Colistin-resistant *Acinetobacter baumannii* recovered from wastewaters in Croatia

Svjetlana Dekić[1], Jasna Hrenović[1], Paul G. Higgins[2,3], Martina Šeruga Musić[1], Blaženka Hunjak[4], Ivana Goić-Barišić[5]

[1] University of Zagreb, Faculty of Science, Zagreb, Croatia
[2] Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Cologne, Germany
[3] German Centre for Infection Research (DZIF), partner site Bonn-Cologne, Cologne, Germany
[4] Croatian Institute of Public Health, Zagreb, Croatia
[5] University Hospital of Split, Department of Clinical Microbiology and University of Split, School of Medicine, Split, Croatia

INTRODUCTION

Acinetobacter baumannii is an emerging human opportunistic pathogen. It causes nosocomial as well as community-aquired infections in immunosupressed patients (Towner, 2009; Dexter et al, 2015). *A. baumannii* expresses resistance to multiple antibiotics and disinfectants and it persists in the environment for several months (Espinal et al, 2012). Due to the growing antimicrobial resistance rates of clinical isolates of *A. baumannii*, colistin is now considered a last resort antibiotic for the treatment of *A. baumannii* infections. Although colistin-resistant clinical isolates have been sporadically reported in Croatia (Seruga Music et al, 2017), our monitoring of *A. baumannii* outside hospitals suggested the presence of colistin-resistant isolates in Croatian wastewaters. In this study, colistin-resistant *A. baumannii* recovered from hospital and urban wastewater are reported.

MATERIAL AND METHODS

Sample of hospital wastewater (HW), activated sludge and treated effluent at the wastewater treatment plant (WWTP) were colected in 2015 and 2016 (Fig.1). *A. baumannii* was isolated on CHROMagar Acinetobacter supplemented with CR102 and 15 mg/L of cefsulodin after incubation at 42 °C/48h. *A. baumannii* was identified by routine bacteriological techniques and MALDI-TOF MS. MLST alleles (Oxford/Pasteur) were determined. Susceptibility to clinically relevant antibiotics was determined by MICs values obtained by Vitek2 system. Colistin resistance was confirmed by gradient dilution E-test and broth microdilution method.

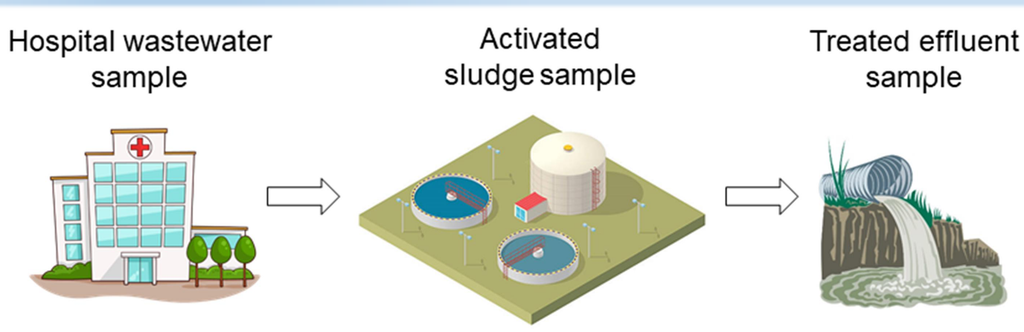

Figure 1. Sampling schematic. Hospital wastewater without pretreatment goes to the wastewater treatment plant where it is treated with the technology of activated sludge and then discharged into the natural recipient.

RESULTS

Three isolates of *A. baumannii* were recovered from untreated HW, one from activated sludge and two from WWTP effluent (Table 1). All isolates were ST-2pas, five were ST-195ox, while one isolate from HW was ST-451ox. These STs correlate with international clonal lineage 2 (IC2). Four isolates were extensively drug-resistant, while two isolates from WWTP effluent were pandrug-resistant (Table 2). Identification of colistin susceptibility by Vitek2 system gave the false negative result for two of six isolates tested. By using the E-test and broth microdilution all isolates were confirmed resistant to colistin.

Table 1. Origin, date of sampling, and MLST results for 6 isolates of *A. baumannii* recovered from untreated hospital wastewater and urban wastewater treatment plant (WWTP).

Isolate	Origin	Date of sampling	ST-Oxford	ST-Pasteur	Clonal lineage
HW2/2	hospital wastewater	27.8.2015	ST-195	ST-2	IC2
HW2/4	hospital wastewater	27.8.2015	ST-195	ST-2	IC2
HW2/10	hospital wastewater	6.10.2015	ST-451	ST-2	IC2
EF7	WWTP effluent	9.9.2015	ST-195	ST-2	IC2
EF31	WWTP effluent	9.3.2016	ST-195	ST-2	IC2
AS14	WWTP activated sludge	24.2.2016	ST-195	ST-2	IC2

CONCLUSION

Colistin-resistant *A. baumannii* were present in untreated HW and WWTP.
The findings suggest the need to disinfect HW and WWTP effluent prior to its discharge into the environment in order to mitigate the propagation of colistin-resistant *A. baumannii*.

Table 2. MIC values of tested antibiotics against isolates of *A. baumannii*.
Carbapenems (MEM-meropenem, IMI-imipenem), fluoroquinolones (CIP-ciprofloxacin, LVX-levofloxacin), aminoglycosides (TOB-tobramycin, GEN-gentamicin, AMK-amikacin), tetracyclines (MIN-minocycline, TGC-tigecycline), penicillins/β-lactamase inhibitors (SAM-ampicillin/*sulbactam,* TIM-ticarcillin/clavulanic acid), SXT- trimethoprim/sulfamethoxazole, CST-colistin (Vitek/E-test/broth dilution). ^{R} - resistant, ^{I} - intermediate according to EUCAST and CLSI criteria.

Isolate	MIC values of antibiotics (mg/L)													Antibiotic susceptibility profile
	MEM	IPM	CIP	LVX	TOB	GEN	AMK	MIN	TGC	SAM	TIM	SXT	CST	
HW2/2	>16^{R}	8^{I}	>4^{R}	>8^{R}	>16^{R}	8^{R}	>64^{R}	2	1	<2	128^{R}	>320^{R}	>16^{R}/64^{R}/80^{R}	XDR
HW2/4	8^{I}	>16^{R}	>4^{R}	>8^{R}	8^{R}	>16^{R}	>64^{R}	4	1	4	64^{I}	>320^{R}	>16^{R}/16^{R}/20^{R}	XDR
HW2/10	8^{I}	>16^{R}	>4^{R}	4^{R}	4	8^{R}	>64^{R}	2	1	4	64^{I}	>320^{R}	>16^{R}/96^{R}/80^{R}	XDR
EF7	>16^{R}	>16^{R}	>4^{R}	>8^{R}	>16^{R}	>16^{R}	>64^{R}	8^{I}	2^{I}	>32^{R}	>128^{R}	>320^{R}	>16^{R}/16^{R}/20^{R}	PDR
EF31	>16^{R}	>16^{R}	>4^{R}	>8^{R}	>16^{R}	>16^{R}	>64^{R}	8^{I}	1	16^{I}	128^{R}	>320^{R}	≤0.5/16^{R}/160^{R}	PDR
AS14	>16^{R}	>16^{R}	>4^{R}	>8^{R}	>16^{R}	>16^{R}	>64^{R}	4	1	16^{I}	128^{R}	>320^{R}	2/3^{R}/160^{R}	XDR

REFERENCES

1. Dexter, C., Murray, G.L., Paulsen, I.T., Peleg, A.Y. (2015) Community-acquired *Acinetobacter baumannii*: clinical characteristics, epidemiology and pathogenesis. *Expert. Rev. Anti. Infect. Ther.,* 13(5):567-73.
2. Espinal, P., Martí, S., Vila, J. (2012) Effect of biofilm formation on the survival of *Acinetobacter baumannii* on dry surfaces. *J. Hosp. Infect.*, 80(1):56–60.
3. Seruga Music, M., Hrenovic, J., Goic-Barisic, I., Hunjak, B., Skoric, D. Ivankovic, T. (2017) Emission of extensively-drug resistant *Acinetobacter baumannii* from hospital settings to the natural environment. *J. Hosp. Infect.,* 96(4):323-327.
4. Towner, K. J. (2009) Acinetobacter: an old friend, but a new enemy. *J. Hosp. Infect.*, 73(4):355–363.

ACKNOWLEDGEMENT

This work has been supported by the Croatian Science Foundation under the project title „Natural habitat of clinically important *Acinetobacter baumannii*“ (project no. IP-2014-09-5656).

Croatian Science Foundation