

Molecular epidemiology of *Mycobacterium* species clinical isolates in Riyadh, Saudi ArabiaNaif A. Al-Dhabi ¹, Basheer A. Al-Sum ¹, Rayed F. Al-Hammad ²¹Department of Botany and Microbiology, College of Science, King Saud University, P. O. Box 2455, Riyadh 11451, Saudi Arabia, ²King Saud Chest Hospital, P. O. Box 7966, Riyadh 11472, Saudi Arabiaaldhabin@gmail.com

Abstract: The aim of this study is to build a future vision in the ability to make important reference Tuberculosis database which would enable us to trace the causes of Tuberculosis and reduce its spread, particularly in Saudi Arabia. Over the previous centuries, *Mycobacterium tuberculosis* caused Tuberculosis was a lethal infectious disease in Saudi Arabia and in many neighboring countries, and still remains as a significant causative agent of morbidity among these population. This study describes the results of a survey work on the cross-section of patients in a health center in Riyadh (Saudi Arabia), in the period between July and September 2011. The survey included twenty-two Saudi patients and forty-eight migrant workers of both sexes and different age groups. Different strains of the microorganism were isolated, including pathogenic strains (70 strains) and non-pathogenic ones 8 strains. The survey concluded that there is a relationship between sex and age group with the incidence of TB bacteria. Twenty-two in patients of 10-40 years, the increase is proportional, while in those of 41-70 years, the increase is inversely proportional. The survey showed also that for female patients, the most affected were at age 65.

[Al-Dhabi N. A, Al-Sum B. A, Al-Hammad R. F. **Molecular epidemiology of *Mycobacterium* species clinical isolates in Riyadh, Saudi Arabia.** *Life Sci J* 2013;10(4):1551-1556]. (ISSN:1097-8135).
<http://www.lifesciencesite.com>. 204

Keywords: *Mycobacterium tuberculosis*, King Saud Chest Hospital, Saudi Arabia

1. Introduction

Tuberculosis (TB) remains as an important public health issue throughout the world. Tuberculosis and other mycobacterial infections have been a major cause of morbidity and mortality throughout the world from time immemorial. In this century, however, the annual infection rate has fallen dramatically in the developed countries and is still declining (Bleiker, 1991). However the resurgence of tuberculosis (TB) has renewed interest in understanding the epidemiology and pathogenesis of the disease. Genotyping of clinical isolates in different parts of the world has shown that global epidemiology of TB is propagated by thousands of different genotypes (Soolingen, et al. 1999; Warren, et al. 1999). The strains occur at different frequencies, and the relative frequencies in different areas vary between districts, cities, countries and continents (Brudey, et al. 2006, Filliol, et al. 2003; Filliol, et al. 2002). The dynamics of TB epidemic in a given area and time frame may therefore be a factor of the different strains circulating in that region. The knowledge of circulating strains can be used for molecular evolutionary and population genetics studies. Saudi Arabia has a moderate incidence of active TB (30 cases per 100,000 inhabitants); however, the infection rate varies between cities. As an example, in 1990, the incidence in Jeddah (Western Province) reached 64 cases per 100,000 compared with 32 per 100,000 in Riyadh. The annual

influx of pilgrims, as well as a high proportion of migrant workers in the country, the majority from countries where TB is endemic, provides an opportunity for the transmission of TB (Al-Hajoj, et al. 2007).

Mycobacterium tuberculosis is one of the leading causes of morbidity and mortality worldwide and infects more than one third of the human population, causing over 2 million deaths. There are >10 million new cases every year (World Health, 2005). 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. Control measures such as health education, active case finding, and prompt and supervised medical treatment are needed (Al-Hajoj, et al. 2007). Infection from *M. tuberculosis* results in the death of three million people worldwide per annum of which an estimated one thousand are in Saudi Arabia. The WHO has set a target for successful treatment of 85% but Saudi Arabia is currently not meeting that target. We believe that the first step in improving the control of tuberculosis in Saudi Arabia is to improve and unify the standards of diagnostic services and laboratories responsible for tuberculosis (Al-Hajoj, 2010). Of all the infected individuals, only 10% will ever develop active disease and half of them may do so during the first 2 years (recent infection with rapid progression to disease) after being infected (Daley, et

al 1992). The Ministry of Health report recently released confirmed the alarming rise in TB. It showed that the total cases of TB in the country have reached 3878 cases in 2007 with increment rate of 0.6 per 100 thousand compared to 2006 (3854 cases). Also the report showed that the percentages of Saudi among the total cases were 52.7 compared to 47.4 of non-Saudis (MOH 2007). Hence better surveys are needed in high burden countries like Saudi Arabia requiring high quality routine surveillance. Ultimately effective control of the disease requires an understanding of the dynamics of disease transmission, implementation of accurate and rapid diagnostics and typing methodologies, and efficacious treatment. The logistics of implementation of these among poor, migrating and rural people further the challenge (Al-Hajoj, 2010; Al-Hajoj et al 2007).

A recent study comprising a total of 1505 clinical isolates of *M. tuberculosis*, isolated between 2002 and 2005 from seven regions of Saudi Arabia partially revealed the interesting pattern of TB transmission. The sample studied showed a male to female sex-ratio of 1.27 with half of the cases among foreign-born and 47% within the age-group of 21—40 years, a total resistance rate of 19.7% and multiple drug resistance of 4.5%. Upon spoligotyping, a total of 387 individual patterns were obtained (clustering rate of 86.4%, 182 clusters containing between 2 and 130 isolates per cluster). A total of 94% of the strains matched to the spoligotype patterns in an international database SpolDB4 database (MOH 2007). Nearly 81% of the isolates of this study were known to belonging established phylogeographic clades. Two clonal complexes with unique spoligotyping signatures specific to Saudi Arabia were identified. The high rate of clustering observed might be an indication of rapid ongoing transmission within certain communities and/or subpopulations in Saudi Arabia (Al-Hajoj, 2010; Al-Hajoj et al 2007).

Number of studies reported difference in transmissibility and virulence among *M. tuberculosis* strains are related to genetic makeup of the organisms (Valway, et al 1998; Caminero, et al. 2001; Lopez, et al 2007). Over representation of clades suggests that they possess biological advantage in specific populations and study of such strains maybe help to understand the characteristics that make *M. tuberculosis* an effective pathogen. Pablos- Mendez (Pablos-Méndez, et al. 1998) who conducted survey on behalf of World Health Organization reported data on drug resistance in 135 countries or regions. Among patients with no history of prior treatment for tuberculosis, a median of 9.9% (range 2–41%) of *M. tuberculosis* (MTB) strains were resistant to at least one drug. Among patients with a history of prior

treatment for more than 1 month, the prevalence of resistance to any drug ranged from 5.3 to 100% and the prevalence of (MDR-TB) from 0 to 54%. Drug resistant (MTB) were encountered in all countries and regions surveyed. The report suggested that global tuberculosis control was seriously threatened. Saudi Arabia was not among the countries surveyed.

The evolution of *Mycobacterium tuberculosis* presents several challenges for public health. The tuberculosis virulence evolution can be affected by treatment success rates, the relative transmissibility of emerging strains, the rate of reactivation from latency among hosts, and the life expectancy of hosts (Basu and Galvani, 2009). Over the last decade there has been worldwide emergence of multi-drug resistant *Mycobacterium tuberculosis* (MDR-TB), in both developed countries and developing countries. Drug resistant MTB were encountered in all countries and regions surveyed. The report suggested that global tuberculosis control was seriously threatened. Saudi Arabia was not among the countries surveyed. But the incidence of drug resistance in *Mycobacterium tuberculosis* (MTB) isolated from King Khalid National Guard Hospital, Jeddah and King Fahad National Guard hospitals between April 1996 and March 1998 when compared with an earlier study (1993–1995) showed that thirty (29.7%) of 101 MTB isolates were resistant to one or more anti-TB drugs and 21 (20%) of 101 were multi-drug resistant *M. tuberculosis* (MDR-TB), (Khan, et al. 2001).

Tuberculosis in Saudi Arabia is an endemic disease and its rate of incidence is increasing. Hence it is clear that tuberculosis in Saudi Arabia warrants attention. We think it is the time to create a knowledgeable, informative guiding database having as far as possible information about all the patients. The aim of this study is to conduct survey on tuberculosis in Saudi Arabia, the follow-up medical conditions increasing tuberculosis and to evolve an action plan to get the high quality results having research and guiding value. The outcome of this research may help to reduce the spread of this disease and to come up with recommendations for scientific management of the disease.

2. Material and Methods

Study population:

Bacterial strains used in this project were isolated at the Mycobacterial Diagnostic Laboratory of King Saud Chest Hospital, from patients who presented for tuberculosis diagnosis between months July to September 2011.

Microbiological Analysis:

Acid-fast smear examination and culture of the sputum samples were performed as described. The samples were digested and decontaminated by the N-acetylcysteine-sodium hydroxide method (Nolte, et al. 1999). The collected samples were then inoculated in Loewenstein-Jensen and BACTEC 12B (BACTEC TB460, Becton Dickinson, Cockeysville, MD) broth. The obtained positive cultures were identified and confirmed as *M. tuberculosis* by conventional biochemical tests.

3. Results

The clinical samples from inhabitants of Riyadh comprising both locals and foreigners were obtained in cooperation with King Saud Chest Hospital in Riyadh and the results of the study period between (May to September of 2011) were presented. The sample isolate include (78) strains of bacteria, out of which (70) are differently diagnosed, identified and found to belong the bacteria that cause tuberculosis, and (8) strains is not a nurse. It is also found from this study that there is a relationship between sex and age group with an increased incidence of TB bacteria, where the number of infected males (49) and the number of female patients (19). The age groups were found to be between the ages of (10-20 years old), the number of patients (2) When the aged (21-30 years), the number of patients (26) and at the ages of (31-40 years), the number of patients (26) and when the aged (41-50 years), the number of patients (8), when the aged (51-60 years) was the number of patients (6) When the aged (61-70 years old), the number of patients (1). It is also found in this study that tuberculosis infection is noted even at the young age (12 years). It is also found during this survey that that aged persons are also living with HIV tuberculosis at the age (65 years old) female. The laboratory survey showing the number of Saudis TB patients (22) and the number of infected non-Saudis foreigners (48) is given (Table 1& Table 2). This study also gives a detailed account on all nationalities from which the TB bacteria had been isolated (Table 3). The overall ratio of SA to NSA nationals was 22 (31.4%): 48 (68.5%). The NSA population included 12 nationalities. More than a third of the non-Saudis were Pakistani (18.7%) followed by Indonesia (14.5%), Filipino (12.5%), Ethiopian (10.4%), and India (10.4%). The remaining patients were from

Nepali, Yemenis, Sudanese, Bangla, Somali, and Morocco.

Although there are health awareness in the Kingdom of Saudi Arabia about TB and to reduce the spread of this disease and low proportion of cases of tuberculosis compared twenty years, but there is concern about the resumption of the spread of TB in the past few years. Saudi Arabia has interesting and special population dynamics. There are up to six million expatriates mainly from endemic regions, in South and South East Asia and over two million pilgrims visiting the holy cities located in the western region of the Kingdom each year, with the majority of pilgrims coming from endemic areas which may be one reason for the spread of this disease (Al-Hajj, 2010; Al-Hajj et al 2007). Infection from Mycobacterium tuberculosis results in the death of three million people worldwide per annum of which an estimated one thousand are in Saudi Arabia. The incidence of drug resistance in Mycobacterium tuberculosis (MTB) isolated from our hospital between April 1996 and March 1998 was compared with an earlier study (1993–1995). Thirty (29.7%) of 101 MTB isolates were resistant to one or more anti-TB drugs and 21 (20%) of 101 were multi-drug resistant *M. tuberculosis* (MDR-TB). Resistance was most common to isoniazid (28.7%), followed by streptomycin (22.8%) and rifampicin (20.8%). Resistance to pyrazinamide and ethambutol was 7.9 and 6.9%, respectively. There was a three-fold increase in resistance compared with the earlier study, (Khan, et al. 2001).

Acknowledgements:

This project was supported by King Saud University, deanship of Scientific Research, College of Science Research Center. I would like to express my sincere gratitude and deep gratefulness to all our colleagues of the Department of Botany and Microbiology, King Saud University for their valuable criticism and advice.

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Table 1. A statistical analysis of the results of the study (May to September 2011).

Month of detection (2011)	Isolates of <i>Mycobacterium</i> strains
May - June	16
June - July	10
July - August	24
August - September	28
Gender	Isolates of <i>Mycobacterium</i> strains
Mal	90
Female	34
Age group (year)	Isolates of <i>Mycobacterium</i> strains
10-20	3
21-30	28
31-40	30
41-50	9
51-60	6
61-70	1
Nationality	Isolates of <i>Mycobacterium</i> strains
Saudi	71
Non Saudi	53
Smear microscopy	Isolates of <i>Mycobacterium</i> strains
Positive	70
Negative	8
The youngest age	Isolates of <i>M. tuberculosis</i>
12 years (Mal)	1
The largest age	Isolates of <i>M. tuberculosis</i>
65 years old (Female)	1

Table 2. Isolates of *M. tuberculosis* strains in this study (July – September 2011).

Nationality	Sex (Mal)	Sex (Female)	Age	Diagnosis
Saudi	3	0	24-42	- ve
Saudi	49	19	12-65	+ ve
non-Saudi	2	3	20-37	- ve
non-Saudi	36	12	20-56	+ ve

- ve: Non *M. tuberculosis*, + ve: *M. tuberculosis*

Table 3. Survey of all nationalities infected- TB and gender.

Lab. Serial No.	Nationality	Sex (Mal)	Sex (Female)	Total
1	Saudi	49	19	68
2	Pakistani	9	-	9
3	Indonesia	2	5	7
4	Ethiopian	3	2	5
5	Filipino	4	2	6
6	India	5	-	5
7	Nepali	4	-	4
8	Yemenis	3	1	4
9	Sudanese	3	-	3
10	Bangla	3	-	3
11	Somali	-	1	1
12	Afghan	1	-	1
13	Morocco	1	-	1
Total	13	87	30	117

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7/1/2013