Reversion to susceptibility in carbapenem-resistant clinical isolate of *Klebsiella pneumoniae* ST258 producing KPC-3 in a kidney-transplant patient

L. Villa[(1)], A.Capone[(2)], D. Fortini[(1)], M. Dolejska[(1)], Irene Rodríguez Fernández[(1)], F. Taglietti[(2)], P. de Paolis[(3)], N. Petrosillo[(2)], and A. Carattoli[(1)]

[1]Department of Infectious, Parasitic and Immune-Mediated Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy
[2]2nd Division of Infectious Diseases, National Institute of Infectious Diseases "Lazzaro Spallanzani", Rome
[3] San Camillo-Forlanini Hospital, Rome, IT

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Abstract

Extremely drug-resistant *Klebsiella pneumoniae* sequence type 258 (ST258) producing the KPC-carbapenemase emerged as one of the most relevant pathogen causing healthcare-associated infections. We report the case of a kidney-transplant patient, suffering recurrent infections by carbapenem-resistant *K. pneumoniae* in a hospital of Rome, Italy. Carbapenem resistant *K. pneumoniae* strain (LS6) was isolated. Under tigecycline treatment, the patient developed a sepsis sustained by a carbapenem-susceptible strain (SC29), which was successfully treated with meropenem.

The reversion of the phenotype allowed the successful therapy of the patient with carbapenems. Complete DNA sequencing of the entire plasmid content of the two strains was obtained applying the 454-Genome Sequencer FLX procedure and demonstrated the loss of bla_{KPC-3} gene.

Objectives

The objective of this study was the characterization of the entire plasmid content of two *K. pneumoniae* ST258 strains, one carbapenem resistant (LS6) and the other susceptible (SC29).

Methods

The entire plasmid content of LS6 and SC29 strains was determined by applying the 454-Genome Sequencer FLX procedure (Roche Diagnostic, Monza, Milan) on a library obtained using plasmid DNA purified from the strains by the Invitrogen PureLink™ HiPure Plasmid Filter Midiprep Kit (Invitrogen, Milan, Italy). At least 30X coverage were obtained from plasmid sequence. Contigs were generated by the gsAssembler software v.2.6. Gene prediction was performed with Artemis Version 8 (Sanger Institute). Pairwise alignment was performed by a BLASTN and BLASTP homology search (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

References

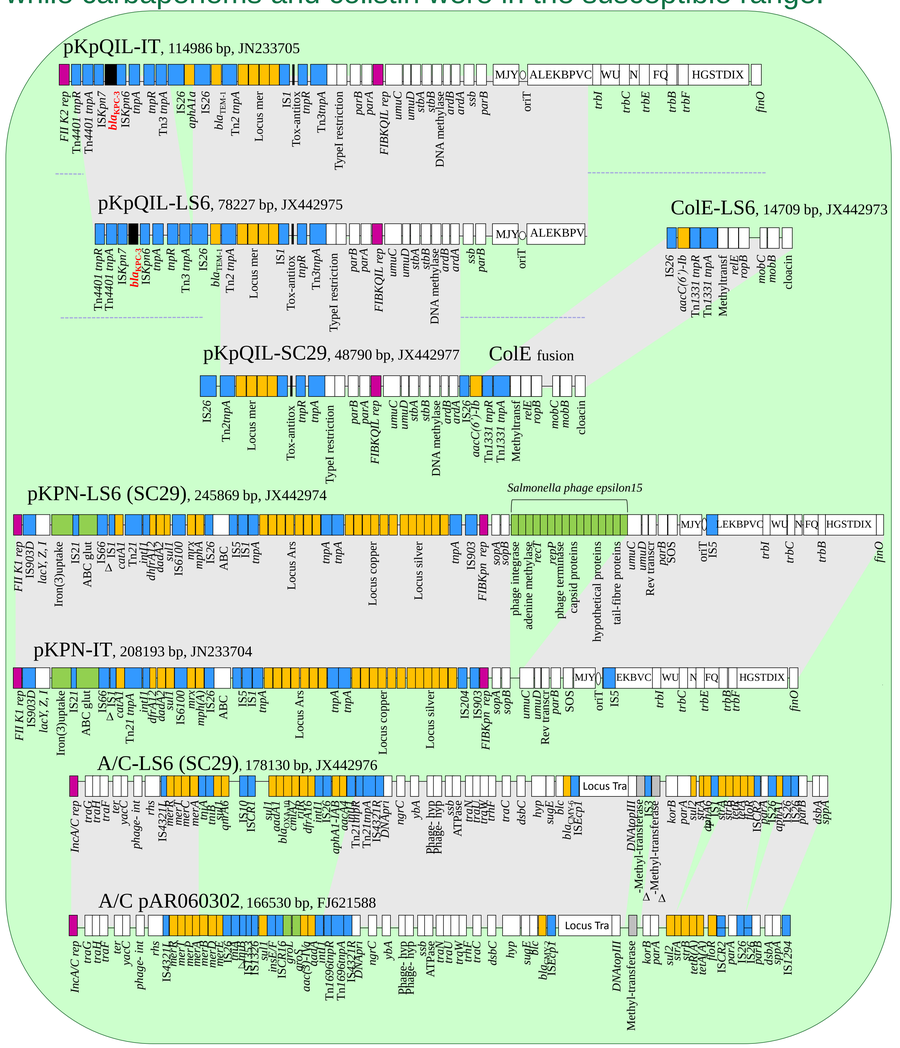
•García-Fernández A, et al. 2012 A. *Klebsiella pneumoniae* ST258 producing KPC-3 identified in Italy carries novel plasmids and OmpK36/OmpK35 porin variants. Antimicrob Agents Chemother; 56:2143-5.

•Chen L, et al. 2012 Partial excision of bla_{KPC} from Tn*4401* in carbapenem-resistant *Klebsiella pneumoniae*. Antimicrob Agents Chemother; 56:1635-8.

Results

Case report

On February 2011, a 57-year-old woman, underwent kidney transplantation. Six weeks after transplantation, the patient was re-admitted to the hospital because of a abdominal abscess at the surgical wound site and a *K. pneumoniae* 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 bla_{KPC-3} gene, and was susceptible to tigecycline and fosfomycin, with MICs of 2 mg/L and 16 mg/L, respectively. The patient was treated with tigecycline plus fosfomycin. After 20 days a carbapenem-susceptible *K. pneumoniae* strain (SC29) was isolated from blood and showed resistance to tigecycline (MIC= 4 mg/L), while carbapenems and colistin were in the susceptible range.



Results

The SC29 strain was assigned to ST258 by MLST and was negative for the bla_{KPC-3} gene. The patient was treated with meropenem plus colistin and was finally discharged 43 days after admission.

Sequence Plasmid analysis :

➢KPC-positive LS6 strain carried four plasmids encoding a total of 24 resistance genes and two putative virulence clusters.

•pKpQIL-LS6 plasmid carried Tn*4401*::bla_{KPC-3} transposon, was highly related to pKpQIL-IT plasmids (identified in ST258 isolates), but lacked the FIIK2 replicon and part of the *tra* locus.

•The pKPN-LS6 plasmid was similar to pKPN-IT but acquired a novel region highly related to the *Salmonella* enteric phage ε-15.

•The plasmid A/C-LS6 showed a backbone similar to other IncA/C plasmids, carrying *qnrA6 gene,* an IS*CR1* class 1 integron and IS*Ecp1*-bla_{CMY-6} module.

•The ColE-LS6 was as identified in other ST258 strains

➢KPC-negative SC29 strains carried three plasmids

•pKpQIL-SC29 derived from a recombination of the pKpQIL-LS6 and the ColE-LS6 plasmid, IS*26*-mediated, and showed a deletion of the entire Tn*4401*::bla_{KPC-3} transposon, with the consequent reversion to carbapenem susceptibility.

•pKPN-SC29 and A/C-SC29 were identical to pKPN-LS6 and AC-LS6, respectively.

Conclusions

The excision of the bla_{KPC-3} gene from the Tn*4401* transposon here reported is not a rare event: partial deletions of this element were previously observed in *K. pneumoniae* and *E. coli* isolates of different genetic backgrounds in the USA. It is plausible that removing the selective pressure of the carbapenems therapy, plasmid plasticity may play in the loss of the KPC-resistance determinant in the ST258 *K. pneumoniae* clone, which is endowed with numerous plasmids, simultaneously resident. The presence of many homologous regions represented by the IS*26* elements scattered on multiple plasmids favored plasmid fusion and recombination events, restoring carbapenem susceptibility in the infectious strain, allowing the successful recovery of the patient by the carbapenem treatment.