

# PRESENCE OF BACTERIUM ACINETOBACTER BAUMANNII IN WASTEWATERS OF THE CITY OF ZAGREB

Jasna Hrenović<sup>a</sup>, Ivana Goić-Barišić<sup>b</sup>, Snježana Kazazić<sup>c</sup>, Blaženka Hunjak<sup>d</sup>, Draženka Stipaničev<sup>e</sup>, Siniša Repec<sup>e</sup>, Marin Ganjto<sup>f</sup>

<sup>a</sup>University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia;

<sup>b</sup>University Hospital Centre Split, Department of Clinical Microbiology and University of Split School of Medicine, Split, Croatia;

<sup>c</sup>Ruđer Bošković Institute, Division of Physical Chemistry, Zagreb, Croatia;

<sup>d</sup>Croatian Institute of Public Health, Zagreb, Croatia;

<sup>e</sup>Croatian Waters, Zagreb, Croatia;

<sup>f</sup>Zagreb Wastewater - Management and Operation Ltd., Zagreb, Croatia

## Background:

*Acinetobacter baumannii* is an emerging hospital pathogen causing outbreaks in Croatia since 2002 and is still present in Croatian hospitals [1]. Clinical isolates of *A. baumannii* in Croatian hospitals are usually multi-drug resistant (MDR), with resistance to carbapenems dramatically increasing from 10% in 2008 to 82% in 2014 [2].

MDR *A. baumannii* has been isolated from patients, hospital environment during outbreaks, and hospital wastewater in Brazil and China [3,4]. However, crucial questions regarding its epidemiology remains incompletely understood [5]: are the infected patients and hospital environment the only sources of *A. baumannii*, at which extent *A. baumannii* are released from hospitals in nature, do they survive or even multiply in nature, do they have natural habitat outside hospitals.

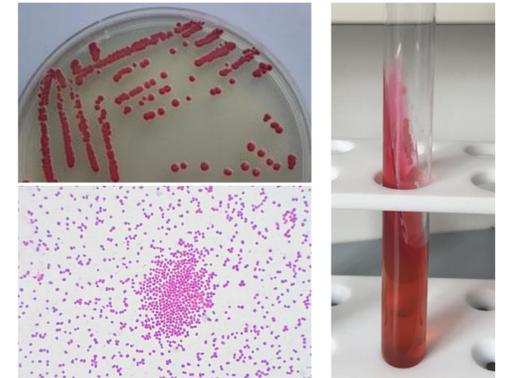
The aim of this study was to screen the hospital and municipal wastewater of the City of Zagreb for the presence of viable *A. baumannii* and carbapenems.

## Material/methods:

Sampling of hospital wastewater was performed on 2 occasions in 2015 at the central manhole of one Zagreb's hospital from which the clinical isolates of *A. baumannii* were recovered. Sampling of municipal wastewater was performed on 6 occasions in 2014/15 at the influent and effluent of the central Zagreb's wastewater treatment plant. This secondary type of wastewater treatment plant receives wastewaters of all 9 clinical hospitals in Zagreb.

Concentration of carbapenems in wastewater was measured by ultra-high performance liquid chromatography - quadrupole time-of-flight mass spectrometry (6550 i-Funnel UHPLC Q-TOF MS, Agilent Technologies). The isolation of *A. baumannii* from wastewater was performed at 42°C/48h on CHROMagar Acinetobacter without or with the addition of commercial supplement CR102 which allows the growth of carbapenem-resistant isolates. Cefsulodin sodium salt hydrate (Sigma-Aldrich) was added at 15 mg/L to suppress the growth of *Pseudomonas* and *Aeromonas* spp. Presumptive *A. baumannii* colonies were characterized phenotypically (Fig. 1).

Further identification was carried out by using Vitek 2 systems (BioMerieux) and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics) on cell extracts [6]. Antibiotic resistance profiles were determined according to MIC values obtained by Vitek 2 system or E-test and interpreted according to EUCAST criteria [7].



**Figure 1:** Presumptive red colonies of *A. baumannii* grown on CHROMagar Acinetobacter phenotypically were Gram negative coccobacilli, with typical negative reaction on the Kligler Iron Agar.

## Results:

Hospital and municipal wastewaters contained high concentrations of imipenem and meropenem which averaged: 894 and 129 ng/L in hospital wastewater, 3060 and 380 ng/L in raw and 497 and 311 ng/L in treated municipal wastewater, respectively (Fig. 2).

From hospital wastewater (Table 2), raw and treated municipal wastewater (Table 3) 8, 30 and 7 isolates of *A. baumannii* were recovered, respectively. All isolates from hospital wastewater and majority (33/37) of isolates from municipal wastewater were resistant to carbapenems and majority of tested antibiotics except colistin. The 7 MDR clinical isolates (Table 4) recovered in the same period showed comparable levels of antibiotic resistance to MDR isolates from hospital and municipal wastewater. These suggest that *A. baumannii* is able to survive in environment outside hospitals. However, 4 isolates from raw municipal wastewater were susceptible to carbapenems and other antibiotics. This finding opens the possibility that *A. baumannii* could have a natural habitat in sewage system.

**Table 2:** Date of sampling, MALDI-TOF MS score values, and antibiotic<sup>a</sup> profile of *A. baumannii* isolates from hospital wastewater.

All isolates were determined by Vitek 2 system as *A. calcoaceticus-baumannii* complex. R - resistant; I - intermediate; S - sensitive according to EUCAST criteria. <sup>a</sup> carbapenems (MEM-meropenem, IMI-imipenem), fluoroquinolones (CIP-ciprofloxacin, LVX-levofloxacin), aminoglycosides (TOB-tobramycin, GEN-gentamicin, AMK-amikacin), SXT- trimethoprim / sulfamethoxazole, CST-colistin.

Sampling date	Isolate name	MALDI TOF score value	Antibiotic profile									
			MEM	IPM	CIP	LVX	TOB	GEN	AMK	SXT	CST	
27.8.2015	Š2/1	2.045	R	R	R	R	R	R	R	R	R	S
	Š2/3	2.101	R	R	R	R	R	R	R	R	R	S
	Š1/1	2.271	R	R	R	R	R	R	R	R	R	S
6.10.2015	Š2/5	2.067	R	R	R	R	R	R	R	S	S	S
	Š2/6	2.232	R	R	R	R	R	R	R	S	S	S
	Š2/7	2.102	R	R	R	R	R	R	R	S	S	S
	Š2/8	2.077	R	R	R	R	R	R	R	S	S	S
	Š2/9	2.041	R	R	R	R	R	R	R	S	R	S

**Table 3:** Date of sampling of influent or effluent wastewater, MALDI-TOF MS score values, and antibiotic<sup>a</sup> profile of *A. baumannii* isolates from municipal wastewater.

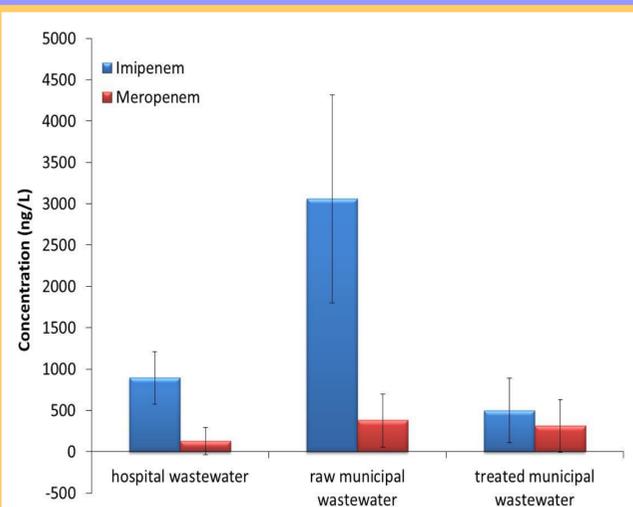
Isolates named as IN were isolated from influent wastewater and isolates named as EF were isolated from effluent wastewater. R - resistant; I - intermediate; S - sensitive according to EUCAST criteria. <sup>a</sup> carbapenems (MEM-meropenem, IMI-imipenem), fluoroquinolones (CIP-ciprofloxacin, LVX-levofloxacin), aminoglycosides (TOB-tobramycin, GEN-gentamicin, AMK-amikacin), SXT-trimethoprim / sulfamethoxazole, CST-colistin.

Sampling date	Isolate name	MALDI TOF score value	Antibiotic profile										
			MEM	IPM	CIP	LVX	TOB	GEN	AMK	SXT	CST		
16.4.2014	EF1	2.262	R	R	R	R	R	R	R	S	S	S	
	EF2	2.352	R	R	R	R	R	R	R	S	S	S	
	EF3	2.329	R	R	R	R	R	R	R	S	S	S	
11.6.2014	IN4	2.231	R	R	R	R	R	R	R	S	S	S	
	IN5	2.085	R	R	R	R	R	R	R	S	S	S	
	IN6	2.157	R	R	R	R	R	R	R	S	S	S	
	IN8	2.168	R	R	R	R	R	R	R	S	S	S	
	IN9	2.167	R	R	R	R	R	R	R	S	S	S	
	IN10	2.193	R	R	R	R	R	R	R	S	S	S	
	IN11	2.409	R	R	R	R	R	R	R	S	S	S	
29.10.2014	EF4	2.191	R	R	R	R	R	R	R	R	S	S	
	EF5	2.161	R	R	R	R	R	R	R	R	S	S	
	EF6	2.219	R	R	R	R	R	R	R	R	S	S	
	IN12	2.190	R	R	R	R	R	R	R	S	S	S	
	IN13	2.118	R	R	R	R	R	R	R	S	S	S	
	IN14	2.213	R	R	R	R	R	R	R	S	S	S	
	IN15	2.121	R	R	R	R	R	R	R	S	S	S	
	IN16	2.244	R	R	R	R	R	R	R	S	S	S	
	IN17	2.163	R	R	R	R	R	R	R	S	S	S	
	IN18	2.048	R	R	R	R	R	R	R	S	S	S	
5.11.2014	IN21	2.328	S	S	S	S	S	S	S	S	S		
3.12.2014	IN22	2.118	R	R	R	R	R	R	R	R	S	S	
	IN24	2.168	R	R	R	R	R	R	R	R	S	S	
	IN25	2.041	R	R	R	R	R	R	R	R	S	S	
	IN26	2.223	R	I	S	S	S	S	S	S	S	S	
	IN27	2.199	I	S	S	S	S	S	S	S	S	S	
	IN28	2.085	R	I	S	S	S	S	S	S	S	S	
23.9.2015	IN31	2.119	S	S	S	S	S	S	S	S	S	S	
	IN32	2.104	R	R	R	R	R	R	R	R	R	S	
	IN33	2.180	R	R	R	R	R	R	R	R	R	S	
	IN34	2.066	R	R	R	R	R	R	R	R	R	S	
	IN35	2.164	R	R	R	R	R	R	R	S	S	R	
	IN36	2.184	S	S	S	S	S	S	S	S	S	S	
	IN37	2.038	R	R	R	R	R	R	R	R	R	S	
	IN38	2.075	R	R	R	R	R	R	R	R	R	S	
	EF9	2.174	R	R	R	R	R	R	R	S	S	R	

**Table 4:** Date of sampling, origin, MALDI-TOF MS score values, and antibiotic<sup>a</sup> profile of *A. baumannii* clinical isolates.

R - resistant; I - intermediate; S - sensitive according to EUCAST criteria. <sup>a</sup> carbapenems (MEM-meropenem, IMI-imipenem), fluoroquinolones (CIP-ciprofloxacin, LVX-levofloxacin), aminoglycosides (TOB-tobramycin, GEN-gentamicin, AMK-amikacin), SXT- trimethoprim / sulfamethoxazole, CST-colistin.

Sampling date	Isolate name	Origin	MALDI TOF score value	Antibiotic profile									
				MEM	IPM	CIP	LVX	TOB	GEN	AMK	SXT	CST	
11.9.2015	OB 3831	Sputum	2.128	R	R	R	R	R	R	R	S	R	S
18.9.2015	OB 3929	Tracheal aspirate	2.000	R	R	R	R	R	R	R	S	R	S
	OB 3930	Bronchial aspirate	2.282	R	R	R	R	S	S	I	R	S	
24.9.2015	OB 4027	Sputum	2.242	R	R	R	R	R	R	R	S	R	S
2.10.2015	OB 4138	Bronchial aspirate	2.021	R	R	R	R	R	R	R	S	S	S
20.10.2015	OB 4358	Bronchial aspirate	2.194	R	R	R	R	R	S	R	S	R	S
22.10.2015	OB 4402	Swab of decubitus	2.019	R	R	R	R	R	S	R	S	R	S



**Figure 2:** Concentrations of imipenem and meropenem in hospital wastewater, raw and treated municipal wastewater.

## Acknowledgements:

This research was supported by the Croatian Science Foundation (grant no. IP-2014-09-5656) and in a part by the University of Zagreb (grant no. 202751).



## Conclusion:

- Viable *A. baumannii* and carbapenems are present in hospital wastewater, as well as in raw and treated municipal wastewater of the City of Zagreb.
- MDR *A. baumannii* are able to survive in environment outside hospitals: in hospital wastewater, sewage system, as well as in the secondary wastewater treatment system.
- *A. baumannii* could have a natural habitat in sewage system.

## References:

- Goić-Barišić I. Multidrug-resistant *Acinetobacter baumannii* (MRAB) - ten years after the onset of these isolates in Croatia. *Infectol Glasn.* 2012; 32(2): 67-70.
- CAMS. Antibiotic resistance in Croatia, 2014. The Croatian Academy of Medical Sciences, Zagreb, 2015.
- Ferreira AE, Marchetti DP, De Oliveira LM, Gusatti CS, Fuentes DB, Corcao G. Presence of OXA-23-producing isolates of *Acinetobacter baumannii* in wastewater from hospitals in southern Brazil. *Microb Drug Resist.* 2011; 17(2): 221-227.
- Zhang C, Qiu S, Wang Y, Qi L, Hao R, Liu X, et al. Higher isolation of NDM-1 producing *Acinetobacter baumannii* from the sewage of the hospitals in Beijing. *PLoS ONE.* 2013; 8(6): e64857.
- Hrenović J, Goić-Barišić, Kazazić S, Kovacic A, Ganjto M, Tonkic M. Carbapenem-resistant isolates of *Acinetobacter baumannii* in a municipal wastewater treatment plant, Croatia, 2014. *Eurosurveillance.* 2016; 21(15): pii=30195.
- Sousa C, Botelho J, Silva L, Grosso F, Nemeč A, Lopes J, et al. MALDI-TOF MS and chemometric based identification of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex species. *Int J Med Microbiol.* 2014; 304: 669-677.
- European Committee on Antimicrobial Susceptibility Testing. EUCAST Reading guide. Version 4.0. 2014.