

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

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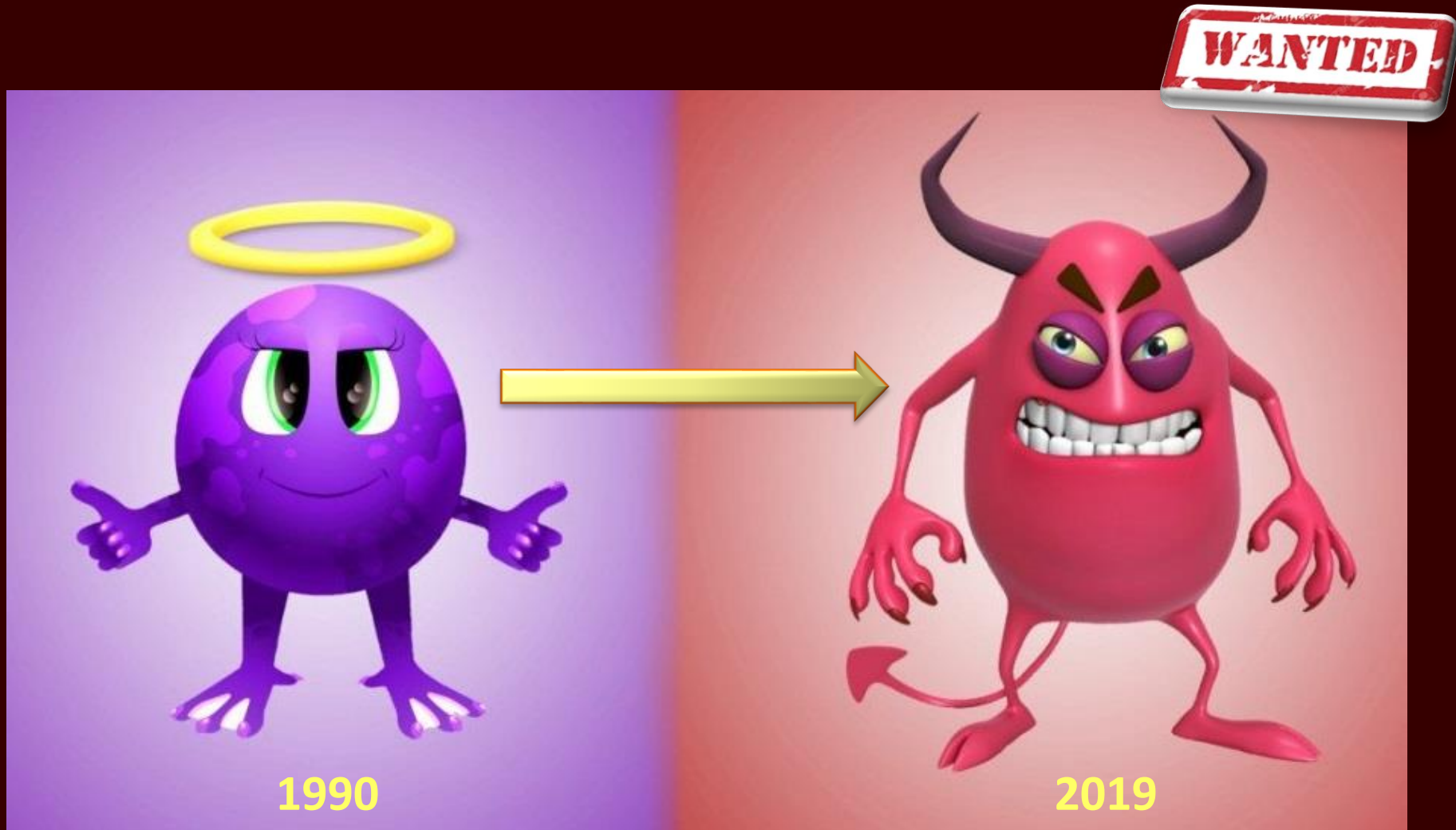
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*Acinetobacter baumannii* is a Gram-negative bacterium with cells of coccobacillus shape (1181 x 996 nm).



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.

*Acinetobacter baumannii*—The New MRSA?



# Surveillance Atlas of Infectious Diseases

Antimicrobial resistance

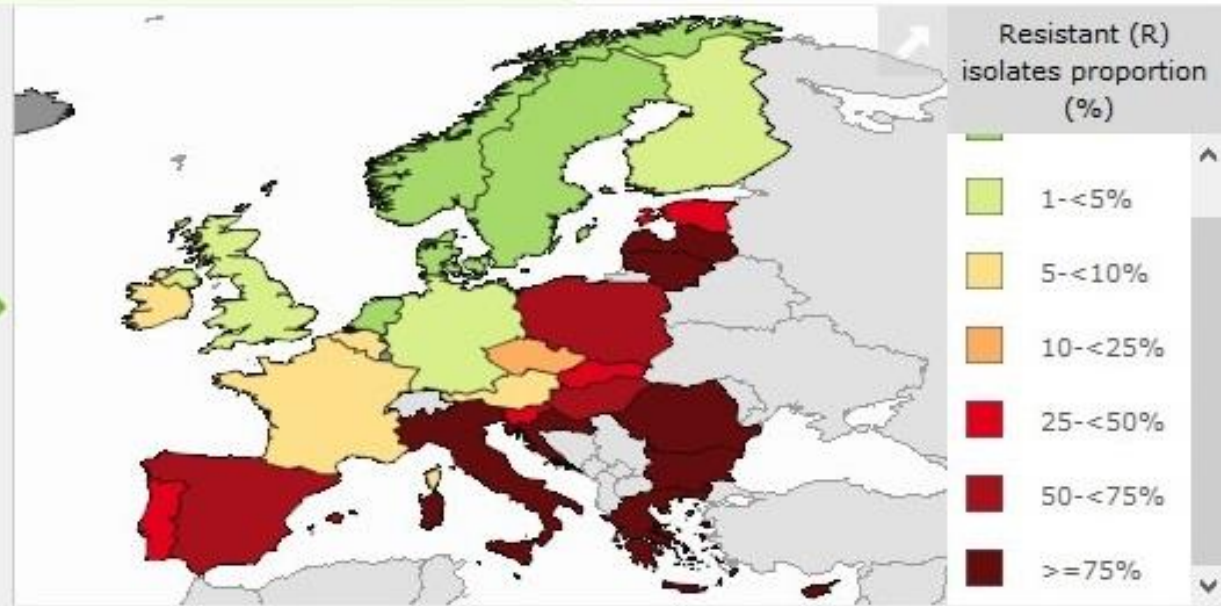
Acinetobacter spp.

Carbapenems

Resistant (R) isolates proportion

2017

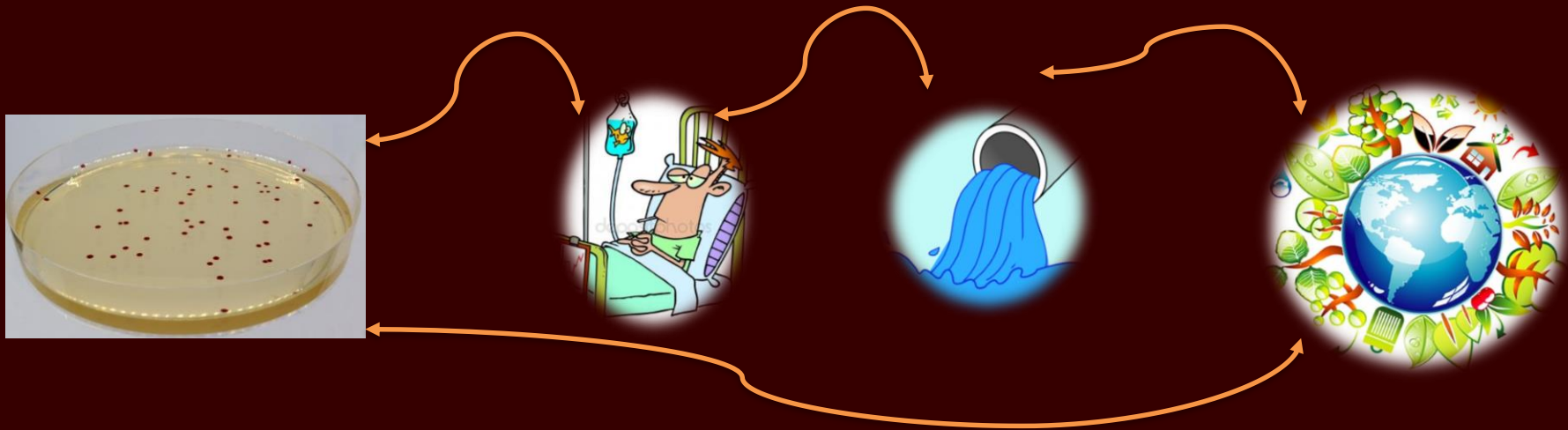
Region	Resistant (R) isolates proportion (%)
Austria	6.7
Belgium	6.9
Bulgaria	80.4
Croatia	96.2
Cyprus	76.0
Czech Republic	12.7
Denmark	0.0
Estonia	33.3



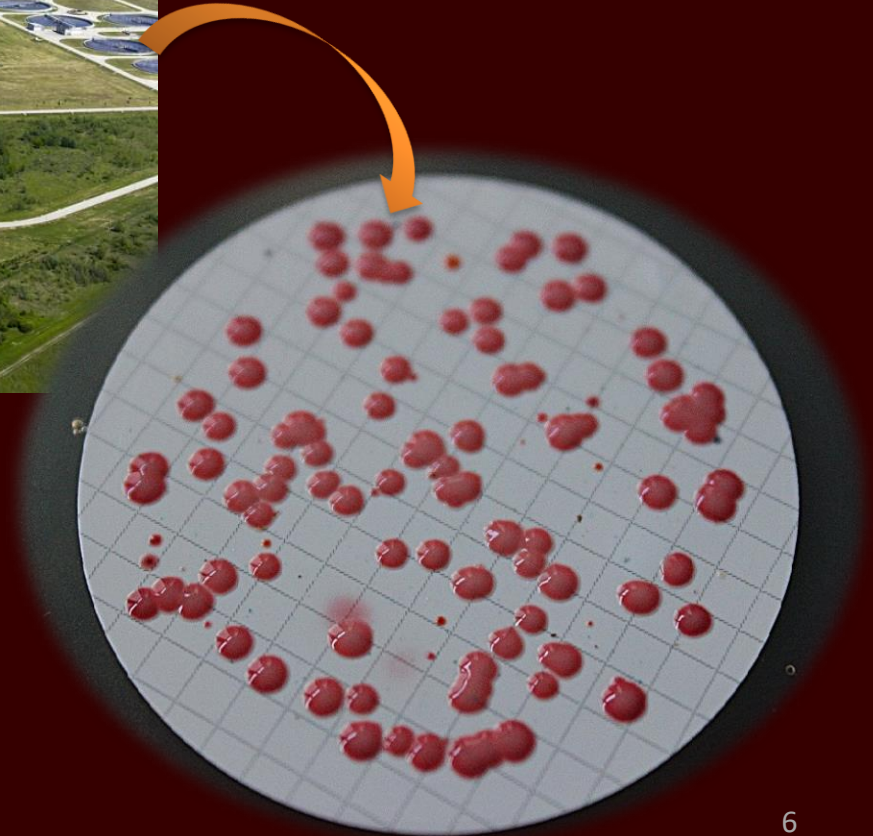
Recently the occurrence of *A. baumannii* in urban wastewaters and rivers influenced by the untreated hospital sewage have been reported.

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 zeolitized 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/ $\beta$ -lactamase inhibitors (SAM-ampicillin/sulbactam, TIM - ticarcillin/clavulanate); folate pathway inhibitors (SXT-trimethoprim/sulfamethoxazole); polymyxins (CST-colistin).

<sup>R</sup> - resistant, <sup>I</sup> - intermediate according to EUCAST or CLSI criteria.

Isolate	MEM	IMI	CIP	LVX	TOB	GEN	AMK	MIN	SAM	TIM	SXT	CST
EF7	R	R	R	R	R	R	R	I	R	R	R	R

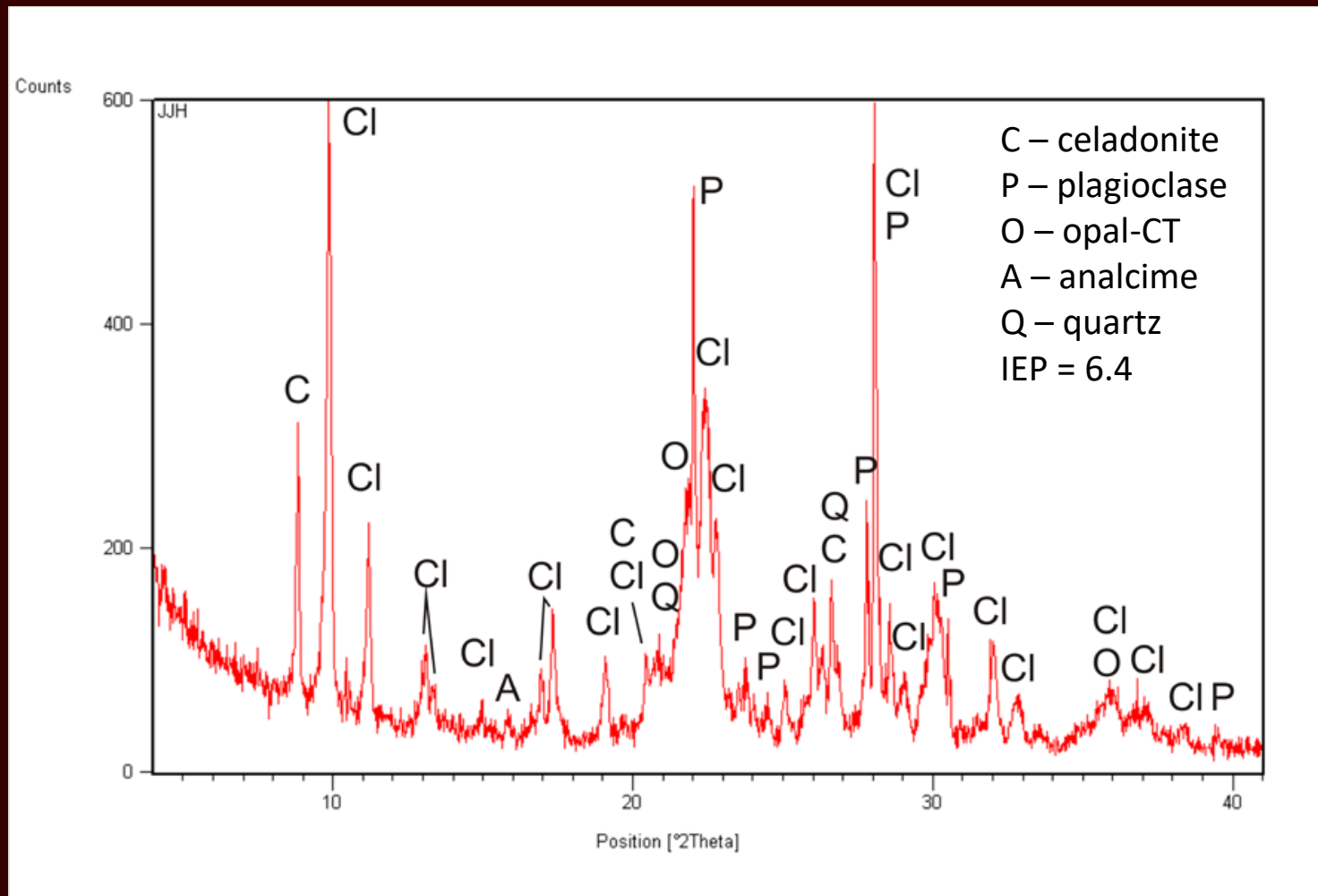
Microb Drug Resist. 2016 Oct 28. [Epub ahead of print]

## Pan Drug-Resistant Environmental Isolate of *Acinetobacter baumannii* from Croatia.

Goic-Barisic I<sup>1,2</sup>, Seruga Music M<sup>3</sup>, Kovacic A<sup>4</sup>, Tonkic M<sup>1,2</sup>, Hrenovic J<sup>3</sup>.



The NZ was obtained from quarry located at Donje Jesenje, Croatia. Dry-autoclaved particles  $\leq 0.122$  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.

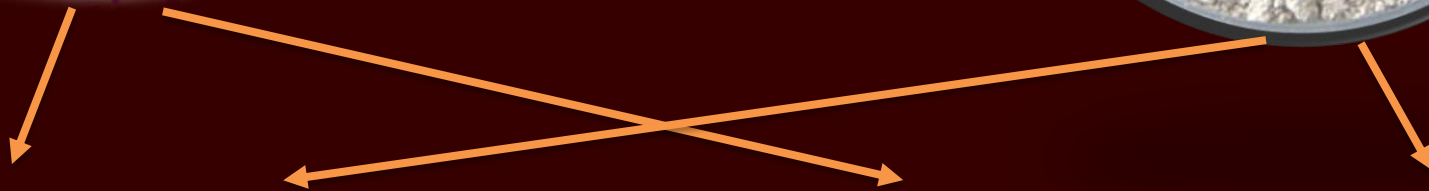




Overnight  
bacterial biomass



1 wt% of dry-  
autoclaved NZ



**nutrient-poor water**  
(commercial spring  
water)

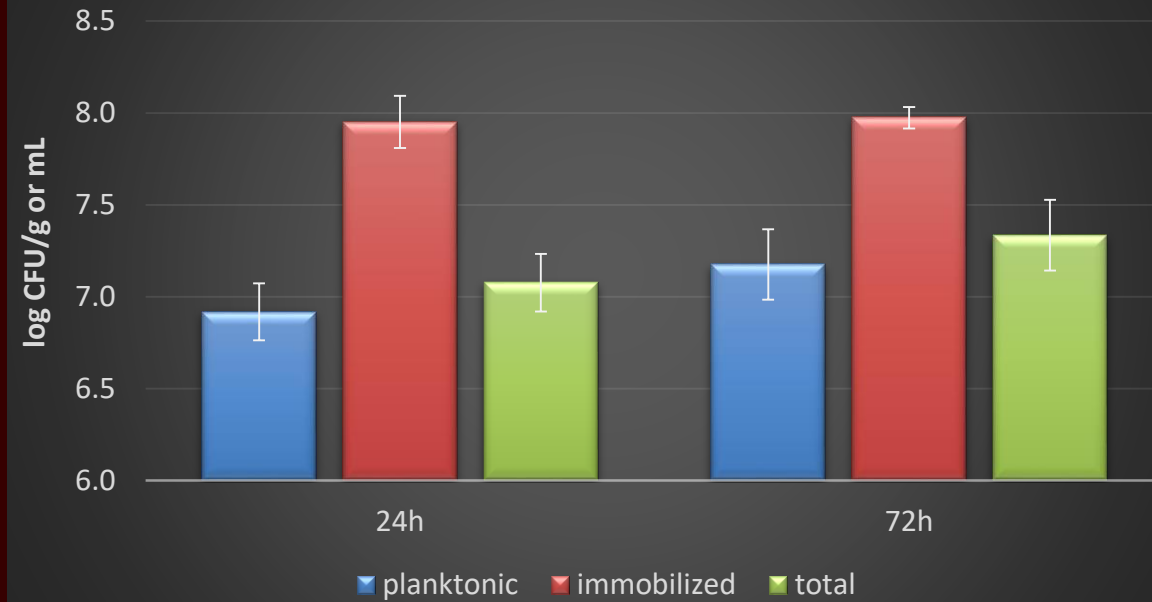
Parameter	
pH	8.1
COD (mg/L)	3
TOC (mg/L)	<1
TN (mg/L)	0.7
TP (mg/L)	0.1

**nutrient-rich water**  
(commercial spring water  
+ 1% nutrient broth)

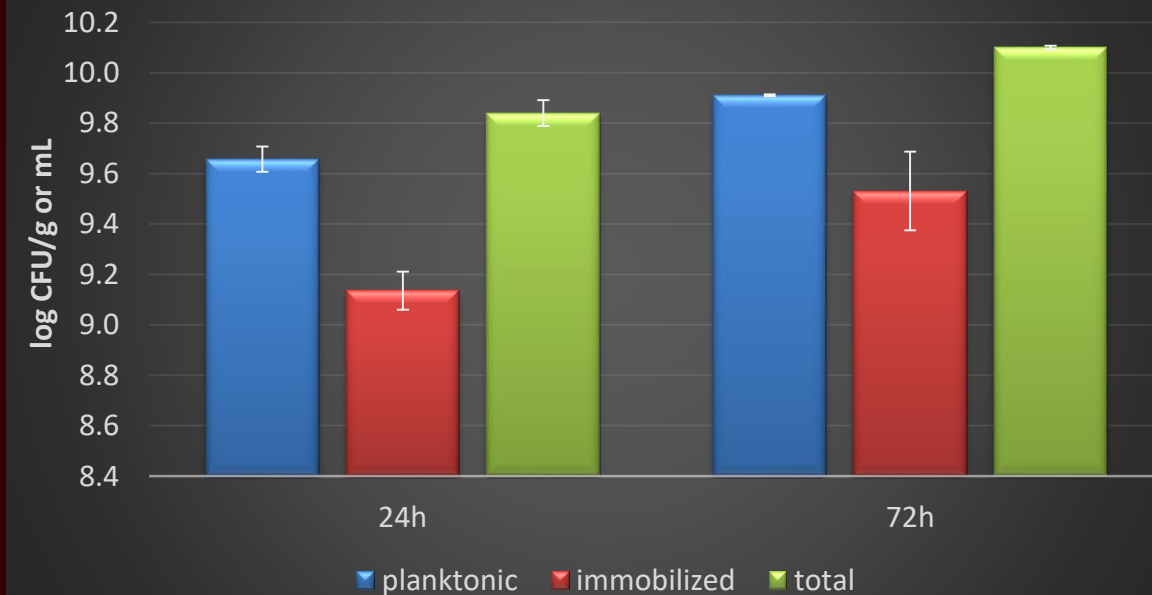
Parameter	
pH	6.9
COD (mg/L)	99
TOC (mg/L)	44
TN (mg/L)	13.2
TP (mg/L)	1.1

Incubation at 35°C , aeration with sterile air (4.6 mg O<sub>2</sub>/L).

### nutrient-poor water



### nutrient-rich water



Numbers of *A. baumannii*  
after 24h and 72h of  
contact with NZ.  
 $c_0$  (log CFU/mL)= $7.3 \pm 0.0$ .

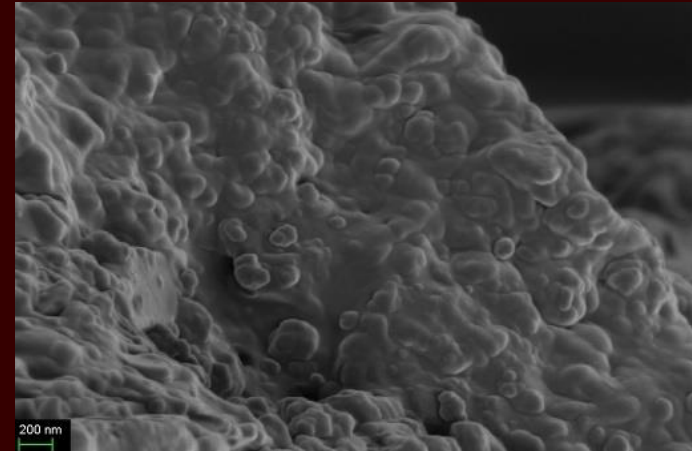
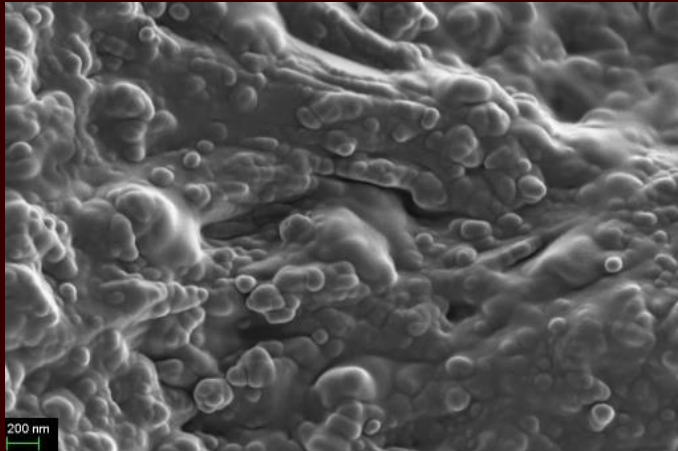
SEM analysis revealed the formation of biofilm on the rough surface of NZ particles within 24h of contact in both nutrient-poor and nutrient-rich water.

Bacteria stayed tightly attached onto NZ and covered by extracellular polymeric substances up to 72h of monitoring.

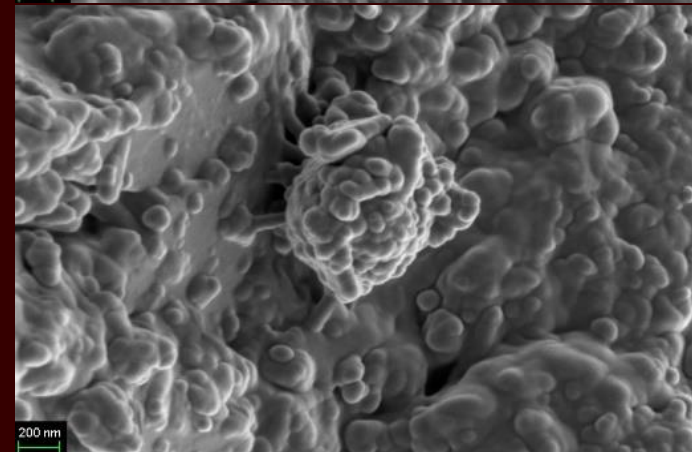
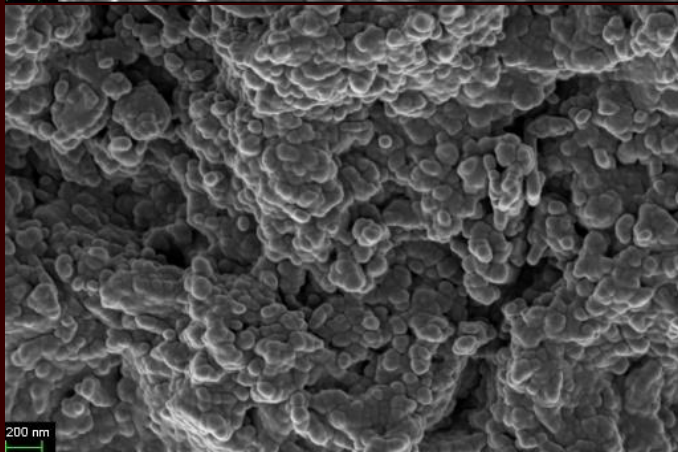
**Nutrient-poor water**

**Nutrient-rich water**

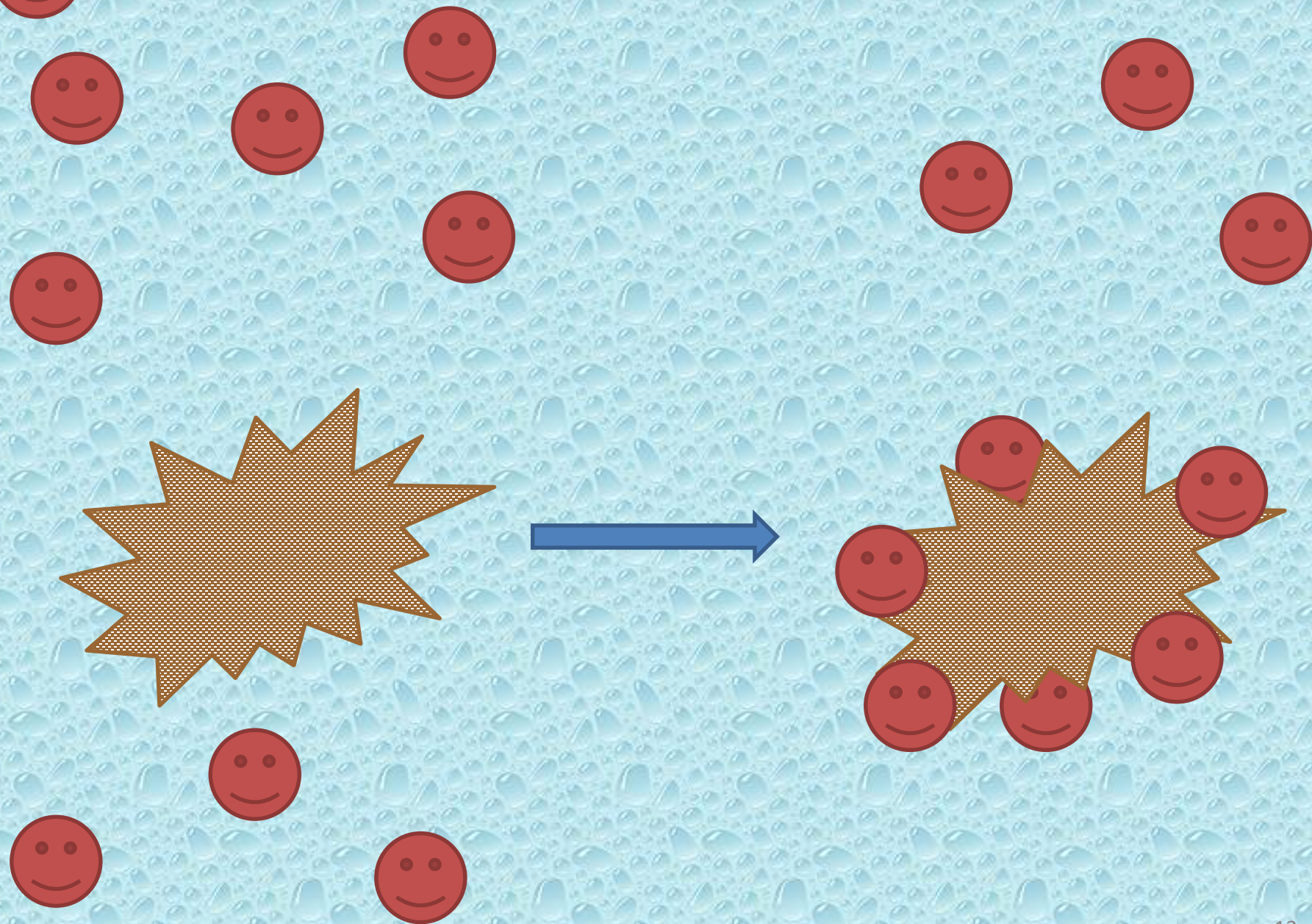
**24h of contact**

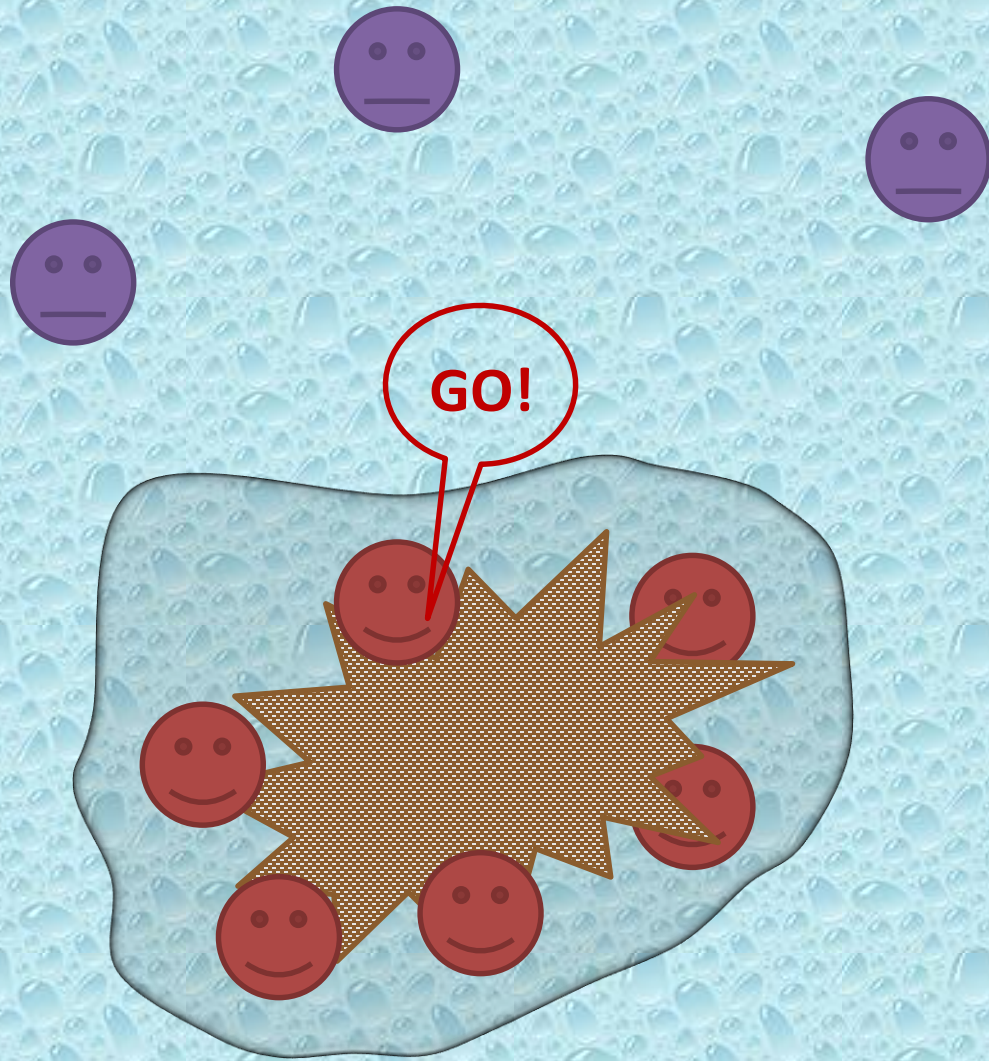


**72h of contact**

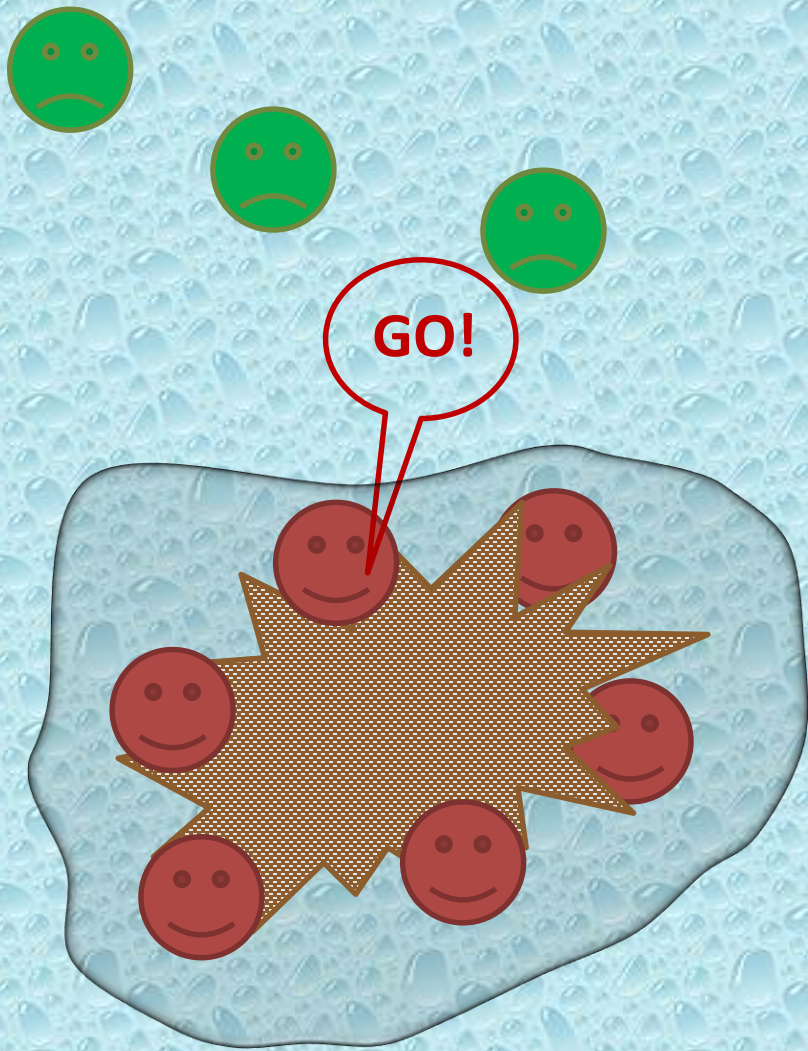


# Mode of *A. baumannii* immobilization onto NZ

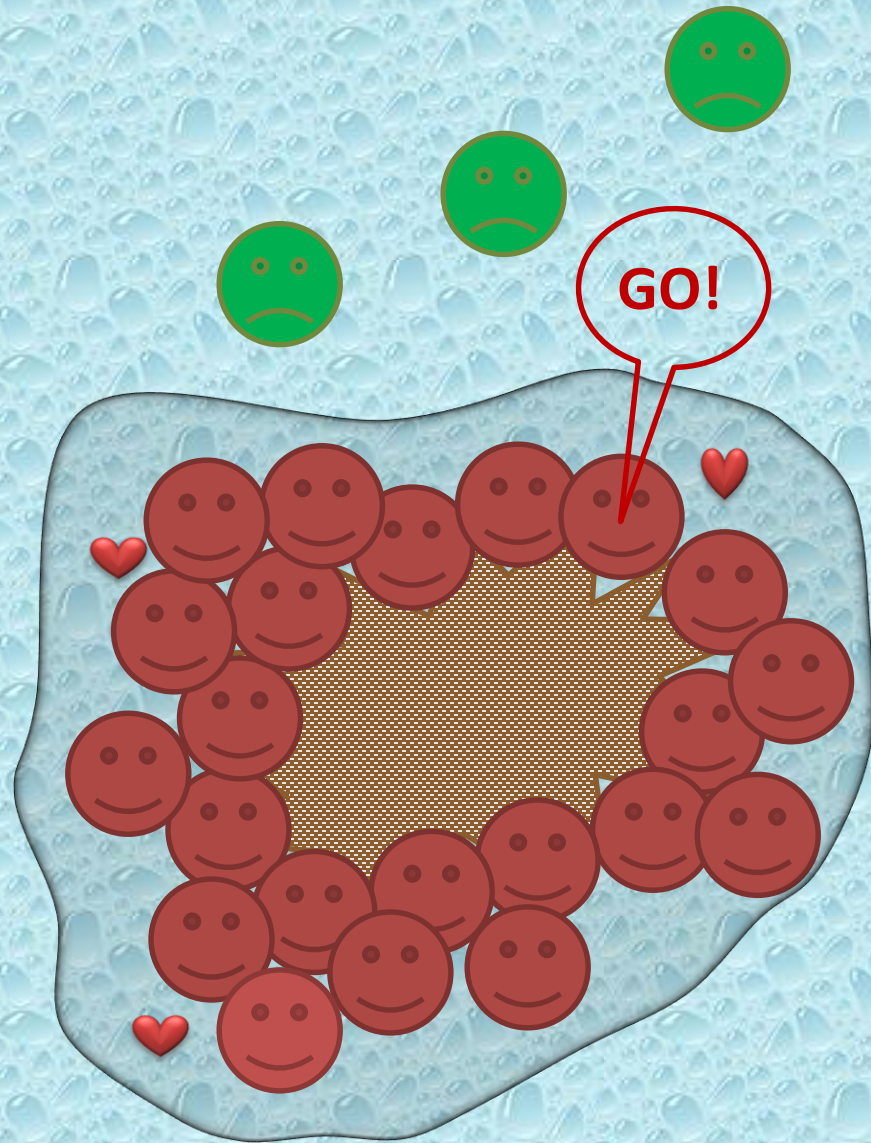




....24h later



**Nutrient-poor water**



**Nutrient-rich water**

....72h later

## 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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<https://www.pmf.unizg.hr/naturaci>

