Reversion to susceptibility of a carbapenem-resistant clinical isolate of *Klebsiella pneumoniae* producing KPC-3

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Objectives: We report the case of a kidney-transplant patient, suffering an intra-abdominal abscess at the surgical site caused by a carbapenem-resistant ST258 *Klebsiella pneumoniae* clone, producing the KPC-3 carbapenemase. Under tigecycline treatment, the patient developed a sepsis caused by a carbapenem-susceptible ST258 *K. pneumoniae* strain. Complete DNA sequences of the plasmids carried by the resistant and susceptible strains from this patient were determined.

Methods: The complete DNA sequences of plasmids were obtained by applying the 454 Genome Sequencer FLX-PLUS procedure on a library constructed of total plasmid DNA purified from the carbapenem-resistant and -susceptible strains.

Results: In the carbapenem-resistant strain, four plasmids encoding 24 resistance genes, including *bla*KPC-3, and two putative virulence clusters were detected. In the susceptible strain, large rearrangements occurred in the KPC-carrying plasmid, causing the deletion of the entire Tn4401::*bla*KPC-3 transposon, with the consequent reversion of the strain to carbapenem susceptibility. The patient was successfully treated with carbapenems and fully recovered.

Conclusions: The description of the plasmid content in these two strains gives interesting insights into the plasticity of KPC-carrying plasmids in *K. pneumoniae*.

Keywords: transplant, plasmids, sequence type 258

Introduction

Solid organ transplant patients are at risk of infections caused by multidrug-resistant bacteria. In recent years, carbapenem-resistant *Klebsiella pneumoniae* emerged as one of the most relevant pathogens causing healthcare-associated infections that in the immediate post-operative period have resulted in poor clinical outcomes after transplantation. The mortality rate and the limited antimicrobial options for treatment adversely affect transplanted patient outcomes. The *K. pneumoniae* clone designated by multilocus sequence typing (MLST) as sequence type 258 (ST258), producing the KPC carbapenemase, has caused epidemics of national and international proportions, with particularly high prevalence in Israel, Greece, Italy and the USA. The genome of this clone has been recently determined, identifying 50 proteins that were unique to ST258, 30 of them located on various plasmids. In a previous work, we characterized the plasmid content of a *K. pneumoniae* ST258 strain isolated in Italy, identifying four plasmids that were also described in other ST258 strains: pKpQIL-IT, carrying the *bla*KPC-3 gene in the Tn4401 transposon; pKPN-IT, conferring arsenic, copper, silver, trimethoprim, streptomycin, chloramphenicol and macrolide resistance; IncXST258; and the ColEST258 plasmid, conferring gentamicin resistance.

Here we describe the clinical course and successful outcome of a patient who underwent kidney transplantation, followed by an intra-abdominal abscess caused by a KPC-3-producing *K. pneumoniae* ST258 strain. Under tigecycline therapy, this strain developed reduced susceptibility to tigecycline, but also reverted the carbapenem resistance. This change in the antimicrobial susceptibility profile of the infectious strain allowed the successful recovery of the patient with a combined carbapenem and colistin treatment and was due to major rearrangements of the KPC-3-carrying plasmid.
Methods

Bacterial typing

The identification and susceptibility of the K. pneumoniae strains were determined by the Vitek 2 system (AST-N089 cards, bioMérieux, Marcy l’Étoile, France), except for colistin, fosfomycin and tigecycline susceptibilities, which were determined by disc diffusion assays and Etests (AB Biodisk, Solna, Sweden and bioMérieux SA, Chemin de l’Orme, France, respectively), following EUCAST guidelines.13

K. pneumoniae strains were assigned to sequence types by MLST, carried out as previously described.14

PCR amplification of the blaKPC gene and sequencing of the amplicon was performed using previously described primers and conditions.15

Plasmid sequencing

The complete DNA sequences of plasmids were obtained by applying the 454 Genome Sequencer FLX-PLUS procedure (http://454.com/applications/whole-genome-sequencing/) on a library constructed of total plasmid DNA purified from the LS6 carbapenem-resistant and SC29 carbapenem-susceptible K. pneumoniae strains by using the Invitrogen PureLink™ HiPure Plasmid Filter Midiprep Kit (Invitrogen, Milan, Italy), according to the manufacturer’s procedure.

De novo assembly of DNA reads and gap closure

Seventy-three and 109 contigs, ranging from 77869 to 73 bp, with ~25-fold coverage were obtained from strains LS6 and SC29, respectively, using the GS-FLX gsAssembler software. Contigs were firstly assembled in silico by the 454 ReadStatus output file, generated by the gsAssembler software, identifying reads overlapping adjacent contigs. Contig assembly and predicted gaps were confirmed by PCR-based gap closure, by Sanger DNA sequencing of the amplicons (Applied Biosystems, Foster City, CA, USA).

Annotation

Gene prediction was performed by the Artemis version 8 software (Sanger Institute). Pairwise alignments were performed by BLASTN and BLASTP (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Nucleotide accession numbers

Plasmids from the LS6 and SC29 strains were submitted to the EMBL GenBank and assigned to the following accession numbers: ColE-LS6, JX442973; pKP3-LS6, JX442974; pKpQIL-LS6, JX442975; A/C-LS6, JX442976; and pKpQIL-SC29, JX442977.

Ethics

The patient gave informed consent to publication after complete and comprehensive reading of the manuscript.

Results and discussion

Case report

In February 2011, a Caucasian patient, HIV negative, underwent kidney transplantation for end-stage kidney disease due to diabetic and hypertensive nephropathy. A ureteric stent was inserted during the surgical procedure and left in place. Six weeks after transplantation, the patient was readmitted to the hospital because of a subcutaneous abscess at the surgical wound site and a deep abscess close to the transplanted kidney and to the pancreas. Percutaneous CT scan-guided abdominal abscess drainage was performed and a K. pneumoniae strain (identified as strain LS6) was isolated from the infected site. The LS6 strain was assigned to ST258 by MLST, showed resistance to carbapenems by the presence of the blaKPC-3 gene (imipenem MIC > 32 mg/L; meropenem MIC > 32 mg/L) and was fully susceptible to fosfomycin (MIC = 16 mg/L), while the tigecycline MIC was at the upper limit of susceptibility (MIC = 2 mg/L). Intravenous tigecycline (100 mg initial loading dose, then 50 mg twice daily) plus fosfomycin (2 g once daily) were administered to treat the severe intra-abdominal infection, considering the optimal tigecycline pharmacokinetic and pharmacodynamic profile in combination with fosfomycin in abdominal tissues. After 20 days of this treatment, the patient experienced a new febrile episode. A carbapenem-susceptible K. pneumoniae strain (identified as strain SC29) was isolated from blood and showed resistance to tigecycline (MIC = 4 mg/L), while carbapenems and colistin were in the susceptible range (imipenem MIC = 0.38 mg/L; meropenem MIC = 0.75 mg/L). The SC29 strain was assigned to ST258 by MLST and was negative for the blaKPC-3 gene. The ureteric stent was removed and tigecycline plus fosfomycin treatment was halted. Meropenem (1000 mg, in prolonged 4 h intravenous infusion, thrice daily) plus colistin (240 mg twice daily after a loading dose of 720 mg) treatment was continued for 18 days. The patient remained afebrile and the abdominal and subcutaneous abscesses disappeared. The patient fully recovered and was finally discharged 43 days after admission.

Plasmids and resistance genes in the LS6 carbapenem-resistant K. pneumoniae strain

The entire plasmid content of the carbapenem-resistant strain was determined. The LS6 strain contained four plasmids with a total of ~500 kb of plasmid DNA, encoding 24 resistance genes and two putative virulence clusters.

The 78227 bp pKpQIL-LS6 plasmid (JX442975) carried the blaKPC-3 gene in the Tn4401 transposon, conferring carbapenem resistance, and the mer operon, conferring resistance to mercuric ions (Figure 1). It was highly related to plasmid pKpQIL-IT (JN233705), but showed a deletion of the FIIK2 replicon, part of the tra locus and the aphA1 gene.8 – 10

The pKPN-LS6 plasmid (245869 bp; JX442974), related to pKPN-IT (JN233704), conferred resistance to arsenic, copper, silver, chloramphenicol, macrolides, trimethoprim and streptomycin by the ars, copper and silver loci and catA1, mph(A), afrmA12 and aadA2 genes, respectively.11 pKPN-LS6 also encoded the Fec iron(III) dicitrate transport system, probably involved in the capacity of the bacterium to acquire iron in the human host, and a completely novel region, whose functions are still unknown, which was highly related to the Salmonella enteric phage eplasion 15 (Figure 2).16

The plasmid A/C-LS6 (178130 bp; JX442976) showed a backbone similar to other IncA/C plasmids (Figure 2). In detail, A/C-LS6 carried the same array of resistance genes previously described in an IncA/C plasmid from Providencia stuartii from Tunisia, including the plasmid-mediated quinolone resistance qnrA6 gene, an ISCR1 class 1 integron conferring streptomycin, β-lactam, chloramphenicol, rifampicin and trimethoprim resistance by the aadA1, blaOXA-10, cmlA7, arr2 and dfrA16 gene cassettes, and a region encoding aminglycoside, chloramphenicol/florfenicol and tetracycline resistance by the...
aphA6, aphA1, aacA4, strA, strB, floR and tet(A) genes. On this plasmid, the mer locus and the IS\text{Ecp1-}\text{bla}_{\text{CMY-6}} module were also found, conferring resistance to mercuric ions and amoxicillin/clavulanic acid, respectively.

Finally, the ColE-LS6 plasmid (14709 bp, JX442973), carried the \text{aac(6\,\prime)}\text{-Ib} gene, encoding gentamicin resistance, as previously described in other ST258 strains (Figure 1). With regard to the number and diversity of plasmids and resistance genes, the plasmid content of the LS6 strain is one of the most interesting described in \textit{K. pneumoniae} so far, showing a formidable set of resistance genes against toxic compounds, metals and all antimicrobial classes, but also containing phage-related and virulence genes, whose function and relevance remain to be ascertained.

**Plasmids and resistance genes in the SC29 carbapenem-susceptible \textit{K. pneumoniae} strain**

Three plasmids were identified in the SC29 carbapenem-susceptible strain: pKPN-SC29 and A/C-SC29 were identical to pKPN-LS6 and A/C-LS6, respectively, while the 48790 bp pKpQIL-SC29 plasmid (JX442977) was a derivative of pKpQIL-LS6 showing important rearrangements. pKpQIL-SC29 derived from a fusion of pKpQIL and the ColE plasmid, probably by an IS26-mediated recombination event in the region adjacent to the \text{araA} gene (Figure 1). Furthermore, pKpQIL-SC29 showed the deletion of the entire \text{Tn4401-}\text{bla}_{\text{KPC-3}} transposon, with the consequent reversion of the strain to carbapenem susceptibility. The deletion also involved the entire transfer locus and the \text{bla}_{\text{TEM-1}} gene. This deletion was probably caused by an IS26-mediated looping out, probably associated with the IS26-mediated fusion of the pKpQIL and ColE plasmids (Figure 1).

The excision of the \text{bla}_{\text{KPC-3}} gene from the \text{Tn4401} transposon was not a rare event; partial deletions of this element were previously observed in \textit{K. pneumoniae} and \textit{Escherichia coli} isolates of different genetic backgrounds in the USA, highlighting the notion that \text{Tn4401} is itself heterogeneous and highly plastic. It is plausible that by removing the selective pressure exerted by the therapy with carbapenems, plasmid plasticity may favour the loss of the KPC resistance determinant in the ST258 \textit{K. pneumoniae} clone. The presence of many homologous regions represented by the IS26 elements scattered on multiple plasmids, simultaneously resident within the same bacterial cell, can cause major rearrangements of the plasmid scaffolds. In this study, plasmid fusion and recombination events favoured by IS26 elements led to the restoration of carbapenem susceptibility in the infectious strain, allowing...
the successful recovery of the patient when treated with carbapenems.

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**References**


Figure 2. Major structural features of pKPN- and IncA/C-type plasmids identified in the carbapenem-resistant and -susceptible K. pneumoniae ST258 strains. Plasmid pKPN-IT (JN233704) identified in K. pneumoniae ST258 in Italy in 2011 is compared with pKPN plasmids, identically identified in strains LS6 and SC29, here indicated as pKPN-LS6-SC29. Plasmid A/C pAR060302 (FJ621588) identified in the USA is compared with A/C plasmids, identified in strains LS6 and SC29, here indicated as A/C-LS6-SC29. The conserved regions among the two plasmids are shaded. Coloured boxes represent different functions/loci: white, plasmid scaffold regions (in the locus tra the white boxes include capital letters indicating the respective tra genes, i.e. J, traJ; D, traD; I, traI etc.); orange, other resistance determinants; blue, transposon-related genes (tnpA, tnpR, tnpM), class 1 integrase and insertion sequences; purple, replicons of the plasmids; pale green, putative virulence genes [Fec iron(III), ABC transporter, prophage-related genes]; and grey, methyl-transferase genes.


