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Comparison of Agar Dilution, Broth Microdilution, Disk Diffusion, E-Test, and BACTEC Radiometric Methods for Antimicrobial Susceptibility Testing of Clinical Isolates of the *Nocardia asteroides* Complex

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An evaluation was undertaken to determine the optimal method for the in vitro susceptibility testing of 26 *Nocardia asteroides* complex isolates to the following antimicrobial agents: amikacin, ampicillin, amoxicillin-clavulanate, ceftriaxone, ciprofloxacin, erythromycin, imipenem, minocycline, and trimethoprim-sulfamethoxazole. Five testing methods were studied including the agar dilution, broth microdilution, and disk diffusion methods, the epsilometer test (E-test), and the BACTEC radiometric method. Results for each antimicrobial agent and each testing method were interpreted as indicating susceptibility, intermediate susceptibility, or resistance according to current guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) for bacteria that grow aerobically and were then compared to a "gold standard" susceptibility test result. The gold standard result for each *Nocardia* isolate was established by a consensus of the results of the majority of testing methods used in the study. When the results were combined for all antimicrobial agents tested against all *Nocardia* isolates by all methods, the BACTEC radiometric method produced the highest level of agreement (97.9%) with the consensus results and had the fewest very major ($n = 1$), major ($n = 2$), and minor ($n = 2$) errors. In contrast, the results of the agar dilution method were in least agreement (93.2%) with the consensus results, and this method also produced the most very major ($n = 8$), major ($n = 4$), and, along with the disk diffusion method, minor ($n = 6$) errors. For all test methods, interpretive errors were most frequent when testing ampicillin or amoxicillin-clavulanate. Moreover, for all *Nocardia nova* isolates tested, ampicillin susceptibility results by any of the testing methods were not in agreement with the results of testing for β-lactamase by the nitrocefin (Cefinase) disk method. We conclude that among the methods evaluated, the BACTEC radiometric method appeared to be the best for determining the in vitro susceptibilities of members of the *N. asteroides* complex to a panel of nine antimicrobial agents. However, none of the test methods, including the BACTEC method, accurately predicted the ampicillin resistance of the *N. nova* isolates tested, all of which produced β-lactamase. Presuming that this β-lactamase hydrolyzes ampicillin, this disparity may relate to the NCCLS breakpoints that were used, which may require modification for this antimicrobial agent when tested against *N. nova* isolates.

In our laboratory we have encountered an increasing demand for the susceptibility testing of clinical isolates of the *Nocardia asteroides* complex (*N. asteroides* sensu stricto, *N. farcinica*, and *N. nova*) against a variety of conventional and newer antimicrobial agents. Because microorganisms of this group can produce significant disease, especially in immunocompromised individuals, and because infections with these microorganisms require extended treatment courses, clinicians are particularly interested in the results of in vitro antimicrobial susceptibility tests. Currently, no standards have been provided by the National Committee for Clinical Laboratory Standards (NCCLS) for testing *Nocardia* spp. Over the years, a few studies have evaluated conventional susceptibility testing methods for these microorganisms, including the disk diffusion (2, 15), broth dilution (2, 15, 17), and agar dilution (2, 4) methods. Recently, two newer methods, the epsilometer test (E-test) (2) and the BACTEC radiometric method (12) have been evaluated. However, no studies which compared all of these methods simultaneously have been performed.

The objective of the current study was to compare the results of three conventional testing methods, the disk diffusion, broth dilution, and agar dilution methods, with two newer methods, the BACTEC radiometric method and the E-test, for testing the in vitro susceptibilities of reference and clinical isolates of the *N. asteroides* complex to a panel of antimicrobial agents. Specifically, 11 isolates of *N. asteroides* sensu stricto, 8 isolates of *N. farcinica*, and 7 isolates of *N. nova* were studied for their susceptibilities to amikacin, ampicillin, amoxicillin-clavulanate, ceftriaxone, ciprofloxacin, erythromycin, imipenem, minocycline, and trimethoprin-sulfamethoxazole.

MATERIALS AND METHODS

Microorganisms. Twenty-six *Nocardia* isolates were tested, including 3 reference strains (*N. asteroides* sensu stricto ATCC 19247, *N. farcinica* ATCC 3318, and *N. nova* ATCC 33726) and 23 clinical isolates. All clinical isolates were confirmed as *N. asteroides* complex strains by their ability to be stained by the modified acid staining method and their failure to decompose casein, xanthine, and/or tyrosine. These isolates were further differentiated as *N. asteroides* sensu

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stricto (n = 10), *N. farcinica* (n = 7), and *N. nova* (n = 6), as proposed by Beaman and colleagues (1), by determining their ability to grow at 45°C, produce arysulfatase at 14 days, and produce opacification on Middlebrook (7H10) agar and by evaluating their susceptibilities to erythromycin, ampicillin, and cefamandole. The following reference microorganisms were used as controls in susceptibility tests: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, and *Enterococcus faecalis* ATCC 29212.

Inoculum preparation. The *Nocardia* isolates were subcultured twice onto sheep blood agar to ensure purity. A large amount of organisms was scraped from the second sheep blood agar plate and was inoculated into test tubes containing Mueller-Hinton broth (MHB). One-millimeter glass beads were added to the MHB to aid in the dispersion of the organisms, and the MHB was incubated at 35°C in ambient air until an optical density approximately equivalent to that of a 1.0 McFarland standard was achieved. The incubation period required for adequate growth ranged from 4 to 9 days. The MHB tubes were periodically vortexed at high speed for 2 to 3 min to achieve a uniform suspension of the organisms, and vortexing was repeated prior to optical density determinations.

Susceptibility testing. (i) Agar dilution. The *Nocardia* isolates were tested by methods published by NCCLS for dilutional susceptibility tests for bacteria that grow aerobically (8, 9). Briefly, Mueller-Hinton agar (MHA) plates with appropriate concentrations of antimicrobial agents were inoculated by using a replicate plating device. A 0.15-μl inoculum with a turbidity equivalent to that of a 0.5 to 1.0 McFarland standard ($\sim 10^8$ CFU/ml), which resulted in $\sim 10^4$ CFU/ml per spot, was delivered, and the MHA plates were incubated at 35°C. The MHA plates were visually inspected after incubation for 3 days. The MIC was the lowest concentration of antimicrobial agent which prevented visible growth. The MHA plates used to test trimethoprim-sulfamethoxazole were supplemented with lysed horse blood.

(ii) Broth microdilution. The *Nocardia* isolates were tested by following NCCLS guidelines for dilutional susceptibility tests for aerobic bacteria (8, 9). Microtiter plates were prepared inhouse and contained all antimicrobial agents except ampicillin and amoxicillin-clavulanate. The plates were stored at −70°C and were thawed at room temperature immediately before use. The appropriate dilutions of ampicillin and amoxicillin-clavulanate were freshly prepared immediately before use and were aliquoted and placed in designated microtiter wells. Ten microliters of an inoculum with a turbidity equivalent to that of a 0.5 to 1.0 McFarland standard was dispensed into each well to give a final concentration of 10^4 to 10^5 CFU/ml. The microtiter plates were incubated at 35°C and were read after 3 days. The MIC was the lowest concentration of antimicrobial agent at which no visible growth could be detected by visual inspection.

(iii) Disk diffusion. The *Nocardia* isolates were tested according to NCCLS guidelines for antimicrobial disk susceptibility tests (9, 10). One hundred fifty-millimeter MHA plates were inoculated by confluent swabbing a suspension of organisms with a turbidity equivalent to that of a 0.5 to 1.0 McFarland standard. Nine antimicrobial agent-containing disks were placed onto each MHA plate, and these plates were incubated at 35°C for 3 days. Inhibitory zones were recorded at 24-h intervals.

(iv) E-test. MHA plates were inoculated in the same way as described above for disk diffusion testing. A maximum of five E-test strips (AB Biodisk, Solna, Sweden) were applied to each MHA plate, and the plates were incubated at 35°C for 3 days. The MICs were determined in accordance with the guidelines provided by the manufacturer. For testing ceftriaxone, high-range strips (0.16 to 250 μg/ml) were used.

(v) BACTEC radiometric method. For testing by the BACTEC radiometric method, a procedure similar to that used by Scopetti and colleagues (12) was followed. One-tenth of a milliliter of the appropriate concentration of antimicrobial agent was injected into vials containing 4 ml of Middlebrook agar (7H12; Becton Dickinson, Sparks, Md.). A suspension of organisms with a turbidity approximately equivalent to that of a 1.0 McFarland standard was diluted 1:1,000, and 0.1 ml of this suspension was inoculated into each BACTEC vial. This created a bacterial density of approximately 10^4 to 10^5 CFU/ml. Two antimicrobial agent-free vials, one with the same density of bacteria as the vials containing antimicrobial agents and one with 100-fold fewer bacteria, were prepared. All vials were incubated at 37°C and monitored on an automated BACTEC 460 instrument (Becton Dickinson, Sparks, Md.) every 24 h until the growth index for the 1:100 control was >10 for 2 consecutive days. The MIC was determined as the minimum concentration which resulted in a lower change in the growth index for the drug-containing sample compared with that for the 1:100 control.

(vi) β-Lactamase testing. β-Lactamase determinations were done by the nitrocefin (Cefinase; Becton Dickinson, Cockeysville, Md.) disk method. All *Nocardia* isolates were checked by examining disks for a color change indicating the hydrolysis of nitrocefin. This examination was done at 5 min, and if no reaction was apparent, the disks were reexamined at 1 h. *Nocardia* spp. with negative nitrocefin disk test results (i.e., no color change after 5 min and 1 h) were subcultured onto MHA plates onto which amoxicillin-clavulanate disks were placed. After adequate growth, the colonies growing closest to the zone of inhibition produced by the amoxicillin-clavulanate disks were then tested for β-lactamase activity by using the nitrocefin disk method described above.

(vii) Interpretation of susceptibility results. Organisms in the categories of susceptible, intermediate, and resistant were determined by using the breakpoints provided in NCCLS guidelines for microorganisms that grow aerobically (8–10). For ampicillin, the breakpoints of ≤2 (susceptible) and ≥4 (resistant) that are recommended for *Listeria monocytogenes* were used. These breakpoints are lower than those recommended by NCCLS for members of the family *Enterobacteriaceae* and lower than those used by some investigators who previously evaluated *Nocardia* isolates for their susceptibility to ampicillin. By using lower breakpoints, we hoped to identify *Nocardia* strains for which MICs were relatively low yet that produced β-lactamase and that may therefore be considered resistant. One previous study by Wallace and Steele (18) indicated that virtually all *Nocardia* isolates that they tested possessed β-lactamase activity. For the purposes of comparing susceptibility testing methods, the reference or "gold standard" susceptibility of each *Nocardia* isolate to which all results were compared was established by using a consensus of the results among a majority of the testing methods in the current study. Interpretive results were compared by determining percent agreement and frequencies of very major (resistant strain called susceptible), major (susceptible strain called resistant), and minor (resistant or susceptible strains called intermediate or intermediate strain called resistant or susceptible) errors.

Statistical analysis. The differences in results for very major, major, and minor errors were determined by a generalized estimating equation model (20).

RESULTS

All *Nocardia* isolates that were tested produced suitable growth for analysis. The results were determined at 72 h, which appeared to be the optimal growth period for all isolates. Table 1 presents the MICs at which 50% of isolates are inhibited (MIC_{50}s), MIC_{90}s, and range of MICs and mean zone diameters and range of zone diameters for all *Nocardia* isolates and all the susceptibility test methods. The rank order for the apparent best in vitro susceptibility results for the antimicrobial agents tested (the antimicrobial agents to which the *Nocardia* isolates were most susceptible) was as follows: amikacin = trimethoprim-sulfamethoxazole > minocycline > imipenem > ceftriaxone > amoxicillin-clavulanate > ciprofloxacin > ampicillin > erythromycin. Not shown in Table 1 are specific results for the organism group (i.e., *Nocardia* species). In this regard, the results for ampicillin testing for all *N. nova* isolates (n = 7) were not in agreement with the results of β-lactamase testing. Using the nitrocefin disk method, 9 of 10 (90%) *N. asteroides* sensu stricto isolates, 8 of 8 (100%) *N. farcinica* isolates, and 5 of 7 (71%) *N. nova* isolates produced β-lactamase. After induction with amoxicillin-clavulanate, β-lactamase activity was also observed by the nitrocefin disk method for all isolates (one *N. asteroides* sensu stricto isolate and two *N. nova* isolates) which did not produce β-lactamase initially by nitrocefin disk testing. For *N. asteroides* sensu stricto and *N. farcinica* isolates, the results of β-lactamase testing agreed with the consensus interpretive results for ampicillin (all isolates were ampicillin resistant) produced by the other susceptibility testing methods. In contrast, all *N. nova* isolates, by consensus, were susceptible to ampicillin, yet they produced β-lactamase. Also not shown in Table 1 is the fact that all *N. nova* and all *N. asteroides* sensu stricto isolates, by a consensus of the results of all methods, showed resistance to amoxicillin-clavulanate.

Table 2 displays the percent agreement between each susceptibility testing method result compared to the consensus interpretive category result for all susceptibility testing methods. Table 3 presents the interpretive category discrepancies (very major, major, and minor errors) for each susceptibility test method, again compared to the consensus interpretive result for all susceptibility testing methods. Of note, all *N. asteroides* sensu stricto, *N. farcinica*, and *N. nova* isolates had the typical consensus susceptibility patterns for ampicillin, amoxicillin-clavulanate, and erythromycin, as described previously by other investigators (1, 2).

When the results were combined for all antimicrobial agents

TABLE 1. MIC_{50}s, MIC_{90}s, and range of MICs determined by agar dilution and broth microdilution methods, E-test, and BACTEC radiometric method and mean zone diameters by the disk diffusion method for 26 isolates of *N. asteroides* complex

Antimicrobial agent	Agar dilution method				Broth microdilution method				E-test				BACTEC radiometric method				Zone of inhibition (mm) by disk diffusion method			
	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)	% Susceptible[a]	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)	% Susceptible[a]	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)	% Susceptible[a]	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)	% Susceptible[a]	50%[h]	90%[i]	Range	% Susceptible[a]
Amikacin	4	4	4–8	100	1	2	0.12–4	100	0.25	0.75	0.032–1	100	1	1	—[b]	100	42	38	33–BM[c]	100
Ampicillin[d]	16	>16	1–>16	27	64	>128	0.5–>128	27	16	NZ[e]	0.064–NZ	27	16	>16	1–>16	27	NZ	NZ	NZ–BM	23
Amoxicillin-clavulanate	8	>16	8–>16	65	16	>128	1–>128	42	16	NZ	0.75–NZ	38	>16	>16	8–>16	38	NZ	NZ	NZ–BM	35
Ceftriaxone	8	>32	8–>32	58	32	>128	0.5–>128	54	6	NZ	0.12–NZ	65	8	32	8–32	58	10	NZ	NZ–50	46
Ciprofloxacin	>2	>2	1–>2	27	8	128	0.12–>128	31	NZ	NZ	0.064–NZ	31	>2	>2	—[f]	31	NZ	NZ	NZ–43	38
Erythromycin	>4	>4	0.5–>4	27	>128	>128	0.25–>128	27	16	NZ	0.032–NZ	27	>4	>4	—[f]	23	NZ	NZ	NZ–BM	27
Imipenem	4	4	—[b]	96	1	8	0.12–16	92	0.19	1.5	0.003–3	100	4	4	—[b]	100	45	38	30–BM	100
Minocycline	2	4	1–4	100	2	4	0.12–8	96	1	2	0.032–6	100	4	4	—[b]	100	35	28	27–BM	100
Trimethoprim-sulfamethoxazole[g]	0.5	0.5	—[b]	92	0.5	1	0.03–8	100	0.064	0.5	0.003–1	100	0.5	0.5	0.5–2	100	34	28	21–BM	100

[a] Susceptibility based on NCCLS interpretive category guidelines for gram-positive bacteria that grow aerobically (8–10).
[b] —, no range resulted because all isolates were susceptible to the following antimicrobial agents at the indicated concentrations: amikacin, 1 μg/ml; imipenem, 4 μg/ml; minocycline, 4 μg/ml; and trimethoprim, 0.5 μg/ml.
[c] BM, zone diameter beyond margins of the Mueller-Hinton agar plates.
[d] Results may be invalid if NCCLS breakpoints for ampicillin and gram-positive bacteria that grow aerobically are used because it has been shown that essentially all *N. asteroides* complex spp. produce β-lactamase (18). In the current study, all *N. asteroides* complex isolates that showed susceptibility to ampicillin were determined to be as *N. nova*. By the nitrocefin disk method, with and without induction, all *Nocardia* isolates possessed β-lactamase activity.
[e] NZ, no zone of inhibition.
[f] —, no range resulted because all isolates were resistant to the following antimicrobial agents at the highest concentrations tested, as indicated: ciprofloxacin, 2 μg/ml; erythromycin, 4 μg/ml.
[g] Results for trimethoprim alone.
[h] Mean zone diameter at which 50% of the isolates are inhibited.
[i] Mean zone diameter at which 90% of the isolates are inhibited.

tested against all *Nocardia* isolates by all methods, the BACTEC radiometric broth method produced results which had the highest level of agreement with the consensus interpretive results and the fewest very major and major errors (combined very major and major errors, $n = 3$). The agar dilution test method produced results which had the least agreement with the consensus interpretive results and the highest number of very major and major interpretive errors (combined very major and major errors, $n = 12$). Minor interpretive errors occurred least frequently with the BACTEC method ($n = 2$) and most frequently with the disk diffusion and agar dilution methods ($n = 6$). The majority of very major errors occurred with susceptibility testing of amoxicillin-clavulanate (14 of 18 [78%]) by any method. Major errors most frequently occurred with testing of ceftriaxone (4 of 10 [40%]) by all methods except the agar dilution method and the E-test. Minor errors were most frequently encountered with testing of erythromycin (6 of 23 [26%]) and ciprofloxacin (6 of 23 [26%]) by all methods. The number of very major errors for amoxicillin-clavulanate was significantly greater for the agar dilution method than for the other methods ($P = 0.03$). Also, for amoxicillin-clavulanate, interpretive errors among the other four methods were not statistically different from each other ($P > 0.05$). For the other antimicrobial agents tested, comparisons for each of the types of interpretive errors were not significant ($P > 0.05$); however, these comparisons may have been limited due to the low number of *Nocardia* isolates studied.

DISCUSSION

The results of our study suggest that the BACTEC radiometric method may be the best method, among conventional and newer testing methods, for determining the in vitro susceptibility of *N. asteroides* complex isolates to the antimicrobial agents commonly used to treat these organisms. If one were to rank the performance of the testing methods that we studied by the number of interpretive errors (least amount of errors ranked as best), the following rank order would occur: BACTEC ($n = 5$) > E-test ($n = 8$) > disk diffusion ($n = 10$) = broth dilution ($n = 10$) > agar dilution ($n = 18$). If one ranked the performance of these methods by the number of combined very major and major errors (least amount of errors ranked as best), the following rank order would be observed: BACTEC ($n = 3$) > E-test ($n = 4$) = disk diffusion ($n = 4$) > broth dilution ($n = 5$) > agar dilution ($n = 12$). Finally, if the performance of these methods is ranked by the percent agreement of the interpretive results with the consensus interpretive results (highest agreement ranked as best), the rank order would be BACTEC (97.9%) > E-test (96.6%) > broth dilution = disk diffusion (95.7%) > agar dilution (92.3%).

The reasons for the poor performance by the agar dilution method are unclear. A recent study by Scopetti and colleagues (12) indicated that the MICs of nine antimicrobial agents that they tested against *Nocardia* isolates by the BACTEC method were consistently lower than those obtained by the agar dilution method. They speculated that these differences may result from less inactivation of antimicrobial agents during prolonged incubation in broth, which is used in the BACTEC system, compared to that on solid media, which is used in the agar dilution method. The time required for adequate growth of *Nocardia* spp. in our study was 72 h. This incubation time exceeds the usual 18-h period used for susceptibility testing of other aerobic bacteria by more than 48 h. It is possible that the bioavailabilities of antimicrobial agents over time are less when they are suspended in solid agar compared to liquid media, as suggested by Scopetti and colleagues (12). Our data do not

TABLE 2. Percent agreement by test methods compared to the consensus interpretive results for 26 *N. asteroides* complex isolates[a]

Antimicrobial agent	% Agreement (no. of isolates with susceptibility result/no. of isolates tested)									
	Disk diffusion method		Agar dilution method		Broth dilution method		E-test		BACTEC radiometric method	
	S	R	S	R	S	R	S	R	S	R
Amikacin	100 (26/26)		100 (26/26)		100 (26/26)		100 (26/26)		100 (26/26)	
Ampicillin	86 (6/7)	89 (17/19)	100 (7/7)	95 (18/19)	100 (7/7)	100 (19/19)	100 (7/7)	95 (18/19)	100 (7/7)	100 (19/19)
Amoxicillin-clavulanate	100 (9/9)	88 (15/17)	100 (9/9)	53 (9/17)	100 (9/9)	88 (15/17)	100 (9/9)	88 (15/17)	100 (9/9)	88 (15/17)
Ceftriaxone	80 (12/15)	100 (11/11)	100 (15/15)	100 (11/11)	87 (13/15)	91 (10/11)	100 (15/15)	82 (9/11)	100 (15/15)	100 (11/11)
Ciprofloxacin	100 (9/9)	88 (15/17)	78 (7/9)	94 (16/17)	89 (8/9)	100 (17/17)	89 (8/9)	100 (17/17)	89 (8/9)	94 (16/17)
Erythromycin	100 (7/7)	100 (19/19)	100 (7/7)	84 (16/19)	100 (7/7)	95 (18/19)	100 (7/7)	89 (17/19)	86 (6/7)	100 (19/19)
Imipenem	100 (26/26)		96 (25/26)		92 (24/26)		100 (26/26)		100 (26/26)	
Minocycline	100 (26/26)		100 (26/26)		96 (25/26)		100 (26/26)		100 (26/26)	
Trimethoprim-sulfamethoxazole	100 (26/26)		92 (24/26)		100 (26/26)		100 (26/26)		100 (26/26)	

[a] The reference or gold standard susceptibility for each *Nocardia* isolate to which all study results were compared was established by a consensus of the results among a majority of the testing methods in the current study. All reference susceptibility results were categorized as either susceptible or resistant; no reference susceptibility results were categorized as intermediately susceptible. Of note, all *N. asteroides* sensu stricto, *N. farcinica*, and *N. nova* strains studied had typical consensus susceptibility patterns for ampicillin, ampicillin-clavulanate, and erythromycin, as described previously (14, 19). Furthermore, breakpoints for categorical interpretation were taken from current NCCLS guidelines for aerobic bacteria (8–10). S, susceptible; I, intermediately susceptible; R, resistant. Overall agreements by the disk diffusion method, the agar dilution method, the broth dilution method, the E-test, and the BACTEC radiometric method were 95.7, 92.3, 95.7, 96.6, and 97.9%, respectively.

directly support this hypothesis. In our study, the percent susceptibility was remarkably similar for all *Nocardia* isolates that we tested against all antimicrobial agents, regardless of the testing method (Table 1). However, we cannot accurately assess from our results whether subtle differences in MICs were present for organisms in one medium versus another because different concentrations of antimicrobial agents were used for some test methods.

The E-test is an appealing new technology which is easily adaptable to most laboratories. In the current study this method resulted in the second most frequent number of very major interpretive errors, after the agar dilution method. In contrast, no major errors were observed by the E-test, and minor errors were relatively infrequent. Biehle and colleagues (2) showed that the results of the E-test agreed 89.4% of the time with those of the broth dilution method and 93.3% of the time with those of the disk diffusion method for a panel of antimicrobial agents that they tested against 52 clinical isolates of *Nocardia* (2). In our study, the overall agreement of the E-test results with the consensus susceptibility results was 96.2%. The very major interpretive errors for the E-test occurred with testing of amoxicillin-clavulanate ($n = 2$) or ceftriaxone ($n = 2$). Modification of NCCLS breakpoints for interpretive categories for these antimicrobial agents when using the E-test method may lessen the number of interpretive errors.

The inconsistencies that we found for the results for β-lactamase testing when compared to the results for all other methods for ampicillin testing of *N. nova* isolates is noteworthy. All seven *N. nova* isolates possessed β-lactamase, as assessed by the nitrocefin disk method at 5 min or 1 h with or without induction. These isolates were susceptible or intermediately susceptible to ampicillin by all other testing methods. This discrepancy may relate to the NCCLS breakpoints that were used, which may require modification for ampicillin when testing *N. nova* isolates. However, further characterization of the β-lactamase produced by *N. nova* isolates, including its effects on ampicillin, is required to understand this discrepancy.

We noted that the majority of *N. nova* isolates and all the *N. asteroides* sensu stricto isolates that we tested were resistant to amoxicillin-clavulanate. Wallace and colleagues (13, 16) have suggested that clavulanate, and not ampicillin, induces a β-lactamase in *N. nova* strains. What is not clear is whether this β-lactamase is produced in quantities that exceed the capacity of clavulanate to neutralize its effect or whether clavulanate is not a suitable substrate for the enzyme. Wallace and colleagues (18) have also shown that essentially all *Nocardia* isolates that they tested possess β-lactamase activity. Detection of these enzymes, especially if they are produced constitutively in small quantities or if their production requires induction, may be difficult in the clinical laboratory. All of the *Nocardia* isolates that we evaluated by the nitrocefin disk method had β-lactamase activity, but this observation only occurred in some instances after reexamining the inoculated nitrocefin disks after 1 h or by inducing the isolate to produce β-lactamase with an amoxicillin-clavulanate disk and then retesting it by the nitrocefin disk test.

Although ampicillin has been used to successfully treat *Nocardia* infections, relapses due to the induction of β-lactamase, to which the ampicillin is susceptible, are theoretically possible. Much remains uncertain about β-lactamases for many *Nocardia* spp., including whether they are constitutively produced or whether their expression can be induced by exposure to β-lactam antimicrobial agents and what substrates (which β-lactams) they hydrolyze. Clearly, different β-lactam resistance patterns have been demonstrated previously for different *Nocardia* spp. (7, 13, 18, 19). Likewise, different β-lactamases have been characterized by isoelectric focusing and antimicrobial substrate activity analyses for isolates of *N. asteroides* sensu stricto (7), *N. nova* (19), and *N. farcinica* (13), which may contribute to differences in the susceptibilities of *Nocardia* spp. to β-lactams or β-lactam plus β-lactamase inhibitor combinations. Our study also suggests that difficulties may be encountered with the susceptibility testing of ceftriaxone when some test methods are used. This may also relate to the susceptibility of ceftriaxone to the β-lactamases produced by these organisms and the ability of the susceptibility test method to detect it.

We demonstrated that by a consensus of the results for all susceptibility testing methods, all *N. asteroides* sensu stricto and *N. farcinica* isolates were resistant to ampicillin. In view of this finding, it may be unnecessary to test these *Nocardia* species for their susceptibilities to ampicillin. We also showed by

TABLE 3. Interpretive inconsistencies by various test methods for 26 *N. asteroides* complex isolates[a]

Antimicrobial agent	Type of error[b]	No. of interpretive inconsistencies by:				
		Disk diffusion method	Agar dilution method	Broth dilution method	E-test	BACTEC radiometric method
Amikacin	Very major	0 (0)[c]	0 (0)	0 (0)	0 (0)	0 (0)
	Major	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Minor	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ampicillin	Very major	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Major	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Minor	3 (11.5)	1 (3.8)	0 (0)	1 (3.8)	0 (0)
Amoxicillin-clavulanate	Very major	1 (3.8)	8 (30.8)	2 (7.7)	2 (7.7)	1 (3.8)
	Major	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Minor	1 (3.8)	0 (0)	0 (0)	0 (0)	1 (3.8)
Ceftriaxone	Very major	0 (0)	0 (0)	1 (3.8)	2 (7.7)	0 (0)
	Major	2 (7.7)	0 (0)	2 (7.7)	0 (0)	0 (0)
	Minor	1 (3.8)	0 (0)	0 (0)	0 (0)	0 (0)
Imipenem	Very major	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Major	0 (0)	1 (3.8)	0 (0)	0 (0)	0 (0)
	Minor	0 (0)	0 (0)	0 (0)	2 (7.7)	0 (0)
Ciprofloxacin	Very major	1 (3.8)	0 (0)	0 (0)	0 (0)	0 (0)
	Major	0 (0)	1 (3.8)	0 (0)	0 (0)	1 (3.8)
	Minor	1 (3.8)	2 (7.7)	1 (3.8)	1 (3.8)	1 (3.8)
Erythromycin	Very major	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Major	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.8)
	Minor	0 (0)	3 (11.5)	1 (3.8)	2 (7.7)	0 (0)
Minocycline	Very major	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Major	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Minor	0 (0)	0 (0)	1 (3.8)	0 (0)	0 (0)
Trimethoprim-sulfamethoxazole	Very major	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Major	0 (0)	2 (7.7)	0 (0)	0 (0)	0 (0)
	Minor	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	Very major	2	8	3	4	1
	Major	2	4	2	0	2
	Minor	6	6	5	4	2

[a] The reference or gold standard susceptibility for each *Nocardia* isolate to which all study results were compared was established by a consensus of the results among a majority of testing methods in the current study. Of note, all *N. asteroides* sensu stricto, *N. farcinica*, and *N. nova* strains studied had typical consensus susceptibility patterns for ampicillin, ampicillin-clavulanate, and erythromycin, as described previously (14, 19). Furthermore, breakpoints for categorical interpretation were taken from current NCCLS guidelines for aerobic bacteria (8–10). The number of very major errors for amoxicillin-clavulanate was significantly greater by the agar dilution method than by any of the other methods ($P = 0.03$); also for this comparison, the other four methods were not significantly different from each other ($P > 0.05$). For all other antimicrobial agents, comparisons for each of the types of interpretive errors were not significant ($P > 0.05$).

[b] Very major error, resistant strain called susceptible; major error, susceptible strain called resistant; minor error, resistant or susceptible strains called intermediate or intermediate strains called resistant or susceptible.

[c] The numbers in parentheses are the percentage of interpretive errors for the total number of *Nocardia* isolates ($n = 26$) tested; no growth failures

a consensus of the results for all susceptibility testing methods that all *N. nova* and all *N. asteroides* sensu stricto isolates were resistant to amoxicillin-clavulanate. It may also be unnecessary to test *Nocardia* species for their susceptibilities to this drug combination. However, determining the susceptibilities of *Nocardia* isolates to ampicillin or amoxicillin-clavulanate may be useful for reasons other than guiding therapy. Wallace and colleagues (19) and Steingrube and colleagues (14) have shown that susceptibility results for these drugs, as well as for erythromycin, can be used to determine that an organism is a member of the species *N. asteroides* sensu stricto (ampicillin resistant, amoxicillin-clavulanate resistant, and erythromycin resistant), *N. farcinica* (ampicillin resistant, amoxicillin-clavulanate susceptible, and erythromycin resistant), and *N. nova* (ampicillin susceptible, amoxicillin-clavulanate resistant, and erythromycin susceptible). In our study, the consensus interpretive results that we generated for these three antimicrobial agents and the *Nocardia* isolates evaluated provided the same species designation as determined by conventional biochemical testing.

The results of the current study, like those of other studies which have recently been published (2, 12), showed that some antimicrobial agents such as amikacin, imipenem, minocycline, and trimethoprim-sulfamethoxazole appear to be highly effective in vitro against *Nocardia* spp. Sulfanomides have been the mainstay of therapy for *Nocardia* infections, although failure rates with these drugs may approach 20% for pulmonary disease and up to 50% for central nervous system infections (18). Case reports suggest that amikacin (3, 6) and minocycline (5, 11) may be quite effective for treating *N. asteroides* complex infections in humans, although large clinical series are lacking.

In conclusion, in our hands, the BACTEC radiometric susceptibility testing method appeared to be the best method for determining the in vitro susceptibilities of clinical isolates of *N. asteroides* sensu stricto, *N. farcinica*, and *N. nova* to amikacin, amoxicillin-clavulanate, ceftriaxone, ciprofloxacin, erythromycin, imipenem, minocycline, and trimethoprim-sulfamethoxazole. None of the test methods that we studied, including the BACTEC method, accurately predicted ampicillin resistance for *N. nova* isolates, all of which produced β-lactamase. Presuming that this β-lactamase hydrolyzes ampicillin, this discrepancy may relate to the NCCLS breakpoints that were used, which may require modification when the susceptibilities of *N. nova* isolates to ampicillin are tested.

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