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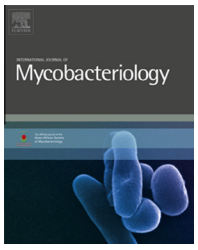

Short Communication

Predominance of Beijing genotype in extensively drug resistant *Mycobacterium tuberculosis* isolates from a tertiary care hospital in New Delhi, India

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ABSTRACT

Out of a total of 311 *Mycobacterium tuberculosis* isolates from sputum specimens subjected to first- and second-line drug-susceptibility testing (DST) at a hospital serving as a referral center for chronic tuberculosis (TB) cases in New Delhi, 232/311 (74.6%) isolates were found to be resistant to isoniazid and rifampicin. Among multidrug-resistant (MDR) isolates, 119/232 (51.3%) were resistant to four first-line drugs (streptomycin, isoniazid, rifampicin and ethambutol). Mono-resistance to isoniazid was observed in 18 (5.7%) isolates, while none of the isolates tested showed mono-resistance to rifampicin. 50/232 (21.5%) isolates met the definition of extensively drug resistant (XDR) TB, i.e., additional resistance to a fluoroquinolone and at least one of the three injectable second-line drugs: kanamycin, capreomycin, or amikacin. Spoligotyping of the XDR-TB isolates revealed 14 patterns; 39/50 (78%) isolates being grouped in three clusters vs. 11/50 (22%) isolates being unique. SIT1/Beijing represented the largest cluster ($n = 21$, 42%), followed by SIT26/CAS1-Delhi ($n = 10$, 20%) and SIT 53/T1 ($n = 8$ isolates; 16%). This study corroborates recent observations from North India suggesting that both Beijing and CAS1-Delhi lineages constitute the bulk of XDR-TB isolates that are disseminating rapidly across a large geographical region in and around the capital city of India.

Introduction

Tuberculosis (TB) continues to be one of the major life-threatening diseases worldwide. In 2009, approximately 2 million TB cases were recorded in India, accounting for one fifth of the global burden of TB [1]. Over the past few years, the control of TB has become more complicated as a result of the emergence of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB); the former is defined by simultaneous drug resistance to isoniazid

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[INH] and rifampin [RIF], while the latter is defined by additional resistance to a fluoroquinolone and at least one of the three injectable second-line drugs: kanamycin, capreomycin, or amikacin [2].

A recently published study showed high levels of MDR-TB in both new and treatment-failure patients from the Revised National Tuberculosis Control Program (RNTCP) in four suboptimally performing municipal wards of Mumbai [3]. The authors determined the extent of drug resistance in 724 cases of pulmonary TB (493 newly diagnosed, and 231 first-line treatment-failure cases, i.e., sputum-smear positive at the fifth month after commencement of therapy), and reported an astoundingly high proportion of MDR-TB strains in both previously untreated (24%) and treatment-failure cases (41%). These numbers are much higher than the World Health Organization (WHO) [4] and the National Reference Laboratory for the RNTCP [5] figures that report MDR-TB amongst new and retreated TB patients in India: around 2.3% (range 1.8–2.8) and 17.2% (range 14.9–19.5), respectively. Hence, the figures generated through sampling of patients accessing a tertiary care center, although "unrepresentative" at the national level, are clearly a cause for concern.

Recent reports have singled out India as having the greatest burden of XDR-TB in the South Asian subcontinent, with a poor prognosis and high mortality among HIV-infected individuals [6–8]. Hence, early detection is essential in arresting further transmission of XDR-TB clones, which is much more expensive to manage owing to prolonged medication and high risk of death. In this grim context, the study of TB epidemiology through DNA fingerprinting is an important tool contributing to the understanding of transmission and control of TB. Spoligotyping targeting the DNA polymorphism at the direct repeat locus (DR locus) of the genome of *Mycobacterium tuberculosis* complex allows simultaneous detection and differentiation of *M. tuberculosis* complex strains. This polymerase chain reaction (PCR)-based method requiring a small amount of DNA is a rapid, robust and cost-effective genotyping technique. Though there have been recent studies characterizing MDR-TB isolates from Delhi [9–11] and they have reported SIT26 as the predominant spoligotype in northern India, studies highlighting genotypic diversity among XDR-TB isolates in India remain rare. Consequently, the present study was aimed at molecular characterization of the XDR *M. tuberculosis* clinical isolates from a tertiary care TB hospital in order to obtain an initial insight into the predominant spoligotypes and to study genetic diversity among the circulating strains.

Materials and methods

Study site

The present study was conducted at the Department of Microbiology, Lala Ram Sarup (LRS) Institute of TB and Respiratory Diseases, New Delhi, India. The Institute is a tertiary care hospital, National Reference Laboratory for the Revised National Tuberculosis Control Program (RNTCP), and also a DOTS-Plus site for the treatment of MDR-TB patients. The Laboratory is efficiently going through the regular rounds of proficiency testing for both first- and second-line drug sensitivity by the WHO Supranational TB Reference Laboratory, Institute of Tropical Medicine; Antwerp, Belgium. Almost all of the cases for which DST was performed were retreatment cases (either relapse or failure cases). The MDR rate among such patients is high and falls between 55% – 60% and XDR rates fall between 15% – 20% [12].

Drug susceptibility testing

During the period July 2010 to June 2011 a total of 311 *M. tuberculosis* isolates from pulmonary specimens (sputum) were subjected to first- and second-line DST as requested by the clinicians. Drug susceptibility testing was performed by MGIT960 liquid culture system (Becton Dickinson, Franklin Lakes, NJ, USA) in a BSL III lab at the following critical concentrations: rifampicin 1.0 μg/ml, isoniazid 0.1 μg/ml, streptomycin 1.0 μg/ml, ethambutol 5.0 μg/ml, capreomycin 2.5 μg/ml, ofloxacin 2.0 μg/ml, amikacin 1.0 μg/ml, and kanamycin 2.5 μg/ml.

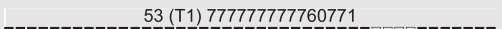
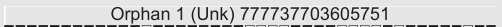
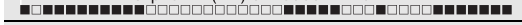
Spoligotyping and database comparison

Spoligotyping was performed with a commercially available kit as described earlier [13]. Spoligotypes in binary format were entered in the SITVIT2 database (Pasteur Institute of Guadeloupe), which is an updated version of the SpolDB4 [14] and SITVITWEB [15] databases. At the time of the present study, SITVIT2 contained more than 3000 SITs with global genotyping information on about 87,000 *M. tuberculosis* clinical isolates from 160 countries of origin. In this database, SIT (Spoligotype International Type) designates spoligotypes shared by two or more patient isolates, as opposed to "orphan" which designates patterns reported for a single isolate. A cluster was defined as two or more strains with an identical genetic pattern. Major phylogenetic clades were assigned according to signatures initially provided in SpolDB4, and slightly revised in SITVITWEB by the addition of five "new rules" for definition of variants within 62 existing lineages/sub-lineages. These include specific signatures for various *M. tuberculosis* complex species, such as *Mycobacterium bovis*, *Mycobacterium microti*, *Mycobacterium caprae*, *Mycobacterium pinipedii*, and *Mycobacterium africanum*, as well as rules defining major lineages/sub-lineages for *M. tuberculosis sensu stricto*. These include the Beijing clade, the Central Asian (CAS) clade and two sub-lineages, the East African–Indian (EAI) clade and nine sub-lineages, the Haarlem (H) clade and three sub-lineages, the Latin American–Mediterranean (LAM) clade and 12 sub-lineages, the "Manu" family and three sub-lineages, the S clade, the IS*6110*-low-banding X clade and four sub-lineages, and an ill-defined T clade with five sub-lineages.

Results and discussion

A total of 232/311 isolates were resistant to at least isoniazid and rifampicin (MDR) and 119 were resistant to four first-line drugs tested. Among the 232 MDR isolates, 50 (21.5%) met the definition of XDR-TB. Nonetheless, since the hospital is a

Table 1 – Description of spoligotype patterns observed among XDR *M. tuberculosis* clinical isolates (*n* = 50) in Delhi, corresponding Spoligotype International Type (SIT) numbers, genotypic lineages, and their worldwide distribution in the SITVIT2 database.

SIT (lineage) octal number spoligotype description[a]	Total (%) in study/vs. database	Distribution in regions with ⩾3% of a given SIT[b]	Distribution in countries with ⩾3% of a given SITs[c]
1 (Beijing) 000000000003771 	21 (42%)/0.22	ASIA-E 34.13, AMER-N 20.95, ASIA-SE 9.46, AFRI-S 8.61, ASIA-N 7.2, ASIA-S 4.88, EURO-N 3.22	USA 20.6, CHN 19.72, JPN 11.97, ZAF 8.61, RUS 7.2, VNM 4.05
26 (CAS1-Delhi) 703777740003771 	10 (20%)/0.78	ASIA-S 53.04, AMER-N 17.06, ASIA-W 6.54, EURO-W 6.0, AFRI-E 5.53, EURO-N 4.52, EURO-S 3.89	IND 30.3, USA 17.06, PAK 11.22, SAU 6.08, BGD 5.84, IRN 4.83, ITA 3.74, NLD 3.35, ETH 3.35
53 (T1) 777777777760771 	8 (16%)/0.14	AMER-N 18.2, AMER-S 13.18, EURO-W 11.3, EURO-S 10.49, ASIA-W 7.59, EURO-N 5.94, AFRI-S 5.54, AFRI-E 5.02, ASIA-E 4.72, AFRI-N 3.92	USA 14.71, ITA 5.94, BRA 5.71, ZAF 5.41, TUR 3.87, AUT 3.82, CHN 3.44, MEX 3.18
11 (EAI3-IND) 477777777413071	1 (2%) / 0.18	ASIA-S 45.26, AMER-N 21.29, EURO-N 11.81, EURO-W 8.23, ASIA-SE 4.83	IND 36.49, USA 21.29, GBR 8.05, NLD 5.72, BGD 4.11, MYS 3.04, DNK 3.04
25 (CAS1-Delhi) 703777740003171	1 (2%)/0.19	AFRI-E 29.09, ASIA-W 18.69, AFRI-N 16.18, ASIA-S 12.14, AMER-N 10.02, EURO-N 5.40, EURO-W 4.43	ETH 27.75, SAU 18.11, SDN 13.30, USA 10.02, IND 6.74, IRN 3.28
247 (CAS1-Delhi) 703777740003471	1 (2%)/3.45	AFRI-E 41.38, ASIA-W 34.48, ASIA-S 10.34, AMER-N 6.90, EURO-W 3.45, AFRI-N 3.45	ETH 37.93, SAU 34.48, USA 6.90, IND 6.90, TZA 3.45, SDN 3.45, PAK 3.45, FXX 3.45
427 (CAS1-Delhi) 703707740003771	1 (2%)/5.88	ASIA-S 52.94, AMER-N 23.53, EURO-W 5.88, EURO-S 5.88, EURO-N 5.88, AFRI-N 5.88	IND 52.94, USA 23.53, TUN 5.88, ITA 5.88, GBR 5.88, DEU 5.88
753 (LAM1) 477777607760771 	1 (2%)/5.0	AFRI-E 30.0, AMER-S 25.0, AMER-N 15.0, EURO-S 10.0, ASIA-S 10.0, EURO-W 5.0, AFRI-S 5.0	ZWE 20.0, USA 15.0, PRY 10.0, MWI 10.0, IND 10.0, ESP 10.0, ZAF 5.0, NLD 5.0, GUF 5.0, COL 5.0, BRA 5.0
1345 (Unk/CAS) 703777700000371 	1 (2%)/12.5	ASIA-S 37.5, AMER-N 37.5, EURO-W 12.5, EURO-N 12.5	USA 37.5, IND 25.0, SWE 12.5, NLD 12.5, BGD 12.5
1926 (T1) 777777777760701 	1 (2%)/16.67	EURO-S 33.33, ASIA-W 16.67, ASIA-S 16.67, ASIA-E 16.67, AMER-S 16.67	ITA 33.33, TWN 16.67, SAU 16.67, IND 16.67, ARG 16.67
1970 (EAI6-BGD1) 777776757413771	1 (2%)/8.33	ASIA-S 50.0, AMER-N 25.0, EURO-W 8.33, CARI 8.33, AFRI-N 8.33	IND 50.0, USA 25.0, TUN 8.33, DEU 8.33
2148 (CAS) 700000000000000	1 (2%)/20.0	ASIA-S 60.0, EURO-S 40.0	IND 40.0, PRT 20.0, ITA 20.0, BGD 20.0
Orphan 1 (Unk) 777737703605751 	1 (2%)/100.0	ASIA-S 100.0	IND 100.0
Orphan 2 (H4) 577600017420771 	1 (2%)/100.0	ASIA-S 100.0	IND 100.0

[a] SIT and lineage designations are shown following SITVIT2 proprietary database of Institut Pasteur de la Guadeloupe. Unk, unknown lineages since the pattern did not correspond to a well-defined signature in the database; note that SIT1345 has a signature close to CAS and was reported as CAS in SpolDB4, but does not follow the rules in a stringent way; hence designated "unknown" in SITVITWEB. Orphan patterns corresponded to following strains: orphan 1, IND12201041090; orphan 2, IND1220104951 (note that lineage interpretations for orphan patterns are based on expert-based visual interpretation of the patterns).

[b] Worldwide distribution is reported for regions with more than 3% of a given SITs as compared to their total number in the SITVIT2 database. The definition of macro-geographical regions and sub-regions (http://unstats.un.org/unsd/methods/m49/m49regin.htm) is according to the United Nations; Regions: AFRI (Africa), AMER (Americas), ASIA (Asia), EURO (Europe), and OCE (Oceania), sub-divided in: E (Eastern), M (Middle), C (Central), N (Northern), S (Southern), SE (South-Eastern), and W (Western). Furthermore, CARIB (Caribbean) belongs to Americas, while Oceania is subdivided in four sub-regions, AUST (Australasia), MEL (Melanesia), MIC (Micronesia), and POLY (Polynesia). Note that in our classification scheme, Russia has been attributed a new sub-region by itself (Northern Asia) instead of including it among the rest of the Eastern Europe. It reflects its geographical localization as well as due to the similarity of specific TB genotypes circulating in Russia (a majority of Beijing genotypes) with those prevalent in Central, Eastern and South-Eastern Asia.

[c] The three letter country codes are according to http://en.wikipedia.org/wiki/ISO_3166-1_alpha-3; countrywide distribution is only shown for SITs with ⩾5% of a given SITs as compared to their total number in the SITVIT2 database.

referral center with a relatively higher proportion of previously treated patients (chronic TB cases), sampling bias cannot be ruled out, and this data may overestimate the percentage of both MDR- and XDR-TB. The majority of the patients (n = 46/50) were in the age group of 15–45. The description of spoligotype patterns observed among XDR *M. tuberculosis* clinical isolates, corresponding Spoligotype International Type (SIT) designations, genotypic lineages, and their worldwide distribution in the SITVIT2 database is summarized in Table 1. Spoligotyping revealed 14 patterns; 39/50 (78%) isolates were grouped in three clusters vs. 11/50 (22%) isolates that were unique. Comparing results with the international spoligotype database categorized 48 isolates in 12 shared types and two isolates as orphans; 78% of the strains clustered were divided into three spoligotype international types: SIT1/Beijing represented the largest cluster (n = 21 isolates; 42%), followed by SIT26/CAS1-Delhi (n = 10 isolates; 20%) and SIT 53/T1 (n = 8 isolates; 16%). One isolate each belonged to SIT11, SIT25, SIT247, SIT427, SIT753, SIT1345, SIT1926, SIT1970 and SIT2148.

With 78% of the isolates grouped in three big clusters, the overall strain diversity among XDR *M. tuberculosis* isolates was lower than in previous reports that included either MDR or pan-susceptible isolates [9–11,16,17]. This study found SIT1/Beijing and SIT26/CAS1-Delhi to be predominant among the XDR isolates in and around the Delhi region, accounting for nearly 65% of the total isolates spoligotyped. Regarding these two most predominant types in this study, previous studies from Delhi have reported their frequent isolation with varying percentages [9,10]. According to a study conducted at the Hinduja Hospital, Mumbai, 62% of all XDR *M. tuberculosis* isolates were found to be Beijing upon spoligotyping [18]. The high prevalence of Beijing and CAS1-Delhi genotypes in the present study and as well as in two recent studies on molecular characterization of *M. tuberculosis* isolates from patients with extrapulmonary tuberculosis in North India [19], and Mumbai [20], underlines the rapid dissemination of these lineages across large geographical regions. As reviewed previously [21], the Beijing clade has been associated with young age (Vietnam) and with drug resistance (Cuba, Germany, Russia, Estonia and Columbia), whereas no statistically significant differences were reported in numerous other countries [11]. Hence, larger prospective studies without selection bias are needed in various geographical regions of India to draw a conclusion on the high incidence of the Beijing genotype observed both among MDR and XDR *M. tuberculosis* isolates in tertiary care centers in Mumbai [22], as well as in New Delhi [this study].

Regarding the second largest family among the XDR *M. tuberculosis* isolates in this study-the SIT26/CAS1-Delhi genotype that belongs to the Central Asian lineage (CAS, n = 11 isolates; 22%)-it has been reported mostly from countries of the Middle-East and Central Asia, or regions that have witnessed an important migration to or from the Indian sub-continent, e.g. Africa, Far-East Asia, Oceania, the United States and Europe [14,15]. This family has been also identified as a predominant strain in Mumbai [22] and in different regions of the country [9,10,23,24].

In conclusion, the association of XDR-TB with these prevalent clades in this study is cause for major concern with implications on TB control in India. Community-based studies with larger recruitment of isolates are now required in order to understand definite transmission patterns and to identify high-risk groups and areas concerned. Careful characterization of the clades with regard to their virulence and transmissibility and rapid diagnosis of drug resistance will be important steps in curtailing chains of transmission and strengthening the TB control program in India.

Conflict of interest

None declared.

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