MOLECULAR CHARACTERIZATION OF *ACINETOBACTER BAUMANNII* FROM ZAGREB WASTEWATER TREATMENT PLANT

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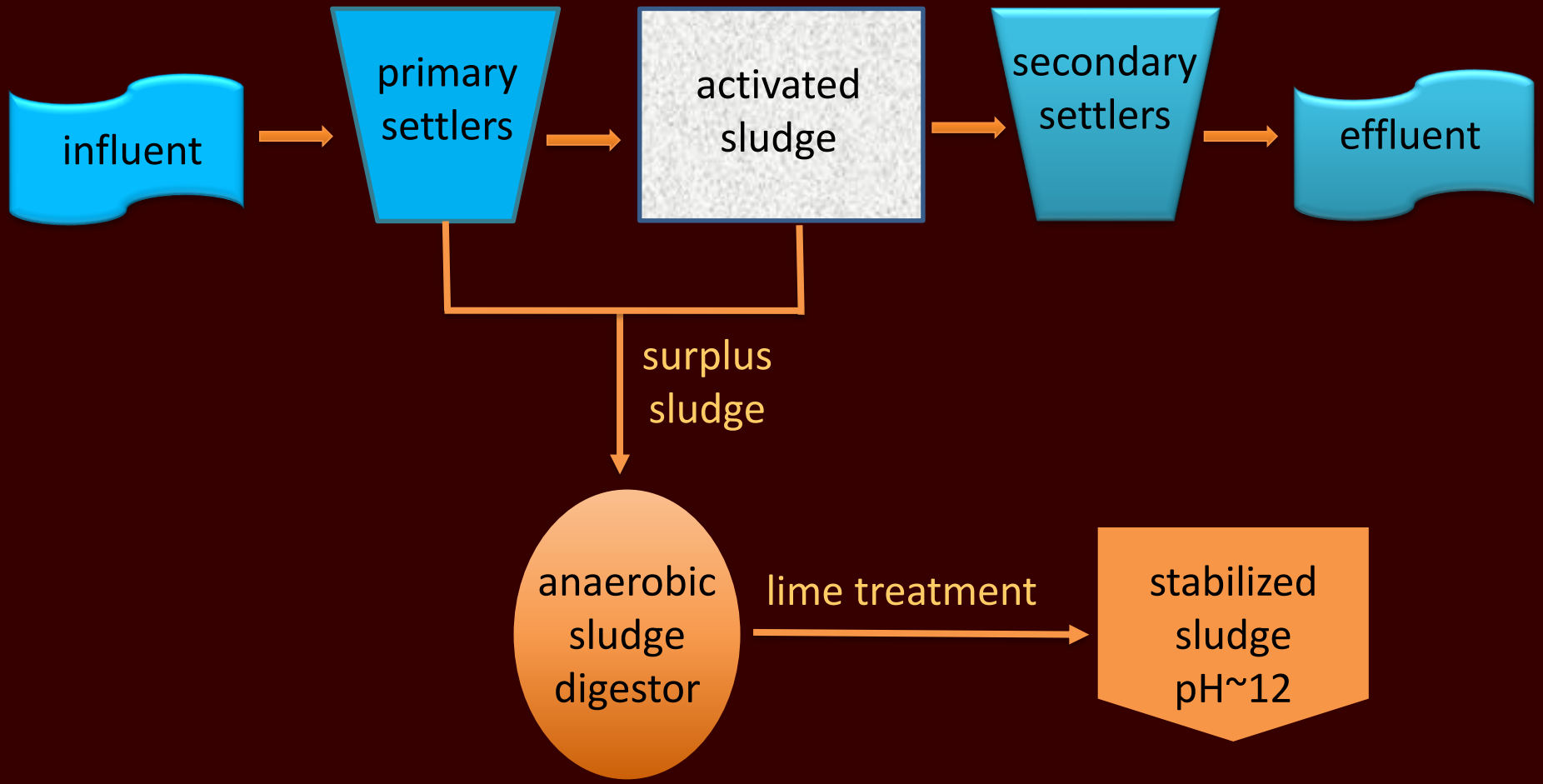
City of Zagreb generates between 880 - 5300 m^3 of hospital wastewaters per day, that are released into the sewage system without pre-treatment.

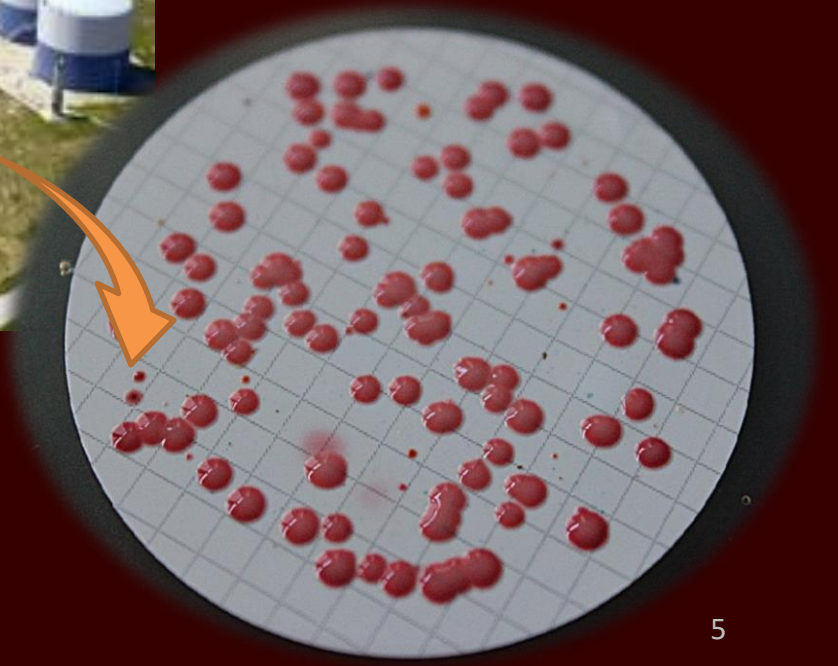
This accounts for 0.3 - 1.7 % of hospital wastewater in urban wastewater.

Urban wastewater is treated at the secondary wastewater treatment plant (WWTP) of capacity 1.2 million population equivalents.

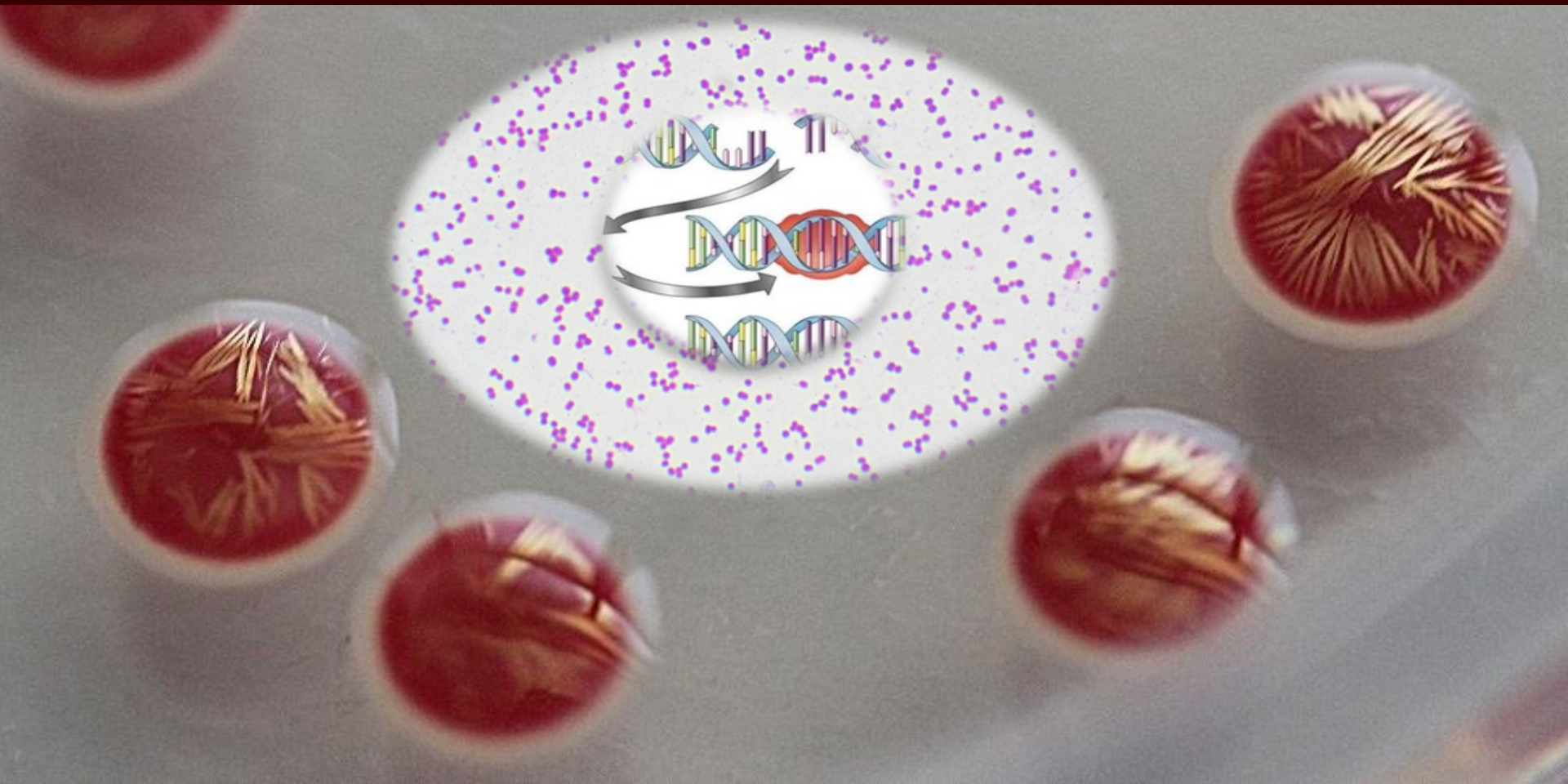
Schematic view of Zagreb WWTP:



Acinetobacter baumannii recovered during one-year monitoring (2015-16) at the main stages of the WWTP were characterized to elucidate the efficiency of WWTP in removal of this emerging hospital pathogen.

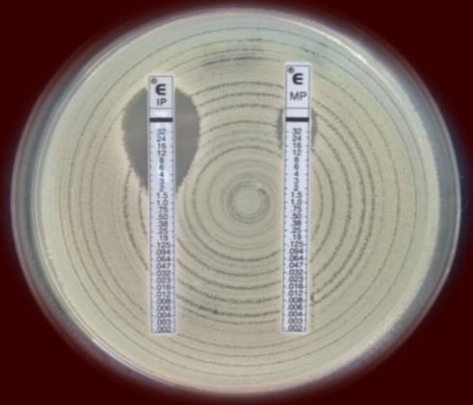


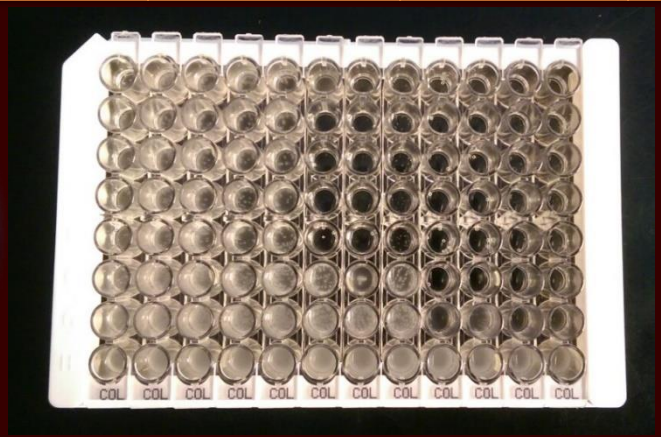
Recovery: CHROMagar Acinetobacter supplemented with 15 mg/L of cefsulodin sodium salt hydrate after incubation at 42 °C/48 h.
Identification: MALDI-TOF MS.
Molecular characterization: whole genome sequencing (WGS) and core genome multilocus sequence typing (cgMLST) on selected isolates.

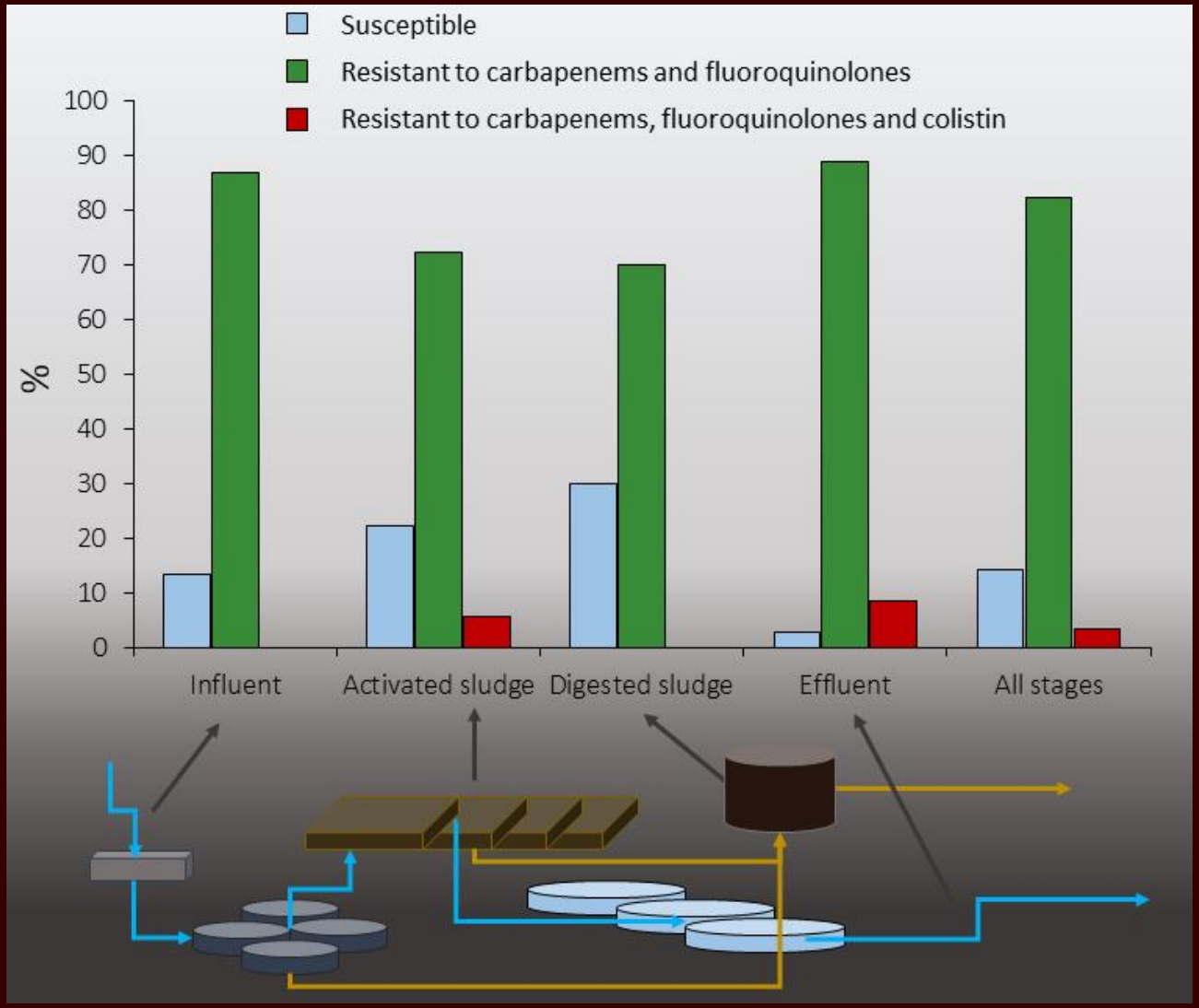


Antimicrobial susceptibility: performed and interpreted according to EUCAST breakpoints for clinical *A. baumannii* isolates.
Isolates were divided into three groups:
S - susceptible to all antibiotics tested;
CFQR - resistant to carbapenems and fluoroquinolones;
PDR - resistant to carbapenems, fluoroquinolones and colistin.

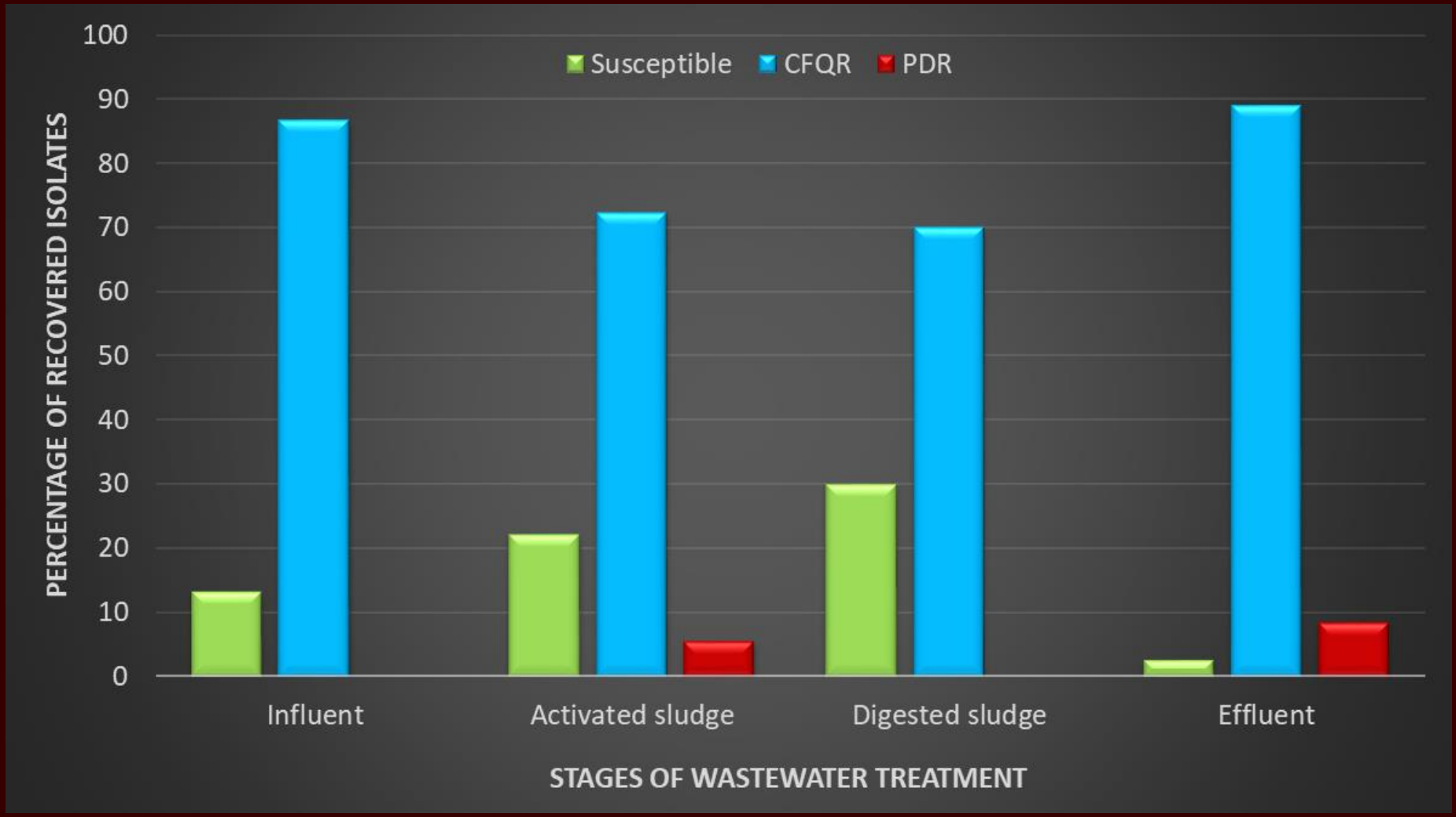
Isolate name	MEM	IMI	CIP	LVX	CST	Antibiotic susceptibility profile
D1	≤ 0.25 S	≤ 0.25 S	≤ 0.25 S	≤ 0.12 S	≤ 0.5 S	S
D11	≥16 R	≥16 R	≥4 R	4 R	≤ 0.5 S	CFQR
EF7	>16 R	>16 R	>4 R	>8 R	20 R	PDR







A. baumannii were recovered from each stage of wastewater treatment, except alkaline lime-treated stabilised sludge (pH 12). Number of isolates: influent 45; activated sludge 18; digested sludge 20; effluent 36; = total 119.



Isolates resistant to carbapenems and fluoroquinolones (CFQR) dominated (82%) in all stages of WWTP, but coexisted with 14% of susceptible isolates.

Pandrug-resistant (PDR) isolates (3%) were detected in activated sludge and effluent wastewater.

Isolate	ST-Oxford /Pasteur	Clonal lineage	Antibiotic susceptibility profile
Influent	195/2	IC2	CFQR
	195/2	IC2	CFQR
	1604/1	IC1	CFQR
	1523/647	unclustered	S
Activated sludge	195/2	IC2	CFQR
	195/2	IC2	PDR
	1524/79	IC5	S
Digested sludge	195/2	IC2	CFQR
	195/2	IC2	CFQR
	231/1	IC1	CFQR
	1525/992	unclustered	S
Effluent	195/2	IC2	CFQR
	195/2	IC2	CFQR
	195/2	IC2	PDR
	195/2	IC2	PDR
	1526/139	unclustered	S

The majority of CFQR and PDR isolates belonged to IC2 (ST-195_{Oxford}/ST-$2_{Pasteur}$), and a minority to IC1. Susceptible isolates belonged to IC5 or were unclustered.

Clonal lineage	Acquired bla_{OXA}	Intrinsic bla_{OXA}
IC2	bla_{OXA-23}	bla_{OXA-66}
	bla_{OXA-23}	bla_{OXA-66}
	bla_{OXA-23}	bla_{OXA-66}
	bla_{OXA-23}	bla_{OXA-66}
	bla_{OXA-23}	bla_{OXA-66}
	bla_{OXA-23}	bla_{OXA-66}
	bla_{OXA-23}	bla_{OXA-66}
	bla_{OXA-23}	bla_{OXA-66}
	bla_{OXA-23}	bla_{OXA-66}
	bla_{OXA-23}	bla_{OXA-66}
IC1	bla_{OXA-72}	bla_{OXA-69}
	bla_{OXA-72}	bla_{OXA-69}
IC5	-	bla_{OXA-65}
unclustered	-	bla_{OXA-51}
	-	$bla_{OXA-208-like}$
	-	$bla_{OXA-117-like}$

Carbapenem resistance was mediated by acquired *bla*$_{OXA-23}$ in IC2 isolates and *bla*$_{OXA-72}$ in IC1 isolates.

The CFQR and PDR *A. baumannii* belonging to ST-195 were previously reported in the same period of monitoring in Zagreb (2015-16) from hospitalised patients, hospital wastewater, and the Sava River.

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Emission of extensively-drug-resistant *Acinetobacter baumannii* from hospital settings to the natural environment

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Summary

Background

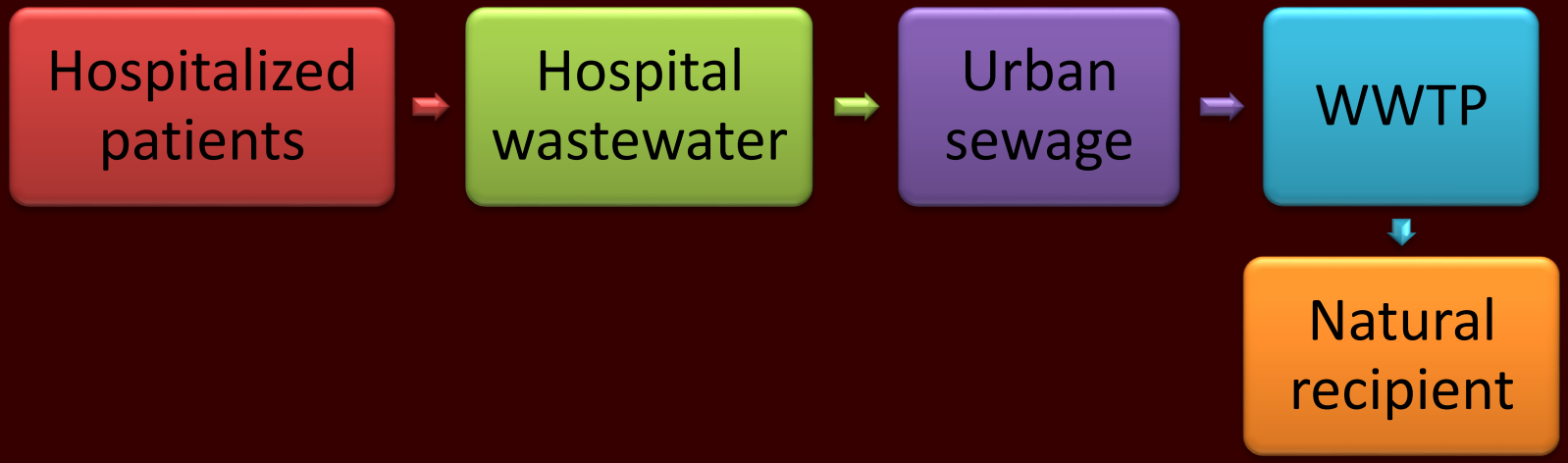
Acinetobacter baumannii is a leading emerging pathogen that is frequently recovered from patients during hospital outbreaks. The role of environmental *A. baumannii* reservoirs is therefore of great concern worldwide.

Aim

To investigate the connection between *A. baumannii* causing hospital outbreaks and environmental isolates from hospital wastewater, urban sewage and river water as the final natural recipient of wastewaters.

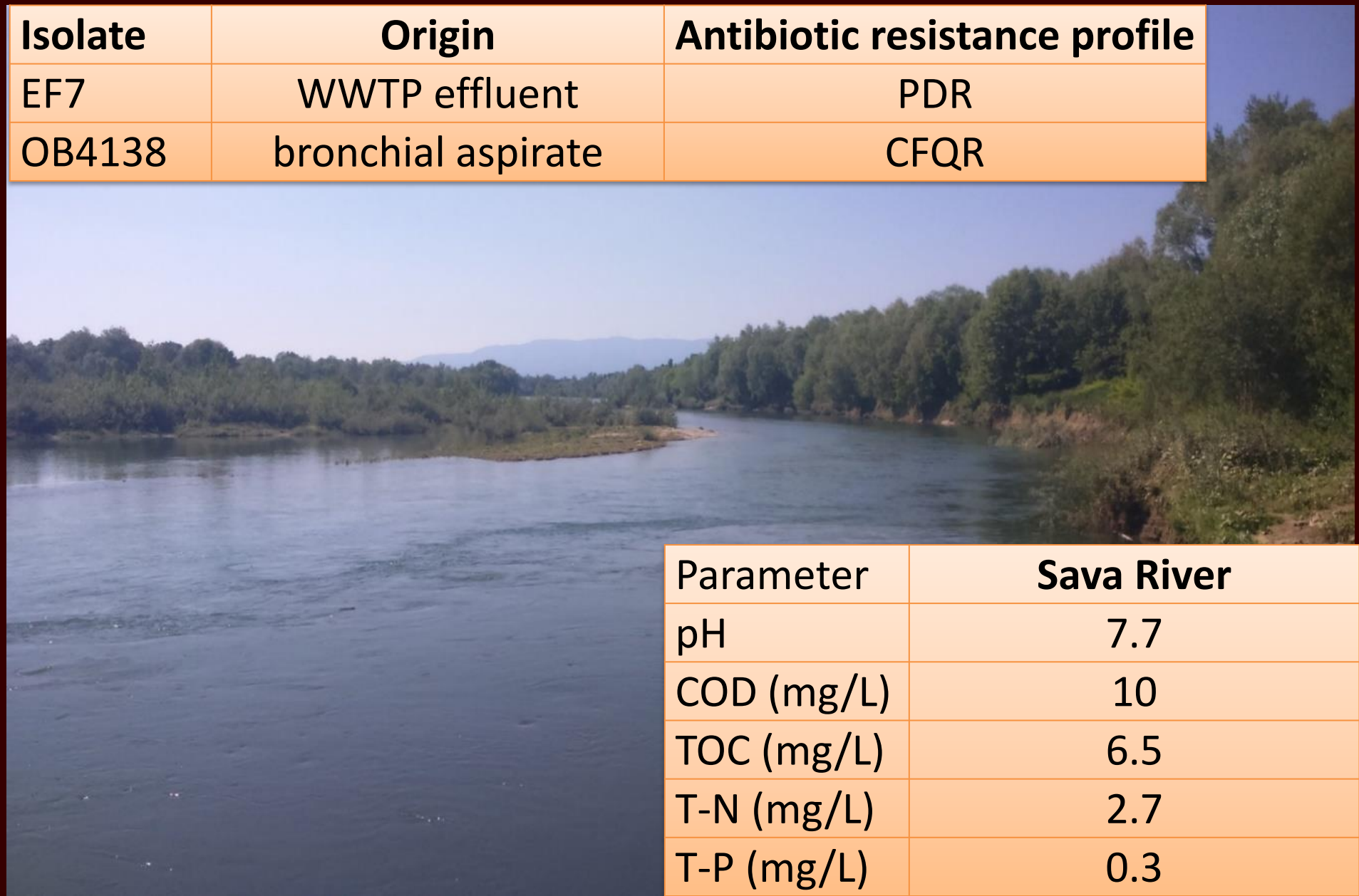
Isolate	ST-Oxford	Clonal lineage	Antibiotic susceptibility profile
Tracheal aspirate	195	IC2	CFQR
Bronchial aspirate	195	IC2	CFQR
Hospital wastewater	195	IC2	CFQR
	195	IC2	CFQR
	195	IC2	CFQR
	195	IC2	CFQR
	195	IC2	CFQR
	195	IC2	CFQR
	195	IC2	CFQR
	195	IC2	CFQR
	195	IC2	PDR
	195	IC2	PDR
Sava River	195	IC2	CFQR

Close relatedness with isolates recovered from Zagreb WWTP suggests dissemination of *A. baumannii* of clinical significance from patients, via untreated hospital wastewater, urban sewage, and WWTP to the Sava River.

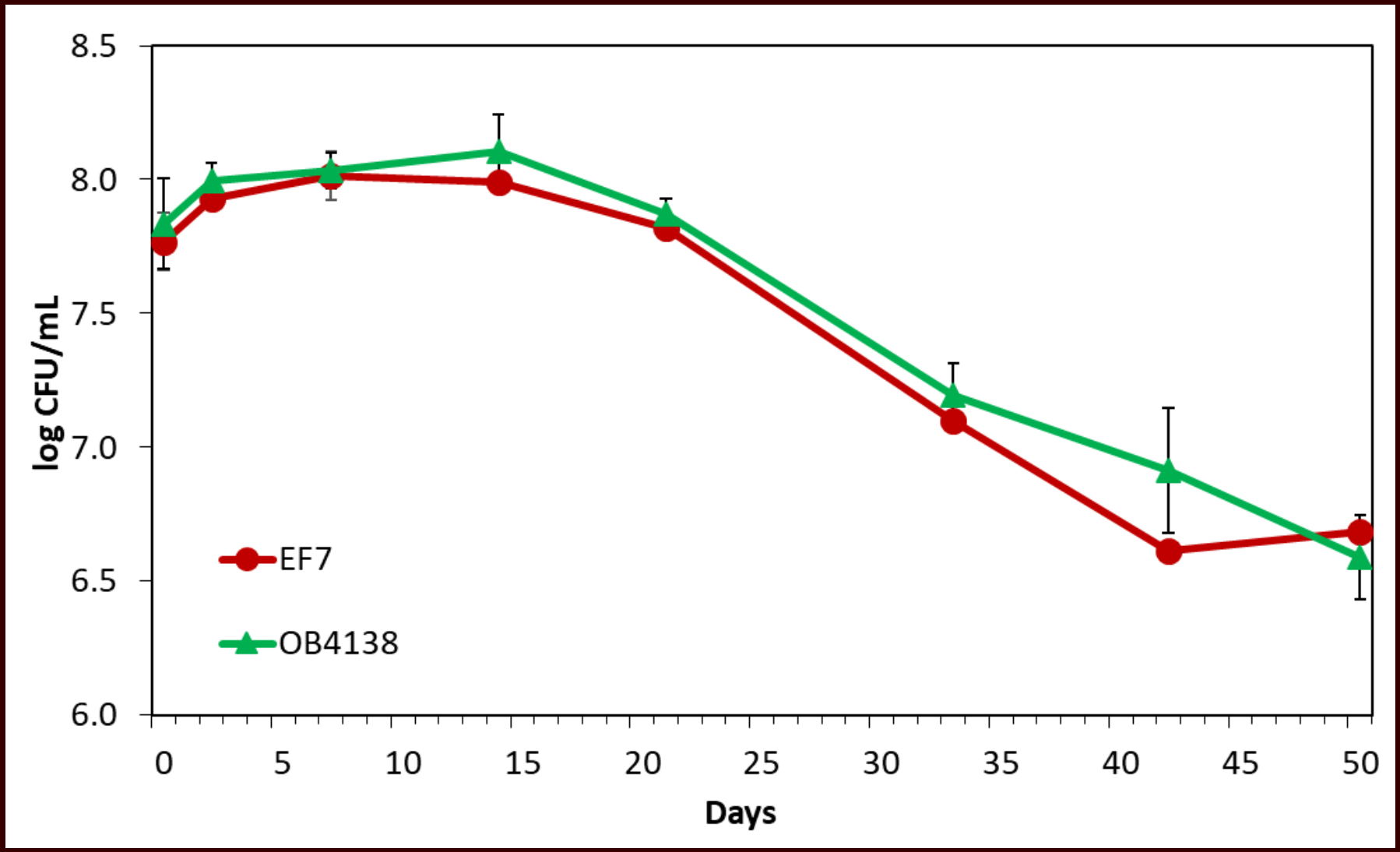


Long-term survival of *A. baumannii* isolates in sample of the Sava River was checked.

Isolate	Origin	Antibiotic resistance profile
EF7	WWTP effluent	PDR
OB4138	bronchial aspirate	CFQR



Parameter	**Sava River**
pH	7.7
COD (mg/L)	10
TOC (mg/L)	6.5
T-N (mg/L)	2.7
T-P (mg/L)	0.3



Two *A. baumannii* isolates multiplied and survived in autoclaved river water during 50 days.

Conclusion:

- Clinically relevant *A. baumannii* persist in secondary WWTP and are emitted via effluent to the Sava River.
- Alkaline lime-treatment renders the waste sludge free of viable *A. baumannii*.
- However, additional disinfection of effluent prior to its discharge into the natural recipient is needed to avoid the possible public-health risk.
- Moreover, disinfection of hospital wastewaters prior to its dilution in the urban sewage system will be promising strategy to mitigate the propagation of *A. baumannii* in environment.

To read more:

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Characterization of *Acinetobacter baumannii* from water and sludge line of secondary wastewater treatment plant

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ABSTRACT

The elimination of potentially pathogenic bacteria in wastewater treatment plants (WWTPs) attracts much attention in public health. Reports on the occurrence of the emerging hospital pathogen *Acinetobacter baumannii* in wastewaters do not include a continuous monitoring at all WWTP stages. The objective of this study was to characterize *A. baumannii* recovered from the water and sludge line of the secondary WWTP in Zagreb, Croatia over the period of one year. Recovery of *A. baumannii* was performed using CHROMagar Acinetobacter plates. Antimicrobial susceptibility testing was performed with broth microdilution and results interpreted using EUCAST breakpoints for clinical isolates of *A. baumannii*. Molecular characterization was performed by WGS and cgMLST. The secondary WWTP treating the urban wastewater is constantly receiving viable *A. baumannii* along with genes encoding carbapenem resistance, and emitting them via effluent into the environment. Furthermore, *A. baumannii* from influent are incorporated into activated sludge flocs in aeration basin. *A. baumannii* can survive the technological process of anaerobic mesophilic sludge digestion, and is finally destroyed in alkaline lime-treated stabilized sludge. The majority (102/119) of *A. baumannii* isolates were carbapenem-resistant, while antibiotic-susceptible isolates (17/119) were rarely recovered from all WWTP stages. Carbapenem-resistant isolates belonged to international clonal lineage IC2 carrying OXA-23 and IC1 carrying OXA-72, while the susceptible isolates belonged to IC5 or were unclustered. Increased resistance to antibiotics, together with the appearance of carbapenem- and even pandrug-resistant isolates in effluent as compared to influent wastewater, suggests the need of additional disinfection of effluent prior to its discharge into the natural recipient.

Thank you for attention!

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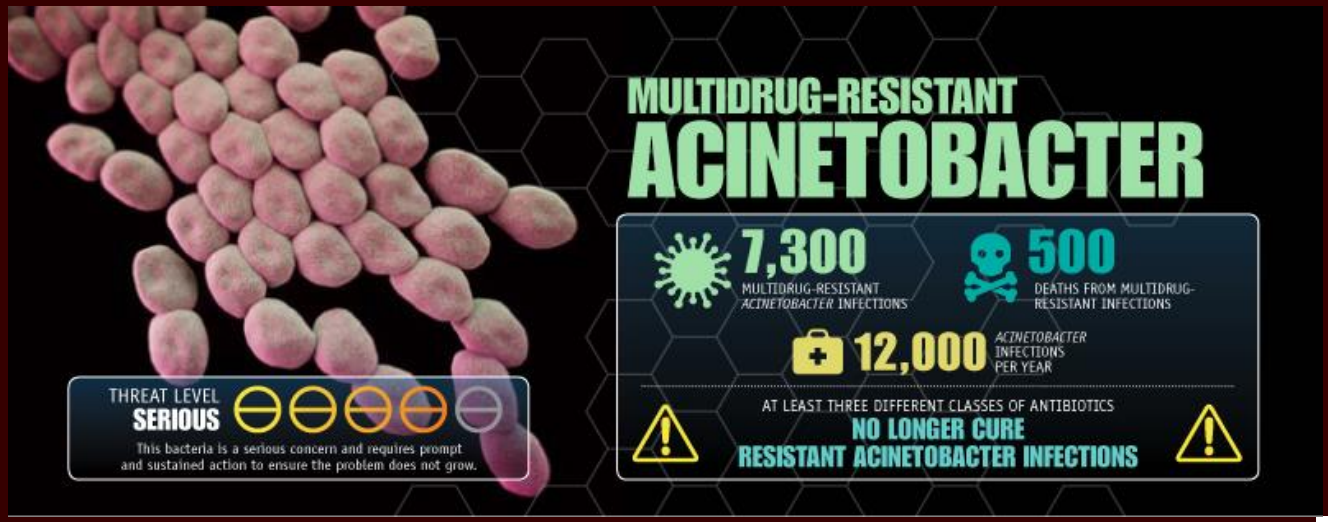
https://www.pmf.unizg.hr/naturaci



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