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Broth Microdilution Testing of Susceptibilities to 30 Antimicrobial Agents of *Mycobacterium avium* Strains from Patients with Acquired Immune Deficiency Syndrome

DAVID M. YAJKO,* PATRICIA S. NASSOS, AND W. KEITH HADLEY

Department of Laboratory Medicine, University of California, San Francisco General Hospital Medical Center, San Francisco, California 94110

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A total of 31 strains of *Mycobacterium avium* complex isolated from patients with acquired immune deficiency syndrome were tested for susceptibility to 30 antimicrobial agents by using microdilution trays containing dried antimicrobial agents. MICs were determined over a period of 7 days of growth in a broth medium (7HSF) that is equivalent to 7H11 agar. MICs obtained by this method showed good agreement with MICs determined by the agar dilution method. Strains could be divided into two groups by their antimicrobial susceptibility patterns. All group 1 strains (8 of the 31 strains tested) were at least moderately susceptible to inhibition by a variety of beta-lactam antimicrobial agents, including amoxicillin-clavulanic acid and cefmenoxime. Group 2 strains (23 of 31) were susceptible only to amikacin (22 of 23 strains). All 31 strains were resistant to oxacillin, clindamycin, erythromycin, tetracycline, chloramphenicol, vancomycin, nitrofurantoin, and aztreonam at the highest concentration of antimicrobial agent present in the microdilution trays. The addition of Tween 80 to 7HSF broth increased the susceptibility of *M. avium* complex to many of the antimicrobial agents tested. Killing of *M. avium* complex (i.e., ≤1% survival after 7 days) was found to vary for different strains and antimicrobial agents. Killing of some strains by amoxicillin-clavulanic acid, carbenicillin, azlocillin, cefmenoxime, cefotaxime, amikacin, and ampicillin occurred at concentrations of antimicrobial agent that are achievable in serum. Further studies are needed to determine whether any of these antimicrobial agents has activity against *M. avium* complex cells that have been ingested by macrophages.

Mycobacterium avium complex is a common cause of infection in patients with acquired immune deficiency syndrome (AIDS) (1, 10). Infections often become disseminated, and the organism has been isolated from blood, bone marrow, lung, liver, spleen, and brain (1, 10, 11). *M. avium* complex is generally resistant to the drugs that are used to treat *Mycobacterium tuberculosis* infections (10; R. C. Good, V. A. Silcox, J. O. Kilburn, and B. D. Plikaytis, Clin. Microbiol. Newsl. 7:133–136, 1985). In vitro, rifabutin (Ansamycin) and clofazimine inhibit the growth of most strains of *M. avium* complex at fairly low concentrations (3, 7, 11, 16). However, clinical response to treatment with these antimicrobial agents has been poor (1, 11). Part of the reason for this failure may be due to the inability of these antimicrobial agents to kill (as opposed to inhibit the growth of) *M. avium* complex at concentrations of drug that are achievable in serum (17).

There are reports on the ability of several beta-lactam and aminoglycoside antimicrobial agents to inhibit or kill *M. avium* complex (12–14). However, widespread screening of antimicrobial agents against *M. avium* complex has not been reported, partly because the agar proportion technique used for testing mycobacteria is so laborious. Recently, broth dilution techniques have been applied to *M. avium* complex, permitting the determination of inhibitory and bactericidal concentrations for a number of antimicrobial agents (3, 5, 7, 15). There are, however, often significant discrepancies in MICs reported by different laboratories for the same antimicrobial agents (5, 6, 15). Differences in the methods and media used may account in part for this lack of agreement. Of particular note is the role played by Tween 80 in culture media. Tween 80 has been shown to affect the permeability of *M. avium* complex cells and to decrease the MIC of certain antimicrobial agents (8, 12). The commercial availability of microdilution trays containing dried antimicrobial agents allowed us to easily test the effect of certain medium supplements on MIC and to screen a large number of antimicrobial agents for activity against strains of *M. avium* complex isolated from patients with AIDS.

MATERIALS AND METHODS

Strains. All strains of *M. avium* complex used in this study were from AIDS patients at San Francisco General Hospital who had disseminated *M. avium* complex infection. Strains were maintained prior to testing on slants of Lowenstein-Jensen medium (Difco Laboratories, Detroit, Mich.). The colonial morphology of each strain was noted both before and after exposure to antimicrobial agents. Serotyping of *M. avium* complex isolates was performed by A. Tsang, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colo.

Media used for growth and susceptibility tests. 7H11 agar supports the growth of *M. avium* complex and is commonly used for susceptibility testing of *M. tuberculosis* (7, 11). This medium, however, is not available commercially in broth form. A broth equivalent of 7H11 agar was prepared by supplementing Bacto Middlebrook 7H9 broth (Difco) with 1 g of pancreatic digest of casein (BBL Microbiology Systems, Cockeysville, Md.) per liter, 0.5% glycerol, and 100 ml of Bacto Middlebrook OADC enrichment (Difco) per liter to

* Corresponding author.

achieve a broth medium (called 7HSF) that is the equivalent of 7H11 agar without malachite green. In experiments designed to test the effect of Tween 80 on MIC, 7HSF broth was supplemented with 0.05% Tween 80 (Difco). These experiments were also performed by using 7H9 broth (containing 0.05% Tween 80) for growth of the organism and for susceptibility testing.

Broth microdilution MIC method. Mycobacteria were inoculated with a cotton swab from Lowenstein-Jensen slants into 4 ml of 7HSF broth and incubated at 35°C for 1 week. The day before the susceptibility test was done, the 1-week-old culture was diluted 1:20 in fresh 7HSF broth and incubated overnight at 35°C. The overnight culture was mixed by inverting the tubes 10 times and then diluted 1:100 in 7HSF broth just prior to the inoculation of microdilution test panels (Sceptor Gram Positive panel and Sceptor Beta-Lactam Plus panel; Johnston Laboratories, Inc., Towson, Md.). This procedure yielded an actively growing culture which reproducibly contained ca. 5×10^5 CFU/ml (17). The dried antimicrobial agents in the microdilution test wells were reconstituted with 0.1 ml of the bacterial suspension in 7HSF broth. After reconstitution, trays were covered with lids, placed in plastic bags, and incubated at 35°C in ambient atmosphere. MICs were determined after 2, 3, 4, 6, and 7 days of incubation. MICs were read in indirect light by using a Dynatech reading stand with mirror (Dynatech Laboratories, Inc., Alexandria, Va.). The MIC was defined as the lowest concentration of antimicrobial agent at which the organism showed no visible growth (17). The criteria used for the interpretation of antimicrobial susceptibility were based upon the achievable levels of antimicrobial agents in serum.

Agar dilution MICs. A total of 15 antimicrobial agents were incorporated into 7H11 agar at concentrations ranging from 0.5 to 256 μg/ml. They were penicillin, ampicillin, cephalothin, amikacin, gentamicin, sulfamethoxazole-trimethoprim (SXT), azlocillin, mezlocillin, piperacillin, ticarcillin-clavulanic acid, cefoperazone, cefotaxime, ceftizoxime, cefuroxime, and netilmicin. *M. avium* complex cultures were grown in 7H9 broth as described for the broth microdilution method. Dilutions of the overnight broth cultures were prepared in 7H9 broth. Mixing of diluted cultures was performed by inverting each tube 10 times. Samples of 0.1 ml of the 10^{-3} dilution were plated by spreading onto 7H11 agar plates containing the antimicrobial agents. This dilution gave an inoculum that contained 1,000 to 3,000 CFU on each plate. The actual size of the inoculum was determined by plating dilutions onto control plates of 7H11 agar without an antimicrobial agent. Plates were incubated at 35°C in ambient atmosphere for 7 to 10 days, and colonies were then counted under a dissecting microscope. The MIC for the agar dilution method was defined as the lowest concentration of antimicrobial agent that yielded fewer than 1% of the number of colonies on the control plate (7).

Measurement of deterioration of antimicrobial activity in microdilution trays during incubation. Tests were performed to determine how much activity of each antimicrobial agent was lost during the period of incubation that occurred before MIC readings were taken. Antimicrobial agents in the microdilution trays were reconstituted with 7HSF broth, and the trays were placed in plastic bags and incubated at 35°C. After 3 or 6 days of incubation, the trays were inoculated with *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, or *Pseudomonas aeruginosa* ATCC 27853 to a final concentration of ca. 5×10^5 CFU/ml. The volume of bacterial suspension to be added to each well was calculated by first determining the amount of evaporation that occurred during incubation (typically 0.008 to 0.010 ml after 6 days of incubation). As a control, trays of freshly reconstituted antimicrobial agents were inoculated with 0.01 ml of the quality control strains. MICs were scored after overnight incubation of all trays at 35°C in ambient atmosphere. All three quality control strains showed good growth in the growth control wells of the microdilution trays after 24 h of incubation. The amount of deterioration of antimicrobial agent was determined by comparing the MIC in the 3- or 6-day-old tray with the MIC obtained by using a freshly prepared tray. The deterioration experiment was performed in triplicate.

MBC(AFB)s. MBCs for acid-fast bacteria [MBC(AFB)s] were determined by subculturing from the microdilution trays after 4 and 7 days of incubation. The concentration of mycobacteria in the suspension used to inoculate the microdilution trays was determined by preparing dilutions of the suspension in 7HSF broth and plating 0.1-ml samples onto 7H10 agar. The MBC(AFB) was determined by removing a 0.01-ml sample from a microdilution tray well that showed no visible turbidity after 4 and 7 days of incubation and spreading this inoculum onto a 7H10 agar plate (17). Prior to sampling, thorough mixing of the contents of the well was accomplished by aspirating and ejecting 0.05-ml samples 10 times. Care was taken to prevent spillovers and contamination. The 7H10 agar plates were incubated in air at 35°C for 7 to 10 days before colonies were counted with the aid of a dissecting microscope. The MBC(AFB) was defined as the lowest concentration of drug that killed at least 99% of the original inoculum (17).

Beta-lactamase tests. Tests for the detection of beta-lactamase were performed by using nitrocefin disks (Cefinase; BBL Microbiology Systems) according to the instructions of the manufacturer. The inoculum was either a loopful of growth from a 1- to 2-week-old culture on Lowenstein-Jensen medium or a drop of sediment from a 1-week-old culture in 7H9 broth. Disks were observed over a period of 60 min.

RESULTS

Growth of all strains of *M. avium* complex was luxuriant in 7HSF broth. Preliminary experiments showed that some strains required 4 days of incubation before there was slight growth in the growth control well of the microdilution trays. Trays were held for 7 days to allow for sufficient growth of these slower-growing strains. In experiments designed to measure changes over time [for example, changes in MIC, MBC(AFB), or antimicrobial deterioration], the readings on days 4 and 7 were compared.

MICs obtained by the broth microdilution method. The susceptibilities of 31 strains of *M. avium* complex to the antimicrobial agents in the Sceptor Gram Positive and Beta-Lactam Plus panels are given in Tables 1 and 2, respectively. The data shown are the MICs obtained after 7 days. There was frequently a one-well increase in MIC between the readings on days 4 and 7. The increase in MIC appeared to be antimicrobial agent dependent. Thus, for most of the strains tested, the MIC increased between days 4 and 7 for erythromycin, tetracycline, netilmicin, moxalactam, cephalothin, SXT, amoxicillin-clavulanic acid, azlocillin, and ceftriaxone. The MIC of imipenem increased by two to four wells between days 4 and 7. In contrast, there was usually no change in MIC between days 4 and 7 for gentamicin, amikacin, penicillin, ampicillin, piperacillin, mezlocillin, cefotaxime, and cefoperazone.

TABLE 1. Antimicrobial susceptibilities of 31 strains of *M. avium* complex by using Sceptor Gram Positive microdilution trays

Antimicrobial agent[a]	No. of strains inhibited by concn (μg/ml)[b]										
	1	2	4	8	>8	16	>16	32	>32	64	>64
Cephalothin (16)	0	0	0	3		5		0	23		
Penicillin (16)	0	2	4	2		0	23				
Ampicillin (16)	0	5	3	0		0	23				
Oxacillin (2)	0	0	0	0	31						
Amikacin (32)	0	0	0	7		12		10		2	
Gentamicin (8)	0	0	0	12		13	6				
Clindamycin (4)	0	0	0	0	31						
Erythromycin (4)	0	0	0	2	29						
Tetracycline (8)	0	0	0	0		0	31				
Chloramphenicol (16)	0	0	0	0		0		0	31		
Vancomycin (16)	0	0	0	0		0		0	31		
SXT (8/152)[c]	5	11	1	4		0	10				
Nitrofurantoin (64)										0	31

[a] The number in parentheses indicates the highest concentration (micrograms per milliliter) for which the interpretation was listed as susceptible or moderately susceptible on the Sceptor scoresheets.

[b] The number of strains for which MICs were greater than the specified concentration reflects the number of strains resistant to the highest concentration of that antibiotic present in the Sceptor trays.

[c] Range, 1/19 to 16/304 μg/ml.

In the Sceptor Gram Positive panel (Table 1), only cephalothin (8 strains), amikacin (29 strains), gentamicin (12 strains), and SXT (21 strains), inhibited growth at a concentration that indicates that the strains were susceptible or moderately susceptible to these antimicrobial agents. Oxacillin, clindamycin, tetracycline, chloramphenicol, vancomycin, and nitrofurantoin were notable in that none of these antimicrobial agents inhibited any of the 31 strains at the highest drug concentration tested.

Many of the antimicrobial agents in the Beta-Lactam Plus panel (Table 2) were inhibitory to some strains at a concentration that indicates susceptibility. Amoxicillin-clavulanic acid, cefmenoxime, and netilmicin appeared to have the greatest activity against the 31 strains tested, while aztreonam had no activity against any of the strains.

The reproducibility of the broth microdilution method was examined by testing each of two strains three times against 24 antimicrobial agents. Of the 144 MIC comparisons, 121 (84%) were identical and 22 (15%) differed by one dilution. A two-dilution discrepancy occurred only once (<1%).

Deterioration of drugs in microdilution trays during incubation. Since drug deterioration over time could affect the interpretation of MICs and MBCs, we determined the extent of deterioration of the drugs in 7HSF broth during the 7 days of incubation. The data showed that after 4 days of incubation, deterioration was ≤50% (i.e., there was either no change or a one-well increase in MIC) for 19 of 22 antimicrobial agents tested. Two antimicrobial agents (cephalothin and tetracycline) lost about 75% of their activity after 4 days (i.e., there was a two-well increase in MIC). One antimicrobial agent, imipenem, lost more than 99% of its activity in 4 days (greater than a seven-well increase in MIC). After 7 days, 9 of 22 antimicrobial agents still retained at least 50% of their activity and 9 others had ≥25% of their activity remaining. Amoxicillin-clavulanic acid retained about 12% of its activity (i.e., there was a three-well increase in MIC)

TABLE 2. Antimicrobial susceptibilities of 31 strains of *M. avium* complex by using Sceptor Beta-Lactam Plus microdilution trays

Antimicrobial agent[a]	No. of strains inhibited by concn (μg/ml)[b]												
	1	2	4	8	16	32	<32	64	>64	128	>128	256	>256
Amoxicillin (16/8)[c]		5	1	3	1	4		15	2				
Ticarcillin (64/2)[d]		0	0	0	2	5		1		8	15		
Carbenicillin (128)			0	0	3	5		0		9		10	4
Azlocillin (64)			0	0	1	3		3		2		3	19
Cefonicid (16)	0	0	0	0	1	6		8	16				
Cefuroxime (16)	0	0	0	2	7	4		15	3				
Cefmenoxime (32)		0	1	5	5	10		10		0			
Ceftizoxime (128)			0	1	5	2		7		11		5	
Ceftriaxone (32)		0	0	1	1	9		7		11	2		
Moxalactam (32)		0	0	0	0	1		1		6	23		
Aztreonam (16)	0	0	0	0	0	0		0	31				
Imipenem (8)	0	0	1	3	3	1	23						
Mezlocillin (64)[e]			0	0	0	3		3		2		7	2
Piperacillin (64)[e]			0	0	0	1		5		0		2	9
Cefoperazone (64)[e]		0	0	0	0	0		10		6	1		
Cefotaxime (32)[e]		0	0	0	9	5		3		0			
Netilmicin (16)[e]	0	0	2	4	5	6							

[a] For explanation of numbers in parentheses, see footnote *a*, Table 1.

[b] For explanation, see footnote *b*, Table 1.

[c] Mixed with clavulanic acid at a ratio of 2:1 in each well.

[d] Mixed with 2 μg of clavulanic acid per ml in each well.

[e] Antibiotic no longer present in the Sceptor Beta-Lactam Plus panel; 17 strains (6 group 1 and 11 group 2) had been tested when the antibiotic was discontinued.

TABLE 3. Comparison of MICs and MBC(AFB)s for three strains of *M. avium* complex[a]

Antimicrobial agent	Strain 10 (group 1)		Strain 11 (group 1)		Strain 14 (group 2)	
	MIC	MBC(AFB)	MIC	MBC(AFB)	MIC	MBC(AFB)
Amoxicillin[b]	≤2	8	≤2	4	(64)	(128)
Carbenicillin	32	64	16	64	(256)	(>256)
Azlocillin	16	32	16	64	128	(>256)
Ampicillin	2	8	2	8	16	(>16)
Imipenem[c]	8	8	4	8	(>32)	(>32)
Cefmenoxime	8	16	8	16	16	32
Cefotaxime	16	(64)	16	(>128)	16	16
Amikacin	32	(>32)	16	(>32)	8	8

[a] Values (micrograms per milliliter) obtained after 7 days of exposure to antimicrobial agent. Parentheses indicate that the concentration is not achievable in serum. Group 1 strains were susceptible to inhibition by a variety of beta-lactam antimicrobial agents. Group 2 strains were resistant to most beta-lactam antimicrobial agents but were usually susceptible to amikacin.

[b] Amoxicillin plus clavulanic acid at a ratio of 2:1.

[c] Values obtained after 4 days of exposure to imipenem. MBC(AFB) after 7 days was >32 μg/ml.

after 7 days. The amount of activity of cephalothin, tetracycline, and imipenem remaining after 7 days of incubation was less than what could be measured by our assay method. For each of the four antimicrobial agents whose deterioration was greater than 75% in 7 days, there was a corresponding increase in the *M. avium* complex MIC on day 7 compared with the MIC on day 4. When deterioration of a drug was less than 50% in 7 days, the MIC of the antimicrobial agent usually did not increase between days 4 and 7. There was a variable increase in MIC between days 4 and 7 for those antimicrobial agents which lost 50 to 75% of their activity in 7 days.

Comparison of agar dilution and broth microdilution MICs. A total of 15 antimicrobial agents were tested by agar dilution and broth microdilution against five strains of *M. avium* complex. The data for the 75 comparisons showed that there was close agreement between broth and agar MICs. However, agar MICs tended to be one dilution higher than broth microdilution MICs. The broth and agar MICs agreed within one dilution in 54 comparisons (72%) and within two dilutions in 69 comparisons (92%). The broth MIC was higher than the agar MIC in only 2 comparisons (3%), while the agar MIC was higher than the broth MIC in 49 comparisons (65%).

Effect of Tween 80 on MIC. In four separate experiments, three strains of *M. avium* complex were tested by using Sceptor microdilution trays reconstituted with 7HSF broth, 7H9 broth (containing 0.05% Tween 80), or 7HSF broth supplemented with 0.05% Tween 80. MICs were scored after 7 days. The MICs of cephalothin, cefmenoxime, vancomycin, and moxalactam were always at least fourfold lower in the presence of Tween 80 than in its absence. The MICs of cefonicid, cefuroxime, ceftriaxone, and amoxicillin-clavulanic acid were always at least twofold lower in the presence of Tween 80. The remaining antimicrobial agents showed no or variable effects of Tween 80 on MIC. These results were obtained regardless of whether the base medium was 7H9 or 7HSF.

Grouping of strains by MIC and serotype. In the Sceptor Gram Positive panel (Table 1), there was a bimodal distribution of MICs of cephalothin, penicillin, and ampicillin. For each of these antimicrobial agents, there was a group of 8 strains with MICs that were at least a dilution lower than the MICs of another group consisting of 23 strains. In the Beta-Lactam Plus panel (Table 2), a bimodal distribution of MICs was seen with ticarcillin-clavulanic acid and carbenicillin, again with 8 strains in one group (MICs, 16 to 64 μg/ml) and 23 strains in another group (MICs, ≥128 μg/ml). Analysis of the MIC data for these five antimicrobial agents showed that the eight strains with the lower MICs constituted a unique group of strains. These strains (group 1) were inhibited by 12 of the 30 antimicrobial agents at concentrations which suggest that they are susceptible or moderately susceptible to these antimicrobial agents. The 12 antimicrobial agents were cefmenoxime, cephalothin, penicillin, ampicillin, carbenicillin, amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, mezlocillin, piperacillin, ceftizoxime, cefotaxime, and cefoperazone. In contrast, the remaining 23 strains (group 2) were uniformly susceptible to none of the 30 antimicrobial agents, although 22 of the 23 strains were susceptible to amikacin. A comparison of the serotypes of group 1 and group 2 strains showed that seven of the eight group 1 strains were serotype 4 strains. Six of these seven strains also showed cross-reactivity with antiserum against *Mycobacterium xenopi*. The other group 1 strain was untypable. The group 2 strains included 12 untypable, 4 type 4, 2 type 9, 2 type 43, and one each type 13 and type 1/43 strains. One group 2 strain reacted only with antiserum against *M. xenopi*.

Determination of MBC(AFB)s. MBC(AFB)s after 4 and 7 days were determined against 12 strains of *M. avium* complex for the following antimicrobial agents: amikacin, ampicillin, azlocillin, amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, carbenicillin, cephalothin, cefotaxime, cefonicid, ceftriaxone, ceftizoxime, cefuroxime, cefmenoxime, moxalactam, and imipenem. The MBC(AFB)s of eight of these antimicrobial agents on day 7 were, for some strains, at levels that are achievable in serum. Representative MICs and MBC(AFB)s of these antimicrobial agents against three strains are presented in Table 3. Strains 10 and 11 are group 1 strains, while strain 14 belongs to group 2. Six of the antimicrobial agents in Table 3 killed the group 1 strains and three antimicrobial agents killed the group 2 strain at concentrations of drug that are achievable in serum. Ticarcillin-clavulanic acid, cefonicid, cephalothin, ceftriaxone, ceftizoxime, cefuroxime, and moxalactam did not kill any of the 12 strains after 7 days at a concentration of drug that can be achieved in serum.

At concentrations of 4 to 8 μg/ml, imipenem killed 99% of strain 10 and 11 cells after 4 days, but after 7 days, the MBC(AFB) for these strains increased to >32 μg/ml. This increase in the MBC(AFB) of imipenem between days 4 and 7 occurred with all 12 strains. For the other drugs tested, the MBC(AFB) typically remained constant (36% of comparisons on days 4 and 7) or decreased by one dilution between days 4 and 7 (53% of comparisons). In no case among the

antimicrobial agents tested was there commonly a wide discrepancy between the MICs and MBC(AFB)s after 7 days of incubation. MBC(AFB)s, however, were frequently two- to fourfold higher than corresponding MICs.

Test for beta-lactamase. The Sceptor Gram Positive MIC trays contain a well for testing for the presence of beta-lactamase activity. Unfortunately, the 7HSF broth medium caused hydrolysis of the beta-lactamase substrate in this well after several hours of incubation. Tests for beta-lactamase production were therefore performed by using nitrocefin disks inoculated with growth from an agar slant or a drop of sediment from a broth culture. None of the 31 strains of *M. avium* complex tested by these methods showed evidence of beta-lactamase activity after 60 min of incubation.

Colonial morphology. The predominant colonial morphology of all strains of *M. avium* complex tested here was smooth, domed, and translucent. Opaque spots usually developed in the center of these colonies during continued incubation. Such colonies are commonly referred to by others as simply domed and opaque. Thin, smooth, transparent, and rough opaque colony types were in a minority both before and after exposure to antimicrobial agents. The relative proportions of the various colonial morphologies of *M. avium* complex did not change markedly after exposure of the organisms to antimicrobial agents in 7HSF broth for 7 days.

DISCUSSION

The absence of effective therapy for the treatment of disseminated *M. avium* complex infection in patients with AIDS underscores the importance of screening a variety of antimicrobial agents for activity against the *M. avium* complex. The susceptibility of some of our strains to beta-lactam and aminoglycoside antimicrobial agents is consistent with the results of other studies (12–14). We were unable to demonstrate beta-lactamase activity in any of the 31 strains tested by using a chromogenic substrate. The resistance of some strains to many beta-lactam antimicrobial agents, however, suggests that beta-lactamase may be present but not detected by present methods (9).

Our finding that broth dilution MICs are lower than agar dilution MICs is in agreement with the findings of Cynamon (3) and Mizuguchi et al. (12). Cynamon suggested that the difference between broth and agar MICs may be due to the presence of Tween 80 in 7H9 broth and the absence of this compound in the agar medium he used (3). Our broth and agar media, however, do not contain Tween 80. When Tween 80 was added to 7HSF broth, there was a reduction in the MICs of some antimicrobial agents. We have also noticed this effect in tube dilution experiments with rifabutine, clofazimine, and ciprofloxacin (data not shown). Data from other workers and our own study suggest that the presence of Tween 80 in media used for susceptibility testing might cause an overestimate of the susceptibility of *M. avium* complex to certain antimicrobial agents.

Another factor that can affect the MIC is the length of incubation before an MIC reading is taken. Our data show that MIC readings taken at day 4 are less affected by drug deterioration than are readings at day 7. On the other hand, MBC(AFB)s often decreased between days 4 and 7. The best correlation between MICs and MBC(AFB)s was obtained when trays were incubated for 7 days. Incubation of MIC trays for periods longer than 7 days could make interpretation of MICs more difficult owing to continued deterioration of drugs and subsequent outgrowth of inhibited cells. This could result in falsely high MICs.

We found that in most cases an increase in MIC between days 4 and 7 correlated with a significant deterioration of antimicrobial activity during the 7 days of incubation. This was most notable with imipenem, amoxicillin-clavulanic acid, and cephalothin. Some strains of *M. avium* complex, however, were inhibited by these antibiotics for 7 days in spite of a significant loss of drug activity during incubation. MIC determinations on any new drugs should take into consideration the possible effect of drug deterioration on MICs.

Unlike rifabutine and clofazimine (17), the antimicrobial agents studied here gave MBC(AFB)s that were only slightly higher than their MICs. For some strains, the MBC(AFB)s of 8 of 15 antimicrobial agents tested were at levels that are achievable in serum. Killing of *M. avium* complex by imipenem was difficult to interpret. Unlike the results for most of the other antimicrobial agents tested, the MBC (AFB) of imipenem increased greatly between days 4 and 7 of incubation. This increase may be due to the rapid deterioration of imipenem that occurred during incubation of the microdilution trays.

Other workers have noted a correlation between the virulence of *M. avium* complex isolates and their colonial morphology (2). Colonies with the thin transparent morphology have been described as being more virulent and more resistant to antimicrobial agents than the domed, more opaque colony type (2, 4). We have observed, however, that few isolates obtained from patients with AIDS are thin and transparent on primary isolation. Upon subculture, occasional thin transparent colonies may arise. We did not notice any increase in the relative proportion of the thin transparent colonies after exposure to antimicrobial agents. Our observations do not support the notion that the thin transparent colony type is more virulent or resistant.

A final note of caution seems appropriate regarding the findings of this study. In vitro tests such as those described here do not yield information on the ability of antimicrobial agents to kill *M. avium* complex in the cells and tissues where it resides. However, the data in this study do show that (i) the simple broth microdilution method used here yields results that are comparable with results obtained by the more traditional agar proportion method; (ii) strains of *M. avium* complex isolated from patients with AIDS differ in their susceptibilities to various antimicrobial agents; and (iii) some strains are killed by antimicrobial agents at a concentration of drug that is achievable in serum. Further work is needed to determine the clinical relevance of these findings.

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