Regression Analysis of MIC Versus Disk Diffusion Zone Diameters for Three Carbapenems Tested Against Enterobacteriaceae Isolates Harboring Serine Carbapenemases with Matched Control Strains

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AMENDED ABSTRACT

Background: KPC serine carbapenemases (SC) were first reported in *Klebsiella* strains in significant numbers from New York City hospitals. More recent reports show increasing prevalence of Enterobacteriaceae (ENT) isolates with KPC-SC outside of that geographic area, and the increasing transmission to other ENT species. We assess the CLSI MIC and disk diffusion (DD) breakpoints for carbapenems and several control agents for SC detection.

Methods: A total of 474 Enterobacteriaceae (ENT) isolates were tested in duplicate by CLSI broth microdilution and disk diffusion susceptibility (S) methods. 948 results were generated for regress-scattergram analyses; 656 from KPC isolates and 292 from hospital-matched control ENT strains showing wildtype (WT) or ESBL patterns of resistance (R). A very small number of metallo-ß-lactamase-producing strains were also tested among carbapenemases. Ertapenem (ETP), imipenem (IPM), meropenem (MER), cefepime (CPM), ceftazidime (CAZ), piperacillin/tazobactam (P/T) and four other non-ß-lactam control drugs were tested.

Results: Percentages of 656 KPC strains results categorized as S (MIC/disk) to each ß-lactam (CLSI M100-S18 criteria) were: ETP (8.5-3.8), IPM (30.2/48.5), MER (30.8/32.8), CPM (25.3/24.8), CAZ (7.6/8.2) and P/T (4.6/4.0). Adjusting ETP-S breakpoints to ≤0.5 µg/ml (≥22 mm correlate zone) reduced false-S very major and major error to only 0.1%. Also, ≤1 µg/ml (≥23mm and ≥21mm, respectively) S criteria for IPM and MER had serious error rates of only 0.2-0.8% without compromising the accuracy against WT or ESBL control strains. High false-intermediate tigecycline DD results (24.4%) were documented for USA-FDA breakpoints.

Table: Current susceptibility and proposed screening breakpoints to optimize detection of KPC-type resistances.

	Current			Proposed		
Carbapenem	MIC (µg/ml)	Disk (mm)	% accuracy	MIC (µg/ml)	Disk (mm)	% accuracy
Ertapenem	≤2	≥19	91.5-96.2	≤0.5	≥19	96.2-99.7
Imipenem	≤4	≥16	51.5-69.8	≤1	≥22	96.2-100.0
Meropenem	≤4	≥16	67.2-69.2	≤1	≥22	98.8

Conclusion: Emerging KPC-SC mediated resistances are suboptimally detected by current CLSI breakpoint criteria with high levels of potential false-S reports. Modest changes correlating with pharmacodynamic features of three carbapenems can minimize serious MIC and DD testing errors (very major and major; ≤3.0%).

INTRODUCTION

The most active broad-spectrum antimicrobial class used to treat infections caused by Enterobacteriaceae isolates are the carbapenems with an advantage of added activity against non-fermentative Gram-negative bacilli including *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains as well as many Gram-positive cocci. The carbapenems are also an important antimicrobial choice for the treatment of infections caused by extended spectrum ß-lactamase (ESBL)-producing Enterobacteriaceae.

Carbapenems are ß-lactam antimicrobial agents with broad antibacterial activity, increased stability to ß-lactamase hydrolysis, and high rates of penetration through the bacterial outer membrane. Two types of ß-lactamases capable of hydrolyzing carbapenems belong to Ambler molecular class A and B, also named serine carbapenemases and metallo-ß-lactamases (MßLs), respectively. Many types of MßLs, such as IMP, VIM, SPM, GIM, and SIM, have been isolated mainly from non-fermentative Gram-negative bacilli, but also less commonly among the Enterobacteriaceae (IMP and VIM types).

Carbapenem susceptibility rates among Enterobacteriaceae isolates have recently been declining due to the spread of serine carbapenemase-mediated resistance mechanisms, especially among *Klebsiella* spp. isolates from medical centers in the New York City area. Currently the additional spread to other geographic regions (inside and outside of the United States [USA]) and into other bacterial species is becoming a serious problem.

We assessed the current susceptibility breakpoints for MIC and disk diffusion testing of the Clinical and Laboratory Standards Institute (CLSI) against several carbapenems, ß–lactams and other control agents using KPC-confirmed, KPC phenotype, MßL, ESBL and wild type Enterobacteriaceae strains for their ability to detect serine carbapenemases, and propose alternative criteria.

MATERIALS AND METHODS

Isolate collection: A total of 328 Enterobacteriaceae isolates were identified from the MYSTIC Program and SENTRY Antimicrobial Surveillance collections with a confirmed KPC serine carbapenemase, a KPC phenotype (≥2 µg/ml for both meropenem and imipenem) or a confirmed metallo-ß-lactamase. Matching wild type or extended-spectrum ß-lactamase-producing controls (146 strains) from the same year, species and medical center were also included in the comparison test set. MIC results from USA Enterobacteriaceae in the SENTRY Program (2006 – 2007) for 5,133 strains excluding known KPC-producing strains and two subsets of indole-positive *Proteus* and *P. mirabilis* were also examined (308 strains).

Susceptibility testing: All isolates were tested for susceptibility by broth microdilution and disk diffusion using reference quality methods (CLSI M2-A9 and M7-A7, 2006) against the ß-lactams; ertapenem, imipenem, meropenem, cefepime, ceftazidime, piperacillin/tazobactam as well as gentamicin, levofloxacin, polymyxin B and tigecycline. All disk diffusion zone diameters were measured in duplicate by two independent readers. CLSI interpretative criteria as published in the M100-S18 were utilized for categorization of susceptibility and resistance for both MIC and disk diffusion tests. Quality control (QC) of the test methods were assured utilizing appropriate American Type Culture Collection (ATCC) strains with all QC results observed within published CLSI ranges.

Regression analysis: Scatter diagrams of broth microdilution MIC results versus disk diffusion zone diameter measurements for each antimicrobial agent tested were plotted. Error rates were calculated using current CLSI (M100-S18, 2008) susceptibility breakpoints and proposed breakpoints to enhance carbapenemase detection.

RESULTS

Table 1. Composition of Enterobacteriaceae sets (474 strains) tested against 10 antimicrobial agents by CLSI broth microdilution and disk diffusion tests.

	Carbapenemase strains (no.)		Control strains (no.)	
Organism group/species	KPC strains	MßL strains	ESBL-type	Wild-type
Citrobacter spp.	15	--	1	6
Enterobacter spp.	65	23	33	26
E. coli	8	--	3	3
Klebsiella spp.	179	26	30	29
Proteus spp.	--	1	--	4
Serratia spp.	11	--	3	8
Total	278	50	70	76

Table 2. Distribution of carbapenem MIC values for KPC-producing and non-producing Enterobacteriaceae.

	Cumulative % of results by carbapenem tested:											
	Ertapenem				Imipenem				Meropenem			
MIC (µg/ml)	KPC[a]	Non-KPC[b]	WT-all[c]	WT-mod[d]	KPC	Non-KPC	WT-all	WT-mod	KPC	Non-KPC	WT-all	WT-mod
≥8	83.5	2.1	0.0	0.0	69.8	0.0	0.1	0.0	69.2	0.0	0.0	0.0
4	91.5	2.7	0.1	0.1	85.7	1.4	0.7	0.1	83.8	0.7	0.0	0.0
2	96.0	6.2	0.5	0.5	100.0	9.6	4.5	2.4	98.8	2.1	≤0.1	≤0.1
1	99.7	10.3	1.6	1.7	-	34.2	14.9	11.4	99.4	4.1	0.2	0.2
0.5	100.0	17.1	2.7	2.9	-	64.4	31.2	27.5	100.0	4.8	0.5	0.5
0.25	-	25.3	4.2	4.5	-	100.0	66.6	64.5	-	8.9	1.3	1.2
0.12	-	38.4	7.1	7.5	-	-	100.0	100.0	-	20.5	100.0	100.0
≤0.06	-	100.0	100.0	100.0	-	-	-	-	-	100.0	NT[e]	NT

a. Includes challenge strains producing metallo-ß-lactamases (50 strains).
b. Includes ESBL-producing and wild type organisms used in scattergram controls.
c. Includes wild type (WT) strains from USA 2006 – 2007 SENTRY Program without KPC enzyme producing strains (5,133 strains; WT-all).
d. Organisms in organism group discussed in C (above), but with 308 *P. mirabilis* and indole-positive *Proteus* spp. deleted (species with inherently elevated carbapenem MIC values; WT-modified [WT-mod]; total strains at 4,825.
e. NT = Not tested.
f. Broken line = proposed MIC screening concentration.

- KPC serine carbapenemase producing strains susceptible to each ß-lactam agent tested using CLSI criteria for MIC/disk diffusion methods were: ertapenem 8.5/3.8%, imipenem 30.2/48.5%, meropenem 30.8/32.8%, cefepime 25.3/24.8%, ceftazidime 7.6/8.2% and piperacillin/tazobactam 4.6/4.0% (Table 2 and 3; data not shown).

Table 3. Distribution of carbapenem zone diameter results for KPC-producing and non-producing strains.

	Cumulative % of results by carbapenem tested:					
	Ertapenem		Imipenem		Meropenem	
Zone diameter (mm)	KPC[a]	Non-KPC[b]	KPC	Non-KPC	KPC	Non-KPC
≤16	88.3	4.5	63.6	1.0	77.3	2.4
17	92.7	5.8	71.6	1.4	84.1	2.4
18	96.2	6.8	81.9	2.1	90.7	2.7
19	98.0	8.2	87.7	2.4	95.0	2.7
20	99.1	11.0	91.6	4.1	97.4	2.7
21	100.0	12.7	96.2	7.5	98.8	2.7
22	-	16.8	97.9	18.2	99.1	3.4
23	-	22.3	99.2	32.5	99.4	5.1
24	-	26.4	99.5	42.3	99.8	9.2
25	-	30.8	100.0	63.0	100.0	19.5
26	-	37.7	-	77.4	-	32.9
27	-	45.5	-	85.3	-	48.3
28	-	53.8	-	92.1	-	65.8
29	-	67.8	-	98.3	-	83.9
≥30	-	100.0	-	100.0	-	100.0

a. Includes challenge strains producing metallo-ß-lactamases (50 strains).
b. Includes ESBL-producing and wild type organisms.
c. Broken line = proposed disk diffusion zone diameter screening breakpoint.

- The ESBL-producing and wild type matched control (2:1 ratio) isolates had MIC and disk diffusion susceptibility rates of 97.3/93.2%, 100.0/99.3%, 100.0/98.6% for ertapenem, imipenem and meropenem, respectively (Table 2 and 3).
- Very major interpretative error rates were 1.4, 5.5 and 10.4% for ertapenem, meropenem and imipenem among KPC strains, but were 0.0% for all carbapenems for control strains. Acceptable major error rates of 0.6 – 2.6% and 0.0 – 0.7% were observed for both KPC and control strains (Table 4).
- Reducing the MIC breakpoint and increasing disk diffusion zone diameter susceptibility/resistance breakpoints to ≤0.5/≥2 µg/ml and ≥22/≤18 mm for ertapenem and to ≤1/≥4 µg/ml and ≥23/≤19 mm for imipenem and to ≤1/≥4 µg/ml and ≥21/≤17 mm for meropenem reduced the very major error rates to ≤0.4% and major error rates to 0.1 – 0.4% for the carbapenems (Table 4).
- Adjusting the MIC and disk diffusion breakpoints (screening criteria) for detecting KPC-producing isolates increased the modified sensitivity for ertapenem (91.5 to 99.7; 96.2%), imipenem (69.8 to 100.0; 51.5 to 96.2%) and meropenem (69.2 to 98.8; 67.2 to 98.8%).

Table 4. Current[a] and proposed MIC and zone diameter interpretative criteria for using carbapenems to accurately detect KPC-producing Enterobacteriaceae.

		Susceptible		Resistant		Error rate (%)		
Carbapenem	Tested organisms (no.)	MIC (µg/ml)	Zone (mm)	MIC (µg/ml)	Zone (mm)	Very major	Major	Minor
Ertapenem	All (948)[a]	≤2	≥19	≥8	≤15	0.9	1.7	12.2
	KPC (656)[a]	≤2	≥19	≥8	≤15	1.4	2.1	15.9
	Controls (292)[a]	≤2	≥19	≥8	≤15	0.0	0.7	4.1
	All (948)	≤0.5	≥22	≥2	≤18	0.0	0.1	6.1
	All (948)	≤0.5	≥21	≥2	≤17	0.2	0.0	7.3
Imipenem	All (948)[a]	≤4	≥16	≥16	≤13	7.2	0.4	21.4
	KPC (656)[a]	≤4	≥16	≥16	≤13	10.4	0.6	30.6
	Controls (292)[a]	≤4	≥16	≥16	≤13	0.0	0.0	0.7
	All (948)	≤1	≥23	≥4	≤19	0.0	0.2	13.4
	All (948)	≤1	≥22	≥4	≤18	0.2	0.2	13.9
Meropenem	All (948)[a]	≤4	≥16	≥16	≤13	3.8	2.0	20.5
	KPC (656)[a]	≤4	≥16	≥16	≤13	5.5	2.6	29.3
	Controls (292)[a]	≤4	≥16	≥16	≤13	0.0	0.7	0.7
	All (948)	≤1	≥21	≥4	≤17	0.4	0.4	13.7
	All (948)	≤1	≥20	≥4	≤16	1.6	0.4	13.8

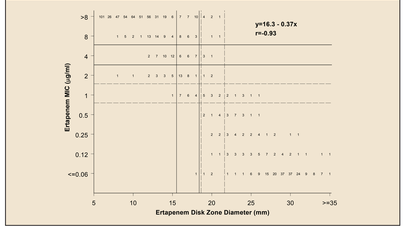
a. CLSI current criteria (M100-S18, 2008).

Table 5. Performance of current CLSI (2008) interpretive criteria for selected non-carbapenems when tested against the challenge collection containing results from 656 tests with KPC-producing Enterobacteriaceae (948 total tests).[a]

	Susceptible		Resistant		Error rates (%)		
Antimicrobial	MIC (µg/ml)	Zone (mm)	MIC (µg/ml)	Zone (mm)	Very major	Major	Minor
Cefepime	≤8	≥18	≥32	≤14	0.7	0.0	22.8
Cefepime[a]	≤4	≥21	≥16	≤17	0.2	0.4	10.9
Ceftazidime	≤8	≥18	≥32	≤14	0.4	0.5	5.1
Gentamicin	≤4	≥15	≥16	≤12	0.6	0.0	6.4
Levofloxacin	≤2	≥17	≥8	≤13	0.0	0.0	7.4
Piperacillin/tazobactam	≤16	≥21	≥128	≤17	0.2	0.8	12.7
Piperacillin/tazobactam[a]	≤8	≥21	≥32	≤18	0.4	0.1	6.1
Polymyxin B[b]	≤2	≥12	-	-	7.7[c]	0.0	NA[c]
Tigecycline[a]	≤2	≥19	≥8	≤14	0.0	0.3	24.4
Tigecycline[a]	≤2	≥16	≥8	≤13	0.0	0.0	2.0

a. Alternative breakpoints were proposed for cefepime and piperacillin/tazobactam. Polymyxin B criteria were those used for susceptibility when testing *P. aeruginosa* and the tigecycline breakpoints are those published in the FDA package insert; proposed changes were suggested to minimize intermethod error.
b. Very major error rate for KPC strains was 7.7%, unacceptable.
c. NA = not applicable.

Figure 1. Scattergram comparing ertapenem MIC and zone diameter results for 948 Enterobacteriaceae determinations including 656 KPC strains. Solid lines are current CLSI breakpoints (M100-S18) and broken lines are proposed / modified criteria to maximize KPC detection and correlate to PK/PD target attainment principals.



- Using the same breakpoints, modified specificity rates were only lowered from 93.2-100.0% down to 89.7-97.9% for this selected collection.
- The modified specificity for detecting KPC-type isolates among the 2006 – 2007 all wild-type Enterobacteriaceae strains using the proposed lower breakpoints was 98.4, 95.5, and >99.9% for ertapenem, imipenem and meropenem, respectively (Table 2).
- The performance of 7 additional antimicrobial agents tested, including three other ß-lactams, had low very major error (0.0 – 0.7%) and major error (0.0 – 0.8%) rates using current breakpoint criteria except for polymyxin B which had a 7.7% very major error rate against this Enterobacteriaceae collection. Tigecycline breakpoints may require adjustment due to elevated (24.4%) minor errors using USA-FDA criteria (susceptible MIC but intermediate zone diameter; Table 5).
- Regression analyses for the three carbapenems tested against the KPC and matched control isolates had good correlation coefficients of 0.86 – 0.93 (see Figure 1; other data not shown).

CONCLUSIONS

- Enterobacteriaceae isolates known to produce KPC serine carbapenemases are suboptimally detected when applying current CLSI susceptibility breakpoint criteria (M100-S18, 2008).
- Applying the current CLSI criteria can lead to high levels of false susceptibility being reported for KPC-producing Enterobacteriaceae strains.
- Modest breakpoint changes correlating with pharmacodynamic parameters for carbapenems would decrease MIC and disk diffusion categorical errors, and significantly enhance the detection of KPC-producing isolates.
- Alternatively, screening concentration or zone diameter could be selected to produce acceptable KPC detection having high (>90%) modified sensitivity and specificity calculations (Tables 2 and 3), example: MIC at ≥1 µg/ml and zone ≤18 mm for ertapenem. Meropenem appears to offer more optimal detection statistics, and by deleting Proteae from the imipenem screening, the specificity was markedly enhanced.

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