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Rapid Broth Macrodilution Method for Determination of MICs for *Mycobacterium avium* Isolates

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A multicenter study was done to investigate the accuracy and reproducibility of a method for determining the MICs of antimicrobial agents against the *Mycobacterium avium* complex in 7H12 broth with the BACTEC system. In phase I, with eight drugs and 10 strains, intralaboratory reproducibility was 95.7 to 100%, allowing a 1-dilution difference upon repeat testing. The results of phase II testing with 41 additional strains were consistent with those obtained in phase I, with good interlaboratory reproducibility. The radiometric method was validated by sampling and plating of the same broth cultures and determining, by the number of CFU per milliliter, the lowest drug concentration that inhibited more than 99% of the initial bacterial population. Three test concentrations of each drug and the tentative interpretation of results are proposed. Radiometric MIC determination has the potential to become the method of choice for clinical microbiology laboratories and evaluation of new agents for the treatment of *M. avium* infections, both pulmonary and disseminated.

Infections caused by mycobacteria other than *Mycobacterium tuberculosis* are on the rise. Among mycobacteria other than *M. tuberculosis*, infection due to the *M. avium* complex (MAC) in general and *M. avium* in particular is the most important and most prevalent one (11, 14). MAC has been known to cause pulmonary disease without detectable predisposing conditions. The increasing frequency of disseminated MAC infection in immunocompromised patients, particularly those with human immunodeficiency virus infection, has drawn attention throughout the world (12, 15, 21). The therapeutic efficacy of antimicrobial therapy against such infections has yet to be established. There are conflicting reports on the susceptibility of these organisms to antituberculosis drugs (1, 10, 20), and many newer antimicrobial agents are being evaluated for in vitro activity (2, 5–8).

One of the problems in studying the therapeutic efficacy of antimicrobial agents or the emergence of drug resistance among MAC organisms is the lack of a standard technique for in vitro susceptibility testing. The proportion or critical-concentration method originally developed for *M. tuberculosis* is often applied to MAC susceptibility testing, which may yield misleading results. There is wide strain-to-strain variation in MAC susceptibility to antimicrobial agents, and therefore, testing against a single drug concentration, as is done with *M. tuberculosis*, does not seem to be suitable (6).

The introduction of the rapid radiometric (BACTEC) method for detection of mycobacterial growth and susceptibility testing of *M. tuberculosis* (13, 16–18) provided a unique opportunity for MAC susceptibility testing in a short time with a wide range of concentrations of antimicrobial agents in liquid medium. MIC determination was the approach that appeared to meet the requirements for testing of conventional antituberculosis drugs, as well as experimental drugs. The MIC, established for other aerobic bacteria, is the lowest concentration of a drug, tested in twofold doubling concentrations, that inhibits more than 99% of the bacterial population. In 7H12 broth, the MIC could be interpreted by the radiometric growth index (GI) and validated by the conventional reference method of plating the broth cultures and counting the number of CFU per milliliter. MIC determination and the advantages of this approach compared with other procedures have been discussed at length by Heifets in a recent publication (4). The purpose of the present multicenter study was to investigate the accuracy and reproducibility of the radiometric method for determination of the MICs of various drugs for *M. avium* and to standardize the method.

MATERIALS AND METHODS

Study sites. The study was done at five sites, the Veterans Affairs Medical Center, Syracuse, N.Y.; the National Jewish Center for Immunology and Respiratory Medicine, Denver, Colo.; the National Reference Center for Tuberculosis, Ottawa, Ontario, Canada; the Maryland State Health Department, Baltimore; and Research & Development, Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.

Test cultures. We tested 51 clinical isolates of *M. avium*, of which 50 were isolated from AIDS patients with disseminated infection and 1 was from a nonimmunocompromised patient with a pulmonary infection. All isolates were confirmed to be *M. avium* by Gen-Probe assay (Gen Probe, San Diego, Calif.).

The cultures were supplied to each site by the National

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TABLE 1. Concentrations of antimicrobial agents tested

Antimicrobial agent	Concns tested (μg/ml)	
	Phase I	Phase II
Amikacin	2.0, 4.0, 8.0	1.0, 2.0, 4.0, 8.0
Ciprofloxacin	0.5, 1.0, 2.0, 4.0, 8.0	0.5, 1.0, 2.0, 4.0, 8.0
Clofazimine	0.12, 0.25, 0.5	0.03, 0.06, 0.12, 0.25
Cycloserine	7.0, 14.0, 28.0	4.0, 8.0, 16.0
Ethambutol	1.0, 2.0, 4.0, 8.0	1.0, 2.0, 4.0, 8.0
Ethionamide	1.2, 2.5, 5.0	1.0, 2.0, 4.0, 8.0, 16.0
Rifampin	0.5, 1.0, 2.0, 4.0, 8.0	0.5, 1.0, 2.0, 4.0, 8.0
Streptomycin	2.0, 4.0, 8.0	1.0, 2.0, 4.0, 8.0

Jewish Center for Immunology and Respiratory Medicine. From a 7H10 agar plate culture, transparent colonies were transferred to 7H9 broth and incubated at 37°C. Once growth was observed, the broth was subcultured to 7H10 agar slants which were shipped to each participating site in five separate shipments. On receipt, these cultures were incubated at 35 to 37°C and tested once sufficient growth was obtained.

Testing schedule. In phase I, 10 cultures were tested at each site at least in duplicate on different days. After completion of testing, results from all five testing sites were collated and compared. If at any site the MICs of a particular antimicrobial agent differed significantly from those reported by the other sites, the test was repeated and results with the two closest MICs at that site were used in the analysis. Phase I data were analyzed to evaluate intralaboratory variations and also to establish the drug concentrations to be tested in phase II.

Phase II included 41 cultures distributed in four shipments. These cultures were tested only once to establish the interlaboratory reproducibility of the test.

Antimicrobial agents and concentrations. The antimicrobial agents tested in this study and the various concentrations used in phases I and II are summarized in Table 1. After the results of phase I were reviewed, minor adjustments in the concentrations were made for phase II. Stock solutions were prepared after factoring in the potency of each antimicrobial powder. Working solutions were made, the concentrations of which were 40-fold greater than the desired concentration in the medium so that when 0.1 ml was added to 4 ml of medium the required final concentration was obtained.

Amikacin, streptomycin, ciprofloxacin, and ethambutol were dissolved in processed water. Clofazimine was dissolved in dimethyl sulfoxide, cycloserine was dissolved in water with the pH adjusted to 10.0 with 0.1% Na_2CO_3, ethionamide was dissolved in ethylene glycol, and rifampin was dissolved in methanol. After the drugs were completely dissolved, the solutions were filter sterilized through 0.2-μm-pore-size polycarbonate filters (Nalgene), except for those drugs dissolved in organic solvents, which were incubated at 37°C overnight for self-sterilization. Further dilutions were made in sterile, processed water, except for clofazimine, which was diluted in dimethyl sulfoxide. Working solutions were frozen in aliquots at −70°C, except for clofazimine, which was stored at room temperature in the dark. At the time of testing, one frozen vial of each test solution was thawed and 0.1 ml was aseptically added to a 12B medium vial. One 12B vial was used for each concentration of each test drug.

Culture media. Standard Middlebrook radiometric 7H12 broth medium (BACTEC 12B; Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) was used for inoculum preparation and susceptibility testing. The medium was tested on a BACTEC 460 instrument for detection of growth radiometrically as indicated by GI units. Middlebrook 7H9 broth (Becton Dickinson Microbiology Systems, Cockeysville, Md.) was used to prepare the stock suspension. Middlebrook 7H10 agar slants were used for subculturing and distribution of cultures, and 7H10 agar plates were used for estimation of CFU.

Preparation of mycobacterial inoculum. Once there was satisfactory growth on the 7H10 agar slants, a few colonies were scraped off and a suspension was made in BACTEC diluting fluid (Becton Dickinson Diagnostic Instrument Systems), homogenized, and adjusted to the density of a McFarland no. 1 standard, and 1-ml aliquots were frozen at −70°C. If growth on 7H10 slants was not sufficient, a few colonies were inoculated into a tube of 7H9 broth and incubated at 35 to 37°C. The tube was examined visually for growth (approximately 3 to 6 days), and once turbid, the growth was diluted with the diluting fluid to adjust the turbidity to approximate a McFarland no. 1 standard. This adjusted suspension was divided into 1-ml aliquots and frozen at −70°C. Before freezing, one vial of the final suspension was tested for quality control by inoculating 0.1 ml into a 12B vial, incubating it at 35 to 37°C, and testing it daily on a BACTEC 460 instrument. For a suspension to be considered satisfactory, inoculated 12B vials should reach a GI of 999 within 24 to 48 h.

Prior to testing, a frozen culture was thawed and mixed thoroughly with an allergist syringe (BD-5540) and 0.1 ml was inoculated into a 12B vial (seed vial). After 24 h of incubation at 35 to 37°C, this vial was tested on a BACTEC 460 instrument and used to inoculate the susceptibility test vials when the GI reached 999 (24 to 48 h).

Inoculation. Prior to inoculation, the 12B vials were tested on a BACTEC 460 instrument to establish a 5% CO_2 atmosphere inside the bottles. The culture seed vial was homogenized thoroughly with an allergist syringe and then diluted 1:100 by adding 0.1 ml to 9.9 ml of BACTEC diluting fluid; this dilution constituted the working suspension. After the working suspension was mixed thoroughly, 0.1 ml was inoculated into each of the drug-containing vials and one vial with no drug (undiluted control). The working suspension was then diluted 1:100, and 0.1 ml was inoculated into another 12B vial with no drug (1:100-diluted control); this amount represented 1% of the bacterial population.

Testing and quality controls. Inoculated vials were incubated at 35 to 37°C and tested on a BACTEC 460 instrument at approximately the same time each day. The MIC was interpretable when, on the day of interpretation, the GI of the 1:100 control read 20 or more for 3 consecutive days while the GI of the undiluted control reached 999. These requirements had to be met between days 4 and 8 of incubation (the day of inoculation is considered day 1) for a test to be considered valid.

If the GI of the undiluted control reached 999 earlier than day 4, the test was considered to be overinoculated and was repeated. Similarly, if the GI did not reach 999 or the GI of the 1:100 control did not reach 20 or greater for 3 consecutive days by day 8 of incubation, the test was considered to be underinoculated and was repeated.

Interpretation. The test was interpreted on the day the GI in the 1:100 control was 20 or more for 3 consecutive days. For a susceptible culture, the final GI in the drug-containing medium at the time of interpretation should be less than 50. Initially, three GI cutoff points, 20, 50, and 100, in the drug-containing vial were considered for determination of

susceptibility. Subsequently, a GI cutoff of 50 was selected for the final analysis. In this study, the MIC was considered the lowest concentration of the test antimicrobial agent that inhibited more than 99% of the bacterial population. On the basis of GI values, the MIC was the lowest concentration of a drug in the presence of which the daily GI increases were less than those in the 1:100 control and the final GI reading in the drug vial was not greater than 50 at the conclusion of the test.

MIC validation. At one site (the National Jewish Center for Immunology and Respiratory Medicine), samples were plated on 7H10 agar to establish the CFU counts on various days during MIC testing. This was done to verify that the MIC was the lowest concentration that inhibited the growth of more than 99% of the bacterial population. A limited number of isolates (usually two) were set up in replicate, and the vials were tested daily on a BACTEC 460 instrument. At the same time, one vial from the other set was sampled at various times to determine the CFU counts. The lowest concentration of a drug which inhibited more than 99% of the bacterial population as determined by CFU counts was designated the MIC and compared with the radiometrically determined MIC.

Analysis of data. In phase I, intralaboratory variation of MICs for each isolate was established by analyzing the duplicate testing of the first 10 isolates. Interlaboratory variation was evaluated by analyzing the MICs for each antimicrobial agent among the five study sites. Median or modal MICs were taken into consideration for this analysis. The modal value was the MIC which was found in most of the testings (three of five sites). In a few cases in which there was no clear majority, the median value was considered for analysis. Numbers and percentages of findings which were within 1 dilution (±1 dilution) of the modal MICs were calculated. A more-than-1-dilution difference was considered a disagreement.

RESULTS

The reproducibility of MIC testing results in phase I is summarized in Tables 2 and 3. Wide strain-to-strain variations in the MICs were observed. However, the reproducibility (within a 1-dilution difference) of the test for each isolate varied from 90 to 100%. Among the five test sites, depending on the antimicrobial agent studied, 50 to 100% of the MICs were exactly the same on repeat testing, except for rifampin, for which the values were lower (Table 2). If a 1-dilution difference was considered to be an agreement, the variation was negligible and in most cases there was 100% agreement between the two testings (Table 2). The interlaboratory frequency distribution of the MICs within ±1 dilution of the modal value is summarized for each antimicrobial agent in Table 3. In most of the cases, the results fell within ±1 dilution of the modal value. With rifampin, clofazimine, and ethionamide, there were a few instances of a more-than-1-dilution difference. However, there was good overall agreement on replicate testing at each of the five sites, considering a 1-dilution difference as an agreement.

Results of phase II were found to be consistent with those obtained in phase I. The frequency distribution of MICs for 41 isolates illustrates the relative variation of MICs for these isolates with each test antimicrobial agent. The frequency distribution of MICs obtained in phase II is summarized in Fig. 1, while the overall MICs for all of the test isolates in both phases are summarized in Table 4. The MICs of amikacin for 47% of all of the test isolates were 4.0 μg/ml or less, while for 53% of the isolates they were ≥8.0 μg/ml. For ciprofloxacin, the MICs for 65% of the isolates were 1.0 to 4.0 μg/ml, while for 35% they were ≥8.0 μg/ml. The MICs of clofazimine were significantly lower than those of the other antimicrobial agents (0.25 μg/ml or lower), while the MICs of cycloserine and ethionamide were higher; for 14 and 55% of the isolates, the MICs were >16.0 μg/ml, respectively. The ethambutol and streptomycin MICs were 8.0 μg/ml or lower for 100 and 90% of the isolates, respectively. MICs of rifampin had a wider range than those of the other antimicrobial agents. Overall, the MICs of the test drugs for all of the isolates were in good agreement with the modal values, ranging from a low of 83% for rifampin to a high of 100% for amikacin (Table 5).

Validations of MICs are summarized in Fig. 2. The radiometrically determined MICs correlated well with those

TABLE 2. Intralaboratory reproducibility of MICs for *M. avium* determined by multiple testing[a]

Antimicrobial agent	Dilution difference between replicates[b]	% Agreement between duplicate tests at site:				
		1	2	3	4	5
Amikacin	0	50	80	90	100	70
	±1	100	100	100	100	100
Ciprofloxacin	0	67	80	60	50	50
	±1	100	100	100	100	100
Clofazimine	0	100	100	100	90	90
	±1	100	100	100	100	90
Cycloserine	0	83	90	100	80	100
	±1	100	100	100	100	100
Ethambutol	0	67	80	80	50	80
	±1	100	100	100	100	100
Ethionamide	0	83	80	80	70	90
	±1	100	100	100	100	100
Rifampin	0	50	20	90	30	80
	±1	60	100	100	90	100
Streptomycin	0	33	80	70	80	90
	±1	100	100	100	100	100

[a] Phase I, 10 isolates.
[b] 0, no difference in MICs between duplicate testings. ±1, difference between duplicate tests was within 1 dilution.

TABLE 3. Overall interlaboratory reproducibility of MICs for *M. avium* determined by multiple testing[a]

Antimicrobial agent	% of isolates with following dilution difference from modal value:					
	±1	−2	−1	0	+1	+2
Amikacin	100	0	11	80	9	0
Ciprofloxacin	100	0	24	61	15	0
Clofazimine	98	2	0	96	2	0
Cycloserine	100	0	4	91	4	0
Ethambutol	100	0	15	72	13	0
Ethionamide	98	0	2	83	13	2
Rifampin	96.0	0	22	56	17	4
Streptomycin	100	0	9	74	17	0

[a] Phase I, 10 isolates; all sites combined.

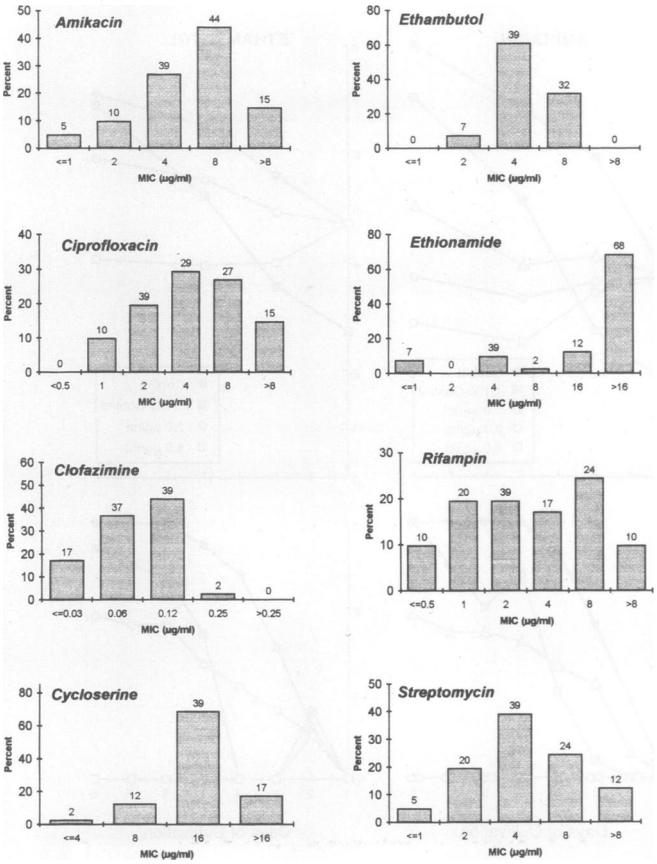

FIG. 1. Frequency distribution of MICs for the test antimicrobial agents in phase II.

based on CFU counts. The concentration of a test antimicrobial agent which met the established MIC criteria by radiometric measurement inhibited 99% of growth in 7H12 broth as determined by CFU counts.

DISCUSSION

This study indicates that drug susceptibility testing of *M. avium* can be performed with reproducible results and that the MICs of different antimicrobial agents can be determined. The results, once the test is set up, can be reported within 6 to 8 days with liquid medium and radiometric detection of growth and its inhibition.

TABLE 5. Overall agreement[a] of MICs of eight antimicrobial agents among five test sites[b]

Antimicrobial agent	% Agreement for test site:					
	1	2	3	4	5	Total % agreement
Amikacin	100	100	100	100	100	100
Ciprofloxacin	94	94	94	98	96	95
Clofazimine	82	90	98	98	98	93
Cycloserine	100	100	100	98	96	99
Ethambutol	98	100	100	100	100	100
Ethionamide	88	94	96	86	90	91
Rifampin	82	80	94	78	80	83
Streptomycin	98	96	94	96	100	97

[a] Agreement was defined as MICs within ±1 dilution of the modal value across all sites.
[b] Total, 51 cultures.

There is an urgent need for effective drugs against MAC infection, particularly for disseminated infection in AIDS patients. Newer antimicrobial agents are being introduced; however, there is no standard procedure for evaluation of their efficacy in vitro. The Centers for Disease Control recently recommended that state health department laboratories discontinue drug susceptibility testing of MAC, partly because the conventional methods established for *M. tuberculosis*, when applied to mycobacteria other than *M. tuberculosis*, yielded results indicating resistance to most of the drugs. The wide range of susceptibility of these organisms makes in vitro susceptibility testing more difficult, especially by the conventional agar plate method. Moreover, little information correlating in vitro susceptibility results with clinical response to therapy is available (9, 19).

Our earlier studies indicated that susceptibility testing of *M. avium* is inoculum dependent. The inoculum had to be well controlled in order to yield reproducible results.

Greater susceptibilities of these organisms to most antimicrobial agents in broth media versus solid media have been reported (3, 4, 10, 20). There are several possible explanations for this difference. The broth susceptibility test, especially the radiometric method, is a rapid test taking only 6 to 8 days, while the agar method requires a longer incubation period. In a separate study, the stability of amikacin, ciprofloxacin, clofazimine, ethionamide, rifampin, and streptomycin was determined by chemical methods (14a). There was no significant loss of the activity of any of these antimicrobial agents in 12B medium incubated for up to 8 days at 37 ± 1°C in the presence or absence of *M. avium*.

During prolonged incubation in an agar medium, there

TABLE 4. Percent occurrence of MICs of each antimicrobial agent for test isolates[a]

Antimicrobial agent	MIC (µg/ml), no. (%) of isolates						
Amikacin	≤2, 10 (20)	4, 14 (27)	8, 21 (41)	≥8, 6 (12)			
Ciprofloxacin	≤0.5, 0	1, 9 (18)	2, 10 (20)	4, 14 (27)	8, 12 (23)	>8, 6 (12)	
Clofazimine	≤0.03, 7 (14)	0.06, 15 (29)	0.12, 28 (55)	0.25, 1 (2)	>0.25, 0		
Cycloserine	≤4, 1 (2)	7–8,[b] 7 (14)	14–16,[b] 36 (70)	>16, 7 (14)			
Ethambutol	≤1, 0	2, 6 (12)	4, 32 (63)	8, 13 (25)	>8, 0		
Ethionamide	≤1–1.25, 4 (8)	2–2.5,[b] 0	4–5,[b] 4 (8)	>5,[b] 9 (17)	8, 1 (2)	16, 5 (10)	>16, 28 (55)
Rifampin	≤0.5, 9 (18)	1, 8 (16)	2, 12 (23)	4, 8 (16)	8, 10 (20)	>8, 4 (8)	
Streptomycin	<2, 17 (33)	4, 16 (31)	8, 13 (26)	>8, 5 (10)			

[a] All sites and phase I and II combined (51 isolates).
[b] Different concentrations were used in phases I and II.

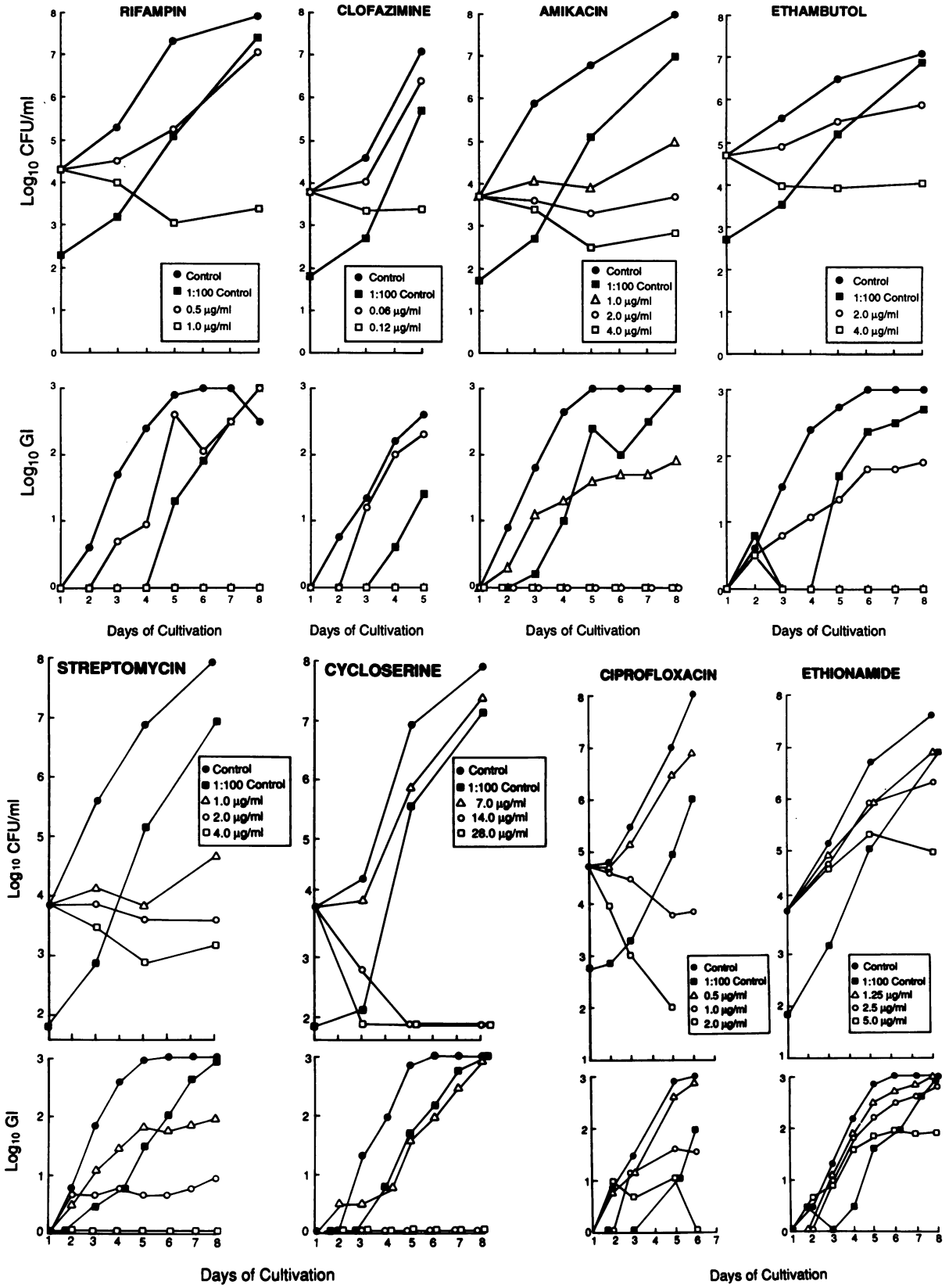

FIG. 2. Correlation between CFU per milliliter and daily GI readings in the control and in the presence of antimicrobial agents for MIC validation.

TABLE 6. Recommended concentrations for determination of MICs for MAC isolates and suggested interpretation of MICs

Antimicrobial agent	Recommended concns (μg/ml)	MIC (μg/ml) for following interpretation:		
		Susceptible	Intermediate	Resistant
Amikacin	2.0, 4.0, 8.0	≤2.0	4.0	≥8.0
Ciprofloxacin	1.0, 2.0, 4.0	≤1.0	2.0	≥4.0
Clofazimine	0.06, 0.12, 0.25	≤0.12	0.25	≥0.5
Cycloserine	4.0, 8.0, 16.0	≤4.0	8.0	≥16.0
Ethambutol	2.0, 4.0, 8.0	≤2.0	4.0	≥8.0
Ethionamide	1.0, 2.0, 4.0	≤1.0	2.0	≥4.0
Rifampin[a]	0.5, 2.0, 8.0	≤0.5	2.0	≥8.0
Streptomycin	2.0, 4.0, 8.0	≤2.0	4.0	≥8.0

[a] Because of the wide range of MICs, fourfold dilutions are recommended for rifampin.

could be some adsorption, as well as breakdown, of a test drug. In liquid medium, there is more cell-to-drug contact as the bacterial population is submerged in the drug-containing medium, while on solid medium bacteria grow on the surface and a concentration gradient could develop during the incubation and growth of bacteria. MAC organisms grow better in liquid media than on solid media and, thus, a test in liquid medium would be more appropriate, especially with the convenience of testing several drug concentrations.

Standardization of the procedure was the most important step for achievement of reproducible results. Inoculum preparation was such that approximately 10^4 to 10^5 CFU were inoculated into each undiluted control and drug-containing vial. Two controls, one undiluted and one diluted 1:100, were necessary to monitor inoculum size and growth patterns. The specified time of 6 to 8 days for interpretation eliminates the impact of over- or underinoculation. Growth in the 1:100-diluted control was needed to determine the time for test interpretation. A GI cutoff of 50 was found to yield more reliable results than a GI cutoff of 20 or 100. A GI cutoff of 20 was too low, because in some situations it could represent background GI readings due to metabolism in the presence of a bacteriostatic agent and could thus lead to the reporting of false resistance. With the cutoff at GI 100, there is a possibility of reporting of false susceptibility because of a gradual increase in the GI on prolonged incubation.

When the test protocol was closely adhered to, the reproducibility of the procedure was excellent. The tests were done by experienced and less trained technicians, yet the results did not differ greatly in all of the test sites. This susceptibility testing procedure was not compared with a solid-medium method because no such standard procedure is available.

A wide range of MICs for MAC organisms has been reported previously (3, 6, 8), which makes it more essential that several concentrations of a drug be tested. On the basis of MIC values, pharmacokinetic parameters, levels achievable in serum, and cost considerations, we recommend the use of three concentrations for each antimicrobial agent, which are listed in Table 6. Clinical isolates could be divided into three tentative categories, susceptible, intermediate (moderately susceptible), and resistant, on the basis of in vitro MIC testing, as indicated in Table 6. It is proposed that the concentration to which the test organisms are found to be completely or moderately susceptible should be considered for future evaluation of therapeutic effectiveness. Our test cultures were analyzed on the basis of these MIC criteria, and the results are summarized in Table 7. This analysis indicates that certain antimicrobial agents, like clofazimine, rifampin, ethambutol, and streptomycin, are active in vitro against most of the test isolates, while cycloserine and ethionamide are not as active against most of these isolates when tested in vitro. History of prior treatment of patients from whom these cultures were isolated was not available.

In vitro susceptibilities of MAC organisms to some of these antimicrobial agents have previously been reported (4, 7, 8, 10, 12, 13, 20). The variation in the results could be due to the different methods used. The clinical relevance of in vitro susceptibility testing of MAC organisms is not well established, in part because of a lack of a standardized procedure for performance of in vitro susceptibility testing and, until recently, the absence of controlled clinical trials. Horsburgh and coworkers studied 75 patients with pulmonary MAC infections and correlated clinical responses with in vitro susceptibility test results (9). Fifty patients responded to the therapy, and 25 were nonresponders. The researchers recommended that therapy be based on a patient's in vitro drug susceptibility test results. An effective clinical trial requires a standardized quantitative method of in vitro susceptibility testing to follow the clinical response and the development of resistance during the course of therapy.

The proposed method in this study is the first step in the establishment of a standardized, rapid, and reproducible technique for MAC susceptibility testing. Further work is needed to evaluate the clinical relevance of MAC in vitro susceptibility test results for both immunocompromised and nonimmunocompromised patients with localized, as well as disseminated, infections. Clinical trials with AIDS patients with disseminated infections are in progress to determine whether a correlation exists.

TABLE 7. Number of test isolates susceptible, intermediate, or resistant on the basis of the proposed criteria

Antimicrobial agent	No. (%) of strains		
	Susceptible	Intermediate	Resistant
Amikacin	10 (20)	14 (27)	27 (53)
Ciprofloxacin	9 (18)	10 (20)	32 (63)
Clofazimine	50 (98)	1 (2)	0 (0)
Cycloserine	1 (2)	7 (14)	43 (84)
Ethambutol	6 (12)	32 (63)	13 (25)
Ethionamide	4 (8)	0 (0)	47 (92)
Rifampin	17 (34)	20 (39)	14 (27)
Streptomycin	17 (33)	16 (31)	18 (35)

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