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Susceptibility of Organisms in the *Mycobacterium fortuitum* Complex to Antituberculous and Other Antimicrobial Agents

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Of 21 antimicrobial agents tested in vitro, amikacin was the most predictably active against clinical isolates belonging to the *Mycobacterium fortuitum* complex; however, only 50% of strains studied were susceptible to clinically attainable concentrations of the drug.

Organisms belonging to the *Mycobacterium fortuitum* complex of rapidly growing mycobacteria have been implicated as causes of bronchopulmonary, wound, and superficial cutaneous infections (1, 3-5, 7, 9). Most clinical isolates have been highly resistant to available antituberculous drugs and other antimicrobial agents (1, 4, 5, 7). The recent report of two outbreaks of postoperative wound infections and a sharp increase in highly resistant isolates from wound specimens submitted to our laboratory from a plastic surgery service highlight the need for antimicrobial agents with activity against these organisms (6). For this reason, we assayed in vitro the activity of 21 drugs against 9 to 11 clinical isolates belonging to the *M. fortuitum* complex.

All isolates were from patients in the state of Florida. Most were repeatedly isolated and implicated as causes of bronchopulmonary disease by the attending physician; however, their etiologic role often was not documented by biopsy or other more definitive procedures. One isolate was responsible for a lethal disseminated infection in a patient with leukemia. Strains were identified by standard methods (8, 11): six were *M. fortuitum*; three, *M. chelonei*; and two, other species. Results did not differ in comparisons between organisms grouped as *M. fortuitum* or other species; therefore, they will be presented for the "*M. fortuitum* complex" only. Immediately after initial isolation, strains were tested for susceptibility to available antituberculous drugs on solid medium with a single concentration of drug and a standardized inoculum (2). They were then stored under refrigeration on Middlebrook 7H10 or 7H11 medium. Susceptibility to isoniazid, rifampin, viomycin, the cephalosporins, everninomicin, streptomycin, kanamycin, gentamicin, sisomicin, amikacin, and erythromycin was also determined by a broth dilution assay, which permitted detection of bacteriostatic and bactericidal end points. The inocula were prepared as follows. Strains were subcultured into Dubos Tween-Albumin liquid medium and incubated for 7 to 10 days at 37°C in 7% CO_2 in air. Each culture was mixed thoroughly (Vortex mixer) once daily. The cultures were then adjusted to comparable optical density (0.48 at 475 nm) with Dubos Tween-Albumin liquid to yield approximately 1 mg/ml wet weight. A 0.1-ml portion of each culture (approximately 5×10^5 colony-forming units) was used as an inoculum for the dilution assay. The drugs were incorporated by serial twofold dilution into Proskauer and Beck liquid medium with glycerol (final volume, 5 ml per tube). Two drug-free control tubes were included for each strain tested. All cultures were examined and mixed thoroughly once daily during incubation at 37°C in 7% CO_2 in air. Results of tests were determined when heavy growth was noted in the drug-free control cultures. End points established by the end of the first week were identical to those obtained after incubation for 2, 3, and 4 weeks. The minimum inhibitory (bacteriostatic) concentration was defined as the lowest concentration of drug that completely inhibited visible growth of the microorganisms. Minimum bactericidal concentration was determined as follows. Portions (0.02 ml) from all clear tubes were subcultured onto Dubos oleic acid-albumin agar plates and incubated for 3 weeks. The lowest concentration of drug that completely prevented growth on the subculture was recorded as the minimum bactericidal concentration. Results obtained by the two methods of susceptibility testing were remarkably consistent. Strains that were considered susceptible

(or resistant) by the standard agar method were uniformly susceptible (or resistant) by the broth dilution procedure.

Each of the 21 drugs studied was selected for one of two reasons: either the drug or one of its congeners was known to be active against at least some mycobacteria, or the drug had not been evaluated previously against rapidly growing mycobacteria in vitro. The range of concentrations of individual drugs to be tested was chosen to ensure inclusion of (i) levels usually attainable in serum (e.g., amikacin) or (ii) the critical concentration for definition of resistance, if well established (e.g., ethambutol).

Fifteen of the drugs were devoid of activity against all isolates as determined by either agar or broth dilution assays (Table 1). The remaining six drugs inhibited growth of one or more of the isolates in concentrations that might be attainable therapeutically (Table 2). Of these, amikacin inhibited the most strains (5 of 10) and was the most active of the aminoglycosides within the ranges of clinically attainable concentrations of each. In general, minimum bactericidal concentrations of the aminoglycosides were equivalent to minimum inhibitory concentrations. Erythromycin was bacteriostatic only. None of the 21 drugs tested was active in vitro against five of the isolates.

Infections with the rapidly growing mycobacteria may pose difficult diagnostic and therapeutic problems. Many of these organisms are ubiquitous in the environment (4, 12). They may colonize body surfaces or contaminate clinical specimens. Some superficial infections have been reported to resolve spontaneously (4). Other infections may progressively invade locally or disseminate. Many of these invasive strains have been resistant to all available antituberculous agents (1, 4, 5). For this reason, surgical excision of lesions, whenever possible, and empiric combinations of antimicrobial agents have been advocated as therapy. Combinations of oxacillin plus kanamycin, rifampin plus erythromycin, and four to six antituberculous agents have been prescribed; none of these appear to have been uniformly successful (1, 4, 6). A few isolates have been reported susceptible to relatively high concentrations of tetracycline, but there is little experience with use of this drug in infections of humans (1). In general, reports of results of drug treatment have been scant. Suffice it to say, no single agent or combination of drugs has been proven to be predictably active in vitro or in therapy.

Results of the present study suggest that amikacin and possibly other aminoglycosides should be included in tests of susceptibility of rapidly growing mycobacteria to antimicrobial agents. Although amikacin has not been used for treatment of infections due to organisms in the *M. fortuitum* complex, physicians may wish to consider use of this drug when (i) antimicrobial therapy is indicated, (ii) the infecting strain is resistant to all available antituberculous drugs, and (iii) informed consent is obtained from the patient. Because of the recent outbreaks of nosocomial infections due to highly resistant strains, every effort should be

TABLE 1. *Drugs that demonstrated no activity at the highest concentration tested against clinical isolates of the M. fortuitum complex*[a]

Drug (no. of strains tested)	Highest concn (μg/ml) of drug used
Isoniazid (11)	25
p-Aminosalicylic acid (11)	10
Rifampin (10)	16
Ethambutol (9)	5
Ethionamide (10)	5
Cycloserine (9)	20
Capreomycin (9)	5
Viomycin (11)	25
Cephalothin (9)	100
Cephalexin (9)	100
Cephaloridine (9)	100
Cephaloglycin (9)	100
Cefazolin (9)	100
Cephapirin (9)	100
Everninomicin (10)	100

[a] Heavy growth of the inoculum was noted in the highest concentration of drug used. Results are presented for broth dilution assays, except for ethambutol, ethionamide, cycloserine, and capreomycin, which were tested in a single concentration in agar only.

TABLE 2. *Minimum inhibitory concentrations (MIC) of six drugs for clinical isolates belonging to the M. fortuitum complex*[a]

Drug (no. of strains tested)	No. of strains with MIC of:							
	0.4	0.8	1.6	3.1	6.2	12.5	25	>25
Streptomycin (10)	0	0	0	1	0	1	0	8
Kanamycin (9)	0	0	0	0	1	1	1	6
Gentamicin (10)	0	0	1	1	1	1	4	2
Sisomicin (10)	0	0	0	1	1	2	2	4
Amikacin (10)	0	3	0	2	0	0	2	3
Erythromycin (11)	0	0	0	0	1	0	1	9

[a] Minimum bactericidal concentrations were equivalent to, or no more than twofold greater than, minimum inhibitory concentrations in the broth dilution bioassay.

made to evaluate potentially useful therapeutic regimens and to report results promptly.

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