

## Colistin-Resistant, *Klebsiella pneumoniae* Carbapenemase (KPC)–Producing *Klebsiella pneumoniae* Belonging to the International Epidemic Clone ST258

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**Five cases of infection due to colistin-resistant, *Klebsiella pneumoniae* carbapenemase–producing *K. pneumoniae* belonging to the international epidemic clone ST258 occurred over a 4-month period. These cases likely represented both emergence of resistance and transmission of resistant organism. The colistin-resistant isolates were able to persist in the absence of selective pressure in vitro.**

*Klebsiella pneumoniae* that produces *K. pneumoniae* carbapenemase (KPC) has rapidly spread across hospitals worldwide in the past decade [1]. KPC-producing *K. pneumoniae* isolates are typically resistant to carbapenems as well as penicillins, cephalosporins, fluoroquinolones, and frequently also aminoglycosides. Infection due to KPC-producing *K. pneumoniae* is therefore commonly treated with a regimen containing colistin [1]. However, development of resistance to colistin has been reported [2–4]. We here report 5 cases of infection due to colistin-resistant, KPC-producing *K. pneumoniae*, 4 of which occurred in the same intensive care unit, over the course of 4 months.

## METHODS

Three colistin-susceptible and 5 colistin-resistant KPC-producing *K. pneumoniae* isolates (including 3 pairs of susceptible and resistant isolates from the same patients) were collected from patients who were admitted to the transplant intensive care unit (TICU; patients A–D) and a patient who was admitted to a medical ward (patient E) between February and May 2010 (Table 1). The TICU and the medical ward are located in 2 hospitals that are connected with each other through several passages.

Minimum inhibitory concentrations (MICs) of various antimicrobials were determined by the agar dilution according to the guidelines from the Clinical and Laboratory Standards Institute [5]. KPC-type  $\beta$ -lactamase gene was identified by polymerase chain reaction and sequencing. Pulsed-field gel electrophoresis (PFGE) was performed using *SpeI* restriction enzyme to determine genetic relatedness of the clinical isolates. In addition, multilocus sequence typing (MLST) was performed as described elsewhere [6]; sequence types were determined using the *K. pneumoniae* MLST Web site (<http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html>).

Growth competition experiments were performed using 2 pairs of colistin-susceptible and colistin-resistant isolates from the same patients (isolates B1/B2 and D1/D2) in Luria-Bertani broth in the absence of colistin. In brief, an equal number of colony-forming units (CFUs) from both isolates ( $\sim 10^6$  CFUs/mL) were mixed at time 0 hours and incubated at 37°C with agitation. Every 24 hours, the bacterial mixture was diluted 1/1000 with prewarmed Luria-Bertani broth, and the percentage of colistin-resistant isolates was estimated by inoculating colistin-free and colistin-containing (8  $\mu$ g/mL) agar plates. As controls, the same experimental procedure was undertaken using pure cultures of colistin-susceptible and colistin-resistant isolates. This procedure was repeated for 10 consecutive days.

## RESULTS

The isolates from all 5 patients harbored the KPC gene. Sequencing confirmed it to be KPC-2 in all cases. These KPC-producing *K. pneumoniae* isolates were resistant to carbapenems (ertapenem, imipenem, meropenem, and doripenem), cephalosporins, penicillins, and aztreonam (Table 2). The 3 KPC-producing, colistin-susceptible isolates exhibited a colistin MIC of 1  $\mu$ g/mL and were also susceptible to tigecycline (MIC,

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**Table 1. Characteristics of the Patients and Isolates**

Patient	Age in years	Sex	Hospitalization dates	Diagnosis	Location (unit, hospital)	Isolate	Specimen	Isolation date	Colistin therapy, days/DDD	MIC, $\mu\text{g/mL}$	Sequence type	KPC	PFGE	Outcome
A	63	Male	13 Jan 2010–10 Mar 2010	Cholangitis, OLTx	Surgical, A	A1	Urine	22 Jan 2010	None	1	258	2	A1	Survived
					TICU, A	A2	Wound	10 Mar 2010	14/19.2	>128	258	2	A1	
B	25	Female	18 Jan 2010–18 Sep 2010	Hemorrhagic pancreatitis, multivisceral Tx	TICU, A	B1	Blood	20 Mar 2010	None	1	258	2	A1	Survived
					TICU, A	B2	Wound	13 Apr 2010	18/48.3	>128	258	2	A1	
C	65	Male	11 Mar 2010–18 Mar 2010, 20 Mar 10–21 Apr 2010	Cholangitis, OLTx	Surgical, A	C	Blood	20 Mar 2010	None	>128	258	2	A1	Survived
D	42	Male	25 Feb 2010–23 May 2010	Intra-abdominal abscess, OLTx	TICU, A	D1	Blood	3 Apr 2010	None	1	258	2	A1	Died
					TICU, A	D2	Blood	17 Apr 2010	12/27.6	128	258	2	A1	
E	41	Female	22 Mar 2010–25 Mar 2010, 16 May 2010–20 May 2010	TBI, UTI	Medicine, B	E	Urine	16 May 2010	None	128	258	2	A2	Survived

**NOTE.** MIC, minimum inhibitory concentration; OLTx, orthotopic liver transplantation; Tx, transplantation; TBI, traumatic brain injury; TICU, transplant ICU; DDD, defined daily dose; ST, multilocus sequence type; PFGE, pulse-field gel electrophoresis. The study patients were located in the same units during the following periods of time:

-A + B: 1/18/10–1/20/10, 2/22/10–2/27/10

-A + B + D: 2/25/10–2/27/10

-B + D: 2/27/10–3/1/10, 3/4/10–3/10/10, 3/16/10–3/17/10, 4/5/10–5/17/10

-A + D: 3/1/10–3/4/10

-B + C: 3/12/10–3/14/10, 3/22/10–3/27/10, 3/28/10–3/30/10

-C + D: 3/14/10–3/18/10, 3/20/10–3/22/10, 3/27/10–3/28/10

-B + C + D: 3/30/10–4/5/10

**Table 2. Minimum Inhibitory Concentrations of Colistin-Susceptible and -Resistant *K. pneumoniae* ( $\mu\text{g/mL}$ ).**

Patient	Isolate	Date	TZP	FEP	ATM	ERT	IPM	MEM	DOR	GEN	AMK	CIP	TGC	CST
A	A1	1/22/10	>128/4	>128	>128	>128	>128	>128	128	16	64	64	2	1
A	A2	3/10/10	>128/4	>128	>128	>128	>128	>128	128	>128	64	128	2	>128
B	B1	3/20/10	>128/4	>128	>128	>128	128	128	128	>128	64	64	2	1
B	B2	4/13/10	>128/4	32	>128	64	16	32	8	>128	64	64	1	>128
C	C	3/20/10	>128/4	32	>128	32	8	8	4	8	64	128	1	>128
D	D1	4/3/10	>128/4	>128	>128	>128	64	128	64	>128	64	64	2	1
D	D2	4/17/10	>128/4	>128	>128	>128	64	128	64	>128	64	64	0.5	128
E	E	5/16/10	>128/4	>128	>128	>128	64	128	64	32	64	128	1	128

**NOTE.** TZP, piperacillin/tazobactam; FEP, cefepime; ATM, aztreonam; ERT, ertapenem; IPM, imipenem; MEM, meropenem; DOR, doripenem; GEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin; TGC, tigecycline; CST, colistin.

2  $\mu\text{g/mL}$ ). Their 3 colistin-resistant counterparts exhibited colistin MICs of  $\geq 128$   $\mu\text{g/mL}$  but remained susceptible to tigecycline.

The isolates from patients A–D were identical by PFGE, including both colistin-susceptible and colistin-resistant isolates (data not shown). Isolate E from patient E was different by 4–6 bands from the isolates from patients A–D and was considered to be possibly related to them. By MLST, isolates from all patients were found to belong to clone ST258.

The in vitro competition experiments demonstrated that both colistin-susceptible and colistin-resistant isolates persisted in cultures in the absence of colistin for the entire 10 days. For the isolate pair B1 and B2, the initial inocula were  $9.2 \times 10^6$  CFUs/mL and  $9.9 \times 10^6$  CFUs/mL, respectively. On day 10 of daily passages without colistin, isolate B1 reached an inoculum of  $9.8 \times 10^8$  CFUs/mL (97%) whereas the inoculum of isolate B2 was  $2.9 \times 10^7$  CFUs/mL (3%). For the isolate pair D1 and D2, the starting inocula were  $8.8 \times 10^6$  CFUs/mL and  $10 \times 10^6$  CFUs/mL, respectively, with isolate D1 growing to an inoculum of  $1.3 \times 10^9$  CFUs/mL after 10 passages (93%) compared with  $9.4 \times 10^7$  CFUs/mL for isolate D2 (7%). Passage of the resistant isolates alone in the absence of colistin in the medium for 10 days showed stable colistin resistance.

## DISCUSSION

KPC-producing *K. pneumoniae* has become a major hospital pathogen. Colistin, which had been in disuse for decades due to concerns about toxicity and availability of safer antimicrobial agents, now constitutes a first-line regimen for treatment of infection caused by this organism. With the increased use of colistin, emergence of colistin resistance has been reported. Multiclonal clusters of colistin-resistant *K. pneumoniae* isolates have been described from hospitals in Greece and South Korea [7, 8]. Emergence of colistin or polymyxin B resistance among KPC-producing *K. pneumoniae* during therapy with these agents has also been reported from hospitals in New York City, New

York, where the organism is epidemic [2, 9]. More recently, clonal outbreaks of colistin-resistant, KPC-producing *K. pneumoniae* were reported from Greece, the United States, and Hungary, the latter of which was attributed to the ST258 clone [3, 4, 10].

This report documents a cluster of colistin-resistant organisms belonging to the international epidemic clone ST258 in the United States. It is increasingly recognized that ST258 is the predominant clone of KPC-producing *K. pneumoniae* that is distributed in the United States as well as worldwide [11, 12]. The use of colistin at our hospital had been stable between 2008 and the time of the cases described here. Colistin use was at 35.06 defined daily doses (DDDs) per 1000 patient-days in 2008 and 30.82 DDDs per 1000 patient-days in 2009. Monthly colistin use from January through May 2010 was as follows: 27.13, 24.82, 33.68, 42.08, and 43.62 DDDs per 1000 patient-days, respectively. Nonetheless, 3 patients grew colistin-resistant KPC-producing *K. pneumoniae* while receiving colistimethate, the intravenous formulation of colistin, for 12–18 days. These events therefore may represent de novo emergence of colistin resistance. However, all of the initial colistin-susceptible and subsequent colistin-resistant isolates had identical PFGE profiles and belonged to the same sequence type, making it difficult to rule out the possibility of horizontal spread of resistant isolates. Our review of the cases showed that, while the patients were not in immediate proximity with each other, the 4 patients in the TICU (patients A–D) were cared for by the same medical teams. The fourth patient (patient C) had no previous colistin exposure and likely acquired a colistin-resistant isolate through transmission in the same unit. Finally, the fifth patient (patient E) also had not been treated with colistin and thus likely acquired the resistant organism from an unidentified source during a previous hospital stay. Whereas patient B remained bacteremic with colistin-resistant KPC-producing *K. pneumoniae* for several months thereafter, no new clusters of colistin-resistant *K. pneumoniae* have been found since. Nonetheless, along with other recent reports of infections caused by KPC-2–producing *K. pneumoniae*

belonging to the ST258 clone from various parts of the world, our findings attest to its high capability for successful spread.

Development of colistin resistance under experimental conditions frequently involves alterations in 2-component regulatory systems and modifications of lipopolysaccharide [13, 14]. These changes have the potential to negatively affect the fitness of colistin-resistant organisms, especially in the absence of selective pressure from this agent. However, the colistin-resistant organisms were not completely overgrown by colistin-susceptible ones after 10 days of passage in vitro in the present study, which implies that colistin resistance in clinical isolates may occur through pathways that do not substantially compromise fitness. This implication, together with our clinical observation, raises the possibility that clinically selected colistin-resistant organisms, once emerged, have a potential to persist in the patients and the hospital environment and cause subsequent transmission. With the continued use of colistin for treatment of infection with various multidrug-resistant Gram-negative pathogens, it is likely that we will see an increasing number of instances of both de novo emergence of resistance and nosocomial spread.

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