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Conversion of OXA-66 into OXA-82 in clinical *Acinetobacter baumannii* isolates and association with altered carbapenem susceptibility

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AMENDED ABSTRACT

- Three clonally related *A. baumannii* clinical isolates were investigated to determine the mechanism responsible for their differences in carbapenem susceptibility. Isolates A, C and B were carbapenem-susceptible, -intermediate and -resistant, respectively.
- Isolate A possessed $bla_{\text{OXA-66}}$ while isolates B and C had $bla_{\text{OXA-82}}$ ($bla_{\text{OXA-51-like}}$ variants) associated with IS*Aba1*.
- Quantitative RT-PCR indicated that expression of $bla_{\text{OXA-82}}$ was >40-fold greater than $bla_{\text{OXA-66}}$ and that expression of the 33-36 kDa *omp* was the major difference between isolates B and C.
- By SDS-PAGE no difference was apparent in the OMP profiles between the isolates.
- Multiple repeating of Etests revealed isolates B and C were carbapenem-heteroresistant.
- Over-expression of $bla_{\text{OXA-82}}$ seems to be the predominant mechanism that mediates carbapenem resistance in these isolates.

INTRODUCTION AND PURPOSE

- Carbapenem resistance in *Acinetobacter baumannii* is most frequently conferred by carbapenem-hydrolysing oxacillinases (OXA)
- In the absence of an acquired OXA, carbapenem resistance is commonly conferred by over-expression of the intrinsic $bla_{\text{OXA-51-like}}$, mediated by an upstream located IS*Aba1*.[1]
- Additionally reduced porin expression (e.g. CarO[2], OprD-like[3], 33-36 kDa Omp[4]) is also associated with carbapenem resistance.
- The aim of this study was to investigate altered carbapenem susceptibilities in three clinical *A. baumannii* isolates which were part of an extended outbreak.

METHODS

- Three *A. baumannii* isolates were recovered from three separate patients in a hospital in Krakow, Poland (Table 1).
- Carbapenem susceptibility was investigated by Etest.
- Presence of bla_{OXA} was tested by PCR and sequencing.
- Molecular epidemiology was investigated by rep-PCR[5] and $bla_{\text{OXA51-like}}$ typing[6].
- Expression of $bla_{\text{OXA-51-like}}$ and three major porins was investigated by quantitative RT-PCR using *rpoB* as a housekeeping gene. Additionally, OMP expression was determined by SDS-PAGE.
- IS*Aba1*-$bla_{\text{OXA-82}}$ was cloned into the shuttle vector pWH1266 and transformed into *A. baumannii* ATCC 17978.

RESULTS

- Multiple testing revealed isolate A remained susceptible to carbapenems, whereas isolates B and C were heteroresistant.
- Using rep-PCR, the isolates were clonally related (> 98 % similarity) and clustered with worldwide clonal lineage 2 (WW 2). This was confirmed by $bla_{\text{OXA-51-like}}$ typing.
- $bla_{\text{OXA-51-like}}$ sequencing revealed conversion of $bla_{\text{OXA-66}}$ (isolate A) to $bla_{\text{OXA-82}}$ (isolates B and C) (L167→V) which was associated with the acquisition of an upstream located IS*Aba1*.
- IS*Aba1*-$bla_{\text{OXA-82}}$ conferred carbapenem resistance in ATCC 17978.
- qRT-PCR revealed over-expression of $bla_{\text{OXA-82}}$ in isolates B and C and reduced expression of the porins *carO* and *oprD-like*. Expression of the 33-36 kDa *omp* was reduced in isolate B but increased in isolate C (Table 1).
- In contrast, SDS-PAGE revealed no change in porin expression.

Table 1: Phenotypical and genotypical characterisation of clinical isolates

Isolate		A	B	C
Date of isolation		30/01/09	09/09/10	15/09/10
OXA-51-variant and presence of IS*Aba1*		OXA-66	*Aba1* OXA-82	IS*Aba1*-OXA-82
MIC (mg/L)	IPM	4	>32‡	8 - >32‡
	MEM	2	>32‡	16 - >32‡
Difference in gene expression* [x-fold]	$bla_{\text{OXA-51-like}}$	1	46.5	46.5
	carO	1	0.22	0.79
	oprD-like	1	0.28	0.64
	33-36 kDa *omp*	1	0.14	2.05

*compared to gene expression in isolate A; IPM, imipenem; MEM, meropenem
‡isolates B and C were carbapenem-heteroresistant

CONCLUSIONS

- Carbapenem resistance in two outbreak-related isolates was associated with conversion of OXA-66 into OXA-82 and acquisition of IS*Aba1*.
- Isolates B and C consistently over-expressed $bla_{\text{OXA-82}}$ compared to isolate A.
- qRT-PCR revealed changes in porin expression that were not seen by SDS-PAGE.
- RT-PCR data should be confirmed by phenotypical methods.

References

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