RESISTANCE TO ETHAMBUTOL AS AN AID TO THE IDENTIFICATION OF *MYCOBACTERIUM FRIEDMANNII* (ABSCUSSUS) AND *M. BORSTELENSE*

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BÖNICKE (1962) and Tacquet and Tison (1963) investigated the sensitivity of several mycobacterial species to ethambutol. When recently a strain of *Mycobacterium friedmannii* was isolated in our laboratory (Pattyn *et al.*, 1971), we had to reinvestigate this problem, particularly in relation to the rapidly growing species of mycobacteria. We took the opportunity to test the sensitivity to ethambutol of some other mycobacterial species not studied in the earlier papers.

MATERIAL AND METHODS

The mycobacterial strains we studied came from several sources. Some were from our own collection, and included a number that have been described previously (Pattyn, 1966, 1967; Pattyn *et al.*, 1967; Pattyn, Hermans-Boveroulle and van Ermengem, 1968), others were from the American Type Culture Collection (ATCC) and the remainder were a series of rapidly growing mycobacteria distributed by Dr G. P. Kubica as part of an international cooperative study of mycobacteria of group IV initiated by the International Working Group on Mycobacterial Taxonomy.

Ethambutol was added in aqueous solution to Loewenstein-Jensen medium before tyndallisation, and sensitivity tests were performed by the proportion method (Buttiaux, Beerens and Tacquet, 1966). The final concentrations of antibiotic in the medium were 1, 5, 25 and 50 µg per ml.

RESULTS

Slowly growing mycobacteria

In accordance with Bönicke, and Tacquet and Tison, we found (table Ia) that slowly growing mycobacteria were inhibited by relatively low doses of ethambutol. Also the more recently described species *M. gastri*, *M. terrae* (Wayne, 1966), *M. xenopei* (Marks and Schwabacher, 1965), species V (Jones *et al.*, 1966) and also *M. microti* were uniformly sensitive to the antibiotic.

Rapidly growing mycobacteria

M. marinum (2 strains), *M. smegmatis* (3 strains), *M. phlei* (1 strain), *M. diernhoferi* (1 strain), *M. parafortuitum* (1 strain), and *M. vaccae* (1 strain) were inhibited by 1 µg ethambutol per ml (table Ib). *M. fortuitum* strains are somewhat more resistant: of 18 strains tested, only 4 were inhibited to a significant degree by 1 µg per ml, and 8 strains grew in the presence of 5 µg per ml, but were inhibited by 25 µg ethambutol per ml.

In contrast to this, *M. thamnopheos* (no. ATCC4445), *M. borstelense* (no. ATCC 19235 and 19237), and *M. friedmannii* (no. ATCC19977, Stanford's strains no. IW124, JB133 and WT58, and our strain WM) stand out as highly resistant because they grew in the presence of 50 µg ethambutol per ml. Only one *M. friedmannii* strain (MP) was inhibited by 25 µg of antibiotic per ml; this strain is described elsewhere (Pattyn *et al.*, 1971).

To establish the value of ethambutol sensitivity in mycobacterial identification, 44 strains of unknown, rapidly growing mycobacteria, distributed by Dr G. P. Kubica to the members of the International Working Group on Mycobacterial Taxonomy, were tested for their

ethambutol sensitivity. Recently, the computerised results of this international collaborative study have become available and can thus be correlated with the ethambutol sensitivity tests.

TABLE I

Ethambutol sensitivity of (a) slowly growing and (b) rapidly growing mycobacteria

(a) Slowly growing mycobacterial species	Number of strains of slowly growing mycobacteria for which the minimum inhibitory concentration* of ethambutol was (μg per ml)					(b) Rapidly growing mycobacterial species	Number of strains of rapidly growing mycobacteria for which the minimum inhibitory concentration* of ethambutol was (μg per ml)				
	≤ 1	5	25	50	> 50		≤ 1	5	25	50	> 50
<i>M. kansasii</i>	4	0	0	0	0	<i>M. marinum</i>	2	0	0	0	0
<i>M. scrotochromogen</i> 1	1	1	0	0	0	<i>M. smegmatis</i>	3	0	0	0	0
<i>M. scrotochromogen</i> 2	2	0	0	0	0	<i>M. phlei</i>	1	0	0	0	0
<i>M. scrotochromogen</i> 3	0	2	0	0	0	<i>M. diernhoferi</i>	1	0	0	0	0
<i>M. scrotochromogen</i> 4	0	2	1	0	0	<i>M. parafortuitum</i>	1	0	0	0	0
<i>M. scrotochromogen</i> 5	3	0	0	0	0	<i>M. vaccae</i>	1	0	0	0	0
<i>M. avium</i>	0	3	0	0	0	<i>M. fortuitum</i>	4	6	8	0	0
<i>M. intracellulare</i>	3	1	0	0	0	<i>M. thamnophaeos</i>	0	0	0	0	1
<i>M. gastri</i>	3	0	0	0	0	<i>M. borstelense</i>	0	0	0	1	1
<i>M. terrae</i>	3	0	0	0	0	<i>M. friedmannii</i>	0	0	1	1	4
<i>M. xenopei</i>	0	3	0	0	0	(abscessus)					
<i>M. "species V"</i>	0	2	0	0	0						
<i>M. microti</i>	1	0	0	0	0						

* Lowest concentration in which the number of colonies was < 10 per cent. of the number in the control tube.

TABLE II

Ethambutol sensitivity of a collection of rapidly growing mycobacteria distributed on behalf of the International Working Group on Mycobacterial Taxonomy

Mycobacterial species	Number of strains of mycobacteria for which the minimum inhibitory concentration of ethambutol was (μg per ml)				
	≤ 1	5	25	50	> 50
<i>M. vaccae</i>	4	0	0	0	0
<i>M. flavescens</i>	5	1	0	0	0
<i>M. smegmatis</i>	4	0	1	0	0
<i>M. phlei</i>	1	0	0	0	0
<i>M. fortuitum</i>	0	2	4	0	0
<i>M. peregrinum</i>	0	4	4	0	2
<i>M. friedmannii</i> (abscessus)	0	0	1	1	6
<i>M. borstelense</i>	0	0	0	1	3

Table II shows that only one strain identified as *M. friedmannii* (abscessus) (no. ATCC 23016) was inhibited by less than 50 μg ethambutol per ml. We were informed by Dr J. Marks, of the Tuberculosis Reference Laboratory, Cardiff, that this culture lacks the specific lipids of *M. friedmannii*; its final identification is therefore in doubt. The remaining seven strains of this species, and all four strains of *M. borstelense*, were more resistant.

Each of the six *M. fortuitum* strains, and eight of the ten *M. peregrinum* strains were sensitive to 50 μ g ethambutol per ml.

If the two sets of cultures are considered together, therefore, high resistance to ethambutol (i.e., a minimum inhibitory concentration of 50 μ g per ml or greater) was observed in at least 18 of 20 (90 per cent.) of strains identified as *M. friedmannii* or *M. borstelense*, but only in two of 34 cultures (6 per cent.) identified as *M. fortuitum* or *M. peregrinum*.

DISCUSSION

Mycobacterium fortuitum and *M. peregrinum* on one hand, and *M. friedmannii* (syn. *M. abscessus*, *M. runyonii*, *M. chelonae*) and *M. borstelense* on the other hand, are closely related species, as was shown in the above-mentioned international collaborative study, in which each pair could be separated into two species at an S value of 85 per cent. but not at an S value of 80 per cent. Members of the two pairs of species also behave similarly in their sensitivity to ethambutol.

High ethambutol resistance can thus be used as further means of separating *M. friedmannii* and *M. borstelense* from *M. fortuitum* and *M. peregrinum*, with which they have a large number of characters in common (Stanford and Beck, 1969; Stanford and Gunthorpe, 1969).

M. thamnophaeos also is highly resistant to ethambutol, but this species is very rarely isolated.

SUMMARY

Sensitivity of atypical mycobacteria to ethambutol is generally high, except in the two species *Mycobacterium friedmannii* and *M. borstelense*, in which at least 90 per cent. of strains are resistant to 25 μ g per ml or more. The character can be used as an aid to the identification of these species.

The single culture of *M. thamnophaeos* examined was also resistant to ethambutol.

REFERENCES

- BÖNICKÉ, R. 1962. *Beitr. klin. Tuberk.*, **126**, 108.
 BUTTIAUX, R., BEERENS, H., AND TACQUET, A. 1966. *Manuel de techniques bactériologiques*, 2nd ed., Paris.
 JONES, W. D., ABBOTT, VELLA D., VESTAL, ANNIE L., AND KUBICA, G. P. 1966. *Amer. Rev. Resp. Dis.*, **94**, 790.
 MARKS, J., AND SCHWABACHER, HERTA 1965. *Br. Med. J.*, **1**, 32.
 PATTYN, S. R. 1966. *Zentbl. Bakt. ParasitKde*, Abt. I, Orig., **201**, 246.
 " 1967. *Z. Tuberk.*, **127**, 41.
 PATTYN, S. R., HERMANS-BOVEROULLE, M. T., MORTELMANS, J., AND VERCRUYSE, J. 1967. *Acta zool. path. antw.*, **43**, 125.
 PATTYN, S. R., HERMANS-BOVEROULLE, M. T., AND VAN ERMENGEM, J. 1968. *Zentbl. Bakt. ParasitKde*, Abt. I, Orig., **207**, 509.
 PATTYN, S. R., VANDEPITTE, J., PORTAELS, F., AND DE MUYNCK, A. 1971. *J. Med. Microbiol.*, **4**, in press.
 STANFORD, J. L., AND BECK, A. 1969. *J. Gen. Microbiol.*, **58**, 99.
 STANFORD, J. L., AND GUNT HORPE, W. J. 1969. *J. Bact.*, **98**, 375.
 TACQUET, A., AND TISON, F. 1963. *Revue Tuberc.*, **27**, 431.
 WAYNE, L. G. 1966. *Amer. Rev. Resp. Dis.*, **93**, 919.