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Multisite Reproducibility of Results Obtained by Two Broth Dilution Methods for Susceptibility Testing of *Mycobacterium avium* Complex

Gail L. Woods,[1]* Natalie Williams-Bouyer,[1] Richard J. Wallace, Jr.,[2] Barbara A. Brown-Elliott,[2] Frank G. Witebsky,[3] Patricia S. Conville,[3] Marianne Plaunt,[4] Geraldine Hall,[5] Priscilla Aralar,[6] and Clark Inderlied[6]

Department of Pathology, University of Texas Medical Branch, Galveston, Texas 77555[1]; Department of Microbiology, University of Texas Health Center at Tyler, Tyler, Texas 75710[2]; Microbiology Service, Department of Laboratory Medicine, W. G. Magnuson Clinical Center, National Institutes of Health, Bethesda, Maryland 20892[3]; StatProbe, Ann Arbor, Michigan 48108[4]; Department of Microbiology, Cleveland Clinic Foundation, Cleveland, Ohio 44195[5]; and Department of Pathology, Children's Hospital, Los Angeles, California 90027[6]

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A multicenter study was conducted to assess the interlaboratory reproducibility of susceptibility testing of *Mycobacterium avium* complex (MAC) by broth microdilution using two different media (cation-adjusted Mueller-Hinton broth with 5% oleic acid-albumin-dextrose-catalase and 7H9 broth with casein) and by macrodilution using the BACTEC 460TB and 12B media at pH 6.8 and 7.3 to 7.4. Ten well-characterized strains of MAC (four macrolide susceptible, six macrolide resistant) were tested against clarithromycin and azithromycin (the latter only by BACTEC 460TB, pH 6.8). At each site, strains were tested against clarithromycin three times on each of three separate days (nine testing events per isolate) by using a common lot of microdilution trays and BACTEC 12B medium, pH 6.8; strains were tested once on three separate days against clarithromycin in 12B medium at pH 7.3 to 7.4 and against azithromycin. Agreement among MICs (i.e., mode ± 1 twofold dilution) was 100% for all strains and both drugs when BACTEC 460TB was used, regardless of the pH of the medium, but varied when microdilution with either medium was used, particularly with susceptible strains. Agreement based on interpretive category, with NCCLS-recommended breakpoints, was 100% for all strains with the BACTEC 460TB method (both drugs and both pH values) and with microdilution using 7H9 broth. With microdilution and Mueller-Hinton broth, agreement by interpretive category was 100% for eight isolates and >90% for two; errors occurred only in laboratories where personnel had minimal experience with this technique. MAC susceptibility testing may be performed by broth macrodilution or microdilution at either pH, with NCCLS-recommended interpretive breakpoints. However, because visual interpretation of broth microdilution end points is subjective, it is more prone to reader error; therefore, this method requires greater expertise than the BACTEC 460TB. Both techniques require appropriate validation and continued documentation of proficiency.

Organisms in the *Mycobacterium avium* complex (MAC) are the most frequently encountered mycobacteria in many clinical laboratories in the United States today. MAC causes a wide range of infections (14, 15, 20, 24, 25). Among the most important are disseminated disease in persons with AIDS, and invasive pulmonary disease, including upper lobe cavitary disease in males who are smokers and often abuse alcohol and nodular interstitial disease in older women with associated cylindrical bronchiectasis and adolescents with cystic fibrosis. Data from several studies have shown that macrolides (azalides) are effective therapy for both disseminated MAC and invasive pulmonary diseases and are effective prophylactic agents for persons with AIDS who are at risk for disseminated MAC (2–10, 17, 23, 24). Indeed, the incidence of disseminated disease has declined dramatically in recent years with the widespread use of MAC prophylaxis and highly active antiretroviral therapy. Additionally, macrolides are the only antimicrobial agents for which a correlation between in vitro susceptibility tests for MAC and clinical response has been demonstrated in controlled clinical trials (3, 21).

Indications for MAC susceptibility testing are not clearly defined or uniformly accepted by all clinicians. Isolates for which testing might be considered include (i) clinically significant isolates from patients who are receiving or have received prior macrolide therapy, (ii) blood isolates from patients who become bacteremic while receiving macrolide prophylaxis, (iii) isolates from patients who relapse while on macrolide therapy, and (iv) initial isolates from blood or tissue of patients with disseminated disease or from respiratory specimens of patients with invasive pulmonary disease to establish baseline values (16, 19, 24). When testing is performed, the recently published guidelines of the NCCLS (formerly National Committee for Clinical Laboratory Standards) for susceptibility testing of the nontuberculous mycobacteria recommend broth microdilution or macrodilution, such as the BACTEC 460TB (19). However, there is controversy regarding the optimal pH of the broth medium. Macrolides are more active in vitro under mildly alkaline conditions (pH 7.3 to 7.4) than under slightly acidic conditions (pH 6.8, which is the pH of the commercially available BACTEC 12B medium). Therefore, testing in broth at pH 7.3 to 7.4 is suggested by some investigators (12, 13). Others

* Corresponding author. Present address: Merck & Co., Inc., 10 Sentry Pkwy., BL3-4, Blue Bell, PA 19422. Phone: (484) 344-2481. Fax: (484) 344-3404. E-mail: gail_woods@merck.com.

advocate the use of a medium at pH 6.8 because some MAC isolates grow poorly at the higher pH and because the BACTEC 12B medium is unstable at pH 7.4 (16; S. Beaty, S. Siddiqi, and M. Gnacek, Abstr. 92nd Gen. Meet. Am. Soc. Microbiol., abstr. U102, 1992). Additionally, the intracellular environment of macrophages infected with MAC is pH 6.0 to 6.5, which suggests that testing the susceptibility of MAC under mildly acidic conditions may be more clinically relevant. Because of the controversy regarding pH, NCCLS-recommended breakpoints differ based on the pH of the medium in which the isolate is tested. The purpose of this multicenter study was to assess a BACTEC 460TB method using 12B medium at pH 6.8 and 7.3 to 7.4 and a microdilution method for their ability to provide reproducible end points and interpretive categories in several laboratories with different levels of experience with susceptibility testing of MAC isolates.

MATERIALS AND METHODS

Organisms. Ten clinical strains of MAC (separate isolates from separate patients) and one American Type Culture Collection strain (ATCC 700898, included as the quality control [QC] strain) were selected for testing. All test strains had previously been studied in depth as a part of a drug discovery project at the University of Southern California Children's Hospital (Los Angeles, Calif.). This included antimicrobial susceptibility testing of several agents as a means to establish mechanisms of action and/or resistance. The test strains used in this study reflect the serotypes commonly isolated from patients with MAC disease and the differences in their susceptibilities to macrolide antimicrobial agents. Of the 10 clinical strains tested, 4 were previously determined to be macrolide susceptible and 6 were resistant, based on susceptibility testing, molecular analysis, and clinical response. Expected clarithromycin MICs by BACTEC 460TB, pH 6.8, against macrolide-susceptible strains were as follows: strain 100, 1 μg/ml; strain 104, 2 μg/ml; strain 116, 1 μg/ml; strain 504, 2 μg/ml. The MIC ranges of other agents against these four strains were 0.5 to 4 μg/ml for ciprofloxacin, 0.5 to 1 μg/ml for rifabutin, and 8 to 16 μg/ml for ethambutol; the amikacin MIC for all four strains was 4 μg/ml. Against each macrolide-resistant strain, strains 101R, 511, 512, 513, JJL, and JWT, the expected clarithromycin MIC result by BACTEC 460TB, pH 6.8, was >64 μg/ml. MIC ranges of other agents against these six strains were 2 to 8 μg/ml for ciprofloxacin, 0.5 to 1 μg/ml for rifabutin, and 4 to 8 μg/ml for ethambutol; the amikacin MIC for all six strains was 2 μg/ml. Thus, the MIC ranges for four nonmacrolides (i.e., ciprofloxacin, amikacin, rifabutin, and ethambutol) varied from 1 to 3 dilutions and the ranges for macrolide-susceptible and macrolide-resistant strains were similar or the same. Therefore, the 10 test strains are highly likely to be representative of macrolide-susceptible and macrolide-resistant MAC isolates from clinical specimens. However, there are no clinical correlates or interpretive criteria for these nonmacrolide antimicrobials, and routine susceptibility testing of agents other than macrolides is not recommended (19, 24).

Isolates on Middlebrook 7H11 agar slants were mailed from the University of Southern California Children's Hospital to the four other participating sites. Upon arrival in the site laboratory, the isolates were suspended in Middlebrook 7H9 broth containing 5% glycerol (BBL, Becton Dickinson Diagnostic Systems, Sparks, Md.) to a turbidity equal to that of a 1.0 McFarland standard ($\sim 10^8$ CFU/ml) and frozen at −70°C. Prior to testing, an aliquot of each frozen stock culture was subcultured onto Middlebrook 7H11 agar plates (BBL, Becton Dickinson Diagnostic Systems).

Antimicrobial agents. Single lots of azithromycin and clarithromycin powders were provided by Pfizer Pharmaceuticals (New York, N.Y.) and Abbott Laboratories (Abbott Park, Ill.), respectively. Powders were stored at 4°C prior to testing. Stock solutions of each drug (azithromycin, 10,000 μg/ml; clarithromycin, 3,333 μg/ml) were prepared fresh in methanol at the time of testing. For the BACTEC 460TB method, final concentration ranges (in doubling dilutions) for azithromycin and clarithromycin were 32 to 512 μg/ml and 4 to 64 μg/ml, respectively, for evaluations of test strains and 8 to 64 μg/ml and 1 to 8 μg/ml, respectively, for evaluations of the QC strain. For broth microdilution testing, a single lot of dried and sealed microtiter trays containing twofold serial dilutions of clarithromycin was provided by Trek Diagnostic Systems (Westlake, Ohio). The final concentration range was 0.25 to 256 μg/ml. Each tray also contained a positive-growth-control well. The trays were stored at ambient temperature until they were used in the study.

Inoculum preparation. For BACTEC 460TB susceptibility testing, MAC colonies (transparent colonies were selected, if possible) from the subculture on Middlebrook 7H11 agar were suspended in sterile saline. The growth suspensions were mixed vigorously on a vortex mixer for 15 to 20 s, and the turbidity was adjusted to match that of a 1.0 McFarland standard. Working inocula were prepared by making a 1:100 dilution of each suspension. The inocula for control vials were prepared by making a 1:100 dilution of each working inoculum. To determine inoculum size, 0.1 ml of a 1:1,500 dilution of each working inoculum was plated onto Middlebrook 7H11 agar plates to obtain ~200 CFU/plate, thus yielding a final inoculum of $\sim 7.5 \times 10^4$ CFU/ml in BACTEC 12B culture vials (Becton Dickinson Diagnostic Systems), which contained 4.0 ml of Middlebrook 7H12 media. Plates were also monitored daily to ensure that each test was not contaminated.

For broth microdilution testing, inocula were prepared by transferring three to five MAC colonies to tubes containing 5.0 ml of demineralized water; subsequent organism suspensions were adjusted to equal the turbidity of a 0.5 McFarland standard ($\sim 10^6$ CFU/ml). The final inocula ($\sim 5 \times 10^5$ CFU/ml) were prepared by transferring 25 μl of the suspension to tubes containing 5.0 ml of either 7H9 broth supplemented with casein (pH 6.8 to 6.9) or cation-adjusted Mueller-Hinton broth supplemented with 5% oleic acid-albumin-dextrose-catalase (OADC) enrichment (pH 7.3 to 7.4; both provided by Trek Diagnostic Systems). Each of the tubes was then inverted 8 to 10 times prior to use.

Susceptibility test methods. (i) BACTEC 460TB method. MIC testing of clarithromycin was performed with BACTEC 12B media at pH 6.8, which is the pH of the commercially available product, and pH 7.3 to 7.4, which was specially prepared by Becton Dickinson Diagnostic Systems for this study; azithromycin MIC testing was performed with media at pH 6.8 only. The procedure used in this study is a modification of that previously described by Siddiqi et al. (22). BACTEC 12B vials were tested on the BACTEC 460TB instrument prior to the addition of drugs and inocula to establish a 5% CO_2 atmosphere in each vial as well as to detect contamination (i.e., vials with a growth index [GI] ≥20 were not used for testing). Vials were injected with 0.1 ml of the well-mixed antimicrobial solution to be tested at each concentration. The working inoculum (0.1 ml) of the QC strain and each test isolate was added to the appropriate drug-containing vials. Drug-free growth control vials for each isolate were inoculated with 0.1 ml of the 1:100 dilution of the working inoculum. Additional test controls included (i) a negative control (no antimicrobial agent and no bacterial inoculum) and (ii) a no-growth control (no antimicrobial agent and heat-killed [100°C] inoculum).

Test vials were incubated at 37 ± 1°C and read daily on the BACTEC 460TB instrument. If the growth control reached a GI of ≥30 within 2 days, the inoculum was too heavy and the test was repeated. For tests with an appropriate organism concentration, the minimum requirement for testing was 4 days (5 days of incubation), and readings were monitored for up to 7 days (8 days of incubation). The test results were interpreted when the GI of the growth control was ≥30 for three consecutive days; an isolate at a particular drug concentration was considered to be susceptible if the GI was <50 when this condition was met. When determined in this manner, the MIC was defined as the lowest drug concentration that gave a final GI reading of <50. Because the organism concentration in the drug-free control vial was 100-fold lower than that in the drug-containing vials, the MIC represents the lowest concentration of drug that inhibits more than 99% of the mycobacterial population tested.

(ii) Broth microdilution method. Broth microdilution testing was performed with clarithromycin only. Testing was performed within 30 min following final inoculum preparation as described by Brown et al. (1). Final inoculum suspensions were poured into sterile disposable reagent reservoirs (Matrix Technologies Corporation, Hudson, N.H.), and 100-μl aliquots were transferred to each well of the MIC tray with a multichannel pipettor. The inoculated trays were covered with an adhesive seal and incubated at 35°C in ambient air. A Middlebrook 7H11 agar plate was also inoculated with a loopful of the final inoculum to check for purity. The trays were first examined following 7 days of incubation. If growth (appearing as turbidity or a deposit of cells at the bottom of the well) in the growth control well was sufficient (i.e., at least 2+ based on the following scale: ± to 1+ growth, a few flecks in the bottom of the well; 2+, moderate growth; 3+ to 4+, a readily visible button in the bottom of the well), the MICs were recorded. If growth in the control wells was insufficient, the trays were reincubated to achieve better growth and read again after an additional 7 days of incubation. The MIC was recorded as the lowest concentration of clarithromycin that inhibited visible growth. If no growth was detected in control wells following reincubation of trays, results were invalid.

Study design and analysis. Five laboratories participated in this study; one tested only clarithromycin (by BACTEC 460TB and broth microdilution), one

TABLE 1. Broth dilution breakpoints for macrolides suggested by NCCLS for MAC

Drug	Method/pH	MIC (μg/ml) for category:		
		Susceptible	Intermediate	Resistant
Clarithromycin	BACTEC 460TB or microdilution/6.8	≤16	32	≥64
Clarithromycin	BACTEC 460TB/7.3–7.4	≤4	8–16	≥32
Clarithromycin	Microdilution/7.3–7.4	≤8	16	≥32
Azithromycin	BACTEC 460TB/6.8	≤128	256	≥512

tested only azithromycin, and three tested both drugs. Four laboratories tested clarithromycin (both methods), and four tested azithromycin. All five laboratories had experience in using the BACTEC 460TB system, although one laboratory (laboratory A) had considerably more experience in using it to test MAC than the others. Two laboratories (A and C) had extensive experience with the microdilution method of MAC susceptibility testing; the other two laboratories that evaluated this method (B and D) had minimal or no experience. When testing clarithromycin by microdilution and by BACTEC 460TB with media at pH 6.8, the four laboratories tested each isolate three times on each of three separate days. When evaluating clarithromycin in the 12B medium at pH 7.3 to 7.4 and azithromycin in 12B at pH 6.8, laboratories tested each isolate only once on each of three separate days. The MIC results and the day of reading were recorded on data sheets and mailed to a coinvestigator (M.P.) for entry into a database. Each test at each site was considered a separate result. Agreement was determined by calculating the percentage of MICs within a 3-dilution range (i.e., mode ± 1 twofold dilution) for each drug. High-off-scale MICs were converted to the next-highest concentration, whereas low-off-scale MICs were left unchanged. The breakpoints for determining susceptibility and resistance (Table 1) are those recently suggested by the NCCLS (19).

RESULTS

The day on which the MICs were considered interpretable varied based on method and medium. For all isolates, BACTEC 460TB results were interpretable earlier than microdilution results, regardless of medium. The average times to results with the BACTEC 460TB were 6.1 days (ranges, 4 to 10 days for clarithromycin and 3 to 10 days for azithromycin) for media at pH 6.8 and 6.5 days (range, 5 to 10 days) at pH 7.3 to 7.4. The average times to results when using microdilution were 8.8 days (range, 6 to 14 days) for 7H9 broth and 9.0 days (range, 6 to 14 days) for Mueller-Hinton broth.

MIC results obtained by BACTEC 460TB are summarized in Table 2. With the BACTEC 460TB, there was 100% agreement on MICs among the four sites that tested clarithromycin when testing was performed at both pH values and among the four sites that tested azithromycin (pH 6.8). However, one isolate at one site did not grow when azithromycin was tested.

Clarithromycin results by microdilution are shown in Tables 3 and 4. Agreement among MICs was more variable than when using the BACTEC 460TB, especially with susceptible strains, and interlaboratory agreement was more variable than intralaboratory agreement. There was less than 100% agreement among the four laboratories for 5 of the 10 strains with Mueller-Hinton broth and for 3 strains with 7H9 broth. Additionally, some strains did not grow in Mueller-Hinton and/or 7H9 broth, although this was a problem at only two sites (laboratories B and D, which had minimal experience with this test method). Of the 36 times each strain was tested, strain 504 did not grow once (2.8%) in Mueller-Hinton broth, strain 116 failed to grow 3 times (8.3%) in Mueller-Hinton broth and 11 times (30.6%) in 7H9, and strain 100 did not grow 9 times (25.0%) in Mueller-Hinton broth.

To assess the potential impact of the variability in MIC results on patient management, we also evaluated percent agreement based on the interpretive category. With BACTEC 460TB, agreement again was 100% for both drugs and for both pH values when clarithromycin was tested. For microdilution, agreement was 100% for all strains when 7H9 medium was used and 100% for eight of the strains when Mueller-Hinton medium was used. For the other two strains, agreement was

TABLE 2. MICs of clarithromycin and azithromycin for MAC reported by four laboratories for each drug using BACTEC 460TB

MAC strain	MIC[a] of:		
	Clarithromycin at pH:		Azithromycin at pH 6.8
	6.8	7.3–7.4	
JWT	128 (128)	128 (64–128)	1,024 (1,024)
JJL	128 (128)	128 (128)	1,024 (1,024)
513	128 (128)	128 (128)	1,024 (1,024)
512	128 (128)	128 (128)	1,024 (512–1,024)
511	128 (128)	128 (128)	1,024 (512–1,024)
504	4 (4)	4 (4)	32 (32)
116	4 (4)	4 (4)	32 (32)[b]
104	4 (4)	4 (4)	32 (32)
100	4 (4)	4 (4)	32 (32)
101R	128 (128)	128 (128)	1,024 (512–1,024)

[a] Values are modes. Values in parentheses are ranges. All values are in micrograms per milliliter.
[b] One isolate at one site did not grow.

TABLE 3. MICs of clarithromycin for MAC reported by four laboratories using microtiter dilution

MAC strain	MIC (μg/ml) for isolate using:			
	Mueller-Hinton broth with 5% OADC		7H9 broth with casein	
	Mode (range)	% Agreement[a]	Mode (range)	% Agreement
JWT	512 (512)	100	512 (512)	100
JJL	512 (128–512)	77.8	512 (512)	100
513	512 (256–512)	100	512 (256–512)	100
512	512 (512)	100	512 (512)	100
511	512 (512)	100	512 (512)	100
504	0.5 (0.25–512)	82.9	2 (1–2)	100
116	0.5 (0.25–512)	90.9	2 (0.5–4)	92.0
104	1 (0.5–2)	100	8 (2–8)	75.0
100	0.25 (0.25–1)	88.9	4 (0.5–16)	72.2
101R	512 (64–512)	91.7	512 (512)	100

[a] % Agreement, percentage of MICs in each 3-dilution range (mode ± $\log_2$) for isolates that produced sufficient growth. High-off-scale MICs were converted to the next-highest concentration; low-off-scale MICs were left unchanged. The following isolates did not grow for one or more tests: strain 504, once in Mueller-Hinton; strain 116, 3 times in Mueller-Hinton and 11 times in 7H9; strain 100, 9 times in Mueller-Hinton.

TABLE 4. MICs of clarithromycin for MAC by microdilution by laboratory

Strain	Modal MIC[a] with indicated medium[h] at laboratory[b]:							
	A		B		C		D	
	MH	7H9	MH	7H9	MH	7H9	MH	7H9
JWT	512 (512)	512 (512)	512 (512)	512 (512)	512 (512)	512 (512)	512 (512)	512 (512)
JJL	512 (512)	512 (512)	512 (512)	512 (512)	256 (128—512)	512 (512)	128 (128–512)	512 (512)
513	512 (512)	512 (512)	256 (256–512)	512 (512)	256 (256–512)	512 (512)	512 (512)	512 (256–512)
512	512 (512)	512 (512)	512 (512)	512 (512)	512 (512)	512 (512)	512 (512)	512 (512)
511	512 (512)	512 (512)	512 (512)	512 (512)	512 (512)	512 (512)	512 (512)	512 (512)
504	—[b] (0.25–1)	2 (2)	— (0.5–512)[c]	2 (1–2)	0.5 (0.5–1)	2 (2)	0.25 (0.25–4)[d]	2 (2)
116	1 (0.5–1)	2 (2)	— (0.25–512)[c,e]	NG[i]	0.5 (0.25–1)	2 (0.5–2)	— (0.25–1)	2 (2–4)[f]
104	1 (0.5–1)	2 (2)	1 (0.5–1)	4 (4)	1 (0.5–2)	8 (4–8)	1 (0.5–2)	8 (8)
100	0.5 (0.25–0.5)	8 (4–16)	0.25 (0.25)[e]	0.5 (0.5–512)[c,e]	0.25 (0.25)	4 (4–8)	1 (1)[g]	2 (2–4)
101R	512 (512)	512 (512)	512 (512)	512 (512)	256 (64–512)	512 (512)	512 (512)	512 (512)

[a] Values in parentheses are ranges. All values are in micrograms per milliliter. —, no modal MIC.
[b] Laboratories A and C had considerable experience with microdilution testing of MAC; laboratories B and D had minimal or no experience.
[c] Results for three tests on 1 day were 512 μg/ml.
[d] No growth for one test on 1 day.
[e] No growth for all three tests on 1 day.
[f] No growth for two tests on 1 day.
[g] No growth for all three tests on 2 days.
[h] MH, Mueller-Hinton broth with 5% OADC; 7H9, 7H9 broth with casein.
[i] NG, no growth.

91.4 (32 of 35 tests [no growth once]; strain 504) and 90.9% (30 of 33 tests [no growth in three events]; strain 116). All three incorrect results for these two strains occurred on 1 day at one site.

DISCUSSION

Susceptibility testing of clinically significant isolates of MAC against a macrolide is useful in certain situations, especially blood isolates from patients with AIDS who are receiving macrolide prophylaxis and become bacteremic and isolates from patients with disseminated or invasive pulmonary disease who relapse while on macrolide therapy. In this study, thoroughly characterized strains of macrolide-susceptible and -resistant MAC were tested by macrodilution and microdilution using media with different pH values. The strains selected were clinical isolates representative of those typically encountered in a clinical mycobacteriology laboratory. In general, MAC isolates from patients never treated with a macrolide are susceptible to the macrolides. After exposure to a macrolide, MAC may acquire a one-step point mutation in the adenine at position 2058 or 2059 in the 23S rDNA, resulting in clinical resistance and MICs in the resistant range (11, 18). MAC isolates with intermediate susceptibility to macrolides are very infrequently encountered and, therefore, were not included in this evaluation.

We found that the overall reproducibility of MICs was better for the BACTEC 460TB, regardless of the pH of the 12B medium, than for microdilution. The variability in MICs when using microdilution was greater among the four laboratories that performed the test than within each laboratory and, in general, was greatest in the two laboratories that had minimal or no experience in using microdilution to test MAC isolates (laboratories B and D; Table 4). This likely reflects the subjective end point of microdilution compared to the objective BACTEC 460TB reading. Additionally, the problem of strains failing to grow occurred only in the two laboratories that had minimal or no experience in using microdilution to test MAC isolates, and the only marked discrepancies (i.e., clarithromycin MIC of 512 μg/ml for a susceptible strain) occurred in one of these two laboratories (laboratory B). This suggests that the lower reproducibility may be more related to the expertise of the testing personnel rather than to a problem with the method itself. All five laboratories participating in this study had experience with the BACTEC 460TB; but if one or more laboratories had had minimal experience, reproducibility might have been less than 100%.

With microdilution testing, lower reproducibility occurred predominantly when macrolide-susceptible strains were tested, especially those with MICs of ≤4 μg/ml, although the categorical agreement was excellent. It is unknown whether similar variability would have occurred with the BACTEC 460TB had lower concentrations of clarithromycin been tested. BACTEC 460TB concentrations were selected based on clinical relevance and recommendations of the NCCLS (19). Additionally, there is no evidence to suggest that MAC isolates for which the MICs were in the 0.5- to 8-μg/ml range respond differently to therapy.

Medium pH influenced MICs of susceptible, but not resistant, strains obtained by microdilution. Microdilution MICs with 7H9 broth (pH 6.8) were generally higher (by two- to eightfold) than values with Mueller-Hinton broth (pH 7.3 to 7.4). This difference is similar to what other investigators have previously reported (13). With BACTEC 460TB, pH had no apparent impact on MICs. It is likely that the range of clarithromycin concentrations tested precluded detection of any pH effect with the BACTEC 460TB and with microdilution when resistant strains were tested.

Results were available earlier (by an average of almost 3 days) with BACTEC 460TB than with microdilution. This difference between BACTEC 460TB and microdilution may, in part, reflect the design of our study. We selected day 7 for the first microdilution reading, but trays could have been examined earlier. Although microdilution results for several tests at all

sites could not be interpreted at 7 days due to inadequate growth, it is possible that results for those with sufficient growth were interpretable earlier. Moreover, inexperienced testing personnel found microdilution end points more difficult to read when using Mueller-Hinton broth than when using 7H9 broth. With BACTEC 460TB, results were available about 10 h sooner when 12B medium at pH 6.8 was used than when 12B medium at pH 7.3 to 7.4 was used, which is consistent with slower growth at the higher pH.

In summary, data from this study suggest that both BACTEC 460TB and microdilution are acceptable methods for testing the susceptibility of MAC to macrolides. Similarly, the test medium used may be either pH 6.8 or pH 7.3 to 7.4, providing the appropriate interpretive breakpoints are used (19). However, with the BACTEC 460TB, using the commercially available 12B medium (pH 6.8) is more convenient than modifying the pH and would appear to be less prone to technical error because fewer manipulations are involved. Although proper training of testing personnel is important for both methods, it appears to be particularly critical for microdilution testing due to the subjective interpretation of the end point compared with an objective reading with the BACTEC 460TB. For laboratories that infrequently have requests for MAC susceptibility testing, therefore, referring those isolates to an experienced laboratory may be most reasonable. If a laboratory chooses to perform testing in house, test performance must be validated and continued proficiency must be documented at regular intervals. The Centers for Disease Control and Prevention performance evaluation program for susceptibility testing of *Mycobacterium tuberculosis* occasionally includes a MAC challenge; but, because MAC is not a consistent part of that program, the best approach to test validation and documenting proficiency may be comparison of results with those of an experienced reference laboratory.

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