Molecular fingerprinting reveals familial transmission of rifampin-resistant tuberculosis in Kuwait

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Tuberculosis (TB) is a major public health hazard killing nearly 2 million people worldwide each year.1 The emergence of multidrug-resistant Mycobacterium tuberculosis strains (MDR-TB) causing increased morbidity and mortality compared to drug-susceptible strains, has become a major obstacle in the control of TB.2 The MDR-TB strains evolve due to sequential accumulation of resistance-conferring mutations in target genes as a result of the selective pressure of anti-TB drugs.3 The resistance develops either during treatment due to poor follow-up and compliance with TB therapy or the patient may be infected with an already drug-resistant strain.2-3 Outbreaks of drug-resistant TB and MDR-TB prior to 1986 were rare but have occurred more frequently since then in hospitals and institutional settings involving both human immunodeficiency virus (HIV)-infected and HIV-negative individuals.4-7 The development of drug resistance during treatment (acquired resistance) and nosocomial transmission of MDR-TB to close contacts was also demonstrated, particularly among HIV-positive patients.4 The mortality rates among HIV-positive patients with MDR-TB have also been reported to be much higher (41 to 72%) compared to those reported for HIV-negative patients (4 to 17%).9

Although resistance to isoniazid evolves first in nearly all cases of acquired drug resistance in TB patients, one previous report has described the development of monoresistance to rifampin in TB patients co-infected with human immunodeficiency virus (HIV) and M. tuberculosis.10 This study reports, for the first time, primary rifampin-resistant TB in a previously treated diabetic male patient and its transmission to his daughter (both HIV-negative) in the familial environment in Kuwait. Molecular fingerprinting confirmed the clonal relatedness of the isolates recovered from the two patients.

Patients and Methods:
The first patient, a 33-year-old Kuwaiti (Middle Eastern) female was referred to the TB Control Unit of the Chest Diseases Hospital in January 2003 with the diagnosis of cold abscess of the right gluteus medius muscle with adjacent trocentric involvement. Both the hip joints and psoas muscles were normal. Ultrasonic guided aspiration from the abscess yielded thin pus that was positive for acid-fast bacilli. The culture was grown from the aspirate (isolate Kw 176) and identified as M. tuberculosis.11 The drug susceptibility performed with BACTEC 460 TB system showed that the bacilli were sensitive to isoniazid (INH), streptomycin (SM), pyrazinamide (PZA) and ethambutol (EMB) but resistant to rifampin (RMP).11 She was treated with a regimen of INH, SM, PZA and EMB for seven months and fully recovered.

The second patient, a 63-year-old diabetic Kuwaiti (Middle Eastern) male was the father of the first patient and lived with his daughter. He was diagnosed with pulmonary TB nearly two years earlier (June 2000). The M. tuberculosis isolate recovered from this patient in the year 2000 was susceptible to INH, SM, EMB and PZA but resistant to RMP. He was treated with a four-drug regimen of INH, SM, PZA and EMB for six months. This patient was admitted to the Chest Diseases Hospital in February 2003 with relapsing pulmonary tuberculosis with cough, fever, loss of appetite and loss of weight. The chest radiogram showed pulmonary infiltrates. The sputum was positive for acid-fast bacilli. The drug susceptibility performed with BACTEC 460 TB system on the M. tuberculosis strain (isolate Kw 180) grown from sputum exhibited sensitivity to INH, SM, EMB and PZA but resistance to RMP.11 A repeat isolate recovered 15 days later (isolate Kw 181)
yielded the same profile. The patient was started on daily therapy with INH, SM, PZA, EMB and ciprofloxacin (CIP). However, he could not tolerate PZA, so the treatment was continued with the regimen of SM, INH, EMB and CIP for 75 days after which the sputum was negative for acid-fast bacilli. The treatment was continued with the regimen of INH, EMB and CIP for four months. The patient was successfully treated and the last culture result at the end of therapy was negative.

Most (>95%) rifampin-resistant strains of M. tuberculosis contain mutations within an 81-base pair rifampin-resistance-determining region (RRDR) of the rpoB gene encoding the β-subunit of RNA polymerase. The presence of rifampin resistance conveying mutations in the rpoB gene in clinical M. tuberculosis isolates (Kw 176, Kw 180 and Kw 181) was determined by direct DNA sequencing of the PCR amplified DNA fragments containing RRDR as described previously. The fingerprinting of the isolates was carried out by the modified double repetitive element (DRE)-PCR as described in detail elsewhere. The isolates were assigned to one of four genetic groups on the basis of the polymorphisms at katG codon 463 and gyrA codon 95. The presence of R463/L463 in the katG gene and S95/T95 in the gyrA gene were determined by amplification of the corresponding katG and gyrA gene DNA regions by PCR followed by restriction endonuclease digestion of the PCR amplified fragments with Nci I and Alu I, respectively, to generate restriction fragment length polymorphism as described previously.

Results

The DNA sequencing data showed that the molecular basis of resistance to rifampin in isolate Kw 176 was due to a rare mutation (insertion TTC at codon 514) in RRDR of the rpoB gene. The DNA sequencing data for the isolate Kw 180 also revealed the presence of TTC insertion at codon 514 of RRDR of the rpoB gene. The repeat isolate recovered from the second patient (Kw 181) also yielded similar results (Table 1). The presence of this rare mutation in RRDR of the rpoB gene in isolates (Kw 176, Kw 180 and Kw 181) recovered from two related patients (father and daughter who lived in the same premises) suggested that the isolates were clonally related. This aspect was studied further. The isolates recovered from both the patients exhibited identical patterns of DRE-PCR amplified DNA fragments (Figure 1). The repeat isolate (Kw 181) also yielded similar results. Additionally, isolate Kw176 as well as isolates Kw180 and Kw181 belonged to the same genetic group (Group 2) based on polymorphisms at katG codon 463 and gyrA codon 95 (Table 1) further indicating clonal relatedness among these isolates.

Discussion

The incidence of TB varies considerably among Middle Eastern countries. Kuwait with nearly 30 cases per 100 000 population is a low incidence country among Arabian countries in the Persian Gulf region. However, it has a large expatriate population originating from TB endemic countries of South Asia (largely India), Southeast Asia (mainly Philippines) and some Middle Eastern countries (mainly Egypt). Although all expatriates entering Kuwait are screened for TB (chest radiograph) and visas are granted only to those individuals who have had no prior exposure to active disease, nearly 80% of all active TB cases in Kuwait occur in the expatriate population. Approximately 600 patients are diagnosed with active disease every year with nearly 1% and 7% of the M. tuberculosis isolates being resistant to rifampin and isoniazid (the two most effective anti-TB drugs), respectively. Nearly all rifampin-resistant strains are also resistant to isoniazid and/or other first-line anti-TB drugs. However, drug-resistant TB and MDR-TB are predominantly found among expatriate patients. The incidence of M. tuberculosis strains resistant to several anti-TB drugs or MDR-TB among Kuwaiti patients is extremely rare and the isolation of M. tuberculosis strains monoresistant to rifampin has not been described previously.

Table 1. Clinical background and genotypic characteristics of M. tuberculosis isolates recovered from two related patients.

| Isolate | Clinical specimen | rpoB gene mutation | DRE-PCR fingerprinting | katG gene codon 463 | gyrA gene codon 95 |
|---------|------------------|-------------------|-----------------------|-------------------|------------------|
| Kw 176  | Pus               | Ins. 514 TTC      | Identical pattern     | R (CGG)           | T (ACC)          |
| Kw 180  | Sputum            | Ins. 514 TTC      | Identical pattern     | R (CGG)           | T (ACC)          |
| Kw 181  | Sputum            | Ins. 514 TTC      | Identical pattern     | R (CGG)           | T (ACC)          |

Ins., insertion; R, arginine; T, threonine
FAMILIAL TRANSMISSION OF RIFAMPIN-RESISTANT TB

The two *M. tuberculosis* isolates mono-resistant to rifampin reported in this study were recovered from two Kuwaiti nationals who were also related (father and daughter) and lived in the same premises. The male patient was previously treated in the year 2000 for infectious (sputum positive for acid-fast bacilli) primary rifampin-resistant pulmonary TB for six months. He most likely infected his daughter at that time since he had a highly infectious form of the disease. The daughter, however, was asymptomatic for nearly two years probably due to acquired immunity, but remained latently infected with an *M. tuberculosis* strain mono-resistant to rifampin, which re-activated two years later with an unusual presentation. It is unlikely that the daughter infected her father since she became ill two years later and had a non-infectious form of the disease. Other family members including the male patient’s wife, six other daughters and a son (all native Kuwaiti nationals) who also live in the same premises have, however, not shown any signs or symptoms of the disease so far.

Mono-resistance to rifampin occurs less frequently than to isoniazid since spontaneous mutations conferring resistance to rifampin occur nearly 100 times less frequently than those causing isoniazid resistance in *M. tuberculosis*. The rifampin-resistant TB in the two cases described above was documented by both phenotypic drug susceptibility testing and by direct sequencing of RRDR of the *rpoB* gene of the *M. tuberculosis* isolates. This rarely occurring mutation in RRDR of the *rpoB* gene (insertion TTC at codon 514) has previously been shown to be associated with rifampin resistance in *M. tuberculosis*. Rifampin mono-resistant TB has been observed among patients co-infected with HIV. Other risk factors for acquiring rifampin-resistant TB besides HIV-positive status of the individual, are malabsorption and low serum levels of orally administered anti-TB drugs. Low serum levels of rifampin have also been noted in some non-HIV infected individuals and were found to be associated with some particular combinations of anti-TB drugs and their formulations. The sub-therapeutic levels of rifampin in the serum of a patient were also noted in another study. This patient with fulminant pulmonary TB with no obvious signs of malabsorption died despite intensive care of 41 days. The data reported in this study demonstrate that primary rifampin-monoresistant TB infection may occur in HIV-negative individuals even in low incidence countries such as Kuwait and be transmitted in the familial environment. However, the source of infection in the male patient who was diagnosed with primary rifampin-monoresistant TB in the year 2000 could not be ascertained due to lack of epidemiological records.

Although this is the first report describing transmission of *M. tuberculosis* strain mono-resistant to rifampin among HIV-negative individuals, the increasing incidence of drug-resistant TB across the globe suggests that such cases may be encountered with greater frequency if urgent remedial measures are not taken to control the evolution and spreading of drug-resistant strains of *M. tuberculosis*. Further, since such strains remain infectious, they may also acquire further resistance to other front-line anti-TB drugs such as isoniazid and evolve into MDR-TB strains. Infections with MDR-TB strains are extremely difficult and costly to treat.

![Figure 1. Agarose gel of touchdown DRE-PCR generated DNA fragment patterns from the isolate Kw 176 (Lane 1) and Kw 180 (Lane 2). The patterns obtained from the repeat isolate recovered from the second patient (Kw 181) are shown in Lane 2'. Lane M is 100 bp DNA ladder and the positions of migration of 100 bp and 600 bp fragments are marked.](image-url)
The DNA sequencing data reported in this study has been deposited to EMBL (European Molecular Biology Laboratory) under the accession nos. AJ870394 and AJ870395.

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