A study on Beijing genotype in the clinical isolates of pulmonary drug-resistant tuberculosis

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ABSTRACT

Background: Mycobacterium tuberculosis (MTB) Beijing strains are spread worldwide and are responsible for major outbreaks of tuberculosis (TB), sometimes spreading multidrug resistance (MDR). Aim: The aim of this study was to explore clinical features associated with the infection with Beijing strains among MDR patients of pulmonary TB in Lucknow and surrounding areas. Materials and Methods: It was a hospital-based epidemiological study. Our study population was selected from all the newly diagnosed patients attending outpatient department and admitted patients of Department of Respiratory Medicine, King George’s Medical University, Lucknow, Uttar Pradesh, India. Those isolates which were characterized to be MTB by morphological and molecular techniques were tested for their resistance against the first-line drugs; after which each patient’s isolate was genotyped. Results: The results suggested that the presence of Beijing genotype in 31.78% of strains. Conclusion: Our results predicted that genotypic patterns reveal a large diversity among the MTB Beijing strain population. Increasing frequency of Beijing strains demands further research to unravel the factors behind its propensity to prevail.

KEY WORDS: Beijing strains, genotyping, multidrug resistance, polymerase chain reaction

INTRODUCTION

Tuberculosis (TB) is a disease evident from ancient time. It is a widespread, preventable, and treatable infectious disease caused by acid-fast bacilli Mycobacterium tuberculosis Complex (MTBC). Multidrug-resistant pulmonary TB (MDR-PTB) remains relatively low, at around 4%, among new patients and 12%–19% in re-treatment cases.[1] The resistance is also caused by the genetic alterations that make a drug ineffective against the mutant bacilli. Infection with drug-resistant (DR) strains is also believed to be one of the major source of DR.[2-4]

Spoligotyping could be major assistance in cases when results are required quickly such as during TB dissemination, disease outbreaks, especially, in high-risk areas.[3]

The continuous emergence of Beijing genotype possesses a serious threat. In Beijing, China, a large proportion of the MDR-PTB strains were reported to have mutually highly similar multi-banded restriction fragment length polymorphism patterns; these strains were also present in many other populations. It has high virulence and causes large outbreaks, sometimes involving MDR of MTB strains.[8]
The MTB Beijing strains were responsible for widespread resistance against antibiotics commonly used to treat TB. However, this association varies in different countries. This may be due to the heterogeneity in adaptation and prevalence of Beijing strains in the local population.\cite{6-8}

The characterization of MTB genotype could be done to analyze the association between various MTB strains and drug susceptibility patterns with focus on Beijing strains, giving an insight into the biology of the organism that relates to its clinical phenotype.\cite{9}

**MATERIALS AND METHODS**

**Study population**

This study was conducted in all newly diagnosed patients attending outpatient department of Department of Respiratory Medicine, King George’s Medical University, Lucknow, Uttar Pradesh and admitted in the hospital from March 2014 to November 2014 registered under Revised National Tuberculosis Control Programme (directly observed treatment, short-course) treatment. The recruited cases were positive for chest X-ray diagnosis, sputum microscopy, cartridge-based nucleic acid amplification test of MTB culture.\cite{3}

MTB isolates were recovered from 107 HIV-negative and smear-positive cases of both genders falling into the age group from 18 to 65 years with MDR-TB that was refractory to chemotherapy given during recruiting time.

All MTB strains positive for culture were included in the study and were subjected to drug susceptibility testing. MTB isolates were used for genotyping by molecular methods including DNA fingerprinting after signed informed consent by the participants.

**Drug susceptibility testing**

Clinical specimen processed using the NaOH method. Susceptibility to the first-line antitubercular drugs was tested for all the clinical isolates. Drugs used for the procedure were rifampicin (R), isoniazid (H), ethambutol (E), and streptomycin (S). The phenotypic resistance against all drugs was determined at baseline.\cite{7}

**DNA isolation**

DNA was extracted from clinical isolates grown on Lowenstein–Jensen medium as described earlier.\cite{10}

**Polymerase chain reaction amplification**

Conventional polymerase chain reaction (PCR) was performed with each DNA being amplified independently with three sets of primers: Beijing (BjF and BjR), non-Beijing (nBjF and nBjR), and insertion sequence (IS) 6110 [Table 1].\cite{9}

**RESULTS**

**Clinical characteristics of study population**

A total of 107 sputum samples were collected among the DR-PTB cases recruited. Patients presented the symptoms before diagnosis, which were cough production, fever, weight loss, chest pain, loss of appetite, fatigue, and weakness. HIV test was done on all the patients, and all patients were confirmed HIV negative. Furthermore, none of the patients had extraPTB (EPTB) and diabetes.

**Demographic characteristics of study population**

These patients were grouped in three groups, 16 (15.0%) patients had not received any previous treatment, 69 (64.5%) patients had received inadequate previous treatment, and rest 22 (20.6%) patients received adequate previous treatment [Table 2 and Figure 1]. A majority of patients in our study were inadequately treated during earlier treatment. A number of male (72/107) patients are approximately double of female patients (35/107).

All the samples eligible to be included in the study were PCR positive for IS6110. Once MDR-TB was diagnosed, the patients were switched to treatment regimens tailored for phenotypic drug susceptibility profile of their isolates.

**Table 1: Primers sequences used for identification of Beijing strains**

| Primer (name) | Sequence (5'-3')                  | Product size (bp) |
|--------------|-----------------------------------|------------------|
| Beijing      | F-5’-CTCGGCAGCTTCCTCGAT-3’        | 129              |
| (BjF; BjR)   | R-GAACTCGAGGCTGCCTACT             |                  |
| Non-Beijing  | F-GGCCATGACTCGAAAGAAG             | 95               |
| (nBjF; nBjR) | R-AAGCATTCCCTTGACAGTCGGAA        |                  |
| IS6110       | F-5’-CTCGGCCAGCGCTCCGATG-3’      | 123              |
| IS: Insertion sequence |

**Table 2: Distribution of cases according to history of previous treatment status**

| Previous treatment history | Number of cases (%) |
|----------------------------|---------------------|
| Not treated                | 14 (15.0%)          |
| Inadequately treated       | 69 (64.5%)          |
| Adequately treated         | 22 (20.6%)          |

**Figure 1:** Distribution of cases according to history of previous treatment status
Phenotypic resistance profile of *Mycobacterium tuberculosis*

In the present study, 107 sputum smear-positive genotypically RIF resistance patients were included in the study. All samples were found resistant to the majority of first-line drugs. Some strains even showed resistance to more than one drug.

Beijing genotype prevalence

Genotypic results power to the approach based on the detection of prevalence of Beijing strains among populations. In our study, Beijing strain was found to be dominating (31.7%) among them. Next to Beijing, other family present included H₃₇Rv was 07.14%.

| MTB spoligotype | Number of strains | Prevalence | MTB family prevalence |
|-----------------|-------------------|------------|-----------------------|
| Beijing         | 34                | 31.78      | 34 (31.78)            |
| H₃₇Rv           | 12                | 7.14       | 12 (7.14)             |
| IS6110          | 107               | 107        | 107 (100)             |

Table 3: Genotype of *Mycobacterium tuberculosis* strains isolated from multidrug-resistant pulmonary tuberculosis patients

DISCUSSION

The study was conducted to detect the mutations occurring in MDR-TB-associated Beijing strains and their correlation with DR. The application of DNA fingerprinting can provide valuable insights into the pathogenesis of TB and may help in identifying strains of MTB with specific properties such as latent infection, strain-specific patterns, virulence, and failure of drug response. Most of the epidemiological applications of RFLP analysis have used an *IS6110*. In recent years, the Beijing genotype of MTB has attracted special attention because of its global emergence. The ubiquity of the Beijing strain and its frequent appearance in outbreaks, particularly of DR-TB, suggests that it may have the potential to spread. In Estonia, although there was no association found between Beijing strains and age. In addition, the MTB population from urban areas was more diverse than that of the population found in patients living in rural areas in which some distinct genotypes were recognized.

The prevalence of Beijing strains observed in our study was 31.78% and 7.14% associated with H₃₇Rv strain. We saw that the MTB population was more diverse and the Beijing family was the most prevalent 31.78% (34/107). Patients who were diagnosed with MDR-TB or resistance to any of the given anti-TB drugs had a higher possibility of being infected with the Beijing genotype.

INH and RIF resistance in MTBC isolates are mainly based on mutations in a limited number of genes. However, mutation frequencies vary in different mycobacterial populations. Some studies found the percentage of Beijing genotype to be 6%. As mentioned above, MTB generally acquires DR through *de novo* nonsynonymous single nucleotide polymorphism, small deletions, or insertions in specific chromosomal loci, unlike most other pathogenic bacteria, which often acquire DR through horizontal transfer. In a review of the Indian situation, the magnitude of the DR problem is principally due to acquired resistance replaced by the term DR among previously treated cases.

In developing countries, the diagnosis of MTB with conventional diagnostics methods is a greater challenge. Recent advances in the field of molecular biology and progress in the understanding of the molecular basis of DR in MTB have provided new tools for its rapid diagnosis.

Figure 2: Genotype of *Mycobacterium tuberculosis* strains isolated from multidrug-resistant pulmonary tuberculosis patients

Figure 3: Primer insertion sequence *6110*, M-DNA ladder, lane 1-17-clinical isolates *IS6110*, C - positive control

Figure 4: Primer Beijing M-DNA ladder, lane 1-17-clinical isolates; C - positive control
by molecular methods. Some reports have evaluated that the role of PCR is the diagnosis of MTB and in EPTB with high sensitivity and specificity.\[13\]

Our work demonstrates that regional and cross-border tracing research might pave the way to monitor the further spread of MDR-TB. In general, it suggests that MTB populations were possibly derived from different ancestor strains that diversified from each other by both the number of IS6110 insertion and their transposition sites throughout the genome. Beijing strain was most common in the Beijing area of China, accounting for 92% of strains. However, there is no apparent evidence that the Beijing strains have high mutation frequency or some unique drug efflux system which may confer a selective advantage. Despite these facts, the Beijing strains continue to dominate over a wide range which demands the need for further research to unravel the factors behind the propensity of Beijing strains to prevail.\[14,15\]

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Conflicts of interest
There are no conflicts of interest.

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