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Comparison of Broth Microdilution, Agar Dilution, and Etest for Susceptibility Testing of Doripenem against Gram-Negative and Gram-Positive Pathogens[▽]

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The susceptibility of 513 clinical isolates to doripenem was determined by broth microdilution, agar dilution, and Etest. Overall agreements for Etest and agar dilution MIC values compared to reference broth microdilution at ±1 $\log_2$ dilution were 88% and 94%, respectively. Etest MIC values demonstrated 98% agreement within ±2 $\log_2$ dilutions compared to the reference broth microdilution method.

Doripenem is a broad-spectrum carbapenem recently approved in the United States for complicated intra-abdominal and complicated urinary tract infections, including pyelonephritis, and in Europe for the same two indications and for nosocomial pneumonia, including ventilator-associated pneumonia. Doripenem has been shown to have *in vitro* activity against nonfermentative Gram-negative bacilli, such as *Pseudomonas aeruginosa* and *Acinetobacter* spp., *Enterobacteriaceae*, and Gram-positive cocci (except for *Enterococcus faecium* and methicillin-resistant staphylococci) (3, 7, 8, 9). MIC susceptibility testing for doripenem as for other agents can be performed by a variety of test methodologies, such as broth

TABLE 1. Doripenem MIC distributions determined by broth microdilution MIC method

Organism (description)[a]	No. of isolates											
	Total	With doripenem MIC (μg/ml):										
		≤0.015	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	>16
Gram negative													
Enterobacteriaceae[b] (18 ESBL$^+$)	100	14	44	11	16	10	3	2					
Non-*Enterobacteriaceae*	100		2	4	16	21	14	12	9	8	5	4	5
Pseudomonas aeruginosa	80		1	4	15	17	11	10	6	7	4	1	4
Acinetobacter baumannii (10 MDR)	20		1		1	4	3	2	3	1	1	3	1
Gram positive													
Enterococcus spp.[c]	49								15	21	5	1	7
Staphylococcus spp.	100	13	35	7	6	16	7	3	3	2	3	3	2
S. aureus (26 MR, 35 MS)	61	9	23	5	3	10	5		2	1		2	1
CoNS (20 MR, 19 MS)[d]	39	4	12	2	3	6	2	3	1	1	3	1	1
Streptococcus spp., not *S. pneumoniae*	61	43	9	3	2	2				2			
Streptococcus spp., beta-hemolytic group[e]	39	35	4										
Streptococcus spp., viridans group[f]	22	8	5	3	2	2				2			
Streptococcus pneumoniae	103	39	8	12	4	7	25	7	1				

[a] ESBL$^+$, extended-spectrum β-lactamase positive; MDR, multidrug resistant; MR, methicillin resistant; MS, methicillin susceptible; CoNS, coagulase-negative staphylococci.

[b] *Enterobacteriaceae* included *Citrobacter braakii*, $n = 2$; *Citrobacter freundii*, $n = 6$; *Enterobacter cloacae*, $n = 10$; *Enterobacter sakazakii*, $n = 2$; *Escherichia coli*, $n = 31$; *Hafnia alvei*, $n = 1$; *Klebsiella oxytoca*, $n = 7$; *Klebsiella pneumoniae*, $n = 14$; *Morganella morganii*, $n = 9$; *Proteus mirabilis*, $n = 10$; *Proteus penneri*, $n = 2$; *Proteus vulgaris*, $n = 2$; and *Serratia marcescens*, $n = 4$.

[c] Enterococci included *Enterococcus faecalis*, $n = 39$, and *Enterococcus faecium*, $n = 10$, including 1 vancomycin-resistant strain.

[d] Coagulase-negative staphylococci included *Staphylococcus capitis*, $n = 1$; *Staphylococcus epidermidis*, $n = 24$; *Staphylococcus haemolyticus*, $n = 9$; *Staphylococcus hominis*, $n = 3$; *Staphylococcus saprophyticus*, $n = 1$; and *Staphylococcus simulans*, $n = 1$.

[e] *Streptococcus* beta-hemolytic group included *Streptococcus agalactiae*, $n = 21$, and *Streptococcus pyogenes*, $n = 18$.

[f] *Streptococcus* viridans group included *S. anginosus*, $n = 2$; *Streptococcus bovis*, $n = 1$; *Streptococcus constellatus*, $n = 5$; *Streptococcus mitis*, $n = 3$; *Streptococcus oralis*, $n = 3$; *Streptococcus salivarius*, $n = 1$; and *Streptococcus* viridans group, $n = 7$.

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TABLE 2. Doripenem MICs determined by various susceptibility methods

Organism group (*n*)	MIC determination method	MIC (μg/ml)				% susceptible[a]
		Range	MIC_{50}	MIC_{90}	Mode	
Enterobacteriaceae (100)	BMD	≤0.015–1	0.03	0.25	0.03	98
	Agar D[b,c]	0.015–1	0.06	0.25	0.03	99
	Etest	0.015–1	0.06	0.25	0.06	99
Non-*Enterobacteriaceae* (100)	BMD	0.03–>16	0.5	8	0.25	—
	Agar D	0.015–>16	0.5	8	0.25	—
	Etest	0.03–>32	0.5	32	0.25	—
P. aeruginosa (80)	BMD	0.03–>16	0.5	8	0.25	80
	Agar D	0.06–>16	0.5	4	0.25	80
	Etest	0.06–>32	0.25	8	0.25	78.8
A. baumannii (20)	BMD	0.03–>16	1	16	0.25	55
	Agar D	0.015–8	2	8	2	40
	Etest	0.03–>32	2	>32	2	40
Enterococcus spp. (49)	BMD	2–>16	4	>16	4	—
	Agar D	1–>16	4	>16	2	—
	Etest	0.5–>32	4	>32	4	—
Staphylococcus spp. (100)	BMD	≤0.015–>16	0.06	2	0.03	—
	Agar D[e]	≤0.015–>16	0.06	2	0.03	—
	Etest	0.004–>32	0.125	4	0.06	—
S. aureus (61)	BMD	≤0.015–>16	0.03	0.5	0.03	—
	Agar D	0.03–16	0.03	2	0.03	—
	Etest	0.03–>32	0.125	2	0.06	—
CoNS[d] (39)	BMD	≤0.015–>16	0.125	8	0.03	—
	Agar D	≤0.015–>16	0.06	2	0.03	—
	Etest	0.004–>32	0.125	4	0.06	—
Streptococcus spp., other than *S. pneumoniae* (61)	BMD	≤0.015–4	≤0.015	0.06	≤0.015	—
	Agar D	≤0.008–4	0.015	0.06	≤0.008	—
	Etest	0.002–2	0.03	0.125	0.03	—
Beta-hemolytic group (39)	BMD	≤0.015–0.03	≤0.015	≤0.015	≤0.015	—
	Agar D	≤0.008–0.03	0.015	0.015	≤0.008	—
	Etest	0.002–0.125	0.03	0.03	0.03	—
Viridans group (22)	BMD	≤0.015–4	0.03	0.25	≤0.015	—
	Agar D	≤0.008–4	0.06	0.25	0.06	—
	Etest	0.008–2	0.06	0.25	0.06	—
Streptococcus pneumoniae (103)	BMD	≤0.015–2	0.06	0.5	≤0.015	—
	Etest	0.008–1	0.06	1	0.008	—

[a] Doripenem FDA susceptible breakpoints: *Enterobacteriaceae*, ≤0.5 μg/ml; *P. aeruginosa*, ≤2 μg/ml; *A. baumannii*, ≤1 μg/ml. —, doripenem FDA breakpoints have not yet been determined for these organism groups.
[b] Agar D, agar dilution.
[c] The number of *Enterobacteriaceae* tested by agar dilution was 98.
[d] CoNS, coagulase-negative staphylococci.
[e] The number of staphylococci tested by agar dilution was 97, including 60 *S. aureus* isolates and 37 coagulase-negative staphylococci.

microdilution (BMD), agar dilution, and Etest. As laboratories may use any one or multiple methods, it is of interest to know how MIC results for a drug will compare across methods. In this study the *in vitro* activity of doripenem against relevant Gram-negative and Gram-positive pathogens was determined by three MIC methods: broth microdilution or the standard reference method, agar dilution, and Etest. The BMD MICs were compared to results from agar dilution and Etest.

(This work was presented in part at the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 2007 [1].)

Recent clinical isolates (n = 513) were studied from eight major organism groups based on the current groupings in the Clinical and Laboratory Standards Institute (CLSI) M100 supplement (6). The organism groups were *Staphylococcus* spp., *Streptococcus pneumoniae*, *Streptococcus* beta-hemolytic group, *Streptococcus* viridans group, *Enterococcus* spp., *Enterobacteriaceae*, *P. aeruginosa*, and *Acinetobacter* spp. The appropriate CLSI quality control (QC) strains *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *S. pneumoniae* ATCC 49619, *Escherichia coli* ATCC 25922, and *P. aeruginosa* ATCC 27853 were evaluated concurrently with every test.

Broth microdilution MIC testing followed CLSI methodology (4, 5) using cation-adjusted Mueller-Hinton (MH) broth (Sensititre; Trek Diagnostic Systems, Cleveland, OH) with or without the addition of 3% lysed horse blood (Hardy Diagnostics, Santa Maria, CA). Custom susceptibility panels in the frozen format were obtained from Trek Diagnostic Systems (Cleveland, OH).

Agar dilution MIC testing was performed on MH II agar (BD BBL, Sparks, MD) according to CLSI methodology (4, 5).

TABLE 3. Distribution of doripenem $\log_2$ dilution differences between agar dilution and BMD methods

Organism group	No. of isolates							% agreement	
	Total	With $\log_2$ dilution difference[a]:							
		−2	−1	0	1	2	≥3	Same	±1	±2
Enterobacteriaceae	98		18	59	19	1	1	60.2	96.8	99.0
Non-*Enterobacteriaceae*	100	5	15	52	26		2	52.0	93.0	98.0
P. aeruginosa	80	3	11	44	22			55.0	96.3	100.0
A. baumannii	20	2	4	8	4		2	40.0	80.0	90.0
Enterococcus spp.	49	3	16	24	6			49.0	93.9	100.0
Staphylococcus spp.	97	7	12	40	32	6		41.2	86.6	100.0
S. aureus	60	1	8	23	22	6		38.3	88.3	100.0
CoNS[b]	37	6	4	17	10			45.9	83.8	100.0
Streptococcus spp., not *S. pneumoniae*	61		8	41	11	1		67.2	98.4	100.0
Streptococcus spp., beta-hemolytic group	39		3	33	3			84.6	100.0	100.0
Streptococcus spp., viridans group	22		5	8	8	1		36.4	95.5	100.0
Total	405	15	69	216	94	8	3	53.3	93.6	99.3

[a] Negative number, agar dilution result was lower than BMD result. Positive number, agar dilution result was higher than the BMD result.
[b] CoNS, coagulase-negative staphylococci.

Organisms were inoculated onto 100-mm agar plates prepared on the day of testing with a replicator (Craft Machine Inc., Chester, PA) that delivered 2 μl per spot. For streptococci other than *S. pneumoniae*, MH agar was supplemented with 5% sheep blood (Hardy Diagnostics, Santa Maria, CA).

MIC testing by the doripenem Etest (AB Biodisk, Solna, Sweden) was performed and results were interpreted according to the manufacturer's instructions. MH II agar plates (BD BBL, Sparks, MD) and plates of MH with 5% sheep blood agar (BD BBL, Sparks, MD) were used. Etest MICs were rounded up to the nearest 2-fold dilution in a standard MIC test, and these values were used in comparisons between the Etest and the reference BMD method.

MIC values for isolates to doripenem were generated by BMD, agar dilution, and Etest methods. Agar dilution MICs were not determined for *S. pneumoniae*, since agar dilution is not a recommended reference method for this organism. The MIC results from agar dilution and Etest were compared to those from the standard reference BMD MIC method. For all isolates, the comparator carbapenem class agent, meropenem, was tested in parallel with doripenem. In testing of the appropriate CLSI QC strains, both doripenem and meropenem performed within acceptable CLSI QC limits (data not shown).

The distribution of doripenem MIC values against Gram-negative and Gram-positive clinical isolates is shown in Table 1. The isolates were chosen to provide a range of MIC values and were not selected at random. Thus, the number of resistant isolates is higher than those that would be found in a random population. The percentages of doripenem-susceptible clinical isolates were 98% (98/100) for *Enterobacteriaceae*, 80% (64/80) for *P. aeruginosa*, and 55% (11/20) for *Acinetobacter baumannii* based on FDA breakpoints. Of the viridans group streptococci, seven isolates were identified as *Streptococcus anginosus* group, and of these seven isolates 100% were doripenem susceptible. The FDA susceptible MIC breakpoints for doripenem are ≤0.5 μg/ml for *Enterobacteriaceae*, ≤2 μg/ml for *P. aeruginosa*, ≤1 μg/ml for *A. baumannii*, and ≤0.12 μg/ml for *S. anginosus* group. At this time, susceptible breakpoints for doripenem have not been determined for the other organism groups.

The BMD, agar dilution, and Etest doripenem MIC values are summarized in Table 2. Among the Gram-negative isolates, all methods yielded a doripenem MIC_{90} for *Enterobacteriaceae* of 0.25 μg/ml. For *P. aeruginosa* the BMD and Etest MIC_{90}s were 8 μg/ml and the agar dilution MIC_{90} was 4 μg/ml for doripenem. For *A. baumannii*, the BMD, Etest, and agar dilution MIC_{90} values were 16, >32, and 8 μg/ml, respectively.

Against 49 enterococci, the MIC_{50} and MIC_{90} were 4 and ≥16 μg/ml, respectively, by all three methods. For all staphylococci, the doripenem MIC_{90}s were 2 μg/ml by BMD and agar dilution and 4 μg/ml by Etest. The *S. pneumoniae* doripenem MIC_{90} was 0.5 μg/ml by BMD and 2-fold higher by Etest. The MIC_{90} for beta-hemolytic streptococci was ≤0.015 μg/ml by BMD and agar dilution and 0.03 μg/ml by Etest; for viridans group streptococci the MIC_{90} was 0.25 μg/ml for all test methods.

As doripenem has only susceptible FDA breakpoints, an analysis of very major test errors was conducted for organisms that were defined as nonsusceptible by the reference method, BMD, and categorized as susceptible by agar dilution or Etest. Major errors were determined for organisms that were susceptible by BMD and nonsusceptible by agar dilution or Etest. For the *Enterobacteriaceae* group, there was one very major error for both agar dilution and Etest methods. Among the *P. aeruginosa* isolates tested, there was one major error by Etest. For *A. baumannii*, there were three major errors by both agar dilution and Etest methods.

Doripenem MICs for 60% (59/98) of the *Enterobacteriaceae*, 55% (44/80) of the *P. aeruginosa* isolates, and 40% (8/20) of the *A. baumannii* isolates tested were identical for BMD and agar dilution within each group. Among the Gram-positive isolates tested, 50.7% (105/207) had identical MIC results for BMD and agar dilution (Table 3). Etest and BMD tests had a lower percentage of identical MIC results for many of the organism groups, especially the *Enterobacteriaceae*, staphylococci, and non-*S. pneumoniae* isolates. Etest and BMD MICs were identical for only 28% (28/100) of the *Enterobacteriaceae*, 45% (36/80) of *P. aeruginosa* isolates, 45% (9/20) of *A. baumannii*

TABLE 4. Distribution of doripenem $\log_2$ dilution differences between Etest and BMD reference methods

Organism group	No. of isolates								% agreement	
	Total	With $\log_2$ dilution difference[a]:								
		≤−3	−2	−1	0	1	2	≥3	Same	±1	±2
Enterobacteriaceae	100		1	7	28	57	7		28.0	92.0	100.0
Non-*Enterobacteriaceae*	100		1	27	45	19	6	2	45.0	91.0	98.0
P. aeruginosa	80		1	26	36	13	4		45.0	93.8	100.0
A. baumannii	20			1	9	6	2	2	45.0	80.0	90.0
Enterococcus spp.	49	1		8	26	13	1		53.1	95.9	98.0
Staphylococcus spp.	100		2	3	16	45	29	5	16.0	64.0	95.0
S. aureus	61				7	27	24	3	11.5	55.7	95.1
CoNS[b]	39		2	3	9	18	5	2	23.1	76.9	94.9
Streptococcus spp., not *S. pneumoniae*	60			4	30	20	5	1	50.0	90.0	98.3
Streptococcus spp., beta-hemolytic group	39				22	16		1	56.4	97.4	97.4
Streptococcus spp., viridans group	21			4	8	4	5		38.1	76.2	100.0
Streptococcus pneumoniae	103			6	78	19			75.7	100.0	100.0
Total	512	1	4	55	223	173	48	8	43.6	88.1	98.2

[a] Negative number, Etest result was lower than BMD result. Positive number, Etest result was higher than the BMD result.
[b] CoNS, coagulase-negative staphylococci.

isolates, and 48.1% (150/312) of the Gram-positive isolates tested (Table 4).

Comparison of doripenem agar dilution MIC results to Etest MIC values (Table 5) showed >92% essential agreement (within ±1 $\log_2$ dilution) for *Enterobacteriaceae*, *P. aeruginosa*, enterococci, and streptococci that were not *S. pneumoniae*. *A. baumannii* and staphylococci demonstrated lower essential agreements (within ±1 $\log_2$ dilution) of 60% and 84.5% for doripenem agar dilution MIC and Etest MIC comparisons, respectively.

The essential agreement (within ±1 $\log_2$ dilution) of doripenem BMD results for all organisms tested compared to agar dilution MICs was 93.6%, and that compared to Etest MICs was 88.1%. *A. baumannii* and staphylococci demonstrated slightly lower essential agreements (within ±1 $\log_2$ dilution) of 80% and 86.6% by agar dilution and 80% and 64% by Etest, respectively (Tables 3 and 4). *A. baumannii* and staphylococci also demonstrated lower essential agreement when Etest results were compared to agar dilution MICs. Organism groups with >93% essential agreement (within ±1 $\log_2$ dilution) of doripenem BMD and agar dilution MICs were *Enterobacteriaceae*, *P. aeruginosa*, enterococci, and viridans group streptococci, and the group with 100% essential agreement (within ±1 $\log_2$ dilution) was beta-hemolytic streptococci. In comparing doripenem BMD to Etest, organism groups with >92% essential agreement (within ±1 $\log_2$ dilution) of doripenem BMD and agar dilution MICs were *Enterobacteriaceae*, *P. aeruginosa*, enterococci, and beta-hemolytic streptococci, and *S. pneumoniae* showed 100% essential agreement (within ±1 $\log_2$ dilution). Within ±2 $\log_2$ dilutions, the agreements of BMD results for all organisms tested compared to agar dilution and to Etest were 99.3% and 98.2%, respectively.

As commercial methods for susceptibility testing of doripenem are becoming more available, it is important to know how results determined by alternate methods compare to the standard reference method, BMD. This study demonstrated that agar dilution MICs were comparable to BMD MICs with ≥93% essential agreement and that 99% were within ±2 $\log_2$

TABLE 5. Distribution of doripenem $\log_2$ dilution differences between Etest and agar dilution methods

Organism group	No. of isolates								% agreement	
	Total	With $\log_2$ dilution difference[a]:								
		≤−3	−2	−1	0	1	2	≥3	Same	±1	±2
Enterobacteriaceae	98			4	40	49	5		40.8	94.9	100.0
Nonfermenters	100	3	3	26	50	8	5	5	50.0	84.0	92.0
P. aeruginosa	80	2	2	26	39	7	3	1	32.5	90.0	96.3
A. baumannii	20	1	1		11	1	2	4	55.0	60.0	75.0
Enterococcus spp.	49	1		5	19	22	1	1	38.8	93.9	95.9
Staphylococcus spp.	97			1	25	56	13	2	25.8	84.5	97.9
S. aureus	60				14	38	8		23.3	86.7	100.0
CoNS[b]	37			1	11	18	5	2	29.7	81.1	94.6
Streptococcus spp., not *S. pneumoniae*	60			1	37	18	3	1	61.7	93.3	98.3
Beta-hemolytic group	39				23	13	2	1	59.0	92.3	97.4
Viridans group	21			1	14	5	1		66.7	95.2	100.0
Total	404	4	3	37	171	153	27	9	42.3	89.4	96.8

[a] Negative number, Etest result was lower than agar dilution result. Positive number, Etest result was higher than the agar dilution result.
[b] CoNS, coagulase-negative staphylococci.

dilutions. By Etest, most MICs, including the MIC mode and MIC_{90} values for all organisms examined, tended to be 1 $\log_2$ dilution higher than the BMD values. This trend was also observed in a previous study with *P. aeruginosa* isolates at or near the doripenem breakpoint (2 μg/ml) (2).

In this study, the Etest method performed comparably to the standard reference methods with >96% of the doripenem MICs within ±2 $\log_2$ dilutions of the BMD and agar dilution methods. Given the overall comparability of these three methods, laboratories should be comfortable in using their method of choice for MIC testing of doripenem. However, it should be recognized that there may be inherent, although sometimes subtle, differences that may occur in MIC testing, depending on the method chosen.

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