Reduction of *Acinetobacter baumannii* biofilm formation by natural zeolite

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**Acinetobacter baumannii**

- Gram negative coccobacillus
- Emerging human pathogen isolated mainly from hospital setting
- Pneumonia, bloodstream infections, urinary tract infections, wound infections in immunosuppressed patients
- Environmental isolates related to clinical isolates were found in soils and waters under the influence of human solid and liquid waste (Seine River, Sava River, acid paleosol from Croatia)
- Survives adverse environmental conditions for several months
Acinetobacter baumannii

- Antibiotic resistance and virulence factors contribute to *A. baumannii* success as a pathogen
- Surface motility on solid/semi-solid media
- Biofilm formation on various surfaces
  - respiratory devices, intravenous devices, catheters, furniture, linen
  - human epithelial cells

Bacteria on human alveolar epithelial cells
**Acinetobacter baumannii**

- **Biofilm** - an assemblage of cells enclosed in an extracellular matrix formed on different interfaces (solid-liquid, air-liquid)
- **Pellicle** - highly organised form of biofilm formed at the air-liquid interface
Experimental

- Wastewater treatment plant in Zagreb
- Combined sewage of domestic, hospital, industry and storm wastewater
- Samples of influent, efluent, fresh activated and digested sludge were analysed
Experimental

- Isolation on commercial agar CHROMagar Acinetobacter at 42°C/48h
- Identification with Matrix assisted laser desorption ionisation with time of flight (MALDI TOF) on ribosomal proteins
Experimental

- Antibiotic resistance profile (Vitek2 system, EUCAST and CLSI criteria for clinical isolates)
- Hydrophobicity (BATH assay)- affinity of bacteria for organic hydrocarbon
- Biofilm formation (Crystal violet assay)
  - $\text{OD}_{550} < 0.3$ poor
  - $\text{OD}_{550} 0.3-1.0$ intermediate
  - $\text{OD}_{550} > 1.0$ strong
- Pellicle formation
  - No pellicle (0)
  - Poor (1)
  - Strong (2)
Natural zeolite (NZ)

- Quarries at Donje Jesenje, Croatia
- Clinoptilolite (50-55%), celadonite, plagioclase feldspars and opal-CT (10-15% each), analcime and quartz in traces
- <0.122mm
- dry NZ was sterilized by autoclaving
- Experiments with 1 and 10 wt % NZ
24 isolates recovered
14 MDR, 10 sensitive to 12 antibiotics tested
9/24 isolates hydrophobic
Majority of isolates intermediate biofilm and poor pellicle formers
Antibiotic sensitive isolates more hydrophobic and stronger biofilm and pellicle formers

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Antibiotic resistance</th>
<th>Hydrophobicity (%)</th>
<th>Pellicle formation</th>
<th>Biofilm formation (OD&lt;sub&gt;550&lt;/sub&gt;)</th>
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IN- influent,  EF- effluent, S - fresh sludge, D - digested sludge
MDR (multi-drug resistant)- resistance to three or more classes of antibiotics

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<th>Hydrophobicity</th>
<th>Biofilm</th>
<th>Pellicle</th>
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Biofilm formation

OD values 0.3 and 1.0

0% NZ
1% NZ
10% NZ
Pellicle formation

1 wt % NZ

Pellicle of the same consistency

Control

Lower consistency pellicle

10 wt % NZ
Cells of *Acinetobacter baumannii* immobilized onto NZ particles
Conclusions

- Cell surface hydrophobicity is an important feature which determines biofilm and pellicle formation of *A. baumannii*.
- Isolates sensitive to antibiotics form stronger biofilm and pellicles than MDR isolates.
- NZ successfully reduces biofilm and pellicle formation due to the immobilization of bacteria onto the NZ particles.
Conclusions

- NZ is a promising material for the reduction of *A. baumannii* virulence factors.
- NZ could find application in control of this emerging pathogen in the form of cleaning product where *A. baumannii* could be captured by NZ and safely removed from the contaminated environment.
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THANK YOU!