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Detection of the IncX3 plasmid carrying bla_{KPC-3} in a *Serratia marcescens* strain isolated from a kidney–liver transplanted patient

Floriana Gona,[1,*] Carla Caio,[2] Gioacchin Iannolo,[1] Francesco Monaco,[1] Giuseppina Di Mento,[1] Nicola Cuscino,[1] Ignazio Fontana,[3] Giovanna Panarello,[4] Gaetano Maugeri,[5] Maria Lina Mezzatesta,[5,*] Stefania Stefani[5] and Pier Giulio Conaldi[1,2]

Abstract

Dissemination of resistance to carbapenems among *Enterobacteriaceae* through plasmids is an increasingly important concern in health care worldwide. Here we report the first description of an IncX3 plasmid carrying the bla_{KPC-3} gene in a strain of *Serratia marcescens* isolated from a kidney-liver transplanted patient at the transplantation centre ISMETT (Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione, Palermo, Italy). To localize the transposable element containing the resistance-associated gene Next-Generation Sequencing of the bacterial DNA was performed. *S. marcescens* was positive for bla_{KPC-3} and bla_{SHV-11} genes. The molecular analysis demonstrated that the bla_{KPC-3} gene of this bacterial strain was located in one copy of the Tn-3-like element Tn*4401-a* carried in a plasmid that is 53 392 bp in size and showed the typical IncX3 scaffold. Our data demonstrated the presence of a new bla_{KPC-3} harbouring the IncX3 plasmid in *S. marcescens*. The possible dissemination among *Enterobacteriaceae* of this type of plasmid should be monitored and evaluated in terms of clinical risk.

The worldwide dissemination of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Enterobacteriaceae* has caused a public health crisis as hospital-acquired pathogens are associated with high mortality of infected patients [1]. KPC production is mostly related to *Klebsiella pneumoniae* isolates but bla_{KPC} genes have also been found in other microbial species such as *Escherichia coli*, *Proteus mirabilis*, and *Serratia marcescens*. At least 30 variants of bla_{KPC} genes have been described [2] which are generally carried on a highly conserved transposon, Tn*4401-like*, detected on different transferable plasmids with a narrow (IncFIIk, ColE, IncX) or broad (IncN, IncL/M and IncA/C) host range [3]. Plasmids of the IncX family, subdivided into 5 different groups (IncX1-IncX5), are usually self-transmissible and often identified in *E. coli, Salmonella* spp. and *Klebsiella* spp. These plasmids have recently been analysed for the range of adaptive and drug resistance genes that are able to transfer among members of *Enterobacteriaceae* [4].

The carbapenem-hydrolyzing activity in *Serratia marcescens* was generally related to the production of KPC-2 [5]; in few cases KPC-3 and only in one KPC-4, within a truncated Tn*4401,* were found [6, 7]. Here we report the detection, for the first time in *S. marcescens,* of the bla_{KPC-3} gene carried on a plasmid belonging to the IncX3 group. The bacterial strain was isolated from a patient who had undergone kidney–liver transplantation in February 2013 at the Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione (IRCCS ISMETT, Palermo, Italy). After a prolonged hospital stay for several critical treatments, in October 2013 the patient developed pneumonia and a carbapenem-resistant *S. marcescens* was isolated from bronchoalveolar lavage (BAL). The patient was treated with gentamicin, first in combination with meropenem and ertapenem and then as monotherapy. After several months, the patient was discharged in good condition (part of this study was presented at the 26th European Congress of Clinical Mcicrobiology

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Author affiliations: [1]Department of Laboratory Medicine and Advanced Biotechnologies, IRCCS-ISMETT (Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione), Palermo, Italy; [2]Fondazione Ri.MED, Regenerative Medicine and Biomedical Technologies Unit, Palermo, Italy; [3]Institute of Biosciences and Bioresources, National Research Council (IBBR-CNR), Palermo, Italy; [4]Department of Intensive Care, IRCCS-ISMETT, Palermo, Italy; [5]Department of Biomedical and Biotechnological Sciences, Section of Microbiology, University of Catania, Italy.

***Correspondence:** Floriana Gona, floriana61180@hotmail.it; Maria Lina Mezzatesta, mezzate@unict.it

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Abbreviations: KPC, *Klebsiella pneumoniae* carbapenemase; MIC, minimum inhibitory concentration; ORF, open reading frame.

Submission of sequence data to GenBank accession number: pIncX-3-KPC-3: KU934011.

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The *S. marcescens* isolate was identified using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (Vitek AMS; bioMérieux Vitek Systems, Hazelwood, MO, USA) and tested for antibiotic susceptibility with Phoenix Automated Microbiology System (Becton Dickinson, Franklin Lakes, NJ, USA). Minimum inhibitory concentration (MIC) breakpoints were interpreted using standards from the European Committee on Antimicrobial Susceptibility Testing [8]. The strain was resistant to aztreonam (MIC, 128 μg ml^{-1}), cefepime (MIC, 64 μg ml^{-1}), cefotaxime (MIC, 128 μg ml^{-1}), ceftazidime (MIC, 32 μg ml^{-1}), imipenem (MIC, 64 μg ml^{-1}), meropenem (MIC, 32 μg ml^{-1}), and ertapenem (16 μg ml^{-1}), while it was susceptible to gentamicin, amikacin, ciprofloxacin and tigecycline. It was screened by PCR and direct sequencing (3500 Genetic Analyzer, Applied Biosystems, Foster City, CA, USA) for metallo-β-lactamases (MBLs), KPCs, extended-spectrum β-lactamase (ESBL) genes and the Tn*4401* transposon as previously described [9, 10]. In order to firmly establish the localization of the transposable element containing the resistance genes we analysed the total DNA (chromosome and plasmid) extracted from the bacterial strain by Next Generation Sequencing (NGS, Illumina MiSeq). Briefly, 1 ng of total DNA was processed usin g the Nextera XT library and the library was loaded in an Illumina MiSeq sequencer running a 151 bp paired-end reads. (Illumina, San Diego, CA, USA). The reads were assembled by SPAdes Genome Assembler (version 3.6.1). The gaps were closed by PCR and Sanger sequencing (Applied Biosystems). The plasmid was annotated by the Prokka program version 1.11 and each predicted open reading frame (ORF) was further blasted against the NCBI non-redundant protein database using BLASTP.

Our results revealed the presence of bla_{SHV-11} and bla_{KPC-3} genes and indicated that the bla_{KPC-3} gene was located on Tn*4401a,* an isoform of Tn*4401*. The Tn*3*-like element was carried on a single plasmid showing a typical IncX3 scaffold: replication (replication initiation protein, pir; replication accessory protein, bis), partitioning (parA), plasmid maintenance (a putative DNA-binding protein, hns; a putative type III topoisomerase, topB), conjugation/type IV secretion system (T4SS, with 11 genes, pilX1 to pilX11), transcriptional activator (actX) and putative DNA transfer proteins (taxA and taxC).

The plasmid identified was 53 392 bp in size and the assembled sequences were contained in a single contig with a coverage of 243X. The complete sequence was reconstructed by PCR and Sanger sequencing and then analysed. The linear map of the plasmid (GenBank accession number KU934011), with the indication of the open reading frames (ORFs), is shown in Fig. 1. It must be pointed out that the plasmid that we found in *S. marcescens* is quite similar to the novel variant plasmid p45-IncX3 detected in a recently reported strain of *K. pneumoniae* [4]. As mentioned above, the plasmid scaffold presents homologous regions for the replicase gene, *tax* and *pilX* gene clusters according to IncX3 plasmid structure. However, differently from p45-IncX3, we found the presence of only 1 copy of the Tn*4401a* containing bla_{KPC-3} gene (Fig. 1).

Conjugation experiments were carried out using *E. coli* HB101 as a recipient and following a previously published protocol [11]. Transconjugants were selected on Mc Conkey agar containing meropenem (8 mg l^{-1}) and streptomycin (100 mg l^{-1}) and identified. Gene transfer experiments demonstrated that the plasmid was transferred from *S. marcescens* to *E. coli* HB101 with a frequency of 10^{-3} (transconjugants per recipient). The presence of the plasmid and the bla_{KPC-3} gene was confirmed by a PCR assay.

Resistance to carbapenems has occasionally been reported in *S. marcescens*, due either to production of plasmid-mediated Ambler class B metallo-β-lactamases such as IMP-1, IMP-6, and VIM-2, or to chromosomally encoded

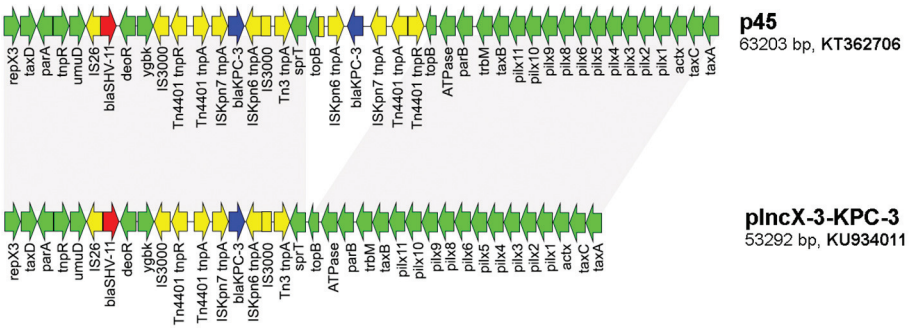

Fig. 1. Linear maps of plasmids pIncX-3-KPC-3 and p45. Green arrows represent predicted open reading frames (ORFs) deduced from nucleotide sequences. The ORFs of pInX-3-KPC-3 were identified in this study, the ORFs of p45 were deduced from GenBank accession number KT362706. The transposase genes of the Tn*44011a* and genes of insertion sequences are indicated by yellow arrows, the bla_{KPC-3} genes inside Tn*4401a* are indicated by blue arrows, red arrows bla_{SHV-11}. The presence of only 1 copy of the Tn*4401a* indicates the difference of plasmids.

SME-type Ambler class A β-lactamases [12]. Few cases of KPC production by *S. marcescens* have been reported, mostly related to KPC-2 gene expression as above cited. Concerning the presence of plasmids related to antimicrobial resistance in *Serratia* spp. a recent report describes the detection of the IncX3 in an *S. marcescens* isolate [13]. To the best of our knowledge, for the first time a carbapenem-resistant strain of *S. marcescens* with the $bla_{\text{KPC-3}}$ gene on a conjugative IncX3 plasmid was found and characterized.

It is remarkable that a similar plasmid, carrying two copies of the $bla_{\text{KPC-3}}$ gene, was recently found in a *K. pneumoniae* isolated in 2014 in Palermo. This finding confirms the occurrence of horizontal transmission of IncX3 plasmids harbouring the $bla_{\text{KPC-3}}$ gene through *Enterobacteriaceae* species within our healthcare region, or it may suggest contact with patients infected or colonized with KPC-producing bacteria carrying these plasmids within the hospital setting [14].

In our centre a complex epidemiological picture of KPC-producing *K. pneumoniae* was detected. Since 2008 an ST258 carrying the pKpQIL-IT plasmid was demonstrated (data on file). This clone was predominant until 2012 when the appearance of a new gentamicin-resistant clone (ST307), with a novel profile of resistance genes including CTX-M-15 carrying new plasmids, may have competed and prevailed over the previously successful ST258 clone [15, 16].

In order to strictly monitor the evolution of carbapenem-resistance for the high clinical relevance of this phenomenon, epidemiological and molecular studies are required to better understand the dynamics of transmission, the risk factors and the reservoirs for *Enterobacteriaceae* with the $bla_{\text{KPC-3}}$ gene harbouring IncX3 plasmids.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Tängdén T, Giske CG. Global dissemination of extensively drug-resistant carbapenemase-producing *Enterobacteriaceae*: clinical perspectives on detection, treatment and infection control. *J Intern Med* 2015;277:501–512.
2. Naas T, Oueslati S, Bonnin RA, Dabos ML, Zavala A *et al.* Beta-lactamase database (BLDB) – structure and function. *J Enzyme Inhib Med Chem* 2017;32:917–919.
3. García-Fernández A, Villa L, Carta C, Venditti C, Giordano A *et al.* *Klebsiella pneumoniae* ST258 producing KPC-3 identified in italy carries novel plasmids and OmpK36/OmpK35 porin variants. *Antimicrob Agents Chemother* 2012;56:2143–2145.
4. Fortini D, Villa L, Feudi C, Pires J, Bonura C *et al.* Double copies of $bla_{\text{KPC-3}}$::Tn*4401a* on an IncX3 plasmid in *Klebsiella pneumoniae* successful clone ST512 from Italy. *Antimicrob Agents Chemother* 2015;60:646–649.
5. Lin X, Hu Q, Zhang R, Hu Y, Xu X *et al.* Emergence of *Serratia marcescens* isolates possessing carbapenem-hydrolysing β-lactamase KPC-2 from China. *J Hosp Infect* 2016;94:65–67.
6. Leung V, Loo VG, Frenette C, Domingo MC, Bourgault AM *et al.* First Canadian outbreak of Enterobacteriaceae-expressing *Klebsiella pneumoniae* carbapenemase type 3. *Can J Infect Dis Med Microbiol* 2012;23:117–120.
7. Bryant KA, van Schooneveld TC, Thapa I, Bastola D, Williams LO *et al.* KPC-4 Is encoded within a truncated Tn4401 in an IncL/M plasmid, pNE1280, isolated from *Enterobacter cloacae* and *Serratia marcescens*. *Antimicrob Agents Chemother* 2013;57:37–41.
8. European Committee on Antimicrobial Susceptibility Testing. *Breakpoint Tables for Interpretation of Mics and Zone Diameters, Version 4.0*. Sweden: EUCAST; 2016.
9. Mezzatesta ML, Gona F, Caio C, Adembri C, Dell'utri P *et al.* Emergence of an extensively drug-resistant ArmA- and KPC-2-producing ST101 *Klebsiella pneumoniae* clone in Italy. *J Antimicrob Chemother* 2013;68:1932–1934.
10. Perilli M, Mezzatesta ML, Falcone M, Pellegrini C, Amicosante G *et al.* Class I integron-borne $bla_{\text{(VIM-1)}}$ carbapenemase in a strain of *Enterobacter cloacae* responsible for a case of fatal pneumonia. *Microb Drug Resist* 2008;14:45–47.
11. Baral P, Neupane S, Marasini BP, Ghimire KR, Lekhak B *et al.* High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal. *BMC Res Notes* 2012;5:38.
12. Bush K, Pannell M, Lock JL, Queenan AM, Jorgensen JH *et al.* Detection systems for carbapenemase gene identification should include the SME serine carbapenemase. *Int J Antimicrob Agents* 2013;41:1–4.
13. Chen L, Peirano G, Lynch T, Chavda KD, Gregson DB *et al.* Molecular characterization by using next-generation sequencing of plasmids containing $bla_{\text{NDM-7}}$ in *Enterobacteriaceae* from Calgary, Canada. *Antimicrob Agents Chemother* 2015;60:1258–1263.
14. Gona F, Barbera F, Pasquariello AC, Grossi P, Gridelli B *et al.* *In vivo* multiclonal transfer of $bla_{\text{KPC-3}}$ from *Klebsiella pneumoniae* to *Escherichia coli* in surgery patients. *Clin Microbiol Infect* 2014;20:O633–O635.
15. Kim JO, Song SA, Yoon EJ, Shin JH, Lee H *et al.* Outbreak of KPC-2-producing Enterobacteriaceae caused by clonal dissemination of *Klebsiella pneumoniae* ST307 carrying an IncX3-type plasmid harboring a truncated Tn4401a. *Diagn Microbiol Infect Dis* 2017;87:343–348.
16. Geraci DM, Bonura C, Giuffrè M, Saporito L, Graziano G *et al.* Is the monoclonal spread of the ST258, KPC-3-producing clone being replaced in southern Italy by the dissemination of multiple clones of carbapenem-nonsusceptible, KPC-3-producing *Klebsiella pneumoniae*? *Clin Microbiol Infect* 2015;21:e15-e17.