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CASE REPORTS

Transfer of KPC-2 Carbapenemase from *Klebsiella pneumoniae* to *Escherichia coli* in a Patient: First Case in Europe▽

Sara N. Richter,[1,2]* Ilaria Frasson,[1] Cristina Bergo,[2] Saverio Parisi,[1,2] Antonietta Cavallaro,[2] and Giorgio Palù[1,2]

Department of Histology, Microbiology and Medical Biotechnologies, via Gabelli 63, 35121 Padua, Italy,[1] and Azienda Ospedaliera di Padova, Microbiology and Virology Unit, via Giustiniani 2, 35121 Padua, Italy[2]

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The first case in Europe of *Klebsiella pneumoniae* carbapenemase (KPC) 2 transfer from *K. pneumoniae* to *Escherichia coli* in the same patient is described. KPC-positive plasmids from the two species were identical, indicating horizontal plasmid transfer. Selection of the KPC-producing *E. coli* strain was triggered by therapy with meropenem.

CASE REPORT

In April 2010, a 55-year-old male Italian patient with a history of epilepsy, alcoholism, and hypertension was admitted to the intensive care unit of the Saint Antony Hospital (ICU-SAH) of Padua after suffering a stroke. The patient had extensive left frontoparietal hemorrhage, which required evacuative craniotomy and a transitory tracheotomy. Microbiological analysis isolated *Klebsiella pneumoniae* and *Escherichia coli* strains from a blood sample. MIC values were measured by the Vitek 2 automated system (bioMérieux, Hazelwood, MO) and are reported in Table 1. According to the CLSI standards (4), the *K. pneumoniae* isolate was fully resistant to carbapenems (imipenem and meropenem MICs of ≥16 mg/liter, 26 April, Table 1), while the *E. coli* isolate was fully susceptible (imipenem MIC of ≤1 mg/liter, 26 April, Table 1). The patient, who had not undergone any previous antimicrobial therapy, was treated with gentamicin (240 mg/day for 13 days). After 2 days of therapy, the patient's hemoculture yielded no microbial growth.

In August 2010, the patient was transferred to the intensive care unit of the Teaching Hospital (ICU-TO) of Padua for respiratory arrest, which required endotracheal intubation. At that time, microbiology tests revealed the presence of *Staphylococcus aureus* and carbapenem-susceptible *E. coli* in bronchoalveolar lavage (BAL) fluid and nasal swab samples, respectively (23 August, Table 1). At the beginning of September, the patient developed hyperthermia (>38°C), and blood and urine cultures grew *Staphylococcus epidermidis* and carbapenem-susceptible *E. coli* (imipenem MIC of ≤1 mg/liter), respectively (2 September, Table 1). To cure these infections, the patient initially received ceftriaxone (2 g/day for 3 days), which was replaced with meropenem (3 g/day for 7 days) and teicoplanin (400 mg on the first day and 200 mg/day for the next 4 days). Six days later, surveillance microbiological tests of a skin sample were positive for both *Pseudomonas aeruginosa* and *E. coli* (8 September, Table 1). The latter isolate had an increased imipenem MIC (4 mg/liter) that, interpreted according to CLSI criteria (4), indicated resistance to the drug. At that time, the presence of *K. pneumoniae* carbapenemase (KPC) was phenotypically confirmed by the modified Hodge test (5). The patient was isolated and treated with a wide-spectrum empirical therapy of daptomycin and fluconazole (500 and 200 mg/day, respectively, for 10 days). In addition, the bladder and central venous (right subclavian) catheters were replaced.

At the end of September, a BAL fluid culture still grew *P. aeruginosa* and *E. coli*. They were both resistant to imipenem (MICs of ≥8 and 4 mg/liter, respectively, 27 September, Table 1). Nine days later, the *E. coli* isolate in the BAL fluid showed further increased resistance to imipenem (MIC of ≥16 mg/liter, 6 October, Table 1). The patient was treated with levofloxacin (750 mg/day for 10 days) and up to December 2010, clinical samples showed no clinically relevant microbial growth.

The first isolate of *K. pneumoniae* (26 April) and the last isolates of *E. coli* and *P. aeruginosa* (6 October and 27 September, respectively) were genotypically analyzed using specific primers (12) to check for the presence of carbapenemase-mediated resistance; amplicon sequencing confirmed the presence of the bla_{KPC-2} gene in both the *K. pneumoniae* and *E. coli* strains but not in the *P. aeruginosa* strain. The three strains were further analyzed for the presence of additional mechanisms of resistance to β-lactams. In particular, the *bla* genes for TEM-, SHV-, CTX-, IMP-, VIM-, NMC/IMI-, SME-, SPM-, and OXA-type carbapenemases and β-lactamases were tested by PCR amplification and sequencing (21). The bla_{TEM-1} and bla_{OXA-9} genes of class A and D β-lactamases, respectively (2), were found in both *K. pneumoniae* and *E. coli*; the former also contained the bla_{VIM-1} gene, while *P. aeruginosa* did not present any of the β-lactamases tested for.

* Corresponding author. Mailing address: Department of Histology, Microbiology and Medical Biotechnologies, University of Padua, Via Gabelli 63, 35121 Padua, Italy. Phone: 390498272346. Fax: 390498272355. E-mail: sara.richter@unipd.it.

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FIG. 1. Plasmid analysis of KPC-2-positive *K. pneumoniae* and *E. coli* isolates. Plasmids from clinical strains were purified and transformed into *E. coli* Top10 cells. After plasmid purification of the transformed KPC-2-positive colonies, plasmids were loaded onto 1% agarose gel before (nondigested, ND lanes) or after (digested, D lanes) digestion with EcoRI and HindIII. K stands for *K. pneumoniae*, and E stands for *E. coli*. M is a molecular size marker of 75 to 20,000 bp (GeneRuler 1 kb Plus DNA Ladder; Fermentas).

Multilocus sequence typing analysis was performed according to reported guidelines (11) on *K. pneumoniae* and *E. coli*, which were shown to belong to sequence type 147 (ST147) and ST9, respectively. The former was circulating in the ICU-SAH, where the patient had been initially hospitalized (unpublished data), and many of these strains were KPC positive. No information about the presence of *E. coli* ST9 in our hospital was available, but no other phenotypically imipenem-resistant strains were isolated during that period of time in the two hospitals.

Purified plasmids from *K. pneumoniae* and *E. coli* were transformed into an *E. coli* Top10 (Invitrogen) recipient strain and selected on plates containing 100 mg/liter ampicillin; screening for bla_{KPC}-positive colonies showed that, in both cases, *E. coli* Top10 was transformed with a 20-kb plasmid, which encoded KPC-2, bla_{TEM-1}, and bla_{OXA-9}, and not bla_{VIM-1}, which was located on a different plasmid. Plasmids from the transformed colonies were purified and digested with the HindIII and EcoRI restriction enzymes (Fermentas Life Sciences, Milan, Italy). The restriction patterns were identical, indicating that the *K. pneumoniae* and *E. coli* clinical isolates contained the same plasmid that bore the KPC-2 resistance gene (Fig. 1).

The presence of this plasmid increased the MICs of imipenem and meropenem, measured by Etest (AB Biodisk, Solna, Sweden), from 0.25 and 0.32 mg/liter to 3 and 1 mg/liter, respectively. In addition, transformed Top10 gained resistance to all β-lactam antibiotics.

Bacteria producing KPCs are rapidly emerging as a cause of multidrug-resistant infections worldwide. Bacterial isolates harboring these enzymes are capable of hydrolyzing a broad spectrum of beta-lactams, including penicillins, cephalosporins, carbapenems, and monobactams (19).

TABLE 1. Antimicrobial susceptibilities of bacterial strains found from April to October 2010 in the indicated samples from the case patient

Date of isolation	Species	Sample	MIC (mg/liter)[a]																
			IPM	MER	AMP	AMX	FEP	CFZ	CTX	CAZ	PIP	TZP	OXA	TGC	GEN	AMK	SXT	NIT	CIP	LVX	NOR	ERY	CLI
26 Apr	*K. pneumoniae*	Blood	≥16	≥16	≥32	≥32	≥64	≥64	≥64		≥128	≥128		2	4	≥64	≥320			≥8			
26 Apr	*E. coli*	Blood	≤1	≤0.25	≥32	8		≤4			≥128	≤4			≤1		≥320			1			
23 Aug	*S. aureus*	BAL fluid											≥4		≤0.5		≤10			≥8		≤0.25	≤0.25
23 Aug	*E. coli*	BAL fluid	≤1	≤0.25	≥32	≥32	≤1				≥128	≥128			≤1		≥320						
2 Sep	*S. epidermidis*	Blood						≤2					≤0.25		≤2		≤10			≤1		≤0.25	≤0.5
2 Sep	*E. coli*	Urine	≤1	≤0.25	≥32	≥32	8	≥64	≥64		≥128	≥128			≥16	16	≤20	≤16			≥16		
8 Sep	*P. aeruginosa*	Skin	≥8	8			8			4	16	8			≤1	≤2	160		1				
8 Sep	*E. coli*	Skin	4	1	≥32	≥32	4	≥64	4	4	≥128	≥128		≤0.5	≤1	4	160	≤16		1	2		
27 Sep	*P. aeruginosa*	BAL fluid	≥8	8			8			4	16	8		≤1	≤1	≤2	160		2				
27 Sep	*E. coli*	BAL fluid	4	1	≥32	≥32	4	≥64	4	4	≥128	≥128		≤0.5	≤1	4	≥320	≤16		1	2		
6 Oct	*E. coli*	BAL fluid	≥16	1	≥32	≥32	4	≥64	4	4	≥128	≥128		≥0.5	≤1	4	≥320	≥16		1	1		

[a] IPM, imipenem; MER, meropenem; AMP, ampicillin; AMX, amoxicillin-clavulanic acid; FEP, cefepime; CFZ, cefazolin; CTX, cefotaxime; CAZ, ceftazidime; PIP, piperacillin; TZP, piperacillin-tazobactam; OXA, oxacillin; TGC, tigecycline; GEN, gentamicin; AMK, amikacin; SXT, sulfamethoxazole-trimethoprim; NIT, nitrofurantoin; CIP, ciprofloxacin; LVX, levofloxacin; NOR, norfloxacin; ERY, erythromycin; CLI, clindamycin.

In the last few years, KPCs have been detected in nosocomial *Enterobacteriaceae* (mainly *K. pneumoniae*) and *P. aeruginosa*. Epidemiological studies have highlighted that the great majority of KPC-producing isolates of *K. pneumoniae* are clonally related (17). However, bla_{KPC} has been reported to usually reside on a transmissible element (Tn*4401*) (15) and has been recovered in other genera of bacteria (20). In *E. coli*, KPCs have been described in only a few cases, especially in the United States, where they were reported first and subsequently, (1, 7, 10, 13), in Israel (9, 16), in China (3, 14), and very recently in Brazil (6).

To our knowledge, this is the second report of a KPC-positive *E. coli* in Europe; the first one was reported in France from a patient initially hospitalized in Israel (18). In addition, *K. pneumoniae* belonging to ST147 has been shown for the first time here to bear KPC-mediated resistance. Transfer of KPC-3 from *K. pneumoniae* to *E. coli* within the same patient has been reported only once and very recently in Israel (8). Here we show the transmission of KPC-2 from *K. pneumoniae* to *E. coli* within the same patient. Interestingly, resistance of the *E. coli* strain to imipenem could be found only 5 months after the initial detection of a blood infection by the KPC-positive *K. pneumoniae* strain. The patient was transferred from ICU-SAH to ICU-TO, which is located in a different hospital complex within the city of Padua. *K. pneumoniae* with KPC-2 has been isolated in ICU-SAH (unpublished data), while no such strains have been found in ICU-TO so far. Hence, we argue that the horizontal transfer of a KPC-2 plasmid between *K. pneumoniae* and *E. coli* happened in April, while the two strains coexisted in the blood of the patient. They were subsequently both cleared from the blood, but *E. coli* probably colonized other organs, where it was later found. *E. coli* initially retained susceptibility to carbapenems, but subsequent therapy with meropenem selected those *E. coli* cells that contained the KPC-2 plasmid (found 6 days after the beginning of carbapenem therapy).

Finally, although an increasing number of reports have detected the acquisition of KPC-mediated resistance by the non-enterobacterial species *P. aeruginosa* (17), no sign of horizontal transfer of a KPC plasmid from *E. coli* was found in a patient coinfected with these two bacterial species.

Treatment of infection caused by KPC-positive bacteria is particularly worrisome, as the carbapenems are often regarded as agents of last resort for resistant Gram-negative infections; optimal treatment of infections caused by KPC-positive bacteria is not well established yet, and clinical outcome data remain scarce. In addition, the phenotypic detection of carbapenemase production remains difficult, a fact that has undoubtedly contributed to KPC dissemination. So far, only molecular techniques can positively detect the presence of bla_{KPC} genes in clinical isolates. Rapid routine detection of KPCs is not only particularly needed for *K. pneumoniae* but should be promptly extended (in an infected patient, as well as in patients hospitalized in the same care unit) to all bacterial species reported to bear this resistance mechanism in order to optimize antibiotic therapy, limit KPC-mediated resistance spread worldwide, and therefore increase patient survival.

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