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 Shriniwas 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 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></td>
<td></td>
<td>1312*</td>
<td>Type 1</td>
<td></td>
<td>Sensitive</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>1404</td>
<td>Type 24</td>
<td>Same ward; subsequent hospitalization terms</td>
<td>Not available†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1421*</td>
<td>Type 25</td>
<td></td>
<td>Sensitive</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>1413</td>
<td>Type 23</td>
<td>Same ward; overlapping hospitalization terms</td>
<td>Not available†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1416*</td>
<td>Type 23</td>
<td></td>
<td>PAS resistant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1417*</td>
<td>Type 23</td>
<td></td>
<td>Not available†</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>1388*</td>
<td>Type 18</td>
<td>Same ward; overlapping and subsequent hospitalization terms</td>
<td>INH strep resistant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1392*</td>
<td>Type 18</td>
<td></td>
<td>INH strep resistant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1401*</td>
<td>Type 18</td>
<td></td>
<td>INH strep resistant</td>
</tr>
</tbody>
</table>

PAS = p-aminosalicylic acid; INH = isonicotinic 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.

**RESULTS**

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 invariant 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-
butol. 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-
viduals are available, especially in institutional set-
tings. 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 clus-
tered patients. Majority of patients were infected with unre-
related strains of \textit{M. tuberculosis}, despite being patients in two
wards of the urban hospital. 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 sus-
pected transmission were not available because of their abra-
ciliary 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 transmissions 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 sus-
pected reinfection and the disease. However, it appears to be
approximately five weeks, which is less than the least incuba-
tion period of seven weeks reported by Wallgren. It is pos-
sible that the original strain and the reinfecting strain coex-
isted 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 intrigu-
ing aspect in the present study is the rapid progression of the
disease in the patients suspected to be involved in trans-
mision. The supposed rapid proliferation of the organism in
the contact patients suggests the absence of inhibition by the
immune system. In immunocompetent adults, approximately
9% of progressive tuberculosis develops 10 years after infec-
tion; this incubation period could be dramatically abbrevi-
ated in immunocompromised patients. Although the human
immunodeficiency virus (HIV) status of the patients in clus-
ters 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. Kenyon and others have re-
ported that severe immunodeficiency among HIV-infected in-
dex cases was protective against transmission. 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, 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% re-
ported among low incidence populations by other investiga-
tors. The frequency of acquisition of new strains by pulmo-
ary tuberculosis patients in endemic regions is unknown.
Since a period of two years after acquiring infection is gen-
erally acknowledged as the risk period for developing tuber-
culosis, 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}. In health care facilities in developed
countries, the importance of isolation of smear-positive pa-
tients to avert nosocomial (patient to patient) and occupa-
tional transmission (patient to health care worker) is recog-
nized. 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, iso-
late from both patients in cluster A were sensitive to the
rifampicin, INH, ethambutol, streptomycin, PAS, and amika-
cin. 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. More-
over, 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 clus-
ters (A-D), \textit{M. tuberculosis} isolated from two patients in clus-
ter B had similar IS6110-RFLP patterns, while their spolio-
type patterns were different. Similarly, the two isolates be-
longing 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 Tennes-
see. However, the RFLP pattern using pTBN12 typing in-
dicated these strains to be non-O type. Thus, the use of ap-
propriate secondary and tertiary typing markers ascertains
custering, besides unequivocally establishing the veracity of
high transmissibility of certain genotypes, especially when re-
ported 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, incrimi-
nating 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 pa-
tients 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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