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Molecular characterization of an IncX3 Plasmid carrying blaKPC-3 and SHV-11 in a *Citrobacter freundii*

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Background: Carbapenem-resistant Enterobacteriaceae (CRE) have become a global problem in recent years, especially *Klebsiella pneumoniae* carrying the KPC Class A Carbapenemase. Although KPC enzymes have been mainly identified among *Klebsiella pneumoniae*, carbapenem resistance has emerged in a wide variety of species of Enterobacteriaceae including *Citrobacter freundii* and *Escherichia coli*. The blaKPC gene has been reported on different incompatibility group plasmids (Inc), IncFII, ColE, IncN, IncL/M, IncA/C, IncX or untypable plasmids. Here we report the complete nucleotide sequence of an IncX3 plasmid, carrying the blaKPC-3 and blaSHV-11 genes in a *C. freundii* clinical isolate.

Material/methods: In 2014 *C. freundii* and *E. coli* strains were isolated from the rectal swab of a sixty-year-old male treated at the L. Spallanzani Institute, during a routine surveillance programme following kidney transplantation. Identification and antimicrobial susceptibility testing of the two strains were performed using the Vitek-2 System (bioMérieux, France) and Maldi ToF analysis (Brucker Daltonics, Germany). MIC breakpoints were interpreted according to the EUCAST recommendations. Carbapenemase genes and extended-spectrum β -lactamases (ESBLs) were detected by PCR (Progenie Molecular) and sequencing. Genotyping of *C. freundii* and *E. coli* isolates was performed using multi-locus sequence typing (MLST). Plasmid replicons were typed using the PCR-based method PBRT (DIATHEVA, Italy). Plasmid DNA from *C. freundii* and *E. coli* strains were used to transform competent *E. coli* DH5 α cells (Invitrogen, USA); EcoRI and PstI restriction patterns were obtained from transformants and compared. Complete sequence of the plasmid from *C. freundii* was obtained using the 454 GS FLX system (Roche, USA).

Results: The sequence type 91 (ST91) *C. freundii* and ST5 *E. coli* isolates carried the same IncX3 plasmid, as shown by restriction analysis. The plasmid from *C. freundii* (pCfr-30) was completely sequenced: it was 53292bp in size, showed the characteristic IncX3 scaffold and carried the blaKPC-3 and blaSHV-11 genes. The best homology was found with the *K. pneumoniae* plasmid pIncX-SHV pKpS90 (GenBank accession no. JX461340.1). The pKpS90 plasmid was also used for *in silico* analysis reconstruction.

Conclusions: Our study reports the presence of IncX3 plasmid carrying blaKPC-3 and blaSHV-11 genes in *C. freundii* and *E. coli* strains isolated from the same patient and highlights the potential dissemination of this type of plasmid among different species of Enterobacteriaceae, other than *K. pneumoniae*.

Keywords: KPC, IncX3, *Citrobacter freundii*