JCM Journals.ASM.org

Production of KPC-2 Alone Does Not Always Result in β-Lactam MICs Representing Resistance in Gram-Negative Pathogens

In 2010, the CLSI lowered the susceptibility breakpoints for many cephalosporins and the carbapenems to streamline the screening of potential carbapenemase-producing *Enterobacteriaceae*. Despite this effort, cases of carbapenemase-producing *Enterobacteriaceae* that demonstrate susceptibility to the cephalosporins and the carbapenems have been reported (4). The existence of such isolates suggests that lowering the β-lactam breakpoints is insufficient to detect potential carbapenemase producers, a finding which is important for selection of appropriate therapy. Clinically encountered KPC-producing isolates often produce multiple β-lactamases and may have other resistance mechanisms, such as porin modifications that impact susceptibility to β-lactam antibiotics. This makes it difficult to assess the specific contribution of bla_{KPC} to β-lactam susceptibilities (5, 7, 10). The effect of inoculum concentration on KPC producers has been addressed for the drugs imipenem and meropenem but only using clinical isolates that typically possess multiple β-lactam resistance mechanisms (1, 9). Therefore, the purpose of this study was to examine the impact of KPC-2 alone on β-lactam MICs at both standard and higher inocula.

bla_{KPC} transformants were constructed by cloning the bla_{KPC} structural gene and its upstream promoter region into a low-copy-number, broad-host-range vector before transformation into β-lactam-susceptible strains of *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa* as described previously (8). To examine the influence of bla_{KPC} on antimicrobial susceptibilities, the MICs of ceftazidime, ceftriaxone, cefepime, imipenem, and ertapenem were determined using the CLSI agar dilution method and results were interpreted according to 2010 CLSI breakpoints (2, 3). The observed β-lactam MICs for the bla_{KPC-2} transformants at the recommended inoculum of 10^4 CFU per spot and a high inoculum of 10^6 CFU per spot are shown in Table 1. Although in tests with the standard inoculum, the MICs of cefepime increased for the *Enterobacteriaceae* transformants and the MICs of ceftazidime increased for the *K. pneumoniae* transformant, the increases were not sufficient for the transformants to be categorized as resistant. For all transformants, the MICs of the five β-lactams tested increased above the resistance breakpoint when the higher inoculum was used. An inoculum effect (fold changes ranging from 8- to >64-fold) was observed for the *Enterobacteriaceae* transformants when tested against ceftriaxone, cefepime, imipenem, and ertapenem. An inoculum effect for ceftazidime was observed with *E. coli*, *K. pneumoniae*, and *P. aeruginosa* but not with *E. cloacae*, which demonstrated only a 2-fold increase in MIC. Ceftazidime and imipenem MICs increased 8-fold for the *P. aeruginosa* transformant, but the MICs of ceftriaxone, cefepime, and ertapenem exceeded the upper limit of this assay (512 μg/ml) and the fold increase was not able to be determined.

This study indicates that the activity of the five β-lactam drugs studied, including ertapenem, which is the most frequently used drug for KPC screening, can be affected by variations in inoculum density when testing KPC-producing *E. coli*, *K. pneumoniae*, *E. cloacae*, and *P. aeruginosa*. These data suggest that the MIC of these antibiotics may not be a reliable indicator for the presence of bla_{KPC}. The most recent CLSI guidelines remove the need for clinical laboratories to screen isolates for carbapenemases for therapeutic purposes, recommending such testing as optional for only epidemiological and infection control inquiries. For laboratories that continue to follow the 2009 CLSI guidelines, the marginal increases in ceftazidime and cefepime MICs observed in this study are of particular concern, as resistance to an expanded-spectrum cephalosporin is used as a criterion for further carbapenemase screening. Thus, clinical isolates with similar susceptibility patterns would not meet the criteria for additional screening if ceftazidime or even cefepime were used for initial susceptibility testing. The data presented in this report indicate that clinical isolates that produce only a KPC enzyme in the absence of other mechanisms conferring resistance to oxyimino-cephalosporins and/or carbapenems may not be recognized as possible KPC producers during susceptibility testing. Undetected KPC-only-producing isolates

TABLE 1 Antimicrobial susceptibility with recommended and increased inocula, determined by agar dilution of bla_{KPC} transformants

Species[a]	Inoculum amt	β-Lactam MIC (μg/ml)[b]				
		CAZ	CRO	FEP	IPM	ERT
E. coli	10^4	0.06	0.007	0.06	0.12	0.007
	10^6	0.25	0.015	2	0.25	0.015
E. coli + KPC-2	10^4	16	64	8	8	4
	10^6	256	512	>512	128	256
K. pneumoniae	10^4	0.12	0.015	0.03	0.12	0.007
	10^6	0.25	0.03	0.06	0.5	0.03
K. pneumoniae + KPC-2	10^4	4	32	4	8	4
	10^6	64	>512	512	512	128
E. cloacae	10^4	1	4	0.06	0.12	0.06
	10^6	16	16	0.12	2	0.5
E. cloacae + KPC-2	10^4	16	32	4	2	2
	10^6	32	>512	256	32	64
P. aeruginosa	10^4	1	32	1	1	8
	10^6	2	128	4	2	16
P. aeruginosa + KPC-2	10^4	64	>512	256	4	512
	10^6	512	>512	>512	32	>512

[a] *E. coli* K-12 259, *K. pneumoniae* KP23, *E. cloacae* ATCC 13047, *P. aeruginosa* PAO1.
[b] CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; IPM, imipenem; ERT, ertapenem.

Published ahead of print 12 September 2012
Address correspondence to Nancy D. Hanson, ndhanson@creighton.edu.

doi:10.1128/JCM.02194-12

increase the risk of spread for these isolates and/or the transposon carrying bla_{KPC}. These data support the argument that lowering the breakpoints of certain β-lactam antibiotics may not be sufficient for preventing the spread of KPC-mediated carbapenem resistance (6). Therefore, it is necessary for laboratories to screen for the presence of KPC rather than simply lowering susceptible breakpoints.

ACKNOWLEDGMENT

This research was conducted with support from the Investigator-Initiated Studies Program of Merck & Co., Inc.

REFERENCES

1. **Bratu S, et al.** 2005. Rapid spread of carbapenem-resistant *Klebsiella pneumoniae* in New York City: a new threat to our antibiotic armamentarium. Arch. Intern. Med. **165**:1430–1435.
2. **Clinical and Laboratory Standards Institute.** 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Clinical and Laboratory Standards Institute, Wayne, PA.
3. **Clinical and Laboratory Standards Institute.** 2010. Performance standards for antimicrobial susceptibility testing; 20th informational supplement. CLSI M100-S20. Clinical and Laboratory Standards Institute, Wayne, PA.
4. **Daikos GL, et al.** 2009. Prospective observational study of the impact of VIM-1 metallo-β-lactamase on the outcome of patients with *Klebsiella pneumoniae* bloodstream infections. Antimicrob. Agents Chemother. **53**: 1868–1873.
5. **Kitchel B, et al.** 2010. Genetic factors associated with elevated carbapenem resistance in KPC-producing *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **54**:4201–4207.
6. **Livermore DM, et al.** 2012. Are susceptibility tests enough, or should laboratories still seek ESBLs and carbapenemases directly? J. Antimicrob. Chemother. **67**:1569–1577.
7. **Moland ES, Hong SG, Thomson KS, Larone DH, Hanson ND.** 2007. *Klebsiella pneumoniae* isolate producing at least eight different β-lactamases, including AmpC and KPC β-lactamases. Antimicrob. Agents Chemother. **51**:800–801.
8. **Roth AL, Kurpiel PM, Lister PD, Hanson ND.** 2011. bla_{KPC} RNA expression correlates with two transcriptional start sites but not always with gene copy number in four genera of Gram-negative pathogens. Antimicrob. Agents Chemother. **55**:3936–3938.
9. **Tenover FC, et al.** 2006. Carbapenem resistance in *Klebsiella pneumoniae* not detected by automated susceptibility testing. Emerg. Infect. Dis. **12**: 1209.
10. **Yigit H, et al.** 2001. Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **45**:1151–1161.

Amanda L. Roth
Kenneth S. Thomson
Philip D. Lister
Nancy D. Hanson
Department of Medical Microbiology and Immunology
Creighton University
Omaha, Nebraska, USA