



The Sensitivity of Tubercle Bacilli from Ceylonese Patients with Pulmonary Tuberculosis to Cycloserine and Ethionamide

by

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SUMMARY A study of drug sensitivity of *Myc. tuberculosis* from patients with pulmonary tuberculosis in Sri Lanka to cycloserine and ethionamide is reported. It was found that there is no significant incidence of resistance to these drugs among strains isolated in this country.

INTRODUCTION

Cycloserine and ethionamide are two antituberculosis drugs used widely in Sri Lanka in patients presenting for retreatment of tuberculous disease and also in the initial treatment of previously untreated patients who for some reason cannot be given the standard first line drugs. Cycloserine was introduced for use in Sri Lanka in 1959 and ethionamide in 1961. However no surveys of the patterns of sensitivity and resistance of strains of tubercle bacilli from Ceylonese patients with pulmonary tuberculosis to these drugs have been made. In this paper, a survey of drug sensitivity of tubercle bacilli in Sri Lanka to cycloserine and ethionamide is reported.

MATERIALS AND METHODS

The patients investigated were those with pulmonary tuberculosis, 12 years of age and above, of both sexes. Specimens of sputa (obtained before the institution of antituberculosis chemotherapy in those with no previous history of such treatment) were decontaminated by the standard modified Petroff's method (Cruikshank, 1965), inoculated on to Lowenstein-Jensen (L.J.) medium without potato starch (Jensen, 1955) and incubated at 37°C. Of all strains of acid fast bacilli isolated, only the results of those identified as *Myc. tuberculosis* ("human type" tubercle bacilli) are presented here. An acid fast bacillus was identified as *Myc. tuberculosis* if it produced eugonic growth on glycerol containing egg medium, was a slow grower at 37°C, produced no growth in 4 weeks at 22°C and 42°C, produced no pigment in the dark or on exposure to light, and was niacin positive.

All drug sensitivity tests were done with actively growing 4 week old cultures using solid L. J. medium without potato starch. Drug solutions were prepared using sterile distilled water in the case of cycloserine, and ethylene glycol in the case of ethionamide, and sterilised by filtration using membrane filters. The drug solutions were added to the L. J. medium before inspissation using a double dilution technique to yield the following concentrations:

Cycloserine	2.5 80	5 160	10 320	20 640	40 1280	µg/ml.
Ethionamide	5 160	10 320	20 640	40 1280	80 2560	µg/ml.

The drug containing slopes were prepared in $\frac{1}{2}$ oz. or 1 oz. screw capped containers, and were used within 7-10 days of preparation; storage was at 4°C.

The bacillary inoculum used was a homogeneous 1 mg (moist weight) per ml suspension in sterile distilled water prepared by shaking vigorously in a ball mill [an one oz. screw-capped bottle with approximately 20 (diameter 4 mm glass) beads] for 30-40 seconds. One loopful (3-4 mm internal diameter) of this suspension was inoculated on each L. J. slope. The slopes were then incubated at 37°C and read at four weeks, to determine the minimal inhibitory concentration (MIC—which was defined as the lowest drug concentration which showed a growth of less than 20 colonies). In each set of ethionamide slopes was included one drug-free L. J. slope containing an equal volume of the solvent added before inspissation. In addition, for each inoculum (strain), a neat L. J. slope was also included. The results of each set was evaluated only if the drug-free L. J. slopes showed a confluent or near confluent growth (usually over 200 colonies). In the first instance, usually only the upper row of drug concentrations was inoculated, but if a strain had a MIC higher than that of the highest drug concentration in the upper row, the test was then repeated using the entire range of drug concentrations. With each batch of sensitivity tests was also inoculated several sets with the reference strain—H37Rv, to determine the modal MIC, for the calculation of the resistance ratio (RR—which is defined as the ratio of the MIC of the test strain to the MIC of H37Rv).

The criterion of resistance adopted in this study was determined by the RR,—a RR of 2 or less being considered to indicate sensitivity; and of 8 or more or 4 repeatedly to indicate resistance.

The patients studied were unselected consecutive cases (seen by one of the authors) from whom acid fast bacilli identified as *Myc. tuberculosis* could be cultured. They were of two groups—those with and without a previous history of antituberculosis chemotherapy. The former included patients who had relapses after continuous or interrupted chemotherapy.

RESULTS

In the table are presented the sensitivity patterns to the two drugs shown by the strain of tubercle bacilli culture from the patients studied. There is no significant incidence of resistance to either drug in both groups of patients. With both drugs the incidence of resistance appears to be higher in strains showing resistance to one or more of the first line drugs.

In the case of cycloserine cross resistance to the first line drugs (streptomycin, isoniazid (INAH) and para-amino-salicylic-acid, PAS) has not been reported. In the case of ethionamide, however, cross-resistance has been reported with INAH (Tan Thiam Hok 1964 ; Canetti, Kreis, Thibier, Gay & Le Lirzin, 1967) and with thioacetazone (Bartmann, 1960 ; Rist, 1960 ; Lefford, 1969). Examination of the pattern of resistance shown by the ethionamide resistant cultures to streptomycin, INAH, PAS and thioacetazone—(with the same bacillary inoculum using the criterion of a MIC of 0.2 $\mu\text{g}/\text{ml}$ or more to indicate resistance to INAH and a RR of 8 or more or 4 repeatedly to indicate resistance to the other three drugs) showed, that of the six ethionamide resistant cultures, three were resistant to all four drugs, one was resistant to streptomycin, INAH and PAS, one to streptomycin, INAH and thioacetazone and the sixth to INAH and thioacetazone.

DISCUSSION

The findings of this study show that there is no significant incidence of drug resistance to either cycloserine or ethionamide in strains of tubercle bacilli isolated from patients with pulmonary tuberculosis in Sri Lanka. Hence, these drugs are suitable for use in previously untreated cases in whom the standard first line regimen could not be used.

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TABLE

Pattern of Drug Sensitivity of *Mycobacterium tuberculosis* in Sri Lanka to Cycloserine and Ethionamide

CATEGORY OF PATIENTS (Strains) (Number tested, given within brackets)	<i>Approximate Percentage Strains</i>			
	Cycloserine (Cy)		Ethionamide (E)	
	Sensitive	Resistant	Sensitive	Resistant
With no previous history of antituberculosis chemotherapy: Total (Cy — 171 ; E — 163)	95	5	99	1
Strains with sensitivity test results to first line drugs available: Sensitive to all three (Cy — 110 ; E — 114)	95	5	100	0
Resistant to one or more (Cy — 41 ; E — 42)	94	6	98	2
With a past history of antituberculosis chemotherapy: Total (Cy — 87 ; E — 88)	95	5	95	5
Strains with sensitivity test results to first line drugs available: Sensitive to all three (Cy — 28 ; E — 27)	100	0	100	0
Resistant to one or more (Cy — 59 ; E — 60)	93	7	91	9
Total patients (Cy — 258 ; E — 251)	95	5	98	2