

# *Klebsiella pneumoniae* Carbapenemase-Producing *Enterobacteriaceae* Testing Susceptible to Cefepime by Reference Methods

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**β-Lactam susceptibility of 499 *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Enterobacteriaceae*, determined by reference broth microdilution, demonstrated that 14.4% of isolates were categorized as cefepime susceptible according to current CLSI breakpoints. Ceftazidime- and meropenem-susceptible isolates were also observed (2.6 and 3.0%, respectively). Cefepime-susceptible KPC-producing isolates may confuse laboratory staff and clinicians in their therapeutic choices.**

Since their first description in 2001, *Klebsiella pneumoniae* carbapenemase (KPC)-producing bacteria have been isolated in microbiology laboratories from health institutions worldwide (1, 2). This carbapenem-hydrolyzing β-lactamase is commonly identified in *Klebsiella* clinical isolates, but it can also be found among other *Enterobacteriaceae*, *Pseudomonas* spp., and *Acinetobacter* spp. (2–4). Several phenotypic methods to detect carbapenemases such as KPC have been proposed (5, 6); however, these methods can generate false-positive results due to concomitant hyperproduction of chromosomal cephalosporinases, antimicrobial inhibitory effects of the enzyme inhibitor alone, or other technical artifacts (6, 7). Furthermore, some of these methodologies are not suitable to identify all carbapenemases, and different tests for detection of serine carbapenemases and metallo-β-lactamases might need to be applied (6).

Evidence that the MICs have a greater significance in predicting clinical outcomes than the presence or absence of β-lactamases and the absence of simple and reliable phenotypic methods to detect carbapenemase-producing *Enterobacteriaceae* clinical isolates prompted the Clinical and Laboratory Standards Institute (CLSI) to modify the carbapenem breakpoints for interpreting susceptibility test results (8, 9). Lower carbapenem MIC breakpoint values were recently recommended, aiming to categorize the vast majority of carbapenemase-producing *Enterobacteriaceae* isolates as resistant to these compounds and eliminating the need for additional phenotypic tests, such as the modified Hodge test (8). However, breakpoints for cefepime and some β-lactam/β-lactamase inhibitor combinations were not changed, and KPC producers might be categorized as susceptible to these antimicrobials, generating confusion for the interpretation of isolates now considered resistant to carbapenems but susceptible to other β-lactam agents (10).

In this study, we evaluated the MICs and current CLSI breakpoints for eight β-lactams tested against 499 KPC-producing enterobacterial isolates collected during an 11-year period as part of two international surveillance studies. Nonduplicated clinical isolates originating from medical centers located in Europe, Asia, and North and Latin America and recovered from bloodstream, respiratory tract, or skin/skin structure infections were included according to defined protocols used by the SENTRY Antimicrobial Surveillance Program and the MYSTIC Program USA. All isolates were identified in the participating medical center and forwarded to a central laboratory (JMI Laboratories, North Liberty, IA), where species identification was confirmed by standard biochem-

ical tests and by using the Vitek 2 systems (bioMérieux, Hazelwood, MO) when necessary. PCR experiments targeting *bla*<sub>KPC</sub> were conducted for all isolates by using previously described primers and cycling conditions (11). Isolates belonged to nine bacterial species: *Citrobacter freundii* (*n* = 13), *Enterobacter cloacae* (*n* = 54), *Enterobacter gergoviae* (*n* = 2), *Escherichia coli* (*n* = 16), *Klebsiella oxytoca* (*n* = 18), *Klebsiella pneumoniae* (*n* = 389), *Raoultella ornithinolytica* (*n* = 1), *Raoultella planticola* (*n* = 2), and *Serratia marcescens* (*n* = 4).

All isolates were susceptibility tested by the CLSI broth microdilution method (12) using validated panels (ThermoFisher Scientific [formerly TREK Diagnostics], Cleveland, OH). Results were interpreted using CLSI criteria (13). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were tested for quality assurance.

All KPC-producing organisms were resistant to aztreonam; however, isolates showing susceptibility to piperacillin-tazobactam (0.6%), ceftriaxone (0.2%), ceftazidime (2.6%), ertapenem (0.4%), imipenem (0.6%), and meropenem (3.0%) were observed (Table 1). Of note, 72 (14.4%) KPC producers were categorized as susceptible to cefepime, including *K. pneumoniae* (*n* = 31), *K. oxytoca* (*n* = 13), *C. freundii* (*n* = 10), *E. cloacae* (*n* = 8), *E. coli* (*n* = 8), and *R. planticola* (*n* = 2), in which cefepime MIC results were as low as 0.5 μg/ml. Cefepime-susceptible isolates included five *E. coli*, four *K. oxytoca*, and four *K. pneumoniae* isolates that were also categorized as ceftazidime susceptible. Among these, two *E. coli* and two *K. pneumoniae* isolates showed meropenem susceptibility, and one *K. pneumoniae* strain was also susceptible to imipenem using the revised CLSI breakpoints (13). All strains showing low MICs for the β-lactam agents tested had the presence of *bla*<sub>KPC</sub> confirmed by multiple PCR experiments (11).

Susceptibility data interpreted using the EUCAST criteria revealed susceptibility rates to cefepime (0.8%) and ceftazidime (0.4%) that were substantially lower than those obtained with

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TABLE 1 MIC distributions for eight broad-spectrum  $\beta$ -lactams tested against 499 KPC-producing *Enterobacteriaceae* isolates collected in the SENTRY Antimicrobial Surveillance Program and MYSTIC Program USA

Antimicrobial agent and organism (no. tested)	No. (cumulative %) inhibited at MIC <sup>a</sup> ( $\mu$ g/ml):										
	$\leq 0.25$	0.5	1	2	4	8	16	32	64	$>64^b$	
<b>Aztreonam</b>											
All species (408)	<b>1</b>	<b>0 (0.0)</b>	<b>0 (0.0)</b>	0 (0.0)	0 (0.0)	3 (0.7)	3 (1.5)	— <sup>c</sup>	—	402 (100.0)	
<i>K. pneumoniae</i> (306)	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>0 (0.0)</b>	0 (0.0)	0 (0.0)	1 (0.3)	2 (1.0)	—	—	303 (100.0)	
Non- <i>K. pneumoniae</i> (102)	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>0 (0.0)</b>	0 (0.0)	0 (0.0)	2 (2.0)	1 (2.9)	—	—	99 (100.0)	
<b>Cefepime</b>											
All species (499)	<b>0 (0.0)</b>	<b>1 (0.2)</b>	<b>3 (0.8)</b>	10 (2.8)	18 (6.4)	40 (14.4)	337 (82.0)	—	—	90 (100.0)	
<i>K. pneumoniae</i> (389)	<b>0 (0.0)</b>	<b>1 (0.3)</b>	<b>0 (0.0)</b>	1 (0.5)	9 (2.8)	20 (8.0)	275 (78.7)	—	—	83 (100.0)	
Non- <i>K. pneumoniae</i> (110)	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>3 (2.7)</b>	9 (10.9)	9 (19.1)	8.0 (37.3)	62 (93.6)	—	—	7 (100.0)	
<b>Ceftazidime</b>											
All species (499)	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>2 (0.4)</b>	3 (1.0)	8 (2.6)	19 (6.4)	28 (12.0)	—	—	439 (100.0)	
<i>K. pneumoniae</i> (389)	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>0 (0.0)</b>	1 (0.3)	3 (1.0)	11 (3.9)	12 (6.9)	—	—	362 (100.0)	
Non- <i>K. pneumoniae</i> (110)	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>2 (1.8)</b>	2 (3.6)	5 (8.2)	8 (15.5)	16 (30.0)	—	—	77 (100.0)	
<b>Ceftriaxone</b>											
All species (499)	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>1 (0.2)</b>	0 (0.0)	5 (1.2)	8 (2.8)	—	—	—	485 (100.0)	
<i>K. pneumoniae</i> (389)	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>1 (0.3)</b>	0 (0.0)	0 (0.0)	1 (0.5)	—	—	—	387 (100.0)	
Non- <i>K. pneumoniae</i> (110)	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>0 (0.0)</b>	0 (0.0)	5 (4.5)	7 (10.9)	—	—	—	98 (100.0)	
<b>Ertapenem</b>											
All species (481)	<b>0 (0.0)</b>	<b>2 (0.4)</b>	8 (2.1)	12 (4.6)	34 (11.6)	58 (23.7)	—	—	—	367 (100.0)	
<i>K. pneumoniae</i> (381)	<b>0 (0.0)</b>	<b>1 (0.5)</b>	4 (1.6)	4 (2.6)	12 (5.8)	27 (12.9)	—	—	—	332 (100.0)	
Non- <i>K. pneumoniae</i> (100)	<b>0 (0.0)</b>	<b>0 (0.0)</b>	4 (4.0)	8 (12.0)	22 (34.0)	31 (65.0)	—	—	—	35 (100.0)	
<b>Imipenem</b>											
All species (497)	<b>0 (0.0)</b>	<b>2 (0.4)</b>	<b>1 (0.6)</b>	<b>0 (0.0)</b>	54 (11.5)	124 (36.4)	—	—	—	316 (100.0)	
<i>K. pneumoniae</i> (387)	<b>0 (0.0)</b>	<b>2 (0.5)</b>	<b>0 (0.0)</b>	<b>0 (0.0)</b>	22 (6.2)	89 (29.2)	—	—	—	274 (100.0)	
Non- <i>K. pneumoniae</i> (110)	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>1 (0.9)</b>	<b>0 (0.0)</b>	32 (30.0)	35 (61.8)	—	—	—	42 (100.0)	
<b>Meropenem</b>											
All species (499)	<b>1 (0.2)</b>	<b>8 (1.8)</b>	<b>6 (3.0)</b>	<b>29 (8.8)</b>	56 (20.0)	76 (35.3)	—	—	—	323 (100.0)	
<i>K. pneumoniae</i> (389)	<b>1 (0.3)</b>	<b>5 (1.5)</b>	<b>3 (2.3)</b>	<b>11 (5.1)</b>	20 (10.3)	51 (23.4)	—	—	—	298 (100.0)	
Non- <i>K. pneumoniae</i> (110)	<b>0 (0.0)</b>	<b>3 (2.7)</b>	<b>3 (5.5)</b>	<b>18 (21.8)</b>	36 (54.5)	25 (77.3)	—	—	—	25 (100.0)	
<b>Piperacillin-tazobactam</b>											
All species (499)	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>(0.0)</b>	<b>0 (0.0)</b>	<b>3 (0.6)</b>	0 (0.0)	3 (1.2)	13 (3.8)	480 (100.0)	
<i>K. pneumoniae</i> (389)	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>3 (0.8)</b>	0 (0.0)	2 (1.3)	3 (2.1)	381 (100.0)	
Non- <i>K. pneumoniae</i> (110)	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>0 (0.0)</b>	0 (0.0)	1 (0.9)	10 (10.0)	99 (100.0)	

<sup>a</sup> Susceptible MIC ranges by CLSI criteria are highlighted in gray; EUCAST susceptibility MIC ranges are in boldface.

<sup>b</sup> Greater than the highest dilution tested.

<sup>c</sup> —, untested concentration.

CLSI criteria (Table 1), while the meropenem susceptibility rate increased considerably (8.8%) (Table 1).

Temporal analysis of susceptibility rates showed that isolates recovered between years 2000 and 2005 were significantly more susceptible to cefepime and ceftazidime than those recovered during the 2006-2010 period ( $P < 0.05$ ) (Table 2). In addition, more recent isolates showed meropenem and piperacillin-tazobactam resistance rates that were significantly higher than those of isolates recovered between years 2000 and 2005 ( $P < 0.05$ ) (Table 2). These results indicate increased extended-spectrum  $\beta$ -lactamase (ESBL) production among isolates recovered during the 2006-2010 period, likely due to *bla*<sub>CTX-M</sub> dissemination in more recent years (14).

The CLSI decision to maintain current cefepime breakpoints was based on reports showing that current standard (or usual)

doses of intravenous cefepime ( $\geq 3$  to 4 g/day) have a high likelihood of achieving optimal exposure against *Enterobacteriaceae* categorized as susceptible to this drug (15, 16). Additionally, it has been reported recently that KPC-2 expression in *Enterobacteriaceae* is not enough to confer cefepime resistance (17). On the other hand, cefepime therapy of infections due to ESBL-producing *Enterobacteriaceae* was recently associated with lower survival rates among patients infected with isolates showing cefepime MICs of  $>1$   $\mu$ g/ml, within the CLSI susceptibility range (18). Therefore, more studies in animal models, pharmacokinetic/pharmacodynamic modeling, and/or focused clinical trials appear necessary to provide conclusive evidence that cefepime is a useful treatment option for infections due to KPC-producing isolates that demonstrate *in vitro* susceptibility to this agent under current CLSI breakpoints.

TABLE 2 Number tested and percentages of KPC-producing isolates according to susceptibility categories assigned using the current CLSI breakpoints

Antimicrobial agent	No. tested (all species/ <i>K. pneumoniae</i> only)		Comparison of percentages by category <sup>a</sup> (all species/ <i>K. pneumoniae</i> only)			
	2000–2005	2006–2010	Susceptible		Resistant	
			2000–2005	2006–2010	2000–2005	2006–2010
Aztreonam	80/42	328/264	0.0/0.0	0.0/0.0	97.5/100.0	99.7/99.6
Cefepime	80/42	419/347	<b>32.5/14.3</b>	<b>11.0/7.2</b>	0.0/0.0	21.5/31.1
Ceftazidime	80/42	419/347	<b>7.5/0.0</b>	<b>1.7/1.2</b>	88.7/95.2	94.5/96.3
Ceftriaxone	80/42	419/347	0.0/0.0	0.2/0.3	100.0/100.0	99.8/99.7
Ertapenem	62/34	419/347	0.0/0.0	0.5/0.6	96.8/100.0	98.1/98.3
Imipenem	80/42	417/345	1.3/0.0	0.5/0.6	98.7/100.0	99.5/99.4
Meropenem	80/42	419/347	2.5/0.0	3.1/2.6	<b>81.2/97.6</b>	<b>93.1/94.5</b>
Piperacillin-tazobactam	80/42	419/347	0.0/0.0	0.7/0.9	<b>91.2/100.0</b>	<b>97.1/97.7</b>

<sup>a</sup> Results in boldface were considered significant ( $P < 0.05$ ); statistical significance of percentages between groups was assessed using the chi-square test.

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We have no speaker bureaus or stock options to declare.

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