Detection of the IncX3 plasmid carrying \textit{bla}_{KPC-3} in a \textit{Serratia marcescens} strain isolated from a kidney–liver transplanted patient

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Abstract

Dissemination of resistance to carbapenems among \textit{Enterobacteriaceae} through plasmids is an increasingly important concern in health care worldwide. Here we report the first description of an IncX3 plasmid carrying the \textit{bla}_{KPC-3} gene in a strain of \textit{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. \textit{S. marcescens} was positive for \textit{bla}_{KPC-3} and \textit{bla}_{SHV-11} genes. The molecular analysis demonstrated that the \textit{bla}_{KPC-3} gene of this bacterial strain was located in one copy of the Tn-3-like element Tn4401-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 \textit{bla}_{KPC-3} harbouring the IncX3 plasmid in \textit{S. marcescens}. The possible dissemination among \textit{Enterobacteriaceae} of this type of plasmid should be monitored and evaluated in terms of clinical risk.

The worldwide dissemination of \textit{Klebsiella pneumoniae} carbapenemase (KPC)-producing \textit{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 \textit{Klebsiella pneumoniae} isolates but \textit{bla}_{KPC} genes have also been found in other microbial species such as \textit{Escherichia coli}, \textit{Proteus mirabilis}, and \textit{Serratia marcescens}. At least 30 variants of \textit{bla}_{KPC} genes have been described [2] which are generally carried on a highly conserved transposon, Tn4401-like, detected on different transferable plasmids with a narrow (IncFIIk, CoE, 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 \textit{E. coli}, \textit{Salmonella} spp. and \textit{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 \textit{Enterobacteriaceae} [4].

The carbapenem-hydrolyzing activity in \textit{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 Tn4401, were found [6, 7]. Here we report the detection, for the first time in \textit{S. marcescens}, of the \textit{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 \textit{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 Microbiology and Infection diseases, 2016, Berlin).
Our results revealed the presence of resistant to aztreonam (MIC, 128 µg ml⁻¹) on Antimicrobial Susceptibility Testing [8]. The strain was interpreted using standards from the European Committee on Antimicrobial Susceptibility Testing (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 (MIC, 32 µg ml⁻¹), and tigecycline. It was screened by PCR and direct 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 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 Tn4401a 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

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**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 pIncX-3-KPC-3 were identified in this study, the ORFs of p45 were deduced from GenBank accession number KT362706. The transposable genes of the Tn4401a and genes of insertion sequences are indicated by yellow arrows, the bla_KPC-3 genes inside Tn4401a are indicated by blue arrows, red arrows bla_SHV-11. The presence of only 1 copy of the Tn4401a 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*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*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*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*KPC-3 gene harbouring IncX3 plasmids.

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

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