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CASE REPORT

Isolation of KPC 3-producing *Enterobacter aerogenes* in a patient colonized by MDR *Klebsiella pneumoniae*

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SUMMARY

We describe the interspecies transmission of the plasmid-mediated bla_{KPC-3} gene, which confers carbapenem resistance, between clinically relevant gram-negative bacteria in a single patient. A KPC-3 producing *Enterobacter aerogenes* was isolated from a hospitalized patient previously colonized and then infected by a *Klebsiella pneumoniae* ST101 carrying the bla_{KPC-3} gene. The strains showed identical plasmids. Since intense horizontal exchanges among bacteria can occur in the gut, clinicians should be aware that patients colonized by carbapenem-resistant *K. pneumoniae* could become carriers of other carbapenem-resistant Enterobacteriaceae.

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Carbapenem-resistant Enterobacteriaceae (CRE) are a growing public health problem in Italy (Giani *et al.,* 2013). Resistance to carbapenems in gram-negative bacteria is mostly due to the acquisition of carbapenemase genes belonging to Ambler classes A, B and D beta-lactamases (Queenan *et al.,* 2007). The Class A *Klebsiella pneumoniae* carbapenemases (KPC) are the most common, and were first reported in 2001 (Yigit *et al.,* 2001). While carbapenem resistance among *Klebsiella* species has been widely studied, few clinical and epidemiological investigations have addressed carbapenem resistance in *Enterobacter* spp. (Mezzatesta *et al.,* 2012), despite reports of KPC production in *E. aerogenes* in areas where KPC-producing *K. pneumoniae* are routinely identified (Marchaim *et al.,* 2008).

The aim of the present work was to characterize multidrug-resistant (MDR) K. *pneumoniae* and *E. aerogenes* strains isolated from a single patient, and to highlight the circulation between these isolates of a common plasmid conferring carbapenem resistance.

An active surveillance programme for the detection of CRE has been in place at the 'Lazzaro Spallanzani' National Institute for Infectious Diseases in Rome, Italy, since 2013. According to this programme, patients admitted to the intensive care unit (ICU) and medical wards are screened for CRE colonisation by rectal swabs taken at admission; ICU patients only are subsequently tested once weekly.

Key words:
Magnusiomyces capitatus, Pleural infection, Galactomannan antigen, MALDI-TOF identification, Posaconazole.

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Samples are cultured on a selective medium designed to screen for carbapenemase producers (chromID CARBA, bioMèrieux, Marcy l'Etolie, France). Antibiotic susceptibility is determined by the Vitek-2 System AST-N202 (bioMèrieux, France). Minimum inhibitory concentrations (MICs) are interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations. Confirmatory phenotypic inhibition tests for detection of carbapenemase production are then performed using phenyl boronic acid (PBA) and EDTA (Liofilchem, Italy) as previously described (Tsakris *et al.,* 2009).

Six *K. pneumoniae* and one *E. aerogenes* isolates obtained from a single patient were collected from August 2013 to February 2014. The patient was initially admitted to the neurosurgical ICU of a tertiary care hospital in Rome after undergoing surgical drainage of a spontaneous cerebral haemorrhage. During his hospital stay the patient became an MDR *K. pneumoniae* rectal carrier. He subsequently developed severe carbapenem-resistant *K. pneumoniae* sepsis. MDR *K. pneumoniae* was isolated from multiple sites, including blood, urine and from a subcutaneous groin abscess where a femoral central-vascular catheter used for haemodialysis had been placed (*Table 1*). Carbapenem-susceptible *E. aerogenes* was also isolated from the subcutaneous groin abscess. Subsequently, the patient was transferred to the ICU of 'the Lazzaro Spallanzani' National Institute for Infectious Diseases. During his ICU stay, the patient was also found to be HIV-infected. The antimicrobial treatment for the *K. pneumoniae* sepsis, which included colistin, tigecycline, aminoglycosides, and carbapenems at higher dosages, proved effective, allowing his transfer to a medical unit of the Institute. During his stay in the medical unit, MDR *Enterobacter aerogenes* was detected from a rectal swab, as a consequence of the periodic screening policy. No further infectious complications

Table 1 - *Summary of clinical data and susceptibility pattern of clinical isolates.*

Strain	*Date of isolation*	*Clinical specimen*	*Clinical ward*	*Identification*	*Antibiotic MIC values (mg/L)*[1]														
					AMK	*AMC*	*FEP*	*CTX*	*CAZ*	*CIP*	*CST*	*ERT*	*FOF*	*GEN*	*IMP*	*MEM*	*TZP*	*TGC*	*SXT*
128	06/10/13	Rectal swab	ICU	K. pneumoniae	4	≥32	2	8	≥64	≥4	3	4	32	≤1	≥16	≥16	≥128	1	≥320
134	17/10/13	Rectal swab	ICU	K. pneumoniae	4	≥32	2	8	≥64	≥4	3	4	32	≤1	≥16	≥16	≥128	1	≥320
2060	17/10/13	blood	ICU	K. pneumoniae	≥64	≥32	2	8	≥64	≥4	3	≥8	≤16	≥16	≥16	≥16	≥128	≤0,5	≥320
2105Kp	07/11/13	blood	ICU	K. pneumoniae	≥64	≥32	2	8	≥64	≥4	16	≥8	≤16	≥16	≥16	≥16	≥128	≤0,5	≥320
708	08/12/13	blood	ICU	K. pneumoniae	4	≥32	2	8	≥64	≥4	16	≥8	≤16	≤1	≥16	≥16	≥128	1	≥320
708B	13/12/13	Abscess culture	ICU	K. pneumoniae	≤2	≥32	2	8	≥64	≥4	6	≥8	≤16	≤1	≥16	≥16	≥128	≤0,5	≥320
210	16/01/14	Abscess culture	II	E. aerogenes	≥64	≥32	≤1	≤1	≤1	≤0,25	≤0,5	≤0,5	≤16	≥16	0,5	≤0,25	≤4	1	≥320
163E	06/02/14	Rectal swab	II	E. aerogenes	≥64	≥32	2	8	≥64	≤0,25	≤0,5	≥8	≤16	≥16	≥16	≥16	≥128	1	≥320

[1]MICs were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria. Susceptibility MICs are highlighted in gray shading.
Abbreviations: AMK, amikacin; AMC, amoxicillin-clavulanic; FEP, cefepime; CTX, cefotaxime; CAZ, ceftazidime; CIP, ciprofloxacin; CST, colistin; ERT, ertapenem; FOF, fosfomycin, GEN; gentamicin; IMP, imipenem; MEM, meropenem; TZP, piperacillin-tazobactam; TGC, tigecycline, SXT, trimethoprim-sulfamethoxazole. ICU: Intensive Care Unit.

were observed, and the patient was finally transferred to a rehabilitation unit.

All *K. pneumoniae* isolates were highly resistant to all beta-lactams tested, including carbapenems and penicillin/inhibitor combinations, colistin and ciprofloxacin. Two isolates were also resistant to aminoglycosides (*Table 1*). The *E. aerogenes* was resistant to carbapenems and aminoglycosides, but susceptible to colistin and ciprofloxacin. Identification of genes encoding carbapenemases (bla_{VIM} , bla_{IMP} , $bla_{NDM,}$ bla_{KPC}, bla_{OXA-48}, bla_{BIC}, bla_{SPM}, bla_{SIM}, bla_{GIM}, bla_{DIM}, bla_{AIM} genes), extended-spectrum ß-lactamases (ESBLs) (such as bla_{SHV}, $bla_{TEM,}$ $bla_{C\text{-}TX\text{-}M}$) and plasmid-mediated *ampC* beta-lactamases was performed by PCR as previously described (Mabilat *et al.,* 1990; Pérez *et al.,* 2002; Woodford *et al.,* 2006; Poirel *et al.,* 2011). Sequences were analyzed using the BioEdit software and BLAST tool (http://www.ncbi.nlm.nih.gov/BLAST). As reported in *Table 2*, PCR screening and sequencing results showed that *K. pneumoniae* and *E. aerogenes* isolates carried the $bla_{KPC\text{-}3}$ and $bla_{TEM\text{-}1}$ genes; the six *K. pneumoniae* isolates were positive for the intrinsic, chromosomally located $bla_{SHV\text{-}1}$ gene, as expected. Multilocus sequence typing (MLST) was carried out according to Diancourt *et al.,* (2005) and sequence types were analyzed using the Institut Pasteur database (http://www.pasteur.fr/recherche/genopole/PF8 /mlst/). Analyses revealed that all *K. pneumoniae* isolates belonged to the epidemic clone sequence type 101 (ST101). Further analyses by PCR-based replicon typing (PBRT) (DIATHEVA, Italy) (Carattoli *et al.,* 2005) showed that plasmids carried by the *K. pneumoniae* and *E. aerogenes* isolates were positive for the $IncFII_K$ replicon, and two strains of *K. pneumoniae* (2105Kp and 128) also harboured the IncR-type replicon.

One of the six *K. pneumoniae* isolates (2105Kp) and the only *E. aerogenes* 163E were chosen as prototypes to compare plasmids carrying the $bla_{KPC\text{-}3}$ gene. Plasmid DNAs were purified with the PureLinkTM HiPure Plasmid Filter Midiprep Kit (Invitrogen, USA) and transformed into competent *E. coli* DH5α cells (Invitrogen, USA). PCR analysis (Villa *et al.,* 2010) on transformants (TF) 2105Kp and 163E revealed that the $bla_{KPC\text{-}3}$ and $bla_{TEM\text{-}1}$ genes were located on the $IncFII_K$-type plasmid. In particular, sequence analysis showed that $IncFII_K$ replicon sequences were homologous (100% identity) to those identified in the pKpQIL $IncFII_K$ plasmid (GU595196) (15). The identification of the pKpQIL-like plasmid, carrying the $bla_{KPC\text{-}3}$ in ST101, is an interesting finding. This plasmid is the most frequently identified carrier of bla_{KPC}-like genes in the most commonly spread *K. pneumoniae* clone ST258. Plasmids purified from TF2105Kp and TF163E transformants were then compared by EcoRI and PstI restriction fragment length polymorphism (RFLPs) analysis. The plasmids isolated by transformation from the ST101 *K. pneumoniae*

Table 2 - *Summary of genetic features of clinical isolates and transformants.*

Strain	*Identification*	*Resistance determinats*	*PCR-based replicon typing*	*Sequence Type*
128	*K. pneumoniae*	$bla_{KPC\text{-}3}$, $bla_{TEM\text{-}1}$, $bla_{SHV\text{-}1}$	R, FIIk	101
134	*K. pneumoniae*	$bla_{KPC\text{-}3}$, $bla_{TEM\text{-}1}$, $bla_{SHV\text{-}1}$	FIIk	101
2060	*K. pneumoniae*	$bla_{KPC\text{-}3}$, $bla_{TEM\text{-}1}$, $bla_{SHV\text{-}1}$	FIIk	101
708	*K. pneumoniae*	$bla_{KPC\text{-}3}$, $bla_{TEM\text{-}1}$, $bla_{SHV\text{-}1}$	FIIk	101
708B	*K. pneumoniae*	$bla_{KPC\text{-}3}$, $bla_{TEM\text{-}1}$, $bla_{SHV\text{-}1}$	FIIk	101
2105Kp	*K. pneumoniae*	$bla_{KPC\text{-}3}$, $bla_{TEM\text{-}1}$, $bla_{SHV\text{-}1}$	R, FIIk	101
163E	*E. aerogenes*	$bla_{KPC\text{-}3}$, $bla_{TEM\text{-}1}$	FIIk	ND
TF2105Kp		$bla_{KPC\text{-}3}$, $bla_{TEM\text{-}1}$	FIIk	ND
TF163E		$bla_{KPC\text{-}3}$, $bla_{TEM\text{-}1}$	FIIk	ND

ND: not determined.

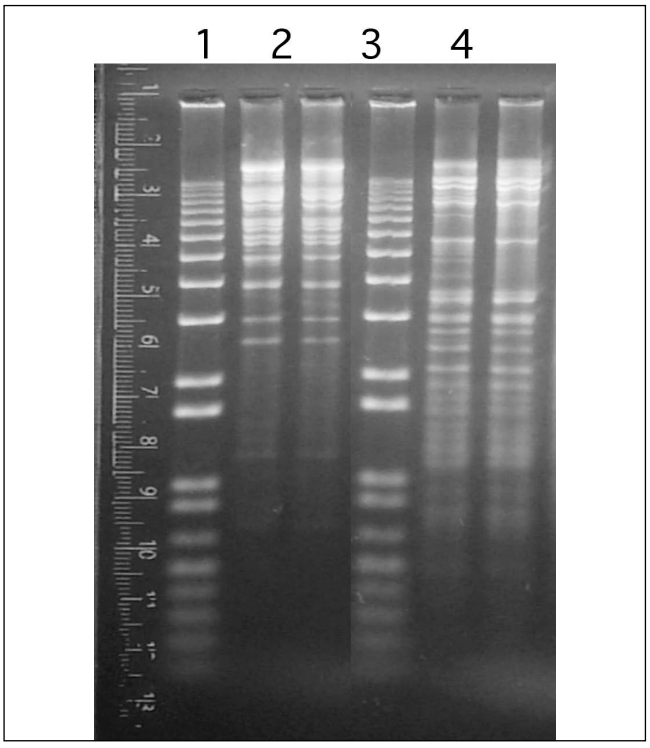

Figure 1 - *Restriction fragment length polymorphisms (RFLPs) of transformants. Restriction pattern of plasmid DNA of transformants TF2105Kp and TF163E obtained by digestion with EcoRI (lanes 2 and 3 respectively) and PstI (lanes 5 and 6), showing identical profiles. A 1Kb plus ladder size standard was run in lanes 1 and 4 (Invitrogen, USA).*

and *E. aerogenes* showed indistinguishable restriction profiles (*Fig. 1*).

Regarding the acquisition of the MDR *E. aerogenes*, it must be pointed out that no carbapenem-resistant microrganisms other than *K. pneumoniae* had been detected in our surveillance system up to that time. Considering that a carbapenem-susceptible *E. aerogenes* strain was previously isolated from a subcutaneous abscess (*Table 1*), we hypothesize that the $IncFII_K$ bla_{KPC-3}-carrying plasmid was transferred *in vivo* between the two species. Coexistence of multiple multidrug-resistant Enterobacteriaceae in one patient and transmission of the bla_{KPC} gene between different strains was reported previously (Tsakris *et al.,* 2010; Kocsis *et al.,* 2014; Ding *et al.,* 2015). So far, the transmission of the plasmid carrying the bla_{KPC-3} gene among *K. pneumoniae* isolates has been shown to occur independently of the sequence types (Corbellini *et al.,* 2014, Manageiro *et al.,* 2015), suggesting that its dissemination is due to lateral gene transfer rather than clonal spread. To our knowledge, this is the first observation of bla_{KPC-3} in ST101 and its possible transmission between *K. pneumoniae* and *E. aerogenes*. The ST101 is one of three clones prevalent in our hospital, together with ST258 and ST512 (data not shown), and has already been reported in Italy: an outbreak of carbapenem-resistant *K. pneumoniae* in Palermo (Mammina *et al.,* 2012), Valle D'Aosta region (Del Franco *et al.,* 2015) and an extremely drug-resistant *K. pneumoniae* in Padua (Frasson *et al.,* 2012). In all cases the bla_{KPC-2} was identified as the mechanism of carbapenem resistance in the *K. pneumoniae* isolates. The ST101 strain gene is likely to be endemic in Italy also in the community, as several strains, most carrying the $bla_{CTX-M-15}$ gene, have been identified in a collection of ESBL producers isolated from animal samples between 2006 and 2012 (Donati *et al.,* 2014). Moreover, international studies have described the dissemination of bla_{KPC-2}, $bla_{CTX-M-15}$ or $bla_{CTX-M-14}$ and bla_{OXA-48} genes in *K. pneumoniae* ST101 (Oteo *et al.,* 2013; Potron *et al.,* 2013; Mshana *et al.,* 2015).

Horizontal transmission of the carbapenemase-resistant plasmid pKpQIL across strains, species, and genera of bacteria has been widely observed (Goren *et al.,* 2010; Mathers *et al.,* 2011), and KPC-producing Enterobacteriaceae such as *K. pneumoniae, Klebsiella. oxytoca, Escherichia coli, Serratia marcescens, Enterobacter cloacae, E. aerogens, Citrobacter freundii* have been detected worldwide (Li *et al.,* 2011, Piazza *et al.,* 2016). In the last countrywide survey in Italy, KPC-type enzymes were the most common carbapenemases and the most affected species was K. *pneumoniae,* while only a minority of cases were *Enterobacter spp* (Giani *et al.,* 2013). However, bla_{KPC-3}-carrying *E. aerogenes* have been isolated in Portugal (Manageiro *et al.,* 2015) and Italy (Kocsis *et al.,* 2014). These observations indicate that KPC-dependent resistance can occur in Enterobacteriaceae other than *K. pneumoniae*, possibly leading to the establishment of further reservoirs of carbapenem resistance genes. Our first finding of a highly resistant *E. aerogenes* strain occurred during a routine surveillance programme aimed at detecting all CRE. Our experience, together with other similar reports (Manageiro *et al.,* 2015; Pesesky *et al.,* 2015), suggests that attention should be given to all relevant KPC-carrying bacteria, including less pathogenic species, as part of an effective surveillance to reduce the number of CRE infections (Chen *et al.,* 2012). Further investigations should focus on the mechanisms that can facilitate the horizontal transfer of plasmids carrying genes conferring antibiotic resistance.

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