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Abstract (poster session)

Description of OXA-233, a novel class D carbapenemase inhibited by clavulanic acid

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Objectives: The worldwide increase of multidrug-resistance in Gram-negative bacteria, especially the resistance to carbapenems, has become an important clinical challenge. Carbapenem resistance can be caused by a variety of mechanisms, however the worldwide spread of carbapenemases is most important. Among the mocular class D beta-lactamases, several carbapenem hydrolyzing enzymes have been described. Here we describe a novel class D carbapenemase that was found in a multidrug resistant Citrobacter freundii isolate from Germany and is inhibited by clavulanic acid. Methods: Susceptibility to antibiotics and inhibition by clavulanic acid was determined by disk diffusion and Etest. A modified Hodge-Test was performed. Whole cell DNA was digested with HindIII and ligated into the HindIII site of the pBK-CMV cloning vector and transformed into E. coli TOP10. Beta-lactam resistant clones were sequenced. The OXA-233 encoding sequence was cloned into the pBK-CMV vector and transformed into E. coli TOP10 for activity analysis. Localisation of the gene was determined by PFGE and Southern blotting. Results: A multidrug resistant Citrobacter freundii isolate (NRZ-02127) was sent to the National Reference Laboratory for Multidrug-resistant Bacteria for further characterisation. The isolate was resistant to penicillins and cephalosporins and also showed reduced susceptibility towards carbapenems. The modified Hodge-test was positive for imipenem, meropenem and ertapenem. Cloning of whole cell DNA led to a beta -lactam resistant clone. The clone harboured a novel class D carbapenemase, OXA-233. E. coli TOP10 producing OXA-233 showed increased resistance to beta-lactams. Unlike most other OXA variants, OXA-233 was inhibited by clavulanic acid. The sequence of OXA-233 showed it to be a OXA-10 variant with one single amino acid substitution at position 117, compared to the next relative OXA-17. Conclusions: Citrobacter freundii NRZ-02127 harboured a new OXA carbapenemase, OXA-233. Its closest relatives are OXA-10 and OXA-17 with 2 and 1 amino acid substitution, respectively. The enzyme confers resistance to penicillins and carbapenems and is inhibited by clavulanic acid. The amino acid substitution at position 117 is a mutation of a highly conserved region of OXA beta-lactamases that has not been reported before.