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O0438 Evolution towards increased carbapenemase activity in the OXA-51-like beta-lactamases of Acinetobacter baumannii

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Background: The OXA-51-like beta-lactamases are a large group of enzymes intrinsic to *Acinetobacter baumannii*. In the absence of an acquired carbapenemase, *A. baumannii* is known to be able to achieve clinical resistance to carbapenem antibiotics by increasing expression of their OXA-51-like beta-lactamase. However, not all *A. baumannii* are carbapenem-resistant, and there is evidence that different OXA-51-like enzyme variants confer different levels of resistance. This study investigated the structural basis and evolution of carbapenem resistance conferred by different OXA-51-like variants.

Materials/methods: The enzyme variant OXA-66 was cloned and purified, and used for solving the enzyme crystal structure. The genes for the variants OXA-64, -65, -66, -69, -71, -107, -108, -110 and -111 were cloned into the plasmid pYMAb2 and inserted into *E. coli* DH5α, *A. baumannii* CIP70.10 and *A. baumannii* BM4547, and the MICs of the carbapenems were measured. The nucleotide sequence for OXA-66 was used to identify all OXA-51-like sequences present in the NCBI genome sequence database. These were extracted, duplicates removed, aligned, and a maximum likelihood phylogeny estimated.

Results: The crystal structure of OXA-66 was solved to a resolution of 2.1 Å and was consistent with that for OXA-51 that has recently been solved. The MICs of meropenem and imipenem showed a greater than 2-fold increase for only three enzyme variants: OXA-107, -108 and -110. These variants carry differences from consensus at positions 129 (OXA-110) and 167 (OXA-107 and -108). Phylogenetic analysis of all 190 known OXA-51-like variants suggests that substitutions at position 129 may have occurred on 10 independent occasions, and at position 167 on 8 occasions, with no enzyme carrying substitutions at both positions. Furthermore, substitutions at positions 226, 222 and 130, previously shown to be responsible for increased carbapenemase activity, may have occurred on 2, 4 and 14 separate occasions.

Conclusions: Amino acid substitutions surrounding the enzyme active site are responsible for increasing the ability of certain OXA-51-like enzyme variants to hydrolyse the carbapenems, resulting in decreases in phenotypic susceptibility. These substitutions have occurred on many separate occasions, representing widespread evolution towards reduced carbapenem susceptibility in *A. baumannii* independent of acquired carbapenemases.