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Rapidly Growing Mycobacteria: Testing of Susceptibility to 34 Antimicrobial Agents by Broth Microdilution

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A total of 18 strains of *Mycobacterium fortuitum*, 15 strains of *M. chelonei*, and 31 strains of *M. chelonei*-like organisms were tested by both broth microdilution and agar dilution to determine their susceptibility to 34 antimicrobial agents. All strains grew well enough in cation-supplemented Mueller-Hinton broth for endpoints to be read after 72 h of incubation. Some strains of *M. chelonei* did not grow on Mueller-Hinton agar. A few discrepancies were noted between the broth and agar procedures. For *M. fortuitum*, doxycycline, minocycline, amikacin, sulfamethoxazole, and sulfamethoxazole-trimethoprim were the most active agents. For *M. chelonei*, amikacin, sisomicin, tobramycin, and erythromycin were the most active agents. The *M. chelonei*-like organisms were most susceptible to ampicillin, doxycycline, minocycline, amikacin, erythromycin, sulfamethoxazole, and sulfamethoxazole-trimethoprim. Broth microdilution appears to be a reliable method for susceptibility testing of rapidly growing mycobacteria, although clinical studies are needed to determine how well in vitro results correlate with therapeutic in vivo outcome.

Mycobacterium fortuitum and *M. chelonei*, two frequently isolated, rapidly growing mycobacteria, have been implicated in several forms of clinical disease (25). Skin and soft tissue infections are the most common of these, but pulmonary, skeletal, and disseminated infections also have been reported (25). The severity of the infections caused by these organisms varies from mild, localized cutaneous disease to serious, potentially fatal pulmonary and soft tissue infections (2, 10). Outbreaks of wound infection after open-heart surgery have also been reported (11).

These organisms are known to be resistant to most of the antimycobacterial agents (25); but until recently, antimicrobial therapy, if used, has usually been with the antimycobacterial agents. Within the past few years, several investigators have reported that other antibacterial agents are active against these organisms (5, 6, 22, 23). Clearly, however, there are problems involved with therapy and diagnosis of infections with the rapidly growing mycobacteria. In some cases, disease caused by *M. fortuitum* or *M. chelonei* has been reported to resolve spontaneously or with only surgical intervention (10), but in other cases, long-standing disease has resolved or improved only when antimicrobial agents that had been shown to be active in vitro were used (8, 12, 15, 22; R. J. Wallace, Jr., E. J. Septimus, V. A. Silcox, and J. Swenson, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., abstr. no. 403, 1980).

Dalovisio and Pankey have reviewed some of the problems with diagnosis and therapy of these infections, and they suggest that treatment with antimicrobial agents may decrease the severity of the infection and that, since these isolates are resistant to many of the antimycobacterial drugs, susceptibility testing may be necessary to choose the most effective and least toxic drug (7).

A variety of methods have been used to test the susceptibility of these organisms to antimicrobial agents, making comparisons of interlaboratory methods and analyses of results difficult.

Because broth microdilution is the method for determining minimum inhibitory concentrations (MICs) that appears to have a potential for clinical usefulness and acceptance by laboratory personnel, we chose to use it to determine the susceptibility of strains of *M. fortuitum*, *M. chelonei*, and a group of organisms designated *M. chelonei*-like (resembling *M. chelonei* but as yet unnamed [18]) to 34 antimicrobial agents. The activity of some antimicrobial agents against the same mycobacterial species was also tested by agar dilution; these results were compared with those obtained by broth microdilution.

MATERIALS AND METHODS

Organisms. A total of 18 strains of *M. fortuitum*, 15 strains of *M. chelonei*, and 31 strains of *M. chelonei*-like organisms were obtained from the Mycobacteriology Branch at the Centers for Disease Control. Sources of the strains (with numbers of strains given parenthetically) were as follows: *M. fortuitum*—sternal wound (5), breast prosthesis (1), sputum (3), urine (1), pacemaker insertion (1), wound or ulcer (4), blood (1), and other (2); *M. chelonei*—sternal wound (3), breast prosthesis (1), sputum (1), heart valve (3), wound or ulcer (3), cornea (2), pulmonary (1), and unknown (1); *M. chelonei*-like strains—peritoneal fluid (19), dialysis machine (6), sputum (2), wound or ulcer (1), water (1), and unknown (2). Most of the *M. chelonei*-like strains were isolated during an outbreak in a peritoneal dialysis unit in Seattle, Wash., as reported by Band et al. (1).

The organisms were maintained on Lowenstein-Jensen slants at room temperature until testing was performed. To prepare the inoculum for both the broth and the agar tests, the strains were subcultured onto Trypticase (BBL Microbiology Systems, Cockeysville, Md.) soy sheep blood agar plates and incubated at 35°C for 2 to 4 days, until good growth and discrete colonies were obtained. Three to five similar colonies (or more, if colonies were very small) were then used to inoculate 5 ml of Mueller-Hinton broth supplemented with 0.02% Tween 80 (Difco Laboratories, Detroit, Mich.). The inoculated broth was incubated at 35°C for 1 to 3 days until the turbidity was equal to or greater than a 0.5 McFarland barium sulfate standard (16). If possible, broth cultures were used when they had reached the correct turbidity, usually after overnight incubation, but if the turbidity exceeded the standard, the cultures were diluted as necessary. If the broth cultures grew much beyond the desired turbidity, they clumped and were difficult to resuspend, even with vigorous mixing. This occurred only if cultures that grew to the correct turbidity in 24 h were left to incubate for an additional 24 h or longer. Therefore, we avoided excessive incubation of the cultures. Before being used in the tests, the broth cultures were shaken on a Vortex mixer.

To determine the number of colony-forming units (CFU) in a suspension equivalent to a 0.5 McFarland standard, counts were done on three to four strains of each of the three species tested. All counts ranged from 1×10^7 to 2×10^8 CFU/ml.

Antimicrobial agents. Antimicrobial powders suitable for susceptibility testing were obtained from the following manufacturers: penicillin G, cephalothin, cefamandole, vancomycin, tobramycin, cycloserine, and capreomycin from Eli Lilly & Co., Indianapolis, Ind.; ampicillin, kanamycin, and amikacin from Bristol Laboratories, Syracuse, N.Y.; tetracycline and doxycycline from Pfizer Inc., New York, N.Y.; erythromycin and clindamycin from The Upjohn Co., Kalamazoo, Mich.; rosaramicin, gentamicin, netilmicin, and sisomicin from Schering Corp., Bloomfield, N.J.; minocycline and ethambutol from Lederle Laboratories, Pearl River, N.Y.; cefuroxime from Glaxo, Ltd., Greenford, Middlesex, England; cefoxitin from Merck, Sharp, & Dohme, West Point, Pa.; josamycin from Endo Laboratories, Inc., Garden City, N.Y.; rifampin from Ciba-Geigy Corp., Summit, N.J.; chloramphenicol from Parke, Davis & Co., Detroit, Mich.; thiamphenicol from USV Pharmaceutical Corp., Tuckahoe, N.Y.; metronidazole from G. D. Searle & Co., Chicago, Ill.; isoniazid and *para*-aminosalicylic acid from Ormont Drug and Chemical Corp., Englewood, N.J.; ethionamide from Ives Laboratories Inc., New York, N.Y.; fortimicin from Abbott Laboratories, North Chicago, Ill.; and sulfamethoxazole and trimethoprim from Burroughs Wellcome Co., Research Triangle Park, N.C.

Broth microdilution. Broth microdilution plates were prepared and inoculated with the MIC 2000 system (Dynatech Laboratories, Inc., Alexandria, Va.). Serial twofold dilutions of antimicrobial solutions were added to Mueller-Hinton broth (Difco) supplemented with calcium and magnesium cations (CSMHB) (16) to achieve final concentrations of antimicrobial agents ranging from 128 to 0.12 μg/ml for all drugs except rifampin (64 to 0.06 μg/ml), sulfamethoxazole (304 to 0.3 μg/ml), trimethoprim (16 to 0.5 μg/ml), sulfamethoxazole-trimethoprim (304:16 to 0.3:0.01 μg/ml), metronidazole (32 to 1.0 μg/ml), and ethionamide (64 to 0.06 μg/ml). Kanamycin was not tested by broth microdilution.

The prepared plates were sealed in plastic bags, stored at −70°C, and used within 4 weeks of preparation. Before inoculation, the plates were removed from the freezer and allowed to reach room temperature.

The plates were inoculated with the MIC 2000 mechanical inoculator. The final inoculum size was between 10^3 and 10^4 CFU per well (10^4 and 10^5 CFU/ml). Inoculated plates were sealed inside plastic bags and incubated at 35°C. MICs were read at 48, 72, and 96 h. An MIC was the lowest concentration that completely inhibited macroscopic growth, except that for sulfamethoxazole, trimethoprim, and sulfamethoxazole-trimethoprim, it was the concentration that inhibited 80 to 90% of the growth as compared with the control well.

Agar dilution. Serial twofold dilutions of appropriate antimicrobial solutions were added to Mueller-Hinton agar (Difco) to achieve final concentrations as used in the broth test. Rosaramicin, josamycin, thiamphenicol, fortimicin, metronidazole, isoniazid, *para*-aminosalicylic acid, ethionamide, and cycloserine were not tested by agar dilution because they had been shown to be relatively inactive by the broth procedure. Kanamycin was tested only by agar dilution.

The agar plates were inoculated with a Steers replicating device (19). The final inoculum was between 10^3 and 10^4 CFU per spot. Plates were incubated at 35°C, and readings were taken at 48, 72, and 96 h.

MICs were read as the lowest concentrations that completely inhibited growth or that allowed no more than one colony to grow. For sulfamethoxazole, trimethoprim, and sulfamethoxazole-trimethoprim, MICs were read as the concentrations inhibiting 80 to 90% of the control growth.

RESULTS

Before beginning the study, we subjectively rated the growth of one strain of each of the three species to be tested in five different broths: Middlebrook 7H9 broth (Difco), Middlebrook

7H9 broth plus 0.2% dextrose, Dubos broth base plus 10% Dubos medium albumin (Difco), Mueller-Hinton broth, and Mueller-Hinton broth plus 0.2% dextrose. Growth in Mueller-Hinton broth alone was as good as or better than growth in the other four media (Middlebrook 7H9 broth plus dextrose may have given slightly better growth of the *M. chelonei* strain).

Subsequently, in the study, the amount of growth obtained in both agar dilution and broth microdilution was subjectively rated at each reading on a scale from 1 to 4+, with 4+ being optimum growth. The *M. fortuitum* strains achieved maximum growth at 72 h for both the broth microdilution and agar dilution tests. For most strains of *M. chelonei*, maximum growth in broth occurred at 72 h also, but some strains (3 of 15) grew to the maximum at 96 h, 14 of 15 strains achieving 3+ or greater growth. The *M. chelonei* strains grew very poorly on agar, most strains achieving only 2+ growth at 96 h; little change occurred between 72 and 96 h. Of the 15 *M. chelonei* strains, 5 did not grow at all on agar. The *M. chelonei*-like strains grew very well at all readings in either broth or agar at 48 h.

Since growth at 72 h appeared to be the optimum for most strains, only results from that reading are reported in the tables.

In general, there was a fairly good correlation between results obtained with broth microdilution and agar dilution for the *M. fortuitum* and the *M. chelonei*-like organisms. For *M. fortuitum*, the most variation in MICs occurred with vancomycin, minocycline, and sulfamethoxazole. Vancomycin MICs obtained with agar dilution were lower than those obtained with broth microdilution; minocycline had lower MICs with agar dilution with the susceptible strains and higher MICs with the resistant strains, but for sulfamethoxazole the reverse was true. For the *M. chelonei*-like strains, the greatest variation occurred with erythromycin and sulfamethoxazole. Erythromycin values obtained by broth microdilution were much lower than those obtained by agar dilution, but the opposite was true for sulfamethoxazole, with MICs from agar dilution being lower than those from broth microdilution.

Comparison of results between broth and agar for *M. chelonei* was difficult because 5 of the 15 strains did not grow on agar, and with some other strains comparisons could not be made because the MICs were greater than the highest concentration tested. For the strains with both MICs within range, agar dilution MICs tended to be one to two dilutions higher than the broth microdilution MICs.

Since the broth microdilution procedure gave the best results in terms of growth, only results for that procedure will be discussed.

TABLE 1. MICs of 17 antimicrobial agents for 18 strains of *M. fortuitum*, after 72 h of incubation

Agent	Medium	MIC_{50}[a]	MIC_{90}[b]	Range
Cefoxitin	Broth	32	≥64	16–≥64
	Agar	16	32	16–≥64
Cefuroxime	Broth	≥64	≥64	≥64
	Agar	≥64	≥64	≥64
Tetracycline	Broth	8	16	4–16
	Agar	8	16	4–32
Doxycyline	Broth	8	32	0.5–32
	Agar	32	≥64	0.5–≥64
Minocycline	Broth	4	16	0.5–16
	Agar	8	16	0.5–32
Amikacin	Broth	1	8	0.5–8
	Agar	1	16	0.5–32
Kanamycin	Broth[c]			
	Agar	8	≥64	4–≥64
Fortimicin	Broth	16	32	8–32
	Agar[c]			
Gentamicin	Broth	8	16	2–32
	Agar	8	16	4–16
Tobramycin	Broth	16	32	4–≥64
	Agar	16	32	8–≥64
Netilmicin	Broth	16	16	4–16
	Agar	32	32	8–32
Sisomicin	Broth	4	8	2–8
	Agar	8	16	2–16
Vancomycin	Broth	64	≥64	32–≥64
	Agar	16	≥64	8–≥64
Chloramphenicol	Broth	64	≥64	8–≥64
	Agar	64	≥64	16–≥64
Ethambutol	Broth	16	≥64	8–≥64
	Agar	8	≥64	4–≥64
Capreomycin	Broth[d]	16	16	4–≥64
	Agar	16	32	4–≥64
Ethionamide	Broth	32	≥64	8–≥64
	Agar[c]			
Sulfamethoxazole	Broth	4.8	152	≤1.2–152
	Agar	9.5	76	2.4–76
Sulfamethoxazole-trimethoprim[e]	Broth	4.8	152	≤1.2–152
	Agar	4.8	76	≤1.2–152

[a] MIC at which 50% of strains were inhibited (micrograms per milliliter).

[b] MIC at which 90% of strains were inhibited (micrograms per milliliter).

[c] Not tested.

[d] Two strains not tested.

[e] Sulfamethoxazole/trimethoprim ratio, 19:1; MIC expressed only as sulfamethoxazole concentration. Endpoints were easier to distinguish in a 96-h reading.

M. fortuitum. Drugs with no useful activity and their MICs (expressed in micrograms per milliliter) were as follows: ampicillin, >32; penicillin, >128; cephalothin, >32; cefamandole, >16; erythromycin, >4; rosaramicin, >64; josamycin, >8; clindamycin, >16; rifampin, >8; thiamphenicol, >32; trimethoprim, >16; metronidazole, >32; isoniazid, >4; *para*-aminosalicylic acid, >128; and cycloserine, >128.

Antimicrobial agents that were active for at least some strains of *M. fortuitum* are shown in

TABLE 2. MICs of 13 antimicrobial agents for 15 strains of *M. chelonei*, after 72 h of incubation

Agent	Medium	IG[a]	MIC_{50}[b]	MIC_{90}[c]	Range
Penicillin	Broth		≥64	≥64	8–≥64
	Agar	5	≥64	≥64	≥64
Cefoxitin	Broth		≥64	≥64	16–≥64
	Agar	5	32	≥64	32–≥64
Cefamandole	Broth		≥64	≥64	16–≥64
	Agar	5	32	32	32
Cefuroxime	Broth		≥64	≥64	8–≥64
	Agar	5	≥64	≥64	≥64
Amikacin	Broth		16	32	4–≥64
	Agar	5	16	32	8–32
Kanamycin	Broth[d]				
	Agar	5	8	≥64	4–≥64
Gentamicin	Broth		8	32	4–≥64
	Agar	5	32	32	16–32
Tobramycin	Broth		8	16	1–32
	Agar	5	8	32	2–32
Netilmicin	Broth		16	32	4–32
	Agar	5	≥64	≥64	32–≥64
Sisomicin	Broth		4	8	1–16
	Agar	5	8	16	2–16
Erythromycin	Broth		4	≥64	2–≥64
	Agar	5	≥64	≥64	8–≥64
Ethionamide	Broth		≥64	≥64	8–≥64
	Agar[d]				
Trimethoprim	Broth		>16	>16	4–>16
	Agar	5	>16	>16	>16

[a] Insufficient growth (number of strains).
[b] MIC at which 50% of strains were inhibited (micrograms per milliliter).
[c] MIC at which 90% of strains were inhibited (micrograms per milliliter).
[d] Not tested.

Table 1. The most active antimicrobial agents based on achievable blood levels were amikacin, sulfamethoxazole, sulfamethoxazole-trimethoprim, doxycycline, and minocycline. None of the *M. fortuitum* isolates tested was resistant to amikacin.

M. chelonei. Drugs with no useful activity and their MICs (expressed in micrograms per milliliter) were as follows: ampicillin, >32; cephalothin, >32; rosaramicin, >4; josamycin, >64; vancomycin, >32; clindamycin, >16; rifampin, >64; chloramphenicol, >16; thiamphenicol, >32; fortimicin, >16; metronidazole, >32; isoniazid, >4; ethambutol, >16; *para*-aminosalicylic acid, >128; capreomycin, >64; cycloserine, >128; tetracycline, >8 (one strain had an MIC of 8 μg/ml in broth); doxycycline, >8; minocycline, >8; sulfamethoxazole, >152; and sulfamethoxazole-trimethoprim, >152:8. Of drugs with some activity against *M. chelonei* (Table 2), the most active were amikacin, sisomicin, tobramycin, and erythromycin.

***M. chelonei*-like organisms.** The *M. chelonei*-like organisms were the most susceptible of the three groups tested (Table 3). Drugs with no activity are not listed in the table, and their MICs (expressed in micrograms per milliliter) were as follows: clindamycin, >4; thiamphenicol, >8; *para*-aminosalicylic acid, >128; ethionamide, >16; cycloserine, >32; and metronidazole, >32.

The most active drugs against the *M. chelonei*-like organisms were sulfamethoxazole-trimethoprim, doxycycline, minocycline, amikacin, and sulfamethoxazole (Table 3). There were no strains resistant to amikacin. More than 75% of the *M. chelonei*-like strains were susceptible to ampicillin, cefoxitin, cefuroxime, erythromycin, tetracycline, doxycycline, minocycline, amikacin, sulfamethoxazole, and sulfamethoxazole-trimethoprim.

DISCUSSION

In 1963, Canetti et al. (4) published the recommendations of the World Health Organization Expert Committee on Tuberculosis for drug susceptibility testing of mycobacteria. These recommendations were made in an attempt to standardize susceptibility testing of mycobacteria and included three different methods using Lowenstein-Jensen medium that had been shown to be reasonably reproducible and accurate. Despite this, many different media and methods have been used to determine the susceptibility of *M. fortuitum* and *M. chelonei* to antimicrobial agents (3, 13, 14, 17, 21). Since 1978, Dalovisio and Pankey (6), Welch and Kelly (24), Wallace et al. (22, 23), and Tice and Solomon (20) have used Mueller-Hinton agar and a method with complete inhibition of growth as the endpoint.

All the methods recommended by Canetti et al. (4) and most of the modifications of those methods used by other investigators allow for resistant mutants in a population of slowly growing mycobacteria when endpoints are read. For slowly growing mycobacteria, an organism is considered resistant to a drug if more than 1% of resistant mutants (as determined by these methods) exist in a population of mycobacteria, although the chance of treatment failure because of these mutants is reduced because combination therapy is used. Methods for susceptibility testing of rapidly growing bacteria are more strict in their definition of endpoints, usually defining them as a complete inhibition of growth. Because these two species grow quickly enough to be tested by the more standardized antimicrobial susceptibility methods used with the more rapidly growing bacteria, we believe that it may be more useful to test their susceptibility to antibacterial agents with standard bacterial methods rather than with mycobacterial methods.

We did, however, extend the incubation peri-

TABLE 3. MICs of 26 antimicrobial agents for 31 strains of *M. chelonei*-like organisms, after 72 h of incubation

Agent	Medium	MIC_{50}[a]	MIC_{90}[b]	Range	Agent	Medium	MIC_{50}[a]	MIC_{90}[b]	Range
Penicillin	Broth	16	≥64	2–≥64	Netilmicin	Broth	16	16	4–16
	Agar	32	≥64	2–≥64		Agar	16	16	8–32
Ampicillin	Broth	4	8	0.5–≥64	Sisomicin	Broth	4	8	2–8
	Agar	8	16	0.5–≥64		Agar	8	8	2–8
Cephalothin	Broth	≥64	≥64	1.0–≥64	Erythromycin	Broth	1.0	2	0.5–4
	Agar	≥64	≥64	1.0–≥64		Agar	16	16	0.5–≥64
Cefoxitin	Broth	8	16	4–≥64	Rosaramicin	Broth	32	≥64	4–≥64
	Agar	16	16	4–≥64		Agar			
Cefamandole	Broth	16	32	1.0–≥64	Josamycin	Broth	16	>16	4–>16
	Agar	32	≥64	2–≥64		Agar			
Cefuroxime	Broth	16	32	2–≥64	Vancomycin	Broth	32	≥64	2–≥64
	Agar[c]	≥64	≥64	2–≥64		Agar	≥64	≥64	2–≥64
Tetracycline	Broth	2	16	1.0–≥64	Chloramphenicol	Broth	32	≥64	4–≥64
	Agar	2	32	2–≥64		Agar	≥64	≥64	2–>64
Doxycycline	Broth	0.5	32	0.5–≥64	Rifampin	Broth	32	32	2–≥64
	Agar	1.0	≥64	0.5–≥64		Agar	≥64	≥64	16–≥64
Minocycline	Broth	0.5	16	0.5–≥64	Isoniazid	Broth	≥64	≥64	2–≥64
	Agar	0.5	≥64	0.5–≥64		Agar[d]			
Amikacin	Broth	2	4	0.5–≥64	Ethambutol	Broth	16	32	0.5–≥64
	Agar	2	2	0.5–≥64		Agar	4	8	0.5–≥64
Kanamycin	Broth[d]				Capreomycin	Broth	≥64	≥64	16–≥64
	Agar	8	8	1.0–8		Agar	≥64	≥64	16–≥64
Fortimicin	Broth	32	32	2–≥64	Sulfamethoxazole	Broth	76	≥304	9.5–≥304
	Agar[d]					Agar	19	152	4.8–≥304
Gentamicin	Broth	8	16	2–32	Sulfamethoxazole-trimethoprim[e]	Broth	9.5	19	2.4–≥304
	Agar	8	8	2–32		Agar	4.8	19	2.4–≥304
Tobramycin	Broth	8	8	2–8	Trimethoprim	Broth	8	16	4–>16
	Agar	8	8	2–8		Agar	4	16	2–>16

[a] MIC at which 50% of strains were inhibited (micrograms per milliliter).
[b] MIC at which 90% of strains were inhibited (micrograms per milliliter).
[c] Two strains not tested.
[d] Not tested.
[e] Sulfamethoxazole/trimethoprim ratio, 19:1; MIC expressed only as sulfamethoxazole concentration.

od of eight strains in broth microdilution plates to 2 weeks to try to pick up the development of resistance (in this case to only the more useful agents, amikacin, doxycycline, erythromycin, sulfamethoxazole, and cefoxitin). MICs for doxycycline, sulfamethoxazole, and cefoxitin did tend to increase, but in no case was there a major change in category of susceptibility from susceptible to resistant, and in only a few instances was there a minor change in category of susceptibility from susceptible to moderately susceptible or from moderately susceptible to resistant: two strains tested against sulfamethoxazole changed from a level we judged susceptible (≤38 μg/ml) to moderately susceptible (38 to 152 μg/ml), and two strains tested against cefoxitin changed from moderately susceptible (2 to 16 μg/ml) to resistant (>16 μg/ml).

Wallace et al. found that MICs for amikacin, gentamicin, and doxycycline on 7H10 agar were two- to fourfold higher than those on Mueller-Hinton agar (23). Gangadharam and Gonzales also showed that 7H10 agar gave higher ethambutol MICs for *M. tuberculosis* when they compared it with Lowenstein-Jensen medium (9). Thus, a medium that was commonly used for antimicrobial susceptibility testing seemed best, especially since we showed that Mueller-Hinton medium, widely used for bacterial susceptibility testing, was capable of supporting good growth of the three species tested.

Broth microdilution appears to have several advantages over agar dilution. *M. chelonei* strains grew much better in broth than on unsupplemented agar. However, Wallace et al. (23) achieved better growth of *M. chelonei* on agar by supplementing Mueller-Hinton agar with 10% OADC (oleic acid-albumin-dextrose-catalase). Broth microdilution is also a method that is very easily adaptable to routine clinical situations. Agar dilution is most useful in testing many strains and is less convenient in testing only a few strains.

In general, the results from broth microdilution and agar dilution showed good agreement. The major discrepancies encountered were with

the sulfonamides and erythromycin. One explanation for the variation between results from broth microdilution and agar dilution for both *M. fortuitum* and *M. chelonei*-like organisms with sulfamethoxazole may be the subjectivity in reading the endpoints at an 80 to 90% inhibition of growth. The reasons for the discrepancies with erythromycin are not readily apparent, but they were not due to differences in pH. These discrepancies with erythromycin seem to be significant in terms of potential clinical use only for the *M. chelonei*-like strains. In later studies in our laboratory, we have noted that erythromycin produces a trailing endpoint for some strains of *M. fortuitum* and *M. chelonei*. This effect is very similar to that which occurs with sulfonamides, where there is an obvious reduction in growth at a certain concentration and then a very small amount of growth that continues out to a higher concentration. Further investigation is needed to determine which method of susceptibility testing will minimize this problem and how that method relates to clinical therapy.

Of the agents tested in this study, few, if any, have wide enough activity to be used empirically without testing to determine susceptibility. Amikacin is the drug most active in general, although a few strains of *M. chelonei* (2 of 15) have amikacin MICs of $\geq$32 μg/ml.

Doxycycline, sulfamethoxazole, and cefoxitin are the only other agents tested that have good potential for treatment of infections caused by *M. fortuitum*; the first two are the only oral agents with good activity. An oral agent is desirable since many of the infected patients are not hospitalized. Wallace et al. (22) have suggested that sulfonamides may be the treatment of choice for *M. fortuitum* infections. Unfortunately, there are no agents except erythromycin that have good activity against *M. chelonei* and can be administered orally. As discussed earlier, however, there are some problems in testing erythromycin that need to be resolved.

Our results are similar to those obtained by other investigators who used Mueller-Hinton broth or agar. However, Wallace et al. (23) and Welch and Kelly (24) reported amikacin MICs of 1 to 2 μg/ml for a few strains of *M. chelonei*, Dalovisio and Pankey (6) and Wallace et al. (23) reported that some strains of *M. fortuitum* are susceptible to erythromycin, and Cynamon and Patapow (5) found that cefoxitin is slightly more active against *M. fortuitum*.

Because these organisms may be susceptible, but not uniformly so, to relatively nontoxic antimicrobial agents that can be given orally, a standard technique for susceptibility testing seems important. Studies to date in which methods similar to ours were used indicate that there is a correlation between in vitro susceptibility and clinical response (6–8, 22). However, more studies need to be undertaken to further substantiate the correlation of this method with clinical outcome.

The incidence of infection with these organisms is not very high, so it would be feasible for a few reference centers to offer the service of susceptibility testing of clinically significant strains, but those laboratories which are now doing MICs by broth microdilution could easily include the testing of these strains with little modification in their technique.

ADDENDUM

Since this study was completed, many additional isolates of *M. chelonei* have been tested by broth microdilution, and only very rarely has it been necessary to vary this technique to achieve good growth of this species.

LITERATURE CITED

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