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Susceptibility of south Indian strains of *Mycobacterium tuberculosis* to tuberactinomycin

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A total of 114 strains of *Mycobacterium tuberculosis* isolated from sputum samples of 114 patients of pulmonary tuberculosis in south India, were coded and tested for their *in vitro* susceptibility to tuberactinomycin (Tum) incorporated in Lowenstein-Jensen (LJ) medium. Of these strains, 95 (83.3%) and 15 (13.2%) were susceptible to Tum at 25 and 50 mg/l respectively. Only 4 (3.5%) strains were inhibited at 100 mg/l or more. Of the 37 drug sensitive strains, 2 (5.4%) were not susceptible to Tum at 25 mg/l compared to 17 (22.1%) of 77 strains-resistant to one or more of antituberculosis drugs (P <0.02). The drug susceptibility pattern of the strains revealed that there was no significant association of resistance between Tum and streptomycin or rifampicin or ethambutol or ethionamide or isoniazid. However, 15 (53.6%) of 28 kanamycin (K) resistant strains were not susceptible to Tum at 25 mg/l. This cross resistance between Tum and K was further studied in 24 and 15 K sensitive and resistant strains respectively, by correlating their proportion resistance at 16 mg/l and it was found to have a significant positive correlation (r = 0.55; X0.01).

Search for new drugs to evolve effective regimens for treating patients who show resistance to various antituberculosis drugs is to be continued and needs no emphasis. Tuberactinomycin (synonym: Enviomycin), is an antituberculosis drug derived from *Streptomyces griseoverticillatus* var. *tuberacticus*[1]. In Japan, this drug is being used for re-treating patients of refractory pulmonary tuberculosis and in lung diseases caused by *Mycobacterium avium* complex[2]. Turn has been reported to be less toxic than kanamycin (K) and capreomycin (Cap)[3]. However, it is important to know the differences, if any, in the susceptibility pattern of local strains to Tum in view of our earlier finding that south Indian strains of *M. tuberculosis* were known to be less susceptible to thiacetazone, although it was observed that strains isolated elsewhere were highly susceptible to this drug[4]. An investigation was therefore undertaken to study the susceptibility pattern of south Indian strains of *M. tuberculosis* to Turn.

Material & Methods

Cultures: A total of 114 strains of *M. tuberculosis* (37 sensitive to available antituberculosis drugs and 77 resistant strains) isolated from 114 patients with pulmonary tuberculosis were investigated. The sensitive and resistant strains were selected in the ratio of 1:2. *M. tuberculosis* H37Rv was included as control throughout the study. All the

strains were coded before they were subjected to drug susceptibility tests.

Drug susceptibility tests : Indirect susceptibility test was performed with the following preinspissation concentrations of Tum in Lowenstein-Jensen (LJ) medium; 10, 25, 50 and 100 mg/l. The test procedures and the determination of minimal inhibitory concentration (MIC) were carried out as described by Canetti *et al*[5]. The proportions resistant to Tum and K | at 16 mg/l were calculated as per the procedures described by Tripathy *et al* [6].

The following are the MIC levels indicating resistance for various drugs - streptomycin (S): > 32 mg/l; isoniazid (I): > 1 mg/l; rifampicin (R): > 128 mg/l; K: > 64 mg/l; etambutol (Emb) : > 8 mg/l; ethionamide (Eth): > 114 mg/l.

Fisher's exact test, Chi square test and Student's 't' test were used for statistical analysis of the data.

Results

The susceptibility of 114 strains to Turn is presented in Table I. Of the strains, 95 (83.3%) were susceptible at 25 mg/l. At 50 mg/l, 110 (96.5%) of the strains were susceptible. Only 4 (3.5%) strains had MIC of 100 mg/l or more.

Of the 37 drug sensitive strains, 2 (5.4%) were not susceptible to Turn at 25 mg/l compared to 17 (22.1%) of the 77 drug resistant strains and the difference was significant (P <0.02). The difference in the susceptibility pattern to Turn at 50 mg/l of sensitive and resistant strains was not significant (P=0.2). Drug susceptibility pattern of the 114 strains revealed that there was no association of resistance between Tum and S or I or R or Emb or Eth (data not presented). But there was a considerable cross resistance between Tum and K as 15 (53.6%) of 28 K resistant strains-were not susceptible to Tum at 25 mg/l (Table II). This was further studied in 24 and 15 K sensitive and resistant strains respectively, by correlating their proportion resistance (Table III) at 16 mg/l and it was found to 'have a significant positive correlation (r=0.55; P<0.01).

Table I. Susceptibility of south Indian strains of *M. tuberculosis* to tuberactinomycin

	MIC of Tum				Total
	10	25	50	> 100	
Sensitive strains	4	31	2	0	37
Resistant strains	7	53	13	4	77
Total	11	84	15	4	114

Table II. Tuberactinomycin susceptibility of *M. tuberculosis* as against kanamycin

MIC of Tum	MIC of K				Total
	8	16	32	> 64	
10	2	1	6	2	11
25	4	34	28	13	79
50	0	1	4	10	15
> 100	0	0	1	3	4
Total	6	36	39	28	109*

*For 5 strains, K results were not available

The results of the tests to assess the reproducibility of the MIC values showed that, of the 53 strains investigated, 38 (71.7%) gave the same MIC in the repeat test. The reproducibility of MIC 125 mg/l was found to be 79 per cent (with 95% confidence limits of 61 to 91%) and with MIC > 50 mg/l it was 75 per cent (with 95% confidence limits of 51 to 91%). All the 3 strains with MIC > 100 mg/l, when retested, showed the same MIC. The standard reference strain *M. tuberculosis* H37Rv consistently showed MIC 125 mg/l on each of 15 occasions tested.

Discussion

In the present study, 95 (83.3%) of 114 selected strains of *M. tuberculosis* obtained from south Indian patients of pulmonary tuberculosis were susceptible to Tum at 25 mg/l of LJ medium as measured by the indirect susceptibility test. Toyohara[7] reported from Japan that 84 per cent of 197 strains were susceptible to Tum at 25 mg/l in Ogawa egg medium. From Korea, Gill Han Bai and Sang Jae Kim[8] tested 333 pretreatment isolates of *M. tuberculosis* and found that 98 per cent of them were susceptible at 25 mg/l and the remaining 2 per cent at 50 mg/l of LJ medium.

Table III. Frequency distribution of *M.tuberculosis* strains according to proportion resistant to Tum and K at 16 mg/l

KAN	Proportion resistant (%) to TUM								Total
	<1.0	1-	5-	10-	15-	25-	50-	NA*	
<1.0	8	9	–		1	–		1	19
1-	1		1				–		2
5-	–	2	1		–		1		4
10-	-	1	1		–	1		–	3
15-	1			1	–				2
25-	1	1		–	1	3			6
50-	–	1			–	–	–		2
NA	–	1		–	–			–	1
Total	II	15	3	1	2	4	2	1	39

* results not available

The standard definition of proportion resistance (1% resistant population at a given concentration) could not be adopted to classify strains into sensitive and resistant as the concentration of Tum (16 mg/l) selected was found to be low and not suitable for the purpose. Proportion resistance pattern at higher concentration of Tum would perhaps have yielded meaningful classification.

The MIC of Tum against *M. tuberculosis* H37Rv and 4 clinical isolates, was estimated in 7H9 liquid medium, Sauton's agar and Middlebrook's 7H11 agar medium and found to be 5.0 mg/l or less. Gill Han Bai and Sang Jae Kims also reported MIC of 4 and 5 mg/l respectively using Kirchner's medium with 10 per cent horse serum and in Dubos medium.

M. tuberculosis H37Rv and 83.3 per cent of the clinical isolates were inhibited by Tum at 25 mg/l in LJ medium. The higher level of concentration for inhibition by aminoglycosides such as Cap, K, lividomycin, viomycin and Tum, is probably due to binding of a major part of the drug to protein in egg based media[9].

The present *in vitro* susceptibility studies of south Indian strains of *M. tuberculosis* indicate that there is very little cross resistance between Tum and other drugs with the exception of K. These findings could form the-basis for further studies.

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