

Interspecies Spread of *Klebsiella pneumoniae* Carbapenemase Gene in a Single Patient

Hanna E. Sidjabat,^{1,4} Fernanda P. Silveira,¹ Brian A. Potoski,^{1,2}
Kareem M. Abu-Elmagd,³ Jennifer M. Adams-Haduch,¹
David L. Paterson,^{1,4} and Yohei Doi¹

¹Division of Infectious Diseases, ²Antibiotic Management Program, and ³Division of Transplant Surgery, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania; and ⁴University of Queensland Centre for Clinical Research and Royal Brisbane and Women's Hospital, Brisbane, Australia

(See the editorial commentary by Munoz-Price and Quinn, on pages 1739–41.)

***Klebsiella pneumoniae* carbapenemase (KPC)–producing *K. pneumoniae*, *Escherichia coli*, and *Serratia marcescens* were sequentially identified in a patient who underwent small bowel transplantation. Molecular typing and plasmid analysis suggested that the KPC gene was acquired by *E. coli*, most likely from *K. pneumoniae*, and was subsequently transferred to *S. marcescens*.**

The emergence and spread of carbapenem-resistant Enterobacteriaceae poses a major clinical and public health challenge [1]. Especially concerning are organisms that produce *Klebsiella pneumoniae* carbapenemase (KPC)–type β -lactamase. After the initial report in 2001 [2], KPC-producing *K. pneumoniae* first spread in the Northeastern United States, then spread worldwide, causing outbreaks in hospitals. At present, KPC-producing Enterobacteriaceae have been reported from at least 10 countries in 4 continents. KPC-producing organisms are typically resistant to multiple classes of antibiotics, including carbapenems, cephalosporins, fluoroquinolones, and aminoglycosides. Infections due to these pathogens have been associated with high mortality rates [3].

Although the majority of cases that have been reported were due to *K. pneumoniae*, KPC-type β -lactamase has also been identified in various species of Enterobacteriaceae, such as *Esch-*

erichia coli and also *Pseudomonas* species [4–7]. The KPC gene is located on transposon *Tn4401* on a transferable plasmid [8]. These genetic attributes likely facilitate dissemination of the KPC gene from *K. pneumoniae* to the aforementioned species.

We here describe a patient who had sequential infections with 3 species of carbapenem-resistant Enterobacteriaceae, all of which produced KPC-type β -lactamase, and characterize the genetic relationship of these isolates.

Case report. The patient was a 44-year-old woman who underwent small bowel transplantation in 2005 for short gut syndrome, which had resulted from dissection of the mesenteric artery during hysterectomy. She had a prolonged hospital course complicated with multiple episodes of infection after the transplantation. Despite immunosuppression with tacrolimus, she experienced 3 episodes of rejection that were treated with corticosteroids, the last of which occurred 9 months before the current presentation. The patient was admitted to our institution in June 2008 with bacteremia due to *E. coli* and *Enterobacter cloacae*. She was initially treated with piperacillin-tazobactam and a single dose of amikacin and was then treated with cefepime. Because of the persistence of *E. coli* bacteremia, antibiotic therapy was later changed to meropenem, which led to clearance of the bacteremia. In July 2008, *E. coli* and *Proteus mirabilis* grew from cultures of urine specimens, and the patient was again treated with cefepime. In August 2008, *E. coli* (EC1) and *K. pneumoniae* (KP1) grew from the same urine specimen. EC1 and KP1 were both resistant to ertapenem, whereas KP1 was also resistant to imipenem. A month later, the patient required graft enterectomy because of rejection. The abdominal collection at the time grew *E. coli* (EC2) and *K. pneumoniae* (KP2), both of which were resistant to ertapenem and imipenem and susceptible to tigecycline. The patient was treated with meropenem and tigecycline for this episode. In November 2008, a sputum sample obtained from the patient grew *Pseudomonas aeruginosa* and later *Serratia marcescens* and *K. pneumoniae*. Piperacillin-tazobactam and cefepime were administered for these episodes. In December 2008, the patient had another episode of *P. aeruginosa* pneumonia. The isolate was resistant to multiple antibiotics, which necessitated treatment with colistimethate, piperacillin-tazobactam, and aerosolized tobramycin. In January 2009, a sputum sample grew *S. marcescens* resistant to ertapenem and imipenem (SM). The patient was successfully treated with tigecycline, to which the isolate was susceptible. In the following month, the patient developed a new episode of pneumonia due to multidrug-resistant *Acinetobacter baumannii* and *Morganella morganii*. Therapy with colistimethate, doripenem, and aerosol-

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Reprints or correspondence: Dr Yohei Doi, Div of Infectious Diseases, Dept of Medicine, University of Pittsburgh Medical Center, S829 Scaife Hall, 3550 Terrace St, Pittsburgh, PA 15261 (yod4@pitt.edu).

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Table 1. Carbapenem Susceptibility of *Klebsiella pneumoniae* Carbapenemase (KPC)-Producing Clinical Isolates and their Transformants

Carbapenem	Minimum inhibitory concentration, $\mu\text{g/mL}$								
	Clinical isolate					Transformant			
	KP1	EC1	KP2	EC2	SM	KP2	EC2	SM	DH10B
Ertapenem	>32	8	>32	>32	>32	16	1.5	8	0.047
Meropenem	>32	0.25	>32	>32	>32	12	1.5	8	0.047
Imipenem	>32	0.75	>32	>32	>32	32	6	16	0.25
Doripenem	>32	1.5	>32	>32	>32	4	2	2	0.047
KPC-3 gene	+	-	+	+	+	+	+	+	-

NOTE. DH10B, the recipient *Escherichia coli* strain; EC1, *E. coli* isolate 1; EC2, *E. coli* isolate 2; KP1, *Klebsiella pneumoniae* isolate 1; KP2, *K. pneumoniae* isolate 2; SM, *Serratia marcescens* isolate.

ized colistimethate was given. In March 2009, the patient had an episode of *S. marcescens* bacteremia. The isolate obtained during this episode was susceptible to cefepime as well as carbapenems, and the patient recovered after a course of cefepime. Another episode of *K. pneumoniae* bacteremia, which was resistant to ertapenem and imipenem and only susceptible to colistin, occurred in April 2009 and was successfully treated with ampicillin-sulbactam and colistimethate.

Methods. Five ertapenem-resistant isolates belonging to 3 different species (2 *K. pneumoniae* [KP1, KP2], 2 *E. coli* [EC1, EC2], and 1 *S. marcescens* [SM]) were collected and characterized. Determination of the minimum inhibitory concentrations of carbapenems (ertapenem, imipenem, meropenem, and doripenem) for these isolates was conducted using Etest (AB Biodisk). Phenotypic detection of KPC production was performed using the modified Hodge test, as well as the method using 3-aminophenyl boronic acid [9, 10].

Polymerase chain reaction (PCR) and sequencing of TEM-, SHV-, CTX-M-, CMY-2-, and KPC-type β -lactamase genes was performed as described elsewhere [9, 11]. These are broad-spectrum β -lactamase genes that are commonly encountered in Enterobacteriaceae. Transfer of KPC-encoding plasmids to laboratory strains of *E. coli* was conducted by both transformation of extracted plasmids and conjugation [11]. *E. coli* DH10B transformants with KPC-encoding plasmids were selected on Luria Bertani (LB) agar plates containing meropenem at 0.5 $\mu\text{g/mL}$. *E. coli* J53 transconjugants with KPC-encoding plasmids were selected on LB plates containing sodium azide at 50 $\mu\text{g/mL}$ and meropenem at 0.5 $\mu\text{g/mL}$. The presence of the KPC gene was confirmed by PCR amplification. The plasmids carrying the KPC gene was digested with HindIII and PstI (New England Biolabs) and subjected to electrophoresis and hybridization using digoxigenin-labeled DNA probe specific for KPC-type β -lactamase gene (Roche Diagnostics). The primer walking method was used to analyze the nucleotide sequences flanking transposon *Tn4401*. Pulsed-field elec-

trophoresis (PFGE) was performed, followed by cluster analysis for quantification of similarities, to determine genetic relatedness of the clinical isolates [11].

Results. Of the 5 ertapenem-resistant isolates that were investigated, all except *E. coli* EC1 were highly resistant to all carbapenems tested (ertapenem, imipenem, meropenem and doripenem), with MICs >32 $\mu\text{g/mL}$. *E. coli* EC1 had an ertapenem MIC of 8 $\mu\text{g/mL}$ but was susceptible to other carbapenems (Table 1). PFGE showed only minor variation of restriction patterns between the 2 *K. pneumoniae* isolates (KP1 and KP2) and between the 2 *E. coli* isolates (EC1 and EC2) (data not shown). All isolates except *E. coli* EC1 had phenotypic test results positive for KPC production and were positive for the presence of the KPC-3 gene by PCR amplification and sequencing. KPC-3 is 1 of the 2 common KPC variants along with KPC-2. This gene was carried on *Tn4401b*, an isoform of transposon *Tn4401* [8]. In addition, EC1 and EC2 were positive for the CMY-44 gene, which encoded a variant of CMY-2. Ertapenem resistance in CMY-2-producing *E. coli* has been reported previously and associated with the loss of major outer membrane proteins [12, 13].

The carbapenem resistance of KPC-producing *K. pneumoniae*, *E. coli*, and *S. marcescens* was successfully transferred to recipient *E. coli* using both transformation and conjugation.

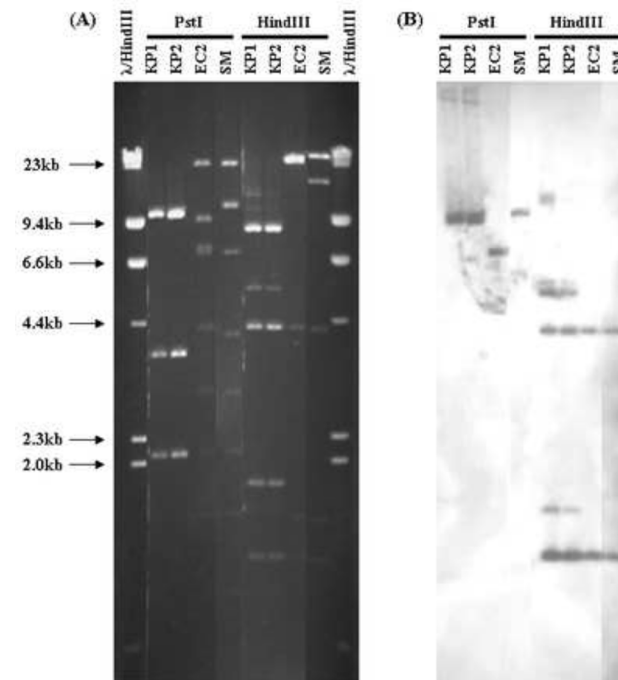


Figure 1. A, Plasmid restriction profile of the 4 *Klebsiella pneumoniae* carbapenemase (KPC)-producing isolates. B, DNA hybridization profile using the KPC gene probe. λ /HindIII, molecular weight marker; EC2, *Escherichia coli* isolate 2; KP1, *K. pneumoniae* isolate 1; KP2, *K. pneumoniae* isolate 2; SM, *Serratia marcescens* isolate.

The *E. coli* DH10B transformants from KP2, EC2, and SM, all producing KPC-3, showed elevated MICs of carbapenems, compared with the recipient strain (Table 1).

The plasmid carrying the KPC-3 gene was identical in the 2 *K. pneumoniae* isolates (KP1 and KP2) but differed from those in the *E. coli* and *S. marcescens* isolates (Figure 1). The plasmids carrying the KPC-3 gene in the second *E. coli* isolate (EC2) and the *S. marcescens* isolate (SM) shared a similar restriction pattern. Hybridization of KPC-bearing plasmids from the 3 species revealed that the restricted plasmid fragments that contained the KPC-3 gene were identical for restriction enzyme HindIII, which is known to digest within the transposon *Tn4401*, and various sizes for PstI, which does not digest within *Tn4401* (Figure 1). Nucleotide sequences flanking *Tn4401* on the *K. pneumoniae* plasmid showed similarity to the KPC-carrying plasmid in *K. pneumoniae* strain S15 that was identified in New York City [14]. In both *E. coli* and *S. marcescens*, *Tn4401* was inserted into another transposon, *Tn3*.

Discussion. We report identification of KPC-3 β -lactamase from 3 species of Enterobacteriaceae obtained from the same patient over a 5-month period. Our experimental evidence, as well as the sequence of clinical events, suggests that the patient initially acquired KPC-producing *K. pneumoniae*, at which time the accompanying *E. coli* in the urine did not produce KPC-3. The KPC-3 gene was then likely mobilized from the plasmid in *K. pneumoniae* to that in *E. coli* as the result of a *Tn4401*-mediated recombination event, given the distinct plasmid profiles. Finally, the gene was likely transferred to *S. marcescens* by conjugation of the KPC-encoding plasmid in *E. coli*.

The global spread of carbapenem-resistant Enterobacteriaceae is increasingly recognized as an emerging threat to public health [1]. Of these pathogens, *K. pneumoniae* that produce KPC-type β -lactamase has spread worldwide within a decade after its discovery. Most recently, KPC-producing isolates that are resistant to colistin and tigecycline have also been reported [15]. On the other hand, under selective pressure from the use of broad-spectrum antibiotics, the KPC gene has spread not only geographically but also horizontally into other species of Enterobacteriaceae. For example, a cluster of KPC-producing *E. coli* has been identified in multiple nursing homes in New York City [5]. It is now well recognized that *E. coli* that produce CTX-M, a specific group of extended-spectrum β -lactamase, has spread into communities worldwide in recent years and are causing community-acquired infections that are difficult to manage [16]. We therefore fear that the capacity of *E. coli* to acquire the KPC gene from *K. pneumoniae*, as observed in the present case, has the potential to lead to dissemination of carbapenem-resistant *E. coli* into the community, as well. Appropriate measures to effectively control the spread of these highly resistant pathogens are needed to prevent significant morbidity and mortality as well as public health consequences.

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