AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

Coproduction of KPC-18 and VIM-1 Carbapenemases by *Enterobacter cloacae*: Implications for Newer β-Lactam–β-Lactamase Inhibitor Combinations

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***Enterobacter cloacae* strain G6809 with reduced susceptibility to carbapenems was identified from a patient in a long-term acute care hospital in Kentucky. G6809 belonged to sequence type (ST) 88 and carried two carbapenemase genes, bla_{KPC-18} and bla_{VIM-1}. Whole-genome sequencing localized bla_{KPC-18} to the chromosome and bla_{VIM-1} to a 58-kb plasmid. The strain was highly resistant to ceftazidime-avibactam. Insidious coproduction of metallo-β-lactamase with KPC-type carbapenemase has implications for the use of next-generation β-lactam–β-lactamase inhibitor combinations.**

Carbapenem-resistant *Enterobacteriaceae* (CRE) has become one of the most urgent threats facing hospitals worldwide due to the limited options available for treatment once infection develops. In the United States, the most common mechanism of carbapenem resistance in *Enterobacteriaceae* is the production of KPC-type carbapenemases (1). Treatment of infections caused by KPC-producing *Enterobacteriaceae* has largely relied on combinations of polymyxins, tigecycline, and other agents, yielding less than optimal clinical outcomes (2). To address this gap, a series of novel β-lactamase inhibitors are in clinical development in combination with various partner β-lactams (3), including avibactam and relebactam, which are diazabicyclooctanes, and RPX7009, which is a cyclic boronic acid compound. They inhibit class A β-lactamases (including KPC-type carbapenemases) and class C β-lactamases (AmpCs) and variably inhibit class D β-lactamases (OXAs), but they do not inhibit class B β-lactamases (metallo-β-lactamases [MBLs]). The ceftazidime-avibactam combination has been approved for the treatment of complicated intra-abdominal infections and complicated urinary tract infections in the United States. Ceftazidime-avibactam is highly active *in vitro* against KPC-producing *Enterobacteriaceae* and has become the standard therapy for CRE infections in some hospitals (4). However, no practical susceptibility testing method has been approved for clinical use; therefore, the agent is used for treatment of CRE infections without susceptibility data in most instances.

Carbapenem resistance due to the production of MBL is still relatively rare in the United States, but hospital outbreaks due to *Pseudomonas aeruginosa* producing VIM-2 MBL and *Klebsiella pneumoniae* and *Escherichia coli* producing NDM-type MBL have been reported (5–7). Since these newer β-lactamase inhibitors do not inhibit MBLs, the β-lactam–β-lactamase inhibitor combinations (BLBLIs) that contain them are not active against MBL-producing bacteria. Here, we report a case of suspected ventilator-associated pneumonia caused by *Enterobacter cloacae* coproducing KPC-type carbapenemase and MBL to highlight this potential caveat.

E. cloacae strain G6809 was isolated from a respiratory specimen of a 58-year-old man who was admitted to a long-term acute

TABLE 1 MICs obtained in tests with *E. cloacae* G6809 isolate (bla_{KPC-18} and bla_{VIM-1} positive) and its bla_{VIM-1}-positive transformant

Drug	MIC[a] with:		
	E. cloacae G6809	*E. coli* TOP10 (pG6809-2)	*E. coli* TOP10
Ticarcillin-clavulanic acid	>128/2	>128/2	≤16/2
Piperacillin-tazobactam	>64/4	>64/4	≤8/4
Cefotaxime	>32	32	≤1
Ceftazidime	>16	>16	≤1
Cefepime	>16	8	≤2
Aztreonam	>16	≤2	≤2
Ertapenem	1.5	0.19	0.004
Imipenem	3	2	0.38
Meropenem	0.75	0.5	0.032
Ceftazidime-avibactam	>256/4	>256/4	0.25/4
Aztreonam-avibactam	0.5/4	≤0.06/4	≤0.06/4
Gentamicin	≤1	≤1	≤1
Tobramycin	8	8	≤1
Amikacin	8	8	≤4
Levofloxacin	>8	≤1	≤1
Ciprofloxacin	>2	≤1	≤1
Trimethoprim-sulfamethoxazole	>4/76	2/38	≤0.5/9.5
Minocycline	8	≤2	≤2
Doxycycline	8	≤2	≤2
Tigecycline	0.5	≤0.25	≤0.25
Polymyxin B	≤0.25	≤0.25	≤0.25
Colistin	≤0.25	≤0.25	≤0.25

[a] The MICs were obtained with broth microdilution (Sensititre GNX2F), except for the carbapenems and ceftazidime-avibactam, which were obtained with Etest, and aztreonam-avibactam, which were obtained with standard broth microdilution.

Received 10 October 2015 **Returned for modification** 3 November 2015
Accepted 22 December 2015
Accepted manuscript posted online 30 December 2015
Citation Thomson GK, Snyder JW, McElheny CL, Thomson KS, Doi Y. 2016. Coproduction of KPC-18 and VIM-1 carbapenemases by *Enterobacter cloacae*: implications for newer β-lactam–β-lactamase inhibitor combinations. J Clin Microbiol 54:791–794. doi:10.1128/JCM.02739-15.
Editor: B. A. Forbes
Address correspondence to Yohei Doi, yod4@pitt.edu.

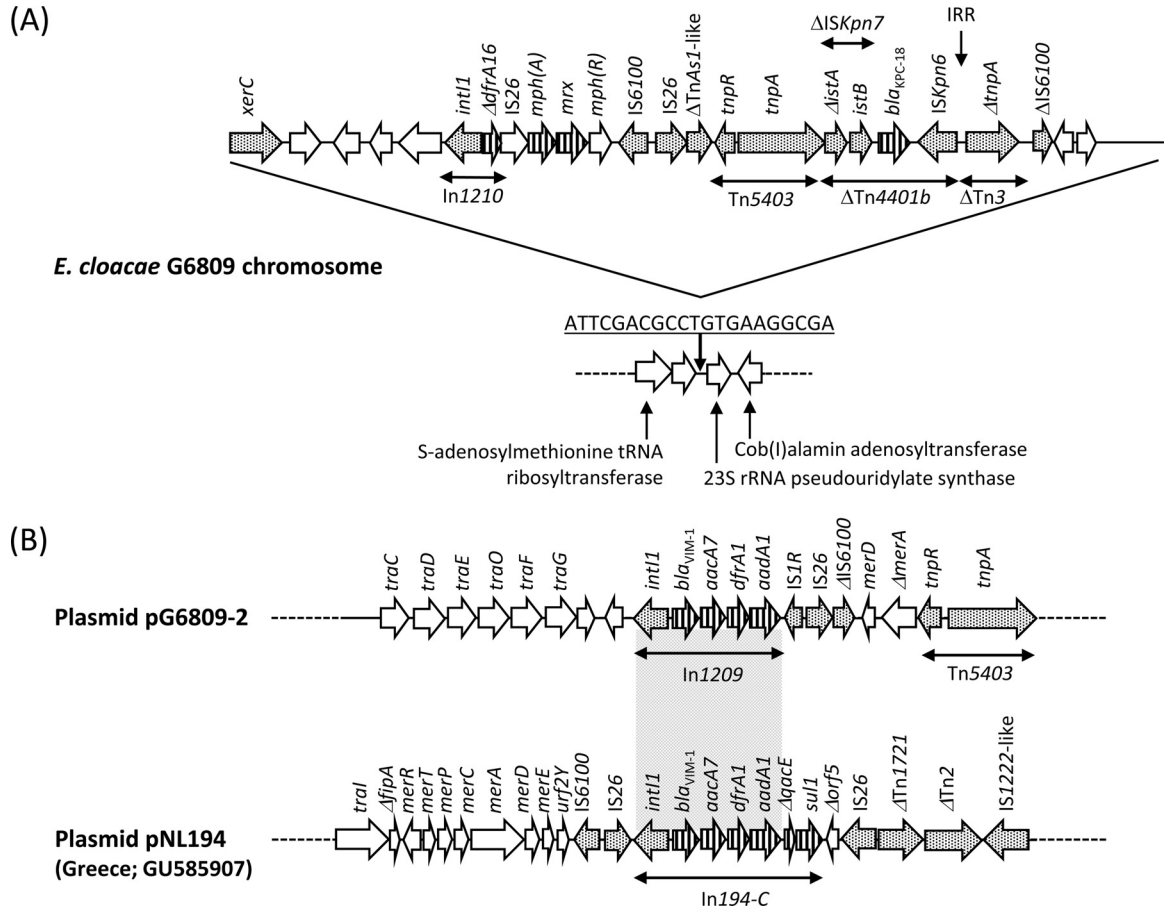

FIG 1 (A) Genetic context of bla_{KPC-18} and bla_{VIM-1} in *Enterobacter cloacae* G6809. Antimicrobial resistance genes are indicated by vertical stripes. Genes involved in transposition are shaded. (B) The region surrounding the class 1 integron in pG6809-2 is compared with that of pNL194, a bla_{VIM-1}-carrying plasmid reported from *K. pneumoniae* in Greece.

care hospital (LTACH) in Kentucky. The strain was subsequently referred to the University of Louisville Hospital for KPC confirmation testing. MICs were determined by a commercial broth microdilution plate (Sensititre GNX2F; Thermo Fisher Scientific, Oakwood Village, OH), except for those of carbapenems, which were tested with Etest (bioMérieux, Durham, NC) (Table 1). *E. cloacae* G6809 was resistant to conventional BLBLIs, including ticarcillin-clavulanic acid and piperacillin-tazobactam and cephalosporins and aztreonam, and to ertapenem and imipenem when applying the latest Clinical and Laboratory Standards Institute breakpoints (8). Notably, the strain was highly resistant to ceftazidime-avibactam with an MIC of >256/4 μg/ml by Etest (bioMérieux). Phenotypic tests with imipenem and Tris-EDTA disks (BD, Franklin Lakes, NJ) were positive for production of both an MBL and a class A or class D carbapenemase (9, 10). The strain was prepared as a 0.5 McFarland standard suspension in saline and was loaded onto a cartridge of a Verigene Gram-negative blood culture test (GN-BC) (Nanosphere, Northbrook, IL) (11). This assay was positive for bla_{KPC} and bla_{VIM} carbapenemase genes. Coproduction of KPC-type carbapenemase and VIM-type MBL has been reported on occasion but is unusual in the United States (12–16). We therefore conducted whole-genome sequencing to elucidate the genetic context of the two carbapenemase genes. The genomic DNA was extracted from the *E. cloacae* G6809 isolate by a DNeasy blood and tissue kit (Qiagen, Valencia, CA), sequenced by RSII (Pacific Biosciences, Menlo Park, CA) at the Yale Center for Genome Analysis, and assembled using SMRT analysis 2.1 (Pacific Biosciences). *De novo* assembly yielded 10 contigs, with an average genome-wide coverage of 172×.

E. cloacae G6809 belonged to sequence type (ST) 88. ST88 was initially assigned to a VIM-1-producing strain found in Greece as a singleton (17). The KPC gene bla_{KPC} was located on a 3.3-Mb contig representing a chromosomal sequence and encoded KPC-18 (GenBank accession no. KP681699; an *E. coli* urinary strain from the United States), which differs from KPC-2 by a single amino acid substitution, V8I. This substitution was located in the signal peptide and was unlikely to affect the kinetic properties of the enzyme. Indeed, when the coding regions of bla_{KPC-18} and bla_{KPC-2} were cloned into pBCSK$^-$ (18), the ertapenem MIC of *E. coli* TOP10 harboring bla_{KPC-18} and bla_{KPC-2} was 0.12 μg/ml for both, compared with 0.003 μg/ml for *E. coli* TOP10 alone. Using *E. cloacae* strain 34977 as the reference genome (GenBank accession no. CP010376), it was apparent that a 29,874-bp sequence was inserted in an intergenic region upstream of the 23S rRNA pseudouridylate synthase gene located on the chromosome, generating 21-bp direct repeats (Fig. 1A). Here, bla_{KPC-18} was located on a Tn*4401b* structure that was truncated at the 5′ end in the *istA* component of IS*Kpn7* by insertion of a Tn*5403*-like trans-

poson. However, the 3′ end of Tn*4401b* was conserved with an intact 32-bp right-inverted repeat sequence (IRR), which in turn truncated Tn*3*. This downstream sequence has been observed in bla_{KPC-2}-carrying IncN plasmids from various *Enterobacteriaceae* species that were involved in an outbreak at the NIH Clinical Center (19), but the upstream sequence consisting of Tn*5403*-like was unique to *E. cloacae* G6809. Chromosomal integration of bla_{KPC} appears to be a relatively rare event overall but has been reported in *K. pneumoniae* (20) and *Acinetobacter baumannii* (21).

E. cloacae G6809 possessed two large plasmids, pG6809-1 (108,462 bp; GenBank accession no. KT345945) and pG6809-2 (58,120 bp; GenBank accession no. KT345946). pG6809-1 was related to p34399, which is a 121-kb plasmid registered from an *E. cloacae* clinical strain from the United States (GenBank accession no. CP010386). It carried a dihydrofolate reductase gene as the only antimicrobial resistance determinant, and the function of the rest of the plasmid was unclear. pG6809-2 was an IncN plasmid that most resembled pNL194, another IncN plasmid that was reported in *K. pneumoniae* from Greece and carried bla_{VIM-1} (22). The structure of the class I integron was similar to that of pNL194 and contained bla_{VIM-1}, *aacA7* [encoding aminoglycoside acetyltransferase AAC(6′)-Ib], *dfrA1b* (encoding dihydrofolate reductase DHFR), and *aadA1b* [encoding aminoglycoside adenylyltransferase ANT(3″)-Ia], whereas the 3′-conserved segment of this integron was missing due to insertion of IS*1R* and IS*26* (Fig. 1B).

E. coli TOP10 transformant carrying pG6809-2 was resistant to BLBLIs, including ceftazidime-avibactam and cephalosporins, and had reduced susceptibility to carbapenems but remained susceptible to aztreonam, which reflected the spectrum of β-lactam hydrolysis by VIM-1 (Table 1) (23). However, *E. cloacae* G6809 was resistant to aztreonam due to the additional production of KPC-18 and the chromosomal AmpC, which together masked this unique susceptibility phenotype that is helpful in identifying MBL-producing organisms. As expected, addition of avibactam restored the susceptibility of *E. cloacae* G6809 to aztreonam with an MIC of 0.5/4 μg/ml, as this combination inhibits MBL, KPC, and AmpC (24).

The advent of a series of novel BLBLIs that inhibit KPC-type carbapenemases has the potential to transform the management of infections caused by KPC-producing *Enterobacteriaceae* for the better. However, this report illustrates the caveat that their activity is lost by coproduction of MBLs, such as VIM-1 in the KPC background, which is difficult to detect in the absence of sophisticated molecular diagnostics. Further complicating this issue is the lack of approved susceptibility testing methods for ceftazidime-avibactam so far, as well as inconsistent breakpoints from CLSI (for ceftazidime, MIC of ≤4 μg/ml for susceptibility) and the FDA (for ceftazidime-avibactam, MIC of ≤8/4 μg/ml for susceptibility). As more novel BLBLI combinations approach late-stage clinical development, we insist that due attention be paid to timely development and validation of the susceptibility testing method along with development of the new agents themselves.

Nucleotide sequence accession numbers. The 100-kb region surrounding bla_{KPC-18} has been annotated and submitted to GenBank under accession number KT884517. The sequences of pG6809-1 and pG6809-2 have been submitted under accession numbers KT345945 and KT345946, respectively.

ACKNOWLEDGMENTS

Y.D. has served on advisory boards for Shionogi, Meiji Seika Pharma, and Tetraphase Pharmaceuticals; consulted for Melinta Therapeutics; and received research funding from Merck and The Medicines Company for studies unrelated to this work. All other authors declare no conflicts of interest.

FUNDING INFORMATION

Office of Extramural Research, National Institutes of Health (OER) provided funding to Yohei Doi under grant number R01AI104895. Office of Extramural Research, National Institutes of Health (OER) provided funding to Yohei Doi under grant number R21AI107302.

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