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Evaluation of the E Test for Antimicrobial Susceptibility Testing of *Pseudomonas aeruginosa* Isolates from Patients with Long-Term Bladder Catheterization

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The E test was evaluated in comparison with reference agar methods (National Committee for Clinical Laboratory Standards) for the susceptibility testing of 248 *Pseudomonas aeruginosa* isolates from bladder-catheterized patients against nine antibiotics. The E-test MICs correlated well with those determined by the agar dilution and disk diffusion reference methods (88 and 92.5% within 1 $\log_2$ dilution step, respectively), confirming that the E test is a reliable method for the determination of MICs of antibiotics for catheterization-associated *P. aeruginosa* isolates.

Catheter-associated urinary tract infections (UTI_c) remain the most common of all nosocomial infections, accounting for approximately 40% of infections in most hospitals (7). In adults, the presence of an indwelling catheter was associated with a decreased incidence of *Escherichia coli* infections and an increased incidence of *Pseudomonas aeruginosa* (9, 15), an opportunistic bacterial pathogen that is becoming increasingly important due to its antibiotic resistance, especially for fluoroquinolones, aminoglycosides, and β-lactams, which has resulted in a high clinical failure rate of approximately 70% (5, 6, 11, 19). Thus, techniques to prevent and control the spread of resistant strains is of great importance. Agar dilution tests are cumbersome to perform and inadequate for routine testing in many clinical laboratories. Disk diffusion tests perform satisfactorily, but they yield categorized qualitative results only and not MICs. The E test (AB Biodisk, Solna, Sweden) is a relatively new agar diffusion-based technology for the quantitative determination of bacterial and fungal susceptibilities (1–3, 14).

We compared the susceptibility results obtained with the E test to those obtained with agar methods approved by the National Committee for Clinical Laboratory Standards (NCCLS) from tests of nine commonly used antibiotics versus 248 UTI_c-associated *P. aeruginosa* isolates.

Two hundred and forty-eight strains of *P. aeruginosa*, which had been isolated from consecutive nonduplicate bladder-catheterized patients and frozen, were thawed, inoculated twice onto Columbia agar supplemented with 5% defibrinated sheep blood, and incubated for 24 h at 37°C. Colonies were suspended in Mueller-Hinton broth to a density of 0.5 McFarland standard. *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were included in the study as control strains.

E-test strips containing amikacin, aztreonam, ceftazidime, ciprofloxacin, gentamicin, imipenem, piperacillin, ticarcillin, and tobramycin were purchased from AB Biodisk. Reagent-grade powders of the same antimicrobial agents were used for agar dilution MIC tests. For the agar disk diffusion method, antimicrobial agent-impregnated disks were purchased from bioMérieux Italia S.p.A. (Rome, Italy).

The E test was performed with Mueller-Hinton agar plates (diameter, 140 mm). The plates were inoculated by confluent swabbing of the surface with the adjusted inoculum suspensions. Inoculated plates were allowed to dry before E-test strips were applied to the medium. After application of the E test (with a maximum of five strips per agar plate), plates were incubated at 37°C. MICs were read after 24 h on the basis of the intersection of the elliptical zone of growth inhibition with the MIC scale on the E-test strip.

Agar dilution tests were performed as described in NCCLS standard M7-A3 (13). Twofold increments (across a range of 0.008 to 128 μg/ml) of the antimicrobial agents incorporated in Mueller-Hinton agar were used. The standardized inoculum was diluted in Mueller-Hinton broth and delivered to the surface of the agar plates with a Steers replicator so that the final concentration was approximately 10^4 CFU per spot.

Disk diffusion tests were performed as described in NCCLS standard M2-A5 (12). The standardized inoculum was inoculated onto Mueller-Hinton agar plates (diameter, 140 mm). Plates were incubated at 37°C, and diameters of inhibition zones were measured after 24 h of incubation. Discrepancies

TABLE 1. Comparison of antimicrobial susceptibility test results obtained by the agar dilution method and the E test for 248 *P. aeruginosa* isolates

Antibiotic	MIC_{90} (μg/ml)		% Resistant	
	E test	AD[a]	E test	AD
Amikacin	8	8	3.2	3.2
Aztreonam	64	64	6.4	6.4
Ceftazidime	16	16	9.6	9.6
Ciprofloxacin	4	4	29.0	32.2
Gentamicin	64	64	24.0	25.8
Imipenem	32	32	3.2	3.2
Piperacillin	>128	>128	54.8	45.0
Ticarcillin	>128	64	35.5	12.9
Tobramycin	>128	64	22.5	22.5

[a] AD, agar dilution method.

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TABLE 2. Distribution of differences in MICs of nine antimicrobial agents for 248 isolates of *P. aeruginosa*: E test versus agar dilution

Antibiotic (no. of strains[a])	No. (%) of E-test MICs within indicated number of $\log_2$ dilutions of agar dilution MICs							% Agreement[b]
	<−2	−2	−1	0	+1	+2	>+2	
Amikacin (248)	0	0	8 (3.3)	80 (32.2)	120 (48.4)	32 (12.8)	8 (3.3)	83.9
Aztreonam (208)	0	16 (7.7)	40 (19.2)	80 (38.5)	48 (23.1)	24 (11.5)	0	80.8
Ceftazidime (175)	2 (1.1)	6 (3.4)	30 (17.1)	76 (43.5)	36 (20.6)	25 (14.3)	0	81.2
Ciprofloxacin (220)	0	8 (3.6)	38 (17.3)	96 (43.7)	72 (32.7)	6 (2.7)	0	93.7
Gentamicin (230)	0	0	50 (21.8)	90 (39.1)	90 (39.1)	0	0	100.0
Imipenem (196)	0	10 (5.1)	44 (22.4)	78 (39.8)	52 (26.5)	10 (5.1)	2 (1.1)	88.7
Piperacillin (111)	8 (7.2)	7 (6.3)	16 (14.4)	64 (57.7)	8 (7.2)	8 (7.2)	0	79.3
Ticarcillin (160)	0	0	40 (25)	96 (60)	24 (15)	0	0	100.0
Tobramycin (208)	0	24 (11.6)	24 (11.6)	56 (26.8)	88 (42.4)	8 (3.8)	8 (3.8)	80.8
Total (1,756)	10 (0.6)	71 (4)	290 (16.5)	716 (40.8)	538 (30.7)	113 (6.4)	18 (1)	88.0

[a] Number of strains for which MICs were within the concentration range of the E test and the agar dilution method.
[b] Percentage of E-test MICs with 1 log dilution of agar dilution MICs.

between the E test and either the agar dilution or disk diffusion reference method were classified as very major (the reference method result was resistant and the E-test result was susceptible), major (the reference method result was susceptible and the E-test result was resistant), or minor (an intermediate result was obtained by only one of the methods) errors (18). The significances of the differences between MICs obtained by two methods were determined by the χ^2 test. A P value of less than 0.05 was considered to represent a statistically significant difference.

The most active compound in vitro was ciprofloxacin (MIC at which 90% of the isolates were inhibited [MIC_{90}], 4 μg/ml for both the E test and agar dilution methods) (Table 1). The highest E-test MIC_{90}s were observed for piperacillin, ticarcillin, and tobramycin (>128 μg/ml). The highest agar dilution MIC_{90} was observed for piperacillin (>128 μg/ml). The E test yielded higher percentages of resistant isolates than did the agar dilution method when ticarcillin ($P < 0.01$), and piperacillin ($P > 0.05$) were tested. The highest percentage of resistant isolates was observed for piperacillin (54.8% for the E test and 45% for the agar dilution method).

The distribution of differences between the E-test and the agar dilution MICs is shown in Table 2. Overall, 88% of results were within 1 $\log_2$ dilution step, and 98.4% of results were within 2 $\log_2$ dilution steps. Excellent correlations (100%) were found for ticarcillin and gentamicin. A total of 186 (8.3%) discrepancies occurred, of which 158 were minor and 28 were major errors.

The comparison of the categories obtained by the E test with those obtained by the disk diffusion method is shown in Table 3. Overall qualitative agreement was 92.5%. A total of 170 (7.6%) discrepancies were observed, of which 162 were minor and 8 were major errors.

No very major errors were found between the E test and the NCCLS reference agar methods. The accuracy of the E-test MICs for *P. aeruginosa* that we found is in agreement with the findings of previous studies (8, 10, 16). Our data indicate that E-test MICs were within 1 $\log_2$ dilution of reference agar dilution results in 88% of instances for the nine antimicrobial agents tested. This good agreement may be due to both the common batch of Mueller-Hinton agar and the common inoculum. However, the E-test MICs tended to be higher than those obtained by the agar dilution method ($P < 0.01$). This is most apparent for the aminoglycosides amikacin and tobramycin. The reason for this effect in the present study is not known. The effect is certainly not due to the cation concentrations or inoculum, since a common batch of Mueller-Hinton agar and the same suspension were used for both methods. However, the higher MICs usually were within 1 $\log_2$ dilution of the agar dilution result and only rarely changed the categorical interpretation of the test. The percentage of resistant isolates determined by the E test was comparable to those obtained by the reference methods. However, for some drugs (amikacin, imipenem, aztreonam, and ceftazidime) the number of strains determined to be resistant was not sufficient to affirm the ability of the E test in predicting antimicrobial resistance.

When interpretive categories are considered, the data of this study are well within the acceptable limits described by Thornsberry (17). Most of the category discrepancies found were minor. The majority of these minor discrepancies occurred with ticarcillin (E test versus both reference methods) and piperacillin (E test versus the agar dilution method). These findings can be explained by the close proximity of the category breakpoints to the usual MICs of these particular antimicrobial agents for *P. aeruginosa*.

In our experience, the E test is much less labor-intensive and is easier to perform than the agar dilution method. The E test uses materials and a methodology which are similar to those of the widely used disk diffusion method. Further, unlike the disk diffusion method, inoculum size, preincubation, and prediffusion do not influence the E-test results because of the stability of the antimicrobial gradient produced by the E test (4).

In conclusion, the present study has demonstrated that the E test could serve as an accurate, easy-to-perform, and time-saving alternative to the reference agar methods for quantitative antimicrobial susceptibility testing of UTI_c-associated *P. aeruginosa*.

TABLE 3. Discrepancies between interpretive categories determined by the E test and the disk diffusion reference method[a]

Antibiotic	% Agreement	% Isolates by error category			
		Very major	Major	Minor	All
Amikacin	100.0	0	0	0	0
Aztreonam	98.4	0	0	1.6	1.6
Ceftazidime	100.0	0	0	0	0
Ciprofloxacin	96.0	0	0	4	4
Gentamicin	92.0	0	0	8	8
Imipenem	96.8	0	0	3.2	3.2
Piperacillin	96.8	0	3.2	0	3.2
Ticarcillin	51.7	0	0	48.3	48.3
Tobramycin	100.0	0	0	0	0
Total	92.5	0	0.4	7.2	7.6

[a] $n = 248$. Values are percentages relative to a total of 2,232 tests.

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