O077
Oral Session
MDR-Acinetobacter baumannii

THE EFFECT OF CLOSELY RELATED OXA-51 VARIANTS ON CARBAPENEM SUSCEPTIBILITY IN ACINETOBACTER BAUMANNII

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Objectives: *Acinetobacter baumannii* harbors the intrinsic OXA-51 subclass of carbapenem-hydrolysing class D beta-lactamases, which is strongly associated with international clonal lineages (IC). For example, OXA-66 correlates with IC2. We previously observed the development of carbapenem resistance in outbreak-related *A. baumannii* isolates whereby OXA-66 was converted into OXA-82 (L167V) and was overexpressed via IS*Aba1*. Alignment of OXA-66-related enzymes has shown two putative evolutionary pathways (Figure 1) from OXA-66 to OXA-201 via OXA-82 or OXA-109 through two stepwise mutational events. To investigate this, the selective advantage of these OXA variants was determined by performing carbapenem susceptibility testing on the basis of an isogenic background.

Materials and Methods: *bla*$_{\text{OXA-66}}$, $_{\text{-82}}$, and $_{\text{-201}}$ were amplified from IC2 *A. baumannii* clinical isolates, while *bla*$_{\text{OXA-109}}$ was generated by site-directed mutagenesis of *bla*$_{\text{OXA-66}}$. The genes were cloned, together with the natural IS*Aba1* promoter, into to ampicillin resistance gene of pWH1266 and transferred into ATCC 17978. The presence of different *bla*$_{\text{OXA-66-like}}$ was confirmed by sequencing and overexpression was confirmed by qRT-PCR. Imipenem and meropenem susceptibility was determined by Etest.

Results: ATCC 17978 transformants showed similar levels of *bla*$_{\text{OXA-51-like}}$ overexpression. Susceptibility testing revealed differences in carbapenem susceptibility. Overexpressed OXA-66 increased imipenem and meropenem MICs in the reference strain from 0.25 mg/L to 1 and 4 mg/L, respectively. OXA-82, OXA-109 and OXA-201 variants recorded carbapenem MICs of >32 mg/L.

Conclusion: In contrast to OXA-66, variants OXA-82, OXA-109 and OXA-201 conferred carbapenem resistance in the reference strain. Therefore conversion of OXA-66 on the basis of an isogenic background seems to provide a selective advantage for the parent strain in the presence of carbapenems. The advantage of OXA-201 over OXA-82 and OXA-109 remains unclear.

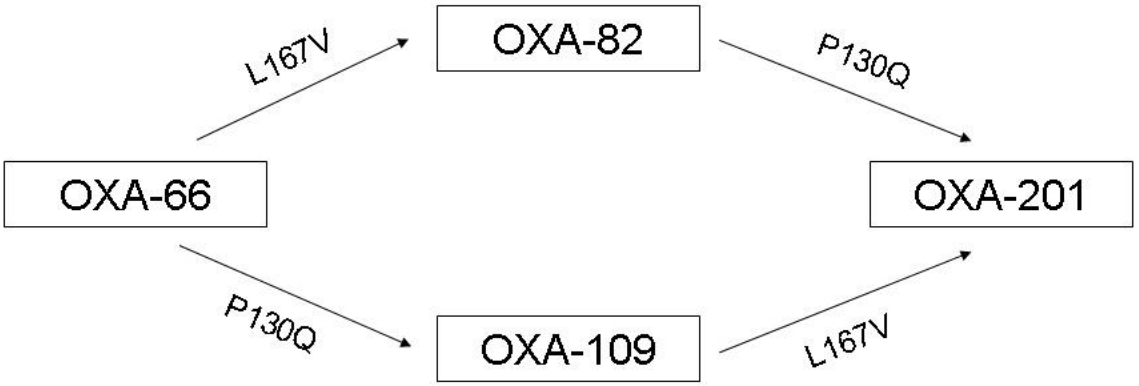

Figure 1: Amino acid substitutions of OXA-82, OXA-109 and OXA-201, compared to OXA-66. L, leucine; P, proline; Q, glutamine; V, valine.