Eight broad-spectrum β-lactam Agents Tested Against a Large Collection of KPC-producing Enterobacteriaceae: Evaluation of Current CLSI Breakpoints

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ABSTRACT

Background: MIC values for the β-lactam agents can vary among strains producing β-lactamases, including KPC serine-carbapenemases, complicating detection by clinical microbiology laboratories. We evaluated the MIC results and current CLSI breakpoints for eight broad-spectrum β-lactams tested against a large collection of KPC-producing Enterobacteriaceae.

Methods: 374 KPC-producing Enterobacteriaceae were susceptibility (S) tested by CLSI broth microdilution method (BMD; M07-A8, 2009). Isolates carrying the KPC gene were screened based on imipenem (IMI) or meropenem (MER) MIC values at ≥2 µg/mL (non-S). Strains showing MIC values below breakpoints for any of the 8 β-lactams were retested by BMD and PCR targeting KPC-encoding genes.

Results: All 374 KPC-producers (283 *K. pneumoniae* [KPN], 41 *E. cloacae* and 7 other species [<20/species]) isolates were categorized using CLSI breakpoints as non-S to aztreonam (MICs, 8-≥32 µg/mL), ceftriaxone (MIC, ≥4 µg/mL), ertapenem (MIC, ≥0.5 µg/mL), IMI (MIC, >2 µg/mL) and piperacillin/tazobactam (MIC, ≥32 µg/mL). 52 strains (13.9%; 20 KPN 11 *K. oxytoca*, 7 *E. coli*, 7 *C. freundii*, 5 *E. cloacae*, 2 *Raoultella planticola*) were categorized as S to cefepime (CEP), with MIC values as low as 1 µg/mL. Eight (2.1%) strains were S to ceftazidime (CAZ; including 4 *E. coli*, 2 *K. oxytoca*, 2 KPN) and 2 KPN were MER-S. All CAZ-S strains were also S to CEP and one was S to MER.

Conclusions: An elevated percentage of KPC-producing isolates were CEP-S by reference BMD (>13%). This compound demonstrated poor prediction of the presence of KPC and revising the current breakpoints might be considered. Infectious disease and clinical microbiology professionals should be aware that depending on the genetic location of *bla*$_{KPC}$, number of copies and presence of other β-lactamases, variations in the β-lactams MICs to S levels may be observed.

Antimicrobial agent (no. tested)	No. (cum. %) inhibited at MIC (µg/mL):						
	≤0.5	1	2	4	8	16	≥32
Aztreonam (286)	0	0	0	0 (0.0)[a]	2 (0.7)	2 (1.4)	282 (100.0)
Cefepime (374)	0	2 (0.5)	4 (1.6)	10 (4.3)	36 (13.9)[a]	61 (30.2)	261 (100.0)
Ceftazidime (374)	0	1 (0.3)	4 (1.3)	3 (2.1)[a]	12 (5.4)	20 (10.7)	334 (100.0)
Ceftriaxone (374)	0	0 (0.0)[a]	0 (0.0)	5 (1.3)	6 (2.9)	19 (8.0)	304 (100.0)
Ertapenem (356)	1 (0.3)	3 (1.1)	8 (3.4)	26 (10.7)	41 (22.2)	-[b]	277 (100.0)
Imipenem (374)	0	0 (0.0)[a]	12 (3.2)	39 (13.6)	89 (37.4)	-	234 (100.0)
Meropenem (374)	2 (0.5)	0 (0.5)[a]	24 (7.0)	42 (18.2)	58 (33.7)	-	248 (100.0)
Pip/Tazo (374)[c]	0	0	0	0	0	0[a]	374 (100.0)

a. Breakpoint concentration.
b. - = untested concentration.
c. Pip/Tazo = piperacillin/tazobactam.

INTRODUCTION

Several methods for detection of carbapenemases have been proposed in recent years. These methods usually generate false-positive results due to hyperproduction of chromosomal cephalosporinases, antimicrobial inhibitory effects of the enzyme inhibitor alone, or other technical artifacts. Furthermore, some of these methodologies are not suitable for detection of all carbapenemases and different tests for detection of serine-carbapenemases and metallo-β-lactamases would need to be applied.

The Clinical and Laboratory Standards Institute (CLSI) had recently changed the carbapenem breakpoints to lower values categorizing the vast majority of carbapenemase-producing Enterobacteriaceae isolates as resistant to these compounds. However, the breakpoints for some cephalosporins and β-lactam/β-lactamase combinations were not re-evaluated generating confusion for the interpretation of isolates now considered resistant to carbapenems and susceptible to other β-lactam agents. Additionally, the application of the lower carbapenem breakpoints replaced the use of the Modified Hodge test in clinical microbiology laboratories that, according to the current recommendation, should only be used in epidemiologic evaluations of carbapenemase-producing organisms.

In this study, we evaluated the MIC results and current CLSI breakpoints for eight β-lactams tested against 374 KPC-producing Enterobacteriaceae collected during two surveillance studies.

MATERIALS AND METHODS

Bacterial isolates. Enterobacteriaceae clinical isolates were collected from medical centers located in Europe, North and Latin America from bloodstream, respiratory tract and skin and skin structure infections according to defined protocols used in the SENTRY Antimicrobial Surveilance Program and the MYSTIC Program (USA). Only clinically significant isolates were included in the study, one per patient episode. Species identification was confirmed by standard biochemical tests and using the Vitek 2 Systems (bioMérieux; Hazelwood, Missouri, USA), where necessary.

Susceptibility testing. All isolates were susceptibility tested by broth microdilution procedure described by the CLSI (2009) using validated panels (TREK Diagnostics, Cleveland, Ohio, USA). Interpretations of susceptibility testing results were by CLSI (2011) criteria. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were concurrently tested for quality assurance; all results were within the published ranges.

Confirmation of *bla*$_{KPC}$. All isolates analyzed were previously tested for the presence of several carbapenemase-encoding genes by multiplex PCR reactions and were positive for *bla*$_{KPC}$, due to reduced susceptibility to imipenem or meropenem (MIC, ≥2 µg/mL). All strains showing low MIC values for the β-lactam agents evaluated were re-tested for the presence of *bla*$_{KPC}$.

RESULTS

- A total of 374 KPC-producing strains were analyzed. Isolates belonged to nine bacterial species: *Citrobacter freundii* (11), *Enterobacter cloacae* (41), *Enterobacter gergoviae* (2), *E. coli* (14), *Klebsiella oxytoca* (16), *Klebsiella pneumoniae* (283), *Raoultella ornithinolytica* (1), *Raoultella planticola* (2), and *Serratia marcescens* (4).
- According to current CLSI breakpoints, all KPC-producing organisms were categorized as non-susceptible to aztreonam (MIC, >4 µg/mL), ceftriaxone (MIC, >2 µg/mL), ertapenem (MIC, >0.25 µg/mL), imipenem (MIC, >1 µg/mL) and piperacillin/tazobactam (MIC, >32 µg/mL; Tables 1 and 2). Meropenem MIC values for KPC-positive strains at ≤1 µg/mL were rare.
- A total of 52 strains (13.9%; Tables 1 and 2) were categorized as susceptible to cefepime and MIC values as low as 1 µg/mL (MIC range, 1-8 µg/mL) were noted. KPC-producing cefepime-susceptible strains were 20 *K. pneumoniae* (7.1% of all *K. pneumoniae*, Table 2), 11 *K. oxytoca*, seven *E. coli*, seven *C. freundii*, five *E. cloacae* and two *Raoultella planticola*.
- Four *E. coli*, two *K. oxytoca* and two *K. pneumoniae* were susceptible to ceftazidime (8 strains; 2.1%; Tables 1 and 2). Among those organisms, all were cefepime-susceptible and two *K. pneumoniae* strains were also meropenem-susceptible.
- The percentages of *K. pneumoniae* strains susceptible to cefepime and ceftazidime were considerably lower than the entire population of KPC-producers: 7.1 compared to 13.9% susceptible to cefepime and 0.7 compared to 2.1% for *K. pneumoniae* and all species, respectively.

Table 2. Percentages of KPC-producing isolates according to susceptibility categories according to current CLSI breakpoints.

Antimicrobial agent (no. tested)	% by category (*K. pneumoniae* only [no.=283])		
	Susceptible	Intermediate	Resistant
Aztreonam (286)	0.0 (0.0)	0.7 (0.5)	99.3 (99.5)
Cefepime (374)	13.9 (7.1)	16.3 (15.5)	69.8 (77.4)
Ceftazidime (374)	2.1 (0.7)	3.3 (1.8)	94.6 (97.5)
Ceftriaxone (374)	0.0 (0.0)	0.0 (0.0)	100.0 (100.0)
Ertapenem (356)	0.0 (0.0)	0.3 (0.4)	99.7 (99.6)
Imipenem (374)	0.0 (0.0)	3.2 (0.4)	96.8 (99.6)
Meropenem (374)	0.5 (0.7)	6.5 (1.8)	93.0 (98.2)
Piperacillin/Tazobactam (374)	0.0 (0.0)	2.9 (0.3)	97.1 (99.7)

Table 1. MIC Distributions for eight broad-spectrum β-lactams tested against 374 KPC-producing Enterobacteriaceae collected in the SENTRY Antimicrobial Surveillance Program and the MYSTIC Program USA.

Antimicrobial agent/ organism (no. tested)	No. (cumulative %) inhibited at MIC (µg/mL):[a]								
	≤0.25	0.5	1	2	4	8	16	32	64	>[b]
Aztreonam										
All species (286)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.7)	2 (1.4)	-[c]	-	282 (100.0)
K. pneumoniae (201)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	1 (1.0)	-	-	199 (100.0)
Cefepime										
All species (374)	0 (0.0)	0 (0.0)	2 (0.5)	4 (1.6)	10 (4.3)	36 (13.9)	61 (30.2)	-	-	261 (100.0)
K. pneumoniae (283)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.1)	17 (7.1)	44 (22.6)	-	-	219 (100.0)
Ceftazidime										
All species (374)	0 (0.0)	0 (0.0)	1 (0.3)	4 (1.3)	3 (2.1)	12 (5.4)	20 (10.7)	-	-	334 (100.0)
K. pneumoniae (283)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)	1 (0.7)	5 (2.5)	6 (4.6)	-	-	270 (100.0)
Ceftriaxone										
All species (374)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (1.3)	6 (2.9)	19 (8.0)	40.0 (18.7)	-	304 (100.0)
K. pneumoniae (283)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)	9 (3.5)	18 (9.9)	-	255 (100.0)
Ertapenem										
All species (356)	0 (0.0)	1 (0.3)	3 (1.1)	8 (3.4)	26 (10.7)	41 (22.2)	-	-	-	277 (100.0)
K. pneumoniae (275)	0 (0.0)	1 (0.4)	1 (0.7)	1 (1.1)	6 (3.3)	17 (9.5)	-	-	-	249 (100.0)
Imipenem										
All species (374)	0 (0.0)	0 (0.0)	0 (0.0)	12 (3.2)	39 (13.6)	89 (37.4)	-	-	-	234 (100.0)
K. pneumoniae (283)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)	9 (3.5)	63 (25.8)	-	-	-	210 (100.0)
Meropenem										
All species (374)	1 (0.3)	1 (0.5)	0 (0.5)	24 (7.0)	42 (18.2)	58 (33.7)	-	-	-	248 (100.0)
K. pneumoniae (283)	1 (0.4)	1 (0.7)	0 (0.7)	5 (2.5)	9 (5.6)	40 (19.8)	-	-	-	227 (100.0)
Piperacillin/Tazobactam										
All species (374)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.8)	8 (2.9)	363 (100.0)
K. pneumoniae (283)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.4)	282 (100.0)

a. Susceptible MIC range is highlighted in blue.
b. Greater than the highest dilution tested.
c. - = untested concentration.

CONCLUSIONS

- Cefepime MIC values for KPC-producing Enterobacteriaceae were the lowest noted among all β-lactam agents tested and almost 14% of the strains (52/374) were categorized as susceptible according to current CLSI breakpoints (MIC, ≤8 µg/mL). A re-evaluation of cefepime breakpoints by CLSI could be considered, however the breakpoint would need to be lowered to ≤0.5 µg/mL to recognize all KPC-producing strains.
- Current CLSI breakpoints seem to perform better against *K. pneumoniae* strains when compared to other species. This could be explained by the fact that *bla*$_{KPC}$ seems to be better adapted in *K. pneumoniae*, generating greater expression of the gene and consequently higher MIC values.
- Further modification of cefepime and ceftazidime breakpoints to lower MIC values would be contrary to PK/PD principles of adequate target attainment.

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