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Abstract (poster session)

Complete sequencing of a novel IncH plasmid carrying the blaNDM-1, blaCTX-M-15 and qnrB1 genes

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Objective: The current emergence of the blaNDM-1 gene is not related to the spread of an epidemic plasmid but rather to multiple events of acquisitions on different plasmid types. In several strains isolated from seepage water and public tap water collected from New Delhi, India, the blaNDM-1 was identified on large (>250 kb) and non-typeable plasmids (Walsh et al. 2011). The complete sequence of pNDM-MAR, a non-typeable plasmid of 250 kb identified from a K. pneumoniae from Morocco and carrying the genes encoding NDM-1, CTX-M-15 and qnrB1, was determined and analyzed. Methods:Plasmid sequencing was performed by the 454-Genome Sequencer FLX procedure on libraries obtained on total plasmid DNA purified from an E. coli J53 transconjugant. 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: Plasmid pNDM-MAR was 267,242 bp in-size and encoded 177 predicted CDS. BLASTN comparison indicated a completely novel plasmid scaffold, showing only small portions of homology with GenBank data. pNDM-MAR possessed novel replicons and transfer loci, defining a new group within the IncH plasmid family. Plasmid pNDM-MAR carried the blaNDM-1 gene into a genetic environment that was different from those previously described for other plasmids encoding NDM-1. In addition, it encoded the ESBL CTX-M-15 and the plasmid-mediated quinolone resistance gene qnrB1. That latter was flanked by an IS26 element and the Tn3 transposase which immediately preceded the groEL-groES genes, flanking the blaNDM-1 gene. The blaCTX-M-15 gene was associated to insertion sequence ISEcp1. Plasmid pNDM-MAR carried additional resistance determinants to tellurite, mercury, quinolones, chloramphenicol, and aminoglycosides. Conclusion: pNDM-MAR is the first identified plasmid carrying the blaNDM-1 gene together with an ESBL encoding gene which is the most widespread worldwide (blaCTX-M-15). It did not harbor any 16S rRNA methylase encoding genes, whereas all the other NDM-1 encoding plasmids did. It is the largest NDM-1-encoding plasmid described so far and interestingly harbors two completely new replicons. The presence of the HIB replicon, and conservation of conjugative pilus proteins, strongly suggest that pNDM-MAR is a novel plasmid type that however derives from the IncHI plasmid family. Our study provides further insights regarding the current successful dissemination of blaNDM-1 gene.