Risk factors for drug-resistant *Mycobacterium tuberculosis* in Saudi Arabia

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ABSTRACT

Objective: To identify rates of primary and secondary drug-resistant *Mycobacterium tuberculosis* and their risk factors from a tertiary-care center in the Kingdom of Saudi Arabia.

Methods: Review of microbiological and clinical data of all patients with positive isolates of *Mycobacterium tuberculosis* between 1995 and 2000 at King Faisal Specialist Hospital and Research Centre, Riyadh, Kingdom of Saudi Arabia.

Results: Susceptibility to antituberculosis agents was tested in 320 isolates from 320 patients. The median age was 50 years. Pulmonary tuberculosis was diagnosed in 106 (33%) patients, extrapulmonary in 183 (57%), and both in 31 (10%) patients. Two hundred forty-six isolates were sensitive to all 5 first line agents. Resistance to at least one of the first line agents was documented in 36 (11.3%) isolates. For the year 2000, resistance rates increased to 17.6%. Monoresistance was noted in 20 isolates (6.3%) and polyresistance in 16 isolates (5.0%) including 9 multidrug-resistant *Mycobacterium tuberculosis* isolates (2.8%). Resistance rates for antituberculosis agents are: Isoniazid, 9.1%; Rifampin, 2.8%; Ethambutol, 1.6%; Streptomycin, 5%; Pyrazinamide, 3.6%. Seventy-eight percent of the resistant isolates are considered primary resistance. History of antituberculosis therapy was the only risk factor associated with drug resistant *Mycobacterium tuberculosis*, odds ratio 19.9 (P< 0.00001). The mean age of patients with resistant isolates was 42 years compared to 49 years in patients with susceptible isolates (P= 0.047).

Conclusion: In a population of mostly Saudi patients, primary and secondary drug-resistant *Mycobacterium tuberculosis* is relatively low but has increased lately. Previous history of antituberculosis chemotherapy and young age are risk factors identified.

Keywords: *Mycobacterium tuberculosis*, epidemiology, drug-resistance, risk factors.

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Resistance of *Mycobacterium tuberculosis (M. tuberculosis)* to antituberculosis agents develops as a gene mutation of the tubercle bacilli.[1] It is amplified by inappropriate drug treatment by selecting for resistant strains.[2] This selection results in acquired or secondary resistance of *M. tuberculosis.* Subsequently, if this strain is transmitted to a new host, it causes a disease by a drug-resistant strain from the outset, primary resistance. The phenomenon of acquiring a primarily drug-resistant *M. tuberculosis* in dramatic outbreaks has drawn international focus on tuberculosis (TB) as a major cause of morbidity and mortality throughout the world.[3-6] Re-emergence of TB is not limited to

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developed countries and may be worse in developing countries.[7] The global resurgence of TB is coupled with increasing number of patients with multidrug-resistant *M. tuberculosis* (MDR-TB).[8] *Mycobacterium tuberculosis* is associated with significantly higher rates of morbidity and mortality and presents a therapeutic as well as infection control challenges.[9] Programs of global survey of drug-resistant *M. tuberculosis* by the World Health Organization (WHO) did not include countries from the Eastern Mediterranean Region.[10] Reports of susceptibility testing of *M. tuberculosis* from the Kingdom of Saudi Arabia (KSA) are limited and few of them addressed risk factors.[11-18] We receive referrals of mostly Saudi patients from all over the country. We present the results of susceptibility testing of *M. tuberculosis* isolated in our hospital and attempt to identify risk factors associated with resistant isolates.

Methods. Clinical data of all patients with positive isolates of *M. tuberculosis* between 1995 and 2000 drug susceptibility were reviewed. The medical record numbers of TB patient seen at King Faisal Specialist Hospital and Research Centre (KFSH & RC) were identified from the microbiology database. Medical records were reviewed for basic demographics such as age, gender, region of residence, documented past history of TB, previous antituberculosis therapy, site of tuberculous involvement, human immuno-deficiency virus (HIV) status, radiological findings of cavitary lung lesions, bacillary load on smears and susceptibility testing results.

Microbiology. Clinical specimens such as sputum, sterile body fluids, pus and tissue received from patients at the KFSH & RC were processed for acid-fast bacilli stains and culture. They were analyzed for the presence of *Mycobacteria* according to the standard procedure of Vestal.[19] To isolate *Mycobacterium* organisms, media such as Lowenstein Jensen (Saudi prepared media laboratories, Riyadh), Bactec 12B vial (Becton, Dickenson, MD, USA) and mycobactosel agar were used. The cultures were incubated at 37 $\pm$ 1^{O}C in 5% CO_2 for 6 weeks. The susceptibility testing was performed according to the criteria provided by Bactec 12B system,[20] and the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia (GA), United States of America (USA). All *M. tuberculosis* isolates were tested against first line antituberculosis drugs (isoniazid, rifampin, streptomycin, ethambutol and pyrazinamide) and based on results or patient tolerance, 2nd line agents were tested on some isolates. The concentrations used are as follows: isoniazid 0.1 µgm/ml, rifampin 2.0 µgm/ml, ethambutol 7.5 µgm/ml, pyrazinamide 100 µgm/ml, and streptomycin 6.0 µgm/ml. For 2nd-line agents the following concentration were used: amikacin 8, 16 µgm/ml, ciprofloxacin 2.0 µgm/ml, cycloserine 50 µgm/ml, ethionamide 5.0 µgm/ml, capreomycin 5.0 µgm/ml, PAS 2.0 µgm/ml.

Definitions. Drug resistance is defined as a decrease in the in-vitro susceptibility of *M. tuberculosis* of sufficient degree to be reasonably certain that the strain concerned is different from a wild strain that has never come into contact with the drug.[10] Monoresistance is resistance to single first line agent, polyresistance is resistance to more than one agent. Multi-drug resistant *M. tuberculosis* in this report is defined as *M. tuberculosis* isolates resistant to both isoniazid and rifampin. Acquired or secondary resistance implies that the isolate was susceptible then developed resistance on treatment. However, this is not available in all patients. For this report acquired or secondary resistance is defined as resistant isolates in patients who had previously received more than one month of TB chemotherapy. Primary resistance is used for resistant isolates from patients who never received antituberculosis therapy.

Data collection and analysis. Epi Info Version 6.04 (Centers for Disease Control and Prevention, Atlanta, GA, USA and WHO, Geneva) was used for data collection. Statistical analysis was performed using Statistica Software package Version 5.0 (StatSoft, Tulsa, Oklahoma (OK), USA). The students t-test was used to calculate continuous variables, and the x^2 or Fisher's exact test was used for proportions. All reported P-values are 2-tailed and a value of ≤ 0.05 was considered significant.

Results. A total of 320 isolates of *M. tuberculosis* were isolated from 320 patients between 1995 and 2000. The mean age of patients was 48 years (median 50, range 1-99), with 162 male and 158 female patients. All patients were nationals of KSA except for 16; 7 Arabs, 6 from Pakistan or India, 2 from the Philippines and one from South Africa. Only one patient from Pakistan had an isoniazid monoresistant isolate. The site of infection was pulmonary in 106 (33%) patients, extrapulmonary in 183 (57%) and both pulmonary and extrapulmonary in 31 (10%) patients. Out of 276 isolates tested against all 5 first line agents, there were 246 (89%) that were fully sensitive to all first line agents. Forty-four isolates were not tested against pyrazinamide due to lack of reagents. Of these isolates, 38 (86%) were fully sensitive to the other 4 first line agents, the remaining 6 isolates were resistant to isoniazid only (3 isolates), streptomycin only (2 isolates), and to both (one isolate). Resistance to at least one of the first line agents was noted in 36 isolates (11.3%). Monoresistance to a first line agent was noted in 20 isolates (6.3%), polyresistance to more than one first line agent was found in 16 isolates (5%) and 9 of them were MDR-TB (2.8%).

Table 1 - First line drug-resistance rates among 320 isolates.

Drugs	Monoresistance N (%)	Polyresistance N (%)	Total (%)
Isoniazid	13 (4.1)	16 (5)	**29 (9.1)**
Rifampin	0 0	9 (2.8)	**9 (2.8)**
Ethambutol	0 0	5 (1.6)	**5 (1.6)**
Streptomycin	4 (1.3)	12 (3.8)	**16 (5)**
Pyrazinamide*	3 (0.9)	7 (2.2)	**10 (3.6)**
Total	**20 (6.3)**	**16 (5)**	**36 (11.3)**

N - number, * - only 276 isolates were tested

Table 2 - Numbers and percentage of resistant isolates to 2nd line agents.

Drugs	Resistant/tested (%)
Ciprofloxacin	1/27 (3.7)
Amikacin	1/23 (4.4)
Cycloserine	13/19 (68.4)
PAS	6/7 (85.7)
Capreomycin	1/19 (5.3)
Ethionamide	10/22 (45.5)

PAS - Para-amino salisalate

There was no polyresistant isolate that was sensitive to isoniazid. **Table 1** summarises the susceptibility results for *M. tuberculosis* isolates. Twenty-seven isolates were tested against one or more of the 2nd line agents for either resistance or intolerance to first line agents. The susceptibility results of 2nd line agents are summarised in **Table 2**. The details of resistance distribution among the 36 isolates resistant to first line agents are outlined in **Table 3.** The average number of isolates annually is 53. During 1995, not all isolates were tested for susceptibility due to shortage of reagents, hence the lower than average tested isolates and higher resistance rates that year. During the year 2000, more cases of TB were diagnosed, more isolates were cultured and resistance rates were significantly higher (11.7% compared to 9.3%, P=0.05) as shown in **Table 4**. Seventy-eight percent of the resistant isolates had primary resistance (28 isolates) and 22% had acquired resistance (8 isolates). Twelve patients gave history of prior TB and antituberculosis therapy, 8 had resistant isolates and 4 had sensitive isolates. This is compared to 28 resistant isolates out of 306 isolates from patients who reported no previous history of TB, odds ratio 19.9 (95% Confidence Interval: 5.0-84.7), Fisher's exact 2-tailed P=0.00001. Rates of resistant isolates based on site involved and origin of the patient are summarised in **Table 5.** The mean age of patients with resistant isolates is 42 years compared to 48.8 years for patients with sensitive isolates to all first line agents (P=0.047). Median age for patients with drug-susceptible isolates was 50 compared to 42.5 for patients with drug-resistant isolates. **Figure 1** depicts the 2 medians, ranges and 25-75th quartiles for the 2 groups. Other factors investigated for association with resistance included, gender, site of tuberculous infection, HIV infection, presence of cavitary lung lesions, and geographic origin. None of these factors was found to be associated with resistance.

Discussion. The decline in reported pulmonary TB in KSA was not related to susceptibility findings.[21] Reporting requirements do not mandate for susceptibility testing or reporting of resistant isolates. So at a community level, the resistance rates are not known. As early as 1985, resistance was alarmingly high at 43.7% to any of the first line agents.[11] Subsequent reports from referral or chest hospitals noted resistance rates between 8.7% and 30% to at least one of the first line agents.[12-18] Many of these reports included foreign-born patients who may have acquired the organism from other countries. Attempting to determine a pattern of increased resistance by time or location could not be made from these reports. The type of resistance whether primary or acquired was not indicated in many of the reports. We treat mostly Saudi patients because of referral requirements. Most of the patients are not referred due to TB but for other medical illnesses. This explains the high proportion of extrapulmonary TB among our patient population. This has been the pattern since early days of the hospital.[22] Even though, the resistance rates among pulmonary and extrapulmonary isolates are not deferent. We attempted to adopt new reporting methods of resistance patterns as in **Table 1.**[23] Results from our patients are higher than the medians for all countries in the WHO survey.

Resistance to isoniazid was 9.1% of isolates followed by streptomycin 5%. This has been the situation in most of the other reports. The rate for MDR-TB remains relatively stable at 2.8% compared to a previously reported period from our facility.[16] Polyresistance was noted in 5% of all isolates. This is very important when considering therapy using less than 4 agents. The finding that deserves consideration is the high rates of primary resistance, 8.8% of all isolates. In fact 3 of every 4 resistant isolates have primary resistance. This reflects a

Table 3 - Details of resistance distribution among 36 isolates resistant to one or more of the first line agents.

Agents to which isolates are resistant	N of isolates
Isoniazid only	12
Streptomycin only	4
Pyrazinamide only	3
Isoniazid, Cycloserine	1
Isoniazid, PZA, PAS, Ethionamide	1
Isoniazid, Streptomycin, Ethionamide	1
Isoniazid, Streptomycin	2
Isoniazid, Streptomycin, PAS, Cycloserine	2
Isoniazid, Streptomycin, PZA, PAS, Cycloserine	1
Isoniazid, RIF, Ethionamide	1
Isoniazid, RIF, Streptomycin, Ethionamide, PAS	1
Isoniazid, RIF, Streptomycin, Ethionamide	1
Isoniazid, RIF, PZA, Ethionamide, Cycloserine	1
Isoniazid, RIF, Ethambutol, Ethionamide	1
All first line, Cycloserine, Ethionamide	2
All first line, Cycloserine, Amikacin, Capreomycin, Ethionamide	1
All first line, Cycloserine, PAS, Ciprofloxacin	1

N - number, RIF - rifampin, PZA - pyrazinamide, PAS - para-amino salisalate

Table 4 - Annual number of isolates and percentage resistant to at least one of the first line agents.

Year	N of isolates tested	(%) isolates resistant
1995	35	11.4
1996	49	8.2
1997	55	9.1
1998	54	9.3
1999	53	9.4
2000*	74	17.6
Total	**320**	**11.3**

*P=0.05 compared to the preceding 5 years, N - number.

Table 5 - Rates of first line agent resistance in various forms of tuberculosis and regional resistance rates.

Type	N of isolates tested	Resistant isolates N (%)
Site involved		
Pulmonary	106	14 (13.2)
Extrapulmonary	183	21 (11.5)
Both	31	1 (3.2)
Regions		
Central	151	13 (8.6)
Western	32	7 (21.9)
South	57	6 (10.5)
East	26	3 (11.5)
North	21	5 (23.8)
Others	33	2 (6.0)
Total	**320**	**36 (11.3)**

N - number

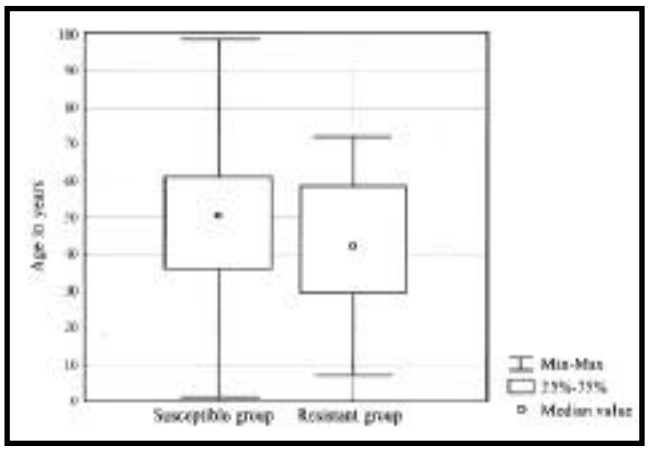

Figure 1 - Box and whisker plots for median age 25th -75th quartiles, and range for patients with drug susceptible isolates and patients with drug-resistant isolates.

relatively high proportion of resistant strains circulating in the community. Second line agents are still active against most isolates especially amikacin, ciprofloxacin and capreomycin. It is not easily explained why resistance rates to para-amino salisalate (PAS) are high even though it is virtually not being used now. The high rate of resistance to cycloserine, 68% has limited its use in our hospital. Again we could not explain the high rate in spite of very limited or non-existent use. We have stopped testing isolates for cycloserine susceptibility after the recent update on susceptibility testing for mycobacteria document M24-T by the National Committee for Clinical Laboratory Standards (NCCLS) was released. In vitro testing is not recommended for cycloserine because of technical reasons.[24]

The trend of resistance rates against antituberculosis agents is an important parameter for control measures and success of treatment programs. Although over a 10-year period, we did not find a pattern of increasing resistance rates among our patient population, last year, 2000, had significantly higher resistance rates compared to the preceding 5 years combined. Undoubtedly, it can be argued that this may be a referral bias related finding, but it occurred after the implementation of the National Tuberculosis Control Program[25] and it is not an isolated phenomenon. This finding was also noted by Khan et al[18] from Jeddah who noted a 3-5-fold increase in resistance rates against isoniazid, rifampin, streptomycin, and any one or more of antituberculosis agents when they compared rates of 1993-1995 period to 1996-1998 period. In Canada, between 1987-1998, more than 8000 isolates were reviewed. Resistance rates have increased particularly against isoniazid.[26] This was also the case in all parts of the world surveyed by WHO except in France and the USA where rates have actually decreased significantly.[23] A finding that should encourage other countries to adopt well supported and structured programs for TB control. Risk factors for drug-resistant *M. tuberculosis* have been evaluated in many studies locally and worldwide.[15,27-30] Our results concur with previous findings of associating drug-resistance and previous therapy with antituberculosis agents. There was almost 20 times the chance of resistance in previously treated patients. A new risk factor that was noted in an earlier cohort of TB patients is young age. In our patients the mean age of patients with resistant isolates was 42 years compared to 49 years in patients with sensitive isolates. The difference was statistically significant. We believe the explanation is that elderly patients have acquired the organisms a long time ago when the circulating bacilli remain sensitive. For younger patients, they have acquired the organism more recently and these are likely to be more resistant. Close follow-up and treatment monitoring can be limited to this high-risk group when resources are scarce. Other risk factors investigated included HIV status, presence of cavitary lung lesions, and bacillary load as noted on smear.

Our findings should alert to the potential increase of drug-resistant *M. tuberculosis* and the need for national surveillance and monitoring of resistance patterns. The identified risk factors may be used to target control and treatment programs and as a parameter of outcome and performance measurements.

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