In vivo multiclonal transfer of bla$_{KPC-3}$ from Klebsiella pneumoniae to Escherichia coli in surgery patients

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Abstract

During active surveillance at the Mediterranean Institute for Transplantation and Advanced Specialized Therapies (ISMETT, Palermo, Italy) with the CARBA screening medium, five pairs of Klebsiella pneumoniae carbapenemase (KPC)-producing K. pneumoniae and Escherichia coli strains were isolated in each of five colonized patients. In each patient, lateral gene transfer was demonstrated by comparing K. pneumoniae and E. coli strains, both possessing KPC-3, Tn4401a and pKpQIL-IT elements. The isolates were found to be multiclonal by multilocus sequence typing (sequence type (ST) 512 related to ST258, and ST307 belonging to a clonal complex different from the habitual sequence clone ST258 isolated in Italy) and pulsed-field gel electrophoresis. The results of our study highlight the easy transfer of KPC among Enterobacteriaceae colonizing the human intestine, and the active and careful surveillance required to identify and prevent the spread of these multidrug-resistant microorganisms.

Keywords: Colonization, Escherichia coli, Klebsiella pneumoniae, KPC, ST307
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Over the last 10 years, multidrug-resistant (MDR) Gram-negative Enterobacteriaceae has posed a substantial challenge to the treatment of nosocomial infections, worsening the outcome of hospitalized patients, particularly in those at high risk, such as organ transplant recipients and patients undergoing specialized surgery. In Italy, from 2009 to 2012, carbapenem-resistant Klebsiella pneumoniae diffusion rose from 2.2% to 19.4%, with a prevalence of K. pneumoniae carbapenemase (KPC) enzymes [1].

The diffusion of KPC-producing K. pneumoniae strains followed two different lines: (i) in the beginning, a unique clone, ST258, was isolated in a number of countries around the world; (ii) recently, new extensively drug-resistant clones have appeared, evidence of the dissemination, by horizontal transfer, of the mobile genetic elements carrying the bla$_{KPC}$ gene. In fact, KPC enzymes are codified by genes frequently located in the Tn4401, which is often carried on conjugative plasmids that vary in size and structure [2,3]. In Italy, the most frequent KPC-carrying plasmid is pKpQIL-IT [4]. The horizontal transferability of these elements, together with clonal expansion of these MDR organisms, poses complex challenges to containment programmes and the planning of correct therapies. In fact, infections sustained by MDR organisms harbouring bla$_{KPC}$ genes are associated with therapeutic failure and high mortality rates [5,6], particularly in high-risk patients.

In the light of this situation, an active search for KPC producers colonizing immunocompromised patients, and patients on the waiting list for transplant, is urgently required.

In May 2013, our institute, the Mediterranean Institute for Transplantation and Advanced Specialized Therapies (IS-METT), following an active rectal swab surveillance programme with chromID CARBA agar (bio-Merieux Clinical Diagnostics, Marcy l'Etoile, France), five pairs of KPC-producing K. pneumoniae (KPC Kp) and Escherichia coli (KPC Ec) strains were isolated from each of five patients (two who underwent organ transplantation, and three who underwent cardiac surgery). At admission, these patients were colonized with KPC Kp, and two developed bloodstream infection with KPC Kp.

All rectal swabs at admission were inoculated on chromID CARBA agar, and the suspected colonies with a pink/brown

TABLE 1. Typing characterization of isolates by pulsed-field gel electrophoresis (PFGE) and multilocus sequence type (MLST)

Patients	Species	PFGE	ST
426814	Escherichia coli	A	131
426814	Klebsiella pneumoniae	B	512
427835	E. coli	B	1672
427835	K. pneumoniae	B	512
427862	E. coli	B	1672
427862	K. pneumoniae	B	512
426010	E. coli	B	1672
426010	K. pneumoniae	A	307
429422	E. coli	C	394
429422	K. pneumoniae	A	307

colour were identified and tested for antibiotic susceptibility with the BD Phoenix™ Automated Microbiology System, (Becton Dickinson, Franklin Lakes, NJ). Susceptibility results of all the strains were confirmed for imipenem, meropenem and ertapenem by both microdilution and gradient test methods (bio-Merieux). MIC breakpoints were interpreted according to the European Committee on Antimicrobial Susceptibility Testing guidelines [7]. All isolates were confirmed by phenotypic testing for carbapenemase with aminophenyl boronic acid and dipicolinic acid (Rosco Diagnostica, Taastrup, Denmark). The results of the phenotypic confirmation test were all positive for KPC. The resistant determinants (bla_{KPC} and extended spectrum b-lactamase-encoding genes), and the localization of bla_{KPC} (Tn4401 and plasmid), were revealed by PCR assay, using previously described primers [3].

Conjugation experiments were carried out on E. coli J-53 as recipient and the transconjugants were selected with meropenem (4 mg/L) and rifampicin (300 mg/L). The identification of b-lactamase genes was performed by PCR [8]. No transconjugants were obtained. In particular, the transconjugant obtained with K. pneumoniae n. 429422 and E. coli J-53 showed a very slight growth, insufficient for molecular verification, so we could not determine conjugation.

Typing characterizations were performed by using pulsed-field gel electrophoresis and multilocus sequence typing, as previously described [9,10].

The five strains of E. coli and K. pneumoniae presented multiclonal macrorestriction profiles by pulsed-field gel electrophoresis, performed after XbaI digestion [11]. The pulsed-field gel electrophoresis clones coincided with sequence types (STs) found by multilocus sequence typing (Table 1).

In E. coli, three pulsed field gel electrophoresis clones and three different STs were found: clone A was ST131, clone B was ST1672, and clone C was ST394 [12]. The two pulsotypes of K. pneumoniae (type A in two strains, and type B in three strains) coincided with sequence types (STs) (clone A ST512 and clone B ST307) [12]. In particular, clone ST512 is a single locus variant of ST258, already found in other Italian hospitals [13], whereas there is no information on the presence of K. pneumoniae ST307 in Italy.

Table 1 also shows the b-lactamase-encoding genes of the isolates. All strains were confirmed to harbour the bla_{KPC-3} and the bla_{TEM-1} genes. The only KPC Ec frankly resistant also contained $bla_{CTX-M-15}$. The same genes were found in the co-cultured K. pneumoniae of the same patient. Plasmid analysis was performed using extraction (QIAGEN Plasmid Mini Kit; Qiagen, Les Ulis, France) and direct sequencing (3500 Genetic Analyzer; Applied Biosystems, Carlsbad, CA), and revealed that bla_{KPC-3} genes were in all cases embedded in a Tn4401a transposon, and plasmid sequences matched for the presence of the pKpQIL-IT in all strains under study [14].

Escherichia coli ST394 was previously not a KPC producer but harboured extended spectrum b-lactamase genes ($bla_{CTX-M-15}$ and bla_{TEM-1}); after 2 weeks the same clone was isolated and was a KPC producer.

Among patients nos 427835, 427862 and 426010, KPC-3-producing E. coli was acquired by cross-transmission

TABLE 2. Susceptibility to carbapenems measured by three methods of Klebsiella pneumoniae carbapenemase (KPC)-producing strains

			MICs of carbapenems (mg/L)								
			Ertapenem BP (0.5/1)			Meropenem BP (2/8)			Imipenem BP (2/8)		
Patients	Species	Isolation date	Ph	BMD	Gradient test	Ph	BMD	Gradient test	Ph	BMD	Gradient test
426814	Escherichia coli	5/3/2013	>1	1	0.75	≤1	0.5	2	≤1	0.5	8
	K. pneumoniae		>1	128	>32	>8	>32	>32	>8	>32	>32
427835	E. coli	5/10/2013	>1	4	1.5	≤1	1	0.75	4	1	3
	K. pneumoniae		>1	128	>32	>8	>32	>32	>8	64	>32
427862	E. coli	5/15/2013	>1	4	2	≤1	4	2	4	1	4
	K. pneumoniae		>1	128	>32	>8	64	>32	>8	64	>32
426010	E. coli	4/30/2013	>1	1	1.5	≤1	2	0.75	4	0.5	3 I
	K. pneumoniae		>1	128	>32	>8	256	>32	>8	256	>32
429422	E. coli	4/27/2013	<1	0.12	0.25	<1	0.12	0.06	<1	0.12	0.12
	K. pneumoniae		>1	128	>32	>8	512	>32	>8	512	>32
	E. coli	5/11/2013	>1	>128	>32	>8	128	32	>8	64	32
	K. pneumoniae		>1	128	>32	>8	512	>32	>8	512	>32

Ph, Phoenix; BMD, microdiluition broth; BP, breakpoint.

during hospitalization in the same ward. The route of acquisition of both KPC producer strains is undetermined for patient no. 426814 because he was positive at the admission date and, moreover, the strain belonged to a different clone (ST131). Table 2 summarizes the susceptibility testing of all isolates. All K. pneumoniae strains were resistant to all carbapenems with all methods used, whereas KPC Ec, possessed lower MIC values compared with those observed in K. pneumoniae. In the KPC Ec strains, the automated Phoenix system showed reduced susceptibility values to meropenem and imipenem, with the sole exception of one isolate, fully resistant to the three carbapenems with all methods. In the other four strains, all susceptible by the Phoenix system, gradient testing revealed a reduced susceptibility to carbapenems, with the exception of ertapenem, which showed a resistant or intermediate level with all methods used. In the light of these findings, this drug was used as the marker of resistance.

Our paper highlights the simple, and worrisome, in vivo inter-species transfer of pKpQIL-IT containing the bla_{KPC} gene. This event can be underappreciated and underreported because of the low expression level of this resistance determinant in a genetic background different from K. pneumoniae, as already observed [15]. Even if little is known about the role that gene expression plays in KPC-mediated resistance, or how the level of expression may affect susceptibility testing, several hypotheses have emerged: (i) some studies have reported a possible presence of different isoforms of Tn4401 with different upstream promoters involved in different degrees of expression [14]; (ii) the KPC gene can be located in a low number of plasmid copies, as reported in previous studies [15]. Further studies will be necessary to fully understand this low level of expression in KPC Ec.

The ease of in vivo transfer of KPC between K. pneumoniae and E. coli is extremely worrisome, and our study strengthens the importance of infection control measures for rapid detection of KPC in nosocomial pathogens, in order to prevent further dissemination and, in case of infection, provide direct targeted therapy.

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Transparency Declaration

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