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Antimicrobial Susceptibility Testing of Carbapenems: Multicenter Validity Testing and Accuracy Levels of Five Antimicrobial Test Methods for Detecting Resistance in *Enterobacteriaceae* and *Pseudomonas aeruginosa* Isolates

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From January 1996 to May 1999, Project ICARE (Intensive Care Antimicrobial Resistance Epidemiology) received 448 nonduplicate clinical isolates of *Enterobacteriaceae* and *Pseudomonas aeruginosa* that were reported to be imipenem intermediate or resistant. However, broth microdilution (BMD) confirmatory testing at the Project ICARE central laboratory confirmed this result in only 11 of 123 (8.9%) *Enterobacteriaceae* isolates and 241 of 325 (74.2%) *P. aeruginosa* isolates. To investigate this overdetection of imipenem resistance, we tested 204 selected isolates from the Project ICARE collection plus five imipenem-resistant challenge strains at the Centers for Disease Control and Prevention against imipenem and meropenem by agar dilution, disk diffusion, Etest (AB BIODISK North America, Inc., Piscataway, N.J.), two MicroScan WalkAway conventional panels (Neg MIC Plus 3 and Neg Urine Combo 3) (Dade MicroScan, Inc., West Sacramento, Calif.), and two Vitek cards (GNS-116 containing meropenem and GNS-F7 containing imipenem) (bioMérieux Vitek, Inc., Durham, N.C.). The results of each test method were compared to the results of BMD testing using in-house-prepared panels. Seven imipenem-resistant and five meropenem-resistant isolates of *Enterobacteriaceae* and 43 imipenem-resistant and 21 meropenem-resistant isolates of *P. aeruginosa* were identified by BMD. For *Enterobacteriaceae*, the imipenem and meropenem test methods produced low numbers of very major and major errors. All test systems in the study produced low numbers of very major and major errors when *P. aeruginosa* was tested against imipenem and meropenem, except for Vitek testing (major error rate for imipenem, 20%). Further testing conducted in 11 of the participating ICARE hospital laboratories failed to pinpoint the factors responsible for the initial overdetection of imipenem resistance. However, this study demonstrated that carbapenem testing difficulties do exist and that laboratories should consider using a second, independent antimicrobial susceptibility testing method to validate carbapenem-intermediate and -resistant results.

Project ICARE is a multicenter study that conducts laboratory-based surveillance for antimicrobial resistance and antimicrobial use at U.S. hospitals participating in the National Nosocomial Infections Surveillance System (1, 10). From January 1996 to May 1999, participating laboratories were asked to send selected antimicrobial-resistant clinical isolates, including imipenem-resistant *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolates, to the Project ICARE central laboratory for confirmatory identification and antimicrobial susceptibility testing. Imipenem-resistant isolates of *Enterobacteriaceae* and *P. aeruginosa* are relatively unusual in the United States, although researchers from several countries report that such strains are increasing in prevalence (11, 18, 25, 28, 29).

Previous studies (5, 12, 17, 23, 35), including a proficiency testing survey of the hospital laboratories participating in Project ICARE (30), have documented imipenem testing problems. To assess the accuracy and validity of carbapenem testing, we tested isolates received from ICARE laboratories by broth microdilution (BMD) in the central ICARE laboratory. For validity testing, the BMD results were then compared with the imipenem susceptibility testing results from the participating hospital laboratories. In addition, a challenge set of 209 isolates was tested at the Centers for Disease Control and Prevention (CDC) by agar dilution, disk diffusion, Etest (AB BIODISK North America, Inc., Piscataway, N.J.), MicroScan WalkAway (Dade MicroScan, Inc., West Sacramento, Calif.), and Vitek (bioMérieux Vitek, Inc., Durham, N.C.) test methods. The results were compared to CDC BMD results to assess the accuracy of the methods most commonly used in U.S. microbiology laboratories.

MATERIALS AND METHODS

Validity testing. One hundred twenty-three nonduplicate isolates of *Enterobacteriaceae* and 325 nonduplicate *P. aeruginosa* isolates collected between January 1996 and May 1999 from 44 hospitals in 24 U.S. states were tested in the Project ICARE central laboratory to confirm the hospital laboratories' imipenem test results (Table 1). All of the isolates were initially reported by the hospital laboratories as imipenem intermediate or resistant. Thirty percent of the *Enterobacteriaceae* isolates sent were *Proteus mirabilis* isolates (Table 1). The Project ICARE protocol did not specify how, or for how long, the isolates were to be

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TABLE 1. *Enterobacteriaceae* isolates reported to Project ICARE as intermediate or resistant to imipenem

Organism[a,b]	No. reported as intermediate or resistant[c]	No. intermediate or resistant by Project ICARE testing[d]	Category agreement	
			No. of major errors	No. of minor errors
Citrobacter freundii complex	1		1	
Citrobacter werkmanii	1			1
Enterobacter aerogenes	7	1	4	3
Enterobacter amnigenus	1	1		1
Enterobacter cloacae	3		2	1
Enterobacter taylorae	1		1	
Escherichia coli	13		13	
Hafnia alvei	1		1	
Klebsiella oxytoca	2	1	1	
Klebsiella pneumoniae	8	2	5	1
Morganella morganii	14		6	8
Proteus mirabilis	37	4	21	14
Proteus penneri	1		1	
Proteus vulgaris	6		5	1
Providencia rettgeri	3		3	
Providencia stuartii	12		8	4
Serratia marcescens	11	2	8	1
Shigella spp.	1		1	
Total	123	11 (8.9%)	81 (65.9%)	35 (28.5%)

[a] If more than one colony type was sent, only the most resistant was included in the analysis.
[b] Identification by Project ICARE central laboratory.
[c] Methods used by hospital laboratories to test isolates were MicroScan and Vitek instruments.
[d] Isolates were tested by the Project ICARE central laboratory using BMD.

stored in the hospital laboratory before shipment. Upon receipt in the Project ICARE central laboratory, the isolates were tested immediately or frozen in defibrinated sheep blood (Lampire Biological Laboratories, Pipersville, Pa.) at −70°C. The frozen isolates were subcultured to Trypticase soy agar plates containing 5% defibrinated sheep blood (blood agar plates) (BD BioSciences, Sparks, Md.) a minimum of two times before being tested against imipenem. In the Project ICARE central laboratory, the BMD reference method was performed using NCCLS procedures (19). BMD panels were prepared in-house (19), using concentrations of imipenem (Merck Research Laboratories, Rahway, N.J.) that ranged from 1 to 32 μg/ml. The panels were stored at −70°C until the day of use. BMD panels were inoculated using MIC-2000 disposable inoculators (Dynex Technologies, Inc., Chantilly, Va.). Quality control strains used for BMD testing included *P. aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, and *Escherichia coli* ATCC 25922. Purity check plates were performed on all isolates tested by BMD.

The identifications of the *Enterobacteriaceae* isolates provided by participating laboratories were confirmed by colony morphology, spot tests (9), and Vitek GNI+ cards. Disagreements were resolved using reference biochemical tests (8). The identifications of *P. aeruginosa* isolates were confirmed by growth at 42°C and pigment production (14). All data analysis was performed using the SAS System for Windows, release 6.12 (SAS Institute, Cary, N.C.).

Analysis of validity testing. If an isolate contained colonies with different morphologies, each morphotype was tested, and the most resistant type was included in the analysis. BMD MICs determined by Project ICARE (reference) and hospital laboratory MICs from MicroScan, Pasco (BD BioSciences), Sensititre (Trek Diagnostics, Westlake, Ohio), or Vitek automated test systems, or zone sizes from disk diffusion testing, were converted into NCCLS category interpretations (21) and compared. An MIC of 8 μg/ml or a zone size of 14 to 15 mm around a 10-μg imipenem disk for an isolate was considered imipenem intermediate, and MICs of ≥16 μg/ml or zone sizes of ≤13 mm were considered imipenem resistant. For category agreement, the number of major errors (where the hospital result was resistant and the BMD reference result was susceptible) and minor errors (where the hospital or reference result was intermediate and the other result was susceptible or resistant) were calculated. Since Project ICARE did not collect imipenem-susceptible strains, the number of very major errors (where the hospital result was susceptible and the reference result was resistant) could not be determined.

Accuracy of test methods. Ninety *Enterobacteriaceae* (18 species among nine genera) and 114 *P. aeruginosa* isolates from 34 hospitals participating in Project ICARE and 5 imipenem-intermediate or -resistant *Enterobacteriaceae* isolates from UCLA Medical Center (two *Citrobacter freundii*, two *Enterobacter cloacae*, and one *Serratia marcescens* isolates), provided by J. Hindler, were tested against imipenem and meropenem at CDC using agar dilution, BMD, disk diffusion, Etest, MicroScan WalkAway conventional panels, and Vitek cards. Project ICARE isolates included 185 organisms sent as imipenem intermediate or resistant and 19 sent as resistant to quinolones or extended-spectrum cephalosporins by the participating hospital laboratories. The latter isolates were screened as potential sources of unrecognized imipenem-resistant strains.

Agar dilution plates were prepared fresh each test day with Mueller-Hinton II powder (BD BioSciences), using solutions of imipenem (Merck Research Laboratories) and meropenem (Zeneca Pharmaceuticals, Wilmington, Del.). The antimicrobial agents had been prepared at 10 times the testing concentrations and were stored at −70°C until they were used. For BMD, panels containing imipenem and meropenem were prepared in-house (19) and stored at −70°C until the day of use. The range of concentrations tested by agar dilution and BMD for both antimicrobial agents was 0.125 to 64 μg/ml.

One commercially prepared 150-mm-diameter Mueller-Hinton II agar plate (BD BioSciences) was inoculated per organism for the disk diffusion and Etest methods. Disks containing 10 μg of imipenem and 10 μg of meropenem (BD BioSciences) and Etest strips (AB BIODISK North America, Inc.) containing imipenem or meropenem (tested MIC range, 0.002 to 32 μg/ml) were placed on each Mueller-Hinton plate. The MicroScan conventional panels were the Neg MIC Plus 3 (MIC) (MIC range, 0.5 to 16 μg/ml for imipenem and 1 to 8 μg/ml for meropenem) and the Neg Urine Combo 3 (Combo) (MIC range, 4 to 8 μg/ml for both imipenem and meropenem). The cards tested on the Vitek instrument included GNS-F7 (imipenem MIC range, 4 to 8 μg/ml) and GNS-116 (meropenem MIC range, 2 to 8 μg/ml).

The agar dilution, BMD, and disk diffusion methods were performed using NCCLS procedures (19, 20). BMD panels were inoculated using MIC-2000 disposable inoculators. Etest, MicroScan, and Vitek testing was performed following the manufacturers' instructions. MicroScan (DMS version 22 software and Vitek R05.03 software were used during the study. In addition to the WalkAway automated reading, manual readings were performed on all MicroScan panels. Quality control strains included *P. aeruginosa* ATCC 27853 (all methods), *E. faecalis* ATCC 29212 (BMD, agar dilution, and Etest), and *E. coli* ATCC 25922 (disk diffusion, MicroScan, and Vitek).

A single colony of each isolate was used to inoculate three blood agar plates. One blood agar plate was used for BMD, disk diffusion, and Etest (all set up

TABLE 2. *P. aeruginosa*: number of errors for imipenem by testing method compared to Project ICARE BMD testing[a]

Hospital testing method	No. of laboratories	Total no. of isolates tested	Category agreement		
			No. of major errors (%)[b]	No. of minor errors (%)[b]	No. correct (%)[b]
Disk diffusion	8	33	5 (15.2)	4 (12.1)	24 (72.7)
MicroScan	22	135	20 (14.8)	40 (29.6)	75 (55.6)
Pasco	1	13	5 (38.5)	3 (23.0)	5 (38.5)
Sensititre	1	4	1 (25.0)		3 (75.0)
Vitek	18	140	35 (25.0)	42 (30.0)	63 (45.0)

[a] If more than one colony type was sent, only the most resistant was included in the analysis.
[b] Percentage is the numerator divided by the total number of isolates tested by that method.

from the same 0.5-McFarland standard suspension), the second blood agar plate was used for Vitek testing, and the third plate was used for MicroScan and agar dilution testing. Purity check plates were performed on all isolates tested from each test system.

BMD was the reference method for this study. All organisms for which very major or major errors were recorded were retested in duplicate by BMD and the test method(s) producing the error.

Analysis of accuracy of test methods. Test method MICs and zone sizes were compared to BMD results directly and by conversion to category interpretations based on NCCLS guidelines (21). Etest MICs that fell between conventional twofold dilutions were rounded up to the next higher twofold dilution before categorization, as described by the manufacturer. The category agreement, or the numbers of very major, major, and minor errors, was calculated using the definitions given above. The denominators used for rate calculations were the number of resistant isolates (very major error rate, when the number of resistant isolates was >20), the number of susceptible isolates (major error rate), and the total number of isolates (minor error rate). These calculations are outlined in the draft document Guidance on Review Criteria for Assessment of Antimicrobial Susceptibility Devices written by the Center for Devices and Radiological Health of the Food and Drug Administration (http://www.fda.gov/cdrh/ode/631.html).

On test systems producing antimicrobial-agent MICs that included five or more testing concentrations of the agent, both overall essential agreement and essential agreement based on evaluable results were calculated. Essential agreement was the number of test method MICs within one doubling dilution of the reference divided by the total number of isolates. Essential agreement based on evaluable results included only on-scale BMD results where the test method MICs could be evaluated as more than one doubling dilution from the BMD result. Acceptable performance for an antimicrobial susceptibility testing device compared to BMD for this study was no very major errors, a <3% major error rate, and >90% overall essential agreement.

Mantel-Haenszel chi-square and Fisher's exact test *P* values were used to determine whether errors were associated with a specific test method (15, 27). The Wilcoxon signed-rank test was performed on the agar dilution versus BMD and the Etest versus BMD MIC distributions to assess any MIC disagreement trends (16). A *P* value of ≤0.05 defined significant associations.

Reproducibility testing. For the accuracy of test methods study, five isolates were tested on each of seven test days by agar dilution and on each of eight test days by all other testing systems to determine reproducibility. For four organisms, the imipenem and meropenem MICs were 4 to 16 μg/ml. The fifth organism was susceptible to both antimicrobial agents.

Proficiency testing. As a follow-up to the Project ICARE validity testing and accuracy of test methods studies, a proficiency testing survey was conducted among 11 Project ICARE laboratories (6 MicroScan users and 5 Vitek users) in eight states. Each laboratory tested four imipenem-susceptible organisms (*Enterobacter aerogenes*, *P. mirabilis*, *Morganella morganii*, and *S. marcescens*) that had been sent to Project ICARE in 1996 as imipenem intermediate or resistant. The participating laboratories were blinded to the reference results of the antimicrobial susceptibility patterns of each organism.

RESULTS

Validity testing. Of the 123 isolates of *Enterobacteriaceae* reported by hospital laboratories as imipenem intermediate or resistant, only 11 (8.9%) were imipenem intermediate (MIC = 8 μg/ml) or resistant (MIC ≥ 16 μg/ml) by the BMD reference method performed in the Project ICARE central laboratory (Table 1). The testing of 81 isolates produced major errors; the errors appeared to be random by species. The imipenem MICs for 65 (52.8%) isolates were ≤1 μg/ml by BMD testing. When analyzed by test method, it was found that major errors were produced by 18 MicroScan users for 70 (76.9%) of 91 isolates and by 10 Vitek users for 12 (37.5%) of 32 isolates tested. Only 4.4 and 9.4% of the results from MicroScan and Vitek, respectively, were concordant with the BMD reference method's interpretive category.

Of 325 isolates of *P. aeruginosa* sent by hospital laboratories as imipenem intermediate or resistant, 241 (74.2%) were imipenem intermediate (MIC = 8 μg/ml) or resistant (MIC ≥ 16 μg/ml) by the BMD reference method. There were 66 and 89 major and minor errors, respectively. By BMD, there were 42 (12.9%) isolates for which the imipenem MICs were ≤1 μg/ml and 165 (50.8%) isolates for which the imipenem MICs were >8 μg/ml. The MicroScan and Vitek instruments produced major errors in 14.8 and 25% of the isolates tested, respectively; other testing methods used for *P. aeruginosa* also produced major errors (Table 2). Compared to BMD, the number of concordant results by testing method used by more than one hospital laboratory ranged from 45.0 (Vitek) to 72.7% (disk diffusion).

Accuracy of test methods. At CDC, 209 isolates were tested against imipenem and meropenem by five different methods. The results were compared to the BMD data. For *Enterobacteriaceae*, the number of very major errors produced by all methods ranged from 0 to 1 for imipenem and from 0 to 2 for meropenem (Table 3). The very major errors for both carbapenems were produced by the testing of four different strains (three species), including *Klebsiella pneumoniae* strain 1534 (36). The testing of another of the four isolates, imipenem-resistant *S. marcescens* strain 525, produced one very major error for meropenem by all testing systems, although manual readings of the MicroScan panels produced a correct MIC result of >8 μg/ml (resistant). The growth in the wells was light but visible. On repeat testing, BMD MICs ranged from 32 to 64 μg/ml, and both MicroScan panel results generated by the WalkAway instrument were >8 μg/ml (no very major errors). However, even on repeat testing, the other five testing systems still produced susceptible MIC results (yielding very major errors).

The major-error rate for both carbapenems ranged from 0 to 2.3% (Table 3). No major errors were produced by agar dilution and Etest. The five major errors for imipenem and mero-

TABLE 3. Number of imipenem and meropenem errors among 95 *Enterobacteriaceae* isolates in the accuracy of test methods study

Parameter	Imipenem[a]				Meropenem[b]			
	Category agreement			% Overall essential agreement (evaluable)[f]	Category agreement			% Overall essential agreement (evaluable)[f]
	No. of very major errors[c]	No. of major errors (%)[d]	No. of minor errors (%)[e]		No. of very major errors[c]	No. of major errors (%)[d]	No. of minor errors (%)[e]	
Agar dilution				91.6 (88.7)				98.9 (71.4)
Initial test	0	0	6 (6.3)		1[g]	0	1 (1.1)	
No. unresolved[h]	0	0			1[g]	0		
Outside ± 1 dilution[i]			2 (2.1)				0	
Disk diffusion				N/A[j]				N/A
Initial test	1	0	5 (5.3)		1[g]	2 (2.3)	4 (4.2)	
No. unresolved	1	0			1[g]	2 (2.3)		
Outside 3 mm[k]			1 (1.1)				0	
Etest				87.4 (84.0)				93.7 (56.3)
Initial Test	0	0	5 (5.3)		1[g]	0	1 (1.1)	
No. unresolved	0	0			1[g]	0		
Outside ± 1 dilution			3 (3.2)				0	
MicroScan Combo				ND[l]				ND
Initial test	0	1 (1.2)	8 (8.4)		2[g]	0	2 (2.1)	
No. unresolved	0	0			1	0		
MicroScan MIC				93.7 (91.4)				ND
Initial test	0	1 (1.2)	8 (8.4)		1[g]	0	3 (3.2)	
No. unresolved	0	0			0	0		
Vitek				ND				ND
Initial test	1	1 (1.2)	5 (5.3)		2[g]	0	4 (4.2)	
No. unresolved	1	1 (1.2)			2[g]	0		

[a] Test results were compared to BMD results. By BMD testing, seven isolates were imipenem resistant and 84 were imipenem susceptible.
[b] Test results were compared to BMD results. By BMD testing, five isolates were meropenem resistant and 88 were meropenem susceptible.
[c] The very major error rate could not be established due to low numbers of carbapenem-resistant isolates.
[d] Major error rate = (number of major errors/total number of carbapenem-susceptible isolates) × 100.
[e] Minor error rate = (number of minor errors/total number of isolates tested) × 100.
[f] Essential agreement = number of test method MICs within one dilution of the reference/total number of results. Only on-scale results are included in the evaluable-result calculation.
[g] One error resulted from testing of the same *S. marcescens* isolate.
[h] Number of very major or major errors persisting upon repeat testing.
[i] Number of minor errors >1 dilution from the BMD MIC.
[j] N/A, not applicable. Essential agreement calculations apply only to testing methods that generate MIC results.
[k] Number of minor errors >3 mm from the categorical disk diffusion breakpoint corresponding to the BMD MIC breakpoint.
[l] ND, not done. Valid essential agreement calculations require that the test panels and cards contain at least five twofold dilutions of the evaluated antimicrobial agent.

penem were produced by the testing of four different strains (four species). Two major errors resolved upon repeat testing (MicroScan MIC and Combo panels). For *Enterobacteriaceae*, the number of minor errors for imipenem and meropenem produced by all testing systems compared to those of BMD ranged from 1 (1.1%) to 8 (8.4%). Minor error rates were higher for imipenem than for meropenem. With the exception of the imipenem results from the Etest method, most of the minor errors by agar dilution, Etest (meropenem), and disk diffusion were less than one doubling dilution from the BMD result or within 3 mm of the appropriate breakpoint. The overall essential agreements for agar dilution, Etest (meropenem), and the MicroScan MIC panel (imipenem) were >90%. Due to limited numbers of dilutions, similar comparisons could not be performed for MicroScan MIC (meropenem) and Combo panels and Vitek card results.

The imipenem and meropenem MICs were the same for BMD and agar dilution for 41 (43.2%) and 86 (90.5%), respectively, of the *Enterobacteriaceae* isolates. By Wilcoxon signed-rank test, the distribution of imipenem agar dilution MICs was significantly lower than the BMD MIC distribution (one-tailed P value < 0.0001); 40 (42.1%) of the MICs were one doubling dilution lower for agar dilution than for BMD. However, this resulted in only three minor errors. The distribution of meropenem MICs was not significantly different in the two methods (one-tailed P value = 0.0767). The Etest and BMD MICs were the same for 44 (46.3%) and 83 (87.4%) of the isolates tested against imipenem and meropenem, respectively. Neither the distribution of imipenem MICs nor that of meropenem MICs was significantly different for Etest and BMD (one-tailed P value = 0.3883 [imipenem]; one-tailed P value = 0.1437 [meropenem]). Due to limited numbers of dilutions, similar comparisons could not be performed for MicroScan and Vitek test results.

For *P. aeruginosa*, the very major error rates for the carbapenems ranged from 0 to 9.5% (Table 4). For one strain tested with meropenem, very major errors were observed with agar dilution, MicroScan MIC, and MicroScan Combo panels. Two

TABLE 4. Number of errors for imipenem and meropenem among 114 *P. aeruginosa* isolates in the accuracy of test systems study

Parameter	Imipenem[a]				Meropenem[b]			
	Category agreement			% Overall essential agreement (evaluable)[f]	Category agreement			% Overall essential agreement (evaluable)[f]
	No. of very major errors (%)[c]	No. of major errors (%)[d]	No. of minor errors (%)[e]		No. of very major errors (%)[c]	No. of major errors (%)[d]	No. of minor errors (%)[e]	
Agar dilution				97.4 (97.4)				93.9 (94.1)
Initial test	0	1 (1.9)	11 (9.7)		2 (9.5)	1 (1.2)	11 (9.7)	
No. unresolved[g]	0	1 (1.9)			1 (4.8)	0		
Outside ± 1 dilution[h]			2 (1.8)				1 (0.9)	
Disk diffusion				N/A[i]				N/A
Initial test	0	2 (3.9)	18 (15.8)		0	6 (7.4)	21 (18.4)	
No. unresolved	0	2 (3.9)			0	5 (6.2)		
Outside 3 mm[j]			2 (1.8)				2 (1.8)	
Etest				64.9 (51.9)				86.0 (83.5)
Initial test	0	2 (3.9)	25 (21.9)		0	4 (4.9)	20 (17.5)	
No. unresolved	0	2 (3.9)			0	2 (2.5)		
Outside ± 1 dilution			9 (7.9)				3 (2.6)	
MicroScan Combo				ND[k]				ND
Initial test	0	1 (1.9)	22 (19.3)		1 (4.8)	0	15 (13.2)	
No. unresolved	0	0			1 (4.8)	0		
MicroScan MIC				95.6 (94.3)				ND
Initial test	1 (2.3)	0	12 (10.5)		1 (4.8)	0	13 (11.4)	
No. unresolved	1 (2.3)	0			1 (4.8)	0		
Vitek				ND				ND
Initial test[l]	0	10 of 50 (20.0)	23 of 111 (20.7)		0	7 of 79 (8.9)	26 of 111 (23.4)	
No. unresolved	0	7 of 49[m] (14.3)			0	6 of 79 (7.6)		

[a] Test results were compared to BMD results. By BMD testing, 43 isolates were imipenem resistant and 52 were imipenem susceptible.
[b] Test results were compared to BMD results. By BMD testing, 21 isolates were meropenem resistant and 81 were meropenem susceptible.
[c] Very major error rate = (number of very major errors/total number of carbapenem-resistant isolates) × 100.
[d] Major error rate = (number of major errors/total number of carbapenem-susceptible isolates) × 100.
[e] Minor error rate = (number of minor errors/total number of isolates tested) × 100.
[f] Essential agreement = number of test method MICs within one dilution of the reference/total number of results. Only on-scale results are included in the evaluable-result calculation.
[g] Number of very major or major errors persisting upon repeat testing.
[h] Number of minor errors >1 dilution from the broth microdilution MIC.
[i] N/A, not applicable. Essential agreement calculations apply only to testing methods that generate MIC results.
[j] Number of minor errors >3 mm from the categorical disk diffusion breakpoint corresponding to the BMD MIC breakpoint.
[k] ND, not done. Valid essential agreement calculations require that the test panels and cards contain at least five twofold dilutions of the evaluated antimicrobial agent.
[l] Three isolates did not grow in the Vitek system in two attempts. Percentages were calculated using numbers of Vitek viable isolates.
[m] One organism that did not grow upon repeat testing was excluded from analysis.

additional very major errors were observed with two unique strains. The major error rates ranged from 0 to 20.0%. Thirty-four major errors (25 of which did not resolve upon repeat testing) were observed for 21 different strains. Three isolates of *P. aeruginosa* did not grow in the Vitek system; another isolate grew initially but failed to grow upon repeat testing. Vitek card GNS-F7 produced more imipenem major errors (20%) than did all other testing systems (Mantel-Haenszel chi-square, 6.411; $P = 0.011$). For each of the 10 major errors, the Vitek imipenem MICs were ≥16 μg/ml and the BMD MICs were either 1 (one isolate), 2 (two isolates), or 4 (seven isolates) μg/ml. Vitek card GNS-116 produced the highest percentage of major errors for meropenem (8.9%) (Fisher's exact test; $P = 0.016$). The major errors for meropenem showed Vitek MICs of ≥16 μg/ml and BMD MICs of 4 μg/ml. The one Vitek major error that resolved demonstrated a Vitek MIC of ≥16 μg/ml and a BMD MIC of 8 μg/ml upon repeat testing (minor error).

For *P. aeruginosa*, the number of minor errors for imipenem and meropenem ranged from 11 of 114 (9.7%) by agar dilution to 26 of 111 (23.4%) by Vitek (Table 4). Many minor errors were noted with imipenem testing by disk diffusion, Etest, MicroScan Combo panels, and Vitek, and they were more common with *P. aeruginosa* than with *Enterobacteriaceae* (Mantel-Haenszel chi-square; $P \leq 0.026$). Similarly, meropenem minor errors were more frequent with *P. aeruginosa* than with *Enterobacteriaceae* isolates by all five test methods (Mantel-Haenszel chi-square; $P \leq 0.026$). For agar dilution, Etest, and disk diffusion, most of the minor errors were within one doubling dilution of the BMD result or within 3 mm of the corresponding categorical disk diffusion breakpoint. However, the overall essential agreements for imipenem and meropenem testing by Etest were 64.9 and 86%, respectively (>90% is considered acceptable by the Food and Drug Administration). Etest categorical errors greater than one doubling dilution from the BMD result included all the major errors (Table 4) but only 12 of the 45 total minor errors.

The MICs for 88 (77.2%) and 66 (57.9%) of the 114 *P. aeruginosa* isolates tested against imipenem and meropenem, respectively, were the same for BMD and agar dilution. By the Wilcoxon signed-rank test, the distribution of imipenem MICs was not significantly different in the two methods (one-tailed *P* value = 0.2000). However, the distribution of meropenem MICs was significantly higher for agar dilution than for BMD (one-tailed *P* value = 0.0026); 31 (27.2%) MICs were one doubling dilution higher for agar dilution than for BMD. In terms of categorical agreement errors, the single major error and 4 of the 11 minor errors produced higher agar dilution MICs (Table 4).

The Etest and BMD MICs were the same for 26 (22.8%) and 40 (35.1%) of the isolates tested against imipenem and meropenem, respectively. The distributions of both imipenem and meropenem MICs were significantly higher for Etest than for BMD (both one-tailed *P* values were <0.0001). For imipenem, 85 (74.6%) of the isolates produced higher MICs by Etest than by BMD; the majority of the Etest MICs were within one doubling dilution (46 isolates, including 15 of 25 minor errors) or two dilutions (32 isolates, including 3 of 25 minor errors) of the BMD MIC result. For meropenem, 57 (50.0%) of the isolates produced higher MICs by Etest than by BMD; most were within one doubling dilution (43 isolates, including 13 of 20 total minor errors) or two dilutions (11 isolates, including 2 of 4 major errors) of the BMD MIC. Twenty-two (88%) of the 25 minor errors for imipenem and 14 (70%) of the 20 minor errors for meropenem had higher Etest MICs than BMD MICs. Of the isolates with Etest MICs greater than one doubling dilution from the BMD result, 93.9 (31 isolates) and 71.4% (10 isolates) had imipenem and meropenem BMD MICs, respectively, of 8 or 16 μg/ml and corresponding Etest MICs of >32 μg/ml.

Reproducibility testing. During the accuracy of test methods study, five isolates (two *P. aeruginosa* and one each of *E. aerogenes*, *K. pneumoniae*, and *M. morganii*) were tested by each method on each of 8 days, except by agar dilution (7 days). In general, the systems performed consistently over all test days, with one- to two-dilution or 1- to 3-mm differences between tests. However, for *E. aerogenes* strain 810 (BMD MICs = 8 [imipenem] and 4 [meropenem] μg/ml) and *K. pneumoniae* strain 1534 (BMD MICs = 16 μg/ml [both imipenem and meropenem]), the Vitek system produced both imipenem and meropenem MICs with three- and four-dilution differences between tests, respectively. For these two isolates, Vitek interpretations were either carbapenem susceptible or resistant, depending on the test day.

Proficiency testing. Of the four imipenem-susceptible *Enterobacteriaceae* isolates sent, all 11 hospital laboratories correctly reported the imipenem-susceptible *E. aerogenes*, *P. mirabilis*, and *S. marcescens* isolates as imipenem susceptible. One MicroScan user reported the imipenem-susceptible *M. morganii* isolate as imipenem resistant (major error).

DISCUSSION

In this investigation, only 8.9% of the *Enterobacteriaceae* isolates and 74.2% of the *P. aeruginosa* isolates reported as imipenem intermediate or resistant by hospital laboratories participating in Project ICARE were confirmed as "nonsusceptible" by BMD testing performed at the Project ICARE central laboratory. Previous proficiency testing surveys have documented carbapenem testing problems (7, 12, 17, 30). However, the extent of the false resistance reported in this study by so many laboratories using a variety of different methods is disturbing, especially for isolates of *Enterobacteriaceae*. For many of the isolates, the imipenem MICs were ≤1 μg/ml by Project ICARE BMD testing but ≥8 μg/ml by hospital laboratory testing. The high number of major errors was not reproducible in the CDC laboratory, even when the same testing systems and the same isolates were used. In the accuracy of test methods study, with the exception of imipenem testing of *P. aeruginosa* by the Vitek system, the five test methods studied produced few very major or major errors. More errors (especially minor errors) occurred with *P. aeruginosa* than with isolates of *Enterobacteriaceae*, and most minor errors were within one doubling dilution of the BMD result (where evaluable) or within 3 mm of the categorical breakpoint. In this study, more isolates of *P. aeruginosa* had MIC test results that clustered around the carbapenem breakpoints than did the isolates of *Enterobacteriaceae*. This could explain the higher percentage of errors observed for *P. aeruginosa*.

The large MIC range tested by agar dilution and Etest allowed more complete comparisons of those data to the results of BMD. The agar dilution imipenem MICs were lower than the corresponding BMD MICs when *Enterobacteriaceae* isolates were tested, and the agar dilution meropenem MICs and Etest MICs for both antimicrobial agents were significantly higher than BMD MICs when *P. aeruginosa* isolates were tested. While the differences between agar dilution and BMD were minimal, the higher Etest MIC distribution (compared to BMD) for *P. aeruginosa* did affect the categorical agreement. All the major errors for imipenem and meropenem and most of the minor errors were produced by the testing of isolates with Etest MICs above the corresponding BMD MIC. The spread of the Etest MICs away from the BMD MICs was reflected in the overall essential agreement scores of <90%.

Due to limited numbers of test dilutions, the MICs of the MicroScan MIC and Combo panels and Vitek cards could not be evaluated much beyond the categorical agreement rates. Meropenem testing of *S. marcescens* strain 525 (H. Yigit, C. D. Steward, J. W. Biddle, and F. C. Tenover, Abstr. 99th Gen. Meet. Am. Soc. Microbiol., abstr. A-61, 1999) and *K. pneumoniae* strain 1534 (36) by MicroScan yielded several very major errors by the automated instrument readings but only minor errors or no errors when the tests were read visually. The reasons for this are unclear but may have to do with the instrument's threshold for identifying growth in the carbapenem wells for these organisms. Both of the organisms contain an enzyme that inactivates carbapenems, in addition to porin changes that make them resistant to carbapenems. Since these organisms were called susceptible by the instrument but were actually resistant, prevention of the few very major errors that occurred in this study would require that clinical laboratories manually read all MicroScan panels, a task that would be too laborious for the small improvement in results.

In the reproducibility portion of the study, Vitek instrument results for *K. pneumoniae* strain 1534 (carbapenem resistant by BMD) and *E. aerogenes* strain 810 (imipenem intermediate and meropenem susceptible by BMD) (37) ranged from car-

bapenem susceptible to resistant. Why the results for these two organisms were so variable is unknown. The other test methods produced remarkably consistent results over all test days. Isolates with carbapenem MICs around the breakpoints may demonstrate varying susceptibilities on retesting because one dilution could change an interpretation (e.g., from susceptible to intermediate).

Since the accuracy of test methods study did not explain the high number of major errors found during validity testing and the subsequent proficiency testing project conducted in 11 of the Project ICARE hospital laboratories failed to pinpoint any factors associated with major errors for imipenem, the observed errors could be due to imipenem degradation in the hospital laboratory test panels (5, 6, 23, 34, 35; R. Grist, Letter, J. Clin. Microbiol. **30:**535-536, 1992); problems with the automated instrument's susceptibility test interpretations (4, 13); improper plate, card, or disk storage conditions (2, 6, 22, 33); or technical errors, such as overinoculation of tests (7, 13). The hospital laboratories were not required to retest resistant isolates before submitting them to the Project ICARE central laboratory. Because errors were observed with a variety of test methods and test panels used by the hospital laboratories, the errors could stem from a combination of the factors listed above.

It is possible that some isolates lost their resistance while in transit to the Project ICARE central laboratory. Bacterial porin channels are in a constant state of flux, and porin changes that contribute to carbapenem resistance have been shown to revert to normal (susceptible) levels in the absence of antimicrobial pressure (24, 26, 37). However, it is unlikely that all isolates reported as imipenem intermediate or resistant by the hospital laboratories but susceptible by the Project ICARE central laboratory were originally imipenem resistant.

Laboratories should be aware that isolates of *Enterobacteriaceae* with decreased susceptibility to carbapenems (MIC $\geq$ 4 μg/ml) are unusual. The carbapenem MICs for most clinical isolates of carbapenem-susceptible *Enterobacteriaceae* are $\leq$1 μg/ml, with a few exceptions, such as *M. morganii* and *Proteus* and *Providencia* spp. (3, 31, 32). In the validity-testing study, 30% of the *Enterobacteriaceae* isolates sent as imipenem intermediate or resistant were *P. mirabilis* isolates, which was not surprising. BMD imipenem MICs for this organism are typically between 1 and 4 μg/ml, which is closer to the imipenem-intermediate breakpoint (8 μg/ml) than are the MICs of most other *Enterobacteriaceae* isolates.

This investigation confirmed seven imipenem-resistant and four imipenem-intermediate isolates of *Enterobacteriaceae* from nine hospitals in seven geographically diverse U.S. states. We remain unable to explain the large number of isolates apparently reported inaccurately as imipenem intermediate or resistant. However, the study did demonstrate that carbapenem testing difficulties do exist and that laboratories should consider using a second, independent antimicrobial susceptibility testing method to validate carbapenem-intermediate and -resistant results.

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