

## Introduction

Acquisition of reduced susceptibility to biocides (RSB) might confer a competitive advantage for *Acinetobacter baumannii* (Ab) exposed to subinhibitory concentrations of biocides. Mechanisms that reduce the permeability of the outer membrane (OM), like overexpression of efflux pumps and/or reduction in the expression of porins, could also confer RSB in Ab, as has been observed for other microorganisms.

The objectives of this study were to evaluate the impact of the acquisition of RSB on the expression of genes encoding proteins (efflux pumps and porins) related with the regulation of the permeability of the OM

## Materials and Methods

Two clinical isolates of Ab (Ab46 and Ab70) representing the same epidemic clone (identical PFGE and ST-2) but differing in the susceptibility to carbapenems, aminoglycosides and minocycline were selected from REIPI-GEIH Ab-2010 project. Susceptibility to these antimicrobials and the biocides Irgasan® (IRG; triclosan), benzalkonium chloride (BZK) and clorhexidine digluconate (CHX) was tested by microdilution (CLSI guidelines). The parental isolates Ab46 and Ab70 were subcultured weekly for one month in presence of IR, BC or CD at sub-MIC concentrations (exposed isolates). The MICs of biocides were determined for exposed isolates and were compared with those of the parental isolates. The relative expression of genes (REG) encoding the efflux pumps *adeB*, *adeJ*, *abeM* and *amvA*, and the porins *OmpA*, *CarO*, *OprD*-like, *Omp 33-36* kDa was determined by RT-PCR (Light Cycler 2.0). The REG was determined using the 2- $\Delta\Delta$ Ct method and the housekeeping gene *rpoB* for normalization. The REG for exposed isolates were compared with those for parental isolates. All experiments were performed in triplicate. Differences in REG greater than 5 times were considered significant.

## References

- Wisplinghoff H, et al. J Hosp Infect. 2007  
Kawamura-Sato K, et al. J Antimicrob Chemother. 2008  
Rajamohan G, et al. J Hosp Infect. 2009  
Rajamohan G, et al. J Antimicrob Chemother. 2010  
Kawamura-Sato K, et al. J Antimicrob Chemother. 2010

## Results

**Table.** Relative gene expression (mean  $\pm$  SD) for isolates exposed to biocides. Gene expression for the parental isolates Ac 46 and Ac 70 was set as 1.

Gene	Isolate Ac 46 exposed to			Isolate Ac 70 exposed to		
	CHX	BZK	IRG	CHX	BZK	IRG
Efflux						
<i>adeB</i>	2.4 $\pm$ 0.9	1.2 $\pm$ 0.1	0.6 $\pm$ 0.1	<b>34.7 <math>\pm</math> 6.7</b>	<b>13.3 <math>\pm</math> 0.8</b>	0.6 $\pm$ 0.1
<i>adeJ</i>	1.5 $\pm$ 0.1	0.7 $\pm$ 0.1	3.4 $\pm$ 0.1	1.1 $\pm$ 0.0	1.0 $\pm$ 0.3	0.9 $\pm$ 0.1
<i>abeM</i>	0.7 $\pm$ 0.4	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1	0.6 $\pm$ 0.2	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1
<i>abeS</i>	3.5 $\pm$ 0.3	2.2 $\pm$ 1.4	2.0 $\pm$ 0.5	2.2 $\pm$ 1.3	4.0 $\pm$ 0.8	2.2 $\pm$ 0.6
<i>amvA</i>	<b>14.2 <math>\pm</math> 3.9</b>	0.6 $\pm$ 0.1	0.3 $\pm$ 0.1	0.8 $\pm$ 0.3	0.5 $\pm$ 0.1	0.3 $\pm$ 0.1
Porins						
<i>ompA</i>	0.8 $\pm$ 0.5	0.5 $\pm$ 0.1	<b>0.2 <math>\pm</math> 0.0</b>	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	<b>0.2 <math>\pm</math> 0.0</b>
<i>carO</i>	0.5 $\pm$ 0.0	0.3 $\pm$ 0.1	<b>0.2 <math>\pm</math> 0.0</b>	0.8 $\pm$ 0.4	<b>0.2 <math>\pm</math> 0.0</b>	<b>0.0 <math>\pm</math> 0.0</b>
<i>omp 33-36</i>	1.5 $\pm$ 0.4	1.3 $\pm$ 0.6	1.5 $\pm$ 0.6	1.1 $\pm$ 0.3	1.5 $\pm$ 0.5	0.3 $\pm$ 0.2
<i>oprD</i>	ND	ND	ND	ND	ND	ND

ND: not determined

## Conclusions

- 1) Biocides can modulate the expression of genes related with the efflux (increasing the expression of some pumps) and influx (reducing the expression of some porins) of substances across the OM.
- 2) Exposition to CHX increases the expression of the genes encoding the efflux pump *AmvA* and the component *adeB* of the efflux pump *adeABC*.
- 3) BZK increases also the expression of the gene encoding *adeB* and has no effect on the expression of porin genes.
- 4) IRG has no significant effect on the expression of efflux genes, but reduces the expression of genes encoding the porins *OmpA* and *CarO*.