

Usefulness of molecular techniques to identify ongoing tuberculosis transmission in Saudi Arabia

Sahal A. Al-Hajoj, PhD, Viqaruddin K. Mohammed, MSc, Abdullah A. Al-Hokail, MD.

ABSTRACT

This study represents the first time that molecular tracing techniques have been used to identify patterns of tuberculosis (TB) infection in Saudi Arabia. The 2 strains were isolated from a socio-economically advantaged family who share a number of common facilities including a car and a driver. There are several factors that may play vital roles in on-going transmission of TB in Saudi Arabia including a high number of expatriates, the Hajj pilgrimage, and the social habits of Saudi citizens. Our sibling case series is believed to be a frequent pattern of disease transmission in this country. Control measures such as health education, active case finding, and prompt and supervised medical treatment are needed. More studies using molecular techniques are recommended to find the incidence of cross infection in Saudi Arabia. In addition, molecular techniques have to be established in all reference laboratories to help the detection of ongoing active transmission, molecular epidemiology and detect sources of infection.

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From the Department of Comparative Medicine (Al-Hajoj, Mohammed) and the Department of Medicine (Al-Hokail), Tuberculosis Research Unit, Infectious Diseases, King Faisal Specialist Hospital and Research Centre, Riyadh, Kingdom of Saudi Arabia.

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Address correspondence and reprint request to: Dr. Sahal A. Al-Hajoj, Department of Comparative Medicine MBC-03, Tuberculosis Research Unit, King Faisal Specialist Hospital and Research Centre, PO Box 3354, Riyadh 11211, Kingdom of Saudi Arabia. Tel. +966 (1) 4424992. Fax. +966 (1) 4427872. E-mail: hajoj@kfsbrc.edu.sa, sahal_abdul@hotmail.com

Tuberculosis (TB) is endemic in Saudi Arabia. Infection rates of up to 64 per 100,000 have been recorded and the problem is compounded by the fact that Saudis live in large family units and share common domestic and social facilities. In addition, the country is home to more than 6 millions immigrants and actually receives more than 2 million Pilgrims. All these factors increase the impact of

ongoing transmission. Availability of molecular techniques to fingerprint *Mycobacterium tuberculosis* (*M. tuberculosis*) has made detection of disease transmission, cross infection, and identification of the source of infection possible. Our aim in the present study was to use molecular techniques, namely restriction fragment length polymorphism IS6110 (RFLP IS6110), and spoligotyping, to genotype 2 isolates that were obtained from a socio-economically advantaged family.

Case Reports. *Patient one.* A 22-year-old healthy female with no significant past medical history presented with a 3 weeks history of fever and cough productive of a small amount of green-yellow sputum occasionally streaked with blood. She had a poor appetite and lost 3 kilogram of body weight over a one month period. Physical examination showed she was not pale or jaundiced, no palpable lymph nodes and chest examination revealed bronchial breathing at the right mid-lung zone. Laboratory investigation showed normal blood [cell] count, erythrocyte sedimentation rate 73, sputum positive 3+ for acid-fast bacilli (AFB), chest x-ray (CXR) showed infiltration in right middle lobe with small cavity.

Patient 2. A 17-year-old healthy male with no significant past medical history presented 3 months after the diagnosis of his sister with a 2 week history of dry cough, fever, anorexia, and night sweat. His temperature was 38.2°C, but he was not pale or jaundiced. Physical examination showed there were no palpable lymph nodes. Chest and abdomen examination was unremarkable. Laboratory investigation showed normal blood count, CXR revealed left epical infiltrate and sputum was positive 2+ for AFB. His purified protein derivation and CXR at the time of his sister's diagnosis were negative and he was given 6 weeks following up appointment but did not attend.

The family's driver is an expatriate worker originating from Sudan, a highly endemic TB area. It

is not clear whether he was the source of the infection, although we know that he was receiving treatment for TB, as it was not possible to track him. No other contacts were suspected sources of transmission. Sputum samples were collected from siblings suspected to have TB. Patients were investigated by routine diagnostic methods. After presumptive identification of the isolated microorganism as *M. tuberculosis* pure cultures were obtained and later confirmed. Samples of pure culture were harvested, DNA extracted as described previously,¹ and then subjected to RFLPI S6110 and spoligotyping. Drug susceptibility test was performed using mycobacterial growth indicator tube (MGIT) 960 (Becton Dickinson-USA). The following concentrations were used: Isonized 0.1 µg/ml, Rifampicin 0.1 µg/ml, Ethambutol 5.0 µg/ml, and streptomycin at 1.0 µg/ml according to manufacturer's instructions.

Restriction fragment length polymorphism IS6110 typing was performed as described by the international standard protocol¹ in which *M. tuberculosis* DNA is digested using the enzyme (*Proteus vulgaris*) PvuII and Southern blot is hybridized with a probe from the right-hand portion of RFLPI S6110. The blot was normalized with the standard *M. tuberculosis* reference strain 14323. Spoligotyping was performed as described.² Briefly, the direct repeat region (DR-region) was amplified by polymerase chain reaction (PCR) with oligonucleotide primers derived from DR sequences. The labeled PCR product was used as a probe to hybridize with 43 synthetic spacer oligonucleotides attached to a carrier membrane (Isogen Bioscience BV, Maarsse, The Netherlands). The 2 isolates were identical by both RFLP and spoligotyping methods **Figure 1**. Drug susceptibility testing (DST) showed that both isolates were sensitive to all 4 first line anti-tuberculosis drugs.

Discussion. Tuberculosis in Saudi Arabia is an endemic disease. The infection rate (according to available information) varies from 32-64 per 100,000 depending on the region. For instance, it is 64 per

100,000 in the western province and 32 in Riyadh, central region.^{3,4} However, little information is available for the rest of the country. The reason for variation is attributed to pilgrims who visit the western region for the Hajj ritual, Omra, and visiting the holy mosque in Madina.⁵ We believe that this is not the only factor that is playing a role in the endemicity of the disease. Several factors may contribute, such as lack of a standardized program to tackle the disease despite the implementation of directly-observed therapy (DOT), a lack of coordination in active case finding, lack of infrastructure in laboratories, and lack of suitably trained individuals.⁶ Successful control programs for TB require patients to be traced and observed until treatment is complete. Active case and contact tracing is difficult among Saudi nationals as medical records are not centralized, and there is no mechanism for medical authorities to trace patients. Patients may be admitted to successive hospitals with new records each time. The infrastructures of the TB laboratories require extensive refurbishment in order for them to safely and efficiently process TB specimens. This study highlights active intrafamilial transmission that we believe is a frequent occurrence in Saudi Arabia. Traditional Saudi families live together in large units sharing domestic facilities, including expatriate-chauffeur driven cars. Immigration from high-risk countries is recognized worldwide to be associated with TB.⁷ Usually the Saudi government requires health certificates for non-national drivers to be obtained from their original country before entering Saudi Arabia. In many cases drivers obtain falsified documents and are then found to develop TB disease soon after arrival. This is evident by presence of many TB clades/families such as Manila, Beijing, Latin America-Mediterranean, and Delhi type (unpublished data). Low rates of success of DOT, below 65%,⁸ frequent migration of people around the country, large family units, employment of immigrant drivers from endemic countries and a low level of education among the majority of citizens are all factors contributing to

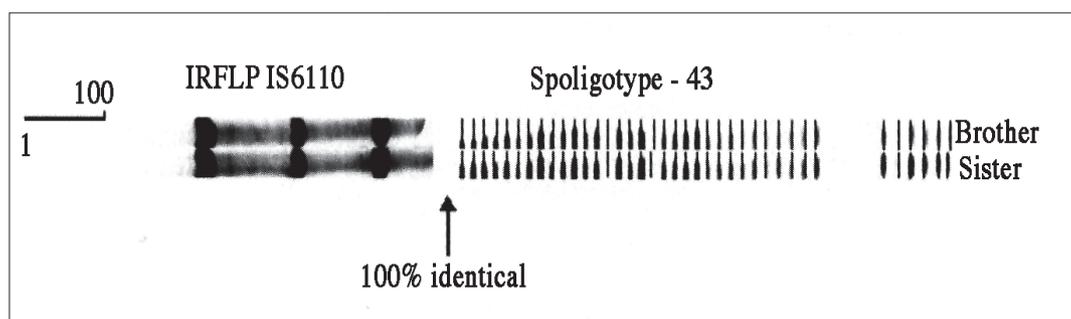


Figure 1 - Restriction fragment length polymorphism (RFLP) and spoligotyping techniques demonstrating identical patterns for 2 isolates of *Mycobacterium tuberculosis* from a brother and sister with pulmonary tuberculosis.

ongoing TB transmission. In addition, Hajj and Omra are 2 Islamic occasions for which millions of potentially TB-infected individuals visit the country each year and congregate in crowded places. Tuberculosis has been previously reported as the main cause of pneumonia during Hajj time,⁹ but no study has yet used molecular epidemiology tools to investigate such places as the Grand Mosque in Makka and Madina. Pilgrims and expatriates who remain in Saudi Arabia illegally do not go to the hospital fearing questioning and deportation.

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Statistics

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Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals). Avoid relying solely on statistical hypothesis testing, such as the use of P values, which fails to convey important information about effect size. References for the design of the study and statistical methods should be to standard works when possible (with pages stated). Define statistical terms, abbreviations, and most symbols. Specify the computer software used.