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Differentiation of *Mycobacterium chelonei* from *M. fortuitum* by ciprofloxacin susceptibility

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SUMMARY

Seventy-five strains of *Mycobacterium fortuitum* were inhibited by 3·0 mg/l ciprofloxacin but 36 strains of *M. chelonei* were resistant. The results correlated well with those obtained by the nitratase test. The ciprofloxin sensitivity test is a useful supplement to the tests used to identify these two species.

INTRODUCTION

Mycobacterium fortuitum and *M. chelonei* are opportunist pathogens, often lumped together in the 'Fortuitum' group. Infections due to *M. fortuitum* are usually localized and self-limiting but although those due to *M. chelonei* may also be limited, more widespread and even fatal infections have been reported; moreover, *M. chelonei* is more resistant to chemotherapeutic agents than is *M. fortuitum* (Grange, 1980). For clinical and epidemiological reasons, therefore, it is desirable that they are identified to species.

The lipid pattern, as demonstrated by thin layer chromatography (t.l.c.) is the most reliable method for distinguishing between the two species (Marks & Szulga, 1965; Jenkins, 1981). It also has the advantage of identifying biotypes but it is expensive and time-consuming.

Carbohydrate utilization (Gordon & Smith, 1953) and the amidase row (Bonicke, 1962) have proved to be of limited use in the diagnostic laboratory (Collins, Grange & Yates, 1985). At that level the nitratase test is the usual tool and is used in this laboratory. *M. fortuitum* gives a positive and *M. chelonei* a negative reaction (Kubica, 1973).

During a recent investigation into the susceptibility of various species of mycobacteria to ciprofloxacin (Collins & Uttley, 1985) we noted that all of 20 strains of *M. fortuitum* were sensitive to 3·0 mg/l and all of 20 strains of *M. chelonei* were resistant. We therefore tested further strains of each species.

MATERIALS AND METHODS

Organisms

A total of 115 strains was examined, including 74 clinical isolates received for routine identification in this laboratory and 41 from the current Study of the International Working Group on Mycobacterial Taxonomy on *M. fortuitum* and

Table 1. *Ciprofloxacin susceptibility of* Mycobacterium fortuitum *and* M. chelonei. (*115 strains*)

Species according to nitratase test	Ciprofloxacin (3 mg/l)	
	Sensitive	Resistant
M. fortuitum	74	2*
M. chelonei	3†	36

* Identified as *M. chelonei* by lipid analysis.

† One strain identified as *M. chelonei*, one with no specific lipid pattern and one with a lipid pattern shown by some other rapidly growing mycobacteria.

M. chelonei. All were non-pigmented, grew within 3 days on subculture at 20 °C but not at 44 °C, reduced tellurite, and gave a strong positive arylsulphatase reaction. Seventy-six had been provisionally identified as *M. fortuitum* and 39 as *M. chelonei* by the nitratase test. They were maintained on Lowenstein–Jensen (LJ) medium.

Ciprofloxacin susceptibility

Ciprofloxacin (BAY 09867) was incorporated into LJ medium at 3·0 and 6·0 mg/l. The stability and resistance to heat of the drug in this medium had already been determined (Collins & Uttley, 1985).

Suspensions of the organisms were prepared in phosphate buffer and tubes of the ciprofloxacin and drug-free medium were inoculated as described by Collins & Lyne (1984). The cultures were incubated at 25 °C for 3 days and growth on the two media compared.

Nitratase test

The organisms were grown for 18 days at 25 °C in 2 ml of Middlebrook 7H9 broth (Difco). Sodium nitrate, 0·1 ml of a 4% (w/v) solution was added; the tubes were reincubated for 4 h and tested for nitrate reduction using sulphanilimide, *N*-1-naphthylethylene diamine di-HCl and zinc dust (Virtanen, 1960; modified by Collins & Lyne, 1984).

RESULTS

All the strains examined were either sensitive or resistant to both ciprofloxacin at 3·0 and 6·0 mg/l in LJ medium.

Table 1 shows that 74 strains identified as *M. fortuitum* by a positive nitratase test were sensitive to ciprofloxacin while 36 strains identified as *M. chelonei* by a negative nitratase test were resistant.

Five strains did not conform to these patterns and Dr P. A. Jenkins, of the Mycobacterium Reference Unit, Cardiff, kindly determined their lipid pattern by t.l.c. The two that gave a positive nitratase reaction but were resistant to ciprofloxacin were shown to be *M. chelonei*. One of the three that were nitratase negative and sensitive to ciprofloxacin was identified as *M. chelonei*, one had no specific lipid pattern and the other a pattern that was not recognized but which had been observed in other rapidly growing mycobacteria.

DISCUSSION

It is unwise to rely on a single test for distinguishing between two species of micro-organisms and the nitratase test is inclined to give equivocal results depending on the technique used (Cowan & Steel, 1965) and the interpretation of the worker. An additional, or 'back-up' test is desirable.

The results of this investigation show that sensitivity to ciprofloxacin satisfies this requirement. In testing 113 strains the nitratase test gave a wrong identification of two and the ciprofloxacin sensitivity test the wrong identification in one. Neither test was of assistance in excluding two strains that were neither *M. fortuitum* nor *M. chelonei*.

The addition of a single tube of LJ medium containing 3·0 mg/l of ciprofloxacin in the routine tests used in the examination of rapidly growing mycobacteria is clearly of value in the identification of these two species. We would suggest, however, that strains of rapidly growing mycobacteria that are (1) nitratase-negative and ciprofloxacin-sensitive, and (2) nitratase-positive and ciprofloxacin-resistant, are submitted to a Reference Laboratory for lipid analysis.

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REFERENCES

Bonicke, R. (1962). Report on identification of mycobacteria by biochemical methods. *Bulletin of the International Union Against Tuberculosis* **32**, 13–68.

Collins, C. H., Grange, J. M. & Yates, M. D. (1985). *Organization and Practice in Tuberculosis Bacteriology*, pp. 74–76. London: Butterworths.

Collins, C. H. & Lyne, P. M. (1984). *Microbiological Methods*, 5th ed. pp. 110, 389–390. London: Butterworths.

Collins, C. H. & Uttley, A. H. C. (1985). *In vitro* susceptibility of mycobacteria to ciprofloxacin. *Journal of Antimicrobial chemotherapy*. (In the press.)

Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. pp. 33–34. Cambridge University Press.

Gordon, R. E. & Smith, M. M. (1953). Rapidly growing, acid-fast bacteria. I. Species description of *Mycobacterium phlei* Lehmann and Neumann and *Mycobacterium smegmatis* (Trevisan) Lehmann and Neumann. *Journal of Bacteriology* **66**, 41–48.

Grange, J. M. (1980). *Mycobacterial Diseases*, pp. 85–86, 98–99. London: Edward Arnold.

Jenkins, P. A. (1981). Lipid analysis for the identification of mycobacteria: an appraisal. *Reviews of Infectious Diseases* **3**, 862–866.

Kubica, G. P. (1973). Differential identification of mycobacteria. VII. Key features for identification of clinically significant mycobacteria. *American Review of Respiratory Disease* **107**, 9–21.

Marks, J. & Szulga, T. (1965). Thin layer chromatography of mycobacterial lipids as an aid to classification. Technical procedures: *Mycobacterium fortuitum*. *Tubercle* **46**, 400–403.

Virtanen, S. (1960). A study of nitrate reduction by mycobacteria. *Acta Tuberculosea Scandinavica* **48**, Supplement 1.