Genotypic Diversity among Isoniazid-Resistant Isolates of *Mycobacterium tuberculosis* from Rashid Hospital in Dubai, United Arab Emirates

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Key Words

Mycobacterium tuberculosis · Isoniazid resistance · Genotyping · *katG* gene · Dubai

Abstract

Objective: To perform molecular fingerprinting for strain relatedness among isoniazid-resistant *Mycobacterium tuberculosis* isolates recovered from tuberculosis (TB) patients in the United Arab Emirates (UAE). **Materials and Methods:** Thirty-two consecutive isoniazid-resistant *M. tuberculosis* strains isolated from TB patients (4 natives and 28 expatriates) at Rashid Hospital, Dubai, UAE were analyzed. The typing was carried out by touchdown double-repetitive-element PCR (DRE-PCR). The status of R463 or L463 polymorphism and the presence of S315T mutation in the *katG* gene were also determined for isolates exhibiting cluster pattern in DRE-PCR. **Results:** All the 32 isolates (28 from expatriate patients and 4 from UAE nationals) exhibited 21 distinct patterns in DRE-PCR with 20 of 32 isolates exhibiting unique patterns and the remaining 12 exhibiting cluster 'A' pattern. All the isolates (19 of 19) yielding two or more DNA fragments in DRE-PCR were unique strains. The genotypic heterogeneity among 10 of the 12 cluster isolates was suggested by the varying susceptibility of these isolates to anti-TB

drugs, presence of R463 or L463 polymorphism in the *katG* gene and the presence or absence of S315T mutation in the *katG* gene. **Conclusion:** The genotypic diversity among isoniazid-resistant *M. tuberculosis* isolates mostly recovered from expatriate patients indicates that most expatriates were infected with a unique strain imported, most likely, from their country of origin and that their latent infection was reactivated in UAE.

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Introduction

Tuberculosis (TB) caused primarily by *Mycobacterium tuberculosis* is an infectious disease of global significance. It is estimated that nearly 2 billion people are already infected with *M. tuberculosis*, 8 million individuals develop active disease and nearly 2 million people die of TB each year, mostly in developing but also in developed countries [1]. The emergence and spreading of drug-resistant strains of *M. tuberculosis* has become a major obstacle in the control and management of TB [2]. A better understanding of transmission patterns will help to control the spread of infections, particularly involving drug-resistant strains of *M. tuberculosis*. Clinical isolates of *M. tuberculosis* are usually differentiated by Southern blot-

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ting of genomic DNA with insertion sequence IS6110, poly-GC-rich sequence (PGRS) or other repetitive elements as a probe [3]. The double-repetitive-element PCR (DRE-PCR) is a rapid typing method based on amplification of segments of DNA located between two repetitive elements (IS6110 and PGRS) on the genome [4, 5].

Although isoniazid is the most potent anti-TB drug, resistance to this drug also develops readily and isoniazid-resistant *M. tuberculosis* strains remain infectious. Thus, improper treatment of infections with isoniazid-resistant strains may lead to further development and spreading of multidrug-resistant strains of *M. tuberculosis* [6]. The resistance of *M. tuberculosis* to isoniazid is caused by mutations in several genes, although, clinically relevant resistance is usually due to mutations within the *katG* gene, particularly involving codon 315 [7]. However, polymorphism at codon 463 (R463 or L463) in the *katG* gene is found in both isoniazid-susceptible and -resistant *M. tuberculosis* strains [7, 8].

Dubai, like many urban centers in the United Arab Emirates (UAE) and other Arabian countries in the Persian Gulf region, has a large expatriate population originating from countries where TB is endemic [8, 9]. All expatriates entering UAE are screened for TB (chest radiogram) and visa is granted only to individuals with no active disease. In 1998, the notification rate of TB for the entire population in UAE was reported as 28 cases per 100,000 population [9]. Nearly 85% of TB cases in urban centers of UAE occur in the expatriate population with 15% of *M. tuberculosis* isolates being resistant to isoniazid [9]. The Rashid Hospital in Dubai, UAE, with 500 beds is a major reference hospital of the Government of Dubai where active TB cases are also diagnosed and treated. The aim of this study was to determine whether infection with isoniazid-resistant *M. tuberculosis* strains in Dubai was due to spreading of epidemic strains so that appropriate control measures could be instituted.

Materials and Methods

Clinical M. tuberculosis Isolates

A total of 32 consecutive isoniazid-resistant strains of *M. tuberculosis* isolated from TB patients at Rashid Hospital, Dubai (representing all isoniazid-resistant strains obtained from January 1999 to January 2001) were analyzed. Seven drug-susceptible strains of *M. tuberculosis* isolated during the same period were also analyzed. Only 4 isolates were recovered from local patients (UAE nationals) while 28 isolates were obtained from expatriate workers or their dependents (table 1). Thirty-one of the 32 patients were adults between 18 and 65 years of age, and 28 of them were male patients. Isolation, identification and drug susceptibility testing were performed using

the BACTEC system as reported earlier [10]. The anti-TB drugs were isoniazid, rifampin, streptomycin and ethambutol. The isolates were considered resistant when bacterial growth occurred at a concentration of 0.1 µg isoniazid per milliliter, 2 µg/ml rifampin or streptomycin, and 2.5 µg/ml ethambutol.

Typing by Touchdown DRE-PCR

Touchdown DRE-PCR was performed for typing the 32 consecutive isoniazid-resistant *M. tuberculosis* isolates and the status of polymorphism at codon 463 as well as the presence of S315T mutation in the *katG* gene were also determined for isolates exhibiting cluster pattern. The genomic DNA, prepared from *M. tuberculosis* H₃₇Rv and isoniazid-resistant clinical isolates of *M. tuberculosis* as described previously [5], was used as template in DRE-PCR. The DNA sequences of the four primers used in DRE-PCR were as follows: IS6110-5'R, 5'-GGCTGAGGTCTCAGATCAGAG; IS6110-3'F, 5'-ACCCCATCCTTTCCAAGAACT; PGRS-5'R, 5'-TCCCCGC-CGTTGCCGTACAG and PGRS-3'F, 5'-CTTGGGAAACCCGG-CCAGCTG. The modified touchdown DRE-PCR was performed as described previously [5]. Briefly, the reaction mixtures in a final volume of 50 µl contained ×1 Perkin-Elmer PCR buffer II, 2.5 mM MgCl₂, 10 pmol each of the four primers, 4 µl of template DNA, 0.2 mM dNTP and two units of AmpliTaq DNA polymerase. The amplified product (20–25 µl) was run on 2% agarose gels and the isolates that yielded varying DNA amplified fragments were considered as genotypically distinct strains.

Detection of R463/L463 Polymorphism in the katG Gene

The presence of arginine or leucine at codon 463 (R463 or L463) within the *katG* gene in isoniazid-resistant *M. tuberculosis* isolates was determined by amplification of the *katG* gene DNA region around codon 463 by PCR followed by restriction endonuclease digestion of the PCR-amplified fragment with *NciI* to generate restriction fragment length polymorphism (RFLP), as described previously [8].

Detection of S315T Mutation in the katG Gene

The presence of S315T substitution, the most common genetic alteration in the *katG* gene in isoniazid-resistant *M. tuberculosis* isolates, was also determined for the isolates exhibiting cluster pattern in DRE-PCR. This was achieved by amplification of the corresponding *katG* gene DNA region by PCR followed by restriction endonuclease digestion of the PCR-amplified fragments with *MspI* to generate RFLP as described previously [11].

Results

The clinical background and resistance patterns of the 32 isoniazid-resistant clinical *M. tuberculosis* isolates recovered from TB patients in Dubai, UAE, are presented in table 1. Nine isolates were resistant to isoniazid only, 12 isolates were resistant to one more drug, 8 were resistant to two other drugs and 3 were resistant to all the four anti-TB drugs (table 1). Eleven isolates were multidrug-resistant TB strains (resistant at least to isoniazid and rifampin).

Table 1. Clinical background and genotyping patterns by DRE-PCR of the 32 isoniazid-resistant clinical *M. tuberculosis* isolates from Dubai

Isolate No.	Year of isolation	Specimen cultured	Resistance pattern	Patient's nationality	Sex	DRE-PCR pattern
DH1	1999	sputum	H, E	Indian	male	'A' pattern
DH2	1999	sputum	H, E, S	Indian	male	'A' pattern
DH3	1999	pleural fluid	H	UAE	female	unique
DH4	1999	sputum	H, S	Indian	male	'A' pattern
DH5	1999	sputum	H, R	Indian	male	'A' pattern
DH6	1999	sputum	H, R	Yemani	male	unique
DH7	1999	pus swab	H, E, S	Indian	male	'A' pattern
DH8	1999	sputum	H	UAE	female	unique
DH9	1999	pus swab	H, E, R	UAE	female	unique
DH10	1999	pleural fluid	H	Indian	male	'A' pattern
DH11	1999	abscess	H	Indian	male	unique
DH12	1999	sputum	H, S, R	Bangladeshi	male	'A' pattern
DH13	2000	pleural fluid	H	Indian	male	'A' pattern
DH14	2000	pus swab	H	Pakistani	male	'A' pattern
DH15	2000	lymph node	H	Pakistani	female	unique
DH16	2000	pus swab	H	Bangladeshi	male	unique
DH17	2000	pus swab	H, R	Indian	male	'A' pattern
DH18	2000	sputum	H, R	Russian	male	unique
DH19	2000	sputum	H, E	UAE	male	'A' pattern
DH20	2000	pus swab	H, E, S	Filipino	male	unique
DH21	2000	sputum	H, E, S, R	Pakistani	male	unique
DH22	2000	sputum	H, E, S, R	Indian	male	unique
DH23	2000	sputum	H, E, S, R	Somalian	male	unique
DH24	2000	pus swab	H, S, R	Somalian	male	unique
DH25	2000	pus swab	H, S, R	Somalian	male	unique
DH26	2000	pleural fluid	H, E, S	Indian	male	unique
DH27	2000	CSF	H	Indian	female	'A' pattern
DH28	2001	BAL	H, E	Nepali	male	unique
DH29	2001	pus swab	H, E	Indian	male	unique
DH30	2001	pleural fluid	H, E	Indian	male	unique
DH31	2001	lymph node	H, E	Pakistani	male	unique
DH32	2001	pleural fluid	H, E	Indian	male	unique

CSF = Cerebrospinal fluid; BAL = bronchoalveolar lavage; H = isoniazid; E = ethambutol; S = streptomycin; R = rifampin.

The agarose gel electrophoresis patterns for 15 selected isolates are shown in figure 1. A single DNA fragment was obtained from 13 isolates and two different patterns were apparent (fig. 1, lanes 1 and 3) with most of the isolates (12 strains) yielding a single DNA fragment of the same size (360 bp) as shown in figure 1, lane 1. This DRE-PCR pattern (fig. 1, lane 1) was labeled as 'A' pattern.

Nineteen isolates yielded two or more DNA fragments and all of these isolates exhibited unique patterns (the patterns for 13 isolates are depicted in fig. 1, lanes 2, 4–15). Thus, the 32 isoniazid-resistant isolates exhibited 21 dis-

tinct patterns in DRE-PCR with 20 of them being unique patterns while 12 isolates exhibited 'A' pattern. All the isolates recovered from patients originating from Yemen, Russia, Philippines, Nepal and Somalia exhibited unique patterns (table 1); 3 of 4 isolates from UAE nationals also exhibited unique patterns. Similarly, 3 of 4, 6 of 15 and 1 of 2 isolates recovered from patients originating from Pakistan, India and Bangladesh, respectively, exhibited unique patterns. The remaining isolates yielded 'A' pattern in DRE-PCR (table 1). When the 7 drug-susceptible strains were analyzed, 2 of 3 isolates recovered from

Fig. 1. The DNA fragment patterns in agarose gel from 15 selected isoniazid-resistant *M. tuberculosis* isolates during typing by touchdown DRE-PCR. Twelve isolates yielded a single DNA fragment of the same size (360 bp, labeled as 'A' pattern and shown for one isolate in lane 1) while the remaining 20 isolates yielded one or more unique DNA fragments (the patterns for 14 isolates are shown in lanes 2–15). Lane M is 100-bp DNA ladder and the positions of migration of 100- and 800-bp fragments are marked.

patients originating from India also exhibited 'A' DRE-PCR pattern (data not shown).

The genotypic relationship among 'A' cluster pattern isolates was further evaluated by incorporating the data on resistance patterns, R463 or L463 polymorphism and presence or absence of S315T mutation in the *katG* gene. These analyses showed that 4 of 12 isolates contained R463 while the remaining isolates contained L463 in the *katG* gene. The S315T mutation was also present in 7 of 12 isolates (table 2). However, when all the 12 isolates were analyzed for drug resistance pattern, amino acid at codon 463 and S315T substitution in the *katG* gene, only 2 isolates (DH10 and DH13) recovered from the 2 patients of Indian origin were found identical (table 2).

Discussion

The Rashid Hospital, Dubai, UAE, is a major reference hospital and one of the hospitals/area clinics where active TB cases are diagnosed and treated. Of the 32 patients from whom the isoniazid-resistant *M. tuberculosis* isolates were obtained, only 4 patients were UAE nationals while the rest were expatriate workers or their dependents originating from countries where TB is en-

Table 2. Genotypic heterogeneity among isolates exhibiting a single DNA fragment of the same size (360 bp, 'A' cluster pattern) in DRE-PCR

Isolate No.	Resistance pattern	Country of origin	Amino acid at codon 463	S315T substitution
DH1	H,E	India	L463	absent
DH2	H, E, S	India	R463	absent
DH4	H, S	India	L463	present
DH5	H, R	India	L463	absent
DH7	H, E, S	India	R463	present
DH10	H	India	L463	present
DH12	H, S, R	Bangladesh	L463	present
DH13	H	India	L463	present
DH14	H	Pakistan	L463	absent
DH17	H, R	India	L463	present
DH19	H, E	UAE	R463	absent
DH27	H	India	R463	present

dem. The data strongly suggest that isoniazid-resistant *M. tuberculosis* infections in Dubai, UAE, are mainly associated with expatriate workers or their dependents.

The typing of 32 isoniazid-resistant *M. tuberculosis* isolates exhibited a total of 21 distinct patterns with 20 of them being unique patterns while 12 isolates exhibited

cluster ('A') pattern. Nine of 12 *M. tuberculosis* isolates exhibiting 'A' pattern were recovered from patients originating from India. The DNA fragment patterns in DRE-PCR depend on the copy number and relative positions of repetitive elements IS6110 and PGRS on the *M. tuberculosis* genome [3]. Thus, the amplification of a single DNA fragment from 'A' cluster pattern isolates may indicate low copy numbers of these repetitive elements. Alternatively, the isolates may represent a genuine cluster implying recent transmission. The copy number of repetitive element IS6110 varies widely among clinical *M. tuberculosis* isolates [3] and nearly 50% of the isolates from India are known to contain less than two copies of IS6110 on their genome [12, 13]. The data on resistance pattern, R463 or L463 polymorphism and presence or absence of S315T mutation in the *katG* gene showed genotypic heterogeneity among 'A' cluster pattern isolates. Although altering of drug resistance patterns of *M. tuberculosis* isolates does not lead to a change in IS6110-based fingerprints [3], the strains spreading locally are expected to exhibit similar polymorphism at codon 463 of the *katG* gene [8, 14]. Further, two of three drug-susceptible strains of *M. tuberculosis*, recovered from patients of Indian origin, also yielded 'A' DRE-PCR pattern. Taken together, the amplification of a single DNA fragment in DRE-PCR from some of the isolates exhibiting 'A' pattern is most likely due to low copy number of IS6110. However, the possibility that some of the cluster isolates were genotypically related, could not be completely ruled out.

Molecular epidemiological studies for TB are carried out to ascertain the transmission of *M. tuberculosis* and are based on the assumption that strains derived recently from a common ancestor exhibit the same fingerprint (cluster pattern) [15]. The term cluster is used to denote *M. tuberculosis* isolates with identical or nearly identical DNA fingerprints [3, 16]. In most low-incidence countries for TB, the typing patterns of *M. tuberculosis* are highly polymorphic due to reactivation of previously acquired infection in the majority of indigenous populations (a reflection of genotypes present in the population over a long period of time) and infection with strains novel to the areas that are imported from the country of origin by expatriate/immigrant populations [3, 16]. On the contrary, in countries with a high incidence of TB, the typing patterns are often less variable due to recent acquisition of infection in a sizeable portion of the population [17]. The incidence of TB is low in UAE (28 cases per 100,000 population) and directly observed chemotherapy short course as recommended by the World Health Organization is fol-

lowed for all patients diagnosed with active disease [9]. Since all the expatriates are screened for TB upon entry into UAE, the active disease in local or expatriate patients could either be due to recent acquisition of infection or due to reactivation of latent infection. The typing carried out by touchdown DRE-PCR, the data on polymorphism at codon 463 in the *katG* gene and the presence/absence of S315T mutation in the *katG* gene showed that most TB patients in Dubai were infected with a unique strain of *M. tuberculosis*. The genetic diversity exhibited by the majority of isoniazid-resistant isolates of *M. tuberculosis* suggests that active disease in most of the patients was likely due to reactivation of latent infection and not due to recent transmission. Although the data on the length of stay in UAE for the expatriates was not available, molecular evidence has recently shown that endogenous reactivation resulting in active disease may occur even after 33 years of latent infection [18].

Recent data from Kuwait, another Arabian country in the Persian Gulf region which also has a large expatriate population originating from TB endemic countries of South Asia and Southeast Asia, have also shown that the majority of isoniazid-resistant *M. tuberculosis* infections occur in the expatriate population [11, 19]. Further, similar to the data reported here, most of the isoniazid-resistant *M. tuberculosis* strains from Kuwait also exhibited unique patterns in typing studies [5, 11]. Molecular epidemiological data from the United States of America (USA) have also shown that active disease in the majority of foreign-born patients is caused by reactivation of latent *M. tuberculosis* infection while many TB cases among USA-born persons are attributed to recent transmission of infection [20]. The reactivation of latent infection in immigrants originating from endemic countries may introduce novel strains among local populations in low-incidence countries. The reactivation of latent *M. tuberculosis* infection with drug-resistant strains in Russian immigrants and their subsequent spread to the local population were recently shown in Germany [21]. These studies have suggested treatment of latent infection in immigrants from endemic countries to avert the introduction of novel strains in low-incidence countries [18, 20, 21].

Conclusion

The results presented in this study showed that most infections with isoniazid-resistant strains of *M. tuberculosis* in Dubai, UAE were associated with expatriates. The

genotypic heterogeneity exhibited by most of the isolates suggests that most expatriate patients were infected with a unique strain most probably imported from their country of origin and that their latent infection was reactivated in Dubai.

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