P1305

Paper Poster Session VI

Analysing and overcoming carbapenemase-mediated resistance

Characterization of the IncN-related plasmid pMB3018 from *Citrobacter freundii* carrying the novel carbapenemase gene *bla*OXA-233

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Objectives

The worldwide increase of multidrug-resistance in gramnegative bacteria has become an important clinical challenge. Carbapenem resistance can be caused by a variety of mechanisms, however the worldwide spread of carbapenemases is especially important. Horizontal transfer of carbapenemase genes is mainly provided by conjugable plasmids. In 2011, isolate *C. freundii* NRZ-02127 was referred to the National Reference Laboratory for Multidrug-resistant Gram-negative Bacteria and harboured the novel Class D carbapenemase OXA-233 located on a conjugable plasmid. The aim of this study was to fully sequence and analyse the plasmid carrying the $bla_{OXA-233}$ gene.

Methods

Plasmid pMB3018 was transconjugated into *E. coli* C600 from the clincial isolate *C. freundii* NRZ-02127. Plasmid DNA was obtained using the NucleoBond PC 100 Kit (Macherey-Nagel, Düren, Germany) and sequencing of pMB3018 was performed on a Roche 454 GS junior system. Sequence analysis was performed using several bioinformatic tools. Replicon typing was performed by PCR.

Results

pMB3018 was a 52.278 bp plasmid that whas not typable by PCR-based replicon typing (PBRT). The entire plasmid was fully sequenced and sequence analysis revealed that it was related to the IncN-type, but the *repA* gene sequence was not identical. Recently, other plasmids with this repA gene, pJIE137 (EF219134.3), p271A (JF785549.1) and pTR3 (JQ349086.2), were described. These plasmids are classified as the N2 variant within the IncN family and cannot be identified by PBRT. The plasmid backbone of pMB3018 was very similar to pJIE137, p271A and pTR3, but showed several reorganizations. Apart from the backbone, pMB3018 showed distinct differences to other IncN2 plasmids. It also harboured a conserved class I integron that carried genes conferring multidrug-resistance: the novel $bla_{OXA-233}$ gene and an *aac(6')* gene. The integron was inserted close to the *repA* gene which is very untypical for IncN2-type plasmids. Several phage integrases and putative integrases were identified in the sequence.

Conclusion

Plasmid pMB3018 represents another member of the novel IncN2 replicon type. This further underlines the importance of IncN plasmid-encoded multidrug-resistance and the distribution of the corresponding genes throughout many Gram-negative species with clincial importance. The identification of several integrases and transposases implies the ability of pMB3018 to spread its resistance genes not only to other species but also to other plasmids.