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Identification and Genotyping of *Mycobacterium tuberculosis* Isolated From Water and Soil Samples of a Metropolitan City

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BACKGROUND: The potential role of environmental *Mycobacterium tuberculosis* in the epidemiology of TB remains unknown. We investigated the transmission of *M tuberculosis* from humans to the environment and the possible transmission of *M tuberculosis* from the environment to humans.

METHODS: A total of 1,500 samples were collected from three counties of the Tehran, Iran metropolitan area from February 2012 to January 2014. A total of 700 water samples (47%) and 800 soil samples (53%) were collected. Spoligotyping and the mycobacterial interspersed repetitive units-variable number of tandem repeats typing method were performed on DNA extracted from single colonies. Genotypes of *M tuberculosis* strains isolated from the environment were compared with the genotypes obtained from 55 patients with confirmed pulmonary TB diagnosed during the study period in the same three counties.

RESULTS: *M tuberculosis* was isolated from 11 of 800 soil samples (1%) and 71 of 700 water samples (10%). T family (56 of 82, 68%) followed by Delhi/CAS (11 of 82, 13.4%) were the most frequent *M tuberculosis* superfamilies in both water and soil samples. Overall, 27.7% of isolates in clusters were related. No related typing patterns were detected between soil, water, and clinical isolates. The most frequent superfamily of *M tuberculosis* in clinical isolates was Delhi/CAS (142, 30.3%) followed by NEW-1 (127, 27%). The bacilli in contaminated soil (36%) and damp water (8.4%) remained reculturable in some samples up to 9 months.

CONCLUSIONS: Although the dominant *M tuberculosis* superfamilies in soil and water did not correspond to the dominant *M tuberculosis* family in patients, the presence of circulating genotypes of *M tuberculosis* in soil and water highlight the risk of transmission.

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ABBREVIATIONS: HGI = Hunter and Gaston index; MDR-TB = multidrug-resistant *Mycobacterium tuberculosis*; MIRU-VNTR = mycobacterial interspersed repetitive units-variable number of tandem repeats; PCR = polymerase chain reaction

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The presence of *Mycobacterium tuberculosis* in the environment and its potential role in the epidemiology of TB has been debated since the early 20th century.[1] Musehold[1] started the debate showing the presence of virulent TB bacteria in the sewage of a TB sanatorium in 1900.

TB is characterized as being transmitted by aerosols as a consequence of direct contact with a patient with pulmonary TB. However, TB transmission could occur without patient contact. Johnson et al[2] demonstrated that exposure to medical waste resulted in TB infection in three patients. To date, few studies have assessed the environment as a source of *M tuberculosis* infection.[3] Transmission of *M tuberculosis* from the environment is possible as TB bacilli have been isolated from sputum or carpet up to 19 days, wood over 88 days, and moist and dry soil up to 4 weeks following contamination.[4,5] Furthermore, not only can *M tuberculosis* survive in soil, but it also remains virulent.[6] As there are 13.7 active and 8.8 million newly diagnosed TB cases each year, it is quite likely that soil and water can become contaminated with *M tuberculosis* through expectoration.[7]

To conduct a systematic study of the potential role of transmission of *M tuberculosis* from the environment to humans, we report the isolation, identification, and genotyping of *M tuberculosis* isolates from soil and water samples from the Tehran, Iran metropolitan area.

Materials and Methods

This study was reviewed and approved by the institutional review board of the National Research Institute of Tuberculosis and Lung Disease of Iran (approval number of MRC-2011/023).

Sample Collection and Preparation

In total, 700 soil samples and 800 water samples were collected from three counties of Tehran metropolitan areas—Robat Karim, Firuzkuh, and Shahr-e-Ray—from February 2012 to January 2014 (Fig 1). We collected 5 to 7 g of soil sample from depths of 3 to 5 cm, suspended it in a 50-mL sterile tube, and processed it using a modified Engbaek method.[8-10] Fifty to 100 mL of water from different sources (200 from damp waters, 100 from tap waters, and 500 samples from running water on raceway systems) were collected. The raceway system is a cement canal (10-70 m length and 20-70 cm width) used for transporting the wastewater or sustainable rainwater to the central recycling and treatment sector. The raceway system is designed for almost all streets, lanes, and alleys in the Tehran metropolitan area.

Water samples were decontaminated with cetylpyridinium chloride (final concentration of 0.05%) for 30 min and digested using a standard protocol.[11,12] The final water and soil sediments were acid-fast stained (Ziehl-Neelsen) and cultured by inoculating three Löwenstein-Jensen medium bottles with sediments (200 μL of sediment/tube). Bottles were sealed and incubated at 37°C, 25°C, and 42°C for 12 weeks. The inoculated cultures were checked for growth every 3 to 4 days. Acid-fast colonies were identified by standard phenotypic tests (including niacin and nitrate tests). A molecular analysis was performed on single colonies derived from subcultures of original isolates as previously described.[13]

Patients

We randomly selected 458 patients with culture-positive pulmonary TB who were residents of the sampled counties and were diagnosed during the study period. Of 458 patients, 25 were from Robat Karim, 10 were from Firuzkuh, 20 were from Shahr-e-Ray, and 413 were from Tehran city. Demographic information including nationality of patients was collected. *M tuberculosis* isolates from patients were genotyped and compared with *M tuberculosis* isolates from water and soil.

DNA Extraction and Species Identification From Acid-Fast Bacilli-Positive Cultures

The DNA was extracted from heat-inactivated colony suspensions using a QIAGEN DNA Extraction kit. Species identification was performed

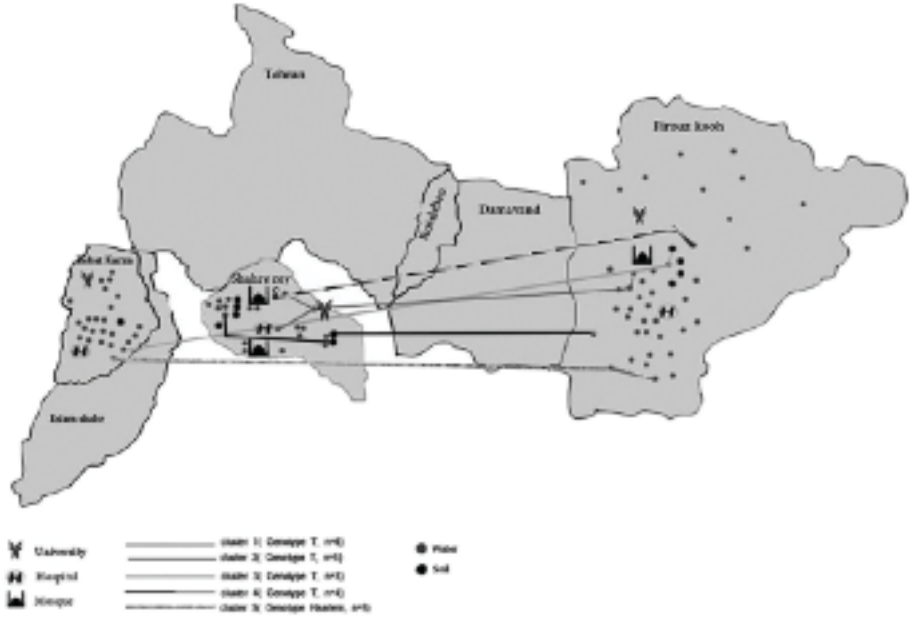

Figure 1 – *Water and soil sampling locations in Tehran metropolitan areas.*