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Ability of Ciprofloxacin but Not Pipemidic Acid To Differentiate All Three Biovariants of *Mycobacterium fortuitum* from *Mycobacterium chelonae*

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When tested against 312 clinical isolates of rapidly growing mycobacteria, the quinolone pipemidic acid correctly separated *Mycobacterium chelonae* (*M. chelonei*) from *Mycobacterium fortuitum* biovar *fortuitum* but not from biovar *peregrinum* or the third biovar complex. The new 4-quinolone ciprofloxacin correctly separated all three biovars of *M. fortuitum* from *M. chelonae* and appears to provide a better taxonomic test.

The observation that there are major differences in antimicrobial susceptibility between the two pathogenic species of rapidly growing mycobacteria, *Mycobacterium fortuitum* and *Mycobacterium chelonae* (formerly *M. chelonei*) (8, 9), has stimulated greater interest in the separation of the two species in the clinical laboratory. The best-known biochemical tests which differentiate these two species are those of nitrate reduction and iron uptake (4, 7). *M. fortuitum* gives a positive reaction to both tests, but *M. chelonae* gives a negative reaction. More recently, disk diffusion susceptibility to polymyxin B and pipemidic acid on Mueller-Hinton agar has also been used. Polymyxin B is a basic peptide antibiotic, and pipemidic acid is a quinolone antibiotic related to nalidixic acid. Several studies of *M. fortuitum* have shown this species to give zones of inhibition of ≥10 mm to polymyxin B, whereas isolates of the two subspecies of *M. chelonae* give no zones of inhibition (11, 12). Casal and Rodriguez tested 37 strains of *M. fortuitum* and found all to be susceptible to pipemidic acid, whereas the 11 strains of *M. chelonae* were resistant. These isolates were identified to the species level only (1, 2). Recently, we noted that not all strains belonging to *M. fortuitum* are susceptible to pipemidic acid, and this difference is related to the biovar group to which the isolate belongs. This difference was not noted, however, with the new related quinolone, ciprofloxacin.

Clinical strains of *M. fortuitum* (n = 162) and *M. chelonae* (n = 150), referred for susceptibility testing between 1979 and 1985, were studied. Identification was performed by the Mycobacteriology Reference Section of the Centers for Disease Control by standard methods (6). Of the 162 isolates of *M. fortuitum*, 120 belonged to biovar *fortuitum*, 9 were biovar *peregrinum*, and 33 belonged to the unnamed third biovar complex (6). The 150 *M. chelonae* isolates included 111 subsp. *abscessus* and 39 subsp. *chelonae*. Five laboratory strains including four type strains were studied: *M. fortuitum* biovar *fortuitum* ATCC 6841, *M. fortuitum* biovar *peregrinum* ATCC 14467, *M. chelonae* subsp. *abscessus* ATCC 19977, *M. chelonae* subsp. *chelonae* ATCC 35752, and *M. fortuitum* biovar *peregrinum* ATCC 35755. No type strain of the third biovar complex of *M. fortuitum* has been identified.

Disk diffusion susceptibility testing was performed as previously described (9, 11) by using Mueller Hinton agar whose surface had been swabbed with Middlebrook OADC (oleic acid, albumin, dextrose, catalase) enrichment. A commercial disk of pipemidic acid (20 μg) (kindly provided by the Mycobacterial Reference Section of the Centers for Disease Control) or ciprofloxacin (5 μg) (Miles Pharmaceuticals, West Haven, Conn.) was added to each plate. After incubation for 72 h in room air at 30 or 35°C, the zone of inhibition around the disk was measured. Resistance was defined by the presence of a <10-mm zone, and susceptibility was defined by the presence of a ≥10-mm zone, according to Casal and Rodriguez (1). Experience with the disks revealed that not all *M. fortuitum* isolates gave clear zones of inhibition to pipemidic acid, but partial zones of inhibition to pipemidic acid were never seen with *M. chelonae*. For this reason, zones of definite but only partial inhibition were considered susceptible.

In contrast, partial zones of inhibition to ciprofloxacin were seen with some isolates of *M. chelonae* but never with *M. fortuitum*. Thus, for ciprofloxacin only clear zones of inhibition were recorded.

A total of 312 clinical isolates were tested with pipemidic acid (Table 1). The 120 strains of *M. fortuitum* biovar *fortuitum* all gave zones of inhibition of ≥10 mm with a mean zone size ± standard deviation of 18.7 ± 5.9 mm (range, 10 to 34 mm). The type strain was susceptible. Thirty-two strains of the third biovar complex of *M. fortuitum* were resistant with no zones of inhibition. Only one strain showed a partial zone of inhibition (6 to 13 mm). There was a variable result among strains of *M. fortuitum* biovar *peregrinum*. The type strain was susceptible, but a Trudeau Culture Collection strain (ATCC 35755) was resistant. All 150 clinical isolates of *M. chelonae* and the type strain of subsp. *abscessus* were resistant with no zone of inhibition, whereas the type strain of subsp. *chelonae* was susceptible.

A total of 134 clinical isolates were tested against ciprofloxacin (Table 2). Isolates from the three biovars of *M. fortuitum* were uniformly susceptible to ciprofloxacin with clear zone diameters of 23 to 78 mm, whereas all isolates of *M. chelonae* were resistant. Of 58 isolates of the latter, 11 gave partial zones, but none gave clear zones. Although all three biovars of *M. fortuitum* were susceptible to ciprofloxacin, there was a difference in the size of the zones of inhibition. The 54 strains of biovar *fortuitum* gave a mean

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TABLE 1. Disk zone diameters to pipemidic acid for 312 clinical isolates of *M. fortuitum* and *M. chelonae*

Organism (no. of strains)	No. of strains for each zone size (mm)					
	No zone	7–9	10–14	15–19	20–29	≥30
M. fortuitum						
biovar *fortuitum* (120)	0	0	34	46	30	10
biovar *peregrinum* (9)	5	0	1	1	2	0
third biovar complex (33)	32	0	1	0	0	0
M. chelonae						
subsp. *chelonae* (39)	39	0	0	0	0	0
subsp. *abscessus* (111)	111	0	0	0	0	0

TABLE 2. Disk zone diameters to ciprofloxacin for 134 clinical isolates of *M. fortuitum* and *M. chelonae*

Organism (no. of strains)	No. of strains for each zone size (mm)					
	No zone	7–20	21–30	31–44	45–59	≥60
M. fortuitum						
biovar *fortuitum* (54)	0	0	0	4	38	12
biovar *peregrinum* (7)	0	0	1	1	4	1
third biovar complex (15)	0	0	0	14	1	0
M. chelonae						
subsp. *chelonae* (18)	18	0	0	0	0	0
subsp. *abscessus* (40)	40	0	0	0	0	0

zone size ± standard deviation of 55.2 ± 6.9 mm (range, 37 to 74 mm), and 15 strains of the third biovar complex gave zones of 39.7 ± 3.8 mm (range, 32 to 47 mm). There was a more variable result with biovar *peregrinum*, which showed zones of 51.4 ± 17.1 mm (range, 23 to 78 mm).

Previous studies of *M. fortuitum* have found all isolates to be susceptible to pipemidic acid (1, 2, 5). In the study by Casal and Rodriguez, the isolates were not identified to the biovar level, so it is not possible to determine whether isolates from biovars other than *fortuitum* were present (1). Lévy-Frébault et al. (5) identified nine isolates of biovar *fortuitum*, eight isolates of biovar *peregrinum*, and two isolates of the third biovar complex (according to our classification scheme) and found all to be susceptible to pipemidic acid. The type strains of biovar *fortuitum* (ATCC 6841) and biovar *peregrinum* (ATCC 14467) were reported as susceptible, a finding confirmed by our study. The reason for the differences in the other isolates of biovar *peregrinum* and the third biovar complex is not known. Most of their isolates other than the type strains were environmental (six of nine), compared with our study, in which no environmental isolates were studied, and the geographic sources (France and the southwestern United States) were different, factors which may be important.

Although all clinical isolates of *M. chelonae* and the type strain of subsp. *abscessus* (ATCC 19977) were resistant to ciprofloxacin, the type strain of subsp. *chelonae* (ATCC 35752) was susceptible. This strain was also tested by the Lévy-Frébault group (as NCTC 946) (5) with the same results. This strain is a laboratory strain that has been passaged multiple times over many years.

In a recent study of 76 disease-producing isolates of *M. fortuitum* from the southwestern United States, 17% belonged to the third biovar complex and 4% belonged to biovar *peregrinum* (10). Based on the prevalence of these biovars in human disease and the frequency with which they are resistant to pipemidic acid (88%), this test cannot be used in the Southwest to differentiate between *M. fortuitum* and *M. chelonae* with an acceptable degree of accuracy.

In contrast to pipemidic acid, ciprofloxacin was uniformly successful in differentiating these two species, although fewer strains were tested. Collins et al. (3) studied 113 isolates of *M. fortuitum* and *M. chelonae* identified to species only and found that susceptibility to an agar dilution concentration of 3 μg/ml accurately separated 99% of the isolates. These results fit well with the results of the current study.

We (R. J. Wallace, Jr., J. M. Swenson, and V. A. Silcox, Antimicrobic Newsl. 2:85–92, 1985) and others (3) have stressed the need for a second test in addition to the usual nitrate reduction for separation of *M. fortuitum* and *M. chelonae*. Both iron uptake and susceptibility to polymyxin B have been used and have been recently reviewed (Wallace et al., Antimicrobic Newsl.). The current study and the previous one by Collins et al. (3) strongly suggest that susceptibility to ciprofloxacin will provide an equally accurate taxonomic test, although it does not appear to improve on the better-known polymyxin disk method.

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