P1259

Abstract (poster session)

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

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Objectives: Carbapenem resistance in Acinetobacter baumannii is most frequently conferred by carbapenem-hydrolysing OXA enzymes. A. baumannii possess an intrinsic blaOXA-51-like, with 68 variants identified. Over-expression of blaOXA-51-like is associated with carbapenem resistance which is commonly mediated by an upstream located insertion element (ISAba1). Reduced expression of the porins CarO, OprD-like and 33-36 kDa Omp is also associated with carbapenem resistance. The aim of this study was to investigate altered carbapenem susceptibility in three clinical A. baumannii isolates which were part of an outbreak. Methods: Three A. baumannii isolates were recovered from three separate patients (Table). Carbapenem susceptibility was determined by Etest. Molecular relatedness was investigated by rep-PCR (DiversiLab) and blaOXA-51-like typing. Presence of acquired OXAs was tested by multiplex PCR. Expression of blaOXA-51-like, carO, oprD-like and 33-36 kDa omp was investigated using qRT-PCR. rpoB was used as a housekeeping control gene. Results: Results are summarised in the table. Clinical isolates A, B and C were clonally related (>98 % similarity) as assessed by rep-PCR. blaOXA-51-like typing confirmed affiliation to worldwide clonal lineage WW2. Isolates B and C showed reduced carbapenem susceptibility compared to susceptible isolate A (Table). Isolate C was resistant to both carbapenems, whereas isolate B was intermediate to imipenem but resistant to meropenem. Isolate A had OXA-66 while isolates B and C had OXA-82 (L167-->V) which was also associated with ISAba1. qRT-PCR revealed blaOXA-82 genes were >40-fold over-expressed compared to blaOXA-66. Comparison of porin expression revealed that isolates B and C had reduced expression of carO and oprD-like compared to isolate A (Table). Expression of 33-36 kDa omp was reduced in carbapenem-resistant isolate C and was increased in isolate B. Therefore the major difference between isolates B and C was expression of 33-36 kDa omp. Conclusions: Decreased carbapenem susceptibility in two outbreak-related isolates was associated with conversion of OXA-66 into OXA-82 and its over-expression mediated by ISAba1. However, carbapenem resistance was only found in the blaOXA-82 over-expresser with reduced expression of the three outer-membrane proteins. Therefore carbapenem resistance was not solely associated with an enzymatic mechanism but with a combination of reduced permeability and over-expression of blaOXA-82.

Table: phenotypical and genotypical characterisation of clinical isolates

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

*compared to gene expression in isolate A; DOI, date of isolation; IPM, imipenem; MEM, meropenem