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Simple, New Test for Rapid Differentiation of the *Mycobacterium fortuitum* Complex

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A simple, new test to differentiate species in the *Mycobacterium fortuitum* complex by determining susceptibility to pipemidic acid is described. A 20-μg pipemidic acid disk provides a rapid and reliable means of distinguishing *M. fortuitum* from *M. chelonei*.

Mycobacterium fortuitum and *M. cheloni* are two species of the *M. fortuitum* complex presently considered to be pathogenic for humans (1). Differential diagnosis between the two species is usually based on two biochemical tests: nitrate reduction (5) and iron uptake (6).

In this communication we describe a possible new differentiating test based on the susceptibility of each strain to pipemidic acid.

Thirty-seven strains of *M. fortuitum* (four strains were received as *M. pereginum*) (1, 3) and 11 strains of *M. chelonei* were included in the study. The cultures used came from our department and from various international collections: the National Collection of Type Cultures (NCTC), London, England; the Trudeau Mycobacterial Culture Collection (TMC), Saranac Lake, N.Y.; and the Czechoslovak Collection of Microorganisms (CCM), Brno, Czechoslovakia. Cultures also came from the collections of investigators in the field (L. Eidus and A. Laszlo, Laboratory Centre for Disease Control, Ottawa, Ontario, Canada; R. Gordon, Rutgers University, New Brunswick, N.J.; P. A. Jenkins, Tuberculosis Reference Laboratory, Wales, Great Britain; J. Viallier, Hôpital J. Courmont P. Bènite, Lyon, France; I. Tarnok, Forschungsinstitüt, Borstel, Germany; H. David, Institut Pasteur, Paris, France; G. Sabater, Hospital Militar, Valencia, Spain; H. Saito, Shimane Medical College, Isumo, Shimane, Japan; E. Mankiewicz, Montreal Centre, Quebec, Canada) and from our own laboratory.

Identification of *M. fortuitum* and *M. chelonei* species was confirmed on the basis of the nitrate reduction and iron uptake tests.

The disks used contained 20 μg of pipemidic acid (Bio-Merieux). Disk diffusion tests were made on plates containing Müeller-Hinton agar (Difco Laboratories). The plate was evenly covered with an inoculum of a 1/1,000 seed suspension. The seed suspension was obtained from an emulsion in distilled water of organisms incubated for 7 days at 37°C on enriched Middlebrook 7H10 agar (Difco) and had an opacity equivalent to 1 on MacFarland's scale. Plates were examined on days 2 and 5. All tests were repeated two or more times to confirm reproducibility.

In the interpretation of results, a strain was considered resistant when its growth was not inhibited by pipemidic acid. Inhibition was measured as the diameter of the inhibitory zone in millimeters. If the diameter of the inhibitory zone was 10 mm or more, we considered that the species was inhibited.

The results are recorded in Table 1. The amount of pipemidic acid on the disk, the number and percentages of strains inhibited, and the range and average diameters of the resulting inhibitory zones are listed.

All strains of *M. chelonei* were resistant to pipemidic acid. In contrast, all strains of *M. fortuitum* were suspectible, with an average inhibitory zone diameter of 21.5 mm.

Antibiotic disk susceptibility is a rapid and convenient method of recognizing numerous bacterial species (4).

The results of this study show that pipemidic acid has the additional advantage of aiding in differentiating *M. fortuitum* from *M. chelonei*. Susceptibility to this agent is markedly different in these two species. Using a concentration of 20 μg of pipemidic acid per disk, *M. fortuitum* complex strains that are sensitive can be presumed to be *M. fortuitum* while resistant strains are

TABLE 1. *Activity of pipemidic acid against the M. fortuitum complex*

Species	Disk content (μg)	No. of strains tested	No. (%) of strains inhibited	Avg diam of inhibitory zone	
				Range (mm)	Mean (mm)
M. fortuitum[a]	20	37	37 (100)	10–36	21.5
M. chelonei[b]	20	11	0 (0)	0	0

[a] All strains of *M. fortuitum* tested for nitrate and iron uptake were positive.

[b] All strains of *M. chelonei* tested for nitrate and iron uptake were negative.

likely to be *M. chelonei*. Differentiation of the two species is important because of the increasing incidence of the *M. fortuitum* complex in human infections (2).

This test is quick, economical, and effective. It does not require the preparation of reagents, and the results are easily interpreted. We believe that it is a valuable test that can be used routinely in any clinical microbiology laboratory that handles mycobacteria.

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