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Comparison of the E Test to Agar Dilution, Broth Microdilution, and Agar Diffusion Susceptibility Testing Techniques by Using a Special Challenge Set of Bacteria

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The E Test (AB Biodisk, Solna, Sweden) is a new method for performing antimicrobial susceptibility tests. It consists of an impervious carrier (5- by 50-mm strip) with a predefined antimicrobic gradient which is placed on an inoculated agar plate and processed like a disk diffusion test. Results are generated directly as MICs from a continuous concentration gradient covering 15 twofold dilutions, and MICs are read where the edge of the inhibition zone intersects the strip. We compared the E Test with disk diffusion, broth microdilution, and agar dilution tests by using a challenge set of 195 gram-positive and gram-negative bacteria for 14 antimicrobial agents. Also, disk diffusion, broth microdilution, and agar dilution tests were compared with each other. All test method comparisons gave >94% agreement for the category of susceptibility. The E Test category agreement with disk diffusion and broth microdilution was 95.1%, and with agar dilution it was 95.2%. The E Test results were as reliable as the results obtained by the standard antimicrobial susceptibility testing methods.

Antimicrobial susceptibility testing may be done by a variety of techniques. The most commonly used methods in the United States have been the high-content disk diffusion method first described by Bauer et al. (4) and later modified by the National Committee for Clinical Laboratory Standards (NCCLS) (8), the broth microdilution technique as described by the NCCLS (9), the agar dilution method described by Ericsson and Sherris (5) and adapted by the NCCLS (9), and, more recently, automated or mechanized susceptibility testing techniques. Automated methods are usually based on standard methods with modifications to fit the special device being used to perform the susceptibility test (10).

The E Test (AB Biodisk, Solna, Sweden), a recently developed technique, is a modification of the disk diffusion and the agar dilution methods mentioned previously. The E Test is an impervious carrier with a continuous gradient of antimicrobial agent applied to one side of the strip. The test is processed in the same manner as the disk diffusion test, but determines a MIC of antimicrobial agent rather than a category result based on a zone size. This study was undertaken to assess the reliability of results obtained by using the E Test methodology. To accomplish this, we compared results from the E Test method with results from disk diffusion, broth microdilution, and agar dilution methods. In addition, disk diffusion and broth microdilution results were compared with agar dilution results and disk diffusion results were compared with broth microdilution results.

(Part of this study was presented at the 90th Annual Meeting of the American Society for Microbiology in Anaheim, Calif. [2].)

MATERIALS AND METHODS

Antimicrobial agents. Standard antimicrobial powders were obtained from various manufacturers for broth microdilution and agar dilution testing. Antimicrobial agent-impregnated disks were purchased from BBL Microbiology Systems, Cockeysville, Md. The E Test strips were supplied by AB Biodisk. Antimicrobial agents tested were ampicillin,

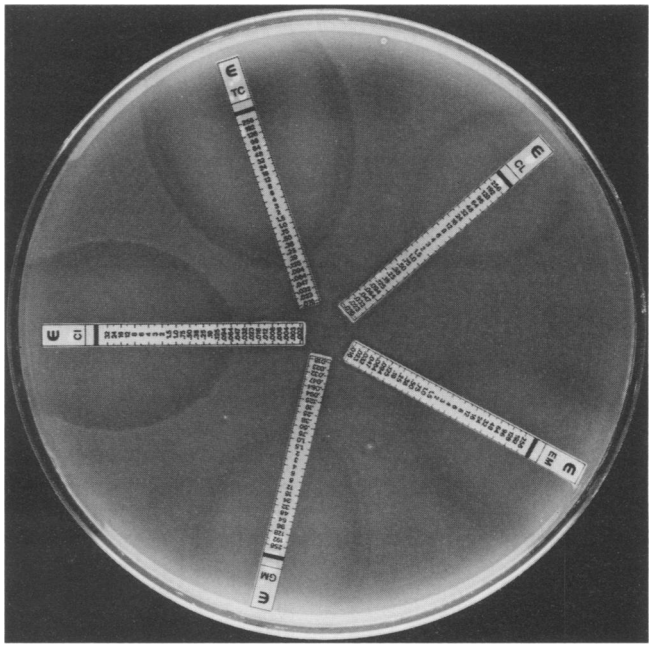

FIG. 1. Photograph of the E Test demonstrating the point at which the zone of inhibition of bacterial growth intersects the antimicrobial strip. The MIC scale is printed on the upper surface of the strip and the antimicrobial agent is on the underside of the strip.

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TABLE 1. Comparison of dilution tests using Pearson correlation coefficients

Antimicrobial agent	Correlation coefficient		
	E Test vs AD[a]	E Test vs MD[b]	MD vs AD
Ampicillin	0.99	0.98	0.99
Cefaclor	0.99	0.98	0.98
Cefoxitin	0.98	0.98	0.99
Cefuroxime	0.98	0.98	0.98
Cephalothin	0.98	0.98	0.98
Ciprofloxacin	0.95	0.95	0.95
Clindamycin	0.99	0.99	1.00
Erythromycin	0.97	0.99	0.97
Gentamicin	0.95	0.93	0.96
Imipenem	0.86	0.87	0.84
Oxacillin	0.96	0.95	0.94
Penicillin	0.97	0.93	0.93
Piperacillin	0.97	0.94	0.95
Vancomycin	0.75	0.68	0.56

[a] AD, Agar dilution antimicrobial susceptibility testing method.
[b] MD, Broth microdilution antimicrobial susceptibility testing method.

piperacillin, imipenem, cephalothin, cefaclor, cefoxitin, cefuroxime, gentamicin, and ciprofloxacin with gram-negative bacteria and oxacillin, penicillin, cephalothin, clindamycin, erythromycin, vancomycin, and ciprofloxacin with gram-positive bacteria. Antimicrobial agent concentrations ranged from 0.016 to 256 μg/ml for all agents except imipenem and ciprofloxacin (0.002 to 32 μg/ml).

Bacterial strains. Fifty-five gram-positive and 140 gram-negative strains were tested, including the following: 12 *Enterococcus faecalis*, 2 *Enterococcus faecium*, 22 *Staphylococcus aureus*, 1 *Staphylococcus capitis*, 13 *Staphylococcus epidermidis*, 2 *Staphylococcus haemolyticus*, 1 *Staphylococcus saprophyticus*, 2 *Staphylococcus warneri*, 6 *Acinetobacter calcoaceticus*, 1 *Aeromonas hydrophila*, 3 *Citrobacter diversus*, 3 *Citrobacter freundii*, 1 *Chromobacterium violaceum*, 10 *Enterobacter aerogenes*, 1 *Enterobacter agglomerans*, 7 *Enterobacter cloacae*, 2 *Edwardsiella tarda*, 21 *Escherichia coli*, 1 *Hafnia alvei*, 2 *Klebsiella oxytoca*, 8 *Klebsiella pneumoniae*, 1 *Morganella morganii*, 12 *Proteus mirabilis*, 4 *Proteus vulgaris*, 4 *Providencia rettgeri*, 2 *Providencia stuartii*, 2 *Providencia* species, 28 *Pseudomonas aeruginosa*, 2 *Pseudomonas cepacia*, 1 *Salmonella arizonae*, 2 *Salmonella paratyphi* A, 1 *Salmonella typhi*, 3 *Serratia liquefaciens*, 6 *Serratia marcescens*, 1 *Serratia odorifera*, 1 *Serratia rubidaea*, 1 *Shigella flexneri*, 1 *Shigella sonnei*, and 2 *Xanthomonas maltophilia*. Identification of these bacteria was done in the following specialty laboratories of the Centers for Disease Control: Special Bacteriology Reference Laboratory, Clinical Bacteriology Laboratory, and the Nosocomial Infections Laboratory Branch, using conventional methodology (7). These strains were from the stock culture collection of the Antimicrobic Investigations Branch and were collected especially for testing new devices and antimicrobial agents. They have a wide variety of known susceptibility characteristics and challenge the accuracy and versatility of a system. The strains were stored in rabbit blood at ≤−120°C in a liquid nitrogen freezer. Control strains used in this study were *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *Pseudomonas aeruginosa* ATCC 27853.

Antimicrobial susceptibility testing. Isolates were removed from storage, streaked onto a Trypticase soy agar plate supplemented with 5% sheep blood (BBL), and incubated for 18 to 24 h at 35°C. One isolated colony was picked from the plate, streaked to a new Trypticase soy agar plate, and incubated for 18 to 20 h. A suspension of growth from this isolate was prepared in 5 ml of Mueller-Hinton broth and adjusted to equal the turbidity of a 0.5 barium sulfate standard. All four susceptibility tests were performed from this adjusted inoculum.

(i) For the E Test, a cotton-tipped swab was dipped into the standardized suspension. Excess liquid was expressed, and a Mueller-Hinton agar plate (15 by 150 mm) was streaked with the suspension to cover the entire surface of the plate. After the surface of the inoculated plate had dried, the E Test strip was placed on the surface of the agar, and the plate was incubated at 35°C in ambient air for 18 h (*Staphylococcus* species, 24 h); the MIC was read at the point where the zone of inhibition intersected the MIC scale on the strip (Fig. 1).

(ii) For the disk diffusion test, from the standardized inoculum, tests were performed as described in NCCLS standard M2-T4 (8).

(iii) In the broth microdilution test, the standardized inoculum was diluted in sterile distilled water to give a final inoculum concentration of approximately 5×10^5 CFU/ml. Tests were performed as described in NCCLS standard M7-T2 (9).

(iv) In the agar dilution tests, the standardized suspension was diluted 1:10 in Mueller-Hinton broth so that the final concentration was approximately 10^4 CFU per spot on the surface of the antimicrobial test plate. Tests were performed as described in NCCLS standard M7-T2 (9).

TABLE 2. Distribution of differences in MICs with seven antimicrobial agents for 55 gram-positive bacteria: E Test versus agar dilution

Antimicrobial agent	% of isolates with the following difference in MIC[a]:							% Agreement[b]	P[c]
	<−2	−2	−1	0	+1	+2	>+2		
Oxacillin	3.6	1.8	16.4	49.1	25.5	3.6	0	90.9 ± 3.9	0.315
Penicillin	0	9.1	27.3	47.3	14.5	1.8	0	89.1 ± 4.2	0.051
Cephalothin	1.8	3.6	16.4	43.6	30.9	3.6	0	90.9 ± 3.9	0.321
Clindamycin	1.8	0	14.5	63.6	18.2	0	1.8	96.4 ± 2.5	0.495
Erythromycin	0	0	10.9	58.2	16.4	10.9	3.6	85.5 ± 4.8	0.002
Vancomycin	0	0	1.8	30.9	67.3	0	0	100.0 ± 0	1.000
Ciprofloxacin	3.6	10.9	65.5	18.2	1.8	0	0	85.5 ± 4.8	0.002
All agents	1.6	3.6	21.8	44.4	24.9	3.1	0.6	91.2 ± 1.5	0.148

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

TABLE 3. Distribution of differences in MICs with seven antimicrobial agents for 55 gram-positive bacteria: E Test versus broth microdilution

Antimicrobial agent	% of isolates with the following difference in MIC[a]:							% Agreement[b]	P[c]
	<−2	−2	−1	0	+1	+2	>+2		
Oxacillin	0	7.3	41.8	21.8	21.8	3.6	3.6	85.5 ± 4.8	0.478
Penicillin	21.8	16.4	36.4	23.6	1.8	0	0	61.8 ± 6.6	<0.001
Cephalothin	0	3.6	7.3	63.6	20.0	5.5	0	90.9 ± 3.9	0.327
Clindamycin	0	1.8	43.6	50.9	3.6	0	0	98.2 ± 1.8	0.159
Erythromycin	0	1.8	25.5	61.8	9.1	0	1.8	96.4 ± 2.5	0.495
Vancomycin	0	0	0	29.1	60.0	10.9	0	89.1 ± 4.2	0.007
Ciprofloxacin	0	18.2	50.9	25.5	5.5	0	0	81.8 ± 5.2	0.001
All agents	3.1	7.0	29.4	39.5	17.4	2.9	0.8	86.2 ± 1.8	<0.001

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

Statistical analysis. Pearson correlation coefficients were calculated for each antimicrobial agent to measure the overall association between the $\log_2$ dilution MIC results of the E Test compared with those of the agar dilution test, the E Test results compared with the broth microdilution test, and the broth microdilution test compared with the agar dilution test. To measure better the degree of agreement between each pair of tests, we looked at the distribution of the difference in the $\log_2$ dilution MIC results among the 55 gram-positive or 140 gram-negative strains tested and calculated the percentage of isolates which yielded identical results within the accuracy limits of standard tests (±1 $\log_2$ dilution). Finally, to see whether one susceptibility method tended to produce significantly lower or higher results than another method, we performed a Wilcoxon signed-rank test (6) on the difference in $\log_2$ MIC results of the two test. MICs within ±1 $\log_2$ dilution were regarded as identical for this hypothesis test.

RESULTS

The Pearson correlation coefficients (Table 1) ranged from 0.75 to 0.99 for the E Test when compared with the agar dilution test; 0.68 to 0.99 for the E Test when compared with broth microdilution; and 0.56 to 1.0 for the broth microdilution test when compared with the agar dilution test. The correlation coefficients for all antimicrobial agents except vancomycin and imipenem were ≥0.90.

The distribution of differences in $\log_2$ MICs, the percentage of agreement, and *P* values from the Wilcoxon signed-rank test for each antimicrobial agent and the various methods are shown in Tables 2, 3, and 4 for gram-positive bacteria and in Tables 5, 6, and 7 for gram-negative bacteria. Overall agreement for the gram-positive bacteria at ±1 $\log_2$ dilution was 91.2% when the E Test was compared with agar dilution and 86.2% when compared with broth microdilution. Broth microdilution compared with agar dilution had this agreement for 86.8% of the strains. Overall agreement for the gram-negative bacteria at ±1 $\log_2$ dilution for the E Test compared with agar was 92.9%, and compared with broth microdilution it was 85.6%. For broth microdilution compared with agar dilution, overall agreement was 90.8%. For gram-positive bacteria, the E Test tended to have lower MICs of ciprofloxacin than either agar or broth microdilution, higher MICs of erythromycin than agar dilution, and lower MICs of penicillin and higher MICs of vancomycin than broth microdilution. However, the percent agreement was >80% for all agents except penicillin when the E Test was compared with broth microdilution. For gram-negative bacteria, the E Test tended to have higher MICs than agar dilution of ampicillin, gentamicin, and cephalothin and lower MICs of imipenem. When compared with the broth microdilution method, the E Test had higher MICs of ampicillin, gentamicin, and cefoxitin and lower MICs of piperacillin and ciprofloxacin. The agreement of all antimicrobial agents was >79% for all gram-negative bacteria. When broth microdilution was compared with agar dilution for gram-positive bacteria, broth microdilution had higher MICs of all agents except vancomycin and ciprofloxacin, which were lower. Penicillin again had the lowest percent agreement. For gram-negative bacteria, broth microdilution had higher

TABLE 4. Distribution of differences in MICs with seven antimicrobial agents for 55 gram-positive bacteria: broth microdilution versus agar dilution

Antimicrobial agent	% of isolates with the following difference in MIC[a]:							% Agreement[b]	P[c]
	<−2	−2	−1	0	+1	+2	>+2		
Oxacillin	1.8	5.5	23.6	20.0	40.0	9.1	0	83.6 ± 5.0	0.382
Penicillin	0	1.8	3.6	27.3	40.0	5.5	21.8	70.9 ± 6.1	<0.001
Cephalothin	5.4	1.8	14.5	54.5	20.0	3.6	0	89.1 ± 4.2	0.194
Clindamycin	0	1.8	1.8	49.1	43.6	3.6	0	94.6 ± 3.1	0.282
Erythromycin	1.8	0	3.6	54.5	21.8	14.5	3.6	80.0 ± 5.4	0.004
Vancomycin	0	3.6	20.0	65.5	10.9	0	0	96.4 ± 2.5	0.079
Ciprofloxacin	0	7.3	18.2	56.4	18.2	0	0	92.7 ± 3.5	0.023
All agents	1.3	3.1	12.2	46.8	27.8	5.2	3.7	86.8 ± 1.7	0.008

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

TABLE 5. Distribution of differences in MICs with nine antimicrobial agents for 140 gram-negative bacteria: E Test versus agar dilution

Antimicrobial agent	% of isolates with the following difference in MIC[a]:							% Agreement[b]	P[c]
	<−2	−2	−1	0	+1	+2	>+2		
Ampicillin	0	1.4	7.9	63.6	20.7	5.0	1.4	92.1 ± 2.3	0.017
Piperacillin	0.7	1.4	22.9	55.7	13.6	3.6	2.1	92.1 ± 2.3	0.066
Imipenem	1.4	10.7	31.4	39.3	14.3	2.1	0.7	85.0 ± 3.0	0.002
Cephalothin	0	0	1.4	68.6	25.7	2.9	1.4	95.7 ± 1.7	0.007
Cefaclor	0	2.1	17.9	69.3	5.7	5.0	0	92.9 ± 2.2	0.103
Cefoxitin	0	2.1	3.6	63.6	25.0	2.1	3.6	92.1 ± 2.3	0.062
Cefuroxime	0.7	0	7.9	57.9	30.0	2.9	0.7	95.7 ± 1.7	0.053
Gentamicin	0	1.4	8.6	42.1	39.3	7.1	1.4	90.0 ± 2.5	0.004
Ciprofloxacin	0	2.1	20.7	62.9	13.6	0.7	0	97.1 ± 1.4	0.159
All agents	0.3	2.4	13.6	58.1	20.9	3.5	1.3	92.5 ± 0.7	0.003

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

MICs of piperacillin and ciprofloxacin. The MICs of ampicillin, imipenem, and gentamicin were lower. The percent agreement for all agents was >80% when broth microdilution was compared with agar dilution.

The MICs for each dilution method and the zone sizes for the disk diffusion method were converted to categories of susceptibility by using the definitions of the NCCLS standards M7-T2 and M2-T4. The percentage of category agreement for each method is shown in Table 8. The category agreement with all antimicrobial agents for the E Test was 95.1% when compared with disk diffusion, 95.1% when compared with broth microdilution, and 95.2% when compared with agar dilution. The category agreement for the disk diffusion test compared with broth microdilution was 94.2%; when compared with agar dilution, it was 95.2%. The category agreement for the broth microdilution test compared with agar dilution was 94.6%. Using the arbitrary definition of minor, major, and very major category discrepancies described by Thornsberry and Gavan (11), there were <0.5% major and <0.7% very major discrepancies observed. Minor discrepancies ranged from 4.9 to 5.6%, major discrepancies ranged from 0.06 to 0.48%, and very major discrepancies ranged from 0.12 to 0.6%.

DISCUSSION

The original purpose of this study was to assess the reliability of the E Test to predict the MICs and interpretative categories of susceptibility as compared with three conventional methods (disk diffusion, broth microdilution, and agar dilution). However, because all test procedures were done from a single inoculum and were carefully controlled, we had a unique opportunity to compare the three standard methods with each other. Comparisons of the E Test, disk diffusion, broth microdilution, and agar dilution methods gave very good agreement regardless of which methods were compared. The few problems encountered with each of these methods were mostly due to inoculum concentration, category breakpoint definitions, instability of a particular antimicrobial agent, or a problem organism-antimicrobial agent combination.

Vancomycin had a lower Pearson correlation coefficient than any other antimicrobial agent when MICs were compared. This was due to a very narrow range of MICs (0.5 to 4 μg/ml) which often tends to reduce the correlation coefficient. A lower correlation coefficient was also observed for imipenem when the E Test was compared with the broth microdilution test (0.87) and agar dilution (0.86). The correlation coefficient was still <0.9 when broth microdilution MICs were compared with agar dilution MICs, which probably indicates a stability problem with the antimicrobial agent rather than a methodology problem. It is well known that this antimicrobial agent may degrade rapidly under in vitro testing conditions (3). Also, most organisms are very susceptible to imipenem, and the scattergram is often con-

TABLE 6. Distribution of differences in MICs of nine antimicrobial agents for 140 gram-negative bacteria: E Test versus broth microdilution

Antimicrobial agent	% of isolates with the following difference in MIC[a]:							% Agreement[b]	P[c]
	<−2	−2	−1	0	+1	+2	>+2		
Ampicillin	0	0.7	2.9	59.3	25.7	7.1	4.3	87.9 ± 2.8	<0.001
Piperacillin	4.2	10.0	22.1	52.1	8.6	2.9	0	82.7 ± 3.2	0.001
Imipenem	0	5.0	17.9	40.7	27.9	6.4	2.1	86.4 ± 2.9	0.118
Cephalothin	0	2.1	4.3	70.0	20.7	2.1	0.7	95.0 ± 1.8	0.350
Cefaclor	2.1	2.9	12.1	64.3	15.7	2.9	0	92.1 ± 2.3	0.176
Cefoxitin	0.7	0.7	9.3	62.1	20.7	1.4	5.0	92.1 ± 2.3	0.017
Cefuroxime	2.1	0.7	11.4	63.6	17.1	4.3	0.7	92.1 ± 2.3	0.194
Gentamicin	1.4	2.9	7.1	37.1	35.0	15.0	1.4	79.3 ± 3.4	0.001
Ciprofloxacin	0.7	6.4	41.4	47.1	4.3	0	0	92.9 ± 2.2	0.001
All agents	1.3	3.5	14.3	55.2	19.5	4.7	1.6	89.0 ± 0.9	0.055

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

TABLE 7. Distribution of differences in MICs with nine antimicrobial agents for 140 gram-negative bacteria: broth microdilution versus agar dilution

Antimicrobial agent	% of isolates with the following difference in MIC[a]:							% Agreement[b]	P[c]
	<−2	−2	−1	0	+1	+2	>+2		
Ampicillin	1.4	5.0	18.6	67.1	6.4	1.4	0	92.1 ± 2.3	0.017
Piperacillin	0	1.4	13.6	45.0	25.7	10.7	3.6	84.3 ± 3.1	<0.001
Imipenem	1.4	15.0	39.3	30.0	11.4	2.1	0.7	80.7 ± 3.3	<0.001
Cephalothin	0.7	0.7	10.0	65.0	17.9	5.0	0.7	92.7 ± 2.1	0.030
Cefaclor	0.7	2.9	20.0	55.7	16.4	2.9	1.4	92.1 ± 2.3	0.377
Cefoxitin	0.7	2.1	9.3	70.0	15.0	2.1	0.7	94.3 ± 2.0	0.500
Cefuroxime	0.7	1.4	8.6	62.9	21.4	2.9	2.1	92.9 ± 2.2	0.102
Gentamicin	0	1.4	24.3	55.7	15.0	2.9	0.7	95.0 ± 1.8	0.127
Ciprofloxacin	0	0	7.1	50.7	35.0	7.1	0	92.9 ± 2.2	0.001
All agents	0.7	3.3	16.7	55.8	18.3	4.1	1.2	90.8 ± 0.8	0.067

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

centrated within a narrow range of MICs, tending to reduce the correlation coefficient (3).

When dilution differences were compared for each method, the most striking effect occurred with β-lactamase-positive *S. aureus* and penicillin. Broth microdilution results for penicillin averaged 3 $\log_2$ dilutions greater than either the E Test ($P < 0.001$) or agar dilution ($P < 0.001$) results. This is probably a methodology problem associated with the small volume of reagents in the test well and the fairly large inoculum used in this method. However, this increased MIC for the broth microdilution method did not cause any interpretative category discrepancies when compared with any of the methods. All β-lactamase-producing strains of *S. aureus* were resistant to penicillin by all methods. Piperacillin also had higher broth microdilution MICs for some strains of *Enterobacter*, *Escherichia*, *Klebsiella*, *Proteus*, and *Serratia* species, organisms known to cause problems with β-lactam antimicrobial agents (1). This could also be due to methodology since small numbers of resistant mutants may be readily discernible in the microdilution wells because of continued growth, whereas, with the E Test and agar dilution, 1 or 2 CFUs may not be visible or may be ignored by the laboratorian reading the test. In general, broth microdilution gave higher MICs of oxacillin, penicillin, piperacillin, cephalothin, imipenem, clindamycin, and erythromycin, antimicrobial agents affected by inoculum, than either the E Test or agar dilution. The E Test averaged 1 $\log_2$ dilution higher with vancomycin than either the broth microdilution or agar dilution, although all results were within the accuracy limits of the test. The E Test results for ciprofloxacin were lower than either broth microdilution or agar dilution. The most probable explanation for the vancomycin and ciprofloxacin differences is a slight concentration difference between the E Test and the broth microdilution tests and agar dilution since the antimicrobic concentrations for broth and agar tests were the same. Gentamicin was usually more active in the broth microdilution test than in the other methods. This probably indicates decreased cation content in the Mueller-Hinton broth (recent NCCLS recommendations have reduced the cation content of Mueller-Hinton broth), which affects results especially with *Pseudomonas aeruginosa* strains (9).

TABLE 8. Agreement of categories of susceptibility[a] for the E Test, disk diffusion, broth microdilution, and agar dilution methods

Antimicrobial agent	No. of strains tested	% Agreement of categories					
		E vs D[b]	E vs MD[c]	E vs AD[d]	D vs MD[e]	D vs AD[f]	MD vs AD[g]
Ampicillin	140	94.3	93.6	95.7	92.8	94.3	95.7
Oxacillin	55	94.5	98.2	94.5	96.4	100.0	96.4
Penicillin	55	100.0	98.2	98.2	98.2	98.2	100.0
Piperacillin	140	92.6	92.1	94.3	92.1	88.6	87.6
Imipenem	140	92.9	93.6	95.7	97.9	93.6	94.3
Cephalothin	195	88.2	93.8	90.3	88.2	94.9	88.2
Cefaclor	140	95.7	93.6	97.1	92.1	96.4	92.9
Cefoxitin	140	95.7	94.3	95.7	94.3	94.3	97.1
Cefuroxime	140	93.6	96.4	94.3	93.6	92.1	93.6
Vancomycin	55	100.0	100.0	100.0	100.0	100.0	100.0
Gentamicin	140	97.1	90.7	92.1	92.9	95.0	95.7
Clindamycin	55	100.0	100.0	100.0	100.0	100.0	100.0
Erythromycin	55	92.7	94.5	92.7	87.3	92.7	87.3
Ciprofloxacin	195	93.8	91.8	92.8	92.8	92.8	95.9

[a] Categories of susceptibility as defined by NCCLS standards M7-T2 and M2-T4.
[b] E Test compared with disk diffusion.
[c] E Test compared with the broth microdilution method.
[d] E Test compared with the agar dilution method.
[e] Disk diffusion compared with broth microdilution.
[f] Disk diffusion compared with agar dilution.
[g] Broth microdilution compared with agar dilution.

When the two NCCLS standards were used to predict categories of susceptibility (8, 9), most of the category discrepancies were minor for all methods. The majority of these minor category discrepancies occurred for *Pseudomonas aeruginosa* and *Providencia* species with gentamicin, *Enterococcus* and *Pseudomonas* species with ciprofloxacin, *Pseudomonas aeruginosa* with imipenem, *Enterococcus faecalis* with erythromycin, and *Enterococcus* species and *Escherichia coli* with cephalothin. These discrepancies occurred with each method compared and can be explained by the close proximity of the category breakpoints to the usual MICs of these particular antimicrobial agents for these organisms. All methods had <6% minor category discrepancies, <0.5% major, and <0.7% very major category discrepancies, which is well within the acceptable limits described by Thornsberry and Gavan (11).

In conclusion, the E Test yielded excellent category agreement results when compared with the disk diffusion (95.1%), broth microdilution (95.1%), and agar dilution (95.2%) tests with a selected set of challenge strains. The E Test, when compared with agar dilution, had the best overall agreement (91.2 ± 1.5% for gram-positive bacteria and 92.5 ± 0.7% for gram-negative bacteria) probably because MICs for both were obtained on agar medium. Category comparisons of disk diffusion, broth microdilution, and agar dilution methods also gave excellent results. The E Test appears to be an excellent addition to the array of methods now available for antimicrobial susceptibility testing.

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