

Immobilization of *Acinetobacter baumannii* onto natural zeolite dependent on the nutrient concentration of water media

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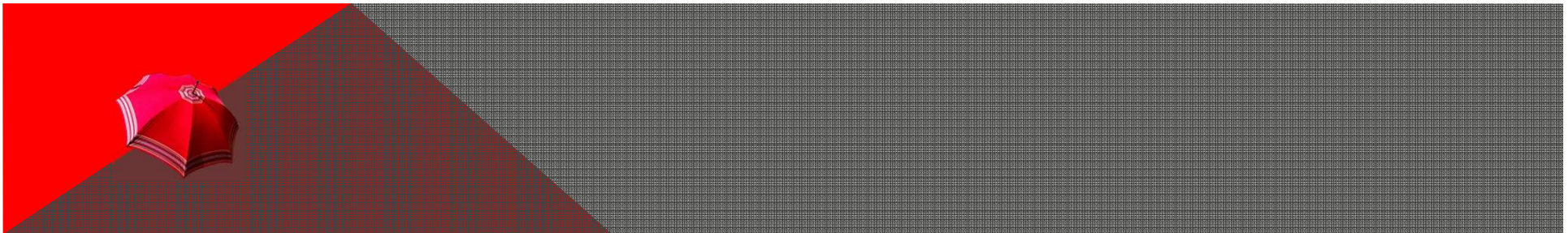
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7th Slovenian-Serbian-Croatian Symposium on Zeolites, Ljubljana, Slovenia,
25th – 27th May 2017

BACKGROUND

- From 2015 - 2019. project „Natural habitat of clinically important *Acinetobacter baumannii*” funded by Croatian Science Foundation, Prof.dr.sc. Jasna Hrenović, project leader.
- *A. baumannii* is an emerging opportunistic pathogen causing hospital-acquired infections, multi-drug resistant, extensive-drug resistant and pan-drug resistant
- *A. baumannii* was found at various sites outside hospital settings



HEALTH

WHO releases list of world's most dangerous superbugs



By HELEN BRANSWELL [@HelenBranswell](#)
FEBRUARY 27, 2017



“Within a generation, without new antibiotics, deaths from drug-resistant infection could reach 10 million a year. Without new medicines to treat deadly infection, lifesaving treatments like chemotherapy and organ transplant, and routine operations like caesareans and hip replacements, will be potentially fatal.”

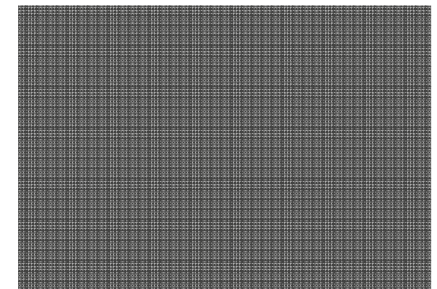
The full list is:

Priority 1: Critical

1. Acinetobacter baumannii, carbapenem-resistant
2. Pseudomonas aeruginosa, carbapenem-resistant
3. Enterobacteriaceae, carbapenem-resistant, ESBL-producing

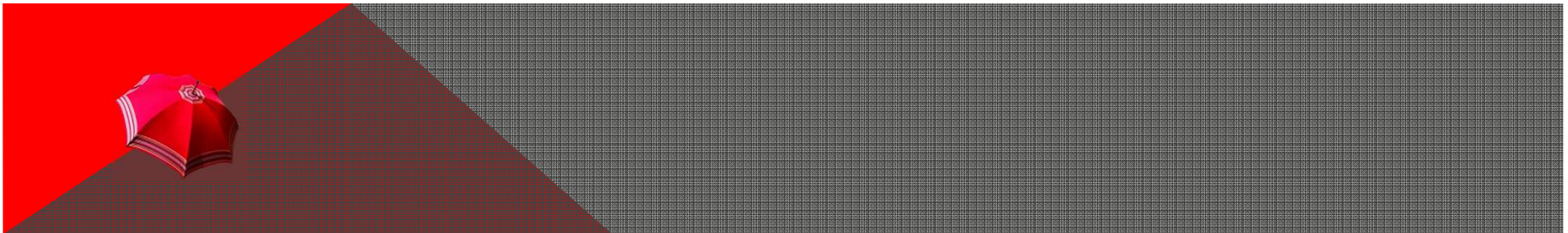
Priority 2: High

4. Enterococcus faecium, vancomycin-resistant
5. Staphylococcus aureus, methicillin-resistant, vancomycin-intermediate and resistant

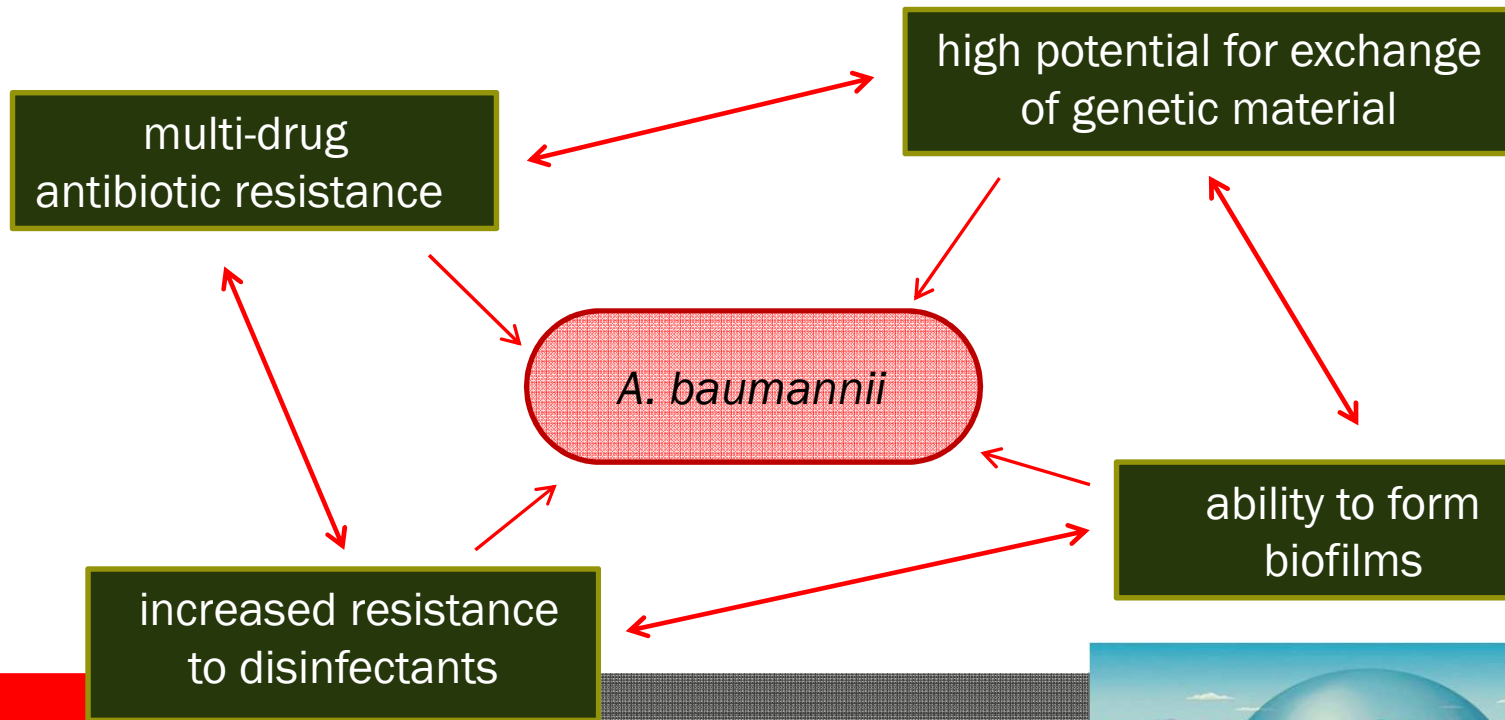


BACKGROUND

- ❑ Why is this bacteria so dangerous?
- ❑ It combines all the virulence factors, which were until now being found scattered over various bacterial species;
- ❑ multi-drug antibiotic resistance
- ❑ high potential for exchange of genetic material
- ❑ ability to form biofilms
- ❑ increased resistance to disinfectants

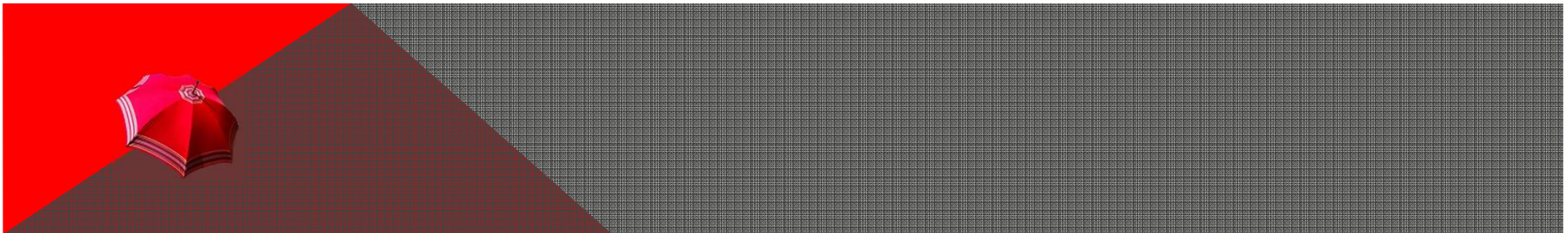


BACKGROUND



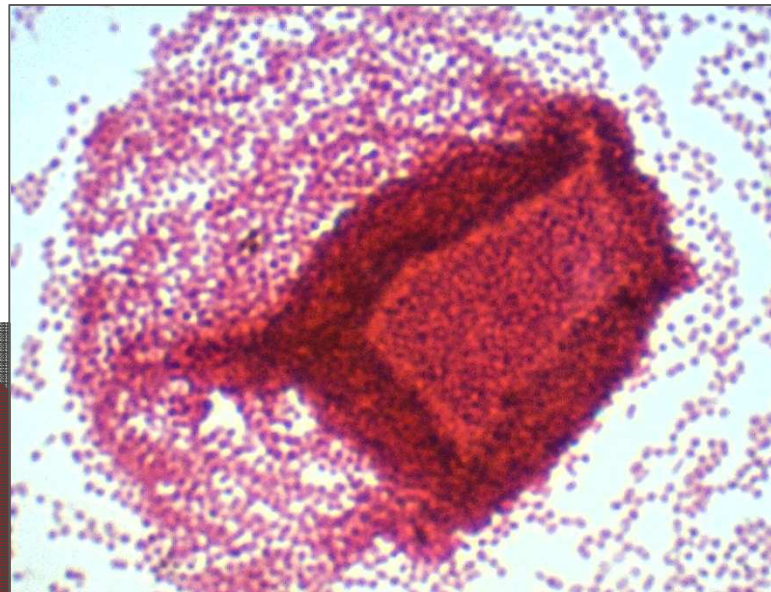
BACKGROUND

- Why did we investigate biofilms on natural zeolites (NZ)?
- Current methodology investigating *A. baumannii* biofilms is almost exclusively oriented on few standard methods;
 - biofilm growth on plastic or glass,
 - and grown in highly nutrient media
- Biofilms grown on NZ represent actual environmental conditions...



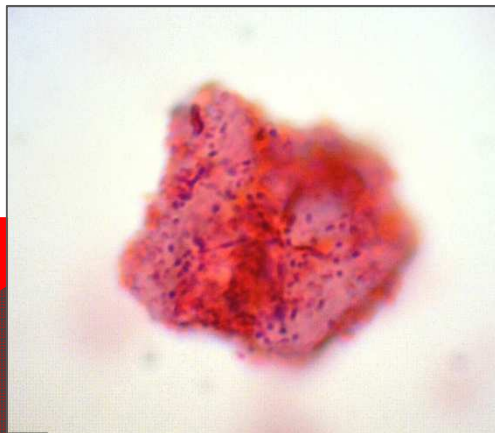
BACKGROUND

- Why did we investigate biofilms on natural zeolites (NZ)?
- NZ was shown to be the optimal support media for bacterial immobilization and biofilm formation



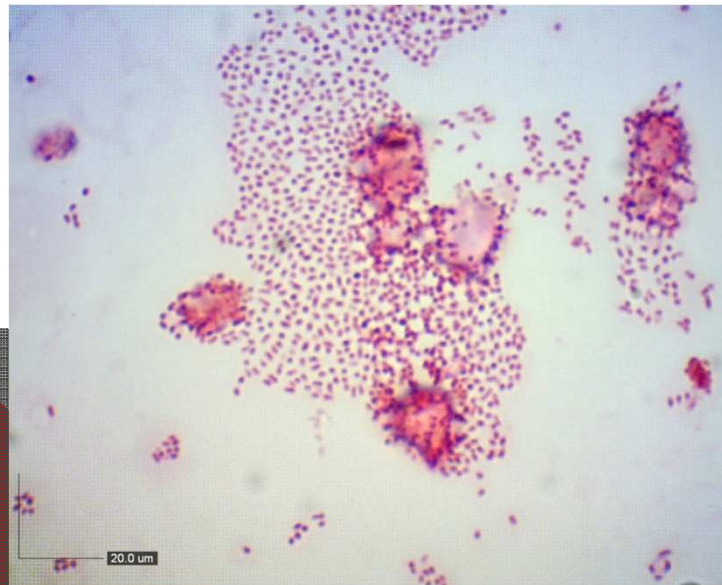
BACKGROUND

- Factors NOT influencing bacterial immobilization on NZ;
 - Mineralogical and chemical composition
 - Surface charge
 - Specific surface area



BACKGROUND

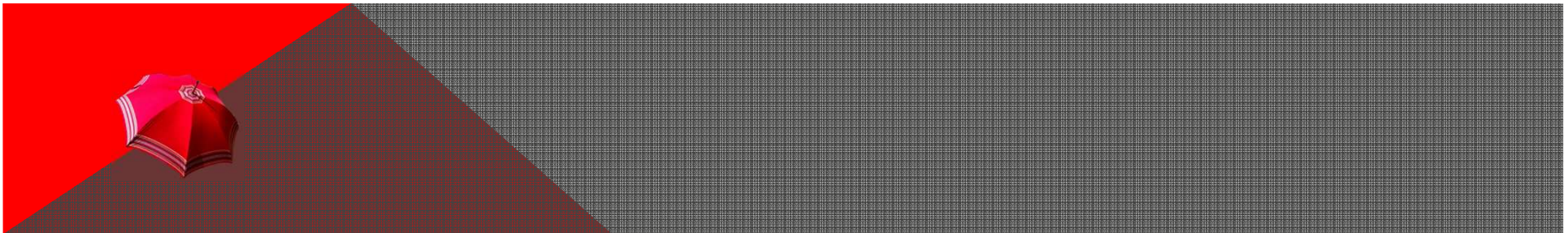
- Factors **INFLUENCING** bacterial immobilization on NZ;
 - Particle size
 - Composition of nutrient/water media?



EXPERIMENT

- Bacteria:
 - *A. baumannii* isolated from acid paleosol influenced by illegally disposed solid waste

- Natural zeolite:
 - Obtained from Donje Jesenje, Croatia
 - Clinoptilolite (50-55%), celadonite, plagioclase feldspars and opal-CT (10-15% each), analcime and quartz in traces
 - size fraction of 0.122 - 0.263 mm



EXPERIMENT

- Growth media:



Spring water

COD:
 $3 \text{ mgO}_2 \text{ L}^{-1}$



Dilluted
Nutrient broth

COD:
 $930 \text{ mgO}_2 \text{ L}^{-1}$



Nutrient broth

COD:
 $93\ 000 \text{ mgO}_2 \text{ L}^{-1}$



COD = Chemical Oxygen Demand

EXPERIMENT

bacteria were added to Schott bottles containing 100 mL of nutrient media

1 g of NZ was added

incubated for 3 days at 20°C/170 rpm

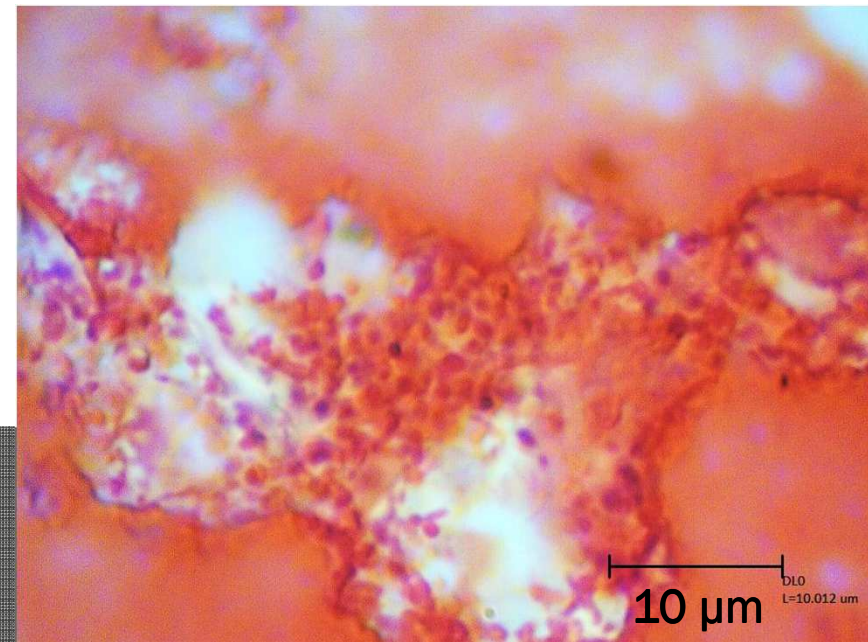
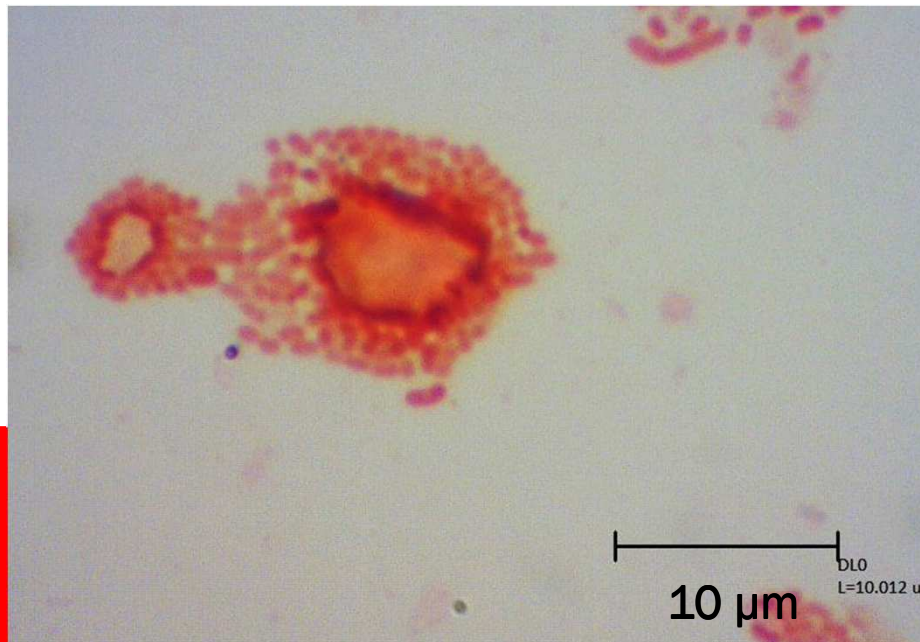
during 3 days, biofilm was formed on the surface of NZ

the number of bacteria in the form of biofilm was determined

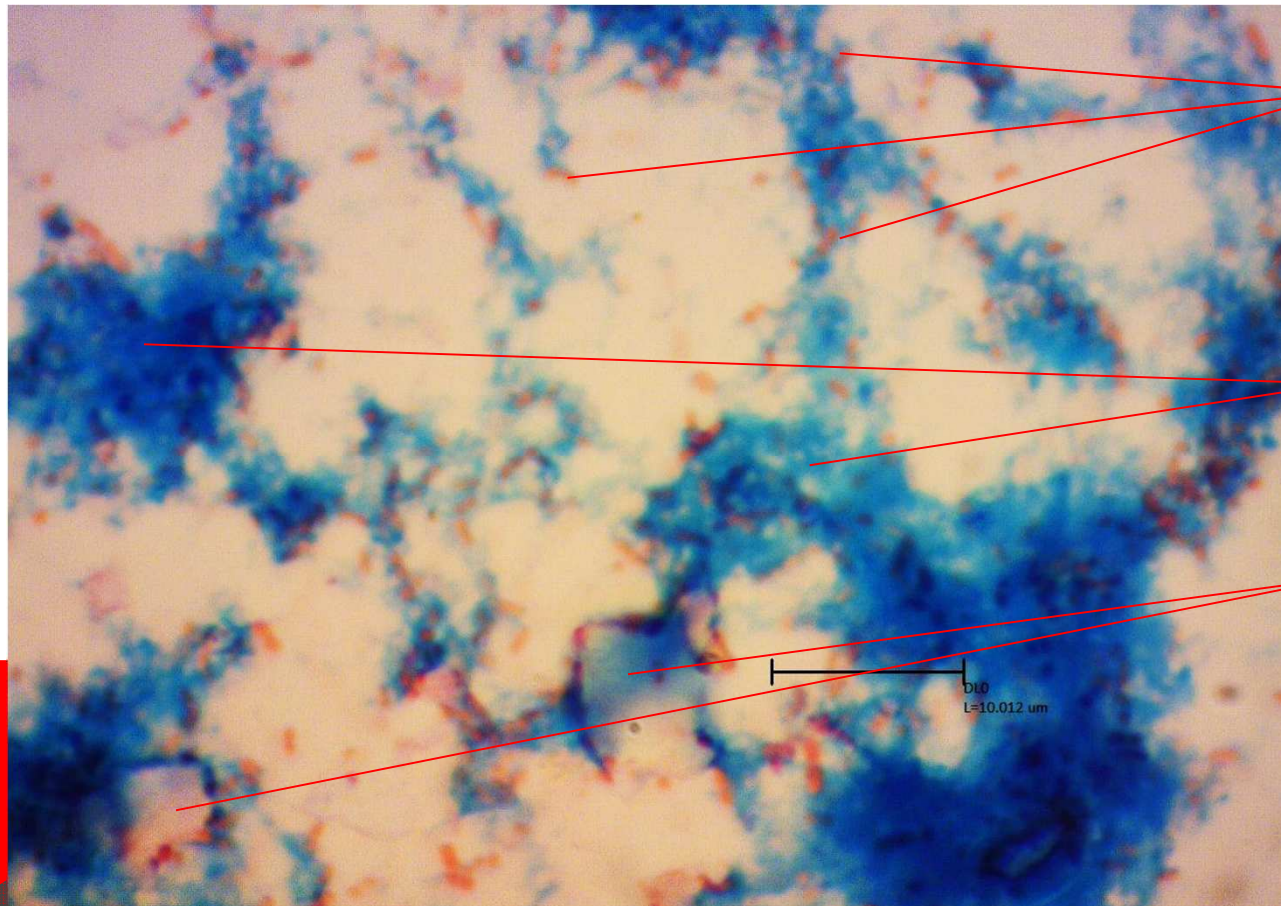


RESULTS

- After 3 days bacteria were immobilized on the surface of NZ and formed a biofilm.



RESULTS



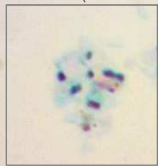
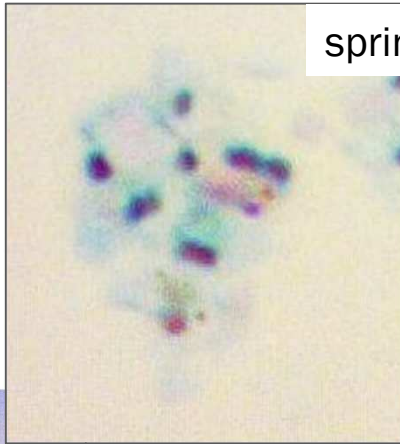
bacteria: red rods

blue: exopolymeric matrix (EPS)

NZ particles:
in these pictures are transparent

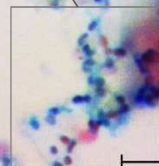
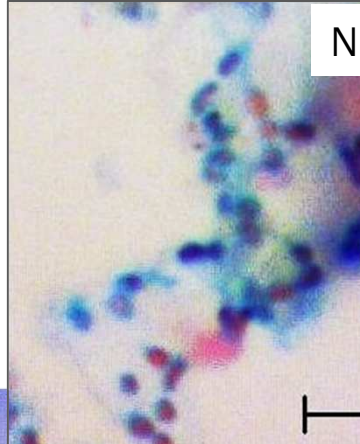
RESULTS

spring water



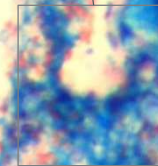
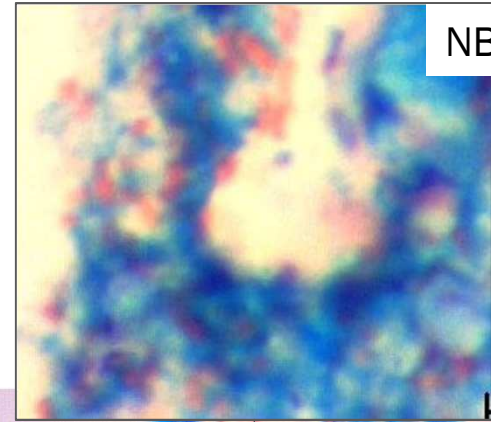
2x0
L=10.012 um

NB/100



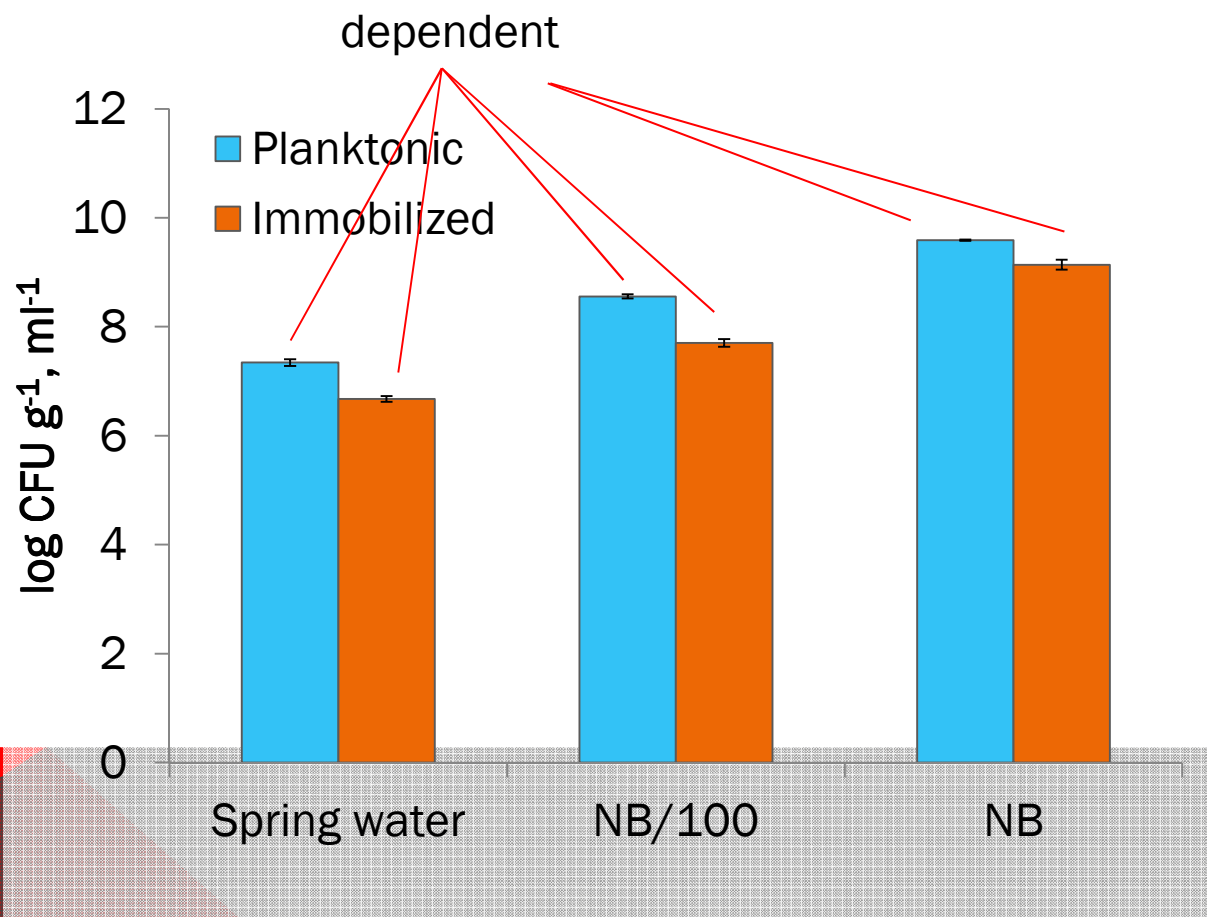
2x0
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NB

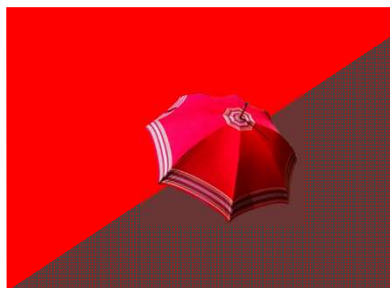
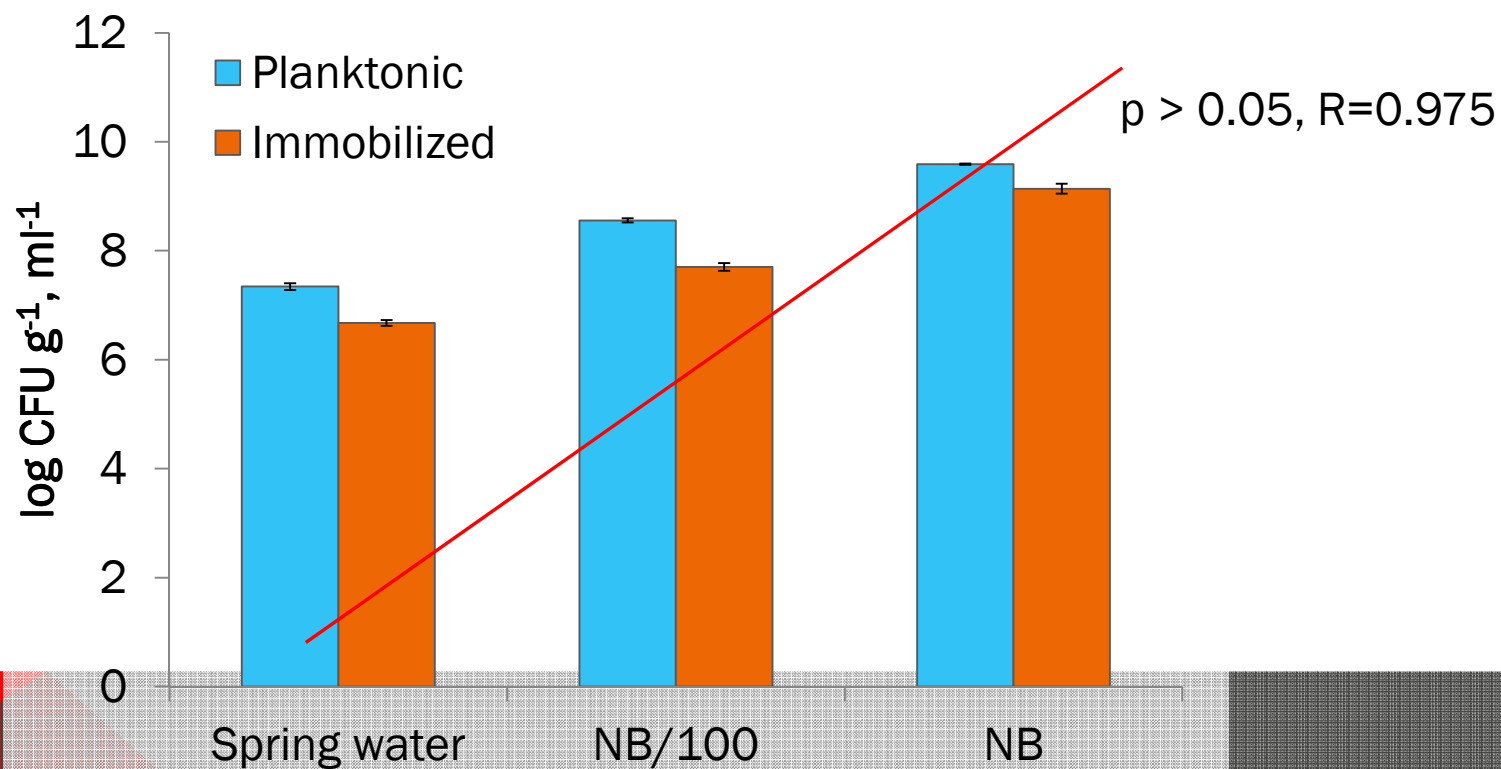


2x0
L=10.012 um

RESULTS

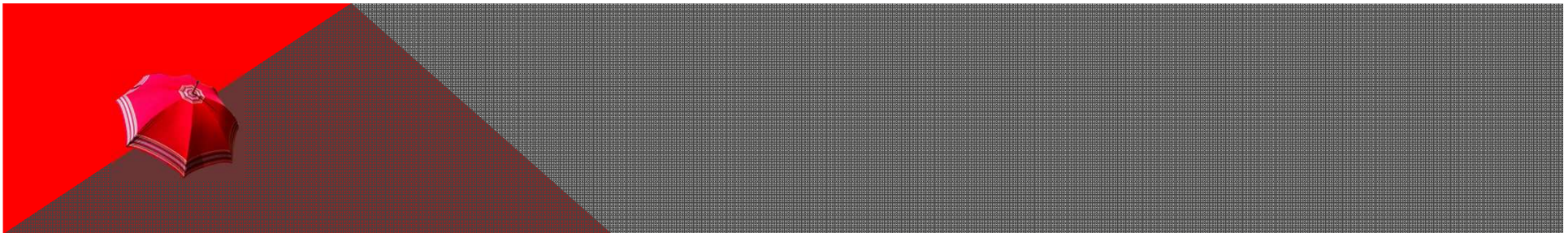


RESULTS



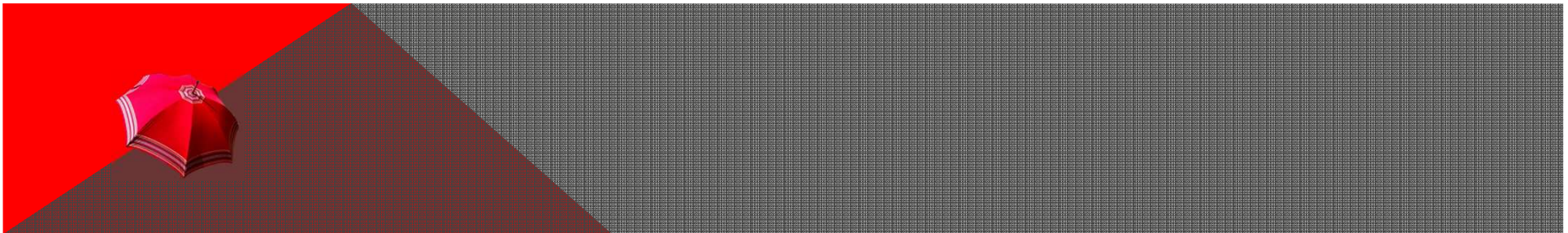
CONCLUSIONS

- The intensity of bacterial immobilization onto the NZ was a function of total bacterial concentration which was determined by COD of surrounding water media.
- Immobilization of *A. baumannii* on NZ was substantially higher than i.e. of *Escherichia coli* or *Enterococcus faecalis*.



CONCLUSIONS

- The *A. baumannii* readily immobilized and formed biofilm on the surface of natural mineral in any water media.
- By formation of extracellular matrix, the *A. baumannii* in the form of biofilm is protected from environmental hazards.
- Soil could be a reservoir of antibiotic-resistant *A. baumannii* in the environment.



Thank You for the attention!

