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Trend in the Molecular Characterization and Geographic Distribution of Mycobacterium tuberculosis Complex Strains: A Systematic Review

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Abstract

This systematic review aims to evaluate the global molecular characterization of *Mycobacterium tuberculosis* complex (MTBC) isolates from 2017 to June 2023, focusing on lineage distribution, drug resistance trends, and diagnostic methods. Following PRISMA guidelines, data from 10 high quality studies comprising 7,848 clinical samples across 10 countries were analyzed, yielding 3,216 confirmed MTBC isolates. Spoligotyping, MIRU-VNTR, and whole genome sequencing (WGS) were used to characterize strains, alongside various drug susceptibility testing (DST) methods. Lineage 4 (Euro-American) was the most widespread globally, especially in sub-Saharan Africa and Europe. Lineage 2 (Beijing), associated with multidrug resistance, predominated in South Asia, while Lineage 3 (CAS/Delhi) was prevalent in Pakistan and Sudan. MDR-TB rates varied widely from 1.9% in Ghana to 75% in Ireland with high rates also in Nepal (56.8%) and India (50.6%). Male patients accounted for 64% of MTBC cases, indicating gender disparities in disease burden. Spoligotyping was the most frequently used molecular method, though WGS is increasingly employed for its higher resolution. DST methods included the proportion method on Lowenstein–Jensen medium, MGIT 960, GeneXpert MTB/RIF, and Line Probe Assays (LPA). Findings reveal significant regional variation in MTBC lineages and resistance rates. To improve TB control, there is an urgent need for standardized molecular diagnostics, enhanced regional surveillance, and

gender-sensitive treatment strategies. Investment in diagnostic infrastructure, particularly in high-burden regions, is critical to curbing the global spread of MDR-TB.

Keywords: Tuberculosis, *Mycobacterium tuberculosis* complex, Molecular characterization, Drug resistance, Whole genome sequencing

Introduction

Since 1900, tuberculosis has been a global public health emergency, accounting for the greatest number of deaths from a single bacterial illness. *Mycobacterium tuberculosis*, the causative agent of tuberculosis (TB), is a member of the *Mycobacterium tuberculosis* complex (MTBC), a genetically well-characterized family of strains that has complicated efforts to eradicate tuberculosis because of pathogen-specific phenotypic differences among its members. The genetically varied human and animal-adapted MTBC strains are members of seven lineages (Euro-American, Indo-Oceanic, East-Asian, East-African Indian, *M. africanum* West Africa 1, *M. africanum* West Africa 2, and Ethopia), as well as the newly discovered Lineages 8 and 9 (Mvubu & Jacoby, 2023). *M. bovis*, *M. canetti*, and *M. africanu*m, which are also common in clinical settings, are included in this complex together with animal-adapted *M. mungi, M. orygis*, *M. suricattae*, *M. microti*, *M. caprae,* and *M. pinnipedi* (rareties) (Prozorov et al., 2014). The World Health Organization (WHO) anticipated that in 2023, there will be 10.8 million new cases and 1.09 million fatalities from tuberculous bacilli, despite increased financing and measures aimed at eliminating them. Aapproximately 400,000 people globally developed multidrug-resistant or rifampicin-resistant TB (MDR/RRTB) in 2023, causing an estimated 150,000 death (WHO, 2024). Extensively drug-resistance tuberculosis (XDRTB**)** poses a growing concern, comprising around 9% of MDRTB cases worldwide (Diriba et al., 2023).

An essential research platform for examining potential correlations between strains and sub-lineages and the clinical and epidemiological features of the disease is created by the integration of molecular characterization of *M. tuberculosis* with clinical and epidemiological data (Dale et al., 2005, Kong et al., 2007, Nava-Aguilera et al., 2011). Molecular strain typing (genotyping) has advanced TB control by revealing transmission patterns, distinguishing reactivation from re-infection, confirming outbreaks, and identifying drug-resistant strain (Gagneux & Small, 2007). Techniques like spoligotyping and MIRU-VNTR are commonly used to characterize MTBC lineages, aiding in TB tracking, control, and understanding host-pathogen interactions (Kamerbeek et., 1997, Brosch et., 2002). The geographical distribution of TB patients, risk factors and medication resistance may be examined using spoligotyping data in conjunction with drug resistance studies and GIS mapping (Luis et al., 2025).

Three molecular typing methods for MTBC were compared. IS6110 RFLP offers high discriminatory power and stability but requires large amounts of DNA, costly software, and technical expertise. Spoligotyping is cost-effective, easy to interpret, and useful for strains with low IS6110 copy numbers, but shows lower discrimination and limited application for Beijing family strains. MIRU-VNTR is simple, fast, reproducible, and cost-effective, yet its discriminatory power (especially with 12- and 15-loci schemes) is lower than IS6110 RFLP, with a slightly slower evolutionary rate. Whole Genome Sequencing (WGS) provides high discriminatory power and precise genetic information but is limited by high cost, challenges in data standardization, and the lack of readily accessible and equitable databases. (Jagielski et al., 2014). In order to identify TB pathogenesis and related diagnostic problems for improved development of therapies and diagnostics to end TB, it is essential to comprehend variations in the lineage makeup of the MTBC complex as well as its drug resistance profiles in various clinical forms of TB (Alemayehu et al., 2024).

DR-TB continues to be a serious public health issue and a challenge to international TB control initiatives. According to WHO estimates, there were 450,000 new cases of RR-TB/MDR-TB worldwide in 2021. The prevalence of MDR-TB was 3.6% in new cases and 18% in patients that had already received treatment (Yenew et al., 2024). WHO (2024) study states that the death rate from MTB illness is a startling 50% if treatment is not received. Although the 88% treatment success rate for drug-susceptible MTB is still impressive, the rate for MDR/RR-TB has increased to 68% from 2022. Nepal reported 593 MDR/RR-TB cases, with 384 patients starting treatment. Among them, 84 were pre-XDR/XDR-TB, and 76 were tested for fluoroquinolone resistance, though exact XDR-TB figures were unavailable (SAARC TB and HIV/AIDS Centre, 2022).

This study aims to provide an overview of molecular techniques used for differentiating MTBC isolates, understand the phylogenetic distribution patterns, assess the trends of drug-resistant TB, and explore the movement and spread of specific MTBC lineages across different regions and populations.

Materials and Methods

Identification Strategy

A systematic review was conducted in accordance with the PRISMA guidelines. The search strategy involved using multiple databases, including PubMed, Web of Science, Scopus, Science Direct, and Google Scholar. Original research articles published between 2017 and June 2023 were considered for inclusion. The search terms used were "Molecular analysis of MTBC," "Molecular epidemiology," "Drug resistant TB (DRTB)," "multidrug resistant TB (MDRTB)" and "Genotypes of MTBC." An initial total of 210 citations were identified across these databases. Following a careful screening of titles

and abstracts, studies focusing on the molecular study, characterization, epidemiology, molecular analysis, and phylogenetic distribution of MTBC across various geographical regions were selected for review.

Data Abstraction

From the selected articles, key information was systematically extracted and organized. The review primarily focused on the molecular techniques employed to differentiate and characterize MTBC isolates, including methods such as spoligotyping, MIRU-VNTR, and whole genome sequencing. Data regarding the number of isolates analyzed in each study were collected, along with the methodologies used for drug susceptibility testing (DST). Particular attention was given to patterns of drug resistance, including DRTB, MDRTB, and extensively drug-resistant TB (XDRTB). Additionally, the phylogenetic classification and geographical distribution of MTBC lineages were examined. The review also highlighted the identification of predominant strains and lineages across different regions, providing insights into the global diversity and spread of MTBC.

Inclusion Criteria

Studies were included in this review if they were original research articles published between 2017 and June 2023, involved molecular characterization and epidemiological analysis of MTBC, reported on phylogenetic distribution and lineage typing of MTBC isolates, and were conducted on human clinical samples or epidemiological populations across different geographical regions. Only articles written in English were considered eligible.

Exclusion Criteria

Studies were excluded if they focused exclusively on Non-Tuberculous Mycobacteria (NTM), lacked molecular analysis or phylogenetic characterization, or were case reports, editorials, or conference abstracts without complete molecular data.

Statistical Analysis

As this study involved a systematic review of published literature, descriptive statistical methods were primarily employed. The frequency and proportion of molecular techniques used, drug resistance patterns (DRTB, MDRTB, XDRTB), phylogenetic lineages, and geographical distribution of MTBC strains were extracted and summarized by using Microsoft Excel software. Data were categorized based on key variables, and results were presented in the form of tables and graphs to illustrate trends and comparisons, as the study focused on summarizing and interpreting existing research findings.

Ethical Considering

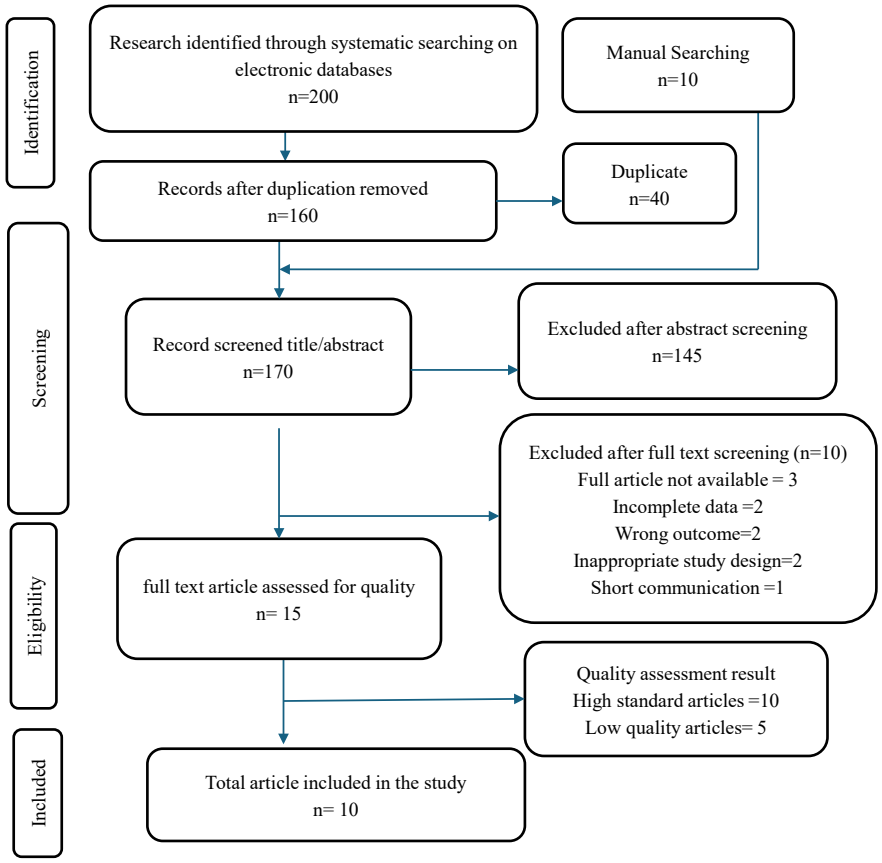
This study, based on a review of published articles without involving new human samples, did not require ethical approval. All data were sourced from publicly available studies and properly cited.

Results

Following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines, a total of 200 studies were initially identified through systematic searches of electronic databases, along with 10 additional records through manual searching. After the removal of 40 duplicate records, 170 unique studies remained. Title and abstract screening were then performed, leading to the exclusion of irrelevant studies. Subsequently, 25 full-text articles were assessed for eligibility. Of these, 10 articles were excluded for various reasons. Quality assessment was conducted on the 15 eligible full-text articles, resulting in 10 high-standard articles and 5 low-quality articles. Ultimately, 10 high-quality articles were included in the final analysis

Figure 1

PRISMA Flow Diagram Showing the Selection Process of Studies Included in the Review



In this systematic review, a total of 7848 samples were analysed from studies conducted across ten countries, resulting in 3216 confirmed isolates of *Mycobacterium tuberculosis* complex (MTBC). The distribution of isolates by country was as follows: Nepal (498 isolates from 877 samples), Nigeria (202/202), India (399/399), Ireland (42/42), Pakistan (116/200), South Africa (184/3810), Ethiopia (323/323), Belgium (954/1342), Sudan (383/383), and Ghana (115/270). Gender distribution analysis showed that 2,058 isolates (64%) were from male patients and 1,158 isolates (36%) were from female patients. In most countries, a male predominance was observed among the isolates: Nepal (71.3% male), Nigeria (65.8% male), Ireland (58.5% male), South Africa (44% male), Ethiopia (64.4% male), Belgium (64% male), Sudan (66% male), and Ghana (69.6% male). Gender-specific data were not available for studies from India and Pakistan. Overall, the findings consistently indicated a higher proportion of tuberculosis cases among males compared to females across different geographical regions.

Table 1

Distribution of isolates by country in 2017 to June 2023

Country	Sample size	No. of Isolates	Male		Female	
			No.	%	No.	%
Nepal	877	498	356	71.3	142	28.7
Nigeria	202	202	133	65.8	69	34.2
India	399	399	NA	NA	NA	NA
Ireland	42	42	25	58.5	17	41.5
Pakistan	200	116	NA	NA	NA	NA
South Africa	3810	184	156	44	28	56
Ethiopia	323	323	208	64.4	115	34
Belgium	1342	954	611	64.0	343	36
Sudan	383	383	253	66	130	34
Ghana	270	115	80	69.6	35	30.4
Total	7848	3216	2058	64	1158	36

Note. NA= Not applicable

A total of 10 studies employing molecular methods for the detection and characterization of MTBC were included (Table 2). Spoligotyping was the most commonly used method, reported in five studies conducted in Pakistan (n = 200), Ghana

(n = 270), Ethiopia (n = 323), Nepal (n = 877), and South Africa (n = 3880). One study from Nigeria (n = 202) employed a combination of Spoligotyping and IS6110-based analysis. Two studies used both Spoligotyping and Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat (MIRU-VNTR) typing methods, conducted in India (n = 399) and Belgium (n = 1342), respectively. A combination of MIRU-VNTR and whole genome sequencing (WGS) was reported in a single study from Ireland (n = 42). Additionally, one study from Sudan (n = 383) solely employed WGS for MTBC detection and characterization.

These findings highlight the global diversity in molecular approaches for MTBC identification, with a growing application of advanced techniques such as WGS, particularly in recent studies. Advanced genotyping methods were also represented across different geographic regions, with spoligotyping remaining the most frequently used method, while WGS is emerging in more recent studies for higher-resolution typing.

Table 2

Molecular methods used for detection of MTBC

Molecular methods	No. of study	Country	Sample size
Spoligotyping	5	Pakistan	200
		Ghana	270
		Ethiopia	323
		Nepal	877
		South Africa	3880
Both Sopligotyping and IS6110	1	Nigeria	202
Both Spoligtyping and MIRU-VNTR	2	India	399
		Belgium	1342
Both MIRU-VNTR and WGS	1	Ireland	42
WGS	1	Sudan	383

The distribution of MTBC lineages across selected studies showed distinct regional patterns. In Nepal (Maharjan et al., 2018) and India (Devi et al., 2021), Lineage 2 (L2) was predominant, representing 48.4% and 62.41% of isolates, respectively. Similarly, L2 dominated in South Africa about 67.4% (Bhembe et al., 2020). In Pakistan (Ali et al., 2019) and Sudan (Shuib et al., 2020), Lineage 3 (L3) was most prevalent, accounting for 82.0% and 73.5% of strains, respectively. In contrast, Lineage 4 (L4) was the major lineage in Nigeria (88.2%) by Pokam et al., 2019, Ireland (54.7%) Roycroft et al., 2018, Belgium (49.36%) Vlugen et al., 2017, Ethiopia (79.0%) Diriba et al., 2020), and Ghana (76.0%) Ameke et al., 2021. Minor representations of Lineages 1, 5, and 6, as well as orphan and newly described strains, were also observed, indicating diverse strain circulation across the regions was given in table 3.

Table 3

Distribution of MTBC lineage in the selected studies

Study and year	Country	Predominant lineage (%)	Lineage Types and Percentage
Maharjan et al., 2018	Nepal	L2 (48.4)	L1 (6.4), L2(48.4), L3(30.7), L4(14.4), Orphan (8.0)
Ali et al. ,2019	Pakistan	L3 (82.0)	L2(5.4), L3(82.0), L4(4.2), Orphan (12.0)
Pokam et al., 2019	Nigeria	L4(88.2)	L4 Cameroon (74.3), L4.4 Uganda I (8.9), L4.1 Harlem (5.0), L5 (3.0)
Devi et al.,2021	India	L2(62.41	L1(5.76), L2 (62.41), L3(19.0), l4(2.5), l5(0.5), Orphan (4.26), other (5.07)
Bhembe et al., 2020	South Africa	L2(67.4)	L1(0.5), L2(67.4), L4(23.1), Orphan (1), Other (8)
Roycroft et al., 2018	Ireland	L4(54.7)	L1(4.8), L2(33.3), L3((7.2), L4(54.7)
Vlugen et al.,2017	Belgium	L4(49.36)	L2(15.02), L3(7.6), L4(49.36), L5(1.76), Orphan (25.53), new (0.11)
Shuib et al., 2020	Sudan	L3(73.5)	L1(2.4), L2(0.6), L3(73.5), L4(23.5)
Diriba et al.,2020	Ethiopia	L4(79.0)	L2(2.0), L3(19.0), L4(79.0)
Ameke et al.,2021	Ghana	L4(76.0)	L1(0.9), L2(2.6), L3(0.9), L4(76), L5(13.6), L6(6.0)

Note. L1: Indo-Oceanic, L2: Beijing), L3: CAS (Delhi), L4: Euro-American, L5: *M. africanum* West Africaan-1

The analysis of MDR-TB cases across various studies reveals considerable variation in the rate of MDR-TB infection among isolates from different countries. The study by Maharjan et al. (2018) in Nepal identified 56.78% of MDR-TB cases among a total of 877 isolates, the highest MDR development rate was observed. In contrast, the study by Ameke et al. (2021) in Ghana found a significantly lower MDR-TB only 1.9% from 111 isolates. Other studies, such as those by Devi et al. (2021) in India and Pokam et al. (2019) in Nigeria, reported moderate MDR-TB of 50.6% and 14.4%, respectively, from 399 and 202 isolates.

On the other hand, the studies by Roycroft et al. (2018) in Ireland and Diriba et al. (2020) in Ethiopia showed relatively high percentages of MDR-TB cases (75% and 9%, respectively), although the number of isolates was lower (42 and 151, respectively). The study in Sudan by Shuib et al. (2020) showed a 12.7% MDR-TB from 383 isolates.

A notable outlier, the study by Vlugen et al. (2017) in Belgium, showed a remarkably low prevalence of 3.3% from 922 isolates. This variation in MDR-TB prevalence across countries may reflect differences in local epidemiology, healthcare infrastructure, and diagnostic capabilities. Further research is needed to explore the underlying factors contributing to these discrepancies.

Table 4

DR-TB and MDR-TB Rates Across Selected Studies

Study (Author, Year)	Country	Total isolate	MDR-TB case (%)
Maharjan et al., 2018	Nepal	877	498 (56.78%.)
Pokam et al., 2019	Nigeria	202	29 (14.4%)
Devi et al., 2021	India	399	202(50.6%)
Roycroft et al., 2018	Ireland	42	32(75%)
Vlugen et al., 2017	Belgium	922	3 (3.3) %
Shuib et al., 2020	Sudan	383	21 (12.7%)
Diriba et al., 2020	Ethiopia	151	14(9%)
Ameke et al., 2021	Ghana	111	2 (1.9%)

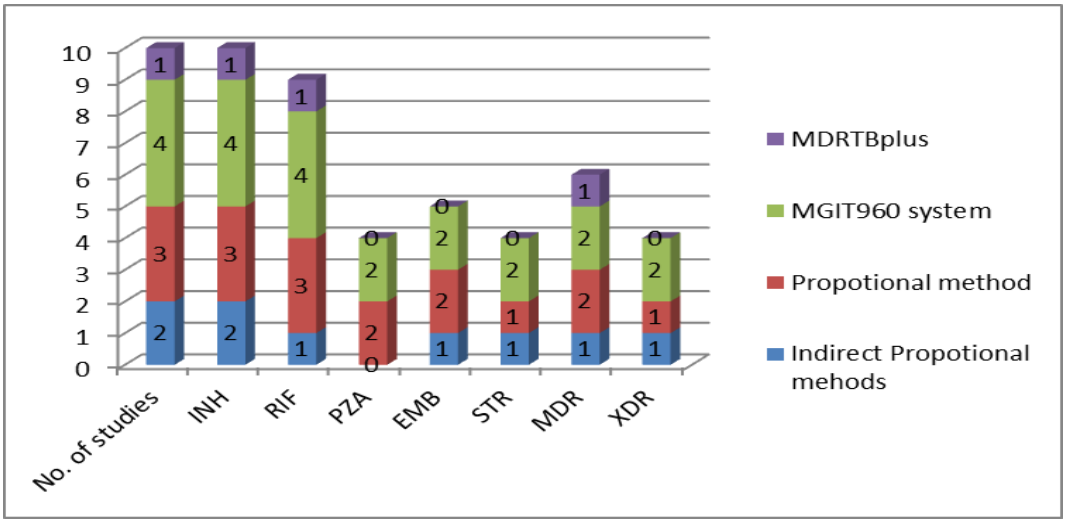
Figure 2 summarizes the methods employed for drug susceptibility testing (DST) across 10 selected studies. The proportion method on Lowenstein–Jensen (LJ) medium was the most frequently used phenotypic technique, applied in 5 studies (50%). Among these, 2 studies (40%) used the indirect proportion method, while 3 studies (60%) utilized the direct proportion method. The BACTEC MGIT 960 liquid culture system was applied in 4 studies (40%) for rapid phenotypic detection of drug resistance. Among molecular methods, the GeneXpert MTB/RIF assay Line Probe Assays (LPA) were performed in 4 studies (40%) to detect mutations associated with resistance to first- and second-line anti-tuberculosis drugs (MDRTBplus) was utilized in 1 study (10%) for the rapid detection of rifampicin (RIF) resistance.

For antibiotic resistance profiling, first-line anti-tuberculosis drug susceptibility testing was performed using all the reported methods. The LPA method was most used, followed by the proportion method, indirect proportion method, and MDRTBplus assay. However, in the case of ethambutol (EMB) and streptomycin (STR), the MDRTBplus method was not employed. This finding indicates that the MDRTBplus assay was limited to the detection of resistance against isoniazid (INH) and rifampicin (RIF) only. Among the 10 studies, 6 studies employed the proportional method and MGIT 960 system as the primary techniques for the detection of MDR-TB, while indirect proportion methods

and the MDRTBplus assay were used less frequently. For the detection of XDR-TB, 4 studies applied the MGIT 960 method, followed by proportional and indirect proportional methods.

Figure 2

Methods used for drug susceptibility testing and antibiotic resistant pattern



Discussion

This systematic review highlights the regional variations in the prevalence MDR-TB and the molecular methods employed to characterize MTBC strains. In this study, study conducted by Maharjan et al., 2018 showed a high MDR-TB in Nepal 56.78%, which is similar to study conducted by Shakya et al., 2021(54%) in Nepal. It is likely to studies from neighboring countries like India by Atre et al.2023, which report similarly high MDR-TB rates due to factors such as inadequate treatment adherence and delayed diagnosis. In contrast, in one study conducted in Ghana show significantly lower prevalence rates (1.9%), but other studies are inline conducted by Sylverken et al., 2021 and Afful et al., 2023 where MDR was 54.1% and 25% respectively. The MDR rate in this study is due to small sample size inconstancy in MDR detection method or likely due to better diagnostic capabilities and more robust TB control programs, as seen in other sub-Saharan African nation. Molecular methods for strain characterization varied, with spoligotyping being the most used technique across studies. Although spoligotyping is cost-effective, it has limitations in identifying closely related strains. This limitation was also reported in a study by Rodriguez-Campos et al (2011).

On the other hand, whole genome sequencing (WGS), used more frequently in Europe and Africa, offers a more precise method for analyzing resistance profiles and

understanding transmission dynamics, echoing trends observed globally toward advanced molecular diagnostics. The distribution of MTBC lineages revealed a clear geographical pattern, with Lineage 2 (Beijing) dominating in Nepal and India, which is consistent with findings from other South Asian countries. Lineage 2 is known for its strong association with drug resistance, particularly MDR-TB, indicating a persistent transmission of resistant strains in this region. Meanwhile, Lineage 4 (Euro-American) was more common in sub-Saharan Africa and parts of Europe, further confirming the region-specific nature of TB transmission dynamics.

Gender differences were also notable, with male patients accounting for 64% of MDR-TB cases, a trend observed globally. This may be due to higher exposure to risk factors such as smoking, alcohol use, and delayed healthcare-seeking behaviors, which are more prevalent in men. This supports the need for gender-sensitive public health interventions. Regarding drug susceptibility testing (DST), while the traditional proportion method on Lowenstein-Jensen medium is still widely used in resource-limited settings, there is an increasing shift toward rapid molecular tests such as GeneXpert MTB/RIF and Line Probe Assay (LPA). These methods offer faster and more accurate detection of resistance, especially to rifampicin, and their growing use in high MDR-TB burden regions aligns with global trends towards improving diagnostic accuracy.

However, this review also highlights significant methodological variations across studies, including differences in sample sizes, study designs, and diagnostic approaches, making cross-country comparisons challenging. These inconsistencies are noted in other reviews as well, underscoring the need for standardized methodologies to ensure more reliable data for global TB control efforts.

In conclusion, this review emphasizes the need for improved regional surveillance, the adoption of advanced molecular diagnostics, and tailored treatment strategies to combat MDR-TB. A coordinated global effort that includes better diagnostic infrastructure, timely treatment, and targeted prevention strategies is essential for addressing the ongoing MDR-TB challenge.

Conclusion

This systematic review provides valuable insights into the global distribution and prevalence of MDR-TB and the molecular techniques used to detect and characterize MTBC strains. The findings underscore significant regional variations in MDR-TB rates, with higher prevalence observed in countries like Nepal and India, and lower rates in nations such as Ghana. The review also highlights the increasing use of advanced molecular techniques like whole genome sequencing (WGS) and Line Probe Assay (LPA), which offer more precise and rapid methods for detecting drug resistance, particularly in regions with high MDR-TB burden. The geographical patterns of

MTBC lineages, with Lineage 2 (Beijing) dominating in South Asia and Lineage 4 (Euro-American) in sub-Saharan Africa and Europe, emphasize the regional specificity of TB transmission dynamics and drug resistance. Furthermore, the observed male predominance in MDR-TB cases aligns with global trends, reinforcing the need for targeted, gender-sensitive interventions.

Overall, the findings highlight the urgent need for standardized diagnostic protocols, improved surveillance systems, and personalized treatment strategies to effectively combat MDR-TB worldwide.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript. The research and findings presented are based solely on the data collected and analysed, without any influence from external organizations, financial interests, or affiliations that could have biased the results.

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