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Comparison of Agar Diffusion Methodologies for Antimicrobial Susceptibility Testing of *Pseudomonas aeruginosa* Isolates from Cystic Fibrosis Patients

JANE L. BURNS,[1]* LISA SAIMAN,[2] SUSAN WHITTIER,[3] DAVISE LARONE,[3]† JAY KRZEWINSKI,[1] ZHENLING LIU,[2] STEVEN A. MARSHALL,[4] AND RONALD N. JONES[4]

Department of Pediatrics, Division of Infectious Disease, University of Washington, Seattle, Washington[1]; Department of Pediatrics, Division of Infectious Diseases, Columbia University,[2] and Department of Pathology, New York Presbyterian Hospital (Columbia Presbyterian Center),[3] New York, New York; and Department of Pathology, University of Iowa College of Medicine, Iowa City, Iowa[4]

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***Pseudomonas aeruginosa* is the most common pathogen infecting the lungs of patients with cystic fibrosis (CF). Improved antimicrobial chemotherapy has significantly increased the life expectancy of these patients. However, accurate susceptibility testing of *P. aeruginosa* isolates from CF sputum may be difficult because the organisms are often mucoid and slow growing. This study of 597 CF isolates of *P. aeruginosa* examined the correlation of disk diffusion and Etest (AB BIODISK, Solna, Sweden) results with a reference broth microdilution method. The rates of interpretive errors for 12 commonly used antipseudomonal antimicrobials were determined. The disk diffusion method correlated well (zone diameter versus MIC) for all of the agents tested. However, for mucoid isolates, correlation coefficients (*r* values) for piperacillin, piperacillin-tazobactam, and meropenem were <0.80. The Etest correlation with reference broth microdilution results (MIC versus MIC) was acceptable for all of the agents tested, for both mucoid and nonmucoid isolates. Category interpretation errors were similar for the disk diffusion and Etest methods with 0.4 and 0.1%, respectively, very major errors (false susceptibility) and 1.1 and 2.2% major errors (false resistance). Overall, both agar diffusion methods appear to be broadly acceptable for routine clinical use in susceptibility testing of CF isolates of *P. aeruginosa*.**

Cystic fibrosis (CF) is a genetic condition affecting approximately 60,000 individuals in North America and Europe. An important cause of morbidity and mortality in CF is chronic endobronchial infection that is most commonly caused by *Pseudomonas aeruginosa* (9). Antimicrobial chemotherapy has made an important contribution to increasing the life expectancy of patients with CF (3) and is one of the mainstays of therapy. However, *P. aeruginosa* isolates from patients with CF frequently have unique phenotypes. They are often multiply resistant and have large amounts of mucoid exopolysaccharide and relatively slow growth rates in the laboratory. These characteristics, thought to be the result of environmental pressure exerted by the milieu in the lungs of patients with CF (4), may adversely affect the performance and interpretation of standardized antimicrobial susceptibility testing.

A consensus conference on CF microbiology was held in May of 1994 (10). This group, composed of microbiologists, infectious disease specialists, and pulmonologists who care for CF patients, evaluated the literature available at the time and recommended the use of disk diffusion susceptibility testing (7) for *P. aeruginosa*. The use of automated microtiter systems was not recommended because of the perceived inaccuracies of these assays for CF isolates. The current study was undertaken to evaluate those recommendations and to test an additional agar-based stable gradient method for MIC determination (Etest; AB BIODISK, Solna, Sweden). We evaluated the susceptibilities of 597 CF isolates of *P. aeruginosa* (which included 99 isolates that were tested in two laboratories) to 12 antimicrobial agents, comparing broth microdilution (12) with the disk diffusion method (7) and the Etest.

* Corresponding author. Mailing address: Children's Hospital and Regional Medical Center, 4800 Sand Point Way N.E., CH-32, Seattle, WA 98105. Phone: (206) 526-2073. Fax: (206) 527-3890. E-mail: jburns@chmc.org.

† Present address: New York Presbyterian Hospital (Weill Cornell Center), New York, N.Y.

MATERIALS AND METHODS

Bacterial strains. Two hundred fifty isolates were randomly selected from the collection of clinical isolates at the CF Center at the Children's Hospital and Regional Medical Center and the University of Washington in Seattle. An additional 250 isolates were selected from strains sent to the CF Referral Center for Susceptibility and Synergy Testing of Multiply Resistant Organisms at Columbia University in New York, N.Y. Strains were stored frozen at −80°C and checked for purity and mucoid phenotype by plating on MacConkey agar, followed by streaking onto Mueller-Hinton agar to confirm purity and detect pigment production. The following strain definition of *P. aeruginosa* was used: oxidase positive, catalase positive, growth at 42°C, and pigment production (5). Of the 500 isolates tested, 147 did not meet these phenotypic criteria for *P. aeruginosa* and thus were further tested by colony hybridization and probing with the exotoxin A gene from *P. aeruginosa* (13). Seven were found to be exotoxin A negative and were further identified using the API 20 NE strip (bioMerieux Vitek, Inc.) in accordance with the manufacturer's instructions. Two of these seven were not identified as *P. aeruginosa* and were excluded from further analysis. The 1.4% (7 of 498) proportion of exotoxin A-negative CF isolates of *P. aeruginosa* is comparable to that reported in the literature (17).

The entire collection of 498 isolates was available at each center and numbered sequentially. Three hundred forty-eight isolates were tested in the laboratory at the Children's Hospital and Regional Medical Center (all odd-numbered isolates and 99 even-numbered isolates), and 249 were tested in the laboratory at Columbia University (even-numbered isolates). An overlap of 99 isolates was tested at both centers to assess the interlaboratory reproducibility identified in an earlier phase of this study using 98 different strains (12).

Antimicrobials tested. The following drugs were obtained from Sigma (St. Louis, Mo.) or from their respective U.S. manufacturers: amikacin, gentamicin, tobramycin, ticarcillin, ticarcillin-clavulanic acid (2-μg/ml fixed concentration), piperacillin, piperacillin-tazobactam (4-μg/ml fixed concentration), ceftazidime, aztreonam, ciprofloxacin, imipenem, and meropenem. The ranges of concentrations tested by the reference broth microdilution method were 0.06 to 8 μg/ml for ciprofloxacin; 0.25 to 16 μg/ml for meropenem and imipenem; 0.5 to 64 μg/ml for gentamicin, tobramycin, aztreonam, and ceftazidime; and 2 to 256 μg/ml for all of the other drugs. For disk diffusion, concentrations were in accordance with the

TABLE 1. Reference broth microdilution MIC and interpretive category results for 498 isolates of *P. aeruginosa* from CF patients

Antimicrobial(s)[b]	MIC (μg/ml) for:		%[a] in interpretive category:		
	50% of isolates	90% of isolates	S	I	R
Amikacin	32	256	39.3	20.2	40.5
Gentamicin	16	>64	28.6	19.0	52.4
Tobramycin	4	32	63.7	14.1	22.2
Ticarcillin	128	>256	47.0		53.0
Ticarcillin-clavulanate	128	>256	46.2		53.8
Piperacillin	64	>256	51.8		48.2
Piperacillin-tazobactam	64	>256	54.7		45.3
Ceftazidime	16	>64	41.8	8.3	49.9
Aztreonam	32	>64	40.6	7.3	52.1
Ciprofloxacin	2	8	41.4	21.2	37.4
Imipenem	4	>16	50.5	9.7	39.8
Meropenem	2	>16	61.4	11.7	26.9
All[c]			47.2	9.3	43.5

[a] NCCLS (1998) interpretive criteria. S, susceptible; I, intermediate; R, resistant.
[b] Ticarcillin-clavulanate, 2 μg/ml; piperacillin-tazobactam, 4 μg/ml.
[c] Mean values.

National Committee for Clinical Laboratory Standards (NCCLS) recommendations. For Etest, the concentration ranges were 0.002 to 32 μg/ml for ciprofloxacin, meropenem, and imipenem and 0.016 to 256 μg/ml for all of the other agents tested.

Susceptibility test methods. The broth microdilution method (6) was selected as the reference method for this study (12). Drug- and cation-adjusted Mueller-Hinton broth medium-containing trays were custom manufactured by PML Microbiologicals (Wilsonville, Oreg.). Trays were stored at −80°C and allowed to come to room temperature prior to use. Testing was performed in accordance with the recommendations of NCCLS document M7-A4 (6). However, inoculated trays were incubated for 48 h and readings were taken between 18 and 24 h, based on growth in the control well, in accordance with the manufacturer's recommendations, and additionally at 48 h. The MIC endpoint was the lowest concentration of an antimicrobial that completely visibly inhibited the growth in a well.

Disk diffusion susceptibility testing was performed in accordance with the recommendations of NCCLS document M2-A6 (7). Cartridges of antimicrobial-containing disks were obtained from BBL (Becton Dickinson Microbiology Systems, Cockeysville, Md.), stored between 4 and −20°C, and allowed to come to room temperature prior to use. Mueller-Hinton plates (PML Microbiologicals) were incubated for a total of 48 h after inoculation with organisms and placement of disks. Zones of inhibition were measured at both 18 to 21 h and again at 48 h.

Etest strips were purchased from AB BIODISK, with the exception of meropenem strips, which were kindly provided by Zeneca Pharmaceuticals (Wilmington, Del.), and strips were used in accordance with the manufacturer's instructions. They were stored at −20°C and brought to room temperature prior to use. Mueller-Hinton agar plates were inoculated as for the disk diffusion method (7); however, in accordance with the verbal recommendation of the manufacturer, the inocula of mucoid isolates were equivalent to 1.0 and were 0.5 McFarland standards for nonmucoid isolates. Six strips were applied radially to the surface of each Mueller-Hinton plate, and plates were incubated for 48 h with readings taken both between 18 and 21 h, in accordance with the recommendations of the manufacturer, and at 48 h.

Interpretive criteria for susceptibility for all of the test methods were in accordance with NCCLS document M100-S9 (8). Results from all of the methods were validated using American Type Culture Collection quality control strains *P. aeruginosa* ATCC 27853 and ATCC 35218 and *Escherichia coli* ATCC 25922.

Retesting of isolates. In the cases in which serious categorical interpretation errors were identified, retesting of the isolates was performed to determine reproducible error. Interpretation errors were classified based on their impact on clinical management. Serious errors included very major errors, defined as susceptible by the test method and resistant by the reference test, and major errors, defined as resistant by the test method and susceptible by the reference method. Minor errors were defined as an intermediate result for either the test or the reference method. For the penicillins, where there is no intermediate category, all errors were either very major or major. All strains with single- or multiple-drug serious errors at the 18- to 21-h incubation were retested in triplicate using the test methodology and the reference broth microdilution test at a third reference laboratory (University of Iowa College of Medicine) by a single experienced technologist. Only retest results are shown in the tables.

Statistical analysis. MICs were converted to a $\log_2 + 9$ values to permit appropriate comparison with each other and with disk diffusion results (millimeters) and entered into the Statistical Analysis System statistical package (SAS Institute, Inc., Cary, N.C.). Pearson correlation coefficients (r values), regression slope equations, and categorical error rates were generated for each antimicrobial agent indexed by susceptibility test method and by mucoid phenotype. A perfect statistical correlation result is 1.00 for the correlation coefficient and/or the slope, and ≥0.90 (r value) is considered desirable and ≥0.80 (r value) is considered an acceptable result.

Limitations of the statistical analysis included exclusion of strains due to insufficient growth for certain test methods (broth microdilution, 3 strains; Etest, 21 strains; disk diffusion, 20 strains), an increased correlation with increased sample size, and inclusion of differing off-scale results (≤ or > values for MIC tests and 6-mm results for the disk diffusion test) from certain antimicrobials in the analysis, the latter usually resulting in a slightly stronger correlation. Off-scale Etest results occurred at the upper end of the tested ranges, while off-scale broth microdilution results occurred at the lower and upper ends of the tested ranges.

RESULTS

Strain characteristics. The strains tested were highly resistant *P. aeruginosa* isolates. The interpretive category results of the CF isolates tested by the reference broth microdilution method are listed in Table 1. Overall, only half (47.2%) of the results were in the susceptible range. Tobramycin and meropenem were the most active agents (63.7 and 61.4% susceptible, respectively), while gentamicin (28.6% susceptible) was the least active. MICs clustered near the respective breakpoints accounted for many nonsusceptible results, especially for the penicillins and β-lactamase inhibitor combinations, which have no intermediate interpretive category (6, 7, 8).

A separate analysis was performed for the MIC and susceptibility category of the 130 mucoid isolates compared with the 368 nonmucoid isolates (Table 2). For every antimicrobial agent tested, the mucoid isolates were less resistant than the nonmucoid isolates. The range for the mucoid isolates was 10.9% resistant to tobramycin to 39.1% resistant to ticarcillin-clavulanate; for the nonmucoid isolates, it was 26.1% resistant to tobramycin to 59.1% resistant to aztreonam (overall means, 27.1% resistant for mucoid isolates and 49.2% resistant for nonmucoid isolates).

Interlaboratory variation. The best comparability between the two laboratories performing initial susceptibility testing was for the broth microdilution method, where overall 88.4% of the results for all of the agents were within 1 $\log_2$ dilution and 96.1% were within 2 dilutions (Table 3). The most consistent results were obtained with ciprofloxacin (98.0 and 100% within 1 and 2 $\log_2$ dilutions, respectively); the least consistent were obtained with piperacillin-tazobactam (81.8 and 89.9%,

TABLE 2. Reference broth microdilution MIC_{50}, MIC_{90}, and percent resistant comparing 130 mucoid and 368 nonmucoid isolates of *P. aeruginosa* from CF patients

Antimicrobial(s)[a]	MIC_{50} (μg/ml)		MIC_{90} (μg/ml)		% Resistant	
	Mucoid	Non-mucoid	Mucoid	Non-mucoid	Mucoid	Non-mucoid
Amikacin	16	32	128	256	28.9	44.6
Gentamicin	8	16	32	>64	35.9	58.2
Tobramycin	2	4	16	32	10.9	26.1
Ticarcillin	64	128	>256	>256	37.5	58.4
Ticarcillin-clavulanate	32	256	>256	>256	39.1	59.0
Piperacillin	16	128	>256	>256	29.7	54.6
Piperacillin-tazobactam	16	128	>256	>256	27.3	51.5
Ceftazidime	8	64	>64	>64	28.1	57.5
Aztreonam	8	64	>64	>64	32.0	59.1
Ciprofloxacin	1	2	4	8	21.9	42.8
Imipenem	2	8	>16	>16	21.9	46.0
Meropenem	1	4	16	>16	11.7	32.2
All[b]					27.1	49.2

[a] Ticarcillin-clavulanate, 2 μg/ml; piperacillin-tazobactam, 4 μg/ml.
[b] Mean values.

TABLE 3. First-run interlaboratory reproducibility results for 99 isolates of *P. aeruginosa* from CF patients

Antimicrobial(s)[a]	% with broth microdilution MIC of:		% with disk zone size of:		% with Etest MIC of:	
	±1 $\log_2$ dilution	±2 $\log_2$ dilutions	±3 mm	±6 mm	±1 $\log_2$ dilution	±2 $\log_2$ dilutions
Amikacin	92.9	99.0	84.0	97.9	89.6	99.0
Gentamicin	93.9	99.0	80.9	96.8	82.1	92.6
Tobramycin	90.9	100.0	70.2	97.9	91.3	97.8
Ticarcillin	84.8	96.0	71.7	85.9	84.6	91.2
Ticarcillin-clavulanate	86.9	93.9	76.3	87.1	86.8	90.1
Piperacillin	85.9	91.9	69.6	79.3	84.8	89.1
Piperacillin-tazobactam	81.8	89.9	61.3	80.6	80.9	89.4
Ceftazidime	85.9	94.9	69.9	84.9	80.0	87.4
Aztreonam	88.9	94.9	68.8	90.3	82.8	89.7
Ciprofloxacin	98.0	100.0	66.3	92.5	71.0	89.9
Imipenem	85.9	96.0	77.4	89.2	87.6	93.3
Meropenem	84.8	98.0	69.7	88.8	77.3	92.4
All[b]	88.4	96.1	72.2	89.3	83.2	91.8

[a] Ticarcillin-clavulanate, 2 μg/ml; piperacillin-tazobactam, 4 μg/ml.
[b] Mean values.

TABLE 4. Disk diffusion results compared with reference broth microdilution MICs for 597 isolates of *P. aeruginosa* from CF patients

Antimicrobial(s)[a]	No. (%) of errors				No. of isolates tested	Correlation coefficient (*r*)
	Very major	Major	Minor	Total		
Amikacin	0	8 (1.4)	122 (21.1)	130 (22.5)	577	−0.89
Gentamicin	1 (0.2)	0	121 (21.0)	122 (21.1)	577	−0.88
Tobramycin	0	1 (0.2)	87 (15.1)	88 (15.3)	577	−0.89
Ticarcillin	4 (0.8)	24 (4.2)		28 (4.9)	577	−0.88
Ticarcillin-clavulanate	1 (0.2)	23 (4.0)		24 (4.2)	576	−0.87
Piperacillin	6 (1.0)	10 (1.7)		16 (2.8)	577	−0.90
Piperacillin-tazobactam	4 (0.8)	6 (1.0)		11 (1.9)	576	−0.90
Ceftazidime	6 (1.3)	1 (0.2)	74 (12.8)	82 (14.2)	576	−0.91
Aztreonam	1 (0.2)	4 (0.7)	102 (17.7)	107 (18.6)	576	−0.90
Ciprofloxacin	0	0	135 (23.4)	135 (23.4)	576	−0.90
Imipenem	1 (0.2)	0	72 (12.5)	73 (12.7)	576	−0.90
Meropenem	2 (0.4)	1 (0.2)	69 (12.0)	73 (12.7)	576	−0.87
All[b]	25 (0.4)	78 (1.1)	782 (11.3)	889 (12.9)	6,917	−0.89

[a] Ticarcillin-clavulanate, 2 μg/ml; piperacillin-tazobactam, 4 μg/ml.
[b] Mean values.

respectively). The disk diffusion test had the least comparable results, with overall 72.2 and 89.3% of results with zones within ±3 and 6 mm, respectively (zone equivalents of ±1 or 2 $\log_2$ dilutions). For this methodology, amikacin results were the least variable (84.4 and 97.9%, respectively) and piperacillin-tazobactam results were the most variable (61.3 and 80.6%). Overall, 83.2 and 91.8% of the Etest results were within 1 and 2 $\log_2$ dilutions, respectively.

Disk diffusion testing. The disk diffusion method results were compared with broth microdilution MICs to examine the frequency of interpretation errors, the correlation coefficient for each tested drug, and the variation of mucoid versus nonmucoid isolates. The analysis of first-run results (data not shown) had an overall 5.5% rate of very major errors, with 6 of 12 agents having unacceptable (>5%) very major errors. When first-run results from incubation for 48 h were compared with those from the standard (18- to 21-h) incubation, there was no clear-cut advantage of one time point over the other.

Those strain-drug combinations with serious errors at the 18- to 21-h incubation times were retested. The frequencies of reproducible interpretation errors for disk diffusion compared with the reference broth microdilution method are listed in Table 4. The highest rates of very major errors were for ceftazidime (1.3%) and piperacillin (1.0%). The rate of very major errors was only minimally influenced by the mucoid phenotype (Table 5); the highest percentage of very major errors for mucoid isolates was also for ceftazidime (1.7%). Overall, the major error rate averaged 1.6% for mucoid isolates and 1.0% for nonmucoid isolates, but for ticarcillin and ticarcillin-clavulanate, the serious error rate exceeded 5% for mucoid isolates (5.5 and 6.2%, respectively).

Upon reference laboratory retesting, the correlation of the disk diffusion method with the broth microdilution method was excellent (≥0.90) for 6 of the 12 drugs tested and acceptable ($r \geq 0.80$) for all (Table 4). The best correlation was for ceftazidime (0.91); meropenem and ticarcillin-clavulanate had the poorest correlation (0.87). The effect of the mucoid phenotype on correlation coefficients was significant. Three drugs, meropenem (0.73), piperacillin (0.79), and piperacillin-tazobactam (0.79), had values in the unacceptable range for mucoid isolates (Table 5).

Etest MIC determination. The Etest method for MIC determination was compared with reference broth microdilution values to examine the frequency of category interpretive errors, the correlation coefficients, and the slope for each drug tested. First-run results (data not shown) for tests incubated for 18 to 21 h had an overall rate of serious errors (very major plus major errors) of 7.1%, with one drug (aztreonam) having a very major error rate of 8.2%. When these results were compared with the results following 48-h incubation, again, no clear-cut advantage was identified for either time point.

All strains with single- or multiple-drug serious errors seen following 18 to 21 h of incubation were reexamined by both methods. After retesting, the overall frequencies of very major and major interpretation errors for Etest results were very similar to those for the disk diffusion method (Table 6). However, different antimicrobials were modestly problematic for the two assays. All agents had low rates of very major errors (0.1% overall) and major errors (2.2% overall). However, ticarcillin had a rate of serious errors of 6.6% while piperacillin,

TABLE 5. Disk diffusion results compared with reference broth microdilution MICs for 160 mucoid isolates of *P. aeruginosa* from CF patients

Antimicrobial(s)[a]	No. (%) of errors				No. of isolates tested	Correlation coefficient (*r*)
	Very major	Major	Minor	Total		
Amikacin	0	1 (0.7)	33 (22.6)	34 (23.3)	146	−0.90
Gentamicin	0	0	38 (26.0)	38 (26.0)	146	−0.87
Tobramycin	0	1 (0.7)	18 (12.3)	19 (13.0)	146	−0.85
Ticarcillin	1 (0.7)	7 (4.8)		8 (5.5)	146	−0.88
Ticarcillin-clavulanate	0	9 (6.2)		9 (6.2)	146	−0.89
Piperacillin	1 (0.7)	5 (3.4)		6 (4.1)	146	−0.79
Piperacillin-tazobactam	0	3 (2.1)		3 (2.1)	146	−0.79
Ceftazidime	2 (1.7)	1 (0.7)	27 (18.5)	30 (20.5)	146	−0.80
Aztreonam	0	1 (0.7)	43 (29.5)	44 (30.1)	146	−0.81
Ciprofloxacin	0	0	34 (23.3)	34 (23.3)	146	−0.81
Imipenem	0	0	14 (9.6)	14 (9.6)	146	−0.82
Meropenem	1 (0.7)	0	17 (11.6)	18 (12.3)	146	−0.73
All[b]	5 (0.3)	28 (1.6)	224 (12.8)	257 (14.7)	1,752	−0.83

[a] Ticarcillin-clavulanate, 2 μg/ml; piperacillin-tazobactam, 4 μg/ml.
[b] Mean values.

TABLE 6. Etest MICs compared with reference broth microdilution MICs for 597 isolates of *P. aeruginosa* from CF patients

Antimicrobial(s)[a]	No. (%) of errors				No. of isolates tested	Correlation coefficient (*r*)	Regression slope
	Very major	Major	Minor	Total			
Amikacin	0	1 (0.2)	96 (16.6)	97 (16.8)	579	0.91	0.74
Gentamicin	0	3 (0.5)	159 (27.5)	162 (28.0)	578	0.89	0.74
Tobramycin	0	1 (0.2)	96 (16.8)	97 (17.0)	571	0.89	0.87
Ticarcillin	1 (0.2)	36 (6.4)		37 (6.5)	566	0.87	0.72
Ticarcillin-clavulanate	0	28 (4.9)		28 (4.9)	569	0.85	0.71
Piperacillin	1 (0.2)	24 (4.2)		25 (4.4)	574	0.89	0.76
Piperacillin-tazobactam	2 (0.4)	24 (4.2)		27 (4.7)	470	0.88	0.75
Ceftazidime	2 (0.3)	3 (0.5)	70 (12.2)	75 (13.1)	572	0.91	0.64
Aztreonam	2 (0.4)	6 (1.1)	63 (11.5)	71 (13.0)	546	0.89	0.64
Ciprofloxacin	0	5 (1.1)	117 (24.7)	122 (25.7)	574	0.86	0.59
Imipenem	0	4 (0.7)	63 (11.4)	67 (12.1)	553	0.91	0.68
Meropenem	0	14 (3.0)	84 (17.8)	98 (20.7)	473	0.90	0.68
All[b]	8 (0.1)	149 (2.2)	748 (11.3)	906 (13.7)	6,625	0.89	0.71

[a] Ticarcillin-clavulanate, 2 μg/ml; piperacillin-tazobactam, 4 μg/ml.
[b] Mean values.

piperacillin-tazobactam, and ticarcillin-clavulanate all had serious error rates of close to 5% (4.4 to 4.9%). These same four agents were more problematic for mucoid isolates (Table 7), although overall serious error rates were comparable between nonmucoid and mucoid isolates. Serious errors were identified for 9.5% of mucoid isolates for ticarcillin, 7.0% for ticarcillin-clavulanate, 6.3% for piperacillin, and 4.9% for piperacillin-tazobactam. No minor errors were possible because of the lack of interpretive criteria (8).

The correlation of the Etest and broth microdilution results was high (≥0.90) for 4 of the 12 drugs tested and acceptable ($r \geq 0.80$) for all (Table 6). The best correlation was for amikacin, ceftazidime, and imipenem (0.91); the worst was for ticarcillin-clavulanate (0.85). The correlation for mucoid isolates was slightly lower than for nonmucoid isolates (0.85 versus 0.89, overall) but was still acceptable for all of the agents tested. The slope of the correlation (0.71, overall) was adversely influenced by the comparative MIC ranges tested by the two methods.

DISCUSSION

This is the most comprehensive study to date to systematically analyze different agar diffusion susceptibility testing methods for *P. aeruginosa* isolates from patients with CF. Half of the isolates used in this study were selected to be highly resistant, and for a high proportion of them the MICs clustered near the breakpoint concentrations for susceptibility and resistance. In addition, more than one-fourth of the isolates were mucoid. Thus, it was anticipated that such strains would be problematic for the performance of accurate susceptibility testing. In this study, both the disk diffusion method and the Etest performed well for CF isolates, although somewhat better for nonmucoid than for mucoid strains. It had been hoped that more prolonged incubation (up to 48 h) would improve the accuracy of the results for slow-growing strains, but this was not seen.

Inaccurate antimicrobial susceptibility testing for *P. aeruginosa* isolates from CF patients may have an adverse clinical impact. Many patients with advanced disease are infected with highly drug-resistant strains, and there are few therapeutic options (11). Timely and accurate performance of susceptibility testing may make a significant difference in the therapeutic management of acute pulmonary exacerbation. In addition, laboratories using different techniques may identify different patterns of susceptibility, potentially influencing eligibility for lung transplantation. Finally, reporting of a multidrug-resistant *P. aeruginosa* strain can affect hospital infection control and isolation policies. A standardized methodology for antimicrobial susceptibility testing is also highly desirable for tracking of resistance patterns in the CF population, especially as new

TABLE 7. Etest MICs compared with reference broth microdilution MICs for 160 mucoid isolates of *P. aeruginosa* from CF patients

Antimicrobial(s)[a]	No. (%) of errors				No. of isolates tested	Correlation coefficient (*r*)	Regression slope
	Very major	Major	Minor	Total			
Amikacin	0	0	28 (18.8)	28 (18.8)	149	0.88	0.80
Gentamicin	0	0	52 (35.1)	52 (35.1)	148	0.86	0.81
Tobramycin	0	1 (0.7)	21 (14.6)	22 (15.3)	144	0.86	1.09
Ticarcillin	1 (0.7)	12 (8.8)		13 (9.5)	137	0.84	0.68
Ticarcillin-clavulanate	0	10 (7.0)		10 (7.0)	142	0.88	0.68
Piperacillin	0	9 (6.3)		9 (6.3)	144	0.80	0.62
Piperacillin-tazobactam	0	7 (4.9)		7 (4.9)	142	0.80	0.64
Ceftazidime	1 (0.7)	1 (0.7)	30 (21.0)	32 (22.4)	143	0.82	0.61
Aztreonam	1 (0.8)	1 (0.8)	21 (16.0)	23 (17.6)	131	0.87	0.64
Ciprofloxacin	0	2 (1.8)	32 (29.1)	34 (30.9)	110	0.84	0.64
Imipenem	0	3 (2.2)	10 (7.3)	13 (9.5)	137	0.88	0.65
Meropenem	0	3 (2.5)	13 (10.7)	16 (13.2)	121	0.81	0.63
All[b]	3 (0.2)	49 (3.0)	207 (12.6)	259 (15.7)	1,648	0.85	0.71

[a] Ticarcillin-clavulanate, 2 μg/ml; piperacillin-tazobactam, 4 μg/ml.
[b] Mean values.

therapeutic agents are developed and traditional antibiotics are delivered to more patients by aerosolization (14).

When the entire population of isolates was examined, both agar disk diffusion and Etest correlation coefficients compared acceptably to the reference broth microdilution methodology. The organisms tested were all from CF patients and were a highly antibiotic-resistant population which mimics those strains that would be problematic. Twenty-seven percent of the isolates were mucoid, which is about half of the percentage (56.7%) reported in a recent study (1). Both tests had poorer correlations for mucoid isolates, although they were still in an acceptable range overall. For disk diffusion, an unacceptable correlation was seen with mucoid isolates for piperacillin, piperacillin-tazobactam, and meropenem. For the Etest, results for mucoid isolates were all within an acceptable range.

Although the correlation with a reference methodology is desirable, the most important information is whether a method of testing can accurately predict susceptibility and resistance. The rate of very major errors (calling an organism susceptible when it is resistant) was, overall, lower with the Etest. The most problematic antimicrobials for the disk diffusion method were ceftazidime, piperacillin, and piperacillin-tazobactam. Overall, major errors (calling an organisms resistant when it is susceptible) for both methods were in an acceptable range (<10%). Because of the lack of an intermediate category for the penicillins, the number of very major and major errors was enhanced for the four penicillin-containing drugs tested (disk diffusion range, 1.9 to 4.9%; Etest range, 4.4 to 6.6%). For both methodologies, the highest rates of serious errors were seen with mucoid isolates. Retesting of strains with minor errors was not performed and might have demonstrated even better performance by each test. However, elevated minor errors were expected due to the clustering of MICs for the CF isolates near the respective breakpoints of the drugs tested.

An important finding in this study was that, overall and for each antimicrobial tested, mucoid isolates in this collection were more susceptible than nonmucoid isolates. There is some controversy in the literature about whether the mucoid phenotype results in increased resistance. A recent study of inhaled-tobramycin therapy examined the susceptibility of 1,240 CF isolates (710 mucoid, 530 nonmucoid) and found that for all seven of the drugs tested, mucoid isolates were more susceptible (14). Several other studies also suggest that mucoid isolates are more susceptible to ciprofloxacin (2) and to the aminoglycosides (16) than are nonmucoid isolates. Another study found that the MIC for 90% of the isolates tested (MIC_{90}) was higher for mucoid versus nonmucoid isolates for ceftazidime, piperacillin, and amikacin but not different for gentamicin, tobramycin, and imipenem (15). However, the concept of increased resistance in mucoid isolates is prevalent in the literature (4) and in clinical microbiology lore.

The results of this study lead to several recommendations regarding susceptibility testing of CF isolates of *P. aeruginosa*. Disk diffusion and Etest performed acceptably compared to the reference broth microdilution methodology, although both methods performed better for nonmucoid than for mucoid isolates. While we tested only CF isolates, these results might also apply to multiply resistant strains of *P. aeruginosa* from non-CF patients. Questions that remain with regard to the performance of susceptibility testing of CF isolates include the applicability of the results for *P. aeruginosa* to other nonfermenting gram-negative bacilli (i.e., *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, and *Achromobacter xylosoxidans*) and what other modalities might improve our ability to test mucoid isolates. Regardless, these two assessed methods were observed to be acceptable for use as routine, practical methods for testing of CF isolates of *P. aeruginosa*.

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