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Random Amplified Polymorphic DNA (RAPD) Analysis of Mycobacterium tuberculosis Strains Isolated from Pulmonary Tuberculosis Patients

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ABSTRACT

Tuberculosis (TB) remains a significant global health challenge, with increasing incidence of multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) posing a threat to public health. This study aimed to analyze the genetic diversity of *M. tuberculosis* strains using RAPD fingerprinting techniques. A total of 100 culture-positive pulmonary TB patients were included, and their isolates were subjected to DNA extraction followed by RAPD-PCR analysis. The genetic diversity among the strains was assessed using dendrogram clustering. The results indicate substantial genetic polymorphism among the isolates, underscoring the need for continuous epidemiological surveillance to track the transmission and evolution of drug-resistant TB strains. The study further highlights the applicability of RAPD-PCR as an efficient molecular tool in epidemiological studies of M. tuberculosis.

Keywords: *Mycobacterium tuberculosis, RAPD-PCR, genetic diversity, pulmonary tuberculosis, molecular epidemiology, drug resistance*

1. INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains a major public health issue, especially in developing nations. It is estimated that nearly one-third of the global population is infected with TB, with a significant proportion being latent carriers. TB primarily affects the lungs but can also spread to other organs, leading to severe complications if left untreated.[1] The emergence of multidrug-resistant TB (MDR-TB) and extensively drug- resistant TB (XDR-TB) further complicates treatment efforts and necessitates advanced diagnostic and epidemiological tools for better disease management [1,2].

Genotyping techniques such as restriction fragment length polymorphism (RFLP), spoligotyping, and variable-number tandem repeat (VNTR) analysis have been used for strain differentiation. However, these methods require sophisticated laboratory infrastructure and extensive sample processing time. Technique like restriction fragment length polymorphism (RFLP), proved to be not useful for Indian isolates because of no or very low copy number have been reported for IS6110.[3] In contrast, RAPD-PCR is a rapid, cost-effective alternative that provides reliable differentiation of *M. tuberculosis* strains based on genomic polymorphisms [3,4]. RAPD-PCR can identify genetic variations in a wide range of bacterial strains and has been successfully applied in molecular epidemiology studies to track TB transmission [5].

Understanding genetic diversity among *M. tuberculosis* isolates is crucial for designing effective public health strategies. Variations in genomic structures influence bacterial virulence, drug susceptibility, and transmission dynamics [6,7]. This study aims to assess the genetic polymorphism of *M. tuberculosis* strains using RAPD-PCR and evaluate its role in epidemiological investigations. By identifying unique fingerprinting patterns among strains, this study contributes to the broader goal of enhancing TB surveillance and control efforts [8,9].

2. MATERIALS AND METHODS

Study Design: This descriptive longitudinal study involved laboratory investigations of *M. tuberculosis* strains isolated from pulmonary TB patients. This study is among the first of its kind conducted in Maharashtra, India [10,11]. Research study was carried out in the department of Microbiology, after obtaining institutional ethical committee approval.

Selection Criteria: Inclusion Criteria: Smear-positive pulmonary TB patients of all ages and genders, irrespective of treatment status; clinically suspected drug-resistant TB cases with proper written consent.

Exclusion Criteria: Smear-negative pulmonary TB cases; extra-pulmonary TB cases and patients not willing participating in the study with proper written consent.

Sample Collection and Processing: Sputum samples were collected from patients attending various medical departments. Two samples (morning and spot) were collected in sterile, leak- proof containers. Samples were processed and cultured on Lowenstein-Jensen (LJ) media using the Kudoh and Kudoh swab method [12,13].

DNA Extraction and RAPD-PCR Analysis:

DNA Extraction: DNA extraction was performed using a modified lysis protocol. A loopful of *M. tuberculosis* culture was suspended in 1 ml of TE buffer and heat-killed at 95°C for 30 minutes. After cooling, 10% SDS and proteinase K were added, followed by incubation at 65°C for 1 hour. The mixture was treated with a phenol-chloroform extraction method to remove proteins, and DNA was precipitated using chilled ethanol. The DNA pellet was washed with 70% ethanol, air-dried, and resuspended in TE buffer for further analysis [14].

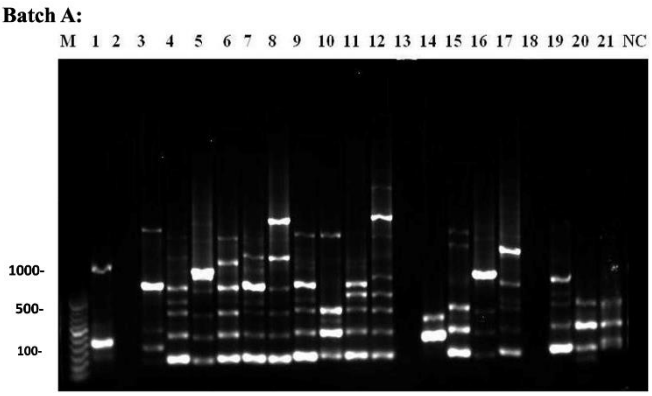
RAPD-PCR Mixture Preparation: The RAPD-PCR reaction was carried out in a 25 µl reaction volume consisting of 2.5 µl 10× PCR buffer, 1.5 mM MgCl2, 200 µM of each dNTP, 1 U Taq DNA polymerase, 1 µl of 20 pmol RAPD primer (INS-1 or INS-2) **(Table No. 1),** and 2 µl of template DNA. The remaining volume was adjusted with nuclease-free water [15].

PCR Cycle Conditions: PCR amplification was performed in a thermal cycler under the following conditions: Initial denaturation at 94°C for 5 minutes; 38 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 2 minutes; final extension at 72°C for 10 minutes; hold at 4°C until further analysis [16]. Appropriate positive and negative controls were always included to assure the quality of PCR and avoid carry over contamination.

Agarose Gel Electrophoresis and Data Analysis: Amplification products were analyzed using 1.5% agarose gel electrophoresis in TBE buffer. The gel was stained with ethidium bromide and visualized under UV light. Banding patterns were scored based on the presence or absence of bands. Dendrogram clustering was performed using Gene Directory software [17,18].

3. RESULTS

The results of this study demonstrated significant genetic polymorphism among the *M. tuberculosis* isolates. Out of 100 isolates, 93 were successfully typed using RAPD-PCR. A high level of genetic polymorphism was observed. It was observed that both the primers INS 1 and INS 2 were good for discrimination of MTB strains, but INS 1 was the best for strain polymorphism **(Figure No. 1 Set A to F).** When RAPD patterns were analyzed visually, it was observed that a large number of different genotypes were present, showing distinctive and unique profiles. About 75% of the isolates exhibited differences, indicating that the source and type of infection within the population is varied. This indicates presence of multiple phenotypes that calls for continuous monitoring of drug resistance and treatment regimen.



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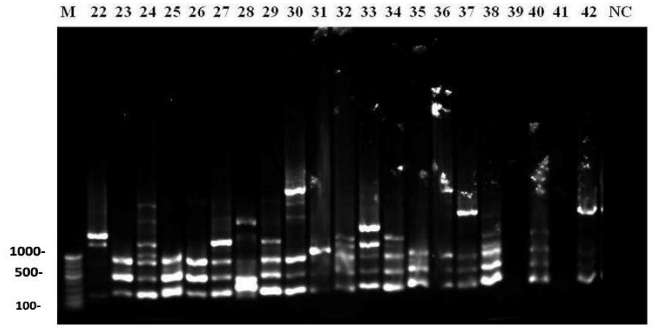

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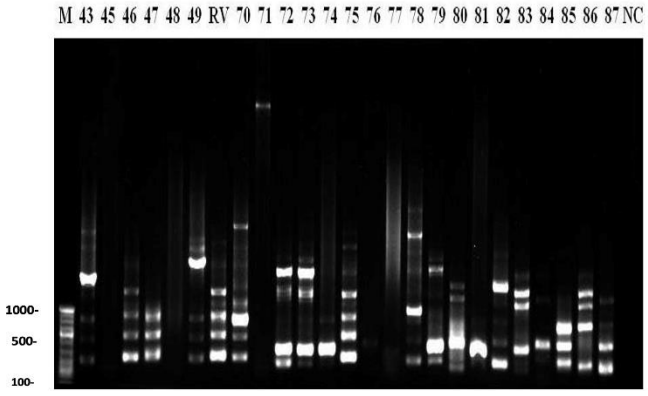

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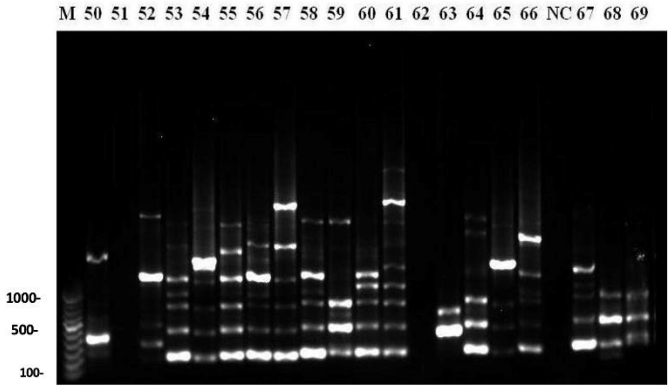

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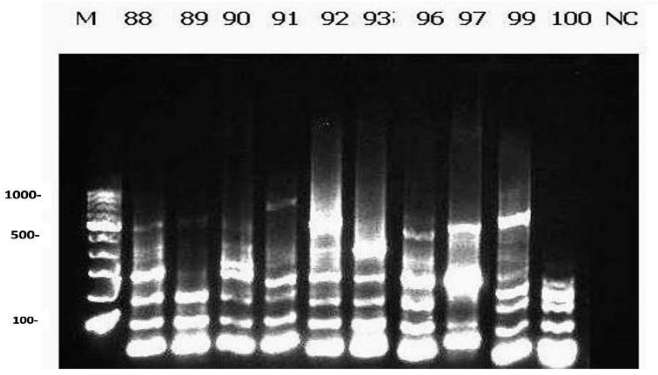

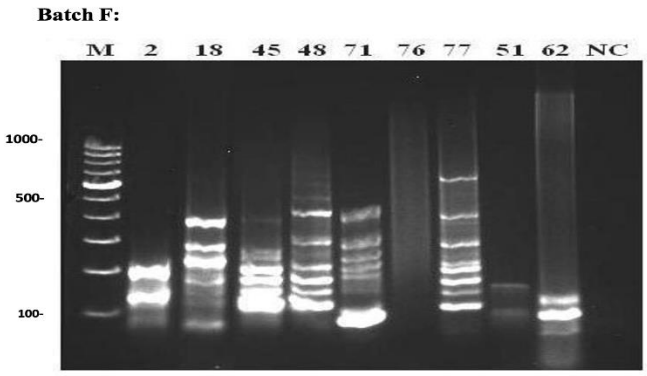

Figure No.1 :- RAPD profile generated from genomic DNA of strains of *M. tuberculosis* processed in batches of strains A to F, by using the oligonucleotide primer INS 1. Lanes:- M- Marker (100 base pair DNA ladder [Merck, India]), NC- negative control, RV- H37Rv *M. tuberculosis* strain and rest all lanes labeled with numbers, representing individual MTB strains.

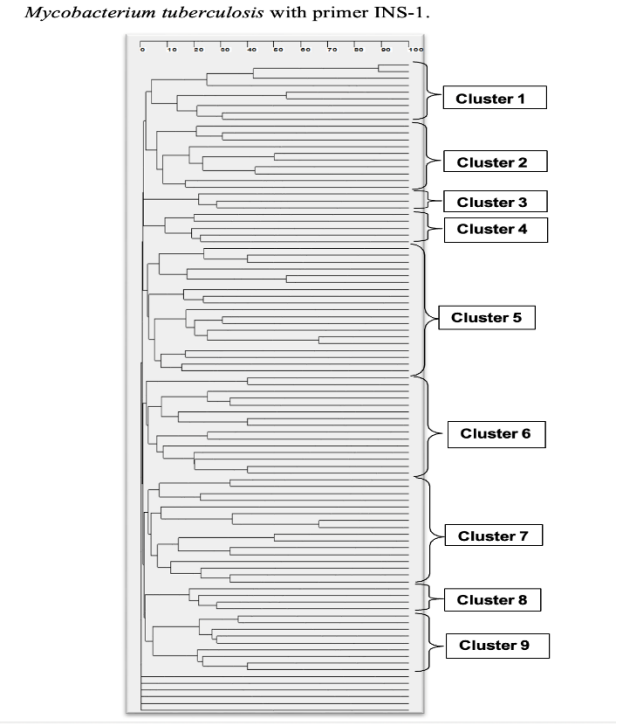

Figure Number 2 :- Dendrogram generated by Unweighted Pair of Group Average Method (UPGMA), showing genetic relatedness among 100 strains of *Mycobacterium tuberculosis* with primer INS-1.

❖ **Table No. 1: Primers used for RAPD-PCR:-**

Primers with 20mer oligonucleotides were obtained from Sigma-Aldrich Pvt. Ltd., Bangalore. Primers obtained from Sigma were de-protected and desalted. These dried DNA oligos were resuspended in 1×TE (10mM Tris, P^H 7.5-8.0, 1mM EDTA) and was used for PCR reaction. Detail descriptions of Primers were as follows:-

Batch	Oligo. Name	G+C%	Sequence (5' to 3')
9616-010	INS-1	70%	CGTGAGGGCATCGAGGTGGC
9616-011	INS-2	60%	GCGTAGGCGTCGGTGACAAA

Upon dendrogram analysis with primer INS 1, no obvious dominating groups of very closely related strains were apparent within these isolates. **(Figure No. 2)** Dendrogram analysis revealed significant clustering, suggesting diverse strain circulation within the community [19]. RAPD banding patterns showed that isolates from this geographical region exhibited distinct fingerprinting profiles, suggesting independent evolutionary lineages [20].Higher degree of genetic variability, indicating a more complex transmission pattern [21]. Several isolates exhibited unique banding patterns not previously reported, which may indicate emerging or localized genetic variations within *M. tuberculosis* populations [22].

4. DISCUSSION

The findings of this study emphasize the importance of molecular typing techniques in understanding TB epidemiology. The high degree of genetic heterogeneity among *M. tuberculosis* strains suggests active and ongoing transmission within the population [23]. RAPD- PCR proved to be a valuable tool for rapid genetic differentiation, providing crucial insights into the spread of TB strains in different communities [24].

Genetic Variability and Transmission Dynamics: The observed genetic variability highlights the adaptability of *M. tuberculosis*, which undergoes mutations that may impact drug resistance and virulence. Such mutations contribute to the emergence of distinct strain lineages, influencing treatment efficacy and disease progression [25]. The presence of multiple genetic clusters among MDR-TB strains suggests that resistance is developing independently in different populations, rather than being transmitted from a single source [26]. **(Figure 1)**

Epidemiological Implications of RAPD-PCR Analysis: The results underscore the significance of RAPD-PCR in tracking TB transmission. This technique allows for rapid and accurate identification of genetic differences among isolates, making it an ideal tool for resource-limited settings [27]. By revealing unique genetic patterns, RAPD-PCR helps in determining the origin and spread of TB strains, facilitating targeted public health interventions [28].

While methods like IS6110 RFLP and MIRU-VNTR have been widely used for TB genotyping, they require sophisticated equipment and longer processing times. RAPD-PCR, on the other hand, is more cost-effective and suitable for routine epidemiological investigations [29]. Our findings align with previous studies that support the broader adoption of RAPD-PCR for TB epidemiology, particularly in settings with limited molecular diagnostic facilities [30]. Although RAPD is relatively simple and useful for epidemiological analysis, standardization of PCR conditions is very important for better reproducibility. To obtain reproducible pattern, duplicate analysis is unavoidable for true profile differences to be evaluated

- **Public Health Considerations and Future Directions:** When RAPD patterns were analyzed visually, it was observed that a large number of different genotypes were present, showing distinctive and unique profiles. About 75% of the isolates exhibited differences, indicating that

the source and type of infection within the population is varied. This indicates, presence of multiple phenotypes as well, that calls for continuous monitoring of drug resistance and treatment regimen.

The study highlights the need for strengthened infection control measures and robust surveillance systems to monitor TB transmission dynamics [31]. MDR-TB strains exhibited extensive genetic diversity, reinforcing concerns about the spread of resistant strains within the community [32]. Future studies should focus on integrating RAPD-PCR with whole-genome sequencing (WGS) to enhance strain characterization and improve TB control strategies [33].

5. CONCLUSION

RAPD-PCR fingerprinting effectively differentiated *M. tuberculosis* strains, demonstrating its potential for epidemiological applications. The observed genetic diversity emphasizes the importance of targeted interventions for TB control. The study recommends further large-scale investigations to validate RAPD-PCR as a standard genotyping tool for TB epidemiology. Strengthening surveillance efforts and incorporating molecular typing techniques can significantly improve TB control and management strategies [34].

Conflict of interest: None.

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