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Evaluation of E-Test for Determination of Antimicrobial MICs for *Pseudomonas aeruginosa* Isolates from Cystic Fibrosis Patients

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We determined the E-Test and National Committee for Clinical Laboratory Standards standardized agar dilution MICs of ceftazidime, ciprofloxacin, piperacillin, and tobramycin for *Pseudomonas aeruginosa* during tests of 100 rough and mucoid *P. aeruginosa* isolates from cystic fibrosis patients. The levels of agreement (±1 $\log_2$ dilution) between quantitative E-Test and agar dilution MIC results were 80, 97, 73, and 89% for ceftazidime, ciprofloxacin, piperacillin, and tobramycin, respectively. Comparison of the results after converting the MIC data to qualitative categories (susceptible, intermediate, and resistant) yielded levels of agreement of 84, 96, 88, and 93% for the same agents, respectively. Of the 39 qualitative discrepancies, 36 were minor and 3 were very major. We conclude that use of the E-Test is easier and more practical than use of the agar dilution method for most laboratories and that the E-Test furnishes results which are at least as accurate as those obtained by the agar dilution method. However, the higher cost of the E-Test method would likely discourage most laboratories from selecting it over disk diffusion for routine antimicrobial susceptibility testing of *P. aeruginosa* isolates from cystic fibrosis patients.

Pulmonary infections are the major cause of morbidity and mortality in cystic fibrosis patients, with *Pseudomonas aeruginosa* serving as the principal pathogen (5, 7). The recovery of exopolysaccharide-producing mucoid strains increases in frequency with disease progression and is associated with marked clinical deterioration (11, 17). Antimicrobial therapy is most frequently used during infectious exacerbations. Effective therapies have included a variety of extended-spectrum penicillins, aminoglycosides, antipseudomonal cephalosporins, monobactams, carbapenems, and fluoroquinolones (10). Some studies have suggested that a possible benefit of early antipseudomonal therapy is postponement of chronic *P. aeruginosa* colonization (17). However, a consensus on therapeutic indications, selection of antimicrobial agents, or dosage schedules does not yet exist (13).

Virtually all *P. aeruginosa* isolates grow well on agar media, but mucoid strains do not grow reliably in broth. This can be a problem for laboratories performing broth microdilution susceptibility test methods. Accordingly, a practical and accurate method for determining antimicrobial susceptibility on agar media would be a valuable addition to the workup of *P. aeruginosa* isolates from cystic fibrosis patients. Disk diffusion tests perform satisfactorily, but they yield categorized qualitative results only. Agar dilution tests function well and provide quantitative data, but they are time-consuming and are often too expensive for typical laboratories to perform them on limited numbers of isolates. The E-Test (AB Biodisk, Solna, Sweden) is an agar diffusion MIC method which uses a thin plastic strip coated with a continuous antimicrobial gradient on one side and a quantitative interpretive scale on the other side. MICs are determined by reading the antimicrobial concentration printed on the test strip at its intersection with the growth inhibitory zone. The ease of performance of the E-Test would be a notable advantage if the MIC results obtained correlate reliably with those of the less convenient agar dilution method. The purpose of our study was to compare the susceptibility results obtained by the E-Test and those obtained by the agar dilution method of the National Committee for Clinical Laboratory Standards (NCCLS) during tests of four commonly used antimicrobial agents versus 100 rough and mucoid *P. aeruginosa* strains freshly isolated from cystic fibrosis patients.

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MATERIALS AND METHODS

The Children's National Medical Center is a referral center for the diagnosis and management of cystic fibrosis patients in the Washington, D.C., region. One hundred rough (59 strains) and mucoid (41 strains) isolates of *P. aeruginosa* freshly recovered from consecutive respiratory cultures of specimens from cystic fibrosis patients were tested by the E-Test and the NCCLS agar dilution method. Care was taken to exclude isolates from replicate cultures of the same patients. The MICs of ceftazidime, ciprofloxacin, piperacillin, and tobramycin were determined. Isolates were stored temporarily as slant cultures on Trypticase soy agar and were subcultured onto 5% sheep blood agar prior to testing. Identification of isolates as *P. aeruginosa* was confirmed with the two-tube N/F Screen (Remel, Lenexa, Kans.). The reference strain *P. aeruginosa* ATCC 27853 was included in the study as a quality control indicator.

E-Test strips containing ceftazidime, ciprofloxacin, piperacillin, and tobramycin were kindly provided by the manufacturer (AB Biodisk North America Inc., Culver City, Calif.). Reagent-grade powders of the same antimicrobial agents (ceftazidime [Glaxo Inc., Research Triangle Park, N.C.]; ciprofloxacin, piperacillin, and tobramycin [Sigma Chemical Co., St. Louis, Mo.]) were used to prepare media for agar dilution tests.

For the E-Test 150-mm-diameter Mueller-Hinton agar plates (Becton Dickinson, Cockeysville, Md.) were inoculated with swabs saturated with suspensions of the study isolates equivalent to a 0.5 McFarland standard. The four antimicrobial agent-coated test strips were placed in separate quadrants on each plate in accordance with the manufacturer's instructions. The antimicrobial concentration ranges tested were 0.016 to 256 μg/ml for ceftazidime, piperacillin, and tobramycin and 0.002 to 32 μg/ml for ciprofloxacin. The results were read after 18 to 24 h of incubation in ambient air at 35°C.

Agar dilution tests were performed as described in NCCLS standard M7-A3

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TABLE 1. Frequency of E-Test results at variance from agar dilution results

Antibiotic	No. of E-Test results with the following $\log_2$ concn variances from agar dilution results:							% Agreement (± 1 $\log_2$ dilution)
	>−2	−2	−1	Same	+1	+2	>+2	
Ceftazidime	4	7	13	64	3	3	6	80
Ciprofloxacin	0	1	25	72	0	2	0	97
Piperacillin	5	16	12	59	2	5	1	73
Tobramycin	0	1	15	74	0	10	0	89
Total	9	25	65	269	5	20	7	

(14). The antimicrobial concentration ranges tested were 0.195 to 50 μg/ml for ceftazidime, ciprofloxacin, and tobramycin and 1.95 to 500 μg/ml for piperacillin. The same bacterial suspensions used for the E-Test were adjusted to a cell density of $\sim 10^7$ CFU/ml for the agar dilution test. Test plates were inoculated with a Steers replicator (Cathra Systems MCT Medical, St. Paul, Minn.) so that the final inoculum approximated 10^4 CFU per spot. The results were read after 16 to 20 h of incubation in ambient air at 35°C.

RESULTS

The MIC determinations obtained for each isolate by the E-Test method were compared with those obtained by the agar dilution method by looking for identical quantitative results within the accuracy limits of each test (± 1 $\log_2$ dilution). Levels of agreements of 80, 97, 73, and 89% were found for ceftazidime, ciprofloxacin, piperacillin, and tobramycin, respectively, with the overall agreement being 85% (Table 1). The results were variant by greater than ± 2 $\log_2$ dilutions for ceftazidime and piperacillin only (10 and 6 isolates, respectively). Separate analyses of rough and mucoid strains revealed no significant differences between the groups.

The MIC results obtained by both methods were converted to qualitative categories (susceptible, intermediate, and resistant) by using NCCLS guidelines (14) and were compared. A very major discrepancy was defined as an isolate which appeared to be susceptible by the E-Test and resistant by the agar dilution method. A major discrepancy occurred when an isolate was susceptible by the agar dilution method and resistant by the E-Test. A minor discrepancy existed when an intermediate result was obtained by only one of the methods. Qualitative agreements were 84, 96, 88, and 93% for ceftazidime, ciprofloxacin, piperacillin, and tobramycin, respectively (Table 2). Three very major discrepancies (0.75%) occurred: one for ceftazidime (11% of ceftazidime-resistant isolates falsely susceptible by the E-Test) and two for piperacillin (11% of piperacillin-resistant isolates falsely susceptible by the E-Test). There were no major discrepancies. Thirty-six minor discrepancies (9%) were noted: 15, 4, 10, and 7 for ceftazidime, ciprofloxacin, piperacillin, and tobramycin, respectively.

Thirteen of 46 isolates yielding discrepant quantitative results (greater than ± 1 $\log_2$ dilutions) with any of the antimicrobial agents were selected at random and retested by both methods with all four antimicrobial agents. The percent agreement between the methods improved for either or both the quantitative and qualitative results with all antimicrobial agents. Among the isolates retested, the E-Test results appeared to be more reproducible than agar dilution results. Changes in quantitative (7 results by the E-Test versus 10 results by the agar dilution method) and qualitative (5 results by the E-Test versus 10 results by the agar dilution method) results were less frequent with the E-Test than with the agar dilution method.

DISCUSSION

The reliability of E-Test MIC results for *P. aeruginosa* that we found are in accord with the findings of previous studies encompassing a variety of other bacteria and fungi (1, 2, 4, 6, 18). The E-Test has been found to be especially useful for susceptibility testing of anaerobes (19), although a problem with cefoxitin results was noted in one study (3). The E-Test has also been found to be a convenient and reliable method for detecting high-level resistance to aminoglycosides among enterococci (16) and for detecting penicillin and cephalosporin resistance among pneumococci (8, 12).

Other investigators have reported an excellent correlation of E-Test results with those obtained by standard methods for *P. aeruginosa*. In a comparative study of five methods, greater than 90% agreement within 1 $\log_2$ dilution was found between the E-Test and reference agar dilution MIC results for amikacin, gentamicin, piperacillin, and ceftazidime (9). Similarly, Rautelin et al. (15) reported 93% agreement of tobramycin MIC results (within 1 $\log_2$ dilution) between the E-Test and the conventional agar dilution method.

In our hands E-Test results were within 1 $\log_2$ dilution of reference agar dilution results in 85% of instances for the four antimicrobial agents tested. However, we did notice an overall trend toward lower quantitative MIC results by the E-Test. The average E-Test MIC was 0.8 $\log_2$ dilution lower than the corresponding agar dilution MIC. This relationship has been noted in a number of other studies as well (8, 15, 18). Very major discrepancies between qualitative E-Test and agar dilution results were rare, occurring in only 3 of 400 (0.8%) result comparisons. We have no explanation other than chance to explain why the three very major discrepancies were observed only with beta-lactam agents. E-Test results appeared to be more reproducible than those obtained by the agar dilution method. Both quantitative and qualitative E-Test results changed less frequently when 13 randomly selected isolates were retested by both methods. Fluctuations in the antimicrobial content of the medium used for the agar dilution method and minor variations in inoculum density may explain the intertest variability observed between sets of results obtained by the agar dilution method.

Once familiarity with the E-Test method was gained, its ease and convenience of use made it more practical and preferable to the agar dilution method for determining the MICs of antimicrobial agents for *P. aeruginosa* isolates from cystic fibrosis patients. Mucoid strains on occasion exhibited an indistinct intersection between the inhibitory zone and the E-Test strip, making precise determination of the MIC problematic. However, isolated antimicrobial agent-resistant colonies within the zones of inhibition were readily identified. On the whole, the advantages offered by the E-Test far outweighed the disadvantages. The E-Test allowed for the simultaneous testing of four

TABLE 2. E-Test results with qualitative variance from agar dilution results

Antibiotic	% Agreement	No. of E-Test results with the following variance from agar dilution results		
		Minor discrepancies	Major discrepancies	Very major discrepancies
Ceftazidime	84	15	0	1
Ciprofloxacin	96	4	0	0
Piperacillin	88	10	0	2
Tobramycin	93	7	0	0
Total	90	36	0	3

antimicrobial agents per 150-mm agar plate. The same Mueller-Hinton agar plates used for disk diffusion testing can be used for the E-Test, eliminating the need to manufacture or purchase limited-shelf-life media containing various concentrations of antimicrobial agents. E-Test strips can be stored at −20°C for at least 1 year and can be used to test single isolates as needed.

The relatively higher cost of the E-Test procedure (list price of ~$2.50 per strip) precludes us from recommending it for routine use. However, we do recommend the E-Test for testing *P. aeruginosa* isolates from cystic fibrosis patients in situations in which quantitative susceptibility data are clinically necessary. An example would be deciding whether to use expensive (e.g., ceftazidime) or potentially toxic (e.g., tobramycin) therapy in patients harboring strains displaying borderline susceptibility by qualitative test methods.

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