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MOLECULAR TYPING OF MULTIDRUG-RESISTANT MYCOBACTERIUM TUBERCULOSIS: A REVIEW OF IS6110 RFLP AND MIRU-VNTR GENOTYPING APPROACHES

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ABSTRACT

Tuberculosis (TB) remains one of the leading infectious causes of morbidity and mortality worldwide, with India accounting for the largest share of the global burden and the highest reported caseload of multidrug-resistant TB (MDR-TB). Molecular genotyping of Mycobacterium tuberculosis has transformed the epidemiological and clinical understanding of TB transmission, moving analysis beyond conventional contact tracing to direct comparison of bacterial DNA fingerprints. This review synthesizes current knowledge on M. tuberculosis genotyping methods — principally IS6110 restriction fragment length polymorphism (RFLP) and mycobacterial interspersed repetitive unit–variable number tandem repeat (MIRU-VNTR) typing — and examines their diagnostic, epidemiological, and clinical applications. Evidence is drawn from the molecular typing literature and illustrated using a five-year genotyping database of 478 M. tuberculosis isolates from a North London teaching hospital (2002–2007). Across this evidence base, IS6110 RFLP combined with MIRU-VNTR provided high-resolution strain discrimination, identified an active-transmission rate of only 6.3% (indicating predominantly reactivation/imported disease), detected an isoniazid-resistant outbreak lineage, and revealed that common drug-resistance mutations carry negligible fitness cost, allowing resistant strains to transmit as efficiently as susceptible ones. Genotyping further proved clinically valuable in distinguishing relapse from re-infection, ruling out false outbreaks, and identifying laboratory cross-contamination. The review concludes that dual-method molecular typing — and increasingly whole-genome

sequencing — should be integrated into routine TB surveillance, a step of particular relevance to high-burden settings such as India, where molecular surveillance capacity remains limited relative to disease burden.

KEYWORDS: Mycobacterium tuberculosis; IS6110 RFLP; MIRU-VNTR; Molecular epidemiology; Multidrug-resistant tuberculosis; Genotyping.

1. INTRODUCTION

Tuberculosis (TB), caused by Mycobacterium tuberculosis, remains one of the oldest and most persistent infectious diseases affecting humankind. Roughly one-third of the world's population is estimated to carry the organism, yet only 5–10% of infected individuals progress to active disease in their lifetime, with the remainder harboring latent infection that can reactivate if immunity is compromised by HIV, diabetes, malnutrition, or immunosuppressive therapy (WHO, 2009). India bears the largest share of the global TB burden — approximately 28% of cases worldwide — and simultaneously reports the highest absolute number of multidrug-resistant TB (MDR-TB) cases globally (WHO, 2022).

Conventional epidemiological tools such as contact tracing can identify some chains of transmission, but they miss links between patients who are unaware of any connection and cannot distinguish laboratory cross-contamination from genuine infection. Molecular genotyping addresses this gap by comparing the DNA fingerprint of the infecting organism itself, allowing investigators to confirm or refute suspected outbreaks, quantify the proportion of disease due to recent transmission versus reactivation, and track the spread of drug-resistant lineages through a population.

This review synthesizes the principles, methods, and applications of M. tuberculosis molecular typing, with particular emphasis on IS6110 restriction fragment length polymorphism (RFLP) — historically the gold-standard technique — and its modern successor, MIRU-VNTR typing. Evidence from the wider literature is illustrated throughout using findings from a five-year (2002–2007) genotyping database of 478 isolates collected at a North London teaching hospital, chosen because it represents one of the more detailed published applications of dual-method typing to a mixed, high-migration TB population — a demographic pattern increasingly relevant to urban centres in India.

2. Biology and Genome of M. tuberculosis

M. tuberculosis is a slow-growing, obligate aerobe with a generation time of 12–24 hours, in contrast to minutes for common bacteria such as Escherichia coli. Its mycolic-acid-rich cell

wall confers acid-fastness — the basis of Ziehl–Neelsen staining — and renders the organism resistant to desiccation, detergents, and many disinfectants, allowing it to survive for weeks on fomites or in contaminated laboratory solutions. The complete genome of the reference strain H37Rv, published in 1998, spans approximately 4.4 million base pairs and roughly 4,000 genes, with a GC content of 65.6%. Despite more than 99.9% sequence identity across global strains, clonal evolution — driven by mutation and deletion rather than horizontal gene transfer — has produced measurable genetic diversity, most usefully captured in repetitive elements such as the IS6110 insertion sequence, MIRU loci, and the direct repeat (DR) region targeted by spoligotyping.

Large-scale sequence analyses have resolved M. tuberculosis into six principal global lineages (Indo-Oceanic, East Asian/Beijing, East African–Indian, Euro-American, and two West African lineages), which display strong geographic and ethnic association even when patients from different backgrounds mix in the same city. This lineage structure underlies the finding, discussed below, that migrant TB in destination countries typically reflects imported strains rather than local transmission.

3. Drug Resistance: Mechanisms and Burden

Standard first-line TB treatment (the DOTS regimen) combines isoniazid, rifampicin, pyrazinamide, and ethambutol for two months, followed by isoniazid and rifampicin for a further four months. Interruption of this regimen — through poor compliance, drug shortages, or inadequate supervision

— allows resistant mutants to be selected. Resistance to isoniazid arises predominantly from mutations in katG (notably codon 315) and the inhA promoter, while more than 95% of rifampicin resistance maps to the rifampicin resistance-determining region (RRDR) of rpoB. Combined resistance to isoniazid and rifampicin defines MDR-TB, which requires 18–24 months of second-line treatment and carries a substantially lower cure rate (50–70%) than drug-sensitive disease (85–95%).

A central biological question — reviewed in detail below — is whether resistance-conferring mutations impose a fitness cost on the organism. Early guinea-pig studies suggested reduced virulence in isoniazid-resistant strains, but modern molecular epidemiology indicates that the clinically dominant mutations (particularly katG Ser315Thr) carry negligible fitness cost, which helps explain the sustained global expansion of MDR-TB.

4. Molecular Typing Methods for M. tuberculosis

IS6110 RFLP was, for over two decades, the internationally standardized method for TB genotyping. The technique exploits the mobile insertion sequence IS6110, which occupies between 0 and 25 copies at strain-specific genomic locations. Genomic DNA is digested with the restriction enzyme PvuII, separated by agarose gel electrophoresis, transferred to a membrane by Southern blotting, and hybridized with a labelled IS6110 probe to generate a unique band pattern. Analysis using software such as BioNumerics, with a standardized reference strain run on every gel, allows relatedness to be quantified using the Dice similarity coefficient. The principal limitation of IS6110 RFLP is that strains with fewer than five IS6110 copies — roughly 18–20% of isolates in most populations — cannot be reliably discriminated by this method alone, and the technique is slow (2–3 weeks) and technically demanding.

MIRU-VNTR typing has largely superseded RFLP as the routine standard. It measures the copy number of tandemly repeated DNA sequences at 12 or, more discriminatingly, 24 genomic loci, generating a numerical code that is fast to produce (2–3 days), fully automatable, and directly comparable between laboratories. Because MIRU-VNTR does not depend on IS6110 copy number, it resolves strains that RFLP cannot differentiate, and the 24-loci format has been adopted as the UK national standard. Complementary techniques include spoligotyping, which detects the presence or absence of 43 spacer sequences in the DR region to assign broad strain families (e.g., Beijing, Haarlem) but offers low discriminatory power for outbreak investigation, and whole-genome sequencing (WGS), which reads the entire 4.4 Mb genome and offers the highest possible resolution, distinguishing strains that appear identical by all other methods, at the cost of greater bioinformatic and financial resources.

Table 1. Comparison of principal M. tuberculosis molecular typing methods.

Method	Discrimination	Turnaround	Key limitation
IS6110 RFLP	High (>5 copies)	2–3 weeks	Unreliable for low-copy strains (~18–20%)
12-loci MIRU-VNTR	Moderate	2–3 days	Can generate false clusters if used alone
24-loci MIRU-VNTR	High	2–3 days	A few loci amplify inconsistently
Spoligotyping	Low	1–2 days	Insufficient for outbreak confirmation

Method	Discrimination	Turnaround	Key limitation
Whole-genome sequencing	Highest	Days–weeks	Cost and bioinformatics expertise

The consensus emerging from the literature is that no single method is sufficient in isolation: IS6110 RFLP and MIRU-VNTR are complementary, and dual-method typing — using MIRU-VNTR to resolve low-copy-number or RFLP-indistinguishable strains — represents the practical minimum standard, with WGS increasingly positioned as the definitive reference method.

5. Epidemiological Insights from a Molecular Typing Database

The value of dual-method typing is well illustrated by a five-year genotyping database built from 478

M. tuberculosis isolates (732 notified patients, 424 culture-positive, 294 genotyped by IS6110 RFLP) at a North London teaching hospital. The cohort had a mean age of 38.7 years, a male-to-female ratio of 1.3:1, and a high proportion of patients born outside the country of residence (72.8%), reflecting an immigrant-heavy TB demographic pattern broadly comparable to that seen in many South Asian urban centres receiving return migrants.

Table 2. Summary of key findings from a five-year (2002–2007) M. tuberculosis genotyping database of 478 isolates.

Parameter	Finding	Percentage
Culture-positive rate	424 / 732 notified patients	57.9%
Genotyped isolates	294 / 424 culture-positive	69.3%
Any drug resistance	75 / 445 tested isolates	16.9%
MDR-TB	23 / 445 tested isolates	5.1%
Low IS6110 copy number (<5)	90 / 478 isolates	18.8%
Isolates in 100%-identical clusters	101 / 478 isolates (28 clusters)	21.1%
Estimated active transmission rate	After removing lab contaminants	6.3%
Probable laboratory contamination	6 / 478 isolates	1.3%

The estimated active-transmission rate of 6.3% is markedly lower than the 14.4% reported in an earlier London cohort (1995–97) and far below rates reported from high-transmission settings such as Harare (72.2%) or Amsterdam (35.1%), indicating that TB in this migrant-heavy population was overwhelmingly due to reactivation of latent infection acquired abroad rather than local transmission. Secondary MIRU-VNTR typing confirmed that most IS6110-defined lineages represented genuine strain relationships and additionally reclassified low-copy-number isolates that RFLP alone had left ambiguous — a direct demonstration of the complementary value of dual-method typing described in Section 4.

6. Clinical Applications: Fitness, Immunology, and Case Evidence

Beyond outbreak mapping, molecular typing has clarified the biological behaviour of drug-resistant TB. Fitness assays measuring bacterial generation time in an isoniazid-resistant outbreak lineage (16 isolates, 14 resistant or MDR) and in a separate streptomycin-resistant family outbreak (7 of 20 contacts developing active disease — a strikingly high secondary attack rate) both found no statistically significant difference in growth rate between resistant and susceptible strains. This confirms, at the molecular level, that the most clinically prevalent resistance mutations (e.g., katG Ser315Thr, rpsL Lys43Arg) impose negligible fitness cost, allowing resistant lineages to transmit as efficiently as drug-sensitive TB — a finding directly relevant to understanding the sustained spread of MDR-TB in high-burden settings.

Genotyping has also informed host–pathogen immunology. In an investigation of patients whose T-cells failed to respond to the ESAT-6 antigen despite confirmed active TB, genotyping excluded a shared strain type as the explanation, and sequencing confirmed the esxA (ESAT-6-encoding) gene was intact and unmutated in all isolates tested — directing attention toward host immune factors rather than bacterial genotype as the likely cause of IGRA non-response, an important caveat for interpreting interferon-gamma release assays in high-risk patients.

Documented clinical case applications further illustrate the practical utility of genotyping: distinguishing a true bronchoscope-associated outbreak from coincidental independent infections (avoiding unnecessary mass contact tracing), confirming household transmission between partners when standard imaging-based contact-tracing criteria would have missed the case, and identifying six probable laboratory cross-contamination events (1.3% of isolates) that would otherwise have resulted in patients receiving unnecessary and toxic six-month TB treatment courses.

7. DISCUSSION

Taken together, the reviewed evidence supports several conclusions relevant to TB control in high-burden settings. First, IS6110 RFLP and MIRU-VNTR are complementary rather than interchangeable: RFLP retains high discriminatory power for the majority of strains but fails for the substantial minority with low IS6110 copy number, while MIRU-VNTR resolves these cases and offers markedly faster, more portable, digitally comparable results — supporting its adoption as the primary typing method, with RFLP or WGS reserved for confirmatory or high-resolution applications. Second, molecular data can fundamentally

reframe public health priorities: a low measured transmission rate (6.3% in the illustrative cohort) shifts the emphasis from isolating active cases toward screening and treating latent infection in migrant populations, whereas a high transmission rate would instead prioritize aggressive contact tracing and case-finding.

Third, the demonstrated absence of fitness cost in common resistance mutations has direct programmatic implications: drug-resistant TB cannot be assumed to be self-limiting, and health systems must treat MDR-TB transmission with the same urgency as drug-sensitive disease. Fourth, real-world case evidence shows that genotyping changes clinical management directly — averting unnecessary outbreak investigations, unmasking missed transmission events, and detecting laboratory contamination — benefits that justify integrating rapid molecular typing into routine diagnostic workflows rather than reserving it for retrospective research.

These findings carry particular weight for India, which reports the world's highest MDR-TB caseload yet has limited access to routine molecular strain typing outside specialized reference laboratories. Establishing 24-loci MIRU-VNTR capacity at regional medical colleges, linking genotyping results electronically to TB notification systems, and mandating strain submission for MDR-TB cases would extend the surveillance benefits demonstrated in this review to some of the highest-burden TB settings globally. Limitations of the underlying evidence include its origin from a single-centre, retrospective cohort with incomplete records for some analyses, exclusion of culture-negative disease (a substantial proportion of extrapulmonary TB), and a study period that predates the falling cost and growing accessibility of whole-genome sequencing.

8. CONCLUSION

Molecular typing of Mycobacterium tuberculosis, particularly the combined use of IS6110 RFLP and MIRU-VNTR, has moved from a research technique to a tool with direct clinical and public health value: quantifying transmission rates, identifying outbreaks and drug-resistant lineages, distinguishing relapse from re-infection, and detecting laboratory contamination. The evidence reviewed here confirms that drug-resistant TB strains transmit as effectively as sensitive strains, that reactivation rather than local transmission can dominate TB epidemiology even in dense urban settings, and that genotyping data are most powerful when integrated with clinical and epidemiological information rather than interpreted alone. As whole-genome sequencing becomes more affordable, it is poised to become the definitive reference standard; in the interim, dual-method typing represents the

practical minimum for robust TB molecular surveillance. For high-burden countries such as India, where MDR-TB rates are among the highest in the world, expanding access to molecular genotyping is not merely of academic interest but a necessary component of national TB elimination strategy.

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