MODE OF ACINETOBACTER BAUMANNII IMMOBILIZATION ONTO NATURAL ZEOLITE IN NUTRIENT-POOR AND NUTRIENT–RICH WATER

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*Acinetobacter baumannii* is a Gram-negative bacterium with cells of coccobacillus shape (1181 x 996 nm).
Although not an obligate pathogen, during the last 30 years *A. baumannii* developed the resistance to commonly used antimicrobial agents.
A. baumannii resistant to last-resort antibiotics is nowadays a leading cause of nosocomial infections worldwide.

Recently the occurrence of *A. baumannii* in urban wastewaters and rivers influenced by the untreated hospital sewage have been reported. This suggest the water as a potential source of clinically relevant *A. baumannii* isolates that poses a threat to people that come into contact with water.

The **goal** of this study was to examine the natural zeolitizied tuff (NZ) as a material for the capture of *A. baumannii* from nutrient-poor and nutrient-rich water.
A. baumannii isolate (named EF7) was recovered from effluent of the Zagreb wastewater treatment plant.
Antibiotic resistance profile of pandrug-resistant isolate of *A. baumannii*.

carbapenems (MEM-meropenem, IMI-imipenem); fluoroquinolones (CIP-ciprofloxacin, LVX-levofloxacin); aminoglycosides (TOB-tobramycin, GEN-gentamicin, AMK-amikacin); tetracyclines (MIN - minocycline); penicillins/β-lactamase inhibitors (SAM-ampicillin/sulbactam, TIM - ticarcillin/clavulanate); folate pathway inhibitors (SXT-trimethoprim/sulfamethoxazole); polymyxins (CST-colistin).

R - resistant, I - intermediate according to EUCAST or CLSI criteria.

<table>
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<tr>
<th>Isolate</th>
<th>MEM</th>
<th>IMI</th>
<th>CIP</th>
<th>LVX</th>
<th>TOB</th>
<th>GEN</th>
<th>AMK</th>
<th>MIN</th>
<th>SAM</th>
<th>TIM</th>
<th>SXT</th>
<th>CST</th>
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<tr>
<td>EF7</td>
<td>R</td>
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The NZ was obtained from quarry located at Donje Jesenje, Croatia. Dry-autoclaved particles ≤ 0.122 mm were used.

X-ray powder pattern of NZ. NZ sample consisted mostly of clinoptilolite (50-55%) with major constituents being celadonite, plagioclase feldspars and opal-CT (10-15% each). Analcime and quartz were present in traces.
Overnight bacterial biomass

Incubation at 35°C, aeration with sterile air (4.6 mg O_2/L).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>nutrient-poor water (commercial spring water)</th>
<th>nutrient-rich water (commercial spring water + 1% nutrient broth)</th>
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<tbody>
<tr>
<td>pH</td>
<td>8.1</td>
<td>6.9</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>3</td>
<td>99</td>
</tr>
<tr>
<td>TOC (mg/L)</td>
<td>&lt;1</td>
<td>44</td>
</tr>
<tr>
<td>TN (mg/L)</td>
<td>0.7</td>
<td>13.2</td>
</tr>
<tr>
<td>TP (mg/L)</td>
<td>0.1</td>
<td>1.1</td>
</tr>
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</table>

1 wt% of dry-autoclaved NZ
Numbers of *A. baumannii* after 24h and 72h of contact with NZ. $c_0 \ (\log \text{CFU/mL})=7.3 \pm 0.0.$
SEM analysis revealed the formation of biofilm on the rough surface of NZ particles within 24h of contact in both nutrient-poor and nutrient-rich water. Bacteria stayed tightly attached onto NZ and covered by extracellular polymeric substances up to 72h of monitoring.
Mode of *A. baumannii* immobilization onto NZ
24h later

GO!

....24h later
Nutrient-poor water

....72h later

Nutrient-rich water

GO!

GO!
Conclusion:

- NZ is a promising material for the immobilization of super-bacterium *A. baumannii* in both nutrient-poor and nutrient-rich water.

- Capacity of the examined NZ for the immobilization of *A. baumannii* could be set at 8 log CFU per one gram of dry weight.

- Higher number of immobilized bacteria could be obtained as a result of bacterial multiplication inside the formed biofilm.

- This feature could find application in the removal of *A. baumannii* from contaminated water, in order to mitigate the propagation of this emerging human pathogen in nature and to avoid the consequent public health risk.
Thank you for attention!

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