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Evaluation of Alamar Colorimetric Broth Microdilution Susceptibility Testing Method for Staphylococci and Enterococci

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We compared the results of the Alamar broth microdilution susceptibility testing method with the results of the National Committee for Clinical Laboratory Standards reference broth microdilution method for 119 gram-positive organisms. The strains were tested for their susceptibilities to 20 antimicrobial agents. Only appropriate antimicrobial agents were evaluated for each species of bacteria. Absolute categorical agreement between the reference method and the test method was 91.5% for enterococci, 99.8% for oxacillin-susceptible staphylococci, and 97.4% for oxacillin-resistant staphylococci. Essential agreement (percent complete agreement plus percent minor errors) was >99% for all organisms tested. The results for enterococci showed no very major errors, one major error with ofloxacin, and numerous minor errors with the quinolones. However, all except one of the minor errors were within ±1 $\log_2$ dilution of the reference result. For staphylococci, only 2 very major errors (one each with chloramphenicol and oxacillin), 1 major error (chloramphenicol), and 15 minor errors (multiple drugs) were observed. The Alamar colorimetric system was easy to use and the results were easy to read. It appears to be an acceptable method for antimicrobial susceptibility testing of staphylococci and enterococci.

The Alamar colorimetric antimicrobial susceptibility testing method uses an oxidation-reduction color indicator (Alamar Blue) to detect bacterial growth in the wells of microtiter plates. The system can be used for susceptibility testing of gram-positive and gram-negative bacteria and for yeasts. It has been evaluated for gram-negative bacteria by Baker et al. (3), for yeasts by Pfaller and colleagues (14, 15), and for enterococci (vancomycin only) by Tenover et al. (18) and Zabransky et al. (22). The evaluations for gram-negative bacteria and yeasts indicated that the Alamar method is a satisfactory alternative to conventional MIC methods (3, 14, 15). However, while one study found that the results of the Alamar method are acceptable for vancomycin-resistant enterococci (18), another study did not report favorable results for testing these organisms (22). The latter study, however, compared the Alamar method with a brain heart infusion agar screening test and used cation-supplemented Mueller-Hinton broth (in place of the recommended cation-adjusted Mueller-Hinton broth) to determine MICs for organisms with discrepant results, and many of the strains with discrepant results were noted to have fastidious growth requirements.

Given the discrepancies in previous studies and our continuing concerns about the ability of conventional antimicrobial susceptibility methods to detect emerging antimicrobial resistance (2, 6, 8, 9, 19, 21), we undertook an evaluation of the Alamar system to determine its accuracy when testing enterococci and staphylococci. We challenged the Alamar test system with 26 clinical isolates and 93 gram-positive bacteria from the Centers for Disease Control and Prevention (CDC) challenge set (5), which is designed to test the limits of accuracy of new antimicrobial susceptibility testing systems. Since the challenge set contains mostly organisms that are difficult to test, additional fresh clinical isolates were added to the study to determine how the test method would perform under more routine laboratory circumstances.

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MATERIALS AND METHODS

Bacterial strains. The organisms used in the study were selected from the CDC challenge set of gram-positive bacteria (n = 93), which have a variety of resistance mechanisms, the MICs for the organisms tend to be at or near the breakpoints for resistance, and in general, the organisms are difficult to test. In addition, fresh clinical isolates (n = 26) collected from hospital microbiology laboratories in metropolitan Atlanta were used. The bacteria were identified at CDC by using conventional biochemical methods (10). The following organisms were selected for use in the study: 2 *Enterococcus casseliflavus*, 24 *Enterococcus faecalis*, 16 *Enterococcus faecium*, 1 *Enterococcus raffinosus*, 54 *Staphylococcus aureus*, 1 *Staphylococcus capitis*, 14 *Staphylococcus epidermidis*, 4 *Staphylococcus haemolyticus*, 1 *Staphylococcus saprophyticus*, 1 *Staphylococcus simulans*, and 1 *Staphylococcus warneri* strains. The strains were stored in defibrinated rabbit blood at or below −120°C in a liquid-nitrogen freezer. The control strains used in the study were *E. faecalis* ATCC 29212, ATCC 51299, and F278 and *S. aureus* ATCC 29213 and ATCC 43300. None of the results from testing the quality control strains were out of the ranges defined by NCCLS (13).

Antimicrobial agents. Standard antimicrobial powders were obtained from various manufacturers for broth microdilution testing; Alamar supplied the prepared antimicrobial agents with the colorimetric growth indicator in dehydrated microdilution trays. We tested the following 20 antimicrobial agents: amoxicillin-clavulanate, ampicillin, ampicillin-sulbactam, cefazolin, cefotaxime, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, imipenem, norfloxacin, ofloxacin, oxacillin, penicillin, tetracycline, trimethoprim-sulfamethoxazole, and vancomycin. Each agent was tested in eight serial twofold dilutions. In addition, we tested gentamicin (500 μg/ml) and streptomycin (1,000 μg/ml) at a single high-level concentration against enterococcal isolates.

Antimicrobial susceptibility testing. Isolates were removed from storage, streaked onto a Trypticase soy agar plate supplemented with 5% sheep blood (Becton Dickinson Microbiology Systems, Cockeysville, Md.), and incubated for 18 to 24 h at 35°C. One isolated colony was picked from the plate, streaked onto a new Trypticase soy plate containing 5% sheep blood, and incubated for 18 to 20 h. A suspension of growth was prepared in 5 ml of Mueller-Hinton broth for the reference broth microdilution tests and in 5 ml of saline diluent (provided with the Alamar test panels) for the Alamar tests (1). The turbidities of these suspensions were adjusted to equal that of a 0.5 McFarland standard for susceptibility testing.

Reference broth microdilution method. The antimicrobial agents were prepared with appropriate solvents and diluents (12). Antimicrobial agent-containing plates were frozen at −70°C until needed and were then removed from storage, warmed to room temperature, and inoculated with a final concentration of approximately 5.0×10^5 CFU/ml. The inoculated broth microdilution plates

TABLE 1. Interpretive categories[a] for the broth microdilution method: errors and agreement between Alamar and broth microdilution methods for enterococci (n = 43)

Antimicrobial agent	No. (%) of strains[b]			No. (%) with errors[c]				No. (%) with agreement	
	Susceptible	Intermediate	Resistant	Minor[d]	Major[e]	Very major[f]	Essential[g]	Absolute[h]	Essential[i]
Ampicillin	64 (74)	0	22 (26)	0	0	0	0	86 (100)	86 (100)
Penicillin	60 (70)	0	26 (30)	0	0	0	0	86 (100)	86 (100)
Gentamicin (high level)	60 (70)	0	26 (30)	0	2 (3.3)	0	2 (2.3)	84 (97.7)	84 (97.7)
Streptomycin (high level)	42 (49)	0	44 (51)	0	2 (4.8)	0	2 (2.3)	84 (97.7)	84 (97.7)
Tetracycline	26 (30)	0	60 (70)	0	0	0	0	86 (100)	86 (100)
Vancomycin	62 (72)	4 (5)	20 (23)	1 (1.2)	0	0	0	85 (98.9)	86 (100)
Ciprofloxacin	39 (45)	25 (29)	22 (26)	18 (20.9)	0	0	0	68 (79.1)	86 (100)
Norfloxacin	51 (60)	21 (24)	14 (16)	21 (24.4)	0	0	0	65 (75.6)	86 (100)
Ofloxacin	41 (48)	31 (36)	14 (16)	21 (24.4)	1 (2.4)	0	1 (1.8)	64 (74.4)	85 (98.8)

[a] Isolates were tested in duplicate; each result was analyzed independently. Categories of susceptibility as defined by NCCLS standards M7-A3 (12) and M100-S5 (13).
[b] Number and percentage of strains that were interpreted as susceptible, intermediate, or resistant by the reference method.
[c] Error classes as defined by Thornsberry and Gavan (20).
[d] Number and percentage of strains with minor interpretive differences compared with the reference method.
[e] Number and percentage of strains that were interpreted as falsely resistant by the test method.
[f] Number and percentage of strains that were interpreted as falsely susceptible by the test method.
[g] Number and percentage of strains with major and very major errors.
[h] Number and percentage of strains for which there was complete interpretive agreement.
[i] Number and percentage of strains for which there was complete interpretive agreement plus those strains with minor errors.

were incubated for 24 h at 35°C in ambient air. Enterococci were reincubated for an additional 24 h if the high-level streptomycin screening test results were negative, and the streptomycin screening test results were reread at 48 h. MICs were read as the lowest concentration at which there was no visible growth.

Alamar broth microdilution method. Twenty-five microliters of the standardized saline inoculum was added to 25 ml of Alamar Mueller-Hinton broth and vortexed. The diluted inoculum was placed in the inoculum reservoir, and 100 μl of this diluted inoculum prepared in saline was added to each well of the microdilution tray by using a multichannel pipettor. The final inoculum was approximately 5×10^5 CFU/ml. The inoculated microdilution trays were covered and incubated for 24 h at 35°C in ambient air. Enterococci were reincubated for an additional 24 h for the high-level streptomycin screening test if the screening test results were negative. The MIC was the lowest concentration of antimicrobial agent at which no color change occurred (red indicates growth and blue indicates no growth). Both the reference broth microdilution tests and the Alamar tests were performed on the same day for each set of test isolates and control strains. Duplicate testing was performed on the following day for each set of test strains and control strains.

Statistical analysis. All tests were performed in duplicate. For comparison and statistical analysis, each pair was treated as an individual isolate. For each species, statistical analysis was performed only for appropriate antimicrobial agents. Interpretive category results (susceptible, intermediate, and resistant) were compared by calculating the minor, major, very major, and essential (major plus very major errors) errors and their rates (11, 16, 20). Since a major error is a categorical change from susceptible to resistant for an isolate determined by the test method, the error rate (percent) was obtained by using the number of susceptible strains determined by the reference method as the denominator; likewise, very major errors were calculated by using the number of resistant strains determined by the reference method as the denominator; minor errors were calculated by using the total number of strains tested as the denominator; and essential errors were calculated by using the total number of susceptible plus the number of resistant strains determined by the reference method as the denominator. To measure the degree of agreement between the Alamar results and the reference broth microdilution method results, we examined the distribution of differences in the $\log_2$ dilution MIC results and calculated the percentage of isolates that yielded identical results within the accuracy limits of the standard test ($\pm 1 \log_2$ dilution). Also, to determine if the Alamar method tended to produce values significantly lower or higher than those produced by the standard method, we performed a Wilcoxon signed-rank test (7) on the difference in $\log_2$ dilution MIC results of the two tests. MICs within $\pm 1 \log_2$ dilution were regarded as identical for this hypothesis test.

RESULTS

We compared the MICs of 20 antimicrobial agents determined by the reference broth microdilution method with the MICs determined by the Alamar method for 43 isolates of enterococci and 76 isolates of staphylococci tested in duplicate. The MICs obtained by the Alamar and broth microdilution methods were converted to interpretive categories of susceptible, intermediate, or resistant by using the definitions of the National Committee for Clinical Laboratory Standards (12, 13). Tables 1 to 3 indicate, respectively, the percentage of category agreement; the number of susceptible, intermediate, and resistant strains; and the minor, major, very major, and essential errors for Alamar results compared with those for the results of the reference broth microdilution method for enterococci, oxacillin-susceptible staphylococci, and oxacillin-resistant staphylococci. Category agreement ranged from 74.4% for ofloxacin to 100% for ampicillin, penicillin, and tetracycline for enterococci, from 98.1% for erythromycin to 100% for the 12 antimicrobial agents tested against oxacillin-susceptible staphylococci, and from 85.4% for chloramphenicol to 100% for the 12 antimicrobial agents tested against oxacillin-resistant staphylococci.

A significant number of minor errors were noted for the quinolones when testing enterococci and for chloramphenicol when testing oxacillin-resistant staphylococci. Major errors for enterococci occurred only with ofloxacin (2.4%) and the high-level aminoglycoside screening tests (gentamicin [3.3%] and streptomycin [4.8%]) and for oxacillin-resistant staphylococci when testing tetracycline (2.9%). No very major errors occurred with the enterococci, one very major error was noted with chloramphenicol for oxacillin-susceptible staphylococci, and one very major error was noted with oxacillin for oxacillin-resistant staphylococci.

Essential errors for all species tested ranged from 0 to 2.3%, and essential agreement for all species tested ranged from 97.7 to 100%. The distribution of differences in $\log_2$ MICs, the percent agreement, and the P values from the Wilcoxon signed-rank test (which indicates whether the MICs produced by the Alamar method are significantly shifted either to lower or higher values) are presented in Tables 4 to 6. Overall agreement at $\pm 1 \log_2$ dilution was 94.2% for enterococci, 90.8% for oxacillin-susceptible staphylococci, and 95.3% for oxacillin-resistant staphylococci. For enterococci, MIC results for ampicillin, penicillin, and tetracycline showed significant shifts to higher MICs by the Alamar system than by the reference broth microdilution method. For β-lactamase-producing strains of staphylococci, penicillin MICs were 2 to 4 dilutions lower by the Alamar test than by the broth microdilution method. For

TABLE 2. Interpretive categories[a] for the broth microdilution method: errors and agreement between Alamar and broth microdilution methods for oxacillin-susceptible staphylococci (n = 52)

Antimicrobial agent(s)	No. (%) of strains[b]			No. (%) with errors[c]				No. (%) with agreement	
	Susceptible	Intermediate	Resistant	Minor[d]	Major[e]	Very major[f]	Essential[g]	Absolute[h]	Essential[i]
Oxacillin	104 (100)	0	0	0	0	0	0	104 (100)	104 (100)
Penicillin	25 (24.0)	0	79 (76.0)	0	0	0	0	104 (100)	104 (100)
Ciprofloxacin	102 (98.1)	0	2 (1.9)	0	0	0	0	104 (100)	104 (100)
Norfloxacin	102 (98.1)	0	2 (1.9)	0	0	0	0	104 (100)	104 (100)
Ofloxacin	102 (98.1)	0	2 (1.9)	0	0	0	0	104 (100)	104 (100)
Clindamycin	96 (92.3)	0	8 (7.7)	0	0	0	0	104 (100)	104 (100)
Erythromycin	68 (65.4)	2 (1.9)	34 (32.7)	2 (1.9)	0	0	0	102 (98.1)	104 (100)
Chloramphenicol	95 (91.4)	0	9 (8.6)	0	0	1 (11.1)	1 (0.9)	103 (99.0)	103 (99.0)
Gentamicin	98 (94.2)	0	6 (5.8)	0	0	0	0	104 (100)	104 (100)
Tetracycline	88 (84.6)	0	16 (15.4)	0	0	0	0	104 (100)	104 (100)
Trimethoprim-sulfamethoazole	102 (98.1)	0	2 (1.9)	0	0	0	0	104 (100)	104 (100)
Vancomycin	104 (100)	0	0	0	0	0	0	104 (100)	104 (100)

[a] Isolates were tested in duplicate; each result was analyzed independently. Categories of susceptibility as defined by NCCLS standards M7-A3 (12) and M100-S5 (13).
[b] Number and percentage of strains that were interpreted as susceptible, intermediate, or resistant by the reference method.
[c] Error classes as defined by Thornsberry and Gavan (20).
[d] Number and percentage of strains with minor interpretative differences compared with the reference method.
[e] Number and percentage of strains that were interpreted as falsely resistant by the test method.
[f] Number and percentage of strains that were interpreted as falsely susceptible by the test method.
[g] Number and percentage of strains with major and very major errors.
[h] Number and percentage of strains for which there was complete interpretive agreement.
[i] Number and percentage of strains for which there was complete interpretive agreement plus those strains with minor errors.

oxacillin-susceptible staphylococci, penicillin, clindamycin, erythromycin, and gentamicin MICs were significantly lower by the Alamar test than by the broth microdilution method; conversely, the MICs of norfloxacin, ofloxacin, and tetracycline were shifted higher. A similar pattern occurred with oxacillin-resistant staphylococci.

DISCUSSION

Achieving accurate and reproducible antimicrobial susceptibility testing results with enterococci and staphylococci continues to be a problem for many clinical microbiology laboratories (2, 6, 8, 17, 18, 21). Testing of staphylococci against oxacillin and enterococci against vancomycin and the quinolones can be particularly frustrating since for many of these organisms MICs appear to be at or near the breakpoints for resistance (2, 9, 18). NCCLS has recommended that only a few critical agents should be tested for these species (12, 13): ampicillin (or penicillin), gentamicin, streptomycin, and vancomycin for enterococci and penicillin, oxacillin, and vancomycin for staphylococci. Additional drugs that would be used for outpatient therapy, such as erythromycin or trimethoprim-sulfame-

TABLE 3. Interpretive categories[a] for broth microdilution method: errors and agreement between Alamar and broth microdilution methods for oxacillin-resistant staphylococci (n = 24)

Antimicrobial agent(s)	No. (%) of strains[b]			No. (%) with errors[c]				No. (%) with agreement	
	Susceptible	Intermediate	Resistant	Minor[d]	Major[e]	Very major[f]	Essential[g]	Absolute[h]	Essential[i]
Oxacillin	0	0	48 (100)	0	0	1 (2.1)	1 (2.1)	47 (97.9)	47 (97.9)
Penicillin	0	0	48 (100)	0	0	0	0	48 (100)	48 (100)
Ciprofloxacin	32 (66.7)	0	16 (33.3)	0	0	0	0	48 (100)	48 (100)
Norfloxacin	32 (66.7)	0	16 (33.3)	2 (4.2)	0	0	0	46 (95.8)	48 (100)
Ofloxacin	32 (66.7)	2 (4.2)	14 (29.1)	0	0	0	0	48 (100)	48 (100)
Clindamycin	22 (45.8)	0	26 (54.2)	0	0	0	0	48 (100)	48 (100)
Erythromycin	1 (2.1)	0	47 (97.9)	0	0	0	0	48 (100)	48 (100)
Chloramphenicol	24 (50.0)	12 (25.0)	12 (25.0)	7 (14.6)	0	0	0	41 (85.4)	48 (100)
Gentamicin	28 (58.3)	6 (12.5)	14 (29.2)	4 (8.3)	0	0	0	44 (91.7)	48 (100)
Tetracycline	35 (72.9)	0	13 (27.1)	0	1 (2.9)	0	1 (2.1)	47 (97.9)	47 (97.9)
Trimethoprim-sulfamethoxazole	44 (91.7)	0	4 (8.3)	0	0	0	0	48 (100)	48 (100)
Vancomycin	46 (95.8)	2 (4.2)	0	0	0	0	0	48 (100)	48 (100)

[a] Isolates were tested in duplicate; each result was analyzed independently. Categories of susceptibility as defined by NCCLS standards M7-A3 (12) and M100-S5 (13).
[b] Number and percentage of strains that were interpreted as susceptible, intermediate, or resistant by the reference method.
[c] Error classes as defined by Thornsberry and Gavan (20).
[d] Number and percentage of strains with minor interpretive differences compared with the reference method.
[e] Number and percentage of strains that were interpreted as falsely resistant by the test method.
[f] Number and percentage of strains that were interpreted as falsely susceptible by the test method.
[g] Number and percentage of strains with major and very major errors.
[h] Number and percentage of strains for which there was complete interpretive agreement.
[i] Number and percentage of strains for which there was complete interpretive agreement plus those strains with minor errors.

TABLE 4. Distribution of differences in MICs of seven antimicrobial agents for enterococci: Alamar method versus broth microdilution method

Antimicrobial agent	No. (%) of isolates with the following differences in MICs[a]:							% Agreement[b]	P[c]
	<−2	−2	−1	0	+1	+2	>+2		
Ampicillin	0	0	0	27 (31.4)	39 (45.4)	18 (20.9)	2 (2.3)	76.7 ± 4.6	0.0001
Penicillin	0	0	5 (5.8)	44 (51.2)	34 (39.5)	3 (3.5)	0	96.5 ± 2.0	0.042
Ciprofloxacin	0	0	23 (26.7)	60 (69.8)	3 (3.5)	0	0	100.0	0.999
Norfloxacin	0	0	3 (3.5)	58 (67.4)	25 (29.1)	0	0	100.0	0.999
Ofloxacin	0	0	3 (3.5)	56 (65.1)	26 (30.2)	1 (1.2)	0	98.8 ± 1.2	0.159
Tetracycline	0	0	0	51 (59.3)	26 (30.2)	9 (10.5)	0	89.5 ± 3.3	0.001
Vancomycin	0	0	13 (15.1)	69 (80.2)	2 (2.3)	1 (1.2)	1 (1.2)	97.7 ± 1.6	0.079
Overall	0	0	47 (7.8)	365 (60.6)	155 (25.8)	32 (5.3)	3 (0.5)	94.2 ± 1.0	0.0001

[a] Zero indicates number and percentage of isolates for which MICs are identical, −1 and +1 indicate −1 and +1 $\log_2$ dilution difference, respectively, etc.
[b] Percentage of isolates within the accuracy limits of the test (±1 $\log_2$ dilution) ± standard error.
[c] *P* values were obtained by the Wilcoxon signed-rank test.

thoxazole, may also be tested for staphylococci. From tests with these limited agents, one can usually predict the in vitro antimicrobial susceptibility profiles of the organism to other clinically useful drugs. However, microbiologists are frequently confronted with commercial susceptibility testing panels that contain multiple drugs, and determining which drugs to report can be a time-consuming problem.

Recently, Tenover et al. (18) and Zabransky et al. (22) reported on the accuracy of the Alamar system for testing enterococci against vancomycin. Zabransky and coworkers (22) reported a major error rate of 4% and a minor error rate of 5%, but they qualified their findings by stating that some of the strains grew poorly and that the growth characteristics of the strains rather than the Alamar system may have been responsible for some of the errors. Tenover et al. (18) reported a minor error rate of 16% and stated that these errors were primarily caused by enterococci containing the *vanB* vancomycin resistance gene or by *E. casseliflavus* strains. In the current study, our error rate for vancomycin (2% minor) was much lower than the error rates obtained by Zabransky et al. (22) (4% major and 5% minor) and Tenover et al. (18) (16% minor), and poor growth of enterococci was not observed. This may be a function of the population of isolates studied. The population that we studied did not contain as large a number of vancomycin-intermediate strains as did the study of Tenover et al. (18). In the current study the Alamar system performed well with enterococci. No differences in error rates were observed between clinical isolates and challenge set isolates.

In addition to vancomycin, we evaluated the Alamar system for its accuracy in testing several other antimicrobial agents and enterococci. Ampicillin results were usually 1 to 2 $\log_2$ dilutions higher than those by the reference method, but this did not cause any interpretation errors. The quality control results for ampicillin were within the range defined by NCCLS (12); however, the quality control results for the enterococcal control strain with the Alamar system were at the upper limits of the range, and the reference method results were in the lower to middle parts of the range. This variation may explain the shift to higher MICs for the Alamar system and may be due to a concentration difference with ampicillin between the two methods. We obtained no very major errors with any antimicrobial agent, two major errors each with high levels of gentamicin and streptomycin and one major error with ofloxacin, only one minor error with vancomycin for *E. faecalis* (the MIC for this strain is 4.0 μg/ml, which is at the breakpoint for susceptibility), and numerous minor errors with the quinolones. All except one of the minor errors were within the ±1 $\log_2$ dilution, which is within the accuracy limits of the test

TABLE 5. Distribution of differences in MICs of 12 antimicrobial agents for oxacillin-susceptible staphylococci: Alamar method versus broth microdilution method

Antimicrobial agent(s)	No. (%) of isolates with the following differences in MICs[a]:							% Agreement[b]	P[c]
	<−2	−2	−1	0	+1	+2	>+2		
Oxacillin	0	0	24 (23.1)	65 (62.5)	14 (13.5)	1 (0.9)	0	99.0 ± 1.0	0.159
Penicillin	44 (42.3)	16 (15.4)	9 (8.7)	32 (30.8)	2 (1.9)	1 (0.9)	0	41.4 ± 4.6	0.0001
Ciprofloxacin	0	0	7 (6.7)	75 (72.1)	22 (21.2)	0	0	100.0	0.999
Norfloxacin	0	0	1 (0.9)	34 (32.7)	60 (57.7)	9 (8.7)	0	91.4 ± 2.6	0.013
Ofloxacin	0	0	0	43 (41.4)	56 (53.8)	5 (4.8)	0	95.2 ± 2.1	0.013
Clindamycin	0	5 (4.8)	70 (67.3)	29 (27.9)	0	0	0	95.2 ± 2.1	0.013
Erythromycin	0	27 (26.0)	41 (39.4)	35 (33.7)	1 (0.9)	0	0	74.0 ± 4.3	0.0001
Chloramphenicol	1 (0.9)	0	6 (5.8)	92 (88.5)	5 (4.8)	0	0	99.0 ± 1.0	0.159
Gentamicin	0	5 (4.8)	25 (24.0)	70 (67.3)	4 (3.9)	0	0	95.2 ± 2.1	0.013
Tetracycline	0	0	0	39 (37.5)	64 (61.5)	1 (1.0)	0	99.0 ± 1.0	0.159
Trimethoprim-sulfamethoxazole	0	0	4 (3.8)	100 (96.2)	0	0	0	100.0	0.999
Vancomycin	0	0	27 (26.0)	68 (65.4)	9 (8.6)	0	0	100.0	0.999
Overall	45 (3.6)	53 (4.2)	214 (17.2)	682 (54.6)	237 (19.0)	17 (1.4)	0	90.8 ± 0.8	0.0001

[a] Zero indicates number and percentage of isolates for which MICs are identical, −1 and +1 indicates −1 and +1 $\log_2$ dilution difference, respectively, etc.
[b] Percentage of isolates within the accuracy limits of the test (±1 $\log_2$ dilution) ± standard error.
[c] *P* values were obtained by the Wilcoxon signed-rank test.

TABLE 6. Distribution of differences in MICs of 12 antimicrobial agents for oxacillin-resistant staphylococci: Alamar method versus broth microdilution method

Antimicrobial agent(s)	No. (%) of isolates with the following differences in MICs[a]							% Agreement[b]	P[c]
	<−2	−2	−1	0	+1	+2	>+2		
Oxacillin	0	3 (6.3)	5 (10.4)	40 (83.3)	0	0	0	93.8 ± 3.5	0.042
Penicillin	2 (4.2)	6 (12.5)	9 (18.7)	24 (50.0)	7 (14.6)	0	0	83.3 ± 5.4	0.002
Ciprofloxacin	0	0	9 (18.7)	32 (66.7)	6 (12.5)	1 (2.1)	0	97.9 ± 2.1	0.159
Norfloxacin	0	1 (2.1)	0	27 (56.3)	16 (33.3)	4 (8.3)	0	89.6 ± 4.4	0.090
Ofloxacin	0	0	0	29 (60.4)	18 (37.5)	1 (2.1)	0	97.9 ± 2.1	0.159
Clindamycin	0	1 (2.1)	15 (31.2)	32 (66.7)	0	0	0	97.9 ± 2.1	0.159
Erythromycin	0	0	3 (6.2)	44 (91.7)	1 (2.1)	0	0	100.0	0.999
Chloramphenicol	0	2 (4.2)	13 (27.0)	30 (62.5)	2 (4.2)	1 (2.1)	0	93.8 ± 3.5	0.282
Gentamicin	0	2 (4.2)	11 (22.9)	33 (68.7)	2 (4.2)	0	0	95.8 ± 2.9	0.079
Tetracycline	0	0	4 (8.3)	19 (39.6)	23 (47.9)	1 (2.1)	1 (2.1)	95.8 ± 2.9	0.079
Trimethoprim-sulfamethoxazole	0	0	1 (2.1)	47 (97.9)	0	0	0	100.0 ± 0.0	0.999
Vancomycin	0	0	16 (33.3)	27 (56.3)	4 (8.3)	1 (2.1)	0	97.9 ± 2.1	0.159
Overall	2 (0.3)	15 (2.6)	86 (14.9)	384 (66.7)	79 (13.7)	9 (1.6)	1 (0.2)	95.3 ± 0.9	0.089

[a] Zero indicates number and percentage of isolates for which MICs are identical, −1 and +1 indicates −1 and +1 $\log_2$ dilution difference, respectively, etc.
[b] Percentage of isolates within the accuracy limits of the test (±1 $\log_2$ dilution) ± standard error.
[c] *P* values were obtained by the Wilcoxon signed-rank test.

method. These errors occurred because the MICs of the quinolones for enterococci fell on or near the breakpoint. Twenty-four to 36% of the enterococci were characterized as intermediate to the quinolones by the reference method, thus making it very difficult to achieve complete agreement for this species.

The antimicrobial susceptibility testing of staphylococci continues to be a problem, particularly with coagulase-negative staphylococci and methicillin-resistant staphylococci (2, 9); therefore, the focus for susceptibility testing of this species should be on critical antimicrobial agents, such as penicillin, oxacillin, vancomycin, and oral antimicrobial agents. We followed NCCLS guidelines (12, 13) in the selection of relevant antimicrobial agents for statistical analysis. For the strains of staphylococci, we evaluated only 2 of the 9 β-lactam agents included on the Alamar panel and 10 other antimicrobial agents. Although concordance with oxacillin results was seen with the results for six of the seven β-lactam drugs not evaluated, ceftriaxone did show 1.9% minor errors (data not shown). Thus, these seven β-lactam drugs should be ignored and only results for penicillin and oxacillin should be reported. For oxacillin-susceptible staphylococci, there were only two minor errors with erythromycin. The erythromycin errors were with erythromycin-inducible strains of *S. aureus*. For oxacillin-resistant staphylococci, there were minor errors with norfloxacin (two), chloramphenicol (seven), and gentamicin (four), a single major error with tetracycline, and a single very major error with oxacillin. The minor errors with norfloxacin and chloramphenicol were all with *S. aureus* strains, and the gentamicin errors were all with coagulase-negative staphylococci. The major error for tetracycline occurred with an *S. aureus* strain, and the very major error for oxacillin occurred with a coagulase-negative staphylococcal strain. The errors were distributed among the clinical and challenge set isolates.

As previously reported for evaluations of other systems (3, 4), we noted that the reference broth microdilution method's MIC results averaged 2 to 4 dilutions higher than the test system's MIC results for β-lactam antimicrobial agents, particularly with penicillin and β-lactamase-producing strains of staphylococci. Also, when there was a shift in the MICs, the reference method's MICs tended to be higher overall than Alamar method's MICs, particularly for those antimicrobial agents affected by enzyme production. Usually, this shift did not cause categorical interpretive errors. Some strains of coagulase-negative staphylococci, particularly oxacillin-resistant strains, are difficult to test for antimicrobial susceptibility without an increased inoculum or prolonged incubation (2). In our study, we had three strains of *S. epidermidis* that did not grow well enough within 24 h to read the antimicrobial susceptibility MIC in the Alamar system; however, two of these strains did grow in the reference system. This information was not included in the study because instructions for performing the Alamar susceptibility test did not indicate prolonged incubation or the use of an increased inoculum.

We believe that the antimicrobial agents used to test enterococcal and staphylococcal species should be limited and carefully selected and that the Alamar antimicrobial susceptibility test system is an acceptable MIC method if appropriate antimicrobial agents are tested and reported.

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