ANTIBACTERIAL ACTIVITY OF METAL-LOADED NATURAL ZEOLITE AGAINST CLINICAL ISOLATES OF *ACINETOBACTER BAUMANNII*

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ABSTRACT

The antibacterial activity of natural zeolite (CLI) containing 2.60 wt. % Cu^{2+} (Cu-CLI) or 5.07 wt.% Ag^{+} (Ag-CLI) were tested against clinical isolates of *Acinetobacter baumannii*. *A. baumannii* belonging to the European clone I and II were isolated in Split University Hospital, Croatia. The isolate from clone II was more sensitive on the effect of the metal-loaded zeolite than isolate of clone I. The antibacterial activity can be ascribed to the metal ions inside the zeolite lattice since the natural zeolite itself does not show antibacterial activity toward the studied bacterial strains.

Keywords: clinoptilolite, copper, silver, antibacterial activity, natural zeolite.

INTRODUCTION

Acinetobacter baumannii is a Gram-negative coccobacilus that are mainly resistant to commonly used antibiotics and may form a reservoir of antibiotic resistance genes, particularly in hospital environments. The resistance is correlated with beta-lactamase production which acts like "sponge" preventing the action of the most common antibacterial agents [1]. These bacteria have been implicated in a variety of food spoilage and human disease processes [2].

Recently, natural zeolites have been extensively studied for medical purposes because of its exchange, adsorptive and biocatalytic activities as well as non-toxic behaviour [3, 4]. Also, several metal-rich modified zeolites have been reported as promising disinfecting materials [5, 6].

In this study, we have investigated the antibacterial activity of copper and silver loaded natural clinoptilolite toward *A. baumannii* isolated from the same hospital and belonging to the two different clones - European clone I and II.

EXPERIMENTAL

The natural zeolite (CLI) containing 72.6 wt % of clinoptilolite, 14.6% of feldspar plagioclase and 12.8% of quartz [7] was used in this work and it was obtained from the sedimentary deposit in Zlatokop mine, Serbia. The CLI (size particle 0.063-0.1 mm) was suspended in solution of NaCl (c= *2 mol* dm^{-3}) in order to improve the cation exchange capacity. Obtained Na-rich sample (Na-CLI) was then used for the preparation of Cu- and Ag-loaded zeolites (Cu-CLI and Ag-CLI, respectively). Cu-CLI and Ag-CLI were prepared by suspending 1.0 g of Na-CLI in 100 cm^3 of 6 mM aqueous solution of $CuSO_4$ or $AgNO_3$. The suspensions were shaken for 24 h in a thermostatic water bath at 25º C, separated by filtration and dried over night in the air. The obtained samples contained 20.33 mg Cu (Cu-CLI) and 50.65 mg Ag per gram of zeolite (Ag-CLI). It has been previously found that both Cu(II) and Ag(I) ions enter the zeolite lattice by an ion-exchange [7]. The results of BET measurements showed that the ion-exchange does not influence the specific area (32 m^2 g^{-1}).

Before testing for antibacterial activity the samples of Cu-CLI were sterilized by autoclaving (121° C, 15 min). The antibacterial activity of Ag-CLI was tested without autoclaving but no contamination of pure cultures was observed during the experiments. Two different isolates of *A. baumannii* were obtained from Split University Hospital with affiliation to European clone I (EU I) and clone II (EU II). *A baumannii* was cultivated in a routine work on blood agar plates (Bio Rad).

The antibacterial activity of Cu-CLI and Ag-CLI was tested in 0.85 % NaCl solution. First, the bacterial biomass was pre-grown on nutrient agar (Biolife, Italy) for 16 h at 36±0.1° C to obtain the cultures in a log phase of growth. The bacterial biomass was suspended in 0.85 % NaCl solution and Cu-CLI and Ag-CLI were added to achieve concentration of 1000 mg zeolite dm^{-3}. Serial dilutions of the suspensions were made according to the standard dilution method [8]. Experimental tubes were incubated in dark conditions during 24 h at 36±0.1° C with shaking at 120 rpm. In additional tubes the experiment with $CuSO_4 \cdot 5H_2O$ and $AgNO_3$ were set up in a same way as described above to examine the antibacterial activity of the free cations in concentration equivalent to the metal amounts loaded onto CLI which showed the minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC). To confirm the absence of the antibacterial activity of Na-CLI itself, the experimental bottles containing 1000 mg L^{-1} of the autoclaved Na-CLI were set up.

The number of viable cells was determined at the beginning of experiment, and then after 1, 3, 5 and 24 h of contact. Tubes were vigorously shaken at 40 Hz/3 minute. A 0.1 cm^3 of sample was plated (spread plate method) directly on a nutrient agar and another 0.1 cm^3 of sample was diluted (10^{-1} – 10^{-8}) and inoculated onto the nutrient agar plates in triplicate. These plates were incubated at 36±0.1° C for 24 h, and after these period the bacterial colonies were counted and the number of viable cells was reported as CFU ml^{-1}. According to the Clinical and Laboratory Standards Institute the minimum inhibitory concentration and minimum bactericidal concentration values were determined after 24 h of experiments. The antibacterial activity was expressed as the percent reduction of log CFU as compared to the corresponding control.

In the tubes showing MIC and MBC (after 24 h) the leaching of Cu^{2+} and Ag^{+} from Cu-CLI and Ag-CLI were examined by atomic absorption spectrophotometry (Varian, AAS 55B).

RESULTS AND DISCUSSION

Antibacterial activity of Cu-CLI and Ag-CLI against *A. baumannii* EU I and EU II are shown in Figure 1. The antibacterial activity of Cu-CLI is increased by increasing the concentration of Cu-CLI and time of contact. The MBC for EU I was 250 mg dm^{-3} and for EU II 125 mg dm^{-3} showing that the EU I is more resistant than EU II to Cu-CLI. The MIC values were one order lower than MBC values for examined concentrations for EU I and II. The results of the antibacterial activity of Ag-CLI shows that the values of MBC and MIC for EU I are 250 mg dm^{-3} and 62.5 mg dm^{-3}, respectively and for EU II 31 mg dm^{-3} and 15.6 mg dm^{-3}, respectively. The results indicate that the EU II is more sensitive to Ag-CLI than EU I.

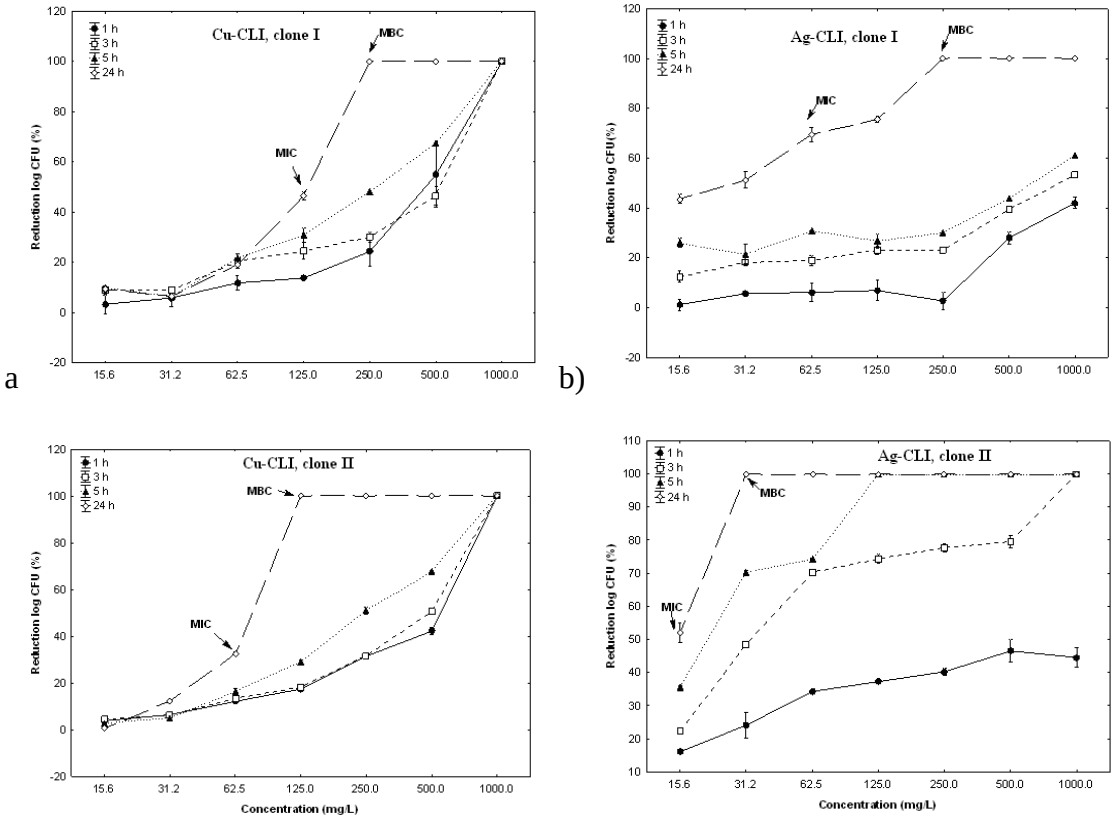

Figure 1. Percent reduction in the number of *A. baumanni* EU I and II during 24 h of contact with a) Cu-CLI and b) Ag-CLI as compared to the corresponding controls. t_0 *A. baumannii* EU I (10^6 CFU cm^{-3}) = 8.80±0.17; t_0 *A. baumannii* EU II (10^7 CFU cm^{-3}) = 1.40±0.17.

In order to examine whether the antibacterial activity could be ascribed to the metal-loaded Na-CLI or to the cations possibly leached from the samples into solutions, leaching tests were also performed. The concentrations of Cu and Ag measured after 24 h exposure in the tubes showing MBC and MIC are given in Table 1.

Table 1. The concentration of the cations in mg dm^{-3} leached from Cu-CLI and Ag-CLI in tubes showing the MBC and MIC.

Sample	Cu-CLI	Ag-CLI
Clone I		
MBC	0.403	0.090
MIC	0.517	0.061
Clone II		
MBC	0.103	0.130
MIC	0.121	0.084

For the both materials amounts of the cations leached from the zeolites were very low (0.7 wt. % Cu and 8.2 wt. % Ag) indicating that the antibacterial activity could be ascribed only to the Cu-CLI and Ag-CLI and not to the cations leached from these materials. It is interesting that Cu concentration in the tubes with clone I is higher than with the clone II whereas concentration of Ag was similar for the both clones.

In this study we also compared the antibacterial activity of free Cu(II) and Ag ions with the Cu-CLI and Ag-CLI. For the free ions 100 % bacterial reduction was found after 1 hour

for both clones. Although the Cu-CLI and Ag-CLI shows reduced antibacterial activity for the same time period their main advantage is their long-term antibacterial activity.

Natural clinoptilolite at concentration 1000 mg dm^{-3} did not show any antibacterial activity against both clones of *A.baumannii*. It could be concluded that the antibacterial activity of Cu- and Ag-loaded zeolite can be ascribed to the modified zeolite but not to natural zeolite itself.

CONCLUSION

Cu-rich and Ag-rich clinoptilolite show good antibacterial activity toward *A. baumannii* belonging to European clone I and clone II. Clinical isolate from the clone II is found to be more sensitive to the metal modified zeolites than clone I. The results indicate that natural zeolite could find very important role in clinical investigations on the higher number of multidrug resistant bacterial isolates on which the bactericidal effect can be expected.

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