SUSPECTED SMALL-SCALE INTERPERSONAL TRANSMISSION OF 
MYCOBACTERIUM TUBERCULOSIS IN WARDS OF AN URBAN HOSPITAL IN 
DELHI, INDIA

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Abstract. Genotypes of Mycobacterium tuberculosis causing disease were investigated in pulmonary tuberculosis patients admitted to two adjacent wards of a tuberculosis hospital in Delhi, India. Genetic markers, the insertion sequence IS6110, a direct repeat sequence, and a polymorphic GC-rich sequence supported the circumstantial epidemiologic link between eight strains of M. tuberculosis, suggesting their possible involvement in small-scale, interpersonal transmission of both drug-sensitive and drug-resistant tuberculosis. This is the first report of a suspected acquisition of M. tuberculosis among hospitalized patients in India. The use of multiple molecular typing markers and techniques unequivocally identified the exact clonality of strains isolated from the hospital. The result of this study emphasizes the need for more comprehensive investigation of high-risk situations for tuberculosis transmission and long-term follow-up analysis for identifying such instances of unsuspected transmission.

INTRODUCTION

Mycobacterium tuberculosis, the pathogen causing tuberculosis in humans, harbors multiple copies of DNA elements interspersed throughout the genome. Among these, the insertion sequence IS6110, a polymorphic GC-rich sequence (PGRS), and a direct repeat (DR) region have been mapped in terms of number and genomic location to elucidate the genetic relatedness among clinical isolates of M. tuberculosis. The widespread use of molecular typing techniques, especially restriction fragment length polymorphism (RFLP) using IS6110 has disclosed the vulnerability of hospitalized patients, especially the immunocompromised, for potential outbreaks of multi-drug resistant tuberculosis. These studies have highlighted the risk factors for nosocomial transmission, namely, delayed diagnosis of the index case, delay in recognizing drug resistance, insufficient isolation procedures, and ambulatory infectious cases, and have adequately drawn the attention of public health authorities to formulate measures arresting such outbreaks.

India accounts for 22% of the world’s smear-positive tuberculosis patients. Conventional epidemiologic studies to control tuberculosis are very limited due to socio-behavioral factors and inadequate governmental policies. Isolated studies from India suggest that reactivation maintains the disease in the community. There is a paucity of studies delineating transmission dynamics of the disease in high-risk groups such as the homeless, alcoholics, and immunocompromised or hospitalized patients in India. In this study, using polymorphism in the IS6110 element as well as the DR element, we describe suspected small-scale transmission of M. tuberculosis among patients admitted to an urban hospital in Delhi.

MATERIALS AND METHODS

The study included all patients admitted to two wards for male patients at the Rajan Babu Tuberculosis Hospital, Kingsway Camp in Delhi during 1995 and 1996. The patients had radiologic suspicion of tuberculosis as well as at least two of the following criteria: a cough of more than four-weeks’ duration, expectoration (mandatory), dyspnea, chest pain, loss of weight, and fever. These two wards admitted patients from Delhi who had no history of tuberculosis, and they had the lowest dropout rates. One hundred sixty consecutive patients admitted to two wards and reported to be smear-positive by the microbiology laboratory of the hospital were enrolled for this study. Additionally, seven outpatients who had been discharged from these wards with smears negative for acid-fast bacilli, but who had expectoration, fever, and weight loss in the follow-up period were also considered. However, the initial cultures of these patients were not available for RFLP. A detailed questionnaire regarding the residence, treatment history, hospitalization, and severity of clinical symptoms was completed for each patient. One to three samples of sputum expectorated early in the morning were collected from each patient in a wide-mouthed, screw-capped container and processed for culture. One hundred eighteen isolates were grown from these patients (both smear-positive and smear-negative in our study), of which 15 had been lost due to contamination. The study was reviewed and approved by the Institutional Committee of the All India Institute of Medical Sciences (New Delhi, India). All patients gave their informed consent to participate in the study.

Thirty-four patients provided a follow-up sample after 1–2 months. Seven patients had a culture-positive sample one month after enrolling in the study (follow-up sample) (Table 1). Of these seven patients, five had been smear-negative but radiologically probable for tuberculosis and two patients were smear-positive before the study period. When the study was initiated, all had completed one month of antituberculosis therapy and had been smear-negative. After being discharged, they were followed-up by the hospital as outpatients. After 1–2 months, these patients became smear-positive and the culture yielded a strain of M. tuberculosis with RFLP patterns matching one of the past or contemporary ward patients.

Of the 103 isolates that survived, 92 were confirmed to be M. tuberculosis by biochemical tests, as well as by growth and colony morphologic characteristics. Of these, DNA suitable for RFLP could be obtained only from 83 isolates.

Sputum was processed by modified Hanks’ flocculation method of Shrinivas and Bhatia. An aliquot of the decontaminated sediment was examined for acid-fast bacilli, and grown on Lowenstein-Jensen medium for eight weeks at
Analysis of IS6110-clustered Mycobacterium tuberculosis strains isolated at various time intervals from pulmonary tuberculosis patients

<table>
<thead>
<tr>
<th>Days 1–3 after admission</th>
<th>Total</th>
<th>Smear+ cultures</th>
<th>Smear− cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days 35–45 after admission*</td>
<td>6 new + 4 follow-up</td>
<td>8</td>
<td>2†</td>
</tr>
<tr>
<td>Days 65–95 after admission†</td>
<td>5 new + 3 follow-up†</td>
<td>4</td>
<td>4†</td>
</tr>
</tbody>
</table>

* One month of standard first-line chemotherapy.
† Patients involved in suspected transmission.
‡ Two months of standard first-line chemotherapy.

37°C. Colony morphology and rate of growth of the cultures were recorded; tests for niacin production and nitrate reduction were performed to establish the species of the isolates. Susceptibility of M. tuberculosis strains to rifampicin, isoniazid, streptomycin, amikacin, and p-aminosalicylic acid (PAS) were determined by an agar proportion method.

DNA was extracted from M. tuberculosis cultures using proteinase K and NaCl-cetyl trimethyl ammonium bromide and digested with Pvu II. Fingerprinting was performed according to the standardized protocol recommended by van Embden and others. The isolates typed for IS6110 were further grouped by the numbers of direct repeat (DR) elements present in them using spoligotyping as described by Kamerbeek and others. Typing patterns sharing 100% homology were considered clustered. Strains bearing single IS6110 element as well as two strains having an IS6110 pattern similar to the virulent O strain were found to cluster in three groups, and were further differentiated for polymorphism of PGRS.

Computer analysis of the DNA banding patterns was done at the National Institute of Public Health and the Environment (Bilthoven, The Netherlands) with Gelcompar software (Applied Math, Kortrijk, Belgium) after scanning the autoradiographs at 74.8 dots/cm (190 dots/inch) (HP Scanjet IIcx/T; Hewlett-Packard, Camas, WA). Patterns were compared using a deviation value of 3%, and the relationship between all the isolates was defined mathematically. The patterns were clustered by the unweighted pair group method using arithmetic averages using the Dice coefficient.

In this study, two genetic markers, IS6110, and DR elements, were exploited to analyze the clonality of isolates. Strains with identical patterns of these two markers were regarded as clustered, while isolates with distinctive fingerprints by both these markers were deemed unique genotypes.

Of 83 isolates of M. tuberculosis, IS6110-RFLP identified 67 different patterns suggesting 80.7% polymorphism. Most isolates showed a multiband pattern characterized by the invariable presence of two high molecular weight DNA fragments bearing IS6110. These M. tuberculosis isolates were designated the Delhi type because of their relatively high prevalence in this region. Other genotypes lacking the typical high molecular weight banding pattern were named the non-Delhi type.

Among these highly polymorphic isolates, 10 M. tuberculosis strains were found to group into four clusters: A-D (Table 2 and Figure 1). Cluster A was composed of M. tuberculosis isolated from two patients (1301 and 1312) that had a unique, non-Delhi type four-band IS6110-RFLP pattern and similar spoligotypes. These isolates were sensitive to the drugs tested. These patients were admitted to adjacent wards and were hospitalized together for 25 days (Figure 2).

Cluster B consisted of isolates from two patients (1404 and 1421) who were infected with M. tuberculosis strains with the Delhi type IS6110-RFLP pattern, but their spoligotypes were discordant. Therefore, these patients were not considered as a part of the suspected nosocomial transmission.

Cluster C included M. tuberculosis isolates from three patients (1413, 1416, and 1427) infected with the Delhi type M. tuberculosis strains. Spoligotyping provided similar patterns for all three patients. These patients had shared various periods of stay in the same hospital ward (Figure 2). The isolate from patient 1416 was resistant to PAS, while drug susceptibilities of the isolates from other two patients could not be estimated because of technical reasons.

Cluster D was composed of three patients (1388, 1392, and 1401) who were infected with the Delhi type M. tuberculosis strains that had similar IS6110 RFLP patterns and identical spoligotypes. These isolates were resistant to INH and streptomycin. Patient 1388 was a chronic defaulter (a patient who did not take their medication) who was partially treated for five months with INH, rifampicin, pyrazinamide, and etham.

### Table 1

<table>
<thead>
<tr>
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* One month of standard first-line chemotherapy.
† Patients involved in suspected transmission.
‡ Two months of standard first-line chemotherapy.

### Table 2

Analysis of IS6110-clustered Mycobacterium tuberculosis strains isolated from adjacent wards of a tuberculosis hospital using spoligotyping, and conventional epidemiologic investigation

<table>
<thead>
<tr>
<th>RFLP cluster</th>
<th>No. of isolates</th>
<th>Patient ID</th>
<th>Spoligotype</th>
<th>Epidemiologic relationship</th>
<th>Drug sensitivity profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2</td>
<td>1301</td>
<td>Type 1</td>
<td>Different wards; overlap of hospitalization terms</td>
<td>Sensitive</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>1312*</td>
<td>Type 1</td>
<td>Same ward; subsequent hospitalization terms</td>
<td>Sensitive</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>1404</td>
<td>Type 24</td>
<td>Same ward; overlapping hospitalization terms</td>
<td>Not available†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1421*</td>
<td>Type 25</td>
<td>Not available†</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>1413</td>
<td>Type 23</td>
<td>Same ward; overlapping and subsequent hospitalization terms</td>
<td>Not available†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1416*</td>
<td>Type 23</td>
<td>PAS resistant</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1417*</td>
<td>Type 23</td>
<td>Not available†</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1388*</td>
<td>Type 18</td>
<td>INH strep resistant</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1392*</td>
<td>Type 18</td>
<td>INH strep resistant</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1401*</td>
<td>Type 18</td>
<td>INH strep resistant</td>
<td></td>
</tr>
</tbody>
</table>

PAS = p-aminosalicylic acid; INH = isoniazid acid; strep = streptomycin.
* Patients 1312, 1421, 1416, 1417, 1392, and 1401 were smear negative and culture negative in samples obtained on days 1–3; however, samples obtained on days 35–45 were smear negative as well as culture positive.
† Persistent contamination of drug susceptibility testing plates.
The second patient (1392) in this group was a newly diagnosed patient with tuberculosis who had occupied the same bed immediately after the earlier occupant (1388) had left the hospital. The third patient (1401) was also a new case of tuberculosis who was three beds away from the second patient (1392) and had spent all 17 days of his hospitalization in the physical presence of the second patient (Figure 2).

The RFLP patterns of the clustered isolates are shown in Figure 1. The durations of hospitalization of the patients suspected to be involved in the interpersonal transmission are shown in Figure 2. The demographic account, drug susceptibilities, as well as spoligotyping results (with PGRS-RFLP for cluster A alone) are shown in Table 2.

DISCUSSION

Delhi is a densely populated city (6,532 persons/km²) with approximately 12.8 million people in urban colonies and 0.9 million in villages (The Provisional Census of India, 2001). The Revised National Tuberculosis Program has been implemented in Delhi, with 14 District Tuberculosis Centers. Kingsway Camp is one such center that serves 0.7 million people in Delhi and admits approximately 7,800 tuberculosis patients every year. Rajan Babu Tuberculosis Hospital is a 1,155-bed tuberculosis hospital. During the study period, 117 patients were admitted to the two wards of this urban hospital in Delhi, and the patients had no social contact before hospitalization.

The ability to genetically characterize different, closely related mycobacterial strains using RFLP has proved to be useful in understanding virulence, resistance to drug therapy, and epidemiologic aspects. Exogenous reinfection found among patients with tuberculosis was a strongly disputed subject before the advent of molecular tools for genotyping. Nevertheless, such instances have been sporadically reported from selected groups such as alcoholics and the homeless. With the availability of RFLP and polymerase reaction–based genotypic analyses, reports of transmission involving both sensitive and drug-resistant strains of M. tuberculosis between immunocompetent and immunocompromised indi-

![Figure 1. Insertion sequence IS6110 restriction fragment length polymorphism (RFLP) patterns and spoligotyping patterns of isolates from 10 pulmonary tuberculosis patients in Delhi, India suspected of having interpersonal transmission. Each patient is denoted by an RFLP number. Mycobacterium tuberculosis isolated from Cluster A patients showed a characteristically virulent O pattern by the IS6110 RFLP. Supplementary polymorphic GC-rich sequence typing revealed a non-O pattern. Cluster B patients had a variant spoligotype and do not represent true cases of transmission. Cluster C and D patients had isolates with identical IS6110-RFLP and spoligotypes.](image1.png)

![Figure 2. Duration of hospitalization and smear status of tuberculosis patients suspected to be cases of reinfection with Mycobacterium tuberculosis in two wards of a hospital in Delhi, India. The duration of the study is plotted. Each patient is denoted by a restriction fragment length polymorphism number and represented as a vertical bar. The solid portion of the bar indicates duration of hospitalization and the dotted extension indicates the duration for which the patient was followed-up as an outpatient. The smear status (+, -, and not known [NK]) of the patients is indicated at different time points (on hospitalization, after one month of antituberculosis therapy on discharge, and after two months as an outpatient). The suspected infectious period is indicated by the horizontal hatched bar. Cluster B (boxed) is differentiated from the other clusters to indicate the absence of 100% homology between the spoligotypes of the patients.](image2.png)
individuals are available, especially in institutional settings.\textsuperscript{2–5,19,20} In this study, we report the incidental finding of small-scale transmission of tuberculosis among hospitalized patients in Delhi.

In the present study among hospitalized patients in Delhi, exogenous reinfection was suspected in the few sparingly clustered patients. Majority of patients were infected with unrelated strains of \textit{M. tuberculosis}, despite being patients in two wards of the urban hospital.\textsuperscript{14} All the patients, except for the suspected index case, had been smear-negative up to 1–2 months after starting antituberculosis treatment, after which they had become smear-positive, which clinically would have meant treatment failure. During the study period, the initial isolates of three of five contact patients implicated in suspected transmission were not available because of their acacillary smears. However, two other patients had been smear and culture positive before the initiation of the present study in their wards. Those early isolates were not included in the RFLP analysis so that a consistently sterile isolation protocol was maintained. Transmission among these patients is further suspected by factors that strongly favor prospective intra- and inter-ward transmission in this setting, namely, the contiguous or overlapping terms of hospitalization, proximity of beds of patients with similar patterns (Figure 1), possibility of social contact, and the uncertainty about the immunologic status of the patients involved.

In the absence of isolates at the time of or just prior to hospital admission for the patients with identical strains, it is difficult to comment on the exact time interval between suspected reinfection and the disease. However, it appears to be approximately five weeks, which is less than the least incubation period of seven weeks reported by Wallgren.\textsuperscript{21} It is possible that the original strain and the reinfecting strain coexisted in the same host during the initial days of exogenous infection, and in course of time, one strain may have become dominant over the other. The validity of this speculation in the present series could have been ascertained had genotyping of single-colony cultures been performed. Another intriguing aspect in the present study is the rapid progression of the disease in the patients suspected to be involved in transmission. The supposed rapid proliferation of the organism in the contact patients suggests the absence of inhibition by the immune system. In immunocompromised adults, approximately 9\% of progressive tuberculosis develops 10 years after infection;\textsuperscript{22} this incubation period could be dramatically abbreviated in immunocompromised patients. Although the human immunodeficiency virus (HIV) status of the patients in clusters A, C, and D was unknown, other immunosuppressive conditions such as alcoholism (cluster A), heavy smoking (cluster C), and diabetes mellitus (cluster D) were noted among these patients, which could have greatly facilitated the transmission and acquisition of new strains of \textit{M. tuberculosis}. These conditions have been recognized as potent risk factors for contracting tuberculosis.\textsuperscript{23–26} Kenyon and others have reported that severe immunodeficiency among HIV-infected index cases was protective against transmission.\textsuperscript{27} Similarly, these investigators also observed that tuberculosis patients with advanced acquired immunodeficiency syndrome (AIDS) may be less infectious than patients in earlier stages of AIDS,\textsuperscript{27} discounting the possibility of HIV being a major risk factor for tuberculosis infection.

Similarly, the present study showed a lower proportion of suspected recent transmission compared with the 30–40\% reported among low incidence populations by other investigators.\textsuperscript{28} The frequency of acquisition of new strains by pulmonary tuberculosis patients in endemic regions is unknown. Since a period of two years after acquiring infection is generally acknowledged as the risk period for developing tuberculosis, a follow-up evaluation of the remaining patients might have provided valuable information about the rates of transmission that occurred in the hospital during the study period.

Droplet nuclei have been known to facilitate transmission of \textit{M. tuberculosis}.\textsuperscript{29} In health care facilities in developed countries, the importance of isolation of smear-positive patients to avert nosocomial (patient to patient) and occupational transmission (patient to health care worker) is recognized.\textsuperscript{30} There is a requirement for studies that reiterate the need to implement such precautions in India. In the present study, the importance of sanitation cannot be underestimated because fomites seem to be the likely sources of infection in cluster D.

Interpersonal transmission of \textit{M. tuberculosis} strains, both drug sensitive and drug resistant, especially in hospitalized patients, is a public health concern. In the present study, isolates from both patients in cluster A were sensitive to the rifampicin, INH, ethambutol, streptomycin, PAS, and amikacin. Conversely, isolates from all three patients in cluster D were resistant to INH and streptomycin. Consequently, these instances of interpersonal transmission of drug-sensitive and drug-resistant strains, however small, are a matter of grave concern.

The possibility of laboratory cross-contamination as the cause of similar RFLP types in the present study could be ruled out since these samples were collected and processed on different days. Furthermore, the absence of identical patterns in consecutive patients is an indication that these patients represent a clear case of interpersonal transmission. Moreover, all patients suspected to be involved in transmission had produced multibacillary sputum from which the cultures were grown.

The present study also emphasizes the necessity of a double-typing strategy to define clustering of \textit{M. tuberculosis} strains in any epidemiologic setting. For example, of four clusters (A–D), \textit{M. tuberculosis} isolated from two patients in cluster B had similar IS6110-RFLP patterns, while their spoligotype patterns were different. Similarly, the two isolates belonging to cluster A had a four-band IS6110-RFLP pattern resembling the O strain, which was reported to have caused an outbreak of tuberculosis in a rural community in Tennessee.\textsuperscript{13} However, the RFLP pattern using pTBN12 typing indicated these strains to be non-O type. Thus, the use of appropriate secondary and tertiary typing markers ascertains clustering, besides unequivocally establishing the veracity of high transmissibility of certain genotypes, especially when reported sporadically and in geographically isolated locations.

In conclusion, the results of this study accentuate the need to investigate the susceptibility of the patients for reinfection, frequency of reinfection in the general population, incriminating settings, and other specific risk factors for transmission of tuberculosis. Such information would promote efforts to be deliberated, not only to detect and cure patients with latent as well as active disease, but also to check smear-negative patients reverting to the infective pool in the community. This is
of special importance in endemic countries such as India, where generation of databases for epidemiologic correlates could potentially influence and maximize the benefits of the tuberculosis control programs.

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