NATURAL ZEOLITE INFLUENCE ON THE SURFACE MOTILITY OF *ACINETOBACTER BAUMANNII*

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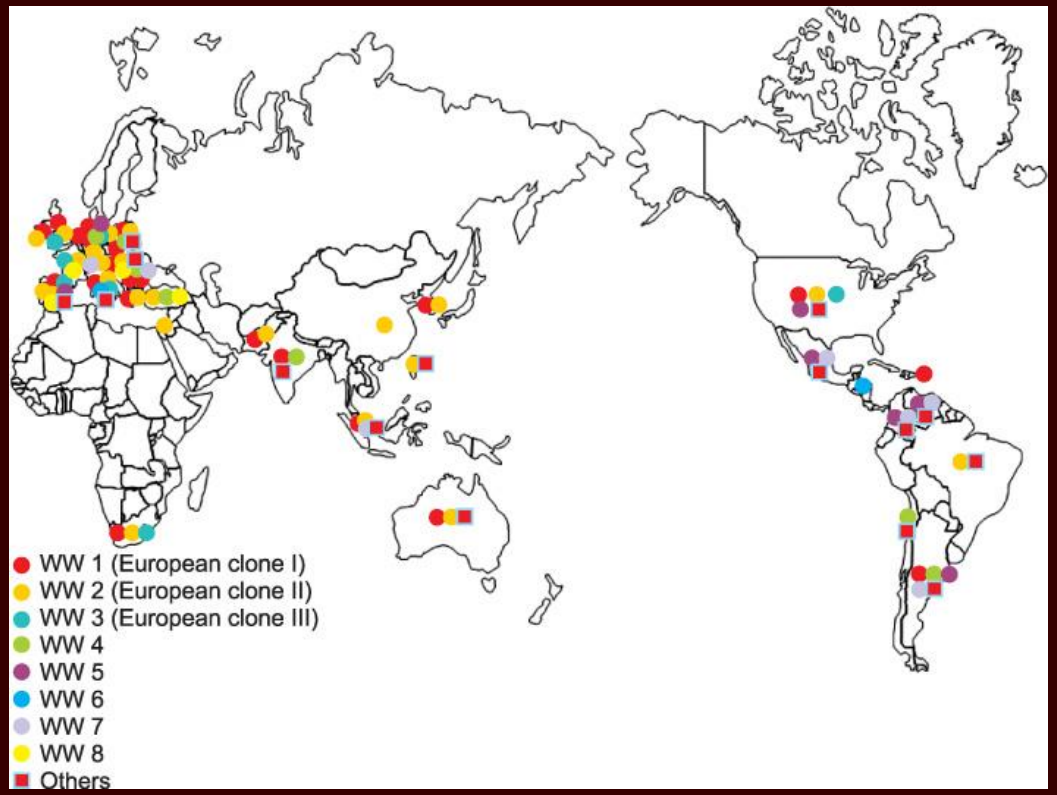
Bacterium *Acinetobacter baumannii* is an emerging hospital pathogen. Globally distributed isolates of *A. baumannii* are designated as international clones (IC) 1-3, while some isolates still remain nonclonally related.

In Croatian hospitals isolates of *A. baumannii*:

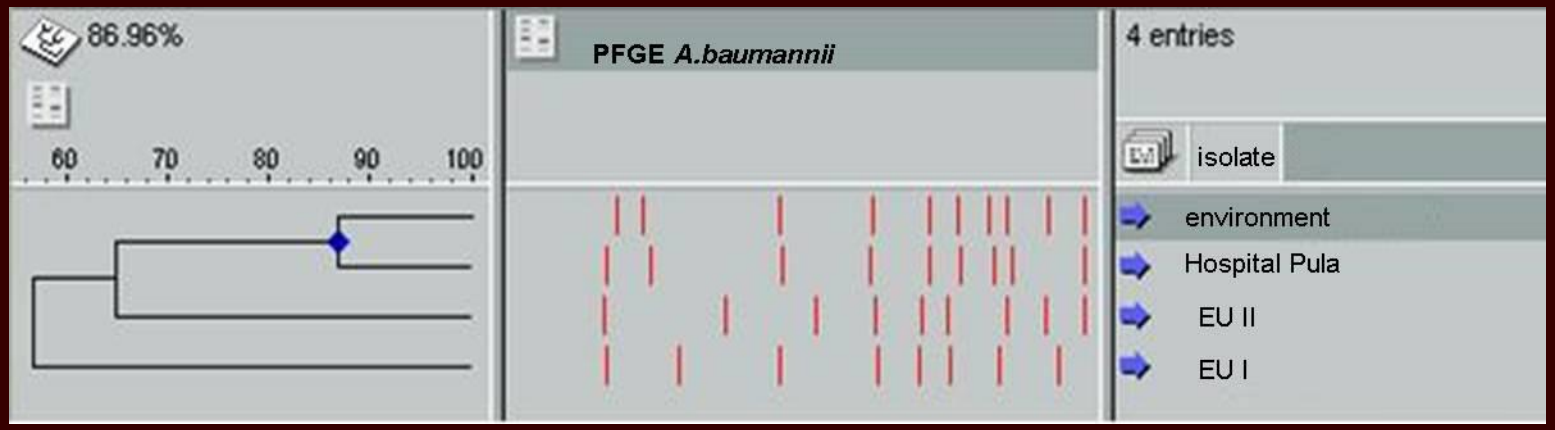
- belonging to IC1 evidenced from 2002,
- belonging to IC2 evidenced from 2009,
- nonclonal isolates also persist.



Reports on the occurrence of clinically important *A. baumannii* in nature are scarce.

Environmental isolates of *A. baumannii* related to clinical isolates were found in:

- wastewaters,
- Seine River,
- acid paleosol from Croatia.



A. baumannii express the:
- resistance to multiple antibiotics (MDR) as well as disinfectants,
- survives in adverse conditions,
- leading to long-term persistence in the hospital environment.

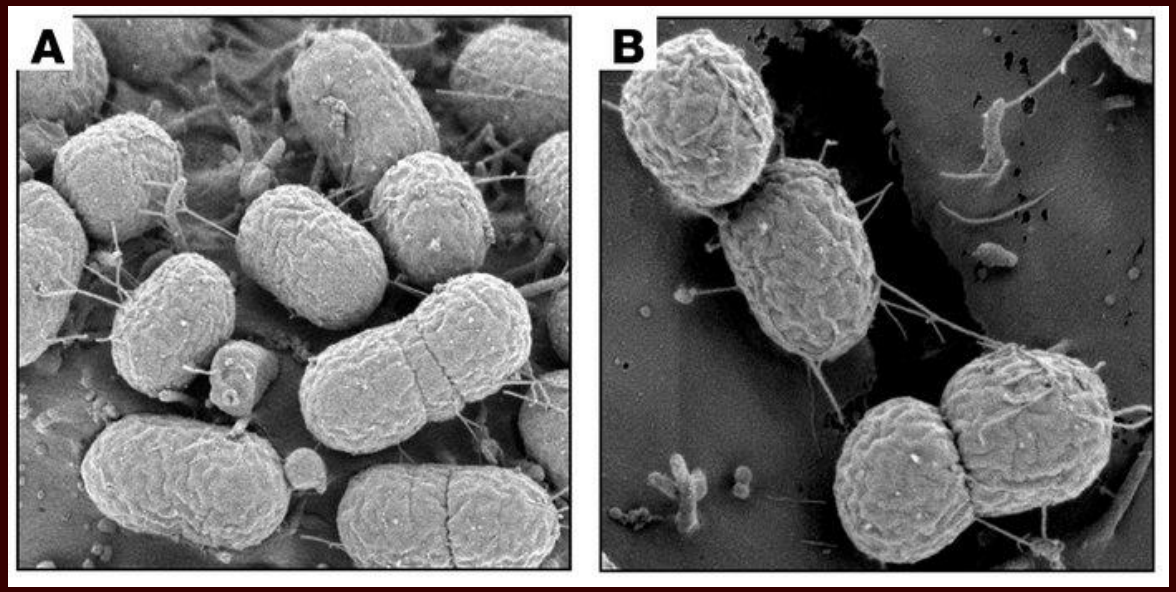
Additionally, virulence factors that influence the success of *A. baumannii* as a pathogen are its:
- surface motility on solid/semisolid media,
- ability to form biofilm on abiotic or biotic surfaces.

Due to the lack of flagella *A. baumannii* is unable to swim in liquid media.

Motility of some isolates is mediated by polar type IV pili.

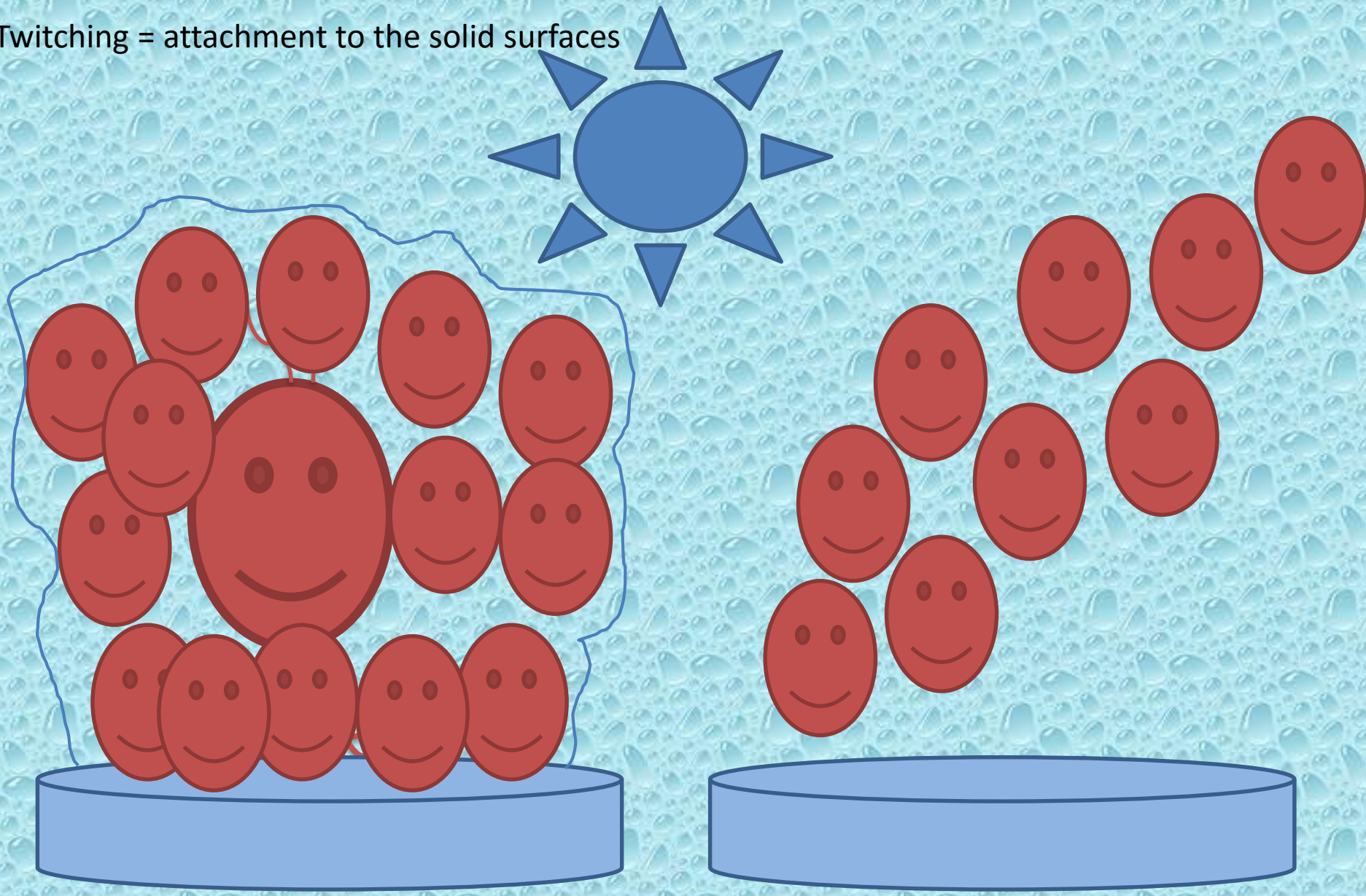
Two distinct forms of phenotypic surface motility of *A. baumannii* are recognized:

- twitching defined as attachment to the solid surfaces,
- swarming defined as surface translocation on the semisolid media.



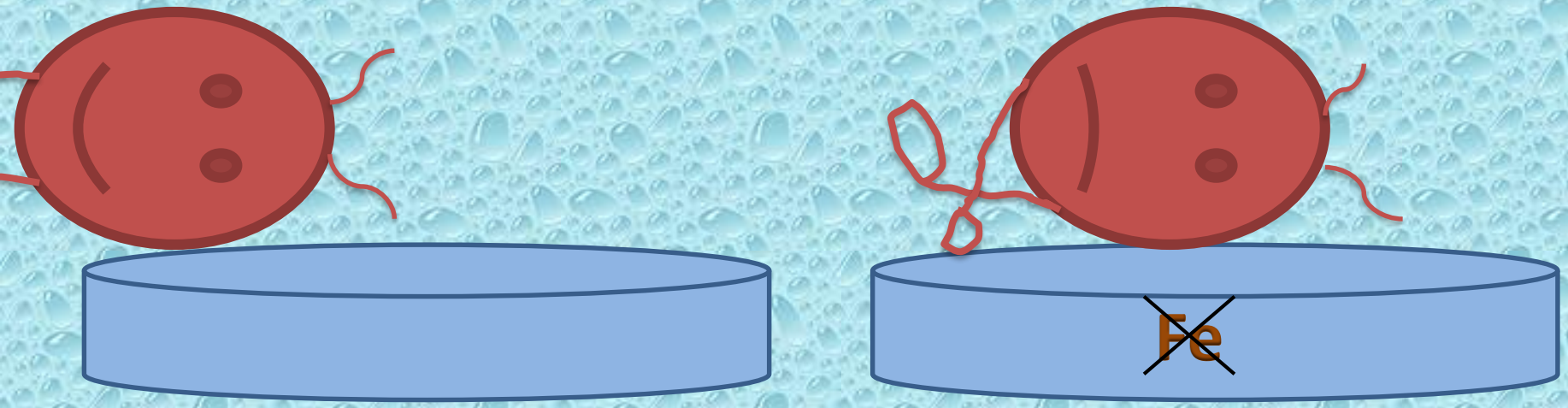
Type IV pili are found on the surface of many Gram-negative bacterial species. Thin, flexible, 6-7nm fibers, composed of proteins, generate motile forces.

Twitching = attachment to the solid surfaces



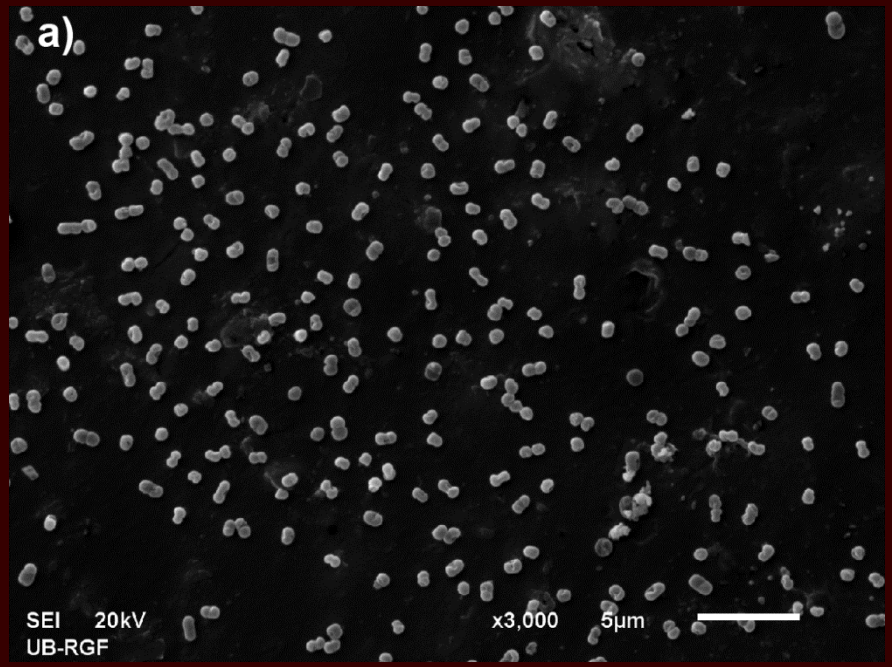
Twitching motility of *A. baumannii* was found to be inhibited by blue light illumination.

Swarming = surface translocation on the semisolid media



Swarming of *A. baumannii* was found to be inhibited by iron limitation.

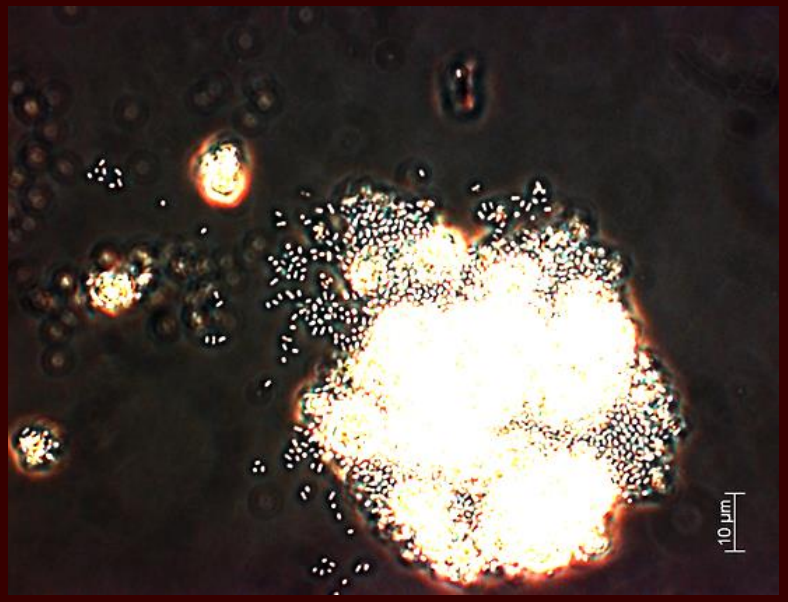
Twitching motility is an important step in colonization and subsequent biofilm formation on medical devices such as ventilator tubing and catheters, which are one of the main sources of hospital infections with *A. baumannii*.



Immobilized cells of *A. baumannii* on the surface of ventilator tube.

Particles of natural zeolitized tuff (NZ) were shown to display a high affinity for the immobilization of *A. baumannii* cells.

Therefore, it was presumed that the addition of NZ into semisolid medium will result in immobilization of *A. baumannii* cells onto NZ particles, thus hindering their twitching motility. Inhibition of twitching motility as a prerequisite for biofilm formation is promising tool to suppress the virulence of *A. baumannii*. The effect of NZ on the surface motility was studied in the same system.

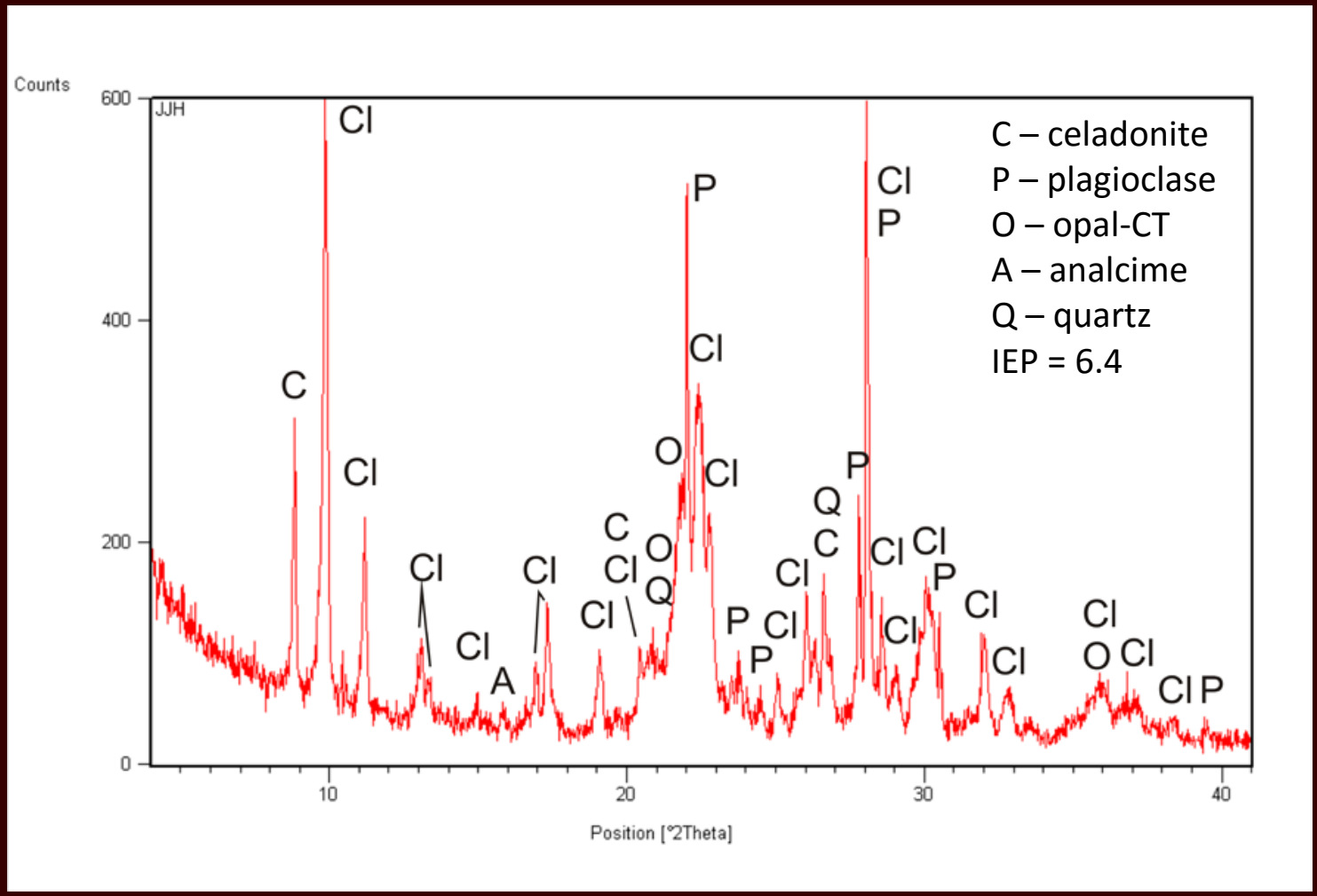


Immobilized cells of *A. baumannii* onto partilcle of NZ.

Mean features of *A. baumannii* isolates used in study.
* resistant to 3 or more classes of antibiotics.

Bacterial isolate	Source	International clonal lineage	Multi-drug resistant*	Biofilm formation (A_{550})
RUH134	reference strain	IC 2	no	1.277
RUH2037	reference strain	IC 1	no	1.474
ST91	wound	IC 2	no	1.250
ST98	eye swab	IC 2	yes	1.417
ST142	catheter swab	IC 2	yes	1.580
ST156	respiratory tract	IC 2	yes	1.561
P25	respiratory tract	IC 1	no	1.387
ŠI125	respiratory tract	non-clonal	no	1.131
Durn	paleosol	IC 1	yes	0.941
IN13	raw sewage	non-clonal	yes	0.979
IN14	raw sewage	non-clonal	yes	0.823
IN16	raw sewage	non-clonal	yes	1.060
IN18	raw sewage	non-clonal	yes	1.560
EF2	treated sewage	non-clonal	yes	1.255
EF4	treated sewage	non-clonal	yes	0.725
EF5	treated sewage	non-clonal	yes	1.594
EF6	treated sewage	non-clonal	yes	0.943

For surface motility assay micronized (5-10µm) particles of NZ from quarry located at Donje Jesenje, Croatia were used.



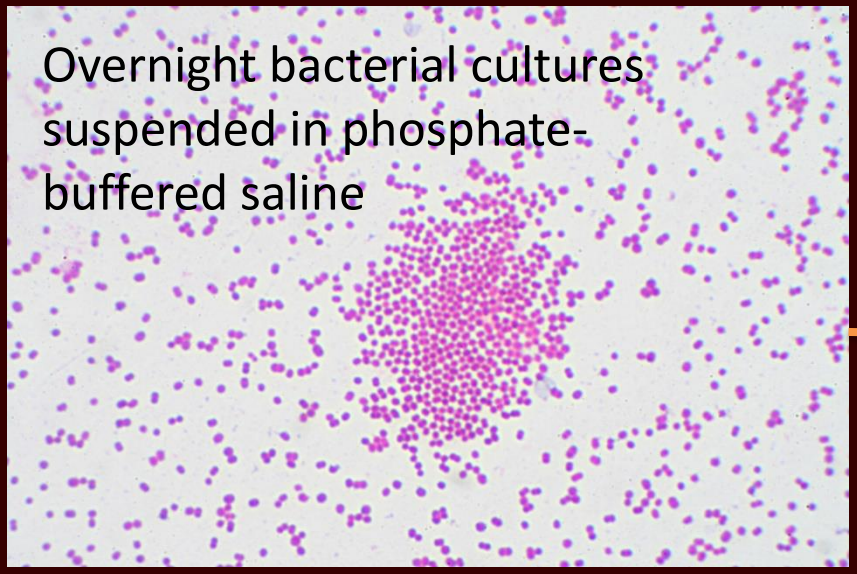
X-ray powder pattern of NZ. NZ sample consisted mostly of clinoptilolite (50-55%) with major constituents being celadonite, plagioclase feldspars and opal-CT (10-15% each). Analcime and quartz were present in traces.

Autoclaved Luria Bertani medium containing 0.5% agarose:

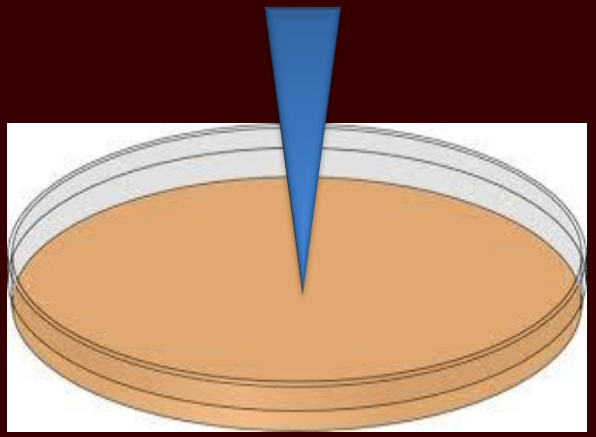
Component	Concentration (g/L)
Tryptone	10
Yeast extract	5
NaCl	10
Agarose	5
pH= 7.0	

+

Homogenized and poured into Petri dishes

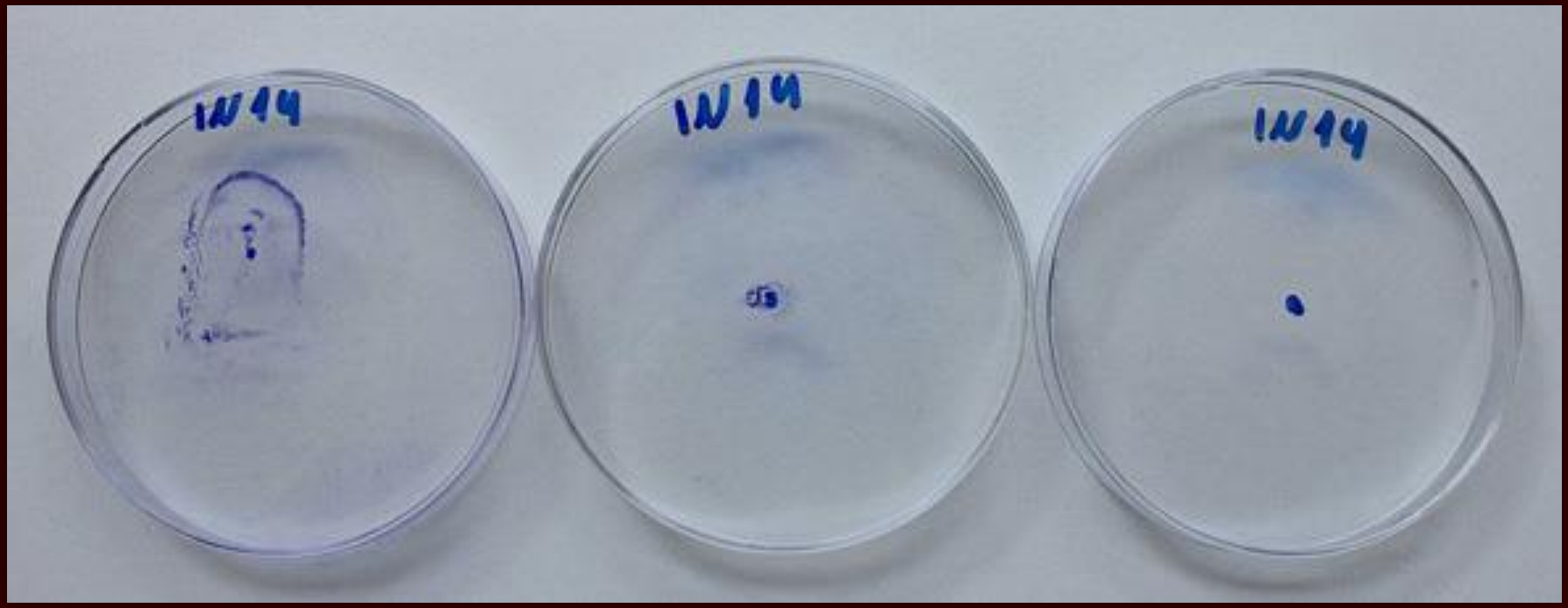


Inoculation with a pipette tip to the bottom of polystyrene Petri dish

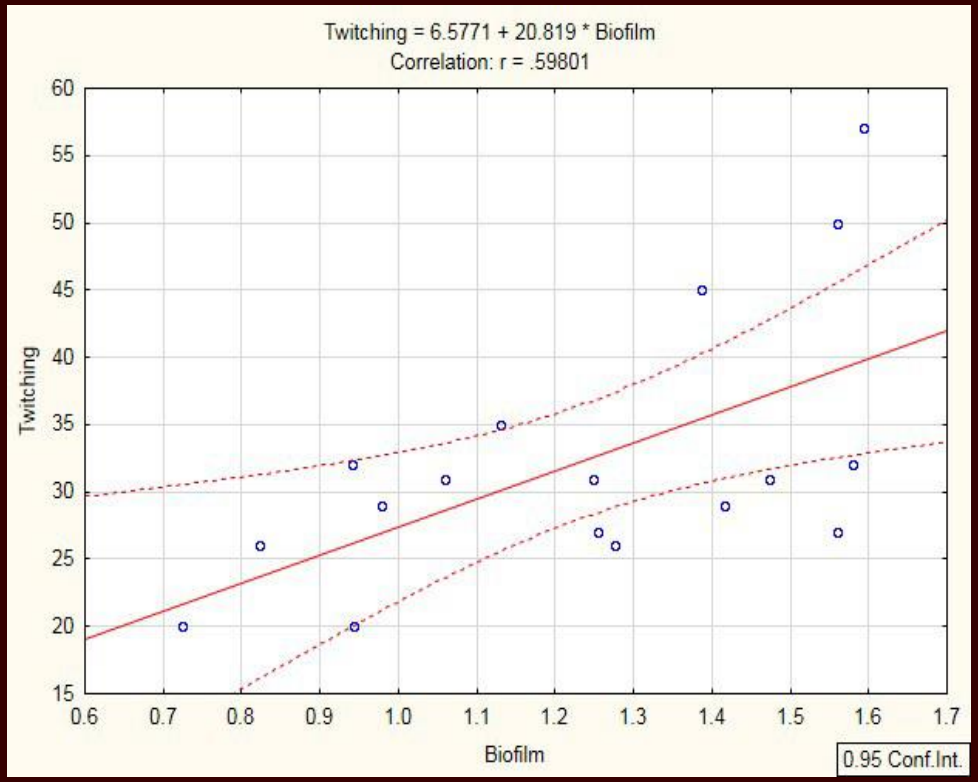


Plates were tightly closed with parafilm to prevent drying and incubated in humid atmosphere at 37°C/24h.

Swarming motility was observed at the air-agarose interface.



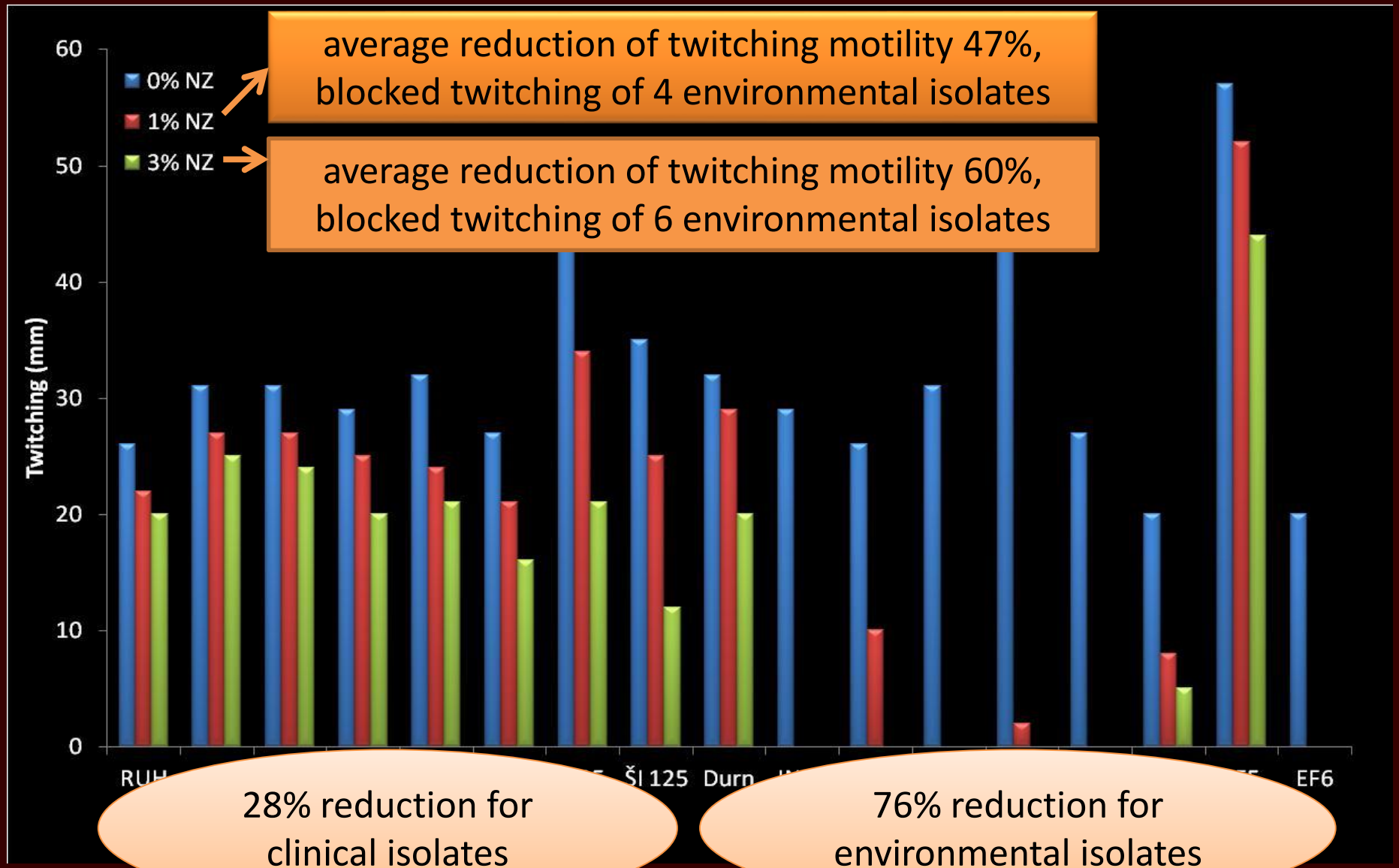
Twitching motility was assessed by removing the agarose layer, staining the dishes with 0.5% crystal violet for 10min, and measuring the longest diameter of motility.



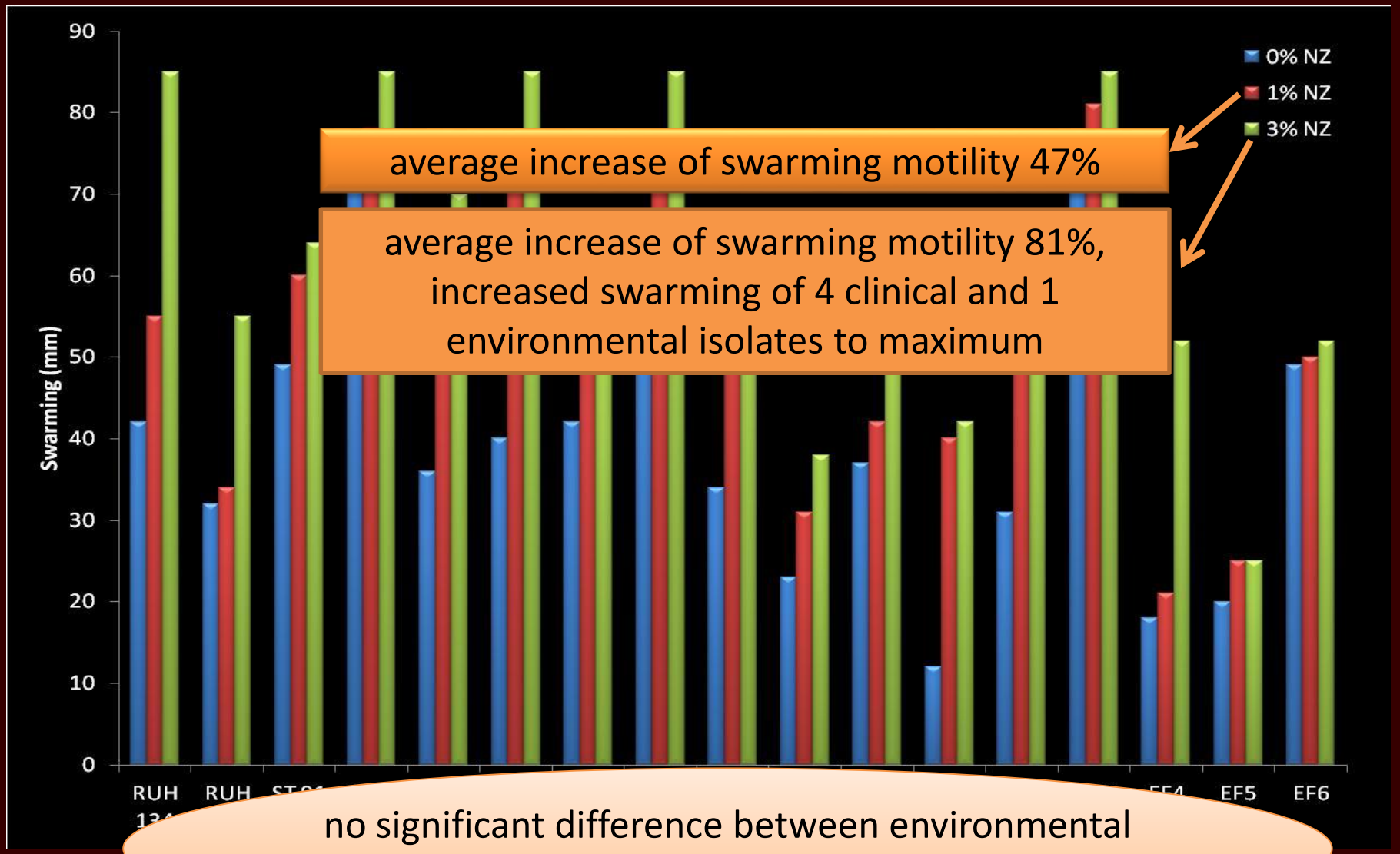
Biofilm formation was quantified in polypropylene tubes after 24h of static incubation at 37°C by crystal violet staining, ethanol solubilisation and measurement of absorbance at 550nm.

Without addition of NZ the intensity of twitching motility showed significantly positive correlation with biofilm formation, while being independent on the swarming motility.

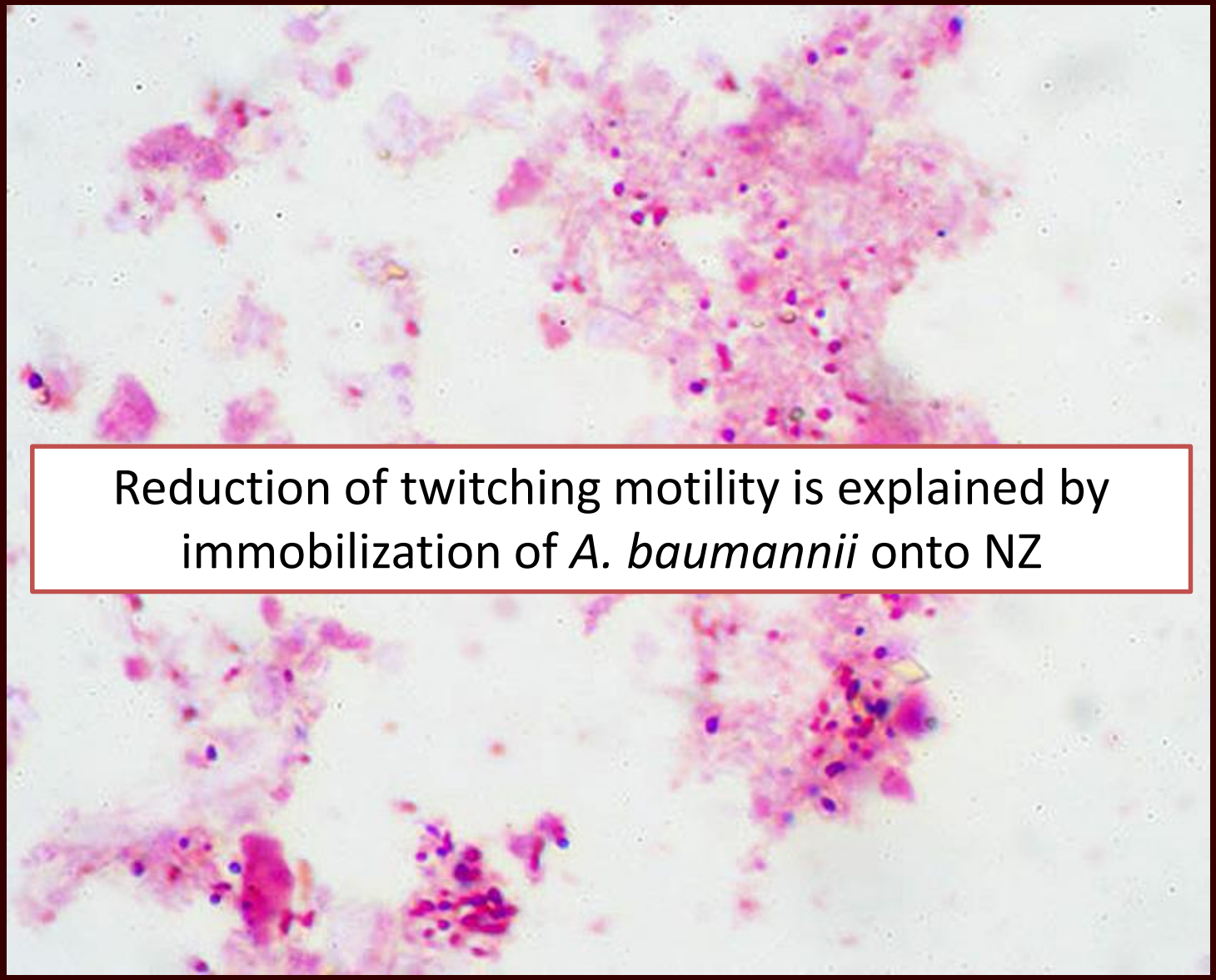
This suggests that the twitching motility is an important prerequisite for biofilm formation of *A. baumannii*.



Effects of different concentrations of NZ on twitching motility of *A. baumannii* isolates. Minimum diameter of twitching zone was 0mm.



Effects of different concentrations of NZ on swarming motility of *A. baumannii* isolates. Maximum diameter of swarming zone was 85mm.



Reduction of twitching motility is explained by immobilization of *A. baumannii* onto NZ

Immobilized cells of *A. baumannii* onto micronized particles of NZ. Light microscopy after staining with carbol fuxin dye.

CONCLUSIONS

✓ No significant differences in antibiotic resistance profiles, biofilm formation, twitching and swarming surface motility were observed among the clinical and environmental isolates, which suggest that the pathogenic potential of *A. baumannii* does not depend on the strain source.

✓ Micronized particles of NZ at concentration of 1-3% reduced or completely blocked the twitching motility of *A. baumannii* on polystyrene.

✓ Bacterial cells were immobilized onto NZ particles, thus hindering the twitching motility of *A. baumannii*.

✓ The swarming motility on the surface of semisolid medium was not reduced by NZ.

✓ Micronized NZ is a promising nontoxic material for prevention of adherence of pathogenic bacteria onto abiotic surfaces, and consequently control of the biofilm formation.