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Genetic and biochemical characterization of OXA-519, a novel OXA-48 variant

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Background: OXA-48 and its derivatives are class D β-lactamases and have disseminated widely, but only in Enterobacteriaceae. OXA-48 was identified from a carbapenem-resistant *Klebsiella pneumoniae* isolate that had been recovered in ITurkey, in 2001. Although OXA-48 hydrolyzes penicillins at high level, it hydrolyzes carbapenems at al low level and shows very weak activity against expanded-spectrum cephalosporin. Some OXA-48 like enzymes, exhibit the same substrate profile as OXA-48, but others OXA-48-like enzymes hydrolyze expanded-spectrum cephalosporins but very weakly carbapenems. Since the discovery of OXA-48, several variants of this enzyme have been reported. The known OXA-48 variants are currently as follows: OXA-162, OXA-163, OXA-181, OXA-204, OXA-232, OXA-244, OXA-245, OXA-247, OXA-370, and OXA-405. The aim of this study was to characterize a novel OXA-48-like β-lactamase named OXA-519 recovered from a clinical strain in Belgium.
Material/methods: The presence of carbapenemases in the clinical isolate was studied by Carba NP and BYG tests. Immuno-chromatographical study was performed using OXA-48 Kset (Coris BioConcept, Gembloux, Belgium). Whole genome sequencing was performed using an Illumina MISeq platform. Antimicrobial susceptibilities were determined by disk diffusion technique and minimal inhibitory concentration (MIC) determination, and interpreted according to the EUCAST breakpoints. Plasmid characterization and mating-out assay were performed. Steady state parameters of the purified enzyme were determined and compared with those of OXA-48 and OXA-163. Structural analysis was also done.
Results: The clinical isolate presented no activity against imipenem (negative for the Carba NP test and negative for the Byg Carba test). The immuno-chromatography was positive for OXA-48.

Sequencing analysis revealed the presence of a novel OXA-48 type β-lactamase gene, named *bla*$_{OXA-519}$. OXA-519 differ from OXA-48 in a single amino acid substitution Val-Leu in the position 120.The *bla*$_{OXA-519}$ gene was located on a ca. 60.7-kb plasmid (pOXA-519) identical to the prototype IncL/M bla$_{OXA-48}$ –carrying plasmid except for a ca.3-kb deletion, and for the insertion of IS1R. The MIC values revealed no expanded-spectrum cephalosporinase activity, and very low carbapenemase activity when p-TOPO(*bla*$_{OXA-519}$) *E.coli* TOP10 was evaluated. The pOXA-519 in *E. coli* HB4 with impaired permeability showed an important increase in MIC values for carbapenems. The substrate specificity was confirmed by determining the steady state parameters of the purified enzyme, which exhibited low catalytic efficiencies for carbapenems and almost no activity for expanded-spectrum cephalosporins.
Conclusions: OXA-519 is an OXA-48 like β-lactamase with a very low carbapenem-hydrolyzing activity and no expanded-spectrum cephalosporin hydrolysis. Although the single amino acid substitution is in a region different from the β5-β6 loop, in the folding protein, it is very close to the active site, which could explain the modification in the protein activity as compared with the one of OXA-48. *bla*$_{OXA-519}$ gene can be detected by PCR and also with immunochromatography assays, but not by its hydrolytic properties.