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Poster Session IV
MDR Gram-negatives - molecular biology of resistance genes

A NOVEL INCN2 PLASMID CONFERRING CARBAPENEM RESISTANCE BY THE BLAVIM-4 GENE IDENTIFIED IN ENTEROBACTER CLOACAE FROM THE SULTANATE OF OMAN

D. Fortini[1], R. Galetti[2], A. Sonnevend[3], A. Ghazawi[3], T. Pal[3], C. Feudi[1], A. Al-Jardani[3], S. Al-Abri[3], L. Villa[1], A. Carattoli[1]

[1]Infectious Diseases, Istituto Superiore di Sanità, Roma, Italy ; [2]Análises Clínicas Toxicológicas e Bromatológicas, Faculdade de Ciencias Farmaceuticas de Ribeirão Preto, Ribeirão Preto- SP, Brazil ; [3]Medical Microbiology and Immunology, United Arab Emirates, Al Ain, United Arab Emirates

Objectives: The aim of this study was to analyse the plasmid carrying *bla*$_{VIM-4}$ gene identified in a carbapenem resistant *E. cloacae* (OM63) isolated in 2011 in the Sultanate of Oman from bile of a 63 year-old Omani female patient with biliary sepsis after cholecystectomy. The patient had no travel history prior hospitalisation.

Methods: The *bla*$_{VIM-4}$ carrying plasmid (pOM63) was non-conjugative, so it was transformed into *E. coli* DH5α and incompatibility typing by PBRT was performed. Complete nucleotide sequencing was determined by the 454-Genome Sequencer FLX procedure on a library constructed on plasmid DNA purified from the transformant.

Results: The plasmid pOM63, identified within the OM63 strain was not typable by PBRT. The entire pOM63 (49,142 kb) was fully sequenced. By nucleotide alignments, the backbone was highly similar to the following plasmids: p271A (JF785549) and pTR4 (NC_019163) identified in *Escherichia coli* and *K. pneumoniae,* respectively, carrying the *bla*$_{NDM-1}$ gene, and with the pJIE137 (EF219134), identified *K. pneumoniae*, carrying the *bla*$_{CTX-M-62}$ gene. All these plasmids can be classified as the N2 variant within the IncN plasmid family. The IncN2 variant shows transfer loci and stabilization systems highly related to the IncN plasmid family, but the replicase gene is divergent and cannot be recognized by the IncN PBRT.

The *bla*$_{VIM-4}$ and *aac(6')-Ib* gene cassettes were identified in a class 1 integron inserted between the *nuc* and *fipA* genes, in a position that is common for integration of integrons in IncN-like plasmids. The integron is similar to the *bla*$_{VIM-4}$ integron found on IncA/C plasmids in *E. cloacae* isolated earlier in the United Arab Emirates. Both integrons contain the ISPa21 transposase integrated in front of the *qacEdelta1* gene at the 3'CS, but carry a different array of genes. Plasmid pOM63 encodes a complete EcoR124 type I site-specific restriction modification system with the methylase and restriction enzyme, flanked by a putative transposase. The cluster is inserted between *fipA* and the *ardRB* antirestriction system, in a region that is inverted with respect to the structure of all the other IncN and IncN2 plasmids. The EcoR124 R-M system was not found in other IncN plasmids but derives from the IncW pR124 plasmid isolated from *Salmonella enterica* serovar Typhimurium.

Conclusions: Plasmid pOM63 represents a novel N2 variant of the IncN group. IncN plasmids represent one of the most frequently encountered resistance plasmid types in *Enterobacteriaceae* of human and animal origin. In the last decade they have been the major vehicles for the spread of the *bla*$_{VIM-1}$ gene in Greece and Italy. This novel plasmid variant has been previously associated with the *bla*$_{NDM-1}$ gene in Australia and Singapore and can be highly diffused in different parts of the world, contributing to the spread of different carbapenemase genes.