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Literature Review On Multi-Extensively Drug-Resistant Mycobacterium Tuberculosis Mutations: Highlighting Molecular Analysis Gaps At Tshepong NHLS Referral Laboratory.

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Abstract
The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) Mycobacterium tuberculosis presents a significant challenge to global public health. This study explores the sensitivity profiling of genes responsible for MDR and XDR mutations in M. tuberculosis isolates from the Tshepong National Health Laboratory Service Referral Tuberculosis Laboratory in North West. A literature review was conducted using databases such as Google Scholar, PubMed, and PubMed Central to identify relevant articles published between 2020 and 2024. The selected studies were analysed to determine the genes involved in resistance mechanisms and to develop a conceptual framework for understanding their interactions with first- and second-line anti-tuberculosis drugs.

According to the literature, more than 95% of rifampicin resistance is associated with rpoB gene alterations within an 81 bp region. Additionally, isoniazid (INH) resistance is more complex and has been linked to multiple genes, particularly katG and the inhA promoter region. The reviewed studies highlight gaps in research and suggest directions for future studies to improve drug susceptibility testing, particularly in regions such as North West Province, South Africa.

Keywords: *Mycobacterium tuberculosis, Multidrug-resistant, extensively drug-resistant, resistance mechanism , multiple gene*

INTRODUCTION

Mycobacteriaceae family comprises a diverse group of bacteria with varying degrees of pathogenicity in humans and animals. These bacteria exhibit distinct host reservoirs and growth dynamics in culture (Karabala et.al., 2021). The Mycobacterium tuberculosis complex (MTBC) is a genetically related group of mycobacteria that includes Mycobacterium tuberculosis (M. tuberculosis), Mycobacterium africanum (M. africanum), Mycobacterium bovis (M. bovis), Mycobacterium canettii (M. canettii), Mycobacterium microti (M. microti), Mycobacterium pinnipedii (M. pinnipedii), and Mycobacterium caprae (M. caprae) (Karabala et.al., 2021).

Tuberculosis (TB), caused by M. tuberculosis, is a major global public health concern. It is a chronic infectious disease primarily transmitted through airborne droplets from coughing or sneezing (Rasool, 2019). TB primarily affects the lungs (pulmonary TB) but can also impact other organs (extrapulmonary TB) (Rasool, 2019). Active TB commonly presents as a persistent cough lasting several weeks, often with blood-streaked sputum. Other classic symptoms include fever, chills, night sweats, weakness, and unintentional weight loss (Moule & Cirillo, 2020). In contrast, latent TB remains asymptomatic, and individuals may be unaware of their infection unless reactivation occurs (Moule & Cirillo, 2020).

Enhancing TB diagnostic efficiency is crucial for improving treatment outcomes (Gill et.al., 2021). Various diagnostic techniques—including microscopy, culture, and molecular methods—are used to detect Mycobacterium in respiratory specimens, each with advantages and limitations (Srivastava et.al., 2020). Culture-based detection remains the gold standard due to its high sensitivity, but it is susceptible to contamination, requiring decontamination steps that can compromise bacterial viability (Srivastava et.al., 2020). The NALC-NaOH decontamination method is widely used, and a cocktail of antibiotics (PANTA: polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin) is employed to suppress non-mycobacterial contaminants (Srivastava et al., 2020). Liquid-based culture systems significantly reduce detection time, averaging 12.8 days compared to 25.1–25.5 days for solid media (Gill et.al., 2021). GeneXpert MTB/RIF is a rapid, automated polymerase chain reaction (PCR) test that detects active TB and rifampicin resistance within approximately two hours, requiring minimal training and biosafety measures (Nogueira et.al., 2022). Smear microscopy, one of the oldest TB detection methods, remains widely used due to its simplicity, speed, and cost-effectiveness. It is a cornerstone of the WHO DOTS (Directly Observed Treatment, Short-Course) strategy, utilizing Ziehl-Neelsen or fluorescence microscopy with light-emitting diodes (Wobudeya et.al.,2022).

Fluorescence microscopy increases mycobacterial detection rates by approximately 10% compared to conventional light microscopy, with sensitivity further enhanced through sputum centrifugation or sedimentation (Silva et.al., 2021).

Serological tests targeting M. tuberculosis antigens, such as trehalose-6,6'-dimycolate (TDM), have demonstrated potential for rapid pulmonary TB diagnosis (Ludi et.al., 2023). Chest X-rays are commonly used to evaluate suspected TB cases, particularly in extrapulmonary or latent infections. However, computed tomography (CT) and positron emission tomography (PET) scans, while offering superior diagnostic accuracy, are not routinely performed due to high costs (Ludi et.al., 2023). The tuberculin skin test (TST), in use for over a century, fails to distinguish latent TB infection (LTBI) from active TB or prior Bacillus Calmette-Guérin (BCG) vaccination effects (Gong & Wu, 2021). To overcome these limitations, newer skin tests and interferon-gamma release assays (IGRAs) have been developed, including the DiaSkin test, C-Tb test, EC-Test, T-SPOT.TB, QuantiFERON-TB Gold In-Tube, QuantiFERON-TB Gold Plus, and LIOFERON TB/LTBI (Huang et.al., 2022). Line probe assays (LPA) enable rapid TB DNA detection and drug resistance profiling, identifying mutations in genes associated with resistance to first-line drugs such as isoniazid (INH) and rifampicin (RIF) (Huang et.al., 2022).

Rapid detection of drug-resistant TB is critical for initiating appropriate treatment (Saderi et.al., 2022). Standard TB treatment for drug-susceptible strains consists of a six-month regimen: two months of isoniazid, rifampicin, ethambutol, and pyrazinamide, followed by four months of isoniazid and rifampicin, achieving an approximately 85% success rate (Saderi et.al., 2022). However, mutations in genes such as rpoB, inhA, katG, pncA, gyrA, and gyrB contribute to resistance against first-line and second-line anti-TB drugs, leading to the emergence of multidrug-resistant (MDR), pre-extensively drug-resistant (pre-XDR), and extensively drug-resistant (XDR) M. tuberculosis strains (Singh, 2021). This review manuscript focuses on the genetic profiling of drug-resistant M. tuberculosis, analysing mutations associated with multidrug-resistant and extensively drug-resistant TB. By evaluating the molecular characteristics of these resistant strains, this review seeks to identify existing gaps in South African TB profiling and contribute to the body of knowledge by showing current trends in TB studies in the development of improved diagnostic and treatment strategies for TB management.

METHODOLOGY

A systematic search was conducted using academic databases such as PubMed, PubMed Central, and Google Scholar to identify relevant studies published between 2020 and 2024. The following keywords were used, Mycobacterium tuberculosis, multidrug-resistant, extensively drug-resistant, resistance mechanism, multiple genes and articles researched in South Africa. Studies were selected based on their relevance to the sensitivity profiling of genes responsible for MDR/XDR-TB. Priority was given to journal articles, meta-analyses, and systematic reviews to ensure the inclusion of high-quality and reliable data.

Topics of interest from the different studies

Studies that were included in the manuscript focused on the following aspects, TB diagnosis and advances in testing such as culture-based detection, GeneXpert MTB/RIF (detects TB and rifampicin resistance), fluorescence microscopy, line probe assay (identifies TB DNA and drug resistance markers), BD MAX MDR-TB assay (detection of inhA, katG, and rpoB mutations). Then further review was on drug resistance trends and treatment challenges studies with main focus on patterns of resistance emergence, current and emerging therapeutic approaches. It was also interesting to expand the focus on common resistance genes on studies that addresses (identification of genetic mutations linked to drug resistance; geographic Trends in TB Drug Resistance; regional differences in resistance prevalence; quantitative analysis of trends; and statistical analysis of MDR/XDR-TB cases over time).

RESULTS

Srivastava et.al. found out that several diagnostics techniques including microscopy, culture and molecular probes are used to evaluate the presence of mycobacteria in respiratory specimens, where each technique has its advantages and disadvantages in the study they did in 2020. Srivastava et.al. further found out that culture-based methods for detecting mycobacteria are highly sensitive but prone to contamination, requiring decontamination steps that can introduce inaccuracies, as some mycobacteria may die during processing. The NALC-NaOH decontamination method is commonly used. To promote mycobacterial growth, antibiotics like PANTA (polymyxin-B, amphotericin-B, nalidixic acid, trimethoprim, azlocillin) are applied to kill other bacteria. Liquid-based systems offer the advantage of faster detection, reducing the time to growth by nearly half, with an average detection time of 12.8 days, compared to 25.1-25.5 days for solid media in the study that was done by Gill et.al.

(2021). GeneXpert MTB/RIF is an automated PCR test that quickly diagnoses Active Tuberculosis (ATB) and detects rifampicin resistance. Unlike traditional nucleic acid amplification tests, it provides results in about 2 hours using automated assays according to the study that was conducted by Nogueira et.al. (2022).

According to Wobudeya et.al. (2022), smear microscopy is the oldest and simplest microbiology test for tuberculosis (TB). It is fast, inexpensive, and a key component of the WHO's DOTS (Directly Observed Treatment, Short-course) TB control strategy. This test relies on visualizing acid-fast bacilli using Ziehl-Neelsen staining or fluorescence microscopy with light-emitting diodes (LEDs). Sputum smear microscopy is crucial for diagnosing TB, as it helps identify patients with active TB who contribute to the disease transmission chain (Silva et.al.,2021). They further found out that Fluorescence microscopy can enhance mycobacteria detection by 10% compared to conventional light microscopy. According to the study that was carried by Huang et.al. in 2022 they discovered that Line Probe Assay (LPA)detects TB DNA and genetic mutations associated with drug resistance, after DNA extraction and PCR amplification. The basis of the LPA is that the pre-labelled amplification product is captured by the DNA probe solidified on the membrane strip and detected by colorimetry, and the results of LPA appear as a linear band (Huang,2022).LPA can detect drug resistance to first-line TB drugs INH and RIF, and there are different version of commercial products, including Geno Type MTBDR plus 1.0 (Hain Life Science) and INNO-LPA Rif TB kit (Innogenetic) (Huang et.al.,2022) . Armstrong et.al. (2023) discovered that the BD MAX™ multidrug-resistant TB (MDR-TB) assay shows high sensitivity and specificity for detecting Mycobacterium tuberculosis complex (MTBC) and resistance to INH and Rifampin (RIF) in pulmonary specimens. In low- and middle-income settings, the BD MAX MDR-TB test demonstrated 93% sensitivity for confirmed pulmonary TB cases, with accuracy comparable to the Xpert MTB/RIF test according to the study that was carried out by Shah et.al. in 2019. A key advantage of the BD MAX assay is its ability to detect mutations in the inhA promoter, katG, and rpoB, unlike other molecular assays that focus primarily on identifying RIF resistance (Shah et.al.,2019). With time, the mutations accumulated within the Mtb genome and specifically in the proteins serving as drug targets, have caused the development of resistance against various first and second lines anti-TB drugs and further its self-transformation into pre-extensively drug resistant (pre-XDR),multi drug resistant (MDR)and extensively drug-resistant according to the study done by Singh in 2021 .Drug resistance –associated genes rpoB, inhA, KatG, pncA,gry A and gry B are known to confer resistance against rifampicin (RIF) ,Isoniazid (INH), pyrazinamide (PZA) and fluoroquinolone (FLQ) ciprofloxacin(CIF), moxifloxacin (MFX), levofloxacin(LFX) and ofloxacin (OFX), respectively (Singh, 2021). More than 95% of RIF resistance is associated with rpoB gene alterations in an 81-bp area (Traore et.al.,2023). INH resistance appears to be more complex and has been linked to numerous genes, most notably katG and the inhA promoter region (Traore et.al.,2023)

In 2021, the majority of TB cases occurred in the WHO South-East Asia region (45%), followed by the WHO African region (23%) and the WHO Western Pacific region (18%) (Alsayed & Gunosewoyo, 2023). India (28%), Indonesia (9.2%), China (7.4%), and the Philippines (7.0%) were the four countries accounting for over half of the global TB burden (Alsayed & Gunosewoyo, 2023).

Drug Resistance Prevalence:

In 2018, an estimated 3.4% of global TB cases were new drug-resistant (MDR/RR), and 18% were from previously treated cases (Gashaw et.al., 2021). WHO's high burden countries (HBCs) accounted for 84% of global TB, 83% of TB/HIV, and 87% of MDR-TB cases (Singh et.al., 2019). These HBCs include countries like India, China, South Africa, and Indonesia.

MDR/XDR-TB Cases and Trends:

-MDR-TB: In 2021, approximately 3.6% of new TB cases and 18% of previously treated TB cases were MDR-TB (Diriba et.al., 2023). MDR-TB is defined as resistance to both rifampicin (RIF) and isoniazid (INH). The global rise in MDR-TB continues, and person-to-person transmission has become the leading driver of its spread (Nandlal et.al., 2022). -XDR-TB: Extensively drug-resistant TB (XDR-TB), defined as resistance to MDR-TB plus resistance to fluoroquinolones and one additional group A drug, is increasingly prevalent. In 2019, 465,000 cases of rifampicin-resistant TB (RR-TB) were reported, with 78% being MDR-TB, and 6.2% of MDR-TB cases had XDR-TB (Diriba et.al., 2023). South Africa's XDR-TB epidemic was driven by Rif resistance mutations (Oostvogels et.al., 2022).

Drug Resistance Mechanisms:

-RIF Resistance: More than 95% of Rif resistance is associated with mutations in the rpoB gene (Traore et.al., 2023).

-INH Resistance: Resistance to INH involves multiple genetic mutations, notably in the katG gene and the inhA promoter region (Traore et.al., 2023).

Global Response and Challenges:
Despite a 20% decrease in TB mortality from 2015 to 2020, the global burden of MDR-TB and XDR-TB continues to rise, with the vast majority of cases occurring in low- and middle-income countries according to the study that was conducted by Nandlal et.al., (2022). There is an urgent need for enhanced diagnostics, better treatment adherence, and more effective strategies to control the spread of drug-resistant TB globally.

DISCUSSION

The identification of resistance-associated mutations is crucial for guiding treatment strategies. Early detection through molecular diagnostic techniques can improve treatment outcomes and curb the spread of drug-resistant strains. Studies indicate that rapid tests like GeneXpert MTB/XDR and BD MAX MDR-TB assays have a high sensitivity and specificity in detecting resistance mutations. However, these methods have limitations, including cost constraints and accessibility issues in resource-limited settings. A critical aspect of MDR/XDR-TB control is understanding the demographic distribution of affected individuals. Studies reveal that males and individuals within the economically active age group (18-45 years) are most affected. This emphasizes the need for targeted public health interventions, including strengthening TB surveillance, promoting adherence to treatment regimens, and improving healthcare infrastructure in high-burden regions.

CONCLUSION

MDR and XDR-TB remain significant threats to TB control efforts. Sensitivity profiling of drug resistance genes plays a vital role in optimizing treatment regimens and preventing the spread of resistant strains. While molecular diagnostic tools have improved TB detection, challenges related to affordability and accessibility need to be addressed. Future research should focus on the development of cost-effective and rapid diagnostic technologies to enhance early detection and effective management of drug-resistant TB. By implementing robust surveillance and diagnostic strategies, countries like South Africa can mitigate the impact of MDR/XDR-TB and work towards achieving global TB elimination goals.

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