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Tentative Interpretive Criteria for In Vitro Antibacterial Susceptibility Testing with Imipenem

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Imipenem is a member of a new class of highly potent β-lactam antibiotics, carbapenems, with a very broad antibacterial spectrum. This study was undertaken to determine tentative interpretive criteria for in vitro susceptibility testing with 10-μg imipenem disks. A careful examination of the zone diameters and the corresponding MICs for 489 clinical isolates by regression-line analysis and the error rate-bounded classification scheme suggested the following guidelines: ≥16 mm with an MIC correlate of ≤4 μg/ml for susceptible, 14 to 15 mm (8 μg/ml) for moderately susceptible, and ≤13 mm (≥16 μg/ml) for resistant. Lack of cross-resistance between imipenem and broad-spectrum cephalosporins such as cefotaxime and ceftazidime argues against their use as class disks to predict in vitro susceptibility of bacterial species to carbapenems.

Imipenem (*N*-formimidoyl thienamycin) is a member of a new class of potent β-lactam compounds, carbapenems, with an unusually broad antibacterial spectrum (2, 3, 6, 7). In addition to the usual bacterial species inhibited by the acylamino penicillins and the broad-spectrum cephalosporins, imipenem has substantial in vitro activity against *Acinetobacter* species, pseudomonads except *Pseudomonas cepacia* and *Pseudomonas maltophilia*, staphylococci including methicillin-resistant strains of *Staphylococcus aureus*, enterococci except *Streptococcus faecium*, and virtually all commonly isolated and clinically significant anaerobic bacterial species.

The study herein reported was undertaken to define appropriate susceptibility criteria for interpreting the results of agar diffusion tests with 10-μg imipenem disks as a guide to therapy.

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MATERIALS AND METHODS

Antibacterial agents. Antibiotics were supplied as follows: imipenem, Merck Sharp & Dohme Research Laboratories, Rahway, N.J.; cefotaxime, Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.; and ceftazidime, Glaxo Group Research Ltd., Greenford, United Kingdom. Two separate lots of paper disks containing 100 to 135% of the stated potency of 10 μg of imipenem were used: lot 26-15 (Merck) and lot 691054 (Difco Laboratories, Detroit, Mich.).

Bacterial isolates. The 489 test strains representing 25 bacterial species (Table 1) included in this study were selected randomly from a stock of clinical isolates acquired within the past 3 years from a variety of hospitals across the United States and stored at −70°C until needed. These bacterial species are routinely encountered in clinical practice and were included in this investigation for their likelihood of being tested by the disk diffusion method. Before testing, each strain was regrown in a blood-based agar medium to check for purity.

Susceptibility testing. Antibiotics were prepared fresh just before use. Imipenem was solubilized in 0.01 M phosphate buffer (pH 7.2). Solutions of cefotaxime and ceftazidime were prepared in distilled water and saturated aqueous sodium bicarbonate, respectively. Inocula were grown in Mueller-Hinton broth and appropriately adjusted to yield approximately 4×10^5 CFU/ml. MICs were determined in divalent cation-supplemented Mueller-Hinton broth by the microdilution broth method (10), using the MIC-2000 system (Dynatech Laboratories, Inc., Alexandria, Va.). The MIC endpoint was defined as the lowest antibiotic concentration resulting in the absence of visible growth after 18 to 24 h of incubation at 37°C. For testing *Listeria monocytogenes*, Mueller-Hinton broth and agar were supplemented with 5% sheep erythrocytes. Disk susceptibility tests with imipenem were performed by the Bauer-Kirby method as modified by the National Committee for Clinical Laboratory Standards (9). Four 10-μg susceptibility disks, two each from lots 26-15 and 691054, were used with each isolate.

The following quality control strains were included with each daily run: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, and *Streptococcus faecalis* ATCC 29212.

Statistical analysis. The MICs and the corresponding arithmetic means of the zone diameters computed from each set of four zone sizes were subjected to regression-line analysis by the method of least-squares. For comparison purposes, the data were also analyzed by the error rate-bounded method of Metzler and DeHaan (8).

RESULTS AND DISCUSSION

The distribution of the MIC and zone diameter test results obtained from in vitro antimicrobial susceptibility testing of 489 bacterial isolates against imipenem is shown in Table 1. These data clearly confirm the unusually broad antibacterial spectrum and increased potency of this new drug compared with other β-lactam antibiotics. Imipenem is shown to be highly active against members of the family *Enterobacteria-*

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TABLE 1. MICs and corresponding zone diameters of imipenem against 489 bacterial isolates

Bacterial species	Code[a]	No. tested	Zone diameter (mm)		MIC (μg/ml)	
			Range	Mean	Range	MIC_{90}
Acinetobacter calcoaceticus subsp. *anitratus*	A	25	26–35	29	0.125–1.0	0.5
Citrobacter diversus	B	14	27–33	30	0.125–0.25	0.25
Citrobacter freundii	C	21	22–35	26	0.125–1.0	1.0
Enterobacter aerogenes	D	25	20–26	23	0.125–2.0	1.0
Enterobacter cloacae	E	20	23–29	26	0.125–2.0	1.0
Escherichia coli	F	35	27–36	30	0.062–0.25	0.25
Klebsiella oxytoca	G	15	25–30	28	0.125–0.5	0.25
Klebsiella pneumoniae	H	25	25–29	27	0.125–0.5	0.5
Listeria monocytogenes	I	15	37–46	41	0.016–0.062	0.062
Morganella morganii	J	25	20–25	22	1.0–4.0	4.0
Proteus mirabilis	K	25	22–29	25	0.125–8.0	4.0
Proteus vulgaris	L	20	21–27	23	0.25–4.0	4.0
Providencia rettgeri	M	15	22–29	26	0.25–2.0	2.0
Providencia stuartii	N	15	22–27	24	0.25–2.0	2.0
Pseudomonas aeruginosa	O	35	12–37	27	0.25–16.0	2.0
Pseudomonas cepacia	P	6	16–29	19	1.0–8.0	8.0
Pseudomonas fluorescens	Q	10	25–36	31	0.5–4.0	1.0
Pseudomonas maltophilia	R	10	6	6	128.0–>128.0	>128.0
Pseudomonas putida	S	10	30–35	32	0.25–0.5	0.5
Serratia marcescens	T	25	23–28	25	0.25–4.0	4.0
Staphylococcus aureus, methicillin susceptible	U	30	45–51	49	0.008–0.031	0.016
Staphylococcus aureus, methicillin resistant	V	10	17–51	36	0.016–1.0	0.25
Staphylococcus epidermidis	W	13	30–51	46	0.008–32.0	0.031
Staphylococcus saprophyticus	X	15	36–49	45	0.016–0.062	0.062
Streptococcus faecalis	Y	30	11–30	26	0.25–16.0	2.0

[a] See Fig. 2 for distribution of letter codes.

ceae, the nonfermenting gram-negative bacilli (except *P. maltophilia*), and gram-positive cocci and coccobacilli. Mean values of the zone diameters for the various test isolates ranged from 6 mm (no zone) for *P. maltophilia* (MIC_{90} >128 μg/ml; MIC_{90}, MIC for 90% of strains tested) to 49 mm for methicillin-susceptible strains of *S. aureus* (MIC_{90}, 0.016 μg/ml). The vast majority of the test isolates had MICs of ≤2 μg/ml. The geometric mean MIC_{90}s of imipenem, in relation to achievable levels of imipenem in plasma based on commonly used dosage rates, against the most frequently isolated bacterial pathogens, taken from recent reviews of worldwide in vitro susceptibility studies (2, 7), are shown in Fig. 1. From the cumulative distribution of in vitro susceptibilities reported in the literature on 30,655 isolates, excluding those of uniformly resistant species (*P. maltophilia*, *P. cepacia, and S. faecium*), 95 and 98% were found susceptible to imipenem at ≤4 and ≤8 μg/ml, respectively (7).

For maximum recovery from urine, imipenem must be coadministered with cilastatin (MK-791), a reversible inhib-

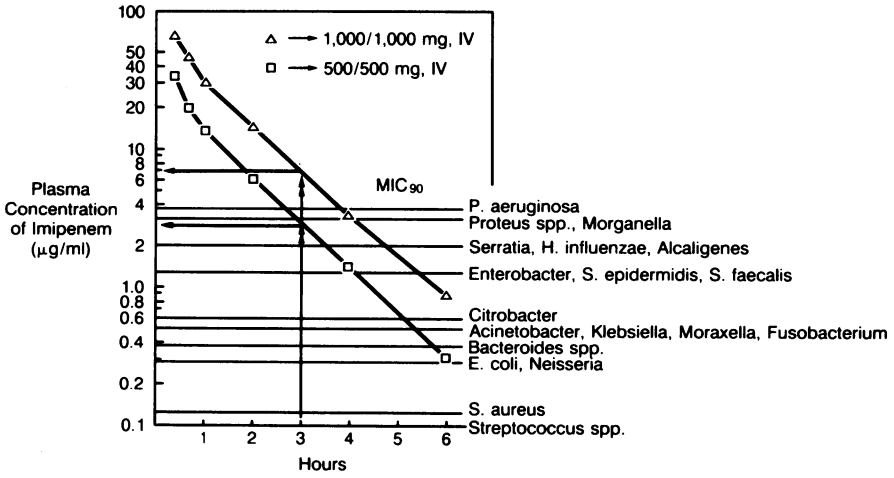

FIG. 1. Mean concentrations in plasma of imipenem administered intravenously in doses of 500 (□) and 1,000 mg (△), combined with equal doses of cilastatin (12; data on file at Merck). Also shown are MIC_{90}s of imipenem against clinically significant gram-positive and gram-negative aerobic and anaerobic bacterial species (2, 7). The pointed lines were used to determine the MIC breakpoint.

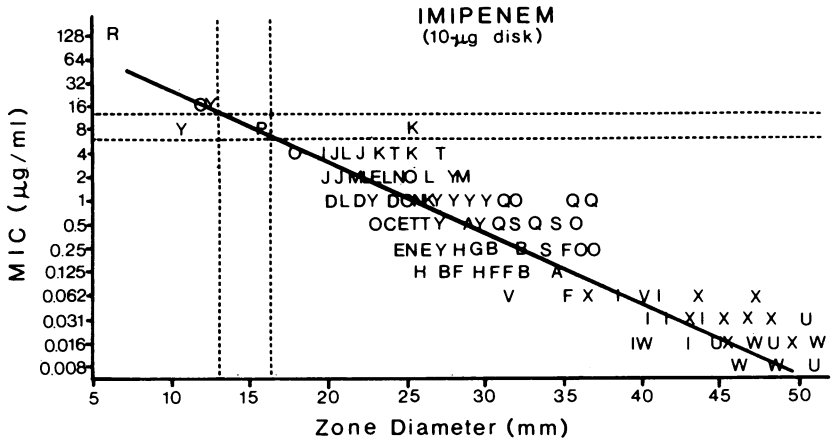

FIG. 2. Scattergram resulting from a regression-line analysis of inhibitory zone diameters and the corresponding MICs of imipenem against 489 bacterial isolates. Broken horizontal lines show the proposed susceptible and resistant MIC breakpoints of ≤4 and ≥16 μg/ml, respectively. Broken vertical lines represent the corresponding susceptible and resistant zone diameter breakpoints of ≥16 and ≤13 mm, respectively. Right broken vertical line also denotes the susceptible breakpoint as determined by the error rate-bounded method. Regression line, $y = 6.42 - 0.265x$; correlation coefficient (r), 0.896. Explanations of letter codes are given in Table 1.

itor of dehydropeptidase-1, in a 1 to 1 ratio (4, 12). Inhibition of this mammalian renal β-lactamase located at the brush border of the proximal tubular cells protects imipenem from extensive enzymatic degradation. Cilastatin is devoid of any antibacterial activity of its own and does not interfere with the intrinsic activity of imipenem. Available human pharmacokinetic data show that imipenem levels in plasma of >32 and >64 μg/ml can be achieved after intravenous administration of single doses of imipenem and cilastatin of 500 and 500 mg and 1,000 and 1,000 mg, respectively (2, 12). With a plasma half-life of approximately 1 h, mean levels in serum of 3.5 μg/ml (500-mg dose) and 7 μg/ml (1,000-mg dose) can be detected 3 h after administration (Fig. 1.).

There is no uniform agreement concerning the minimal ratio between levels in blood and MICs or on how long the level in plasma should exceed the MIC to ensure therapeutic efficacy. However, it is generally believed that with the normally recommended dose, the MIC breakpoint of an antibacterial agent should exceed or equal the expected mean level in serum for at least half the dosage interval (5, 11). Based on these considerations and on a proposed four-or three-times-daily dosage regimen for imipenem, 4 and 16 μg/ml appear to be the most logical choices for susceptible and resistant MIC breakpoints, respectively.

Both the computer-assisted scattergram of MICs versus zone sizes and the related regression-line plot are shown in Fig. 2. From the regression curve alone, corresponding zone diameter breakpoints of ≥16 mm (≤4 μg/ml) for susceptible and ≤13 mm (≥16 μg/ml) for resistant are suggested. Consideration of the error rate-bounded classification scheme (Table 2) also seems to favor ≥16 and ≤13 mm as the susceptible and resistant zone diameter breakpoints, respectively. With ≥16 mm as the susceptible zone diameter breakpoint, only three isolates, *Proteus mirabilis* (25 mm, 8 μg/ml), *P. cepacia* (17 mm, 8 μg/ml), and *P. cepacia* (16 mm, 8 μg/ml) would be classified as susceptible by the disk test and moderately susceptible by the dilution test (0.6% minor error). The ≤13 mm zone diameter breakpoint would yield a 0% false-resistant error rate (major error) (Table 2). One isolate of *S. faecalis* (11 mm, 8 μg/ml) would be classified as moderately susceptible by the dilution test and resistant by the diffusion test (0.2% minor error). With this scheme, there would be a total of 0.8% minor errors and 0% for both major and very major errors.

A summary of the proposed interpretive criteria for in vitro susceptibility testing of imipenem and the statistical distribution of the 489 test strains on the basis of the

TABLE 2. Susceptible and resistant zone diameter breakpoints at selected error rates based on a susceptible MIC breakpoint of 4 μg/ml

Theoretical error rate (%)	Breakpoint	Actual no. of strains and error rate (%)
False-susceptible		
2.5	11	6 (1.2)
1.0	12	5 (1.0)
0.5	17	2 (0.4)
0.0	≥26	0 (0.0)
False-resistant		
2.5	19	5 (1.0)
1.0	18	5 (1.0)
0.5	16	0 (0.0)
0.0	≤16	0 (0.0)

TABLE 3. Distribution of 489 bacterial isolates based on proposed interpretive zone standards for disk susceptibility testing with imipenem

Criteria	Diffusion test categories	Population statistics [no. (%)]	
		All isolates	*P. maltophilia* deleted
Zone diameter (mm)			
≥16	Susceptible	476 (97.3)	476 (99.4)
14–15	Moderately susceptible	0 (0)	0 (0)
≤13	Resistant	13 (2.7)	3 (0.6)
MIC (μg/ml)			
≤4	Susceptible	473 (96.7)	473 (98.8)
8	Moderately susceptible	4 (0.8)	4 (0.8)
≥16	Resistant	12 (2.5)	2 (0.4)

proposed guidelines are provided in Table 3. It can be seen that by either the diffusion or the dilution tests, approximately 97, 0.4, and 2.6% of the organisms tested fell into the susceptible, moderately susceptible, and resistant categories, respectively. Falling into the resistant category by the zone diameter breakpoint were 10 strains of *P. maltophilia*, 2 strains of *S. faecalis*, and 1 strain of *P. aeruginosa*. All 10 strains of *P. maltophilia* and 1 strain each of *P. aeruginosa* and *S. faecalis* would be classified as resistant by the MIC breakpoint. Four bacterial isolates, 1 strain of *P. mirabilis*, 2 strains of *P. cepacia*, and 1 strain of *S. faecalis* would fall into the moderately susceptible category by the MIC breakpoint. The exclusion from analysis of *P. maltophilia*, a bacterial species notoriously resistant to nearly all existing β-lactam antibiotics, would not affect the characteristics of the regression line but would result in a change in the population distribution of the 489 test strains to approximately 99% susceptible, 0.4% moderately susceptible, and 0.5% resistant to imipenem by either the disk or the dilution tests. The correlation coefficient (r) of 0.896, derived from regression-line analysis of the data herein presented and reflecting the degree of correlation between the MICs and the zones of inhibition, was excellent.

Of the 489 clinical isolates included in the present study, 95 were resistant to one or more of the three antimicrobial agents tested. Thirteen of these, or 13.7%, were resistant to imipenem, 67 (70.5%) were resistant to cefotaxime, and 70 (73.7%) were resistant to ceftazidime. On the basis of the MIC_{90} results, cross-resistance between all three antibiotics is suggested only against *P. maltophilia*. All of the test isolates resistant to imipenem were also resistant to cefotaxime and ceftazidime. However, 80.6 and 81.4% of the isolates resistant to cefotaxime and ceftazidime, respectively, were still susceptible to imipenem. Imipenem is unique among all β-lactam antibiotics in displaying substantial in vitro activities against *S. faecalis*, methicillin-resistant *S. aureus*, and *Acinetobacter calcoaceticus* (6, 13). These observations suggest that the 10-μg imipenem disk should be tested separately and that MICs or disk criteria applicable to ceftazidime, cefotaxime, and related compounds cannot be used to predict in vitro bacterial susceptibility to imipenem.

Obviously, the interpretive criteria herein proposed for in vitro susceptibility testing with the 10-μg imipenem disks and which, to a great degree, confirm those proposed previously (1), are only tentative. They are meant for use as a guide to therapy during clinical trials and, therefore, are subject to reevaluation and modification depending on how well they predict bacteriologic eradication and clinical efficacy.

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