ISOLATION, PCR BASED IDENTIFICATION, AND SENSITIVITY PATTERN OF ENVIRONMENTAL MYCOBACTERIA FROM LEPROSY AND TUBERCULOSIS PATIENTS.

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ABSTRACT

We have isolated and identified the biotype of environmental mycobacteria from the expectorate of leprosy patients, their contacts, their drinking water supply and also from the sputa samples of tuberculosis patients. 78% of the isolates from lepromatous leprosy patients and their contacts were *Mycobacterium fortuitum-chelonae* complex (MFC), 9% *Mycobacterium avium* complex (MAC), 9% *Mycobacterium scrofulaceum* and 4% were *Mycobacterium smegmatis*. Among the isolates from tuberculosis patients 63% belonged to *M. fortuitum-chelonae* complex, 19% to *M. avium* complex, 12% to *Mycobacterium Kansasii* and 6% to *M. smegmatis*. All the isolates were multi-drug resistant when tested for sensitivity total of 21 drugs. The *Mycobacterium fortuitum-chelonae* complex organisms from leprosy contacts were more sensitive to rifampicin than those isolated from lepromatous leprosy and tuberculosis patients. Among 23 isolates from leprosy patients one isolate was resistant to 20 drugs, one isolate to 17 drugs and another isolate was resistant to 13 drugs. Among the 18 isolates from drinking water supply six showed resistance to more than 12 drugs. Polymerase Chain Reaction (PCR) and subsequent hybridisation with specific probes confirmed all the isolated strains as non-tuberculous mycobacteria (Using genus primers and probe sensitivity 100%) and none as *M. tuberculosis*, suggesting that PCR could be used to rapidly identify mycobacteria at the genus level and to rule out tuberculosis in leprosy patients at an early stage to decide on appropriate course of therapy.

KEY WORDS: Environmental mycobacteria, leprosy, tuberculosis, drug resistance.

INTRODUCTION

Several saprophytic and environmental mycobacteria have been found to be associated with human diseases either as potential pathogens or

as causative organisms (1). Included in this list are *Mycobacterium fortuitum-chelonae* complex, *M. scrofulaceum*, *M. kansasii*, *M. xenopi* which were once believed to be only environmental. They appear not to be present in the commensal flora of most individuals. Exposure of an individual to mycobacteria is variable and it is greatly influenced by the environment (for example soil and water) and the life style of the individual. The relevance of environmental mycobacteria in human health and diseases lies in the fact that clinically it is difficult to differentiate any one of them as specific etiological agent of pulmonary disease (1-2) and many of these mycobacteria have a positive immunomodulatory role in man (3).

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It has been shown at least in experimental animals that infections with some non-tuberculous mycobacteria can offer same level of protection as BCG against tuberculosis (4-6). Innate resistance to antibiotics has been reported in several of these environmental mycobacteria. Reports from many parts of the world have emphasised that the frequency of mycobacteria other than tuberculosis is increasing in both actual numbers and in the proportion of total mycobacterial disease (7-15). Therefore, the environmental mycobacteria could significantly influence the control strategies of mycobacterial diseases like leprosy and tuberculosis particularly in the context of the global re-emergence of tuberculosis, emergence of multidrug resistance in leprosy and tuberculosis and our lack of knowledge of the exact mechanism of pathogenesis and immune insufficiency in these two dreaded scourges of human health. The etiological role, if any, played by these environmental mycobacteria in the disease process as opportunistic pathogens assume great significance for devising effective control. The various anti-mycobacterial drugs used in this study are 4,4' diamino diphenyl sulphone, strategies. Considering the global re-emergence of tuberculosis, emergence of multi drug resistance in leprosy and tuberculosis, a detailed study of environmental mycobacteria acquires importance. The etiological role, if any, played by these environmental mycobacteria in the disease process as opportunistic pathogen assumes great significance for devising effective control strategies.

In this context a detailed study of environmental mycobacteria, particularly from the inhabited area and drinking water supply, acquires importance. The present study aims at the isolation, polymerase chain reaction (PCR) based identification and characterisation of the biotype of environmental mycobacteria from expectorate of the leprosy patients, their contacts, drinking water supply and from the sputa of pulmonary tuberculosis patients. We have also studied the sensitivity pattern of the isolates to conventional and non-conventional antimycobacterial drugs.

MATERIALS AND METHODS

Expectorates of leprosy patients were collected from the in-patients of the hospital located in Chinglepet, which is an endemic area for leprosy. Sixty-six sputum samples were collected of which 26 were from lepromatous leprosy (LL) patients and 40 from contacts of LL patients who showed no clinical and radiological evidence of pulmonary tuberculosis. 35 water samples collected from the patients'

water supply were also examined for the presence of environmental mycobacteria. 34 sputa samples were collected from patients diagnosed clinically to have tuberculosis. The method of Vestal (16) was followed for the isolation, identification and determination of drug sensitivity of the isolated strains. In the study we included as controls seven non-tuberculous mycobacteria maintained in the laboratory. The percentage resistance of an isolate to a particular drug was calculated as follows:

$$\frac{\text{Colonies on drug plate}}{\text{Colonies on control plate}} \times 100 = \% \text{resistance}$$

clofazimine, rifampicin, isonicotinic acid hydrazide, pyrazinamide, ethambutal and streptomycin. We have also tested other non-conventional drugs as anti-mycobacterials, such as gentamycin, tetracycline, kanamycin, chloramphenicol, ethionamide, methogyl, furoxone, nalidixic acid, para amino salicylate AS, ciprofloxacin, trimethoprim, doxycycline, amoxycilin and perfloxin (Table 1).

Table 1. List of drugs used in this study

No.	Abbreviations	Name of the drug
1.	D	Dapsone,
2.	CL	Clofazimine
3.	R	Rifampicin
4.	I	Isonicotinic acid hydrazide
5.	P	Pyrazinamide
6.	E	Ethambutol
7.	S	Streptomycin
8.	G	Gentamycin
9.	T	Tetracyclin
10.	K	Kanamycin
11.	CLR	Chloramphenicol
12.	ET	Ethionamide
13.	M	Metronidazole
14.	F	Furoxone
15.	N	Nalidixic acid
16.	PA	Paraaminosalicylate
17.	C	Ciprofloxin
18.	TR	Trimethoprim
19.	DO	Doxycyclin
20.	A	Amoxycilin
21.	TCT	Thiacetazone

Sample processing for PCR

The samples were processed for PCR as described earlier (17). The samples were washed twice with the TEX (Tris.Cl buffer 10 mM, pH 8.0; EDTA 5mM; Triton-X 100) buffer and the washed pellets were boiled for 30 min in a final volume of 100 µl of TEX to release the DNA for PCR.

PCR amplification

The processed samples (3-4 µl) were amplified in 50 µl reaction volume containing 1U Taq DNA polymerase (Perkin-Elmer Cetus), 70 mM Tris pH 8.8, 20 mM (NH₄)₂SO₄, 2.5 mM MgCl₂, 1mM DTT, 100 µg ml⁻¹ BSA, 0.1% Triton X-100 with 0.2 mM deoxynucleotide and 50 pmoles of the modified C & D primers (11) to amplify the *M.tuberculosis* specific 383 bp product. The reaction mixture was denatured at 95° C for 5 min and subsequently amplified at 50° C for 1 min, 72° C for 2min and 94° C for 1 min through 30 cycles. Amplification of the *Mycobacterium* genus specific product was performed under identical conditions using the genus primers 17/ & 17B as modified by Sritharan et al. (18).

Analysis of PCR products

Products from PCR amplification were analysed by agarose gel electrophoresis, "Southern Blot" and "Dc Blot" hybridisation. For Southern analysis 15µl of the PCR product were resolved on 1.2% agarose gel and transferred onto hybridisation membrane (Magna NT™ Micron Separations, Inc., MSI, Westboro, MA, USA) and for dot blot analysis 15 µl of the PCR product were denatured with equal volume of 0.5 N NaOH, spotted onto the hybridisation membrane using a vacuum filtration minifold.

Preparation of hybridisation probes

Cloned or amplified *M.tuberculosis* specific 383 bp (TB 400) DNA fragment (17) and *Mycobacterium* genus specific 310 bp (18) DNA fragment were used as probes. The probes were labelled with dig-11- dUTP by PCR under conditions as described above except that dTTP was replaced by a mixture of digoxigenin 11- dUTP and dTTP (molar ratio 0.35 dUTP : 0.65 dTTP). Hybridisation was performed as described by Boehringer-Mannheim (Genius™ kit, Boehringer Mannheim, Indianapolis, IN). The colourimetric substrate of Boehringer-Mannheim was substituted by the chemiluminescent substrate AMPPD, 3-

(2'-spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy)-phenyl-1,2-dioxentane-(Tropix Inc, Bedford, MA) for the detection by exposing filters to X-ray film (Hyperfilm™, Amersham) for 40 min at room temperature.

RESULTS

Environmental mycobacteria were isolated from the expectorate from 9 out of 26 LL patients, 14 out of 40 contacts of leprosy patients and 18 out of 35 water samples. Several isolates belonged to *M.fortuitum* - *chelonae* complex. All the drinking water supply isolates belonged to *M.fortuitum*- *chelonae* (MFC) complex. All the isolates of mycobacteria from the leprosy patients and their biochemical characterisation showed 67% of isolates from LL patients, 79% of isolates from leprosy contacts and 100% of isolates from contacts belonged to MFC complex. The isolates were subsequently tested by genus specific primers and probe (Fig 1).

Fig. 1. Analysis of genus PCR product. Agarose gel electrophoresis (upper rows) and Southern hybridisation (bottom rows). The PCR product (310 bp) was resolved on 1.2% agarose gel and for visualisation it was stained with ethidium bromide (1 µg 100 mL⁻¹) at room temperature for 1 hour and documented under UV illumination. Lane M = Marker DNA ϕ X174 Hae III digest, lanes A-D are amplicons from expectorates of leprosy patients, lanes E-I are amplicons from sputa of TB patients and lane J = negative control.

None of them belonged to *M. tuberculosis*, as determined after PCR amplification and hybridisation to *M. tuberculosis* specific probe TB400 (data not shown).

The sensitivity of all these isolates was tested against 21 drugs. All the isolates and the control organisms were multi drug resistant. The resistance among

Table 2. Percentage resistance of the mycobacterial isolates from leprosy patients to the various anti-leprosy drugs.

S.No	Name of the isolate	%resistance to		
		Dapsone(DDS)	Clofazimine	Rifampicin
1	<i>M. fortuitum-chelonae</i> complex	100	100	100
2	<i>M. fortuitum-chelonae</i> complex	80	100	100
3	<i>M. fortuitum-chelonae</i> complex	100	100	75
4	<i>M. fortuitum-chelonae</i> complex	100	100	75
5	<i>M. fortuitum-chelonae</i> complex	80	80	100
6	<i>M. fortuitum-chelonae</i> complex	75	100	100
7	<i>M. fortuitum-chelonae</i> complex	100	100	100
8	<i>M. scrofulaceum</i>	75	37.5	75
9	<i>M. scrofulaceum</i>	100	75	15
10	<i>M. fortuitum-chelonae</i> complex*	30	30	60
11	<i>M. avium</i> complex*	83.3	75	66.6
12	<i>M. smegmatis</i> *	100	100	100

*Controls.

Table 3. Pattern of drug resistance among isolates from contacts of Lepromatous Leprosy patients.

Isol No.	Percentage resistance				Contami-nation
	0-25%	26-50%	51-75%	76-100%	
1	K,E,C,CL	T,ET,PA,P,D,R	S,DO,ST,F	TR,A,CLR,G,CT,M,N	-
2	E,K	ET,C,CLR,D,R	T,M,PA,P,DO,SA	G,CT,F,N,TR,S,CLR	-
3	ET,K,D,CL	G,T,M,F,P,C,TR,S,DO,A,R,ST,CLR	CT,E,N,PA	-	-
4	CL,R	D	C	G,CT,T,E,M,ET,F,N,PA,P,TR,S,DO,A,K,ST,CLR	-
5	K	D,CL,R	G,E,P,S,CL	CT,T,M,ET,F,N,PA,C,TR,A,DO,ST	-
6	T,E,D	CL,R	T,S,DO,A,ST,F,N,C,K	G,CT,M,ET,P,PA	CLR
7	C,CL,R	T,E,N,DO,A,K	ET,P,TR	G,M,F,P,S,D,ST	CT,CLR
8	TR		A,K,G,T,M,E,ET,PA,CL,C	S,DO,ST,E,F,P,D,R	CLR,CT
9	K,R	E,ET,F,N,DO,P	M,PA,C,A,ST,CL	T,TR,D	G,CT,S,CLR
10	DO,O,E,C,K	G,M,F,P	S,A,ST,ET,PA,N	T,D,CL,R	CLR,CT,T
11	G,CT,T,E,M	R,ET,F,N,PA	D,CL,P,C	TR,S,DO,A,K,ST,CL	CLR
12	R,G,CT	D,C,PA,P,C,S,TR,DO,A	ST,K	CLR,CL	
13	T,E,M,ET,F,N	G,CT,T,E,M,ET,F,N	ST,CLR,CL	D,K	
14	C,R,PA,P,SA,TR,DO	R,T,M,ET,PA,P,C,TR,S,DO,ST,K,CL	D,N	E,F	G,CT,CLR

Isolates 1-11 : *M. fortuitum-chelonae* complex; Isolates 12, 13 : *M. avium* complex

Isolate 14 : *M. smegmatis*. For details of drugs see Table I

Table 4. Percentage resistance of the isolates from the contacts of leprosy patients to main anti-leprosy drugs.

S.No.	Nature of the isolate	Dapsone(DDS)	% resistance to Clofazimine	Rifampicin
1	<i>M. fortuitum-chelonae</i> complex	21.4	14.2	35.7
2	<i>M. fortuitum-chelonae</i> complex	33.3	16.6	33.3
3	<i>M. fortuitum-chelonae</i> complex	33.3	33.3	33.3
4	<i>M. fortuitum-chelonae</i> complex	42.8	17.1	14.2
5	<i>M. fortuitum-chelonae</i> complex	50	50	30
6	<i>M. fortuitum-chelonae</i> complex	20	37.5	37.5
7	<i>M. fortuitum-chelonae</i> complex	100	1	4
8	<i>M. fortuitum-chelonae</i> complex	100	70	100
9	<i>M. fortuitum-chelonae</i> complex	100	75	25
10	<i>M. fortuitum-chelonae</i> complex	77.7	77.7	77.7
11	<i>M. fortuitum-chelonae</i> complex	75	75	37.5
12	<i>M. avium</i> complex	37.5	37.5	0
13	<i>M. avium</i> complex	77.7	16.6	6.6
14	<i>M. smegmatis</i>	75	75	37.5
15	<i>M. fortuitum-chelonae</i> complex*	30	3	60
16	<i>M. avium</i> complex*	83.3	75	66.6
17	<i>M. smegmatis</i> *	100	100	100

*Controls

Table 5. Pattern of Drug Resistance among isolates from Lepromatous Leprosy patients.

Isolate No.	0 - 25 %	26 - 50%	51 - 75%	76 - 100%
1	E,ET,N,PA,P,C, TR,DO,A,S,K	F	TCT,T,M	D,G,CL,CLR,R
2	E,N,K	PA,C	TCT,S	G,T,M,ET,F,P,TR, DO,A,CLR,D,CL,R
3	T,E,ET,C,K	G,TCT,M,S,DO,A,S	F,P,TR,R	N,CLR,D,CL
4	G,T,E,M,ET,F,N, PA,C,S,A,K	TCT,TR,DO	P,R	CLR,D,CL
5	E,M,ET,F,S,K	G,TCT,T,N,P,C, S,DO,A,CLR	PA	TR,D,CL,R
6	G,E,M,ET,C,S,K	TCT,T,F,DO	PA,TR,CLR,SA	N,P,D,CL,R
7	E,ET,C,K	G,TCT,M,N	A,PA	T,F,P,TR,S, DO,D,CL,R
8	E,DO,K	TR,S,CLR,T,M, F,N,PA,P,C,CL	A,S,D,R	ET,G
9	R	-	E,ET,F,N,T, S,A,CLR,CL	G,TCT,M,DO,D

Sensitivity was calculated as described in the Methods. Names of the drugs are abbreviated and for details please see Table 1. Isolates 1 - 7 : *M. fortuitum - chelonae* complex; Isolates 8 & 9 : *M. scrofulaceum*.

the MFC isolates of the LL patients (Table 2) varied from 75% to 100% for dapsone and 80% to 100% for clofazimine and 75% to 100% for rifampicin. This is compared to 30%, 30% and 60% resistance of

the control strains of *M. fortuitum* - *chelonae* for dapsone, clofazimine and rifampicin respectively. The isolates from the leprosy contacts were more sensitive (55%) to these drugs (Table 3 & 4)

Table 6. Pattern of resistance among the isolates from drinking water supply.

Isol. No.	0-25%	26 - 50%	51 - 75%	% resistance 76 - 100%	Contam-ination
1	E,M,ET,F,N,PA,P,C, T,S,DO,A,S,K,CL	DG,T,CLR	None	CT,R	None
2	N,PA,P,C,TR, DO,A,S,R	ET	None	D,G,TCT,T,E,M, F,K,CLR,C	S
3	E,C,TR,K	M,ET,S,CL,R	G,F,P,S	D,TCT,T,N, PA,DO,A,CLR	None
4	E,ET,N,PA,P,C, TR,DO,A,S,K	CL	None	D,G,TCT,T,M,F, CLR,R	S
5	M,K,R	None	D,G,T	TCT,E,ET,F,N,P,C,TR, DO,A,CLR,CL,PA	ST,S
6	TCT,C,CL	D,E,ET,F,S,K	None	G,T,M,N,PA,P,R TR,S,DO,A,CLR,	None
7	C	K	G,ET,CL	D,TCT,T,E,M,F,N,A,P, TR,S,DO,A,R,CLR	None
8	None	None	P,C,K	DG,TCT,T,E,M,ET, F,N,PA,TR,S,DO, A,R,CL,CLR	None
9	None	None	None	D,G,TCT,T,E,M,ETF, N,PA,P,C,TR,S,DO, A,K,CLR,R,CL	None
10	D,TR,K	None	E,M,ET,F, PA,P,C,S, DO,CL	T,N,A,R	G,CT,CLR
11	A	T,M,ET,PA,P,C, TR,S,DO,K,CL,R	D,N	E,F	G,CT,CLR
12	ET,C	E,N,PA,P,K	D,S	T,M,F,TR,DO,A,S,CL	G,CT,T,CLR
13	C	ET,N,PA,P,S,TR, DO,A,K,R,CL D,E		M,F,S	G,CT,T,CLR
14	N,PA,P,C,TR,S,DO,AR	D	TCT,E,K,	G,T,M,ET,F,CL,C,R	None
15	D,G,TCT,E,ET,K,CL,S	F,C	T,M,N,P,TR DO,A,CLR	PA,S,R	None
16	K	E,ET,F,M,P	M,PA,S, DO,A,S	D,T,C,TR,CL,R	G,CT,CLR
17	None	C	F,N,P	D,T,E,M,ET,S,PA, TR,DO,A,K,CLR,R,	G,CT,CLR
18	D,G,TCT,T,EM,PA,P, C,DO,K	ET,F,N	R,S,A,S	CL,TR	CLR

Sensitivity was calculated as described in the Methods. Names of the drugs are abbreviated and for details please see Table I. All isolates are *M. fortuitum* - *chelonae* complex.

compared to those from LL patients (Table 2 & 5). Among the 21 drugs tested, ethambutol, ciprofloxacin and kanamycin were found to be active against the isolates from the LL patients at concentrations recommended by CDC (16). Only ciprofloxacin and kanamycin were found to be active against organisms isolated from water (Table. 6). Among isolates of MOTT from tuberculosis patients (Table 7) 63% belong to *M. fortuitum-chelonae* complex, 19% belong to *M. avium* complex, 12% belong to *Mycobacterium kansasii* and 6% belong to *M. smegmatis*. Two of the three *M. avium* complex isolates were sensitive to streptomycin and ethambutol. Complete resistance (100%) to INH was noticed in 5 of the 10 isolates of *M. fortuitum*, one each of the *M. avium* complex and *M. kansasii*. Four of the 10 isolates of *M. fortuitum* showed complete resistance to rifampicin the remaining isolates exhibiting more than 60% resistance to these drugs. Many of them were sensitive to streptomycin,

pyrazinamide and ethambutol (Table 7). One of the *M. avium* complex isolates and one of the *M. kansasii* isolates showed total resistance to almost all the anti TB drugs. Rifampicin was found to be more active against *M. fortuitum-chelonae* complex from leprosy contacts than from either LL or tuberculosis patients. Among the non-conventional antimycobacterial drugs tested, only kanamycin (5 µg ml⁻¹) and ciprofloxacin (6 µg ml⁻¹) were found to be active against these isolates.

DISCUSSION

Frequency of isolation and tests for identification of non-tuberculous mycobacteria are important for clinicians, epidemiologists and for bacteriologists because patients suspected of having tuberculosis may excrete in the sputum not only tubercle bacilli but also other mycobacteria. These mycobacteria are non-pathogenic to guinea pigs and are

Table 7. Resistance pattern of the MOTT isolates from pulmonary tuberculosis patients to various anti-tuberculosis drugs.

S.No	Name of isolate	INH	Strep.	Pyraz.	% resistance to Etham.	Rif.
1	MFC	100	0	0	0	100
2	MFC	66.6	0.6	100	3.3	100
3	MFC	100	33.3	83.3	100	100
4	MFC	100	85.7	85.7	71.4	85.7
5	MFC	100	0	0	100	0
6	MFC	100	100	100	100	100
7	MFC	77.7	66.6	66.6	33.3	66.6
8	MFC	60	60	80	40	60
9	MFC	75	75	75	50	75
10	MFC	30	5	0	0	5
11	MAC	100	0	100	0	50
12	MAC	83.3	83.3	100	100	100
13	MAC	12.5	0	25	0	0
14	M.kansasii	100	100	100	100	71.4
15	M.kansasii	70	60	60	0	70
16	M.smeg.	83.3	0.6	100	1.3	9.3
17	MFC*	80	60	60	40	60
18	MAC*	83.5	75	91.6	50	66.6
19	M.smeg.*	100	80	100	80	100

* Controls; MFC= *M. fortuitum-chelonae* complex;
MAC= *M. avium* complex; M.smeg.= *M. smegmatis*.

microbiologically and chemically distinct. The non-tuberculous mycobacteria have also been associated with certain other diseases in humans and have been referred to variously as anonymous or other types (14). It is not appreciated how common are the environmental mycobacteria in nature and how they could influence the health of human population (11-14). In the present investigation we have evaluated the possibilities of using PCR to type the mycobacteria at the genus level and also to rule out *Mycobacterium tuberculosis* infection in the leprosy patients. All the isolates tested positive for *Mycobacterium* with the genus specific probe. However, none of them tested positive for *M. tuberculosis* DNA. The usefulness of PCR for direct diagnosis of *M. tuberculosis* and other mycobacteria has already been established (16). The PCR results correlate well with biochemical typing of the cultivated organisms. This suggests that PCR based diagnostics could be used to rapidly detect the mycobacteria at the genus level and only the positive samples need to be investigated further for *M. tuberculosis* or other species identification in leprosy patients. The sensitivity and specificity of sample processing method and the PCR assay have already been validated for direct detection of *Mycobacterium tuberculosis* in sputa from patients (17) and therefore, instead of subjecting every sample to culture, this strategy could be applied particularly for the diagnosis of sub-clinical infection. The negative predictive value of PCR being absolute under optimal conditions, PCR negative samples need not be subjected to culture isolation and analysis. This would also replace the AFB smear which has very limited sensitivity. Considering the sensitivity and specificity of PCR, if PCR is used unequivocal results of samples like, smear positive yet culture negative or smear negative (but clinically showing active disease) yet culture positive may become rare. This strategy would also bring down the cost of culturing by at least a third in endemic population like in India as only the genus PCR positive samples need to be cultured (if required). Further it will reduce the time taken to report the presence of mycobacteria or *M. tuberculosis* in a given sample to two days instead of the conventional 4-6 weeks that is required for culture. Our current knowledge about the drug sensitivity pattern of the non-tuberculous mycobacteria is very limited and is particularly restricted to the conventional anti-

tuberculosis drugs. With some exceptions, non-tuberculous mycobacteria are generally resistant to conventional anti-tuberculosis drugs by *in vitro* standards. Of particular concern are diseases due to the *M. avium* complex and the *M. fortuitum* complex which present the most serious problems in chemotherapeutic management as non-tuberculous mycobacterial isolates have been reported to be highly resistant to isoniazid, PAS and streptomycin (19).

In the current study we investigated the sensitivity pattern of all the non-tuberculous mycobacterial isolates to 21 antibiotics. The isolates from the leprosy patients, their contacts, the drinking water supply and those isolated from the tuberculosis patients sputa showed differences in sensitivity to these drugs. All the isolates were found to be multi-drug resistant and only the extent of resistance to various antibiotics was different. One isolate from water was found to be resistant to all the 21 drugs used in this study. Isolates from the contacts of the leprosy patients were more sensitive to rifampicin than those from leprosy patients probably because the leprosy contacts have not been exposed to rifampicin before. Further, rifampicin is being given to lepromatous leprosy patients at dosages much lower than that given for tuberculosis patients and this could also lead to selection of the resistant mutants of these fast-growers in the lepromatous leprosy patients. However, even among the ten *M. fortuitum chelonae* isolates from tuberculosis patients four were completely resistant to rifampicin which indicates that administration of lower dosage alone may not lead to selection of resistant mutants in leprosy patients. Additional mechanism could be operating in these organisms.

Plasmids have been identified in fast-growing mycobacteria particularly, *M. fortuitum* and *M. chelonae*, isolated from patients or from the environment (20). The innate resistance in these mycobacteria isolated from sputum of leprosy patients or from their drinking water or sputa of TB patients could be either plasmid mediated or mediated through mobile genetic elements like insertion sequence(s) or transposons (21). There has been no direct evidence to demonstrate that the observed drug resistance is conferred by plasmids or mobile genes nor has mobility of these

mobile genetic elements from one mycobacteria to another been demonstrated. However, there could potentially be transfer of antibiotic resistance to the pathogenic mycobacteria from these non-pathogenic species. To understand the implications of the drug resistance of the MOTT organisms in the pathogenesis and the control of leprosy and tuberculosis, molecular mechanisms of resistance to these antibiotics and biochemical changes consequent to the development of resistance to these antibiotics need to be investigated.

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