Effect of Subminimum inhibitory concentrations of different bioactive compounds on biofilm formation and virulence factors of clinical isolates of *Acinetobacter baumannii*

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BACKGROUND

Acinetobacter baumannii is a remarkable hospital pathogen, notably due to the dissemination of highly multidrug resistant isolates and the treatment options for infections caused by multi-drug resistant (MDR) *A. baumannii* strains constitute a strong challenge. The virulence factors of this organism such as biofilm formation, bacterial adherence, bacterial invasion and efflux pumps help the bacterium to survive in adverse environmental conditions and facilitate the development of an infection. Antiseptics are frequently used for the management of MDR pathogens in hospitals and their consistent use in hospitals has elevated concerns about its resistance.

OBJECTIVES

The aim of this study was to determine the effect of subminimum inhibitory concentrations (sub-MICs) of selected antimicrobial agents (Amikacin (AMK), imipenem (IMP), benzalkonium chloride (BZC), and chlorhexidine (CLX)) and natural product (garlic extract) on biofilm formation, bacterial adherence and invasion and the emergence of resistance among *A. baumannii* clinical isolates.

MATERIALS AND METHODS

Susceptibility profiles of 50 non-repetitive *A. baumannii* isolated from admitted patients in two tertiary care hospitals in Cairo, Egypt to eight different antibiotics were investigated. MIC of various antibiotics, antiseptics and garlic were measured by the broth microdilution method. Quantification of biofilm formation after subjecting the isolates to the sub-MIC of the bioactive compounds was carried out using a microtiter plate assay. The ability of test compounds at their sub-MIC to affect the bacterial adherence and invasion was investigated using Type II pneumocyte cell line A549 derived from a human lung carcinoma and the bacterial cells count was determined in each well using flow cytometer. Screening for the presence of antiseptic resistant gene qacA/B was done using PCR.

RESULTS

Table 1: Antibiotic resistance profiles of *A. baumannii* isolates obtained from clinical samples.

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Cefotaxime	1 (2%)		49 (98%)
Meropenem	6 (12%)		44 (88%)
Doxycyclin	6 (12%)		44 (88%)
Imipenem	7 (14%)		43 (86%)
Levofloxacin	4 (8%)	3 (3%)	43 (86%)
Gentamicin	2 (4%)	6 (12%)	42 (84%)
Trimsulfamethoxazole	3 (6%)		37 (74%)
Amikacin	10 (20%)	4 (8%)	36 (72%)

Ten isolates were found to have variation in their susceptibility toward both Amikacin and Imipenem where six isolates were amikacin sensitive and imipenem resistant (ASIR) while four isolates were amikacin resistant and imipenem sensitive (ARIS).

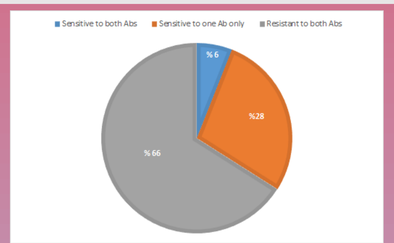


Figure 1: Pattern of cross resistance between carbapenems (Imipenem) and aminoglycosides (Amikacin) among *A. baumannii* isolates.

The obtained MIC values of the bioactive compounds were quite variable and Based on the MICs obtained the sub-MICs were calculated where the 3/4 MIC was considered as sub-MIC.

Table 2 Sub-MICs values obtained for the selected isolates against the bioactive compounds.

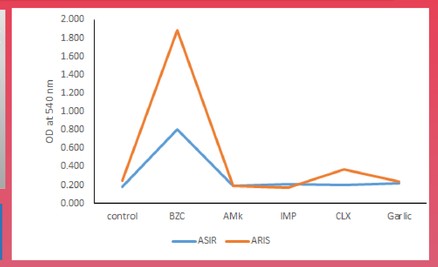
Isolate no.	Gar (µg/ml)	CLX (µg/ml)	AMk (µg/ml)	IMP (µg/ml)	BZC (%w/v)
304	586	7.32	3.66	29.32	0.59
307	2344	3.66	234.38	0.92	0.59
310	2344	3.66	234.38	0.92	0.59
311	1172	7.32	7.32	29.32	0.59
315	293	3.66	7.32	14.65	0.59
316	586	29.30	7.32	29.32	0.59
317	1172	14.65	3.66	58.59	2.34
318	2344	29.30	3.66	58.59	2.34
319	586	14.65	7.32	58.59	2.34
340	586	29.30	29.30	58.59	2.34

CONCLUSION

Sub-MIC of antibiotics and antiseptics can lead to emergence of resistance. Therefore, careful evaluation of sub-MIC effects on bacterial physiology is needed prior to therapeutic use of sub-MICs. Antiseptics are important components of infection control and continuous monitoring of antiseptics use in the hospital is cautioned.

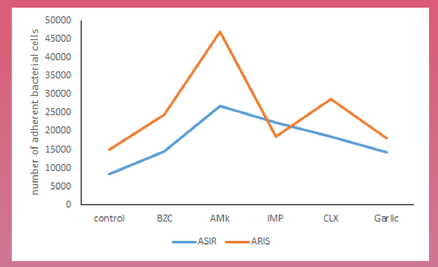
It was found that sub-MIC of BZC markedly increased the biofilm formation by 4.7 folds for ASIR group and 7.8 folds in ARIS group.

Figure 2: Effect of sub-MIC of bioactive compounds on biofilm formation.



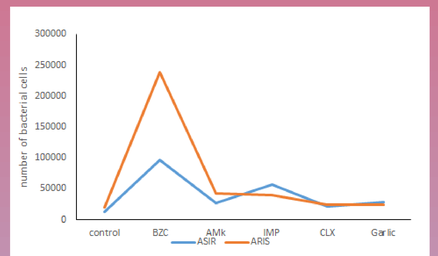
An induction in the bacterial adherence in both groups by 3 folds post treatment with sub-MICs of amikacin.

Figure 3: Effect of sub-MIC of bioactive compounds on bacterial adherence.



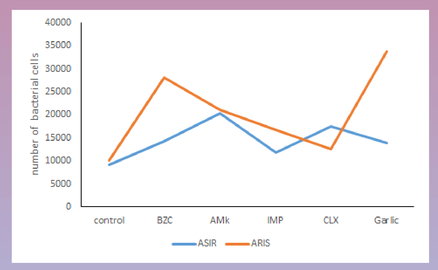
Bacterial invasion was markedly increased where BZC and IMP showed the highest increase in ASIR isolates by 7 and 4 folds respectively. Where in ARIS isolates BZC showed an increase with 12 folds.

Figure 4: Effect of sub-MIC of bioactive compounds on bacterial invasion after 3hr.



In case of 5 hours post treatment, amikacin had the highest effect in ASIR isolates where BZC and Garlic had the highest effect in ARIS isolates.

Figure 5: Effect of sub-MIC of bioactive compounds on bacterial invasion after 5 hr.



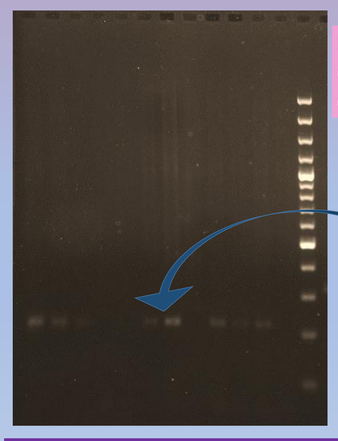


Figure 6: Antiseptic resistant gene *(qacA/B*) was detected in 6 out of 10 isolates using positive control.

Amplification of 217 bp fragment corresponding to *qacA/B*

References

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