BEIJING GENOTYPE AND OTHER PREDOMINANT MYCOBACTERIUM TUBERCULOSIS SPOLOGOTYPES OBSERVED IN MASHHAD CITY, IRAN

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Abstract

Purpose: The purpose of this study was to understand the molecular epidemiology of tuberculosis in Khorasan province of Iran was studied by spoligotyping 113 Mycobacterium tuberculosis isolates. The spoligotyping results were in comparison to the word Spoligotyping Database of Institute Pasteur de Guadeloupe (SpolDB4). Spoligotyping data from Iran has rarely been described and there is limited information on the major circulating clades of M. tuberculosis in Iran. Materials and Methods: Spoligotyping was performed on 113 M. tuberculosis isolates from Mashhad patients between November 2004 and September 2005. Results: The study found 57 spoligopatterns. 17 clusters and 32 true orphan genotype. The biggest cluster with 13 isolates had not been previously reported. The Beijing genotype was seen in eight (7.1%) isolates. Conclusions: Genotyping and Spoligotyping gives a unifying framework for both epidemiology and evolutionary analysis of M. tuberculosis populations.

Key words: Mycobacterium tuberculosis, Beijing genotype, spoligotyping

Introduction

Tuberculosis (TB) has re emerged as one of the leading causes of death. Currently, more than one third of the world’s population is infected by Mycobacterium tuberculosis, and eight million new cases and approximately two million deaths are reported each year.[1] TB may have plagued humans as early as since the Neolithic times.[2] The Khorasan province in Iran has high number of TB cases (26/per 100000).[3] Iran shares geographical borders with three countries where TB is endemic, i.e., Afghanistan, Pakistan and Iraq where TB is endemic. In addition, it is closely associated with other Asian countries where TB is highly endemic, i.e., India, China, Bangladesh, Sri Lanka, and Nepal. Despite a high TB burden in the Middle East region, there is very limited available data on strains circulating in the country. This is because it is still impossible to sequence the whole genome of MTB populations to conduct molecular epidemiology studies. In the last decade, a large number of different molecular methods based on DNA fingerprints have been developed. There are several molecular techniques are available to explore the genetic diversity of MTB populations. They are useful in epidemiological surveillance and understanding of TB transmission. This study has used spoligotyping; the method is based on polymorphism of the chromosomal DR (direct repeat) locus. The DR elements, identified by Hermans et al.[4], contain multiple, wellconserved 36bp DRs interspersed with nonrepetitive spacer sequences (34-41 bp long). Strains vary in the number of DRs and in the presence or absence of particular spacers. Indeed, the spacer oligonucleotide typing (spoligotyping) method described by Kamerbeek et al.[5] detects the presence or absence of spacers of known sequence in an isolate as an alternative to IS6110 RFLP. Contrary to the IS6110 genotyping method, spoligotyping is a technique based on polymerase chain reaction (PCR). The method is simple, rapid, and robust, and only small amounts of DNA are needed.[6] It can be done on clinical samples or on strains shortly after inoculation into liquid culture.[5] When molecular genotyping is applied at the population level, the clustering of isolates can provide important clues about the patterns and dynamics of transmission in the population. The Beijing M. tuberculosis genotype is globally widespread, with the highest prevalence found in Asia and the territory of the former Soviet Union. In some but not all areas, the genotype is associated with drug resistance. There are no reports available on the emergence of the Beijing genotype in China or on its possible association with drug resistance. It is not clear whether the observation in the Beijing area is representative of the whole country. The association of this genotype with HIV was observed in one study[7] and another study showed that mother infant transmission of this genotype was possible.[8]
The present study aimed to determine the proportion of Beijing genotype *M. tuberculosis* and identify predominant spoligotypes with an international designation responsible for transmission and prevalence of TB in Mashhad city, Iran.

**Materials and Methods**

**Patient population and bacterial isolate**

Our analysis was based on 113 *M. tuberculosis* culture isolates which originated from sputum samples of patients from hospitals in Mashhad city. Sputum samples were collected from patients over a period of one year. The mean age of the group was 42.5 years. Among the 113 *M. tuberculosis* cultures, all were selected for spoligotyping analysis.

**MDR status**

The isolates were also tested for drug sensitivity using standard proportion method. Sixty six of the isolates were have resistant to at least one TB drug, five of which belonged to Beijing genotype.

**DNA extraction and spoligotyping**

DNA was extracted from all culture isolates by the standard cetyltrimethyl ammonium bromide (CTAB) method. The DNA was stored in TE1X (10 mM Tris, 1 mM EDTA) buffer at 4°C until use. For spoligotyping, a membrane with 43 oligonucleotides in parallel lines, immobilized covalently on a Biodyne C membrane (Pall Biosystems, Portsmouth, UK.) was performed as previously described by amplifying the spacer regions of the DR locus with primers DRa (forward) and DRb (reverse) (Bioneer company, South Korea). The amplified DNA was hybridized to a membrane containing 43 oligonucleotide probes to detect spacer regions in test strains with a 45 lane blotter (Miniblotter 45, Immunetics, Cambridge, MA). The membrane was incubated with streptavidin peroxidase conjugate and hybridized DNA was detected by enhanced chemiluminescence detection liquid (ECL detection kit, Amersham, Buckinghamshire, UK). The autoradiogram were developed using standard photochemicals after one hour to 18 hour of exposition on X ray films.

**Data analysis**

Spoligopatterns were analyzed using MS Excel data sheets and grouped together for any similarity. The different clusters were labeled with arbitrary numbers and data was further analyzed by comparing with the fourth version of an international spoligotyping database (SpolDB4) which has been created and maintained at the Institute Pasteur of Guadeloupe (IPG). The strains with spoligotype similar to any pattern of an *M. tuberculosis* strain already found in the database were automatically labeled with an already defined ‘shared type’ number, whereas a spoligotype exhibiting a profile not yet found anywhere in the world was termed ‘not seen’.

**Results**

**Spoligopattern**

A total of 113 *M. tuberculosis* isolates were analyzed by spoligotyping. Sixty nine strains (61.1%) out of 113 were present in 17 different clusters. Table 1 shows the spoligopatterns of all strains in this study.

Six clusters of two isolates, four clusters of three isolates and three big clusters (one of 13 isolates, one of nine isolates and one of eight isolates) were observed. The spoligopatterns were further compared with the SpolDB4 database. Among the 57 shared types found, our strains created 42 new spoligopatterns, while the remaining 15 spoligopatterns were already found in the database within various geographic regions. Our strains enriched these already defined shared types. It is clear from Table 1 that the predominant cluster in database, called the Beijing strain, (St33 in this study and type 1 in spolDB4), is present in 7.1% (eight isolates from four Iranian and four Afghani patients) of our study which is comparable to 8.85% of the database. Furthermore, St29 (type 100 in spolDB4) is present in 7.9% of our culture isolates. St4 (not seen in spolDB4), which is a major type in our study (11.5%) is present as unique patterns in our study population, and were declared to be shared types. Another share type (St20 in our study) was not seen in previous studies and was declared to be shared types.

Of the 57 patterns observed in this study, 32 (observed for St 3, 5 to10, 12, 15, 18, 23 to 25, 27 to 28, 30, 34 to 37, 40 to 42, 45, 47, 50 to 53, 55 to 57) were true orphans (no counterpart in the database). In addition, of 17 clusters that observed in this study 10 clusters (44 Isolates) were not present in database. This percentage referred to patterns (42/57, 73.7%) is high and reflects both the current absence of knowledge on the genetic diversity of Iranian *M. tuberculosis* strains and the micro evolutionary genetic driving forces active in TB epidemic dynamics in Iran.

**Drug Resistance Pattern in Beijing Genotypes**

From eight isolates that showed Beijing genotype pattern three isolates were sensitive to all five of the first-line agents tested. Among the five drug resistant isolates, three isolates were MDR (Table 2).

**Conclusion**

Spoligotyping is useful for tracking TB epidemics, detecting new outbreaks, and better defining high-risk populations to focus prevention strategies. Spoligotyping may also constitute a potential tool for global TB epidemiology, population genetics, and phylogeny, although it should be used with another independent genotyping method in many settings to prove clonality. To have a better knowledge of moving and
| St | 1 not seen | 2 not seen | 3 not seen | 4 not seen | 5 not seen | 6 not seen | 7 not seen | 8 not seen | 9 not seen | 10 not seen | 11 not seen | 12 not seen | 13 not seen | 14 not seen | 15 not seen | 16 not seen | 17 not seen | 18 not seen | 19 not seen | 20 not seen | 21 not seen | 22 not seen | 23 not seen | 24 not seen | 25 not seen | 26 not seen | 27 not seen | 28 not seen | 29 not seen | 30 not seen | 31 not seen | 32 not seen | 33 not seen | 34 not seen | 35 not seen | 36 not seen | 37 not seen | 38 not seen | 39 not seen | 40 not seen | 41 not seen | 42 not seen | 43 not seen | 44 not seen | 45 not seen | 46 not seen | 47 not seen | 48 not seen | 49 not seen | 50 not seen | 51 not seen | 52 not seen | 53 not seen | 54 not seen |
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expanding clones of *M. tuberculosis* within the Iranian, we attempted to identify the predominant spoligotypes prevalent in Mashhad city and determine their specific signature. Comparison with the spolDB4.0 database enabled us to compare spoligotypes generated in a Mashhad laboratory with different laboratories around the world.

A study by a deletion targeted multiplex PCR (DTMPCR) method has same results with spoligotyping in identification of Beijing genotype but spoligotyping seems to be both sensitive and specific for the Beijing family and is also easily compared between studies. The prevalence of the Beijing genotype shows strong geographical variation from below 10% to more than 90% of the strains analyzed. High rates of Beijing genotype strains have been reported from some regions in Eastern Europe, such as Estonia, Azerbaijan, and Russia (Samara oblast, Archangel Oblast, north-western region), while in Western European countries the prevalence of Beijing genotype is low. In this investigation we found eight isolates (7.1%); very large number of immigrant population from Afghanistan where TB is endemic. The presence of four of eight Beijing isolates in Afghani patients showed that these populations can influence strain distribution in this region.

This is of importance, since the Beijing genotype was additionally found to be significantly associated with drug resistance and might be a driving force for the spread and emergence of MDRTB. It is well known, that strains of the Beijing genotype have been involved in outbreaks of drug resistant tuberculosis such as in the USA and in Russia. However, there was no consistent association with drug resistance in our study because only a few isolates was investigated.

ST 4 was the major Spoligopattern in our study differ from ST 127 in spolDB4 by only one spacer, and it has been reported that the DR locus was Hotpoint for IS6110; insertion or exiting of IS6110 can affect amplification of spacers. It is thus possible that the ST4 (in our study) and ST127 (in spolDB4) actually share the same direct-repeat locus.

The two another spoligotypes observed in our study were types 26 and 54. ST 26 was initially described in 1997 in a study performed in the United Kingdom. It was later shown that it Belonged to a major genetic group I of *M. tuberculosis* complex organisms. Until now this family of strains has been reported in 11 countries.

Type 54 is also probably belongs to group I organisms. This shared type is less widespread, and its distribution is different. It has been reported to be present in Africa and Europe. This type may be an ancestor of both the CAS and the Beijing Family. Another type that observed in our study was type 381. This type was originally reported in United Kingdom and a prevalent type in India. This genotype also reported from Pakistan.

The present study included a few isolates with no bias for patient selection. Hence, it was truly representative of the community. From 15 spoligopatterns in our study that previously described in spolDB4, eight types have been reported from the United Kingdom (ST 54, 26, 381, 127, 754, 100 and 1). Indian studies have also shown that many of the spoligopatterns that observed in India were originally reported from the United Kingdom. These features could show that relationship between United Kingdom, India and other eastern neighbors of Iran could affect the spoligotypes that observed in Iranian patients. The polymorphism of *M. tuberculosis* from developing countries, where TB is highly prevalent, would provide new insights into epidemiology, transmission, dynamics, phylogenetic analysis, and virulence. Similar studies with detailed epidemiologic data that would reflect on the TB control programs are needed to understand the TB population in Iran.

### References

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