Reduction of *Acinetobacter baumannii* biofilm formation by natural zeolite

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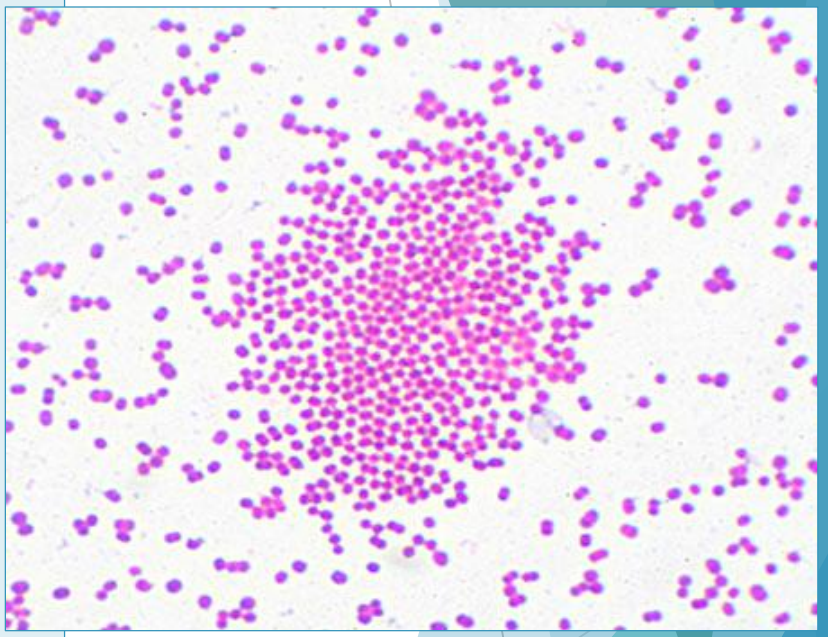
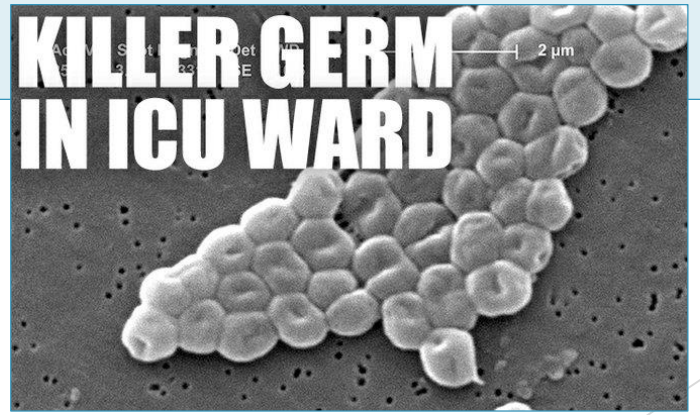
Acinetobacter baumannii

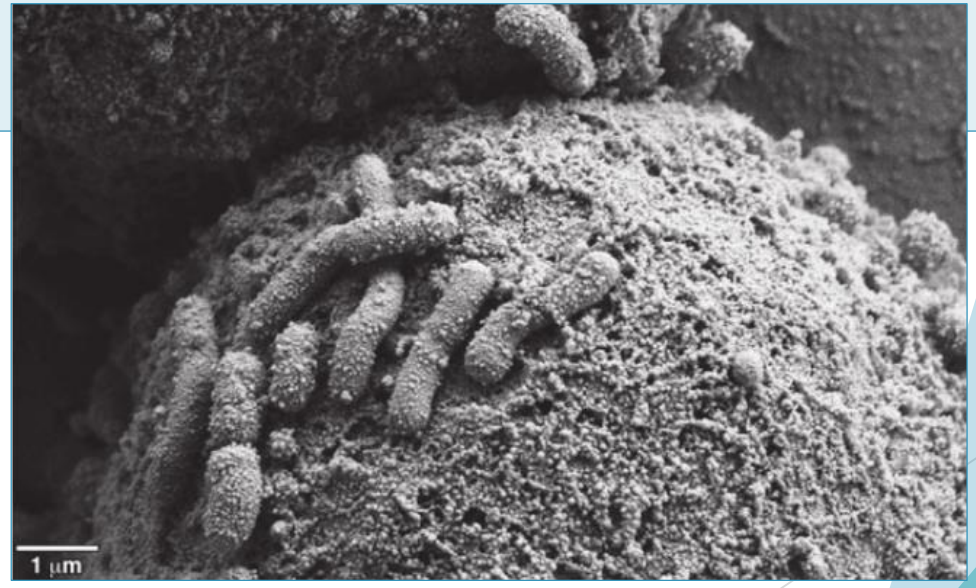
- Gram negative coccobacillus
- Emerging human pathogen isolated mainly from hospital setting
- Pneumonia, bloodstream infections, urinary tract infections, wound infections in imunosupressed patients
- Environmental isolates related to clinical isolates were found in soils and waters under the influence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





Acinetobacter baumannii

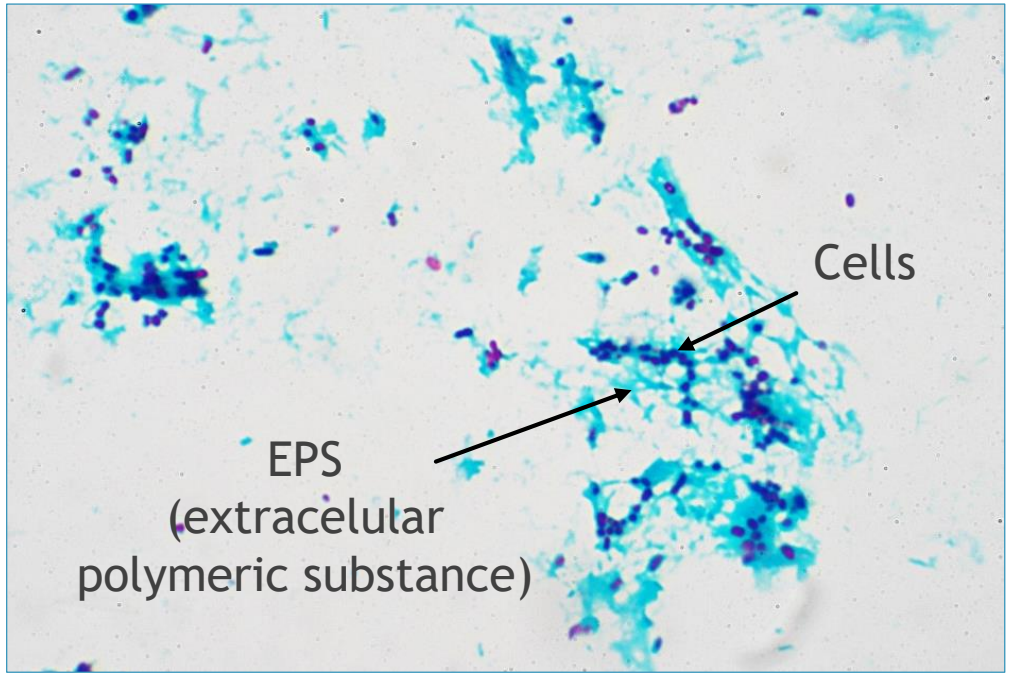
- Antibiotic resistance and virulence factors contribute to *A. baumannii* success as a pathogen
- Surface motility on solid/semi-solid media
- Biofilm formation on various surfaces
  - respiratory devices, intravenous devices, catheters, furniture, linen
  - human epithelial cells

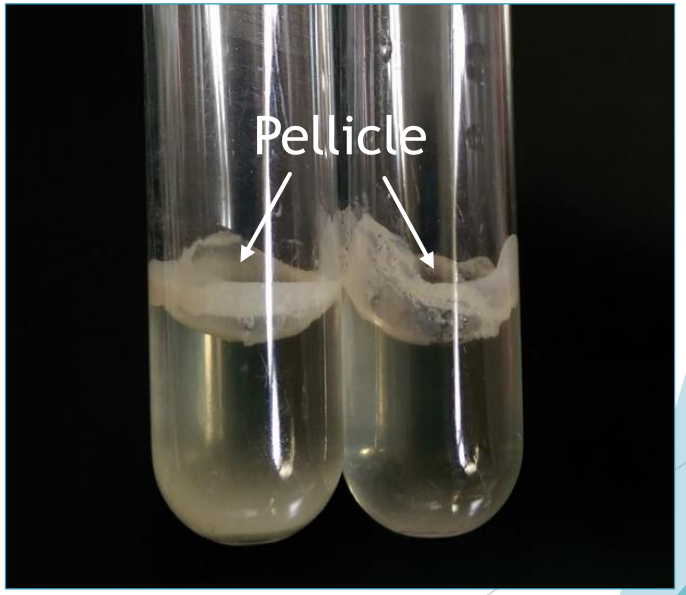


Bacteria on human alveolar epithelial cells

Acinetobacter baumannii

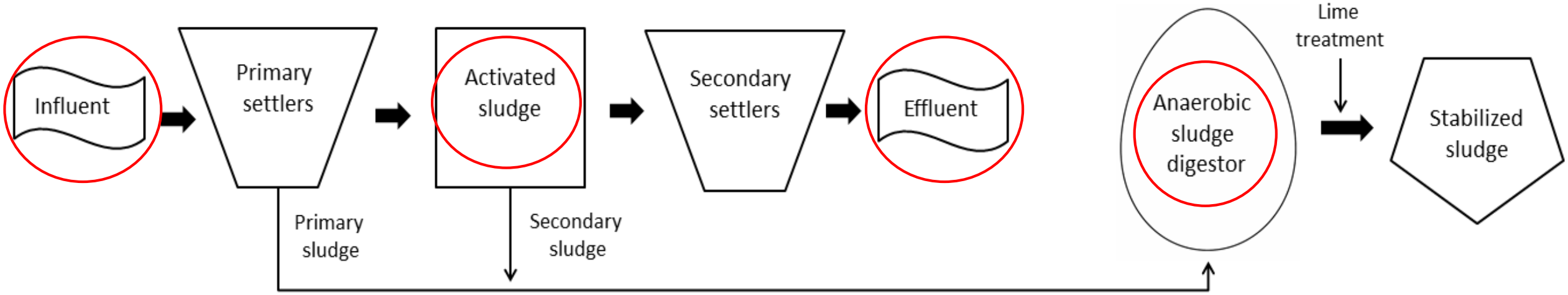
- Biofilm - an assemblage of cells enclosed in an extracellular matrix formed on different interfaces (solid-liquid, air-liquid)
- Pellicle – highly organised form of biofilm formed at the air-liquid interface





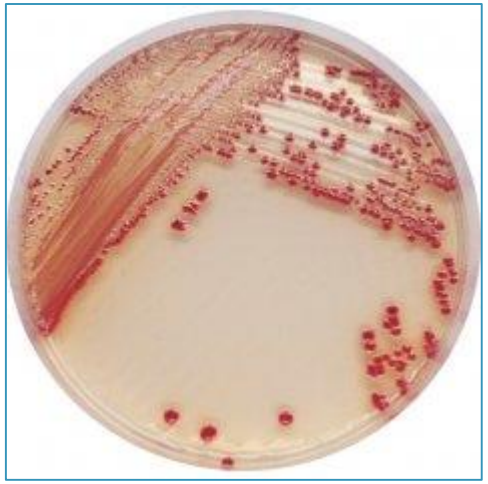
Experimental

- 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

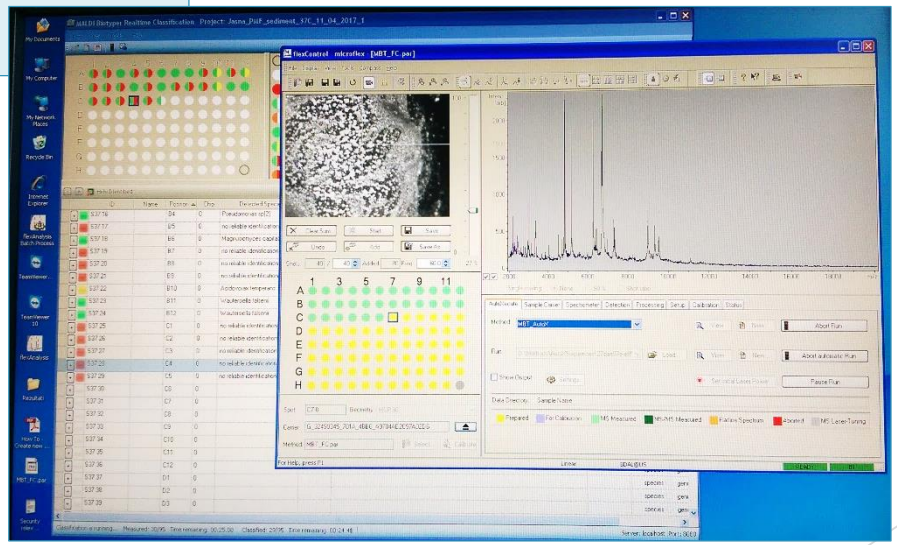


Experimental

- 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



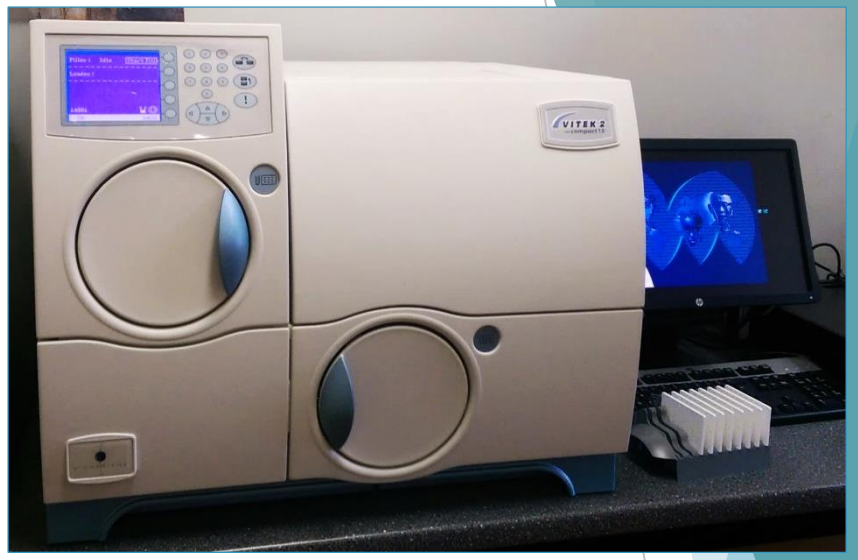
CHROMagar Acinetobacter



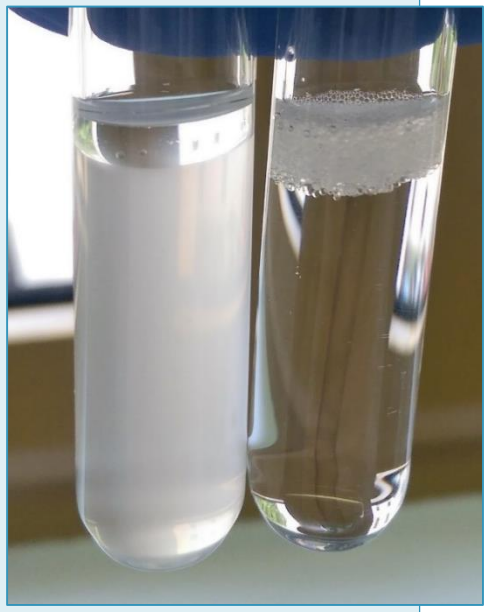
MALDI TOF

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)
  - OD_{550} < 0.3 poor
  - OD_{550} 0.3-1.0 intermediate
  - OD_{550} > 1.0 strong
- Pellicle formation
  - No pellicle (0)
  - Poor (1)
  - Strong (2)



Vitek2



BATH assay

Crystal violet assay

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

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Selective immobilization of *Acinetobacter junii* on the natural zeolitized tuff in municipal wastewater

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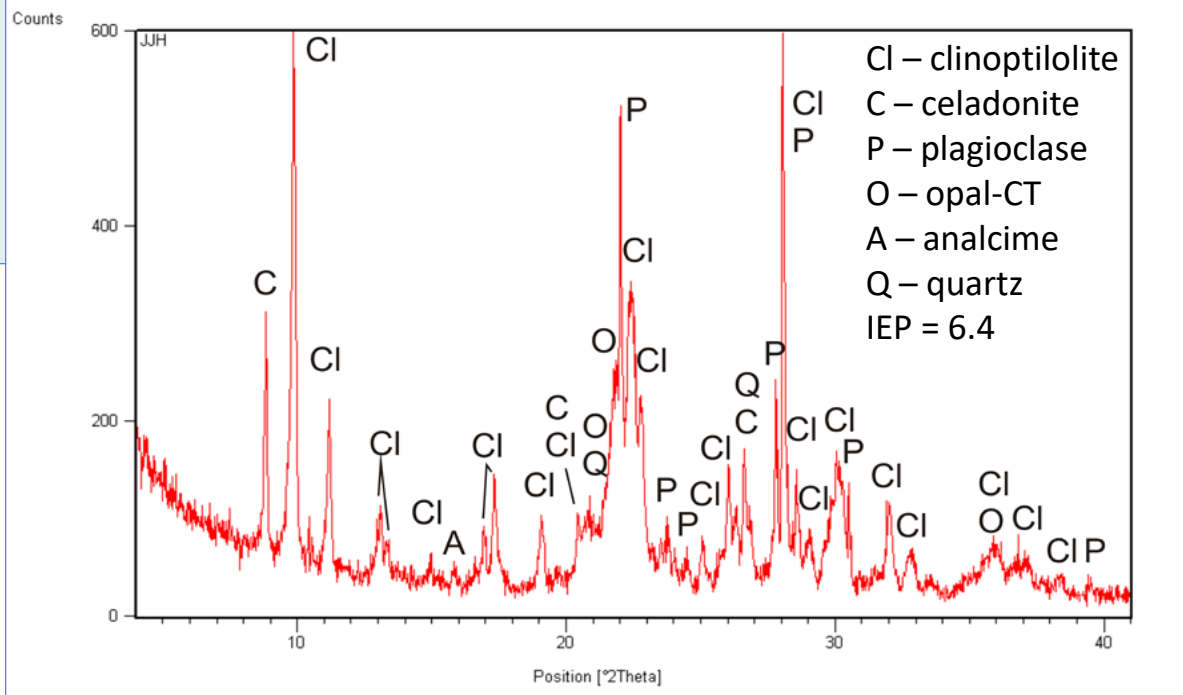
ABSTRACT

The immobilization of desired bacteria onto material was usually performed in synthetic media. The aim of this study was to test the immobilization of phosphate (P) accumulating bacteria *Acinetobacter junii* ... taining the common



Isolate	Antibiotic resistance	Hydrophobicity (%)	Pellicle formation	Biofilm formation (OD_{550})
IN31	sensitive	97	1	1.024
IN34	MDR	1	1	1.006
IN36	sensitive	2	1	1.225
IN41	MDR	0	0	0.138
IN47	MDR	0	1	0.745
IN58	sensitive	93	2	2.497
EF7	MDR	0	1	1.098
EF8	MDR	0	1	1.026
EF11	sensitive	80	2	1.180
EF13	MDR	0	1	0.468
EF22	MDR	0	1	0.483
EF23	MDR	0	1	0.766
S5	MDR	3	1	0.971
S6	sensitive	78	1	0.868
S9	sensitive	8	2	1.274
S10	MDR	2	1	0.364
S11	MDR	0	1	1.005
S15	sensitive	79	1	0.998
D10	sensitive	0	1	0.723
D11	MDR	46	1	0.402
D12	MDR	49	1	0.217
D13	sensitive	0	1	0.267
D16	sensitive	67	1	0.891
D17	MDR	1	2	1.244

IN- influent, EF- effluent, S – fresh sludge, D – digested sludge
MDR (multi-drug resistant)- resistance to three or more classes of antibiotics

24 isolates recovered

14 MDR, 10 sensitive to 12 antibiotics tested

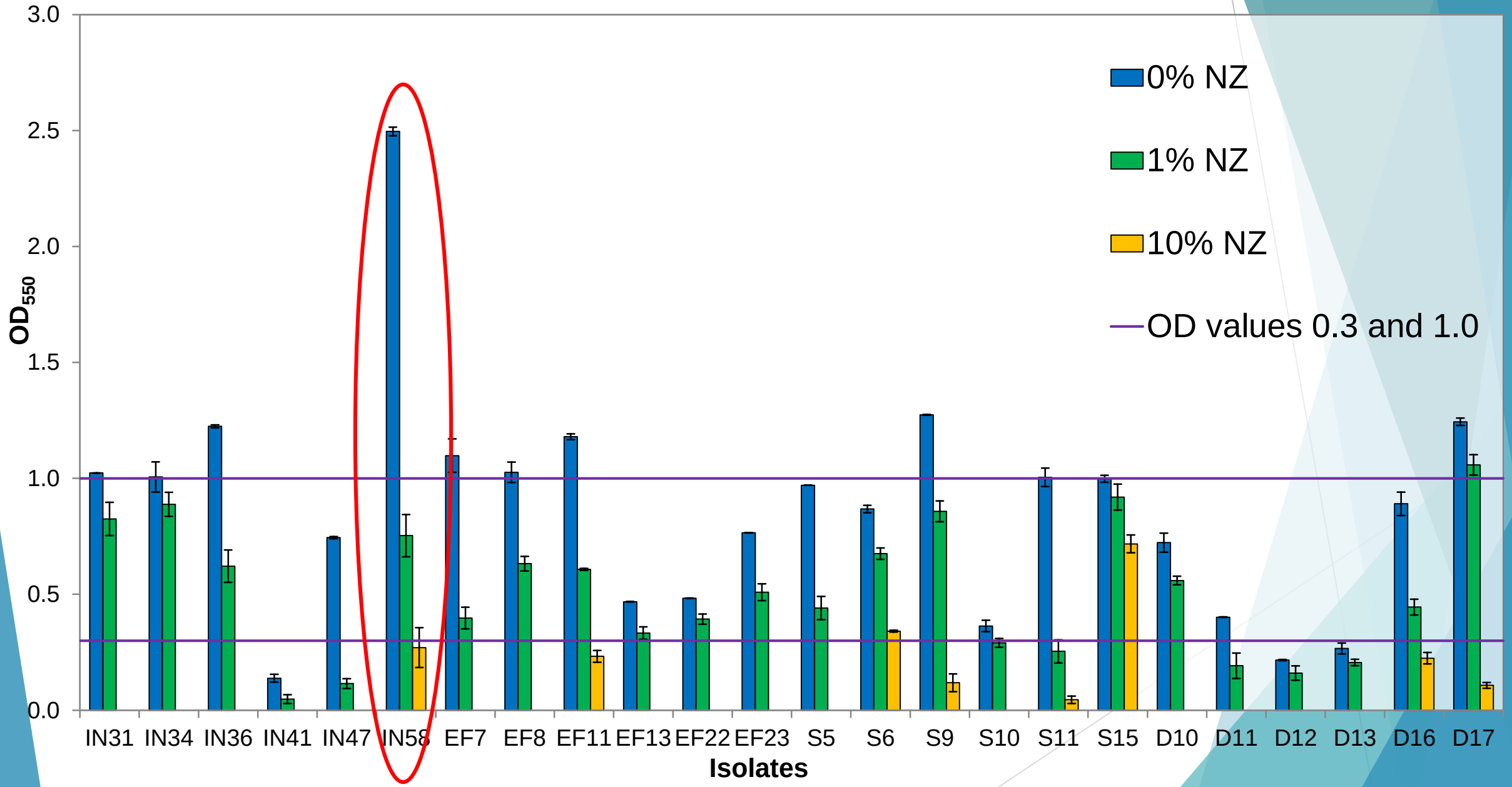
9/24 isolates hydrophobic

Majority of isolates intermediate biofilm and poor pellicle formers

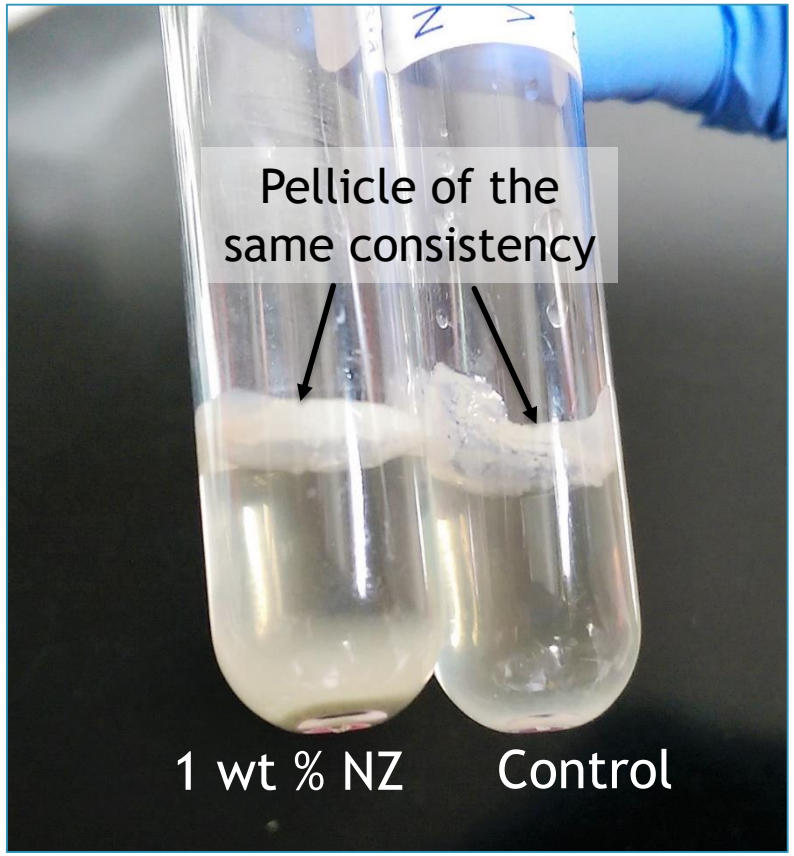
Antibiotic sensitive isolates more hydrophobic and stronger biofilm and pellicle formers

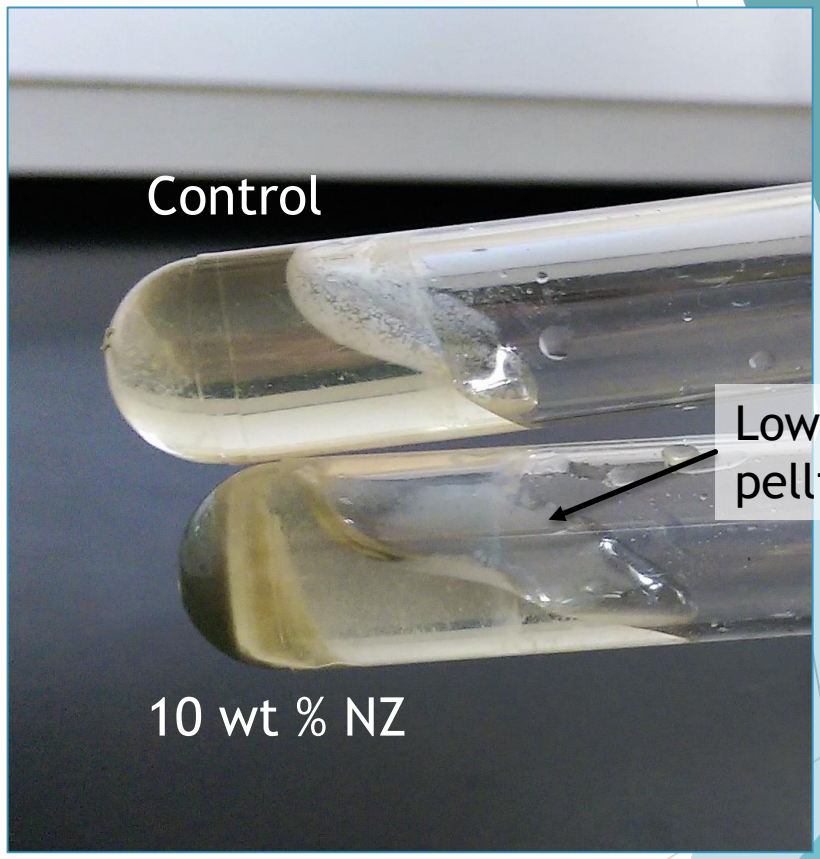
	Hydrophobicity	Biofilm	Pellicle
Hydrophobicity	1.000	r=0.425 p=0.003	r=0.433 p=0.002
Biofilm		1.000	r=0.682 p=0.000
Pellicle			1.000

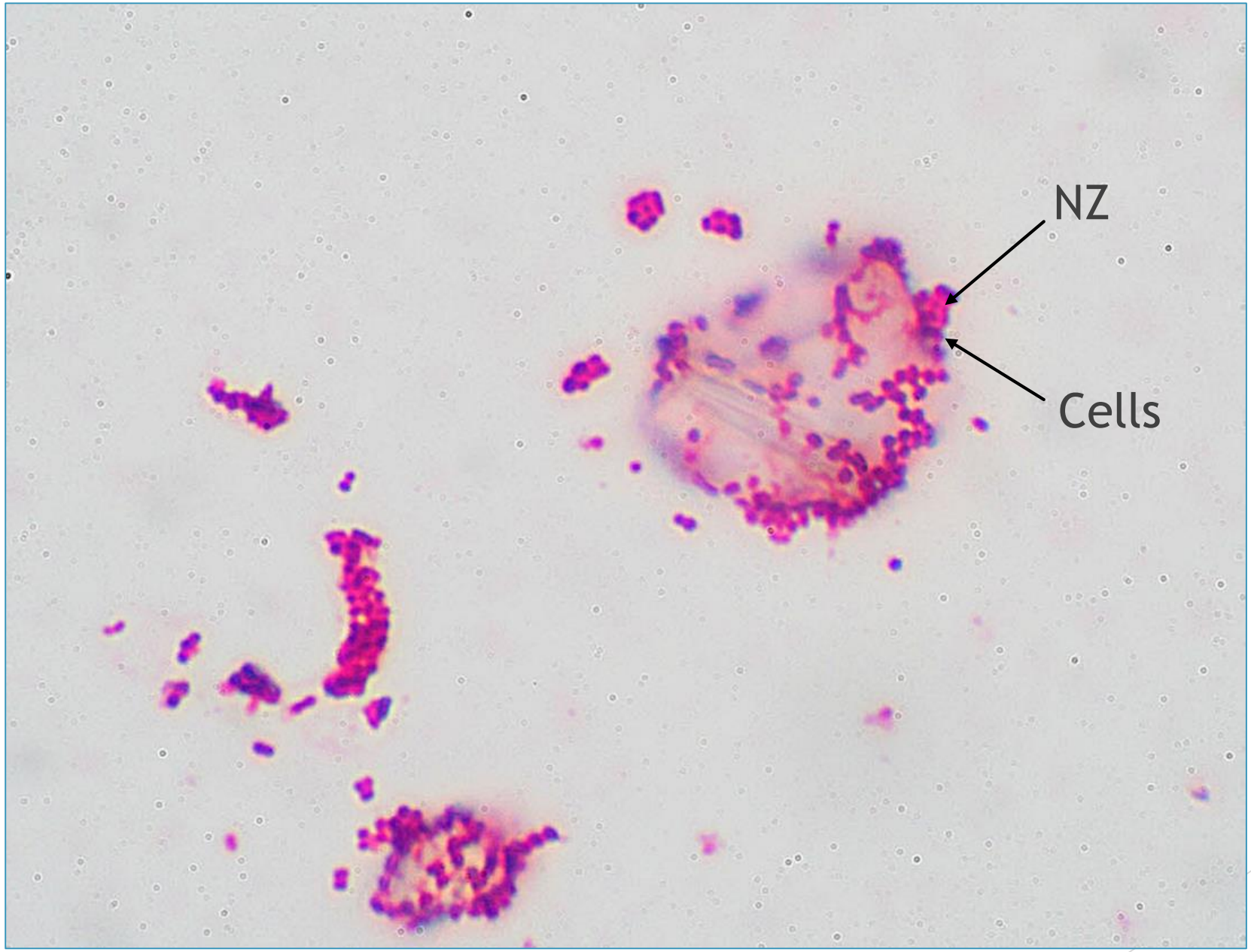
Biofilm formation



Pellicle formation







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!