Original Article

Drug resistance and Susceptibility of Mycobacterium tuberculosis identified at University Kebangsaan Malaysia Medical Centre

S Azura[1], S Hussin[2], Md. Mostafizur Rahman[3]

ABSTRACT

Objective: The prime objective of the study was to evaluate drug resistance and susceptibility of Mycobacterium tuberculosis isolated at University Kebangsaan Malaysia Medical Centre.

Methodology: A total number of 726 specimens in the form of sputum and bronchial lavage obtained from patients suspected to tuberculosis were analysed for confirmatory identification and antibiotic susceptibility testing. The bacteria was identified initially by culture and staining and finally by BDProbeTec™ ET Mycobacteria kits (2008). All Mycobacterium tuberculosis isolates were subjected to antibiotic susceptibility against streptomycin, isoniazid, rifampicin and ethambutol using BACTEC™ MGIT™ 960 system.

Results: Out of 726 specimens 16 (32.65%) were identified as mono drug resistance 7 (14.29%) as poly drug resistance, 4 (8.16%) as multi-drug resistance (MDR-TB). Identified TB bacteria were analysed in the light of sources of samples where brief history of the patients' age, gender and community noted.

Conclusion: Bacteriologic testing with antibiotic sensitivity, standardized treatment with supervision and patient support, provision and management of the drugs used in treatment are necessary for effective treatment of tuberculosis.

KEY WORDS: Mycobacterium tuberculosis, MDR-TB, XDR-TB, Resistance, Susceptibility.

Pak J Med Sci October - December 2011 Vol. 27 No. 5 1107-1111

How to cite this article:

Azura S, Hussin S, Rahman MM. Drug resistance and Susceptibility of Mycobacterium tuberculosis identified at University Kebangsaan Malaysia Medical Centre. Pak J Med Sci 2011;27(5):1107-1111

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* Received for Publication: November 2, 2010
* First Revision Received: November 4, 2010
* Second Revision Received: November 12, 2010
* Final Revision Accepted: August 20, 2011

INTRODUCTION

Tuberculosis is a common and often deadly infectious disease caused by usually Mycobacterium tuberculosis in humans. It has been treated successfully previously using appropriate antibiotic therapy. Recently anti-tuberculosis drug resistance has become a major public health problem that threatens the success of WHO-recommended treatment approach for detection and cure of TB, as well as global tuberculosis control. In the new WHO's Multidrug and Extensively Drug-Resistant Tuberculosis: 2010 Global Report on Surveillance and Response, it is estimated that 440,000 people had MDR-TB worldwide in 2008 and that a third of them died. In sheer numbers, Asia bears the brunt of the epidemic.

Almost 50% of MDR-TB cases worldwide are estimated to occur in China and India. In Africa,

estimates showed 69,000 cases emerged, the vast majority of which went undiagnosed. Multidrug-resistant TB (MDR TB) that are resistant to at least two of the best anti-TB drugs, isoniazid and rifampicin. These drugs are considered first-line drugs and are used to treat all persons with TB disease. MDR-TB was not a serious problem before in Malaysia.[1] However, currently antimicrobial susceptibility testing for Mycobacterium tuberculosis has become an important aspect since the emergence of multi drug-resistant strain is increasing worldwide. Extensively drug resistant TB (XDR TB) is a relatively rare type of MDR TB. It has also been causing problem in controlling TB globally.[2]

Therefore, the present study was aimed to identify Mycobacterium tuberculosis and to determine the multiple drug resistant and susceptibility at UKMMC, Malaysia which would provide guidance to clinicians in treating TB patients effectively.

METHODOLOGY

Study population and selection of subjects: This study was carried out in the laboratory of Medical Microbiology and Immunology, UKMMC from January 2008 to January 2009. A total number of 726 specimens of sputum and bronchial lavage collected from suspected TB patients were sent to the laboratory for processing.

Identification of bacteria: Initially all the specimens obtained from the hospital stained with Kinyon (acid-fast staining). After that these were inoculated in to Lowenstein-Jensen medium. The inoculated media was incubated at 37°C for 8 weeks. The positive cultured were confirmed by BDProbeTecTM ET Mycobacteria kits.[3]

Antibiotic susceptibility study: Mycobacterium tuberculosis organisms identified were subjected to antibiotic susceptibility testing as per procedure described by the authors.[4] All the identified isolates were tested for susceptibility against streptomycin, isoniazid, rifampicin and ethambutol.

Statistical Analysis: The patients' information data were analysed in the light of positive samples using SPSS program version 12.0.1.

RESULTS

A total number of 726 specimens obtained in the form of sputum and bronchial lavaged were analysed for the identification and antibiotic susceptibility testing that received during January 2008 to January 2009. It was observed that out of specimens 53(7.3%) were identified as Mycobacterium. Identification by BDProbeTec™ ET Mycobacteria, kits showed 50 (94.3%) of the 53 as Mycobacterium tuberculosis and 3 (5.7%) recognized as *Mycobacterium* other than tuberculosis.

Community based analysis of the positive Mycobacterium tuberculosis showed that 25 Malays (52%), 16 Chinese (29%), 6 Indians (13%) and 3 others (6%) (Fig.1). Analysis in terms of gender revealed that in the Malay community 12 were males and 13 were females while Chinese community seven were males and other nine were females. Only one male was identified as positive for Mycobacterium tuberculosis in Indian community. In the other community (shiks, tribes, foreigners) five identified tuberculosis patients were female.

The ranges of ages of the patients were from 2 to 78 year-old with mean age was 47.61 year-old (Fig.2).

Twenty-seven of the 50 Mycobacterium tuberculosis isolates showed resistance to anti-tuberculosis drugs. 32% of the resistant *Mycobacterium tuberculosis* were mono resistant, 8% multiple drug resistant and 14% poly resistant (Table-I). Out of the 16 mono resistant isolates, nine were resistant to isoniazid and seven were that of ethambutol. No isolate was resistant to neither rifampicin nor streptomycin.

Distribution of sensitivity pattern by age showed that only one positive isolate was from a patient

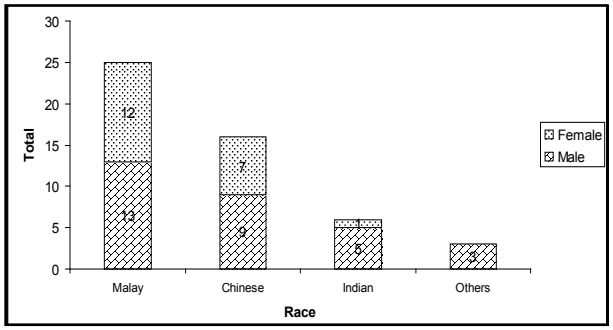

Fig.1: Distribution of Mycobacterium tuberculosis positive cases by community and gender.

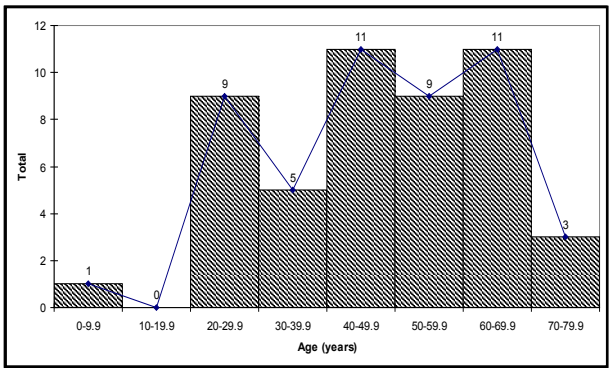

Fig.2: Distribution of Mycobacterium tuberculosis positive cases by age group.

Table-I: Mono, multi and poly drug resistance Mycobacterium tuberculosis during 2008.

Total resistant isolates	*%*	*Mono resistant*	*%*	*MDR*	*%*	*Poly resistant*	*%*
27	54	16	32	4	8	7	14

younger than 20-years-old and it showed mono resistant. For patients between the ages 21 to 40 years old, 14 samples were culture positive, in which 9 isolates were sensitive to all anti-tuberculosis drugs, 2 showed mono-resistance and 3 poly-resistance. The largest numbers of isolates were from patients between the ages of 41 to 60 years old, in which there were 20 culture positive samples. Eight of these isolates were sensitive to all drugs, 7 were mono resistant, 1 poly resistant and 3 MDR. Unfortunately, one of the culture in this age group was contaminated and no result of susceptibility testing was available. This isolate was removed from the analysis. Fourteen positive cultures were from patients 61 to 80-years-old, in whom 4 samples were sensitive, 6 samples were mono resistant, 3 samples were poly resistant and 1 sample was MDR.

Four (8%) cases of MDR-TB were identified, 2 males and 2 females, age ranging from 41 to 77 year-old. The isolates were from Chinese and Malay patients. All of them were new case and HIV status also negative. Three from 4 of MDR-TB cases were sensitive to streptomycin except isolate number 4 which showed resistant to all 4 drugs (Table-II).

DISCUSSION

Analysis based on different community living in Malaysia revealed that Mycobacterium tuberculosis was in 25 Malays (52%), 16 Chinese (29%), 6 Indians (13%) and 3 others (6%). Infection analysis in terms of gender showed that in the Malay community 12 were males and 13 were females while in Chinese community seven were males and other nine were females. Only one male was identified as positive for Mycobacterium tuberculosis in Indian community. In the other community (shiks, tribes, foreigners) five identified tuberculosis patients were female.

As regard racial susceptibility of TB it is generally believed that certain racial groups are highly susceptible to tuberculosis while others have developed natural resistance; epidemiological studies among different racial groups provide inconclusive evidence relating this. Many factors influence the development of active tuberculosis including sex, age, body weight and virulence of the infecting organisms.[5]

Age ranges of the patients under the study were from 2 to 78 year-old with mean age 47.61 year (Fig.2). Age and vulnerability to TB infection reported in a recent study of the authors[6] showed that 28.0% of TB from the suspected cases was positive in the 5-10 year age stratum and 88.0% in the 31-35 year age stratum. This could reflect the year in which effective anti-tuberculosis drugs such as rifampicin was introduced in a particular country. From our study, all MDR-TB cases were aged between 41 to 78 years. No clear association of the present study was observed between MDR-TB and sex. Unemployment, alcohol abuse, low education, and low socioeconomic status were not found to be associated with MDR-TB.

In our study Twenty-seven of the 50 Mycobacterium tuberculosis isolates showed resistance to anti-tuberculosis drugs. 32% of the resistant *Mycobacterium tuberculosis* were mono resistant, 8% multiple drug resistant and 14% poly resistant (Table-I).Out of the 16 mono resistant isolates, nine were resistant to isoniazid and seven were that of ethambutol. No isolate was resistant to neither rifampicin nor streptomycin.

TB and drug resistance is a growing concern worldwide now a days. There is no published data available on the prevalence of drug resistance and susceptibility of Mycobacterium tuberculosis from Malaysia despite the large number of TB cases available here. Drug resistant TB cases have been

Table-II: Multiple Drug Resistant Mycobacterium tuberculosis in respect to age, gender and race.

	Age	*Gender*	*Race*	*Case Status*	*HIV Status*	*Drug Susceptibility Testing*			
						Isoniazide	*Rifampicin*	*Ethambutol*	*Streptomycin*
1	41	Male	Chinese	New case	Negative	Resistant	Resistant	Resistant	Sensitive
2	54	Male	Malay	New case	Negative	Resistant	Resistant	Resistant	Sensitive
3	78	Female	Chinese	New case	Negative	Resistant	Resistant	Resistant	Sensitive
4	46	Female	Malay	New case	Negative	Resistant	Resistant	Resistant	Resistant

reported frequently, including MDR-TB and XDR-TB. When such cases occur, second line drugs were prescribed randomly, without following specific guidelines. This is due to the fact that drug susceptibility testing (DST) towards second line drugs is not available in most countries due to the costly procedures.[7]

WHO[8] estimated 4.8% incidence of MDR among all TB cases globally in the year 2006 of which China and India carrying 50% of the burden. From the Western Pacific region, the MDR-TB burden is 3.9% among new cases and 21.6% among previously treated cases with 85% of incidence arising from China. A study from India, the country with the highest resistance rate globally showed that MDR-TB among new cases is 2.4% and 17.2% among previously treated cases.[9]

Such figure was classified by the WHO as low to moderate MDR-TB burden. Incidence from Malaysia, which was grouped under WHO Western Pacific region, is not available. However, this study showed that there were 4 (8.16%) MDR-TB among new cases and none among previously treated cases. MDR-TB cases may arise by direct transmission of an MDR strain from a patient to another, but also by inadequate treatment of a patient who was initially infected by a fully sensitive strain, or one with only single drug resistance.

HIV and TB have been widely associated. With 33 million cases of HIV globally reported by the WHO, numbers of TB cases have also been rising. The association could be largely accounted by nosocomial outbreaks. In our study however, we did not see any clear association between HIV patients and drug resistance TB because our population of patients do not comprise of a significant number of HIV positive patients as UKMMC is not the national referral centre for HIV positive patients.

Infectivity of MDR strain is higher than was initially thought. Unsuccessful treatment therefore plays a double role with respect to MDR-TB. A review of risk factors for MDR-TB by the authors,[10] they showed that inadequate previous treatment is the strongest risk factor for MDR-TB in European countries. The authors determined the prevalence of MDR-TB up to 10 times higher after unsuccessful treatment Therefore; unsuccessful previous treatment has been widely recognized as inducing MDR-TB. The reasons of inadequate treatment has been identified as delayed diagnosis, delayed recognition of drug resistance, inappropriate chemotherapy regimens, inadequate or irregular drug supply, and poor compliance by both patients and clinicians.[10]

Weekly Report[11] surveyed international network Of TB laboratories on Mycobacterium tuberculosis with extensive resistance to second-line drugs-worldwide, 2000-2004. This report summarized the results of that survey, which determined that, during 2000-2004, of 17,690 TB isolates, 20% were MDR and 2% were XDR. In addition, population-based data on drug susceptibility of TB isolates were obtained from the United States (for 1993-2004), Latvia (for 2000-2002), and South Korea (for 2004), where 4%, 19%, and 15% of MDR TB cases, respectively. The report mentioned that XDR TB emerged worldwide as a threat to public health and TB control, raising concerns of a future epidemic of virtually untreatable TB.

Drug resistance TB aside from previous treatment, immigration has been suggested as a factor leading to an increase of prevalence of MDR-TB.[11] The authors observed that MDR-TB patients were more likely to be foreign born out of Europe, but the relative risk was not as strong as being previously treated. This association between being foreign born and MDR could be due to higher risk of transmission of MDR strains for immigrants, but it may also confounded by previous treatment among immigrants. In the country, it may be due to a surplus of foreign immigrant workers without proper health inspection or adequate immunization.

Since this study is among the first of its kind performed in Malaysia, there are limited data and references available. Therefore, we are unable to compare our results with data on our country. As it was a preliminary study large number of samples could not be included. Thus, for the better understanding of the magnitude of MDR-TB in our country, an extensive study in this regard may be carried out in future with better funding. TB has a great relationship with HIV due to crackdown of immune system of HIV patients become more venerable of getting infected with TB. In our study due to limited number of samples the relationship of HIV and TB could not be established. A further understanding of the sensitivity pattern of these isolates would give us a better view of the magnitude of XDR-TB.

As resistance cases are rising there is need for second line of drugs to be standardized. Many high TB-burden countries do not have access to full range of second line drugs because of financial, regulatory, or other constraints. Cross resistance between drugs in the same group further limits selection of available drugs. The use of appropriate second line

drugs is important not only in treating resistance cases, but to prevent emergence of further resistance. As such, DST against second line drugs in high risk patients such as HIV positive patients is highly recommended. XDR-TB is more expensive and difficult to treat than MDR-TB, and outcomes for patients are much worse. Understanding the magnitude and distribution of XDR-TB is therefore important.[12]

One study[13] recently highlighted the comprehensive remedy programme which correlates the aims of our present study for the MDR-TB. The author mentioned that WHO-recommended Stop TB Strategy provides the framework for treatment, care, and control of drug-susceptible and drug-resistant disease. The directly observed treatment (DOTS) approach, which underpins the Stop TB Strategy, calls for political commitment to control disease by means of early diagnosis with the use of bacteriologic testing, standardized treatment with supervision and patient support, and provision and management of the drugs used in treatment, as well as monitoring of treatment and evaluation of its effectiveness.

ACKNOWLEDGEMENT

Authors acknowledge the help of undergraduate medical students KH Teh, S Firdaus, MS Nora, I Ikhwan and R Hidayah. They also acknowledge the University Kebangsaan Malaysia Medical Centre authority for providing financial support in the form of Fundamental Research Fund (FF-054-2009).

REFERENCES

1. Iyawoo K. Tuberculosis in Malaysia: Problems and prospect of treatment and control. Tuberculosis. 2004;84:4-7.
2. CDC. Emergence of Mycobacterium tuberculosis with extensive resistance to second-line drugs--worldwide, 2000-2004. 2006;55(11):301-305.
3. Gianna M, Laura R, Paola P, Claudio S, Carlo G, Enrico T, Evaluation of the B D Probe Tec ET System for Direct Detection of Mycobacterium tuberculosis in Pulmonary and Extrapulmonary Samples: A Multicenter Study. J Clin Microbiol. 2003;41(4):1779–1782.
4. Winn Jr WC, Allen S, Janda W. Koneman's Colour Atlas and Textbook of Diagnostic Microbiology (Sixth edition). Philadelphia: Lippincott Williams and Wilkins, 2006: 1065-1124.
5. McMillen CW. The red man and the white plague": Rethinking race, tuberculosis, and American Indians. Bull Hist Med Fall. 2008;82(3):608-645.
6. Wood R, Liang H, Wu H, Middelkoop K, Oni T, Rangaka MX, et al. Changing prevalence of tuberculosis infection with increasing age in high-burden townships in South Africa. Int J Tuberc Lung Dis. 2010;14(4):406-412.
7. WHO. 2008a Policy guidance on drug-susceptibility testing (DST) of second-line anti-tuberculosis drugs. (http://www.who.int/tb/publications/2008/who_htm_tb_2008_392.pdf). Accessed on October 20, 2010.
8. WHO. Global tuberculosis Control: Epidemiology, strategy and financing. 2009. http://whqlibdoc.who.int/publications/2009/9789241563802_eng.pdf. Accessed on October 15, 2010.
9. WHO. 2008b. Tuberculosis Control in Western Pacific Region. http://www.wpro.who.int/publications/PUB_9789290613855.htm. Accessed on October 20, 2010.
10. Faustini A, Hall AJ, Perucci CA. Risk factors for multidrug resistant tuberculosis in Europe: A systematic review. Thorax. 2005;61:158-163.
11. WHO. Tuberculosis Control in Western Pacific Region. 2007.http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2636653/Accessed on October 21, 2010.
12. WHO.2008c. Anti Tuberculosis Drug Resistance in the world (Fourth global report). http://www.who.int/tb/publications/2008/drs_report4_26feb08.pdf.Accessed on October 21, 2010.
13. Patrick Wilver. Multidrug resistance tuberculosis. 2010. http://blogs.nejm.org/now/index.php/multidrug-resistant-tuberculosis. Accessed on October 9, 2010.

Authors Contribution:

S Azura: Conducted research with the help of students.

S Hussin: Worked as supervisor of the student and formulated the research program and directly helped in every step of research.

MM Rahman: Helped the research in planning, data compilation, results presentation, editing and over all writing of the paper.

Ethics Committee Approval: The committee approved it as Fundamental Research Fund (FF-054-2009).