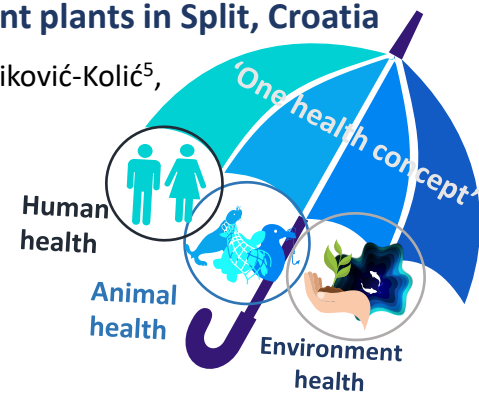


Prevalence and antibiotic resistance determinants of carbapenem-resistant *Acinetobacter baumannii* from wastewater treatment plants in Split, Croatia

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1st International Congress for Sustainable Ecosystems in the Mediterranean Area
October 2-3, 2024.
Split, Croatia



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OBJECTIVES

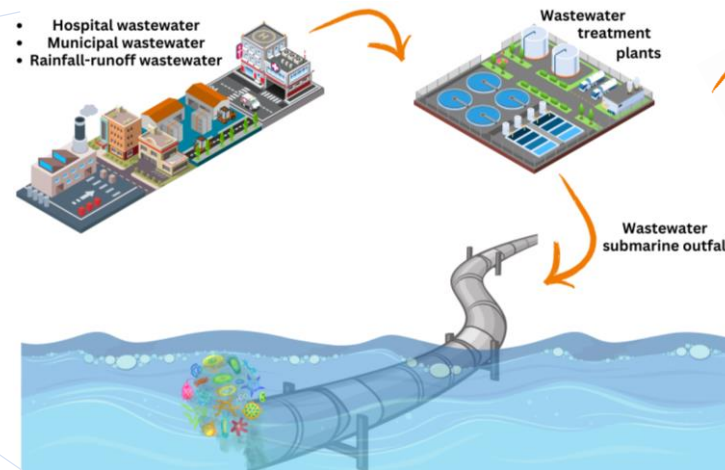
- To investigate the occurrence and antimicrobial susceptibility of carbapenem-resistant *A. baumannii* (CRAB) in the influent and effluent of two wastewater treatment plants (WWTPs) in Split.
- To perform a detailed search of the obtained genome sequences acquired through whole genome sequencing (WGS)

SIGNIFICANCE

- In response to the increasing incidence of CRAB infections, WHO classified it as a critical threat in its 2017 priority pathogens list and maintained this classification in the updated 2024 list, underscoring its persistent and serious threat to public health.
- Considering the importance of CRAB for public health and the One Health approach, we aimed to study their occurrence outside the hospital settings.

FUNDING

This research was funded by Croatian Science Foundation (UIP-2019-04-9778), Croatian Academy of Sciences and Arts, and the Institutional project 'Critical-priority pathogen carbapenem-resistant *Acinetobacter baumannii* in hospitals and wastewater treatment facilities' of Faculty of Science University of Split, Croatia.



MATERIALS AND METHODS

FIELD SAMPLING

- 18 samples (3 influent, 3 effluent, and 3 seawater from 2 locations) were collected over three consecutive days at two WWTPs, Katalinić Brig and Stupe, including their submarine outfalls.

LABORATORY ANALYSES

- Vacuum filtration through membrane filters, which were then placed on *Acinetobacter* CHROMagar to obtain carbapenem-resistant isolates. Selected isolates were tested on 14 antimicrobials. PFGE was performed on CRAB isolates to exclude clonal relatedness, and select isolates for WGS.

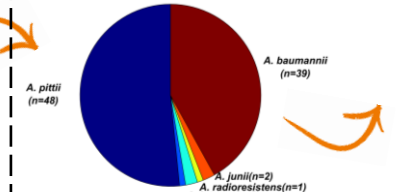


Fig.1: *Acinetobacter* isolates recovered in this study

RESULTS

- Out of 336 Gram-negative isolates, 93 belonged to *Acinetobacter*
- A. pittii* and *A. baumannii* prevailed (Fig. 1)
- 18% of *A. baumannii* species were CRAB isolated from Katalinića Brig influent and effluent, following Stupe influent

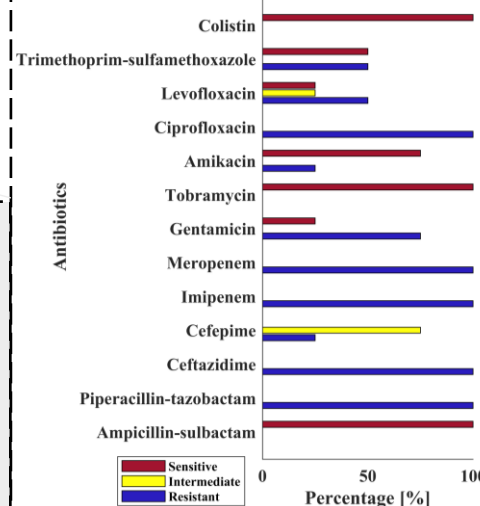


Fig.2: Antibiotic resistance profiles of four CRAB isolates

Pulsed-field gel electrophoresis

Clonal relatedness – selection for WGS

Acquired AMR gene hits of CRAB strain MA1

*bla*_{OXA-51}, *bla*_{OXA-437}, *bla*_{OXA-72}, *bla*_{ADC-25}, *tet*(39)

- bla*_{OXA-51} is intrinsic to *A. baumannii*
- bla*_{OXA-72} detected in *A. baumannii* from WWTPs in Zagreb and hospitals in Croatia
- bla*_{ADC-25} detected in hospital-acquired *A. baumannii* in Croatia
- Tet*(39) found in environmental *A. baumannii* strain

CONCLUSION AND FUTURE WORK

- A. baumannii* propagate from the influent to effluent of both WWTPs
- Detection of both, sensitive and antibiotic-resistant *A. baumannii* in WWTPs indicate these facilities as hotspots for their dissemination outside the hospital settings
- Molecular characterization of clinical and environmental CRAB is to be further done