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Abstract (poster session)

Complete sequence of an IncT-type plasmid carrying the blaOXA-181 carbapenemase gene from Citrobacter freundii

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Objectives: Multiple plasmids were identified in an extensively drug resistant Citrobacter freundii isolate in France from a patient transferred from India. That isolate co-expressed the metallo-beta-lactamases NDM-1 and VIM-1, but was additionally positive for the blaOXA-181 gene, that latter encoding a class D carbapenem-hydrolysing beta-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 blaOXA-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-FLX gAssembler software were assembled in continue plasmid sequences by the PCR-based gap closure method. Results: Sequencing revealed that the blaOXA-181 gene was located onto a 83,557-bp IncT-type plasmid, named pTOXA-181. A fragment of 69 kb of pTOXA-181 derived from the IncT reference Rts1 plasmid (217,182 bp), including the replicon, the origin of transfer and partitioning proteins, but conjugation capability of pTOXA-181 was impaired by the complete loss of the transfer system, with only the TraN and TraG pilus biogenesis proteins remaining. The traG locus was actually truncated through the integration of the blaOXA-181-containing fragment, including a Tn3 transposase similar to a TnpA identified in Acinetobacter baumannii AbaR1 resistance island, the PinR site-specific recombinase, and the ISEcp1 insertion sequence that has mobilized the blaOXA-181 gene by a one-ended transposition process. A 34,828-bp helper IncN-like conjugative plasmid was also identified in the transconjugant, encoding a complete transfer locus. We observed that this latter plasmid had mobilized both pTOXA-181 and a small mobilizable plasmid (8,969 bp) which was also identified in the transconjugant. Conclusion: This study identified the genetic vehicle of the emerging blaOXA-181 carbapenemase gene. Our study shows that the plasmid at the origin of spread of the blaOXA-181 gene was totally different from that of the blaOXA-48 gene known to be vehiculated by an IncL/M plasmid.