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Genetic and biochemical characterization of OXA-535, a novel OXA-48-like enzyme progenitor of OXA-436 from *Shewanella bicestria*

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Background: OXA-48 is the one most commonly identified carbapenemases in Enterobacteriaceae in most European and Mediterranean countries. *Shewanella* species express chromosome-encoded class D β -lactamases and in particular, *Shewanella oneidensis* strain MR-1 was found to naturally harbor the *bla*_{OXA-54} gene, which is closely related to *bla*_{OXA-48}. The aim of this study was to characterize a novel chromosome-encoded OXA-48 like enzyme, named OXA-535, isolate in a *Shewanella bicestria* sp. nov.

Material/Methods: *bla*_{OXA-535} was cloned with pCR-Blunt II-Topo kit (Invitrogen, Eragny, France) and transformed in *E. coli* TOP10. Antimicrobial susceptibilities were determined by disk diffusion technique and minimal inhibitory concentration (MIC) determination, and interpreted according to the CLSI breakpoints. *bla*_{OXA-535} was cloned in an expression vector pET41b(+) and transformed in *E. coli* BL21(DE3). Steady state parameters of the purified enzyme were determined and compared with those of OXA-48.

Results: OXA-535 and OXA-48 share 91, 3% of amino acid sequence identity. OXA-535 is more related to another plasmid encoded OXA-48-like β -lactamase, named OXA-436. OXA-535 and OXA-436 share 98, 9% of identity differing in only three amino acids, Glu125-Gly, Ile226-Val and Ser252-Ile. The MIC values of p-TOPO(*bla*_{OXA-535}) *E. coli* TOP10 revealed carbapenemase activity and no expanded-spectrum cephalosporinase activity. The substrate specificity was confirmed by determining

the steady state parameters of the purified enzyme, which exhibited catalytic efficiencies for carbapenems similar to OXA-48 and no activity for expanded-spectrum cephalosporins.

Conclusions: OXA-535 is the first oxacillinase isolated from *Shewanella bicestrii* sp. nov. It is an OXA-48 like carbapenem-hydrolyzing β -lactamase without expanded-spectrum cephalosporins hydrolysis. OXA-436 has been recently involved in an outbreak in several hospitals in Denmark involving several species of Enterobacteriaceae. It is likely that *Shewanella bicestrii* sp. nov. is the progenitor bacteria of of *bla*_{OXA-436} gene.