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.