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Mycobacterium Group: Identification and Sensitivities of Clinical Isolates Against First Line Drugs. I

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Abstract: Mycobacterium infections play a very important role in the community especially in areas where the disease is endemic. Mycobacterial infections need to be treated with a mixture of agents to improve efficacy, to prevent resistant, or to overcome intrinsic resistant. In order to see the resistant pattern of the local isolates a prospective study was planned. About, 100 clinical isolates of mycobacterium were collected from local clinical laboratories they were confirmed by standard tests recommended by ASM manual and sensitivities were set up by resistant ratio method against the first line drugs, i.e., Streptomycin, Isoniazid, Ethambutanol Rifampicin. and Bac 201 was also found to have antimycobacterial activity. Over all 38% were *Mycobacterium tuberculosis*, 22% *Mycobacterium xenopi*, 16% *Mycobacterium kansasai* and 12% *Mycobacterium thermoresistible* 4% *Mycobacterium merinum*, 4% *Mycobacterium fortutum* and 4% *Mycobacterium terraecomplex* 70% were found to be slow grower, while 28% were fast grower and 2% were found to be moderate grower 22% were Photochromogen and 12% were Scotochromogen, 64% were non chromogenic. About 62% were atypical mycobacteria. Resistance pattern in this study was not very different from other studies. In this study highest resistance was to Rifampicin 30% followed by Isoniazid and Strptomycin 8%. Ethambutanol and Pyrazinamide were found to have relatively lower resistance 6 and 4% respectively. A protein antibiotic i.e., Staphylococcin Bac 201 was also tried which gave some interesting results, its known conc. i.e., 1218 AU mL^{-1}. Sixty two percent were completely resistant to all 6 concs and 38% showed MIC as 1218 AU mL^{-1}, 25-64% resistant to first line drug has been observed. With the emergence of drug resistant mycobacteria it has become essential to carry out the drug susceptibility testing regularly.

Key words: *Mycobacterium* sp., first line drugs sensitivity, staphylococcin

INTRODUCTION

Recent estimates from the World Health Organization (WHO) indicate that there are approximately 8 million new cases of tuberculosis each year, the complications of which results in 2 million deaths annually[1]. The WHO further estimates that, between 2002 and 2020, on the order of 10^9 individuals will be newly infected with *M. tuberculosis* if present control efforts are not improved. The remarkable success of *M. tuberculosis* as a human pathogen is attributable at least in part to the capacity of this organism to persist for months or years within the host even in the setting of a robust host immune response. It is believed that the majority of persons infected with *M. tuberculosis* harbor a latent infection. In approximately 2 to 23% of these latently infected, but otherwise healthy individuals, the infection will reactivate and cause clinically apparent disease within their lifetime[2]. Given that one third of the world's population is thought to harbor latent infection with *M. tuberculosis* and therefore provide a large reservoir for potential disease reactivation[3]. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence and mortality by country[4]. Rapid diagnosis of tuberculosis is important in order to prevent diseases different methods i.e., PCR[5]. (Polymerase chain reaction)[7] and fast plaque are available[6] but still conventional methods have their own values[11]. Most alarming aspect of this is that Tuberculosis is reemerging in developed countries[9] in association of human immunodeficiency virus infection[10]. Identification of mycobacteria in the clinical laboratory

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still remains a difficult and time consuming procedure, the Morphological, Biochemical and cultural tests used for identification require specialized knowledge and well trained laboratory technician[11].

MATERIALS AND METHODS

This was a prospective study in which identification and confirmation of isolates were performed, as acid-fast Mycobacterium and their sensitivity against first line drugs and some protein antibiotics were determined by resistant ratio method. About one hundred isolates were collected (from different pathological labs of Karachi). Identified as acid fast by Ziehl-Nelson Carbol fuschin (Z.N.C.F method)[12], the smears were reported according to CDC (centre for disease control and prevention) method. They were Sub cultured on different media like L.J (Lowenstein Jensen) Medium, MacConkeys without Crystal violet, Blood agar, Urea broth. From Difco Laboratories and Oxoid. All isolates were divided into two groups. First group comprised of *M. tuberculosis* or others while in the second group atypical mycobacteria were included[13].

In identification protocol 1st line of tests comprised of:
Type of colony, Growth rate, Pigment production, Catalase test (heat stable) and Nitrate Reduction test

If according to 1st line tests → Not a *M. tubrrculosis* then → 2nd Line Tests (for other strains) which comprised of[14] Morphology on L.J media, Pigment production, Rate of growth, Growth at different temp, Niacin test, Catalase test, Nitrate reduction test, Tween 80 hydrolysis, Urease test and Pyrazinamidase.

For antibiotic sensitivity medium used was LJ (Lowenstein Jensen). Different first line antibiotics i.e., Streptomycin, Isoniazid, Ethambutanole and Rifampicin were incorporated in to LJ medium for this purpose[14]. Bac201 a bacteriocin was also used.

LJ slopes were prepared from commercially available dehydrated media of Difco Company by the prescribed method. For susceptibility test to perform, drugs were added to the slopes in batches in different concentrations, first concentration of Isoniazid used was 0.4 mg L^{-1}, Rifampicin 25 mg L^{-1}, Streptomycin 40 mg L^{-1}, Ethambutanole 10 mg L^{-1} while Bac201 concentration was in AU (arbitrary unit) which was 1218 AU mL^{-1}. Two fold dilutions of each drug was made about 6 dilutions were used for each drug. After mixing the drugs, it was distributed 5 mL in each screw-capped container, which were labeled for the concentration of drugs[15]. These bottles were then kept in a proper sloping position in the inspissator at 80°C for 1 to 3 h till all eggs were

coagulated. These were then stored at 2°C to 8°C after cooling. These drug containing medias were not stored for more than 3 weeks[16]. For control strains the locally isolated sensitive strains were included in each batch of sensitivity tests.

RESULTS AND DISCUSSION

This was a prospective study over a period of two years. Hundred samples were processed. Hundred percent were smear positive and culture positive.

Total period from positive smear to sensitivity reporting was an average 6-12 weeks. Out of 100 positive cultures 62 were Atypical Mycobacteria i.e., 62 %. Thus in other wise in our population atypical is common. The identified strains were *M. kansasii, M. xenopi, M. thermoresistible, M. merinum, M. fortutum and M. terraecomplex* (Table 2) all of these are atypical mycobacteria with reported cases in literature[17].

Species identification: Over all 38% were *Mycobacterium tuberculosis,* 22% *Mycobacterium xenopi* 16% *Mycobacterium kansasai* 12% *Mycobacterium thermoresistible,* 4% *Mycobacterium merinum,* 4% *Mycobacterium fortutum* and 4% *Mycobacterium terraecomplex,* 70% were found to be slow grower, while 28% were fast grower and 2% were found to be moderate grower 22% were Photochromogen and 12% were Scotochromogen, 64% were non were non chromogenic[18] (Table 1).

Sensitivity: Resistance pattern in our study was not very different from other studies. In this study highest resistance was to Rifampicin 30% followed by Isoniazid and Strptomycin 8%. Ethambutol and Pyrazinamide were found to have relatively lower resistance 6 and 4% respectively[19]. In sensitivity reporting 3 categories had been used, sensitive, intermediate and resistant, but in calculations intermediate is also taken as resistant. Protein antibiotic has also been tried i.e., Staphylococcin some interesting results has been obtained, its known concentration i.e., 1218 AU mL^{-1}(Table 2), this was the first study in which the *in vitro* activity of this protein/peptide antibiotic against clinical isolates of *Mycobacterium tuberculosis* has been investigated. Bac201 appeared to be effective against the clinical isolates of *M. tuberculosis.* Out of 100 clinical isolates, 38 (38%) were found sensitive. Evidently, the antimycobacterial properties of staphylococcin Bac201 allow us to further study its probable application for the prevention and treatment of tuberculosis.

Table 1: Identification characteristics up to species level

	1 Growth rate			2 Pigment production			3 Temperature		
	Slow	Moderate	fast	Ph	Sc	Non	42°C	37°C	22-25°C
Total No. of isolates	84 100	04	12	22 100	12	64	34 100	60	6

4 Catalase		5 Growth on MacConkeys		6 Urease		7 Tween -80 Hydrolysis		8 NA	
+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
HS SQ									
32 46 100	54	6 100	94	74 100	26	72 100	28	60 100	40

HS: Heat Stable Catalase, Ph: Photochromogenic, Sc: Scotochromogenic, +ve: Growth, -ve: No Growth, NA: Niacien Accumulation, Non: Non Chrmogenic, SQ: Semiquantitative Catalase

Table 2: Sensitivities of 100 *Mycobacterium* sp. local clinical isolates against first line drugs

Antibiotics	Rifampicin 40 mg L^{-1}		Streptomycin 25 mg L^{-1}		Isoniazid 0.4 mg L^{-1}		Ethambutanole 10 mg L^{-1}		Bac 201,1218 AU mL^{-1}	
	S	R	S	R	S	R	S	R	S	R
M.tuberculosis (n = 38)	8	30	30	8	30	8	32	6	32	4
M.xenopi (n = 22)	20	2	21	1	21	1	11	11	2	20
M.kansasiai (n = 16)	15	1	16	0	12	4	14	2	2	14
M.thermoresistible (n = 12)	12	0	11	1	12	0	12	0	1	12
M.merinum (n = 4)	3	1	4	0	4	0	4	0	0	4
M.fortutum (n = 4)	0	4	0	4	0	4	0	4	0	4
M.terraecomplex (n = 4)	4	0	4	0	3	1	2	2	0	4

S = Sensitivity, R = Resistant

21-78% resistant to first line drug has been observed. With the emergence of drug resistant mycobacteria it has become essential to carry out the drug susceptibility testing regularly. Bacteriocins also have antimycobacterial activities.

All methods for detecting drug sensitivity have merits and demerits. Mycobacteria are slow growing organisms which have ability to develop inducible resistant during the course of therapy hence one of the method relying on growth on conventional media seems to be most appropriate at least at present.

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