Antimicrobial Susceptibility Testing of Mycobacterium fortuitum Complex

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A total of 24 strains of the Mycobacterium fortuitum complex were tested for susceptibility to antimicrobial agents by the disk diffusion and agar dilution techniques. By comparing zones of inhibition obtained with the disk diffusion technique with results of minimal inhibitory concentration determinations, it was shown that disk diffusion results could predict in vitro susceptibility to selected antimicrobial agents. All of 17 strains of M. fortuitum were susceptible to ≤1 μg of amikacin per ml. The corresponding average zone of inhibition around a 10-μg amikacin disk was 37 mm. Seven M. chelonei strains were more resistant to amikacin, with minimal inhibitory concentrations ranging from 1 to 32 μg/ml, and the corresponding average zone size was 21 mm. Susceptibility of both M. fortuitum and M. chelonei to tetracycline was variable and none of the M. chelonei strains was inhibited by polymyxin B, whereas M. fortuitum strains consistently had zones of inhibition around the polymyxin disk. It appears that identification to species of the M. fortuitum complex may be of importance with regard to antibiotic susceptibility. Separation of M. fortuitum and M. chelonei was readily accomplished in the present study by the nitrate reduction and 3-day arylsulfatase tests.

Organisms belonging to the Mycobacterium fortuitum complex have been implicated as the cause of a variety of conditions. M. fortuitum has been associated with pulmonary infections which are typically present as tuberculous lung disease (6), and, more commonly, with various types of wound infections (8, 10, 12). M. chelonei has been found to be the cause of infections due to implantation of contaminated porcine prosthesis heart valves (3).

Treatment of infections caused by these mycobacteria has been complicated by their resistance to antimicrobial agents. Antituberculous drugs are notably ineffective against M. fortuitum. Recent in vitro studies indicate that amikacin may be effective against organisms of the M. fortuitum complex (5, 11), and amikacin has been successfully used in the treatment of two cases of septic arthritis due to M. fortuitum (2).

The present study was undertaken to determine the susceptibility of additional strains of the M. fortuitum complex to amikacin and other antimicrobial agents. The need for a practical approach to antimicrobial susceptibility testing of clinical isolates representing the M. fortuitum complex was also investigated, and it was found that a disk diffusion method may provide valid antimicrobial susceptibility results.

MATERIALS AND METHODS

Organisms and cultivation. M. fortuitum and M. chelonei strains included in the study were provided by E. Runyon. Five of the strains were originally obtained from Ruth Gordon of Rutgers University. Other cultures, originally obtained through the Center for Disease Control, were isolates from wounds, joint fluid, porcine heart valves, peritoneal fluid, corneas, and environmental sources. The organisms were maintained on Lowenstein-Jensen medium (Pasco Laboratories, Wheatridge, Colo.), and stock slants were stored at 4°C. Inocula were transferred from the stock slants to Dubos broth (Difco Laboratories, Detroit, Mich.), and the cultures were incubated at 35°C for 48 h or until visible growth occurred.

Susceptibility tests. Dubos broth cultures were diluted in Mueller-Hinton broth (Difco) and Kirby-Bauer (1) disk diffusion tests were performed as described by Maizan and Barry (8) on Mueller-Hinton agar plates (Difco medium prepared by Bakke Bennett, Berkeley, Calif.). The media were carefully controlled to insure a depth of 4 to 5 mm and a pH of 7.3. A battery of antimicrobial disks (Baltimore Biological Laboratory, Cockeysville, Md.) routinely used for testing gram-negative organisms was applied to the plates. Zones of inhibition were read after 48 h of incubation (35°C) in most cases. Agar dilution tests were performed by the methods described by Washington and

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Barry (12). Difco Mueller-Hinton agar plates were prepared containing various concentrations of amikacin (Bristol Laboratories, Syracuse, N.Y.); tetracycline (Pfizer Inc., Brooklyn, N.Y.); chloramphenicol (Parke, Davis & Co., Detroit, Mich.), or gentamicin (Schering Corp., Kenilworth, N.J.). These plates were used within 24 h of preparation. Results of agar dilution tests were read after 48 h of incubation except when strains showed unusually slow growth. A faint haze or growth of only one or two colonies was interpreted no growth in determining the minimal inhibitory concentrations (MICs) (13).

Biochemical tests. M. fortuitum and M. chelonei were differentiated on the basis of a rapid nitrate reduction test and the 3-day arylsulfatase test. The nitrate reduction test was performed with Difco nitrate test strips. The Wayne modification of the 3-day arylsulfatase test (14) was employed, utilizing Wayne sulfatase medium (Baltimore Biological Laboratory). If equivocal or questionable results were obtained from the nitrate and 3-day arylsulfatase tests, iron uptake (14) was determined to rule out M. chelonei.

RESULTS

Susceptibility measured by agar dilution tests. All M. fortuitum strains were susceptible to 1 µg of amikacin per ml by agar dilution testing, and 94% of the strains were inhibited by 0.5 µg of amikacin per ml (Table 1). M. chelonei strains were less susceptible to amikacin; a concentration of 32 µg/ml was required to inhibit 100% of the strains tested, and 57% were susceptible to 8 µg/ml. Tetracycline was less effective than amikacin for both M. fortuitum and M. chelonei, and M. chelonei was again more resistant than M. fortuitum. A tetracycline concentration of 16 µg/ml inhibited 100% of the M. fortuitum strains but only 29% of the M. chelonei strains. Chloramphenicol was also tested against M. fortuitum and M. chelonei and found to be relatively ineffective against both species. Gentamicin was less effective than amikacin against M. fortuitum, but 90% of the strains were inhibited by a gentamicin concentration of 8 µg/ml. This concentration of gentamicin also inhibited three of three M. chelonei strains tested.

Susceptibility measured by disk diffusion tests. The average zone sizes around antibiotic-containing disks reflected the susceptibility of the isolates as determined by agar dilution testing (Table 2). The average zone diameter around the amikacin disks was 37 mm for M. fortuitum and 21 mm for M. chelonei. Other antibiotics produced smaller zone sizes when tested against both M. fortuitum and M. chelonei with the exception of kanamycin, which appeared to be more effective against M. chelonei than amikacin.

A linear relationship between disk zone size and agar dilution MIC was observed with amikacin (Fig. 1) and gentamicin (Fig. 2) when

| Table 2. Antimicrobial susceptibility of strains of the M. fortuitum complex as determined by disk diffusion testing |
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| Antimicrobial agent* | Organism | Avg zone diam* |
| Chloramphenicol | M. fortuitum | 6–36 | 12 |
| Tetracycline | | 11–46 | 23 |
| Kanamycin | | 24–39 | 31 |
| Polymyxin B | | 13–19 | 16 |
| Gentamicin | | 16–32 | 22 |
| Amikacin | | 30–44 | 37 |
| Tobramycin | | 10–26 | 16 |
| Chloramphenicol | M. chelonei | 6–22 | 12 |
| Tetracycline | | 6–41 | 20 |
| Kanamycin | | 27–38 | 31 |
| Polymyxin B | | 6–7 | 6 |
| Gentamicin | | 12–21 | 16 |
| Amikacin | | 11–32 | 21 |
| Tobramycin | | 14–29 | 19 |

*Antimicrobial disk content (in micrograms): chloramphenicol, 30; tetracycline, 30; kanamycin, 30; polymyxin B, 300; gentamicin, 10; amikacin, 10; tobramycin, 10.

*Average zone measurements were based on two or more independent determinations.
results with *M. fortuitum* and *M. chelonei* were combined. This relationship indicates a good correlation between agar dilution and disk diffusion susceptibility results for these aminoglycoside antibiotics. Organisms with amikacin MICs of 1 μg/ml or less produced zone diameters of ≥30 mm. Those with MICs of 8 μg/ml or greater produced zone sizes of 16 mm or less. Similar results were obtained with gentamicin; organisms with MICs of 1 μg/ml or less had zone diameters of ≥28 mm. Isolates with MICs of 8 μg/ml or greater produced zones of 18 mm or less. Therefore, for aminoglycoside antibiotics there appears to be a good correlation between disk zone size and agar dilution MIC.

**DISCUSSION**

Recent reports have indicated that strains of the *M. fortuitum* complex may be susceptible to amikacin (4, 10). Sanders et al. (10) found that some strains of the *M. fortuitum* complex were susceptible to ≤3.1 μg of amikacin per ml. They also found that 50% of their isolates were resistant to ≥25 μg of amikacin per ml. Their results are in contrast to those of Dalovisio and Pankey (4), who found that 100% of their strains of the *M. fortuitum* complex were susceptible to ≤4 μg of amikacin per ml. Our results support the findings of Sanders et al. because we found that some *M. chelonei* strains of the *M. fortuitum* complex were resistant to amikacin, whereas all the *M. fortuitum* strains were highly susceptible. Although Sanders et al. did not distinguish between *M. fortuitum* and *M. chelonei*, it is possible that their resistant strains may have been *M. chelonei*.

*M. fortuitum* has also been considered to be susceptible to tetracycline (5). However, we found that 50% of the *M. fortuitum* strains required tetracycline concentrations of ≥8 μg/ml for inhibition, and 70% of *M. chelonei* strains were inhibited only by concentrations of ≥32 μg/ml. In addition, chloramphenicol is relatively ineffective against *M. fortuitum* and *M. chelonei*, but kanamycin and gentamicin may have significant activity against both species. These findings indicate the importance of identification to species and susceptibility testing of isolates of the *M. fortuitum* complex since susceptibility to antimicrobial agents appears to be unpredictable. We found the nitrate reduction and 3-day arylsulfatase tests to be of value for identification to species. Our findings also suggest that susceptibility to polymyxin may provide an aid for the identification of these mycobacteria. *M. fortuitum* strains had zones of inhibition around the polymyxin disks, whereas *M. chelonei* strains were completely resistant to the drug.

Susceptibility testing of *M. fortuitum* and *M. chelonei* isolates can be accomplished by the agar dilution method or possibly by a disk diffusion method. The present studies suggest that the standard disk diffusion method can be used to test rapidly growing mycobacteria for susceptibility to selected antibiotics. In using the disk diffusion method special care must be exercised in preparing standardized suspensions of organisms and in reading the zone sizes. Suspensions made directly from solid media were unsatisfactory, but after growth in Dubos broth, suspensions of uniform turbidity could be made. Most of the strains grew sufficiently well on Mueller-Hinton agar to produce measurable zones within 48 h of inoculation. The aminoglycoside antibiotics and tetracycline gave distinct zones of inhibition, but other agents, including trimethoprim-sulfamethoxazole, chloramphenicol, and erythromycin, produced indistinct zones. Therefore, it is not recommended that these agents be tested by disk diffusion.
Since the zones of inhibition were often large, we recommend that only a few antibiotics be tested on each plate. Amikacin, gentamicin, kanamycin, and tetracycline may be effective agents against the *M. fortuitum* complex. These agents can be conveniently tested on 150-mm plates by using the standard disks available for the Kirby-Bauer procedure. Although additional studies with larger numbers of organisms are needed, our data suggest that disk diffusion susceptibility results may provide a useful guide for antimicrobial therapy of infections due to strains of the *M. fortuitum* complex.

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LITERATURE CITED