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

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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 Tn3-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 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, 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 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 Tn4401, 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 Microbiology...
Our results revealed the presence of resistant to aztreonam (MIC, 128 µg ml\(^{-1}\)) 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-\(\beta\)-lactamases (MBLs), KPCs, extended-spectrum \(\beta\)-lactamases (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 using 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 \(\text{bla}_{3414}\text{SHV-11}\) and \(\text{bla}_{\text{KPC-3}}\) genes and indicated that the \(\text{bla}_{\text{KPC-3}}\) gene was located on Tn\(4401\), an isoform of Tn\(4401\). The Tn3-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, pilX 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, \(\text{tax}\) 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\(4401\) containing \(\text{bla}_{\text{KPC-3}}\) 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 \(\text{bla}_{\text{KPC-3}}\) 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-\(\beta\)-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 transposase genes of the Tn\(4401\)a and genes of insertion sequences are indicated by yellow arrows, the \(\text{bla}_{\text{KPC-3}}\) genes inside Tn\(4401\)a are indicated by blue arrows, red arrows \(\text{bla}_{\text{SHV-11}}\). The presence of only 1 copy of the Tn\(4401\)a 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 blaKPC-3 gene on a conjugative IncX3 plasmid was found and characterized.

It is remarkable that a similar plasmid, carrying two copies of the blaKPC-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 blaKPC-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 and KPC-2-producing Enterobacteriaceae caused by clonal dissemination of Klebsiella pneumoniae ST307 carrying an IncX3-type plasmid [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 blaKPC-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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