Complete sequence of an IncT-type plasmid carrying the *bla*$_{OXA-181}$ carbapenemase gene from *Citrobacter freundii*

P1344

Alessandra Carattoli[1], Patrice Nordmann,[2] Laura Villa[1], Claudio Carta[1] and Laurent Poirel[2]

Istituto Superiore di Sanità, Rome, IT[1] , INSERM U914, Hosp. Bicetre, South-Paris Medical School, FR[2]

Objectives

Multiple plasmids were identified in an extensively drug -resistant *Citrobacter freundii* isolate in France from a patient who had been transferred from India. That isolate co-expressed the metallo-β-lactamases NDM-1 and VIM-1, but was additionally positive for the *bla*OXA-181 gene, that latter encoding a class D carbapenem-hydrolysing β-lactamase. OXA-181 that differs from OXA-48 by four amino acid substitutions hydrolyses penicillins, carbapenems at low level, but spares broad-spectrum cephalosporins.

Methods

Transconjugants expressing OXA-181 were obtained at very low frequency by mating-out assays from the *C. freundii* strain to *Escherichia coli* recipient. One transconjugant contained three plasmids, two non-typeable and one assigned by PCR-based replicon typing to the IncT group. Complete sequencing of the *bla*OXA-181-bearing plasmid was performed using the 454-Genome Sequencer FLX procedure on libraries obtained on total plasmid DNA purified. Contigs with at least 15-fold coverage obtained by GS De Novo Assembler software were assembled in continue plasmid sequences by the PCR-based gap closure method (Panel 1).

Results

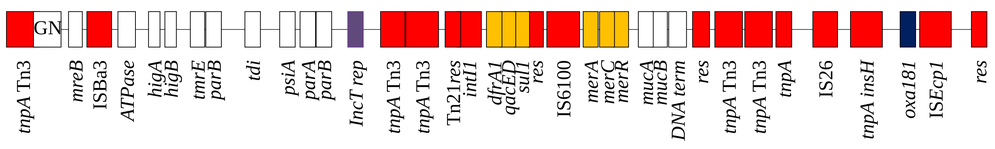
The *bla*OXA-181 gene was located onto a 83698-bp IncT-type plasmid, named pTOXA-181. It derives from the IncT Rts1 plasmid (217,182 bp), but conserving only the replicon, the origin of transfer and partitioning proteins. The conjugation capability of pTOXA-181 was impaired by the complete loss of the transfer system, with only the TraN and TraG proteins remaining (Panel 2). The *traG* locus was actually truncated through the integration of the *bla*OXA-181-containing fragment including a Tn*3* transposase similar to a Tnp*A* identified in *Acinetobacter baumannii* AbaR1 resistance island, the PinR site-specific recombinase, and the IS*Ecp1* insertion sequence that has mobilized the *bla*OXA-181 gene by a one-ended transposition process.

A 34,826-bp IncN-like plasmid, named pN3 was identified in the transconjugant, encoding a complete transfer locus. This plasmid efficiently promoted the conjugation and mobilised *in trans* pTOXA181 and may contribute to the dissemination of the *bla*OXA-181 gene. pN3 belongs to a new IncN subgroup here designated N3, which is different from plasmids p247A and R46, previously classified as IncN2 and IncN1 subgroups, respectively (Panel 3).

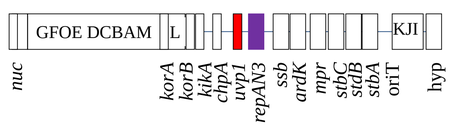
A novel small plasmid, pMobC of 8,969 bp was also mobilized with pTOXA-181 by pN3 and was identified in the transconjugant (Panel 4).

Panel 1: Plasmid content of the OXA-181 transconjugant

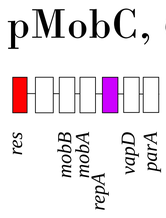
pTOXA-181, *Citrobacter freundii*, 83698 bp



pN3, *Citrobacter freundii*, 34826 bp

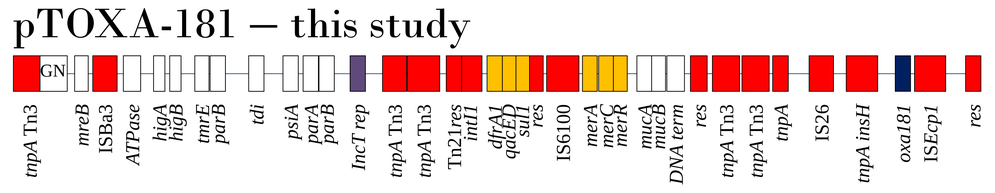


pMobC, *Citrobacter freundii*, 8969 bp

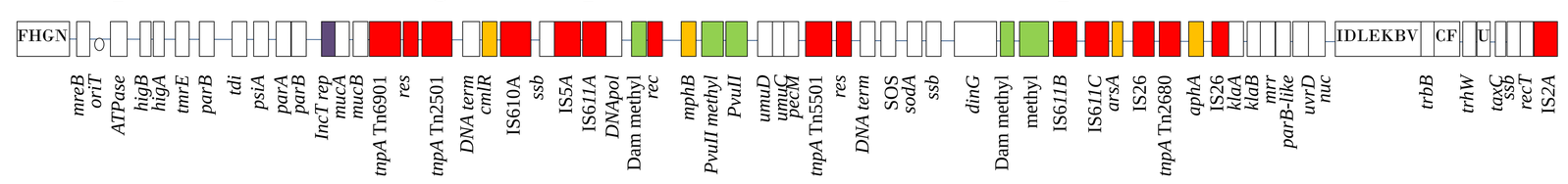


Panel 2: Comparative analysis of IncT plasmids

pTOXA-181 – this study

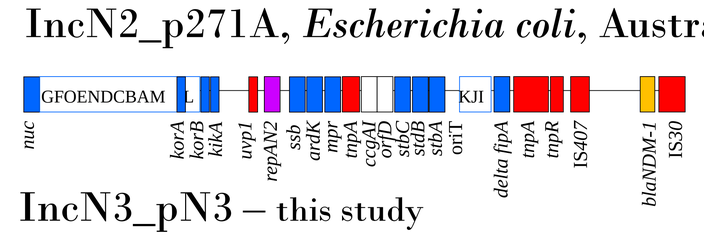


IncT-pRts1, *Proteus vulgaris*, Japan, NC_003905, 217182 bp

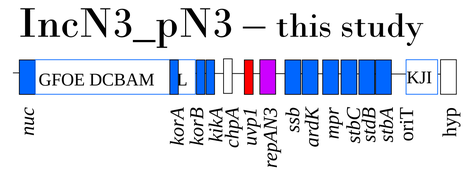


Panel 3: Comparative analysis of IncN plasmids

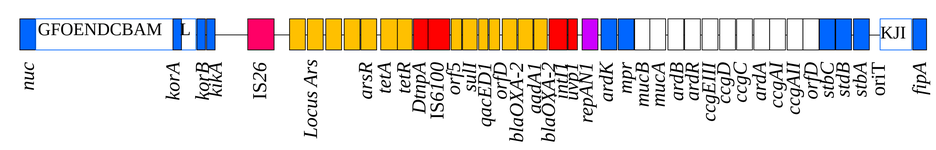
IncN2_p271A, *Escherichia coli*, Australia, JF85549, 35947 bp



IncN3_pN3 – this study

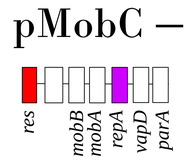


IncN1_R46, *Salmonella typhimurium*, AY046276, 50969 bp

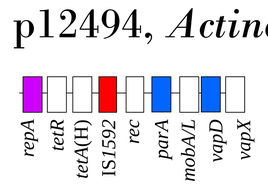


Panel 4: Comparative analysis of Mob plasmids

pMobC – this study



p12494, *Actinobacillus pleuropneumoniae* , 14393 bp, DQ517426



Conclusions

This study identified the genetic vehicle of the emerging *bla*OXA-181 carbapenemase gene that has been found in different enterobacterial species including *K. pneumoniae* from Sultanate of Oman and *Providencia rettgeri* from France. Interestingly, the OXA-181-producing isolate was recovered from a patient with a link with India that co-produced NDM-1 encoded on a different plasmid. Our study shows that the genetic structure at the origin of the mobilization and spread of the b*la*OXA-181 gene was totally different from that of the blaOXA-48 gene known to be widespread and vehiculated by an IncL/M-type 62-kb plasmid.

References

Murata, T, M Ohnishi, T Ara et al. 2002. Complete Nucleotide Sequence of Plasmid Rts1: Implications for Evolution of Large Plasmid Genomes. *J. Bacteriol.* 184, 3194–3202

Poirel L, A Ros, A Carricajo et al. 2011. Extremely Drug-Resistant *Citrobacter freundii* Isolate Producing *NDM-1* and other Carbapenemases Identified in a Patient Returning from India *Antimicrob. Agents Chemother. 55*, 447.

Poirel L, RA Bonnin, and P Nordmann. 2011. Analysis of the Resistome of a Multidrug-Resistant NDM-1-Producing Escherichia coli Strain by High-Throughput Genome Sequencing. *Antimicrob. Agents Chemother.* 55, 4224-4229