GMR
The Original

UNVEILING THE PROTEOMIC LANDSCAPE OF MOLECULAR MECHANISM IN MULTIDRUG-RESISTANT TUBERCULOSIS: A PROMISING APPROACH FOR NOVEL TREATMENT STRATEGIES

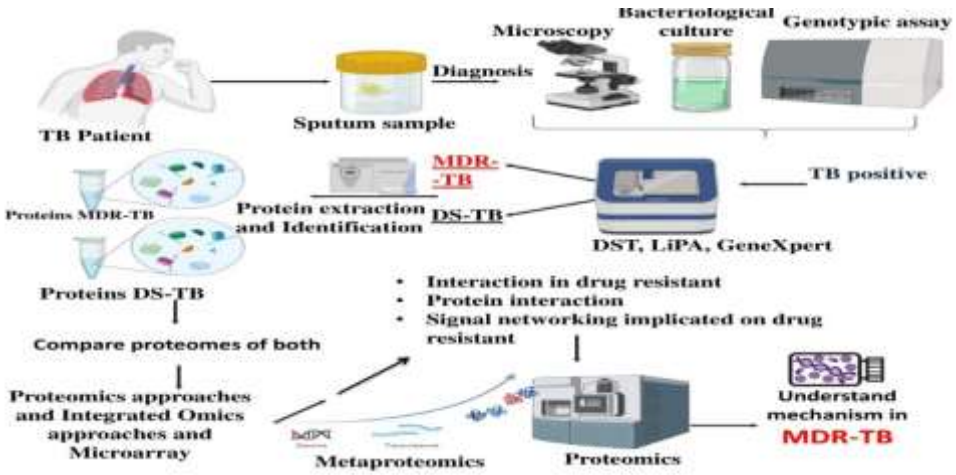
Umesh Kumar[1], Dr. Abhishek Bansal[*2], Dr. Narinder Kaur[3]

[1]Ph.D. Scholar, Department of Biochemistry, Maharishi Markandeshwar Institute of Medical Sciences and Research (MMIMSR), Mullana, Ambala, Haryana, India, Email: Umeshgothwal98@gmail.com
[2*]Assistant Professor, Department of Biochemistry, Maharishi Markandeshwar Institute of Medical Sciences and Research (MMIMSR),Mullana, Ambala, Haryana, India, Email: abhishek.bansal@mmumullana.org
[3]Professor & Head, Department of Microbiology, Maharishi Markandeshwar Institute of Medical Sciences and Research (MMIMSR), Mullana, Ambala, Haryana, India

ABSTRACT
Multidrug-resistant tuberculosis (MDR-TB) is a significant obstacle to global health, requiring innovative approaches for its management and treatment. Proteomics is a very effective method for thoroughly examining the proteins in a cell and understanding their activities. It has become a promising approach for uncovering the molecular pathways concomitant with treatment resistance in Mycobacterium tuberculosis (Mtb). This review examines the modern proteome methodology used to investigate multidrug-resistant tuberculosis (MDR-TB), with a specific emphasis on improvements in mass spectrometry-based techniques, high-throughput proteomic technologies, and integrated omics approaches. Mass spectrometry enables the identification and measurement of proteins, allowing for the comparison of drug-sensitive & drug-resistant Mtb strains. High-throughput proteomic technologies, including protein microarrays and interactomics, provide insights into protein-protein interactions and signaling networks implicated in drug resistance. Integration with multi-omics data sets, such as proteogenomics and Metaproteomics, enhances our understanding of the molecular mechanisms underlying MDR-TB. Furthermore, proteomic studies have identified potential druggable targets and repurposable compounds for overcoming drug resistance in TB. This comprehensive review highlights the significance of proteomics in deciphering the complexities of MDR-TB and underscores its potential for guiding the development of personalized treatment strategies.

KEYWORDS: Tuberculosis, multidrug resistance, proteomics, mass spectrometry, drug resistance pathways, high-throughput proteomics, proteogenomics, metaproteomics, personalized medicine.

Highlights
- Proteomic techniques that integrate omics data to elucidate the complex pathways involved in multidrug resistance mechanisms in tuberculosis.
- Identification of novel protein targets associated with drug resistance pathways, paving the way for developing more effective targeted therapies against tuberculosis.
- Utilization of advanced proteomic technologies to develop phenotypic screening platforms for high-throughput identification of compounds that disrupt drug resistance mechanisms in tuberculosis.



Proteomics approaches to reveal the mechanism of MDR-TB

INTRODUCTION

Globally, TB is becoming an even more significant malady due to the emergence of Mycobacterium TB strains that are resistant to several drug treatments [1]. Despite several efforts to control the disease, public health systems worldwide are extremely concerned about the rising incidence of multidrug-resistant tuberculosis (MDR-TB) [2]. Scientists are looking beyond the current diagnostic and treatment landscape to uncover novel techniques to identify and treat multidrug-resistant tuberculosis (MDR-TB) infections [3].

Proteomics has grown into a crucial tool in TB research, shedding light on the intricate relationship amongst Mycobacterium tuberculosis & the human immune system, along with the processes that cause treatment resistance [4-6]. Proteomics methods provide a thorough examination of protein expression patterns to discover possible biomarkers & therapeutic targets related to multidrug-resistant tuberculosis (MDR-TB). [7, 8].

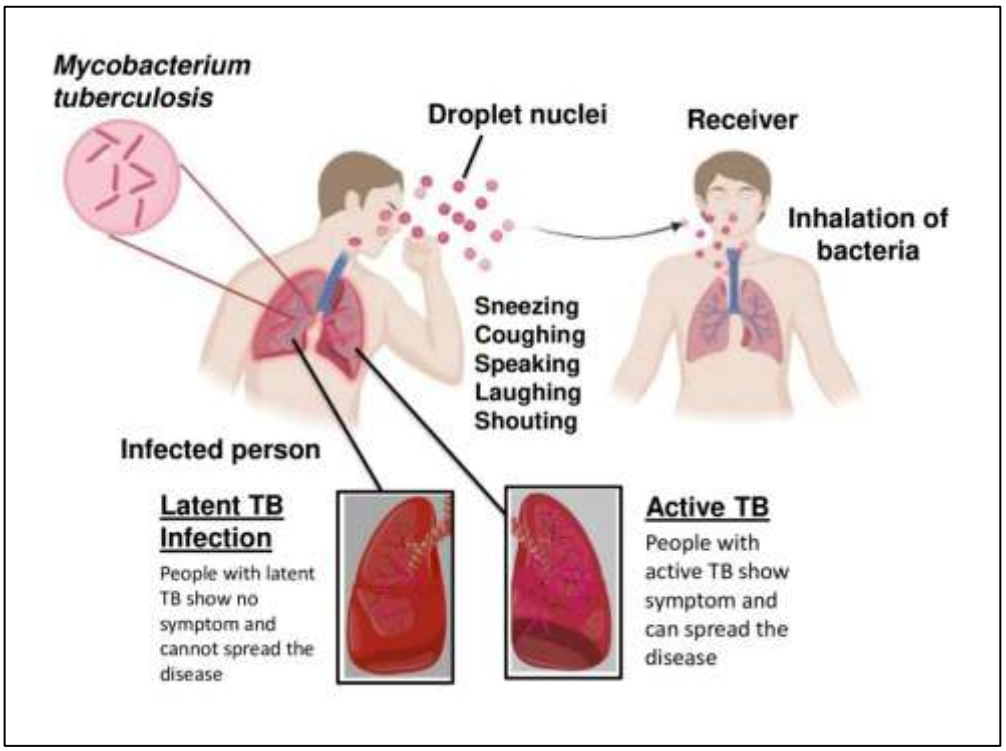


Figure 1 : Transmission of Tuberculosis and type of TB

Proteomics is an effective method for large-scale protein identification of biological materials that are complicated [9]. Proteomics is a technology that has been used to investigate the protein expression profiles of Mycobacterium tuberculosis under various growth conditions, geographic dispersion, genetic backgrounds [10–12]. Researchers have specifically demonstrated that when comparing susceptible & monodrug-resistant strains of M. tuberculosis, 2-DE combined with MS can discover resistance-associated proteins. Global proteome studies of susceptible and multidrug-resistant bacteria's protein profiles may afford additional visions into the mechanisms of resistance & biomarkers linked to multidrug resistance [13, 14]. To develop better drugs, a vaccine, and maybe a method for quickly identifying tuberculosis that is resistant to numerous therapies [15, 16]. Proteomic analysis might prove to be useful as the search for MDR-TB biomarkers progresses. Comparing susceptible Mycobacterium tuberculosis isolates to ofloxacin-resistant isolates, the former showed greater concentrations of 14 proteins [17]. Understanding the makeup of proteins may therefore help clarify the mechanisms underlying multidrug-resistant tuberculosis and open the door to novel approaches to its diagnosis. A subfield of proteomics that focuses on naturally occurring protein fragments, peptidomics has grown in popularity recently [18, 19]. This field is increasingly being used in the study of disease, particularly in monitoring, diagnosis, treatment, and the identification of disease biomarkers. The low-molecular-weight proteome may contain options that have not been discovered before but would be worthwhile investigating. Patients with three different solid tumour types were distinguished from healthy controls using a specific pattern of serum peptides. Peptidoglycanomics also aids in the identification of endogenous peptides specific to ovarian cancer, which is useful for searching for biomarkers.

1.1 Tuberculosis: A Global Health Challenge

Tuberculosis (TB) is the second most deadly contagious illness in 2019 (COVID-19) & the thirteenth prominent killer globally, despite being treated and preventive [20–22]. Although tuberculosis (TB) has been decreasing over the past few decades, the international health community has not yet succeeded in eliminating TB due to the occurrence of COVID-19 & ongoing administrative skirmishes, such as the conflict in Ukraine, which have put healthcare service delivery & TB control initiatives in jeopardy [23]. TB, a deadly infectious disease that continues to rank highly among infectious disease killers worldwide, particularly in developing countries, is caused by Mycobacterium tuberculosis [24, 25]. An estimated 8.6 million people acquire TB infections annually. Isoniazid (INH) & rifampicin (RIF), the two utmost current 1st-line anti-TB drugs, are ineffective against bacteria that are resistant to drugs (MDR-TB) [26]. Multidrug-resistant strains of tuberculosis are becoming more common; 3.6% of newly diagnosed cases worldwide involve them [27]. The WHO [28]

reports that multidrug-resistant tuberculosis (MDR-TB) is present in almost every nation surveyed. 480,000 new instance of MDR-TB were recorded globally in 2013. India is undoubtedly a major actor in the tuberculosis epidemic, accounting for over 25% of global infections and deaths [29]. Approximately 25% of global tuberculosis incidence cases are reported to arise in India, the 2nd most famous country in the globally. Based on the Revised National TB Control Programme RNTCP-TB report [30], 2.2% of new multidrug-resistant tuberculosis cases and 15% of retreated MDR cases were reported from India in 2016. Infection with Mycobacterium tuberculosis has been linked in certain countries to a horrendous and ongoing increase in death rates. In 2021, there are expected to be 10.6 million novel TB cases, 1.6 million deaths from the disease, & 1.7 billion latent infections. TB is a global issue, but it is the eighth biggest killer in low-income countries and the seventh in middle-income countries, which excuse for 80% of TB cases. In 2020, Africa had 39% of TB infections, with South and Southeast Asia accounting for 43%. In terms of health, the WHO oversees these two sectors [32]. Furthermore, about 450,000 new instances of MDR-TB, or rifampicin-resistant tuberculosis, are reported worldwide each year, with 78% of those cases being MDR-TB. Of the cases of tuberculosis that have recently been recorded, 38% in Russia, 35% in Moldova, and 33% in Belarus are MDR-TB cases. One-third of all new cases of tuberculosis are MDR-TB, with 29% occurring in Kyrgyzstan and Tajikistan and 27% in Kazakhstan and Ukraine. These countries follow closely behind. MDR-TB, which accounts for less than 5% of tuberculosis cases, has not changed over the past few years despite still being a serious problem. Finally, it should be noted that 8% of tuberculosis infections globally are associated with HIV; 75% of these cases arise in Africa, and cases from Russia and Ukraine are also rather common. India has joined the World Health Assembly's objective for sustainable development and the worldwide "End TB Strategy" in the fight against tuberculosis. The objectives include eliminating the catastrophic costs brought on by tuberculosis, as well as lowering the disease's incidence and reducing related mortality by 75% by 2025, 90% by 2035, and 95% by 2035 [33].

Challenges

The TB affliction has been dropping at a rate of 1.5-2% each yr. in recent years [34]. A variety of factors contribute to this poor speed. A variety of factors, including a huge pool of latent tuberculosis infections, ageing populations around the world, insufficient and delayed case diagnosis, low cure rates, and treatment resistance, all contribute to tuberculosis' slow decline in occurrence. In addition, socioeconomic factors are highly linked to tuberculosis. People with HIV/AIDS, diabetes, malnutrition, alcoholism, drug and tobacco use, migratory status, incarceration, ethnic minority, and marginalised groups are among the most vulnerable, as are those who live in overcrowded, poorly ventilated, and economically disadvantaged conditions. Tuberculosis (TB) rates are lower in countries with higher GDP, but higher in nations with higher levels of under nutrition [35]. Political disagreements and the current pandemic are two further major disruptive events that are greatly reducing the reduction of the tuberculosis load.

- **The three layers**

The factors influencing the burden of tuberculosis (TB) can be divided into three categories: those occurring surrounded by coast-to-coast TB programmes, those occurring within the general health sector, and those occurring outside of health. Sectors that successfully address discrimination, marginalisation, poor housing, and malnourishment address the latter level (figure 2). It is imperative to use a multi-sectoral approach to eradicate tuberculosis, which involves all pertinent stakeholders, including public and private institutions, community engagement, and survivor associations [36]. The numerous variables that lead to tuberculosis (TB), the health care system overall, and other areas of development aligned with the Sustainable Development Goals (SDGs) must all be considered when formulating this approach. The Western Pacific Regional Framework to End Tuberculosis (TB) identifies three levels of tuberculosis interventions: wellbeing structure base (strong policies), health afar health, and fundamental TB functions [37]. In this approach, classification reflects the global health strategy, which expects non-health sectors to be involved in order to achieve a health outcome, such a reduction in the burden of tuberculosis.

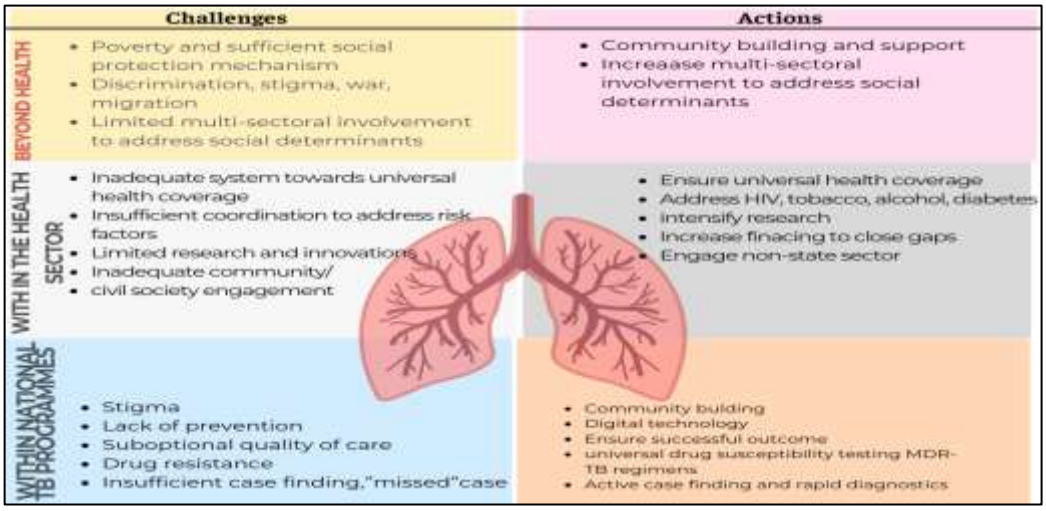


Figure: Challenges and actions on Tuberculosis

1.2 Multidrug Resistance (MDR) in Tuberculosis

The most common causes of MDR-TB among drug-sensitive tuberculosis patients include ineffective drug delivery,

irrational treatment regimens, and anti-TB medication usage [38]. Less than 10% of MDR-TB patients in China receive a diagnosis, & only 19.1% of MDR-TB cases all over the world do. The majority of MDR-TB patients do not acquire a diagnosis within a timely timeframe. As a result, early detection is critical for managing multidrug-resistant TB. Multidrug-resistant TB is identified using phenotypic (conventional) and molecular (genotypic) diagnostic methods. Drug susceptibility testing (DST) is the supreme trustworthy tool for detecting multidrug-resistant tuberculosis (MDR-TB) & this is important component in creating treatment strategies for this disease [39]. Unfortunately, the DST approach's sluggish detection rate and extensive procedure make early diagnosis and therapy impractical. Although false positives are common with microscopic-observation drug susceptibility (MODS) testing, it can reduce the time it takes to culture tuberculosis to one or two weeks [40–41]. Despite its high cost, the fluorophage approach produces results that are identical to those obtained from direct sequencing of first-line anti-TB medicines, and it does so fast. "While mutations in the targeted gene contribute to MDR-TB, the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach is restricted in its ability to detect these alterations". Certain mutations in target genes are widely believed to be the source of tuberculosis treatment resistance [42-45].

Figure 2: "Type of drug resistance"

Nonetheless, some drug-resistant clinical Mycobacterium tuberculosis isolates do not carry mutations [46-48]. According to various pieces of evidence, antibacterial drugs can target a wide range of proteins and enzymes in Mycobacterium tuberculosis [49, 50]. Thus, different pathways may be involved in medicine resistance. Other possible paths for drug resistance in mycobacteria include the creation of enzymes that modify or inactivate medicines, a low cell wall permeability (which restricts medication influx), and efflux mechanisms [51].

2. DIAGNOSIS OF MDR-TB

- **Conventional methods**

Lowenstein-Jensen (LJ) cultures have historically been used to evaluate drug sensitivity using three different techniques: (i) "the proportions technique," (ii) "the resistance ratio method, and" (iii) "the absolute concentration approach" [52]. When following the conventional methods, the results of the sensitivity test are not known for 6–8 weeks. To find the drug's minimal inhibitory concentration (MIC), the absolute concentration approach inoculates the drug-containing (fig.4) and control media with a well calibrated Mycobacterium TB inoculum [53, 54]. A two-fold sequential technique is used to dilute the medications in medium. After four weeks, if there are 20 colonies or more, the minimal concentration of drug required to suppress growth has been reached, showing resistance. The minimum inhibitory concentration (MIC) of the isolate is multiplied by the MIC of a susceptible standard strain that is also acquired at the same time in order for the resistance ratio approach to account for variations between laboratories. Due to the strict control required for the inoculum size in these two processes, unswerving thoughtfulness trying from rigorous medical samplings is not recommended. The proportions technique may be used to determine the percentage of drug-resistant bacilli in a bacterial community by comparing the no. of colonies on a drug-containing mediocre to those on a drug-free media. A strain is considered sensitive if its fraction falls below a certain threshold, and resistant if it exceeds that level.

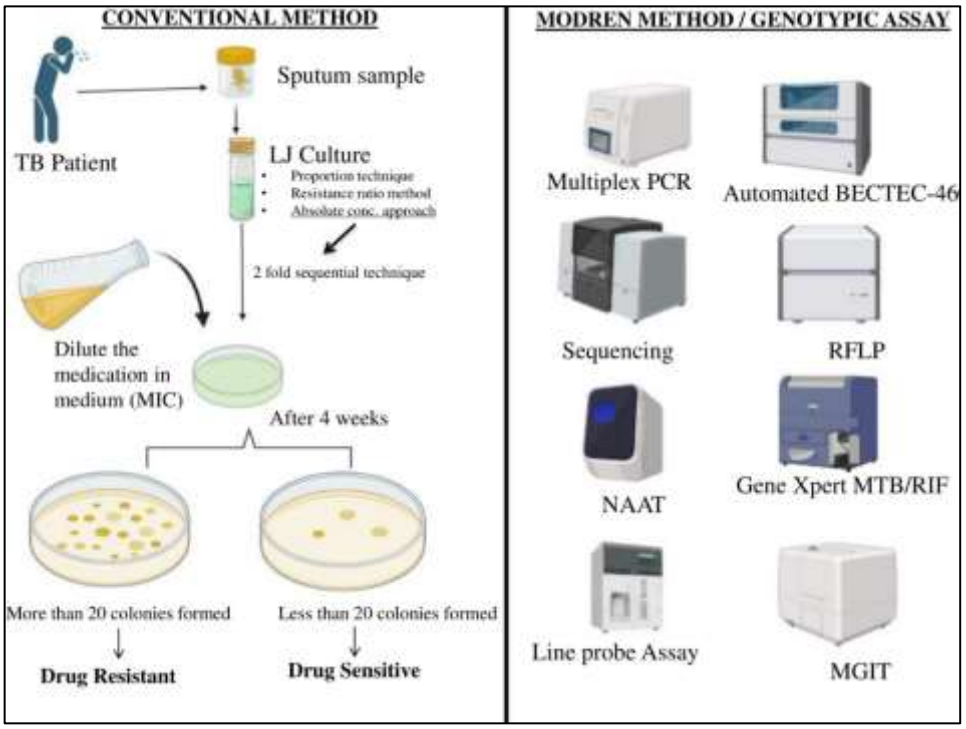


Figure 3 MDR-TB diagnosis conventional and modern methods

Modern methods

Radiometric techniques have made it possible to swiftly and precisely screen for medication susceptibility of Mycobacterium tuberculosis. The radioactive compound 14C-palmitic acid, which is tagged with palmitic acid, is added to 7H12 medium as part of the Beckton-Dickinson-developed BACTEC-460 radiometric method. Measuring the quantity of radioactive carbon dioxide (14CO2) that mycobacteria produce during the breakdown of these fatty acids is one technique to monitor their development. The proportions approach has been modified to substitute the BACTEC procedure for the conventional Lowenstein-Jensen culture. In roughly ten days, the revised sensitivity results ought to be available.The Becton-Dickinson mycobacteria growth indicator tube (MGIT) technology is used to rapidly and safely detect and test for Mycobacterium tuberculosis susceptibility [55]. The MGIT structure uses an oxygen-sensitive fluorescent component & a silicone plug to contain the medium in order to detect mycobacterial development. After a sample of mycobacteria is added to the medium, the bacteria can multiply and use the chemical and oxygen to produce fluorescence. An UV transilluminator can be used to detect the fluorescence that results. Both directly clinical and cultured samples showed that the BACTEC and proportions approaches yielded comparable results.

The classification and comparison of Mycobacterium tuberculosis isolates has been done using restriction-length polymorphisms (RFLPs) in mandate to have a healthier accepting of the genetic epidemiology of tuberculosis strain TB61. This technique is used to isolate genomic DNA from grown bacilli. At base pair 461, restriction endonucleases like as PvuII cleave elements. “DNA fragments are transferred from an electrophoresis gel to a membrane during the southern blotting process”. Using a tagged DNA probe for hybridization and detection is one of the next steps. With X-ray film, biometric fingerprints can be made; these fingerprints display a series of bands that match the DNA of a certain mycobacterial sample. The quantity and spacing of insertion sequences determine the banding pattern, which displays the no. & location of copies of the 1361 base pair attachment system IS6110 inside the chromosomes. Moreover, drug-resistant Mycobacterium tuberculosis germs retain the same DNA fingerprints, according to RFLP research, which means that it is possible to track the bacteria's progress. The molecular epidemiology of Mycobacterium TB may be more comprehensively comprehended by using IS6110 RFLP type in combination with fluorescence amplified fragment length polymorphism (FAFLP) analysis, as shown by recent study. The fundamental basis of the ligase chain reaction (LCR) is the DNA ligase enzyme [57]. This enzyme facilitates the maintenance of two DNA strands as a double helix by connecting them. This procedure can only occur when both ends are matching & identical, since it has the ability to detect even the smallest differences, such as a single nucleotide.. The luciferase reporter assay is one novel reporter gene assay method that can identify medication resistance fast [58]. The luciferase gene, which codes for an enzyme used by fireflies to produce light, is where it all begins. It reacts with luciferin and releases light in the presence of adenosine triphosphate (ATP). The luciferase gene has been introduced into mycobacteriophages. Mycobacterium tuberculosis receives the phage DNA, and once this mycobacteriophage attaches itself to it, viral genes are generated. Mycobacterium tuberculosis-infected luciferase reporter phage organisms can be exposed to antituberculosis drugs, which facilitates the investigation of susceptibility by establishing a correlation between light generation and traditional testing methods. Most strains might be detectable with this approach in 48 hours. The FASTPlaqueTB-RIF test, which utilizes bacteriophages, enables a more expedited detection of multidrug-resistant tuberculosis (MDR-TB). Employ this test to ascertain the susceptibility of clinical strains of TB, cultivated in a semi-automated liquid culture using the bactec-460 method, to rifampicin. Scientists have using polymerase chain reaction (PCR) centered sequencing to investigate the underlying causes of treatment resistance in some mycobacteria. This method can detect any kind of mutation. PCR-based approaches are unsuitable for routine detection of drug resistance mutations due to the need of several sequencing operations on each isolate. However, for targets such as

rpoB, where changes in rifampicin resistance are found in a limited region of the gene, PCR-based sequencing is a very efficient technique. The LiPA test, developed by Inno-Genetics NV in Zwijndrecht, Belgium, is a fast and efficient method for detecting rifampicin resistance. The LiPA methodology employs the converse hybridization process, in which a primer is used for the polymerase chain reaction (PCR). This process entails modifying the structure of biotinylated PCR amplicons and combining them with capture probes that are linked to a nitrocellulose strip. The captured amplicons are then identified using a colorimetric response. Analyzing the banding pattern on the strip allows for the identification of rpoB mutations and the M. tuberculosis complex. The use of DNA microarray technology has facilitated the prompt identification of mutations associated with treatment resistance in tuberculosis [59–61]. Initially, this method was designed to differentiate between different types of mycobacterial species.

3. NOVEL PROTEOMIC TECHNIQUES AND TECHNOLOGIES MDR IN TUBERCULOSIS

Novel proteomic techniques and technologies have revolutionized our understanding of multidrug resistance (MDR) in tuberculosis (TB) by enabling comprehensive profiling of the proteome of Mycobacterium tuberculosis (Mtb), the connective agent of TB [62-64]. These advanced methodologies provide intuitions into the molecular mechanisms fundamental drug resistance, detect potential biomarkers, and uncover novel therapeutic targets. Here, we delve into detail about some of the key proteomic techniques and technologies employed in studying MDR in TB:

Mass Spectrometry-Based Proteomics: "Mass spectrometry (MS)" lies at the heart of proteomic analyses, enabling the identification and quantification of proteins within a biological sample [65]. In the context of MDR-TB, MS-based proteomics facilitates the comparative analysis of protein expression profiles between drug-sensitive and drug-resistant strains of Mtb. Several MS-based approaches are utilized:

Shotgun Proteomics: In shotgun proteomics, proteins are enzymatically consumed into peptides, which are then analyzed by MS. This approach permits for the empathy of a extensive collection of proteins present in a sample & is particularly useful for exploring global changes in the Mtb proteome associated with drug resistance.

Targeted Proteomics: Targeted proteomics techniques, such as selective reaction monitoring (SRM) or parallel reaction monitoring (PRM), allow for accurate measurement of certain proteins or peptides that are of interest. This method is very beneficial for confirming potential biomarkers or tracking the levels of established drug resistance-related proteins.

Label-Free and Isotope Labeling Methods: Label-free quantification methods directly measure the abundance of peptides from MS data, whereas isotope labeling techniques, such as SILAC or iTRAQ/TMT, allow for multiplexed quantification of peptides from different samples. The use of quantitative proteomics techniques is crucial for finding proteins that are expressed differently in drug-sensitive and drug-resistant Mtb strains.

High-Throughput Proteomic Technologies [66]:

Protein Microarrays: Protein microarrays allow for the simultaneous profiling of protein-protein interactions, protein-drug interactions, and protein expression levels. In the context of MDR-TB, protein microarrays can be used to identify novel drug targets or potential inhibitors of drug resistance-associated proteins.

Interactomics: Interactomics methods, such as yeast two-hybrid (Y2H) screening or affinity purification combined with MS (AP-MS), enable the systematic mapping of protein-protein interactions within the Mtb proteome. These studies provide vital knowledge on the molecular networks and signaling pathways involved in drug resistance.

Integration with Multi-Omics Data [67]:

Proteogenomics: Proteogenomic analyses integrate proteomic data with genomic information to refine genome annotations, identify novel protein-coding genes, and validate gene predictions. By correlating protein expression data with genomic mutations or transcriptomic profiles, proteogenomic studies shed light on the genetic basis of drug resistance in Mtb.

Metaproteomics: Metaproteomic approaches explore the functional potential of microbial communities within TB lesions or sputum samples. By characterizing the Mtb proteome within its host environment, metaproteomics offers insights into the host-pathogen interactions and the adaptive responses of Mtb to antimicrobial treatment.

Identification of Druggable Targets and Drug Repurposing:

Proteomic studies have identified potential druggable targets within the Mtb proteome, including enzymes involved in metabolic pathways, membrane transporters, and regulatory proteins [68]. Moreover, proteomic analyses have facilitated the screening of compound libraries for inhibitors of drug resistance-associated proteins, leading to the repurposing of existing drugs for TB treatment.

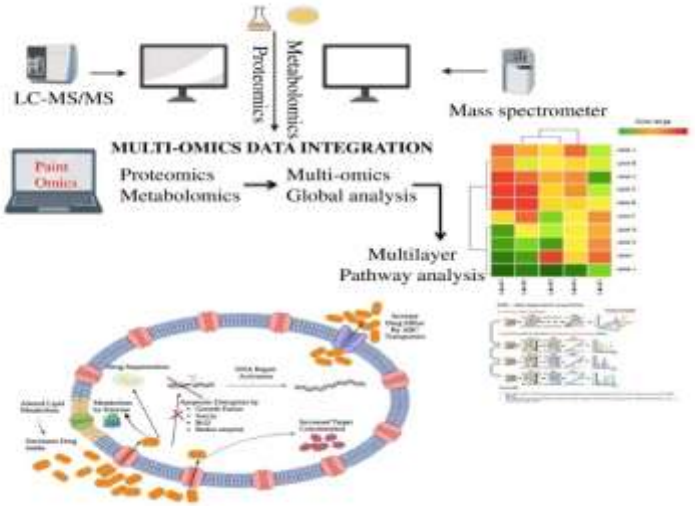


Fig. 2: Proteomics and metabolomics workflow of MDR

4. A MODERN PROTEOMIC AND BIOINFORMATIC ANALYSIS OF MYCOBACTERIUM TUBERCULOSIS

Proteomics and bioinformatics approaches have developed as cutting-edge tools for deciphering the resistome and its known components [69–70]. These reports are based on both discovery-based (expression proteome) and targeted proteomics. Mass spectrometry and two-dimensional gel electrophoresis (2DE) are the gold standard for protein separation and identification in the field of discovery/expression proteomics. These proteins could tragedy a role in virulence or opposition. These potential virulence and resistance components have been extensively investigated in bioinformatics, with studies such as interscan analysis, molecular modelling and docking, pupylation analysis, and protein-protein interactions providing additional evidence of their roles in virulence and drug resistance. Previous research used proteomic and bioinformatic techniques to identify a panel of proteins with putative roles in resistance and virulence; some of these proteins' activities were previously unknown or deemed speculative. Furthermore, these proteins and the pathways that connect them may serve as markers or therapeutic targets in the fight against tuberculosis that has gained resistance. One putative role for proteins in resistance and virulence is to reveal the cell's true status [71]. The first possible application for these proteins is as resistance diagnostic indicators in the future. The potential that these proteins and the mechanisms that regulate them may be therapeutic targets is part of the drug targets strategy for combating resistance. Furthermore, stress proteins, cell wall-related proteins, and membrane-related proteins are among the most important virulence antigens produced in response to stress (such as medicine) and essential for attachment, entrance, and survival in distinct cellular microenvironments. This fatal scenario could be mitigated by finding and producing anti-virulence factors, which are proteins that act as virulence factors [72, 73].

DIAGNOSTIC APPLICATION OF PROTEOMIC BIOMARKERS FOR TUBERCULOSIS

Proteomics has advanced to the point that thousands of proteins may be identified simultaneously, leading to new and more effective approaches to finding tuberculosis biomarkers [74–77]. Recent years have seen the emergence of new proteomic markers for the separation of TB from healthy or disease-free people [78, 79]. This achievement is made possible by the classification of the biomarkers' sources in the human bodily fluids like blood or urine.

5.1 Identification of Biomarkers for Tuberculosis in Human Blood Specimens

Human blood & its derivatives are suitable for TB diagnosis because they are simple, practical, and readily available in large quantities. Furthermore, the host's immune responses to Mycobacterium tuberculosis (Mtb) infection primarily include the transportation of cytokines and other molecules in the circulation. Blood samples are often considered the most dependable and precise approach for proteomic biomarker research focused on identifying TB, due to their many benefits. ESAT6, IP-10 [80], and CD161 have been verified and validated as biomarkers for the detection of Mycobacterium tuberculosis (Mtb) & TB. These markers are produced by either the host or the Mtb. However, it is important to conduct a more thorough examination into other possible biomarkers for diagnosing tuberculosis due to the low probability of detecting leftover traces of Mycobacterium tuberculosis in blood samples.

Developing diagnostic biomarkers for this population is essential in order to decrease mortality rates, since persons who are HIV-positive are disproportionately affected by co-infection with tuberculosis (TB) [81, 82]. The research had 30 individuals from South Africa and 24 people from the United States. The study demonstrated that the plasma host proteins CD14, A2GL, NID1, SCTM1, and A1AG1 were found to be common across individuals from both nations [83]. In contrast to the United States, tuberculosis is quite widespread in South Africa. Afterwards, the scientists used cross-validation to evaluate the diagnostic capacities of the host proteins. The study revealed that panels consisting of 5-12 proteins had a high level of accuracy (AUC 0.74) in both the populations of the United States and South Africa. In addition, these panels demonstrated outstanding precision (AUC 0.93) in predicting tuberculosis (TB) diagnosis 6-12 months before, with a slightly reduced precision (AUC 0.86) as the diagnosis approached. The protein markers AMACR, LDHB, and RAP1B have been discovered as possible biomarkers for tuberculosis (TB) in patients who are HIV-positive. The finding is derived by analyzing 200 plasma samples from persons who are HIV-positive using a data-independent acquisition MS-based

proteomics method [84]. The coexistence of HIV infection often complicates the diagnosis of tuberculosis (TB). To get reliable results, it is important to carry out an extensive investigation that involves persons from various backgrounds. Recently, there has been an increase in the publication of novel proteomic biomarkers for the diagnosis of tuberculosis (TB) in several body fluids, including blood and urine [85]. These putative biomarkers need substantial refinement before they can be used for clinical diagnosis. The majority of proteome biomarkers discovered in various body fluid samples were identified during initial study. Multiple studies have revealed proteomic markers generated by Mycobacterium that may be used for the identification of active tuberculosis [86]. This is likely linked to the bacterium's capacity to survive within cells and its other adaptive traits.

5.2 Identification of Tuberculosis Biomarkers in Human Urine Samples

Proteomic indicators obtained from blood and urine specimens are often used in anthropological studies to diagnose tuberculosis. Extensive research has been conducted in this area. The feces proteome profiles of both healthy persons & patients with TB were analyzed in 2021. The LC-MS/MS technology was used to analyze a total of 20 TB samples & 20 samples from persons without the disease. The objective was to detect potential biomarkers. Afterwards, a total of 52 samples were used, comprising of 52 TB (tuberculosis), 52 LTBI (latent tuberculosis infection), & 52 healthy control samples, to authenticate the previously established proteomic markers. Their research indicates that an amalgam of hemicentin-2 (Q8NDA2), neurotrimin (Q9P121), poliovirus receptor (P15151), signaling lymphocytic activation molecule family 1 (Q13291), & glutathione peroxidase 3 (P22352) might be used to precisely identify tuberculosis in the latent tuberculosis infection (LTBI) group. The sensitivity of this panel is 82.7% & the specificity is 92.3% [87]. In addition, urine samples collected from patients living in Zimbabwe had a unique 21-mer Mtb peptide motif (VVLGLTVPGGVELLPGVALPR). The clinical isolate MTB423, discovered in Kerala, India, has a 95% similarity in genetic sequence to Mtb oxidoreductase (MRGA423_21210). Further investigation is necessary to validate the importance of this seldom identified protein biomarker generated from Mtb.

5.3 Identification of Tuberculosis Biomarkers in Other Human Body Fluids

Saliva and mucus contain many proteins, mRNA, & diverse bacterial species, making them ideal samples for biomarker research focused on identifying and evaluating illnesses. Saliva or sputum specimen are preferable due to their convenience, reliability, & widespread recognition for further testing. Saliva analysis using proteomics methods has shown many biomarkers that might potentially be used in the diagnosis of TB. The proteins Q99574, A0A2Q2TTZ9, P01011, Q8NCW5, P28072, & Q99574 have just been found via the use of a QExactive Orbitrap mass spectrometer. Following leave-one-out cross validation, the collective bio-signature of five proteins demonstrated a sensitivity of 100% (95% confidence interval: 76.2-100%), a specificity of 90% (95% confidence interval: 58.7-99.8%), & an area under the curve (AUC) of 1.00 (95% confidence interval: 1.00-1.00) for tuberculosis diagnosis [89]. By combining multiplex spectrometry with a salivary authentication, it is possible to correctly differentiate between individuals with TB and those without TB. This distinction is measured by the area under the curve (AUC), which has a value of 0.75 [90]. The profile included several proteins, such as cathelicidin antimicrobial peptide, uteroglobin, vitamin D-binding protein, & β-integrin. A separate investigation employing salivary proteome analysis revealed that individuals afflicted with tuberculosis exhibited elevated levels of proteins associated with the regulation of immune response, inflammation, & complement activation. Conversely, they displayed reduced concentrations of proteins related to glucose and lipid metabolism. Potential biomarkers for TB diagnosis include haptoglobin, immunoglobulin gamma 4 chain, fibrinogens, dermcidin, protein disulfide isomerase, triosephosphate isomerase, and ras GTPaseactivating-like protein [91].

CONCLUSION

In conclusion, the incorporation of sophisticated proteomic technologies into the construction of phenotypic screening platforms offers a revolutionary strategy to report the matter of tuberculosis drug confrontation. Through the utilisation of proteomics, scientists are able to methodically deconstruct the complex molecular pathways that underlie mechanisms of drug resistance, thereby providing invaluable knowledge regarding prospective therapeutic targets. Phenotypic screening is a high-throughput technique that enables the expeditious assessment of extensive collections of compounds, thereby facilitating the detection of potentially effective candidates with the ability to disrupt mechanisms of drug resistance. Furthermore, the capacity to establish correlations between alterations in protein expression profiles and phenotypic responses offers a holistic comprehension of the effectiveness and mechanism of action of compounds. This novel approach not only accelerates the identification of previously undiscovered anti-tuberculosis medications but also presents a more comprehensive strategy for addressing drug resistance, thus creating opportunities for the advancement of more efficacious treatment methods. In conclusion, the application of sophisticated proteomic technologies in phenotypic screening presents tremendous potential for tackling the ongoing issue of drug-resistant tuberculosis, thereby instilling optimism regarding enhanced patient prognoses and worldwide endeavours to control tuberculosis.

REFERENCES

1. Stephanie, F., Saragih, M., & Tambunan, U. S. F. (2021). Recent progress and challenges for drug-resistant tuberculosis treatment. Pharmaceutics, 13(5), 592.
2. Husain, A. A., Kupz, A., & Kashyap, R. S. (2021). Controlling the drug-resistant tuberculosis epidemic in India: challenges and implications. Epidemiology and Health, 43.
3. Chopra, H., Mohanta, Y. K., Rauta, P. R., Ahmed, R., Mahanta, S., Mishra, P. K., ... & Dhama, K. (2023). An insight into advances in developing nanotechnology based therapeutics, drug delivery, diagnostics and vaccines: Multidimensional applications in tuberculosis disease management. Pharmaceuticals, 16(4), 581.

4. Bisht, D., Sharma, D., Sharma, D., Singh, R., & Gupta, V. K. (2019). Recent insights into Mycobacterium tuberculosis through proteomics and implications for the clinic. Expert Review of Proteomics, 16(5), 443-456.
5. Kanabalan, R. D., Lee, L. J., Lee, T. Y., Chong, P. P., Hassan, L., Ismail, R., & Chin, V. K. (2021). Human tuberculosis and Mycobacterium tuberculosis complex: A review on genetic diversity, pathogenesis and omics approaches in host biomarkers discovery. Microbiological research, 246, 126674.
6. Gupta, A. K., Sharma, D., & Singh, A. (2020). Proteomics of Mycobacterium Tuberculosis: An Overview. Microbial Proteomics: Development in Technologies and Applications, 1, 179.
7. Ullah, N., Hao, L., Banga Ndzouboukou, J. L., Chen, S., Wu, Y., Li, L., ... & Fan, X. (2021). Label-free comparative proteomics of differentially expressed Mycobacterium tuberculosis protein in rifampicin-related drug-resistant strains. Pathogens, 10(5), 607.
8. Capela, R., Félix, R., Clariano, M., Nunes, D., Perry, M. D. J., & Lopes, F. (2023). Target Identification in Anti-Tuberculosis Drug Discovery. International Journal of Molecular Sciences, 24(13), 10482.
9. Messner, C. B., Demichev, V., Wang, Z., Hartl, J., Kustatscher, G., Mülleder, M., & Ralser, M. (2023). Mass spectrometry-based high-throughput proteomics and its role in biomedical studies and systems biology.
Proteomics, 23(7-8), 2200013.
10. Birhanu, A. G. (2019). Unveiling phenotypic differences among the genetically conserved Mycobacterium tuberculosis complex: Characterization of proteomes and post-translational modifications.
11. Liu, Y., Chen, T., Zhu, Y., Furey, A., Lowary, T. L., Chan, J., ... & Achkar, J. M. (2023). Features and protective efficacy of human mAbs targeting Mycobacterium tuberculosis arabinomannan. JCI insight, 8(20).
12. Hammarén, M. M., Luukinen, H., Sillanpää, A., Remans, K., Lapouge, K., Custódio, T., ... & Parikka, M. (2023). In vitro and ex vivo proteomics of Mycobacterium marinum biofilms and the development of biofilm-binding synthetic nanobodies. Msystems, 8(3), e01073-22.
13. Rahmat Ullah, S., Irum, S., Mahnoor, I., Ismatullah, H., Mumtaz, M., Andleeb, S., ... & Jamal, M. (2024). Exploring the resistome, virulome, and mobilome of multidrug-resistant Klebsiella pneumoniae isolates: deciphering the molecular basis of carbapenem resistance. BMC genomics, 25(1), 408.
14. Torres-Corral, Y., & Santos, Y. (2022). Predicting antimicrobial resistance of Lactococcus garvieae: PCR detection of resistance genes versus MALDI-TOF protein profiling. Aquaculture, 553, 738098.
15. Muthukrishnan, L. (2021). Multidrug resistant tuberculosis–Diagnostic challenges and its conquering by nanotechnology approach–An overview. Chemico-biological interactions, 337, 109397.
16. Dheda, K., Mirzayev, F., Cirillo, D. M., Udwadia, Z., Dooley, K. E., Chang, K. C., ... & Lange, C. (2024). Multidrug-resistant tuberculosis. Nature Reviews Disease Primers, 10(1), 22.
17. Hameed, H. A., Islam, M. M., Chhotaray, C., Wang, C., Liu, Y., Tan, Y., ... & Zhang, T. (2018). Molecular targets related drug resistance mechanisms in MDR-, XDR-, and TDR-Mycobacterium tuberculosis strains. Frontiers in cellular and infection microbiology, 8, 114.
18. Han, T., Cong, H., Yu, B., & Shen, Y. (2022). Application of peptide biomarkers in life analysis based on liquid chromatography–mass spectrometry technology. Biofactors, 48(4), 725-743.
19. de Castro Brás, L. E., & Lindsey, M. L. (2019). Using Peptidomics to Identify Extracellular Matrix-Derived Peptides as Novel Therapeutics for Cardiac Disease. Fibrosis in Disease: An Organ-Based Guide to Disease Pathophysiology and Therapeutic Considerations, 349-365.
20. Kiziltaş, Ş., & Babalik, A. (2023). Tuberculosis: An Overview. Airway diseases, 1-25.
21. Ledesma, J. R., Ma, J., Zhang, M., Basting, A. V., Chu, H. T., Vongpradith, A., ... & Fagbamigbe, A. F. (2024). Global, regional, and national age-specific progress towards the 2020 milestones of the WHO End TB Strategy: a systematic analysis for the Global Burden of Disease Study 2021. The Lancet Infectious Diseases.
22. Villar-Hernández, R., Ghodousi, A., Konstantynovska, O., Duarte, R., Lange, C., & Raviglione, M. (2023). Tuberculosis: current challenges and beyond. Breathe, 19(1).
23. Castro, K. G., Ditiu, L., Sahu, S., Ntoumi, F., Tiberi, S., O'Kane, C. M., ... & Zumla, A. (2022). Optimising tuberculosis care for refugees affected by armed conflicts. The Lancet Respiratory Medicine, 10(6), 533-536.
24. Obeagu, E. I., & Obeagu, G. U. (2023). Human Immunodeficiency Virus and tuberculosis infection: A review of prevalence of associated factors. Int. J. Adv. Multidiscip. Res, 10(10), 56-62.
25. Ratnatunga, C. N., Lutzky, V. P., Kupz, A., Doolan, D. L., Reid, D. W., Field, M., ... & Miles, J. J. (2020). The rise of non-tuberculosis mycobacterial lung disease. Frontiers in immunology, 11, 303.
26. Bereda, G. (2022). Management of drug resistant tuberculosis: isoniazid resistant, rifampicin resistant, multi drug resistant, and extensively drug resistant. J Lung Pulm Respir Res, 9(2), 46-50.
27. Chowdhury, K., Ahmad, R., Sinha, S., Dutta, S., & Haque, M. (2023). Multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) among children: Where we stand now. Cureus, 15(2).
28. Ntshanka, N. M., & Msagati, T. A. M. (2023). Trends and Progress on antibiotic-resistant mycobacterium tuberculosis and genes in relation to human immunodeficiency virus. The Canadian Journal of Infectious Diseases & Medical Microbiology= Journal Canadien des Maladies Infectieuses et de la Microbiologie Médicale, 2023.
29. Padhi, A., Agarwal, A., Bhise, M., Chaudhary, A., Joshi, K., & Katoch, C. D. S. (2024). Progress and challenges in achieving tuberculosis elimination in India by 2025: A systematic review and meta-analysis. Plos one, 19(3), e0301060.
30. Sharma, D., Bisht, D., & Khan, A. U. (2018). Potential alternative strategy against drug resistant tuberculosis: a proteomics prospect. Proteomes, 6(2), 26.
31. Williams, P. M. (2024). Tuberculosis—United States, 2023. MMWR. Morbidity and Mortality Weekly Report, 73.
32. Chakaya, J., Petersen, E., Nantanda, R., Mungai, B. N., Migliori, G. B., Amanullah, F., ... & Zumla, A. (2022). The WHO Global Tuberculosis 2021 Report–not so good news and turning the tide back to End TB. International Journal of

Infectious Diseases, 124, S26-S29.
33. Parums, D. V. (2021). updates from the World Health Organization (WHO) on global treatment recommendations for drug-susceptible and multidrug-resistant tuberculosis. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research, 27, e934292-1.
34. Ding, C., Hu, M., Shangguan, Y., Guo, W., Wang, S., Feng, X., ... & Xu, K. (2022). Epidemic trends in high tuberculosis burden countries during the last three decades and feasibility of achieving the global targets at the country level. Frontiers in medicine, 9, 798465.
35. Wang, L., Xu, C., Hu, M., Qiao, J., Chen, W., Li, T., ... & Yan, M. (2021). Spatio-temporal variation in tuberculosis incidence and risk factors for the disease in a region of unbalanced socio-economic development. BMC Public Health, 21, 1-11.
36. Kilima, S., Mubyazi, G., Moolla, A., Ntinginya, N., Sabi, I., Mwanyonga, S., & Evans, D. (2022). Knowing and working on care-seeking barriers are vital towards ending TB by 2035: Lessons from a qualitative study on stakeholders' perceptions and experiences of the social and financial consequences of Tuberculosis Care-seeking in Mbeya and Songwe Regions, Tanzania.
37. World Health Organization. (2022). Western Pacific regional framework to end TB: 2021-2030.
38. Yang, Y., & Wu, J. (2019). Significance of the differential peptidome in multidrug-resistant tuberculosis. BioMed Research International, 2019.
39. Nandlal, L., Perumal, R., & Naidoo, K. (2022). Rapid molecular assays for the diagnosis of drug-resistant tuberculosis. Infection and Drug Resistance, 4971-4984.
40. Owusu, E., & Newman, M. J. (2020). Microscopic Observation Drug Susceptibility (MODS) Assay: A Convenient Method for Determining Antibiogram of Clinical Isolates of Mycobacterium tuberculosis in Ghana. Medical Sciences, 8(1), 5.
41. Alcántara, R., Fuentes, P., Marin, L., Kirwan, D. E., Gilman, R. H., Zimic, M., & Sheen, P. (2020). Direct determination of pyrazinamide (PZA) susceptibility by sputum microscopic observation drug susceptibility (MODS) culture at neutral pH: the MODS-PZA assay. Journal of clinical microbiology, 58(5), 10-1128.
42. Swain, S. S., Sharma, D., Hussain, T., & Pati, S. (2020). Molecular mechanisms of underlying genetic factors and associated mutations for drug resistance in Mycobacterium tuberculosis. Emerging microbes & infections, 9(1), 1651-1663.
43. Arif, F., Tahir, T., Suhail, S., Anees, R., Nadeem, I., & Hafeez, M. M. (2021). genetic factors associated with mutations of molecular mechanism and drug resistance in Mycobacterium tuberculosis. Biological and Clinical Sciences Research Journal, 2021(1).
44. Singh, R., Dwivedi, S. P., Gaharwar, U. S., Meena, R., Rajamani, P., & Prasad, T. (2020). Recent updates on drug resistance in Mycobacterium tuberculosis. Journal of applied microbiology, 128(6), 1547-1567.
45. Singh, R., Dwivedi, S. P., Gaharwar, U. S., Meena, R., Rajamani, P., & Prasad, T. (2020). Recent updates on drug resistance in Mycobacterium tuberculosis. Journal of applied microbiology, 128(6), 1547-1567.
46. Matsui, T., Pinhata, J. M. W., Rabello, M. C. D. S., Brandão, A. P., Ferrazoli, L., Leão, S. C., ... & Oliveira, R. S. D. (2020). Frequency of first and second-line drug resistance-associated mutations among resistant Mycobacterium tuberculosis clinical isolates from São Paulo, Brazil. Memórias do Instituto Oswaldo Cruz, 115, e200055.
47. Tilahun, M., Shimelis, E., Wogayehu, T., Assefa, G., Wondimagegn, G., Mekonnen, A., ... & Aseffa, A. (2020). Molecular detection of multidrug resistance pattern and associated gene mutations in M. tuberculosis isolates from newly diagnosed pulmonary tuberculosis patients in Addis Ababa, Ethiopia. PLoS One, 15(8), e0236054.
48. Singh, P. K., Singh, U., & Jain, A. (2021). Emergence of specific gyr A mutations associated high-level fluoroquinolone-resistant Mycobacterium tuberculosis among multidrug-resistant tuberculosis cases in North India. Microbial Drug Resistance, 27(5), 647-651.
49. Miryala, S. K., Basu, S., Naha, A., Debroy, R., Ramaiah, S., Anbarasu, A., & Natarajan, S. (2021). Identification of bioactive natural compounds as efficient inhibitors against Mycobacterium tuberculosis protein-targets: a molecular docking and molecular dynamics simulation study. Journal of Molecular Liquids, 341, 117340.
50. Kumar, M., Singh, S. K., Singh, P. P., Singh, V. K., Rai, A. C., Srivastava, A. K., ... & Kumar, A. (2021). Potential anti-mycobacterium tuberculosis activity of plant secondary metabolites: insight with molecular docking interactions. Antioxidants, 10(12), 1990.
51. Sabet, M., Daef, E., Ghaith, D., Agban, M., & Aly, S. A. (2020). HOW DOES QUINOLONES ANTIBOTIC RESISTANCE DEVELOP?. Bulletin of Pharmaceutical Sciences. Assiut, 43(1), 95-104.
52. Zhu, Y. M., Liu, H. M., Wang, F., Shan, W. S., Yin, Q., & Lu, K. Z. (2024). Laboratory Examination for Drug-Resistant Tubercle Bacillus. In Diagnostic Imaging of Drug Resistant Pulmonary Tuberculosis (pp. 19-27). Singapore: Springer Nature Singapore.
53. Fowler, P. W., Wright, C., Spiers, H., Zhu, T., Baeten, E. M., Hoosdally, S. W., ... & CRyPTIC Consortium. (2022). A crowd of BashTheBug volunteers reproducibly and accurately measure the minimum inhibitory concentrations of 13 antitubercular drugs from photographs of 96-well broth microdilution plates. Elife, 11, e75046.
54. Lelovic, N., Mitachi, K., Yang, J., Lemieux, M. R., Ji, Y., & Kurosu, M. (2020). Application of Mycobacterium smegmatis as a surrogate to evaluate drug leads against Mycobacterium tuberculosis. The Journal of antibiotics, 73(11), 780-789.
55. Chopra, K. K., Sidiq, Z., Hanif, M., & Dwivedi, K. K. (2020). Advances in the diagnosis of tuberculosis-Journey from smear microscopy to whole genome sequencing. indian journal of tuberculosis, 67(4), S61-S68.
56. Anyansi, C. A. (2021). Mycobacterium tuberculosis genomics: The Next Generation.
57. Li, Y., Wang, X., Wang, M., Liu, M., Wang, H., Xia, W., & Liu, L. (2023). Advances in ligase-based nucleic acid

amplification technology for detecting gene mutations: a review. Molecular and Cellular Biochemistry, 478(7), 1621-1631.
58. Braun, P., Raab, R., Bugert, J. J., & Braun, S. (2023). Recombinant reporter phage rTUN1:: nLuc enables rapid detection and real-time antibiotic susceptibility testing of Klebsiella pneumoniae K64 strains. ACS sensors, 8(2), 630-639.
59. Yang, H., Li, A., Dang, L., Kang, T., Ren, F., Ma, J., ... & Zhang, T. (2023). A rapid, accurate, and low-cost method for detecting Mycobacterium tuberculosis and its drug-resistant genes in pulmonary tuberculosis: applications of MassARRAY DNA mass spectrometry. Frontiers in Microbiology, 14, 1093745.
60. Jain, B., & Kulkarni, S. (2023). Development of DNA bio-chip for detection of mutations of rpoB, embB and inhA genes in drug-resistant Mycobacterium tuberculosis. Indian Journal of Clinical Biochemistry, 38(2), 242-250.
61. Chen, S., Liu, H., Li, T., Lai, W., Liu, L., Xu, Y., & Qu, J. (2023). Using Microfluidic Chip and Allele-Specific PCR to Rapidly Identify Drug Resistance-Associated Mutations of Mycobacterium tuberculosis. Infection and Drug Resistance, 4311-4323.
62. Giddey, A. D. (2021). Differential Lipidomic and Proteomic Responses Induced by Sub-lethal Drug Challenge in Susceptible and Drug Resistant Mycobacterium smegmatis.
63. Huang, H., Han, Y. S., Chen, J., Shi, L. Y., Wei, L. L., Jiang, T. T., ... & Li, J. C. (2020). The novel potential biomarkers for multidrug-resistance tuberculosis using UPLC-Q-TOF-MS. Experimental Biology and Medicine, 245(6), 501-511.
64. Cao, T., Lyu, L., Jia, H., Wang, J., Du, F., Pan, L., ... & Zhang, Z. (2019). A two-way proteome microarray strategy to identify novel mycobacterium tuberculosis-human interactors. Frontiers in Cellular and Infection Microbiology, 9, 65.
65. Dapic, I., Baljeu-Neuman, L., Uwugiaren, N., Kers, J., Goodlett, D. R., & Corthals, G. L. (2019). Proteome analysis of tissues by mass spectrometry. Mass spectrometry reviews, 38(4-5), 403-441.
66. Cui, M., Cheng, C., & Zhang, L. (2022). High-throughput proteomics: a methodological mini-review. Laboratory investigation, 102(11), 1170-1181.
67. Goff, A., Cantillon, D., Muraro Wildner, L., & Waddell, S. J. (2020). Multi-omics technologies applied to tuberculosis drug discovery. Applied Sciences, 10(13), 4629.
68. Bhattacharya, S., Ghosh, P., Banerjee, D., Banerjee, A., & Ray, S. (2020). In Silico Drug Target Discovery Through Proteome Mining from M. tuberculosis: An Insight into Antivirulent Therapy. Combinatorial Chemistry & High Throughput Screening, 23(3), 253-268.
69. Kwon, Y. W., Jo, H. S., Bae, S., Seo, Y., Song, P., Song, M., & Yoon, J. H. (2021). Application of proteomics in cancer: recent trends and approaches for biomarkers discovery. Frontiers in medicine, 8, 747333.
70. Peng, B., Li, H., & Peng, X. (2019). Proteomics approach to understand bacterial antibiotic resistance strategies. Expert Review of Proteomics, 16(10), 829-839.
71. Denzer, L., Schroten, H., & Schwerk, C. (2020). From gene to protein—How bacterial virulence factors manipulate host gene expression during infection. International Journal of Molecular Sciences, 21(10), 3730.
72. Fleitas Martínez, O., Cardoso, M. H., Ribeiro, S. M., & Franco, O. L. (2019). Recent advances in anti-virulence therapeutic strategies with a focus on dismantling bacterial membrane microdomains, toxin neutralization, quorum-sensing interference and biofilm inhibition. Frontiers in cellular and infection microbiology, 9, 74.
73. Stewart, D., Anwar, F., & Vedantam, G. (2020). Anti-virulence strategies for Clostridioides difficile infection: advances and roadblocks. Gut Microbes, 12(1), 1802865.
74. Fulton, K. M., Baltat, I., & Twine, S. M. (2019). Immunoproteomics methods and techniques. Immunoproteomics: methods and protocols, 25-58.
75. Kitata, R. B., Yang, J. C., & Chen, Y. J. (2023). Advances in data-independent acquisition mass spectrometry towards comprehensive digital proteome landscape. Mass spectrometry reviews, 42(6), 2324-2348.
76. An, Y., Zhou, L., Huang, Z., Nice, E. C., Zhang, H., & Huang, C. (2019). Molecular insights into cancer drug resistance from a proteomics perspective. Expert review of proteomics, 16(5), 413-429.
77. Lill, J. R., Mathews, W. R., Rose, C. M., & Schirle, M. (2021). Proteomics in the pharmaceutical and biotechnology industry: a look to the next decade. Expert review of proteomics, 18(7), 503-526.
78. Yong, Y. K., Tan, H. Y., Saeidi, A., Wong, W. F., Vignesh, R., Velu, V., ... & Shankar, E. M. (2019). Immune biomarkers for diagnosis and treatment monitoring of tuberculosis: current developments and future prospects. Frontiers in microbiology, 10, 2789.
79. Nogueira, B. M., Krishnan, S., Barreto-Duarte, B., Araújo-Pereira, M., Queiroz, A. T., Ellner, J. J., ... & Andrade, B. B. (2022). Diagnostic biomarkers for active tuberculosis: progress and challenges. EMBO Molecular Medicine, 14(12), e14088.
80. Estévez, O., Anibarro, L., Garet, E., Pallares, Á., Pena, A., Villaverde, C., ... & González-Fernández, Á. (2020). Identification of candidate host serum and saliva biomarkers for a better diagnosis of active and latent tuberculosis infection. PLoS One, 15(7), e0235859.
81. Sullivan, A., & Nathavitharana, R. R. (2022). Addressing TB-related mortality in adults living with HIV: a review of the challenges and potential solutions. Therapeutic Advances in Infectious Disease, 9, 20499361221084163.
82. Liebenberg, C., Luies, L., & Williams, A. A. (2021). Metabolomics as a tool to investigate HIV/TB co-infection. Frontiers in molecular biosciences, 8, 692823.
83. Singer, S. N., Ndumnego, O. C., Kim, R. S., Ndung'u, T., Anastos, K., French, A., ... & Achkar, J. M. (2022). Plasma host protein biomarkers correlating with increasing Mycobacterium tuberculosis infection activity prior to tuberculosis diagnosis in people living with HIV. EBioMedicine, 75.
84. Shen, Y., Xun, J., Song, W., Wang, Z., Wang, J., Liu, L., ... & Lu, H. (2020). Discovery of potential plasma biomarkers for tuberculosis in HIV-infected patients by data-independent acquisition-based quantitative proteomics. Infection and Drug Resistance, 1185-1196.
85. Correia-Neves, M., Fröberg, G., Korshun, L., Viegas, S., Vaz, P., Ramanlal, N., ... & Källenius, G. (2019). Biomarkers

for tuberculosis: the case for lipoarabinomannan. ERJ open research, 5(1).
86. Chen, J., Han, Y. S., Yi, W. J., Huang, H., Li, Z. B., Shi, L. Y., ... & Li, J. C. (2020). Serum sCD14, PGLYRP2 and FGA as potential biomarkers for multidrug-resistant tuberculosis based on data-independent acquisition and targeted proteomics. Journal of Cellular and Molecular Medicine, 24(21), 12537-12549.
87. Liu, L., Deng, J., Yang, Q., Wei, C., Liu, B., Zhang, H., ... & Jin, Q. (2021). Urinary proteomic analysis to identify a potential protein biomarker panel for the diagnosis of tuberculosis. IUBMB life, 73(8), 1073-1083.
88. Pollock, N., Dhiman, R., Daifalla, N., Farhat, M., & Campos-Neto, A. (2018). Discovery of a unique Mycobacterium tuberculosis protein through proteomic analysis of urine from patients with active tuberculosis. Microbes and Infection, 20(4), 228-235.
89. Mutavhatsindi, H., Calder, B., McAnda, S., Malherbe, S. T., Stanley, K., Kidd, M., ... & Chegou, N. N. (2021). Identification of novel salivary candidate protein biomarkers for tuberculosis diagnosis: a preliminary biomarker discovery study. Tuberculosis, 130, 102118.
90. Bishwal, S. C., Das, M. K., Badireddy, V. K., Dabral, D., Das, A., Mahapatra, A. R., ... & Nanda, R. K. (2019). Sputum proteomics reveals a shift in vitamin D-binding protein and antimicrobial protein axis in tuberculosis patients. Scientific reports, 9(1), 1036.
91. Mateos, J., Estévez, O., González-Fernández, Á., Anibarro, L., Pallarés, Á., Reljic, R., ... & Carrera, M. (2019). High-resolution quantitative proteomics applied to the study of the specific protein signature in the sputum and saliva of active tuberculosis patients and their infected and uninfected contacts. Journal of proteomics, 195, 41-52.