

# Presence of carbapenem-resistant bacteria in the environment

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Environmental Biotechnology Lab, Ben-Gurion University, June 3, 2018



Zagreb – capital city, population ~ 800 000

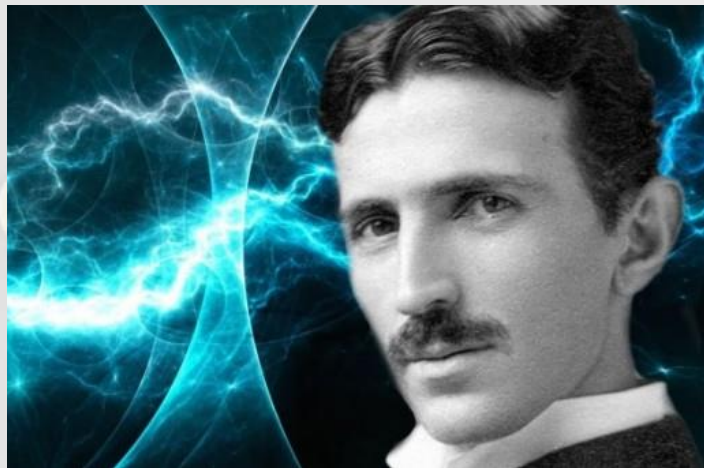


Croatia – small country in South Europe/Balkans, population ~ 4 million





# Fun facts about Croatia:





## University of Zagreb

- Founded in 1699, 30 faculties and 3 academies (Art, Music, Dramatic Arts)
- ~ 70 000 students
- Biggest and best University in Croatia
- Greatest problem is high number of faculties and spatial dispersion

## Faculty of Science

- Founded in 1876 / 1946
- 7 Departments: Biology, Physics, Mathematics, Geology, Geography, Chemistry, Geophysics
- ~ 5 000 students, 35 undergraduate and graduate studies







Main building



Academy of Music



Faculty of Science

*Bacteriological laboratory, Faculty of Science, University of Zagreb*



My visit was funded by ERASMUS+ program for partner countries



Erasmus+

Ben-Gurion University

- Faculty of Education and Rehabilitation Sciences
- School of Medicine
- Faculty of Science

**You are invited and welcome to visit our University!**  
**...even if it's just to see our city 😊**



# background

- the carbapenem-resistant bacteria in the environment.....
- „Natural habitat of clinically important *Acinetobacter baumannii*” a project funded by Croatian Science Foundation (2015-2019), leader Prof. Jasna Hrenovic
- Objective: to isolate viable multiple-drug-resistant *A. baumannii* from the environmental samples of wastewater and soils. The environmental isolates will be genotyped and their phylogenetic relation to human isolates will be determined, as well as the profiles and genes responsible for their antibiotic resistance.



# background

- Carbapenems are considered to be „the last resort” antibiotics
- The incidence of carbapenem-resistant bacteria (CRB) among clinical isolates in Croatia has increased dramatically over the last decade:
  - from 34 to 86 % for *A. baumannii*,
  - from 12 to 21 % for *Pseudomonas aeruginosa*,
  - from none to detectable (1%) for *Enterobacteriaceae*
  - data shown for 2010 and 2016.
- Increasing reports of MDR *A. baumannii* isolated from wastewater (hospital, municipal)





# results

- **2014** (published in 2016: Hrenovic *et al.* Carbapenem-resistant isolates of *Acinetobacter baumannii* in a municipal wastewater treatment plant, Croatia, 2014. Eurosurveillance 21.)
- 21 *A. baumannii* were isolated from influent and effluent of Zagreb wastewater treatment plant (WWTP)
- All were multi-drug resistant (including carbapenems)
- All isolates harbored constitutive ***bla*<sub>OXA-51-like</sub>** gene
- In addition 13 isolates carried acquired ***bla*<sub>OXA-23-like</sub>** or ***bla*<sub>OXA-40-like</sub>** genes
- Findings of MDR *A. baumannii* with acquired OXA-<sub>23/40</sub>-like genes indicated significant presence of this bacterium in wastewater, most probably of anthropogenic origin (from hospital wastewater)



# results

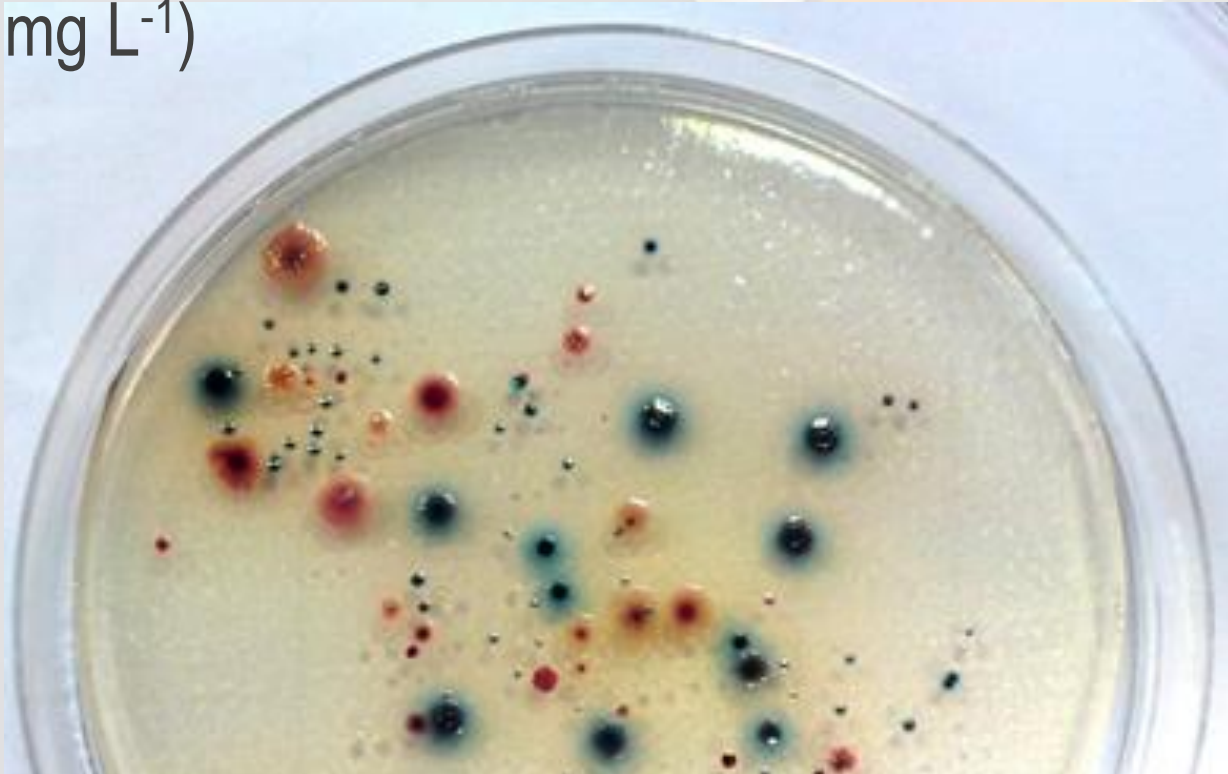
- **2015** (published in 2017: Seruga Music *et al.* Emission of extensively-drug-resistant *Acinetobacter baumannii* from hospital settings to the natural environment. J Hosp Inf 96)
- Occurrence of *A. baumannii* was tracked for 2 months from clinical isolates (patients) to hospital wastewater, to sewage system, to recipient river
- According to MLST analysis the isolates from hospital wastewater, sewage, recipient river and patients belonged to same sequence type (ST 195/ST 1421) and all belonged to same clonal complex (International Clone 2)
- Environmental isolates were all extensively-drug-resistant, same as hospital isolates
- Confirmation that *A. baumannii* originating from hospital are present through entire sewage line.





# materials & methods

- Isolation and identification of *A. baumannii* was performed using CHROMagarAcinetobacter medium (Biomérieux, France) supplemented with CR102 (allowing growth of carbapenem-resistant bacteria – imipenem, meropenem MIC  $\geq 8$  mg L<sup>-1</sup>)



CHROMagar Acinetobacter™ plate inoculated with wastewater sample after incubation at 37°C for 48 hours.

The blue colonies are usually *Enterobacteriaceae* and red colonies are *Stenotrophomonas* sp., *Acinetobacter* sp., *Pseudomonas* sp. or other Gram-negative bacteria.



# materials & methods

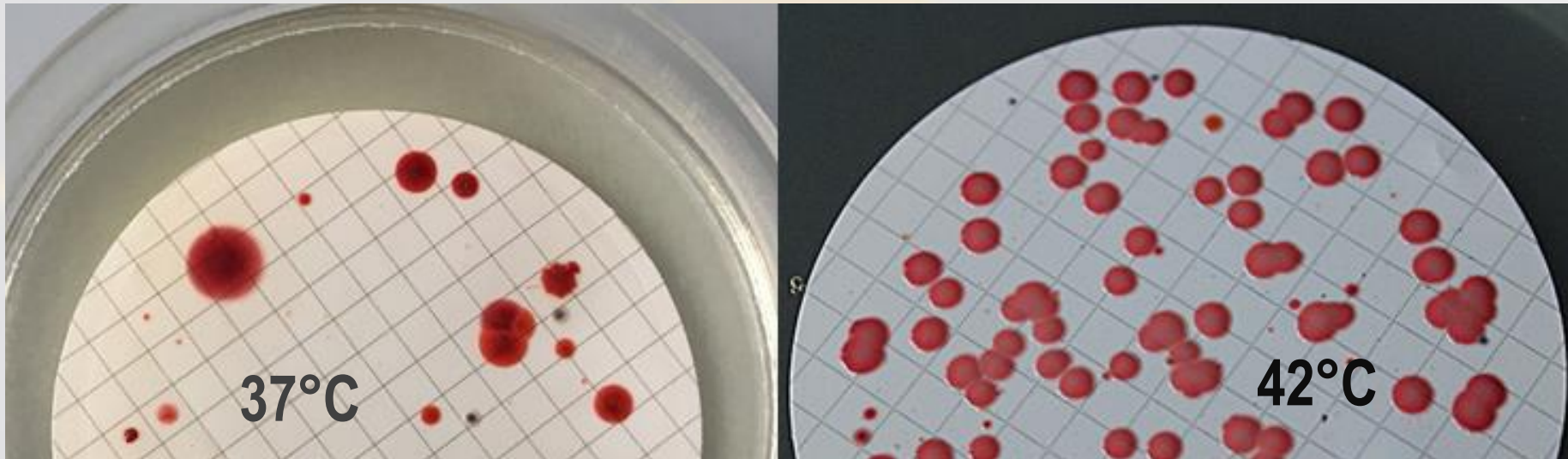
- Presumptive colonies of *A. baumannii* were re-inoculated on the same media and identified via MALDI-TOF technology and/or sequencing.
- During the experiments it was noticed that it is practically impossible to isolate *A. baumannii* when incubation was at 37°C because almost all of the colonies were *Stenotrophomonas*
- Only when incubation was at 42°C the *A. baumannii* started to be detected – at this temp. the growth of *Stenotrophomonas* was inhibited.





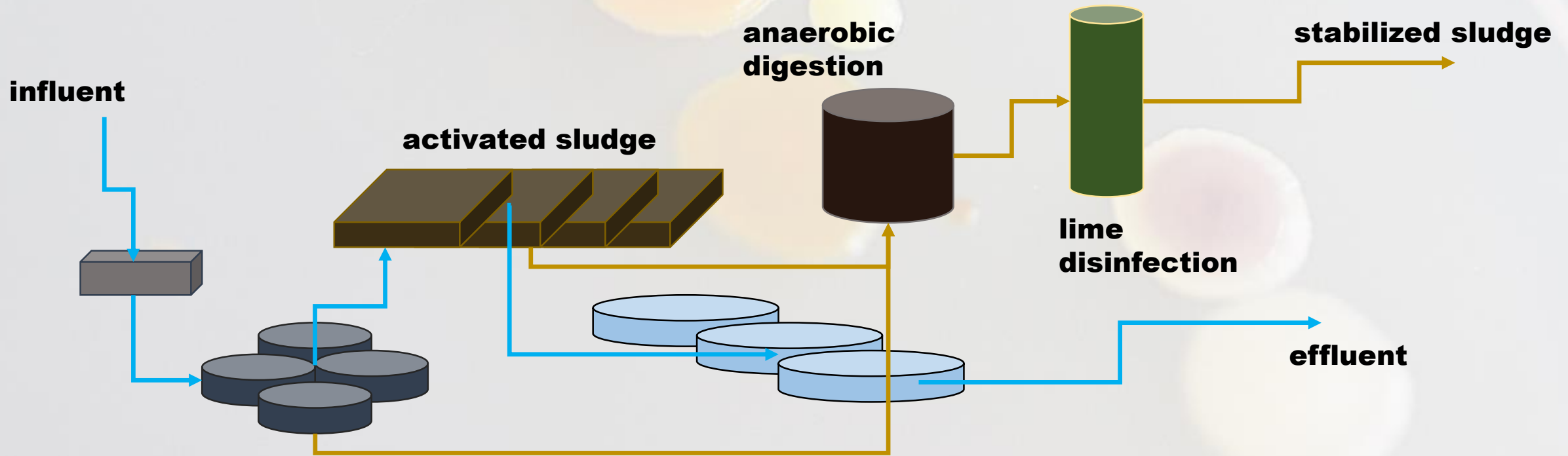
# materials & methods

- In our next experiments, where we investigated total CRB populations, we incubated environmental samples at 37 and 42°C
- This enabled distinguishing between presumably intrinsic CRB – **grown at 37°C**
- and presumably human-associated and clinically relevant, carrying acquired resistance – **grown at 42°C**



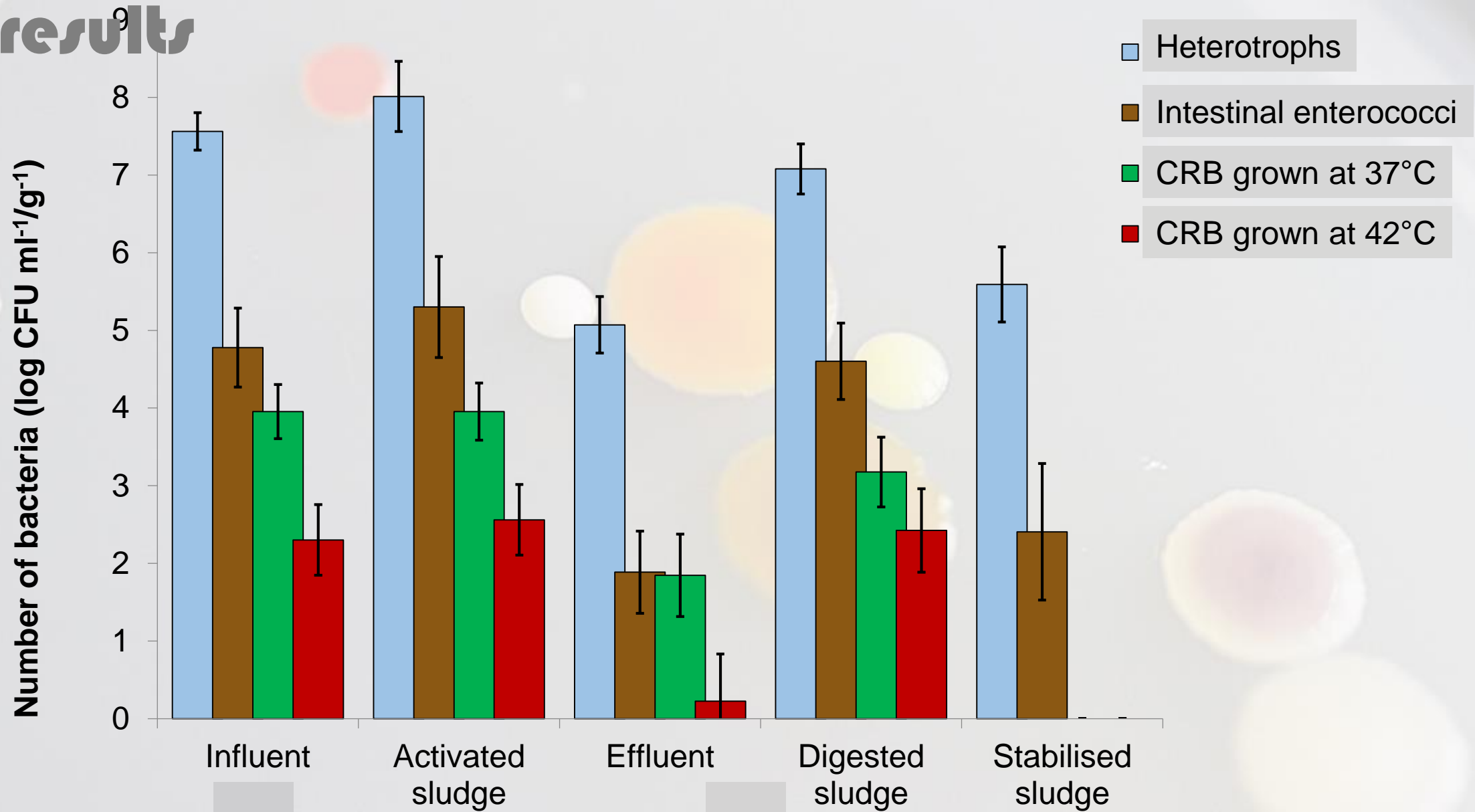
# results

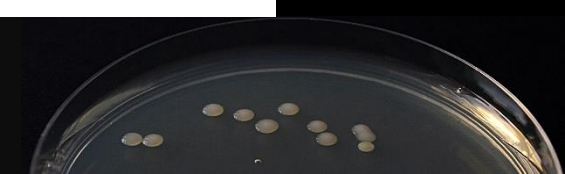
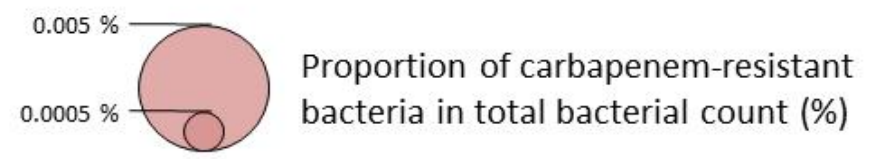
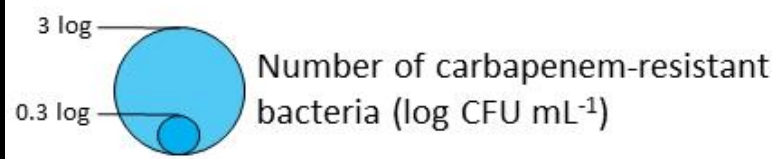
- **2015 – 2016** (published in 2017: Hrenovic *et al.* The fate of carbapenem-resistant bacteria in a wastewater treatment plant. Wat Res 126.)
- The abundance of carbapenem-resistant bacteria (CRB) was monitored for 10 months through major stages of Zagreb WWTP.





# results<sup>9</sup>

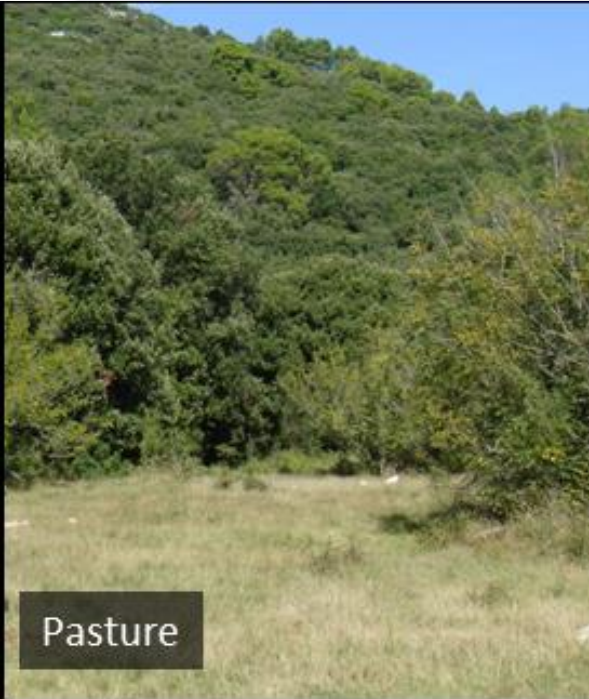






# results

- **2014 – 2017** (submitted in May 2018)
- The CRB were searched for in soil samples across Croatia



Pasture



Illegal dump site



Poultry farm



# results

- Intrinsically resistant CRB were found in all soil samples, and clinically relevant ones were found in most samples from illegal dump sites
- Among intrinsically resistant CRB *Stenotrophomonas* sp. was dominant, and among clinically relevant CRB we identified mostly *Acinetobacter* sp., *Burkholderia* sp. and *Enterobacteriaceae*.
- We proposed cultivation/enrichment of environmental samples at 37 and 42°C to get a better insight into the diversity of CRB in the environment, since *Stenotrophomonas* seems to „shade” other CRB when cultivation was at 37°C.





# results

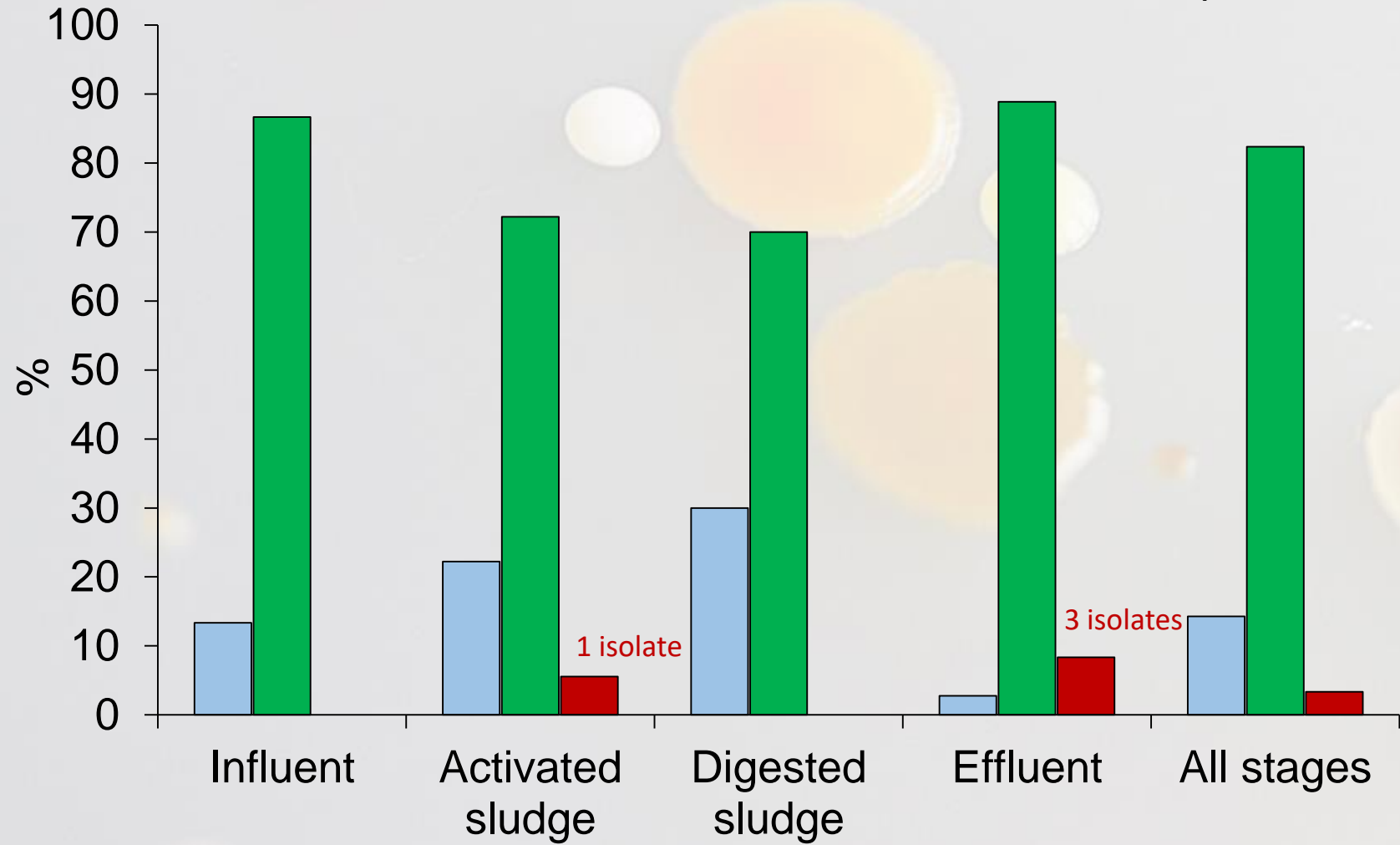
- **2015 - 2016** (published in 2018: Hrenovic *et al.* Characterization of *Acinetobacter baumannii* from water and sludge line of secondary wastewater treatment plant. Wat Res 140)
- Total of 119 *A. baumannii* isolates from WWTP was compared and analyzed
- Confirmation that *A. baumannii* were continuously fed to WWTP and emitted to natural recipient via effluent; **none were found in lime-treated sludge**
- **102/119** were MDR (including carbapenems) and belonged to **IC2** (OXA-23) or **IC1** (OXA-72)
- 17/119 were susceptible, belonged to **IC5** or unclustered
- The ratio of hospital wastewater in total influent of Zagreb WWTP is quite high (approx. 0.3 – 1.7 % daily) and all MDR isolates are probably originating from hospitals





Percentage of *A. baumannii* isolates recovered from different stages of wastewater treatment plant, that were:

- Susceptible
- Resistant to carbapenems and fluoroquinolones
- Resistant to carbapenems, fluoroquinolones and colistin



# what's next...

- **2018 – 2019**
- To determine virulence factors (biofilm formation, surface motility, hydrophobicity) and influence of ecological conditions (pH, temperature, oxygen, nutrients, desiccation, competition with other microorganisms) on the survival of *A. baumannii* in the environment
- So far.....seems that *A. baumannii* is extremely resilient and survives everything....
- different coping strategies – dormant cells, bust and boom, colony dimorphism....



# what's next...

- **2018 – 2019**
- Disinfection and soil bioremediation based on modified natural zeolites
- Zeolites with nanoparticles (Cu, Zn, Ag, Se) and cationic surfactants
- Cost effective and environmental friendly disinfection and bioremediation technology tested specifically against *A. baumannii* (previously collected environmental isolates)
  
- **2020** – collaboration with Ben-Gurion University?







THANK

YOU FOR

YOUR

ATTENTION