, 2015). The emergence of MDR drug-resistant strains of the bacteria has rendered INH and RIF therapeutic agents ineffective due to the acquisition of mutations in INH and RIF enzyme targets.

1.3 Drug resistance tuberculosis

Bacteria have developed several mechanisms to overcome antibiotic treatments, these include physiological, acquired, and intrinsic mechanisms, which have been reviewed extensively (Khawbung et al., 2021). Intrinsic resistances are imparted through cell-wall impermeability, drug efflux systems, drug target modification, and drug neutralization by enzymes, while acquired resistance is due to the acquisition of mutations (Khawbung et al., 2021).

The conventional treatment for drug-sensitive tuberculosis is the use of first-line drugs for the first 2 months, followed by INH and RIF for the next 4 months. However, due to patient noncompliance longer treatment regimens were needed, necessitating the use of directly observed therapy (DOT), in which healthcare workers supervise dose intake (Volmink and Garner, 2007; Nahid et al., 2016). Gastrointestinal intolerance, neuropathy, arthralgia, skin rash, haemolytic anaemia, kidney failure, neuropathies, immune thrombocytopaenia, and agranulocytosis are all side effects of long-term medication (Macalino et al., 2020).

TB disease relapse and drug resistance are attributed to the failure to complete the standard TB treatment regimen and the inappropriate use of antibiotics. Multi-drug resistance tuberculosis (MDR-TB)—a form of TB caused by *M. tb* that has developed resistance to the two first-line drugs, INH and RIF. MDR-TB can be treated with second-line drugs, however, these treatments are often longer (18 months or more) and more expensive with uncertain efficacy and high toxicity, resulting in low compliance and undesirable outcomes in patients (Mirzayev et al., 2021). Furthermore, there is another form of drug resistance known as extensively drug-resistant tuberculosis (XDR-TB), by which patients do not respond to even the second line of treatment, leaving patients with no alternative options for treatment. INH, PZA, EMB, ciprofloxacin, ofloxacin, and kanamycin, are among the medications to which the mycobacteria develop resistance (Muthukrishnan, 2021).

Only 50% of MDR-TB cases respond to treatment, and the global burden of MDR-TB surges at a rate of more than 20% each year. Nearly half of the global burden of drug-resistant tuberculosis (approximately 47 percent) is found in India, China, and Russia, whereas South Africa as of 2018 has the highest number of MDR and XDR tuberculosis cases *per capita* (Ismail et al., 2018). Despite the

global death rate declining by 3% per year, antimicrobial drug resistance TB remains a public health problem. Drug-resistant TB strains are on the rise, with 500,000 cases of MDR-TB in 2019 (Chakaya et al., 2021). Until the recent approval of bedaquiline, there has been a decrease in the antimicrobial drug discovery pipeline, particularly for mycobacteria, where no new medication had reached clinical trials since the 1960s (Waman et al., 2019). MDR and XDR-TB are major challenges and difficult to treat with the currently available drugs. Despite a slower rate of TB decline, the COVID-19 pandemic has delayed the WHO's limited progress toward attaining the "End TB Strategy" milestone of a 20% reduction in TB by 2035 (World Health Organization, 2020; Jeremiah et al., 2022).

With the rise of MDR-TB strains, it is more important than ever to identify novel pharmacological targets and potential hits that can be advanced to new leads. As a complement to wet-lab approaches, subtractive genomics, and metabolic pathways analysis, structural bioinformatics are currently being used to further investigate and elucidate the suitability of molecular targets to develop new therapeutics and combat antibiotic resistance. Additionally, this review focuses on the *M. tb* metabolic network as a drug target hub for novel target identification and highlights a step-by-step subtractive genomics approach for target prioritization.

2 Metabolic pathway regulation in *M. tb*

Metabolism is an intricate process that comprises a network of pathways, reactions, and enzymes that are essential for the biochemical and physiological well-functioning of the cell and the overall homeostasis of the system. Metabolism plays a significant role in *M. tb* development, infection establishment, and persistence in the host. Mycobacteria and other infections' susceptibility to antibiotic treatment has also been connected to their metabolic states (Stokes et al., 2019). Several studies have demonstrated the significance of mycobacteria metabolism during infection and dormancy (Chang and Guan, 2021). *M. tb* must adapt to the harsh environment of changing acidity, osmolarity, and nutrient-deficient microenvironment to establish an infection in the host. *M. tb* has evolved metabolic adaptability, allowing it to withstand the harsh environment within the host by transitioning from replicative to non-replicative states.

As a heterotroph, *M. tb* makes use of a carbon source that is either produced by the bacterium or obtained from the host cells (Pandey and Sassetti, 2008; De Carvalho et al., 2010; Beste et al., 2013). Because its metabolic activity is minimal, the dormancy state, also known as the non-replicative state in bacteria, suffers from unfavourable growth conditions such as limited nutrients or low oxygen. The bacterium can avoid the effects of anti-TB drugs that target actively replicating bacteria in this stage (Lipworth et al., 2016; Caño-Muñiz et al., 2018). Once the bacterium returns to its favourable conditions, the *M. tb's* metabolic rate returns to normal, and bacilli growth is revived, which might lead to an active infection. Persistent bacteria are bacilli that continually operate at low metabolic levels, even when conditions are unfavourable that is, during nutrient starvation and hypoxia. Antibiotics have little effect on these persisters (Lewis, 2010). With the crucial role metabolism play in *M. tb survival*, the pathogen's metabolic route serves as a promising target hub for drug development. Below are some of the *M. tb* metabolic pathways that have been targeted by antibiotics.

2.1 Oxidative phosphorylation and ATP production

All bacteria use ATP as their primary source of energy (Mackieh et al., 2023). ATP production differs significantly amongst bacteria. *M. tb's* development is entirely dependent on oxidative phosphorylation for growth (Cook et al., 2017). *M.* tb continues to produce ATP during dormancy, even at low metabolic rates, to maintain its survival. As a result, the oxidative phosphorylation pathway represents a prospective therapeutic target for both replicating and non-replicating *M. tb*. Bedaquiline, a drug that targets the electron transport chain's F_0F_1 ATP synthase, is effective against MDR-TB (Pym et al., 2016). Q203, an inhibitor that targets the electron transport chain cytochrome bc_1 complex, has passed phase II clinical trials and is being developed further (De Jager et al., 2020). The oxidative phosphorylation protein cytochrome bc1 complex cytochrome b subunit, QcrB has also been reported as an attractive therapeutic target in *M. tb* (Foo et al., 2020).

2.2 Lipid metabolism

M. tb requires lipids (fatty acids and cholesterol) as a carbon source during infection. INH, a commonly used first-line anti-TB medication, targets *M. tb* lipid metabolism. INH inhibits the synthesis of mycolic acid, a key component of mycobacterial cell walls. Ethionamide (ETH), isoxyl (ISO), and thioacetazone (TAC) are other drugs that exert anti-TB effects on *M. tb* lipid metabolism. Apart from lipid production and energy conversion, the transport of lipids inside *M. tb* is also an attractive target. MmpL3 is an inner membrane transporter that facilitates the transfer of mycolic acids to the periplasmic area during mycobacterial cell wall synthesis (Chang and Guan, 2021). Studies have reported that Q109, a 1,2-diamine, structurally similar to ethambutol, inhibits the activities of MmpL3 (Borisov et al., 2018). Another lipid transporter that is a therapeutic target of interest is the lipoarabinomannan carrier protein LprG, which is responsible for the export of triglycerides and lipoglycans to the mycomembrane. *In vivo* studies have demonstrated the significance of LprG in *M. tb* infection, highlighting its relevance as a therapeutic target for the treatment of MDR-TB (Martinot et al., 2016). Fatty acids adenylate enzymes (FadDs), fatty acid and metabolite degradation, and isocitrate lyases are some of the other lipid metabolism targets. Several investigations have indicated that compounds including 3-bromopyruvate, phthalazines, 3-nitropropionate, hydrazones, and 5-nitro-2,6-dioxohexahydro-4-pyrimidinecarboxamides inhibit isocitrate lyases in the lab. Although these compounds have not yet been evaluated clinically (Chang and Guan, 2021).

3 Subtractive genomics approaches in drug target discovery

Subtractive genomics paves the way for novel and distinct drug targets thanks to advances in computational biology and bioinformatics techniques. The subtractive genomics approach analyzes the genomes of the pathogen and its host, prioritizing the pathogen's unique and essential genome as a drug target (Uddin and Saeed, 2014). This approach identifies genes that are not present in the host, referred to as "non-host" genes, yet are necessary for the pathogen's growth, replication, and persistence. Furthermore, these non-host genes are essential and play significant roles in the pathogen's metabolic pathways. As a result, when therapeutic compounds reach the pathogen's metabolic targets optimally, the compound must alter the pathogen's metabolic activity without affecting the host biology. This could result in the disruption of essential gene function/s of the pathogen thereby terminating its pathogenicity. Several studies have applied this approach to identify potential drug targets in *Pseudomonas aeruginosa* (Uddin and Jamil, 2018), *Acinetobacter baumannii* (Uddin et al., 2019), and others (Ahmad et al., 2019; Nayak et al., 2019; Prabha et al., 2019). These computational studies target only essential genes of the pathogen and reduce experimental efforts, saving time and cost.

3.1 Metabolic pathway analyses in target identification

Reconstructed metabolic modelling has proven to be a valuable tool for understanding *M. tb* metabolism by combining data from an experimental model with omics technologies. High-throughput omics data has aided in the simulation of bacterial metabolic pathways, the formulation of hypotheses, and informing drug development (López-Agudelo et al., 2020). BioCyc (Karp et al., 2019) and KEGG (Kanehisa, 2017) databases contain draft metabolic reconstructions for *M. tb*, which comprise metabolism, biosynthesis, biodegradation, and information processing pathways. This compendium can be used during the target identification phase of the prioritization process to rank proteins that play crucial metabolic roles or function as key intermediaries in several pathways. For instance, Gupta et al. (2019) carried out a KEGG metabolic analysis of host-pathogen pathways of *Leptospira* and mapped sixteen pathways that are unique to the pathogen, eight of these sixteen pathways were specific for the viability of 15 strains of *Leptospira* and shared common drug targets.

Exploring the *M. tb* metabolic pathway as an entity (holistically) and not as an individual pathway to identify choke point reactions is a crucial step to elucidating *M. tb*-specific targets responsible for bacterium development, growth, survival, and infection.

3.2 Choke point reaction in target identification

In the metabolic network, a choke point reaction is a biochemical process in which a unique substrate is used up in a reaction to produce a unique product which is further used up in other reaction(s) and not a dead-end metabolite. Targeting choke point enzymes may induce pathogen death by causing cell toxicity due to the build-up of specific metabolites in a pathway thereby disrupting essential cell function (Yeh et al., 2004). Identifying choke point reactions using the conventional method may not be entirely impossible but difficult and time-consuming. However, the reconstructed metabolic pathway in databases such as KEGG and BioCyc contains the updated metabolic pathways of pathogens. Kaur and colleagues predicted phoB, ompR, rstA, cusR, and ddl as putative drug targets in *Acinetobacter* baumannii using this approach (Kaur et al., 2021). In *Leptospira*, *in silico* metabolic choke point analysis identified thiL and cobA as promising therapeutic targets (Gupta et al., 2019).

Targeting choke point reactions in the metabolic network could be a game-changer in the fight against drug resistance *M. tb*. These unique targets (enzymes) will not suffer the same (mutation) fate as other drug targets as there is no alternative reaction or pathway to these targets. This hypothesis is predicated on the assumption that existing pharmacological targets are considerably less likely to represent choke points in the metabolic network.

3.3 Target prioritization by gene essentiality

The availability of mycobacteria genome sequences and pathway databases enables study in which metabolomics and bioinformatics techniques can be coupled to identify essential genes, resulting in an accelerated discovery of viable therapeutic targets. Experimental approaches including gene knockouts, saturation transposon mutagenesis, and RNA interference, scattered shortgun, genetic imprinting are some of the methods used to identify essential genes. The abundance of data from these experiments drives the construction of many essential gene databases (Zheng et al., 2015). In the case of *M. tb*, essential genes have been identified experimentally using high density mutagenesis (Sassetti et al., 2003) and saturation transposon mutagenesis (DeJesus et al., 2017). The proteins encoded by essential genes may exhibit lethal phenotypes in *M. tb* upon inactivation or deletion, making these gene products excellent targets for drug development.

Targeting and inhibiting the essential genes or proteins of the *M. tb* metabolic pathway will produce the desired bactericidal effect. Flux balance analyses, comparative genomics, and machine learning are computational tools that facilitate the identification of essential genes *in silico* and are fast and less expensive than experimental methods (Peng et al., 2017). Different online tools have been developed, and they include the Database of Essential Genes (DEG) (Zhang et al., 2004) and Online Gene Essentiality (OGEE) (Chen et al., 2016), Mycobrowser (Kapopoulou et al., 2011) are all platforms for identifying and prioritizing essential therapeutic targets in many species, including *M. tb*. These online resources include some of the experimentally determined important genes. Cloete et al. (2016) used metabolic pathway mapping and the Tuberculist webserver to prioritize 17 potential targets that were essential for the survival of *M. tb* out of the 39 potential drug targets that were identified through literature mining and the TB Structural Genome Consortium (TBSGC). To automate the process of *M. tb* drug target identification, Hasan et al. (2006), created an online server that prioritizes pathogen unique and growth essential genes to generate a list of *M. tb* specific targets.

Once the set of essential genes has been identified, viable drug targets can be prioritized further by utilizing various methods such as druggability analysis, protein-protein interactome, and sequence structural analysis, as most essential targets are predicted to be evolutionarily conserved.

3.4 Protein-protein interactions (PPI) for target prioritization

Protein sequences are made up of amino acids, which are the fundamental key component of life. Genes code for amino acids, which form peptides, peptides then form various proteins, and proteins produce biological systems. Furthermore, proteins play a significant role in biological activities such as catalysis, transport molecules, immunological responses to various pathogens, and cell-to-cell signaling (Lu et al., 2020). Protein-protein interactions (PPI) form networks that convert a variety of chemical inputs into physiological responses that keep intracellular homeostasis in balance (Cong et al., 2019).

In cells, PPIs form an intricate network known as an "interactome." The interactome plays a function in a variety of physiological and pathological processes, including cell differentiation, cell growth, signal transduction, cell proliferation, and apoptosis (Lu et al., 2020). Several human diseases, including cancer, infectious diseases, and neurodegenerative diseases, are consequently associated with aberrant PPIs (Rosell and Fernández-Recio, 2018).

Proteins involved in PPIs offer novel therapeutic targets because the most frequent pharmacological targets are enzymes, ion channels, and receptors. PPIs have attracted a lot of interest recently and have become attractive therapeutic targets (Lu et al., 2020). For instance, the NusB-NusE PPI, plays a crucial role in facilitating the formation of stable antitermination complexes. These complexes, in turn, enable consistent RNA transcription in all bacteria (Cossar et al., 2020). The pharmacophore screening of mini-Maybridge chemical library, which contained 56,000 compounds identified *N*,*N*′-[1,4-butanediylbis(oxy-4,1-phenylene)]bis(*N*-ethyl) urea as a hit compound. Furthermore, competitive enzyme-linked immunosorbent assay (ELISA)-based screening validated the compound as a potent (20 μM) inhibitor of the NusB-NusE PPI in *Escherichia coli* (Cossar et al., 2017).

In a PPI network, the protein represents the node while interactions between two proteins and more represents the edges. Proteins with high number of edges with other proteins are said to be functionally active and are potential drug target because the deactivation of such protein could lead to loss of connectivity in the network topology which could cause cell function disruption (Uddin and Jamil, 2018). PPIs within the interactome have been identified using a variety of physiochemical experimental techniques such as yeast two-hybrid (Y2H), DNA and protein microarray and mass spectroscopy (MS) (Shoemaker and Panchenko, 2007). However, these technologies are costly and time-consuming. In the post-genomic era, computational techniques is preferred for PPI determination as it is cost-effective and faster (Wang et al., 2020).

A range of methodologies, such as sequence-based approaches, structure-based approaches, chromosome proximity, gene fusion, and gene expression-based approaches, have been used to develop *in silico* methods for predicting PPI (Dong et al., 2019). The Database of Interacting Proteins (DIP) (Xenarios et al., 2000), the Biological General Repository for Interaction Datasets (BioGRID) (Stark et al., 2006), and STRINGv11 are all available databases for PPI. Uddin and Jamil (2018) prioritized drug targets in *Pseudomonas aeruginosa* using protein-protein interaction network. With the aid of the STRING database v10.5, the study identified 8 out of 18 protein as putative drug targets as they are classified as hub proteins with high number of interactions coupled with their involvement in metabolic pathways.

Over the last decade, the continuous research efforts in PPI drug development have produced five small-molecule PPI modulators approved by the U.S. Food and Drug Administration (FDA) for clinical use in the treatment of cancer, dry eye syndrome, autoimmune illnesses, and as immune suppressants with more in various stages of clinical development (Cossar et al., 2020). Comparatively, the use of PPIs as a target for the discovery of antibacterial drugs is still in its infancy.

3.5 Druggability analysis for target prioritization

Target validation has thus become an important part of drug development in recent years. It is well known that traditional target validation methods investigate the connection between modifications in protein biological functioning and pharmacological outcomes. Owing to the lack of identifiable ligand-binding pockets and non-catalytic PPI functionalities, therapeutically relevant pharmaceutical targets that are difficult to drug or have not yet been drugged using conventional procedures are referred to as "undruggable" targets (Zhang et al., 2022). The conventional drug discovery process is time-consuming and expensive, with a staggering failure rate of about 96 percent in different drug development endeavours because of the "undruggability" of many identified protein targets, among other issues (Hingorani et al., 2019). Druggability is the ability of a protein to bind a drug-like molecule, which then modifies its activity in a "desired" manner. Differential scanning fluorimetry and X-ray diffraction data are methods used in experimental druggability studies to discover binding sites on protein crystals. Although the experimental method is a dependable and validated approach, crystalline protein is needed (Handing et al., 2018; Michel et al., 2019). The use of computational techniques serves as an alternative to the experimental method to determine whether any protein with a known three-dimensional structure is druggable and whether or not small drug-like compounds have the inherent ability to bind to and change the functions of proteins (Agoni et al., 2020).

A protein that can bind to drugs (druggable protein) should have a clearly defined pocket with the necessary physicochemical characteristics. Several computational techniques have been developed to evaluate a target's druggability based on the availability of the 3D structure of a protein. The structural properties of proteins, such as surface polarity, surface hydrophobicity, and pocket size, are considered by several of these methods within the pocket prediction algorithms of webservers like, DOGSiteScorer (Volkamer et al., 2012),

Metapocket (Huang, 2009), and PockDrug Server (Hussein et al., 2015). Several software programs and online platforms have been created to assist in the accurate identification of binding pockets, including SiteMap (Halgren, 2009), Sitehound (Hernandez et al., 2009), and Open Targets (Koscielny et al., 2017).

These online resources offer vital information on binding pockets that can determine if a biological target is druggable. To investigate the druggable pockets of interleukin-4R with a shallow pocket and no known inhibitor, Naz et al. (2019) used the DOGSite software where seven druggable sites were identified by the software. The site with the highest score (0.81) represents the location with the highest tendency to bind small molecules. Furthermore, in the analysis of active sites of 82 protein tyrosine phosphatases (PTP) structures using SiteMap, it was found that 18 PTP structures in the open conformation had a higher druggability score (0.79) than the 64 PTP structures in the closed conformation (D-score = 0.69). This finding suggests that while the closed conformation can interact with small organic molecules, it is less able to do so with drug-like molecules than the open conformation (Ghattas et al., 2016). More importantly, some of these compendia (software and online resources) can predict cryptic and allosteric sites on protein structure while some are unable to because most active sites are predicted by sequence homology which makes allosteric sites difficult to detect because evolutionary pressure for sequence conservation is lower and if at all present in allosteric sites that is, allosteric sites are less evolutionary conserved across protein families than active sites (Chatzigoulas and Cournia, 2021).

3.6 Non-ortholog protein identification

The identification of non-orthologous genes is crucial because it minimizes drug cross-reactivity by preventing the drug from interacting with the human host's homologous protein. Orthology is defined as variables and the relationship in the makeup of genomes from distinct species. Orthologues can be traced back to an ancestral gene found in the common ancestor of the species under consideration (Wolf and Koonin, 2012). Gene orthology is fundamental to evolutionary, comparative, and subtractive genomics (Li et al., 2003). Biologists frequently rely on orthology to transfer functional knowledge from experimentally described genes in model species to unknown genes in newly sequenced genomes (Doyle et al., 2010). The validity of such transfer of functional annotation is premised on the idea that orthologues perform equivalent tasks in other organisms, or more specifically, equivalent functions that are biologically related (Doyle et al., 2010). Anti-bacterial drugs need to selectively inhibit or kill the pathogen while remaining non-toxic to the host (Shanmugham and Pan, 2013). Consequently, focusing on the bacterium genes lacking host orthologues will aid in drug target prioritization.

The BLAST is an online resource that helps in identifying orthologue genes between the host and pathogen. This resource relies on the expectation value of alignment, sequence similarity, and query coverage to infer orthology (Hussain et al., 2020). Uddin et al. (2020), performed a BLASTp search against the non-redundant database of *H. sapien* with an anticipated threshold of 0.005 to prioritize therapeutic targets in *Mycobacterium avium* subsp. *Hominissuis*, where proteins with keywords "no hits found" were maintained for further analysis, and proteins that are orthologues in the host proteome were discarded. Likewise, in a study by Cloete et al. (2016), nine putative drug targets were prioritized out of 17 in *M. tb* that are non-orthologues to human proteins using the NCBI Blastp algorithm with an expectation-value of 0.0005.

4 Conclusion

Tuberculosis continues to be a public health concern. Antibiotic overuse, chromosomal gene mutation together with patient noncompliance resulted in the development of resistance. MDR-TB is on the rise due to mutation acquisition in known *M. tb* drug targets and is becoming challenging to treat with available medications. As a result, novel targets and newer medications are required. Several studies have demonstrated the importance of metabolism in *M. tb* development, growth, survival, and infection. Therefore, targeting the *M. tb* metabolic network is an effective way to uncover new anti-TB medication targets using a subtractive genomic analysis approach. The subtractive genomic approach may be effective in the development of novel anti-TB drugs since it can be used to discover viable, *M. tb*-specific therapeutic targets that will not suffer the same fate as other targets. In addition, the approach highlighted in this review can be utilized to identify therapeutic targets in other pathogens.

Author contributions

AA: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing–review and editing, Writing–original draft. SE: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing–review and editing. RC: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing–review and editing, Supervision.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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