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# Clustering analysis of *Mycobacterium tuberculosis* isolates obtained from Sethi Colony slum area of Jaipur City by using RAPD fingerprinting

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**Abstract**

Slums are "*hot spots"* for transmission of tuberculosis (TB) especially in developing nations with high TB prevalence. Molecular typing of strains can be used for studying the recent transmission dynamics of TB. Random Amplified polymorphic DNA (RAPD) analysis was used to study transmission dynamics of TB in Sethi Colony slum of Jaipur city, Rajasthan. Out of 98 Sputum samples MTB growth was obtained in 24 (24.48%) samples. Transmission and Diversity of tuberculosis in the study area was 41.66% and 58.34% respectively.

**Keywords:** *Mycobacterium tuberculosis*, Molecular typing, RAPD analysis, Transmission, Clustering, Fingerprinting

## Introduction

Random Amplified polymorphic DNA (RAPD) analysis provides a rapid and easy means of identifying polymorphism in *M. tuberculosis* (MTB) isolates. The ability to distinguish strains of *M. tuberculosis* can be useful for investigating the source of infection, relation of strains recovered from different patients and the identities of multiple strains recovered from the patients from close by localities. Infections caused by mycobacterium are known to be transmitted from human to human. For the emergence of multi-drug resistant tuberculosis disease, it is important to know whether the disease is due to a new strain or relapse of a known strain. (Singh *et. al.*, 2006) RAPD also referred as "arbitrary primer PCR" has been used for typing of various organisms (Akopyanz *et al.*, 1992), without the knowledge of genetic structure of an organism. RAPD has been successfully applied to differentiate strains of MTB in epidemiological analysis (Harn *et al.*, 1997; Richner *et al.*, 1997; Singh *et al.*, 2002), including strains associated with temporal and geographical cluster of tuberculosis in United Kingdom and those from India, Africa and South Africa (Linton *et al.*, 1994), Taiwan (Harn *et al.*, 1997) and South Africa (Richner *et al.*, 1997). The study by Singh and co-workers (2002), have reported the usefulness of RAPD analysis using arbitrary primer *986*F Primer for the typing of Indian strain of MTB.

Slums are "*hot spots"* for transmission of tuberculosis (TB) especially in developing nations with high TB prevalence Moreover,

there are limited studies delineating transmission dynamics of the disease in high risk groups such as slum areas, homeless, alcoholics and immune-compromised or hospitalized patients in India. In view of the global prevalence of tuberculosis, there is an urgent need to develop techniques that not only identify and characterize tubercular bacilli, but also facilitate epidemiological studies to trace the source of infection thereby facilitating formulation of effective control strategies. Molecular typing of strains can be used for studying the recent transmission dynamics of TB. Standard fingerprinting methods have their own limitations and to overcome that many fast PCR based typing methods have been used. This study was planned to study transmission dynamics and clustering between *M. tuberculosis* isolates obtained from Sethi Colony slum area of Jaipur city by using RAPD molecular fingerprinting technique.

**Materials and methods**

The study was conducted at Mycobacteriology and Advance Research lab, Department of Microbiology and Immunology, SMS Medical College, Jaipur. A total of 98 early morning sputum samples were collected in sterile disposable sputum collection container from the Sethi Colony, slum area of Jaipur city, and were stored at 4 ºC until processed.

**Culture of *M. tuberculosis*:**

Sputum samples were processed by (Modified Petroff's method) with 4% sodium hydroxide in 1:1 ratio for 20 minutes in the 50 ml falcon tube with frequent vortexing. Tubes were filled with sterile distilled water up to the mark of 50 ml and mixed well. After centrifugation for 15 minutes at 3000g, the supernatant was discarded and the pellet was dispersed in 1 ml of distilled water and inoculated on two labeled Lowenstein Jensen (LJ) media slants using 5 mm inoculating loop (Kubica and David, 1980). The inoculated LJ slopes were incubated at 37 ºC up to 8 weeks and observed every week for growth.

**DNA Isolation:**

The DNA from cultures was extracted by standard physio-chemical method (van Soolingen *et al.,* 1994). The culture isolates were heated at 95ºC in boiling water bath for 7 minutes and were snap chilled for 30 minutes. Briefly, the steps involved addition of 40 µl of lysozyme, incubation for 2 hours at 37ºC, followed by 5 µl of proteinase K (10 mg/ml) and 56 µl of 10% SDS and incubation at 65ºC for 30 minutes. 80 µl of 5M sodium chloride was added along with 64 µl of CTAB-NaCl and incubated at 65ºC for 30 minutes. Equal volume of chloroform-isoamyl alcohol (24:1) was added and centrifuged at 8000 g for 5 minutes. Upper layer was transferred to fresh tube. 0.6 volume of isopropanol was added. Tubes were incubated at -20ºC for 1 hour and centrifuged at 8000 g for 15 minutes at room temperature. The supernatant was discarded.150 µl of ice-cold 70% ethanol was added gently side-by-side to DNA pellet and centrifuged at 8000 g for 5 minutes. Supernatant was discarded and pellet air dried at room temperature. DNA was finally dissolved in 30 µl of TE buffer; quality of DNA was checked by Nanoquant spectrophotometer (Tecan, Frence) and stored at -20 ºC for RAPD analysis.

**RAPD Analysis:**

RAPD analysis of MTB isolates was done by standardized protocol (Linton *et al.,* 1994, Singh *et al.,* 2002). PCR was performed using 50ng of MTB DNA, 1.5U/µl of *Taq* polymerase (NEB) and forward primer of IS*986* gene (5'-ACGCTCAACCCCAGAGACCA -3'). PCR was performed on ABI PCR system 9700 (Applied Biosystems, US) using a 36 cycle profile of 94 ˚C for 1', 36 ˚C for 1' and 72 for 2' and a final extension at 72 ˚C for 10'. The amplicons were run on 2% agarose gel

with 1 kb DNA marker (G-Biosciences) for 3 hr at 50V and visualised on gel documentation system (Bio-Rad, USA). The bands from the gels were analyzed by software (Quantity One, Bio-Rad, USA) and dendrogram constructed using the UPGMA online available software for clustering analysis of MTB isolates.

## Results

Among the 98 collected sputum samples 73(74.49%) were from males and 25(25.51%) were from females. From the collected 98 sputum samples MTB growth was obtained in 24 (24.48%), three (3.06%) were contaminated, and 71 (72.44%) samples were culture negative.

All the 24 isolates were analyzed for RAPD with IS*986* forward primer, $H_{37}$Rv was used as control strain. Several fragments were amplified in each sample, and most of these fragments were observed to be common to different strains. The standard $H_{37}$Rv showed uniform pattern of two bands at 350 bp and 800 bp. RAPD-PCR revealed the presence of amplicons of a variety of sizes in MTB isolates.

The dendrogram constructed for the study areas also revealed clustering patterns (Fig.1) with the Cophenetic Correlation Coefficient (Cp) values of 0.41129788225458 in Sethi Colony. Among the 24 MTB isolates, 14 (58.33%) isolates were in four clusters and 10 (41.66%) were unique. These patterns show the transmission of tuberculosis in the study area was 41.66% and rate of diversity was calculated as 58.34%.

## Discussion

The ability to distinguish strains of MTB would be useful for investigating the source of outbreaks of infection, the relatedness of strains recovered from different patients and the identities of multiple strains recovered from the patients from close by localities. Infections caused by mycobacterium are known to be transmitted from human to human (Blazquez *et al.*, 1997). In an outbreak investigation of tuberculosis, it is often important to know whether the disease is due to a new strain or relapse of a known strain.

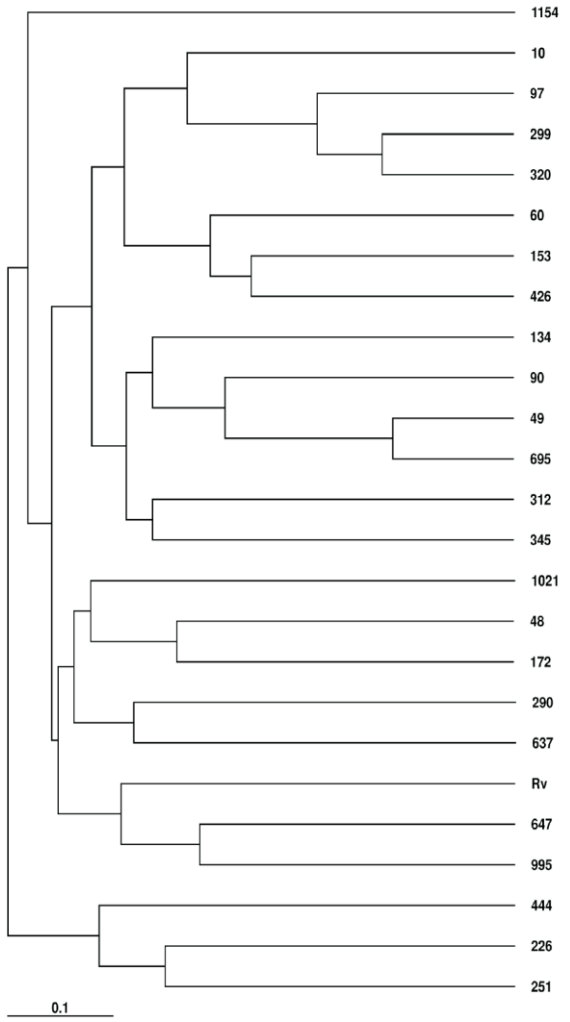


**Fig. 1: Dendrogram showing clustering pattern in MTB isolates of Sethi Colony area by RAPD using IS 986 forward primer.**

Clusters of *M. tuberculosis*, consisting of the largest number strains, showed a possible close genetic relationship. There were no clear patterns of relationships to geographical area in most of the clusters (Singh *et al.*, 2002). The results of the present study also showed the heterogeneity in the *M. tuberculosis* strains in the population studied.

From these patterns, 14 isolates were in four clusters and the transmission of tuberculosis from the study area was calculated as 41.66% and rate of diversity was calculated as 58.34%. Similar findings were observed in another study done at Agra (Singh *et al.,* 2002) and Izatnagar (Singh *et al.,* 2006). In another hospital based study using different sets of primers performed at Olsztyn, Poland revealed 32 different phenotypes with a mean total gene diversity of 0.34 (Korzekwa *et al.,* 2006). However, in a study, use of longer RAPD primers in RAPD yielded similar polymorphism as that of smaller primers (10 nt). The same study also revealed that IS*6110*, OPD01 and OPD02 were best for strain (Korzekwa *et al.,* 2006). About 76% similarity was observed in a study carried out by Rehim and co-workers (2011), where all strains belonged to very close genotypes and the similarity coefficient was more than 75% according to the set of primers used. RAPD was earlier reported to be discriminatory in isolates from Britain, Gambia and Tibetan refugees in India, Malawi, Kenya, Saudi Arabia, Taiwan and South Africa (Singh *et al.,* 2002). As per some studies, the degree of polymorphism obtained by RAPD was almost the same as that obtained by RFLP (Linton *et al.,* 1995).

The variation obtained by RAPD fingerprints may reveal that the chance of transmission of tuberculosis among unrelated cases would be unlikely. Similarly, tuberculosis caused by the strains with identical RAPD patterns are caused by recently transmitted disease and those cases caused by the unique fingerprints may be due to reactivation of infection but it is difficult to comment as exogenous infection may have been acquired from area/population other than the presently studied area as TB is endemic in India (Harn *et al.,* 1997). However, it has been found that the fingerprints generated by more than one primer are necessary to render the method sufficiently differential (Linton *et al.,* 1994).

This method uses short oligonucleotide primers of an arbitrary sequence and low-stringency PCR, to amplify discrete DNA fragments that can be used as molecular markers. RAPD analysis is rapid, inexpensive, easy to perform and can be used for determination of genetic heterogeneity based on DNA sequence diversity (Bowditch *et al.,* 1993; Welsh *et al.,* 1990; Williams *et al.,* 1990). In limitations of this study, results generated by RAPD technique should be compared with other molecular methods.

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