PRESENCE OF BACTERIUM *ACINETOBACTER BAUMANNII* OUTSIDE THE HOSPITAL ENVIRONMENT IN CROATIA

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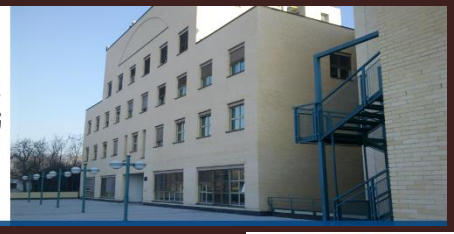
Structure of the Faculty of Science:



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Structure of Department of Biology:

1) Division of Botany
2) Division of Zoology
3) Division of Animal Physiology
4) Division of Molecular Biology
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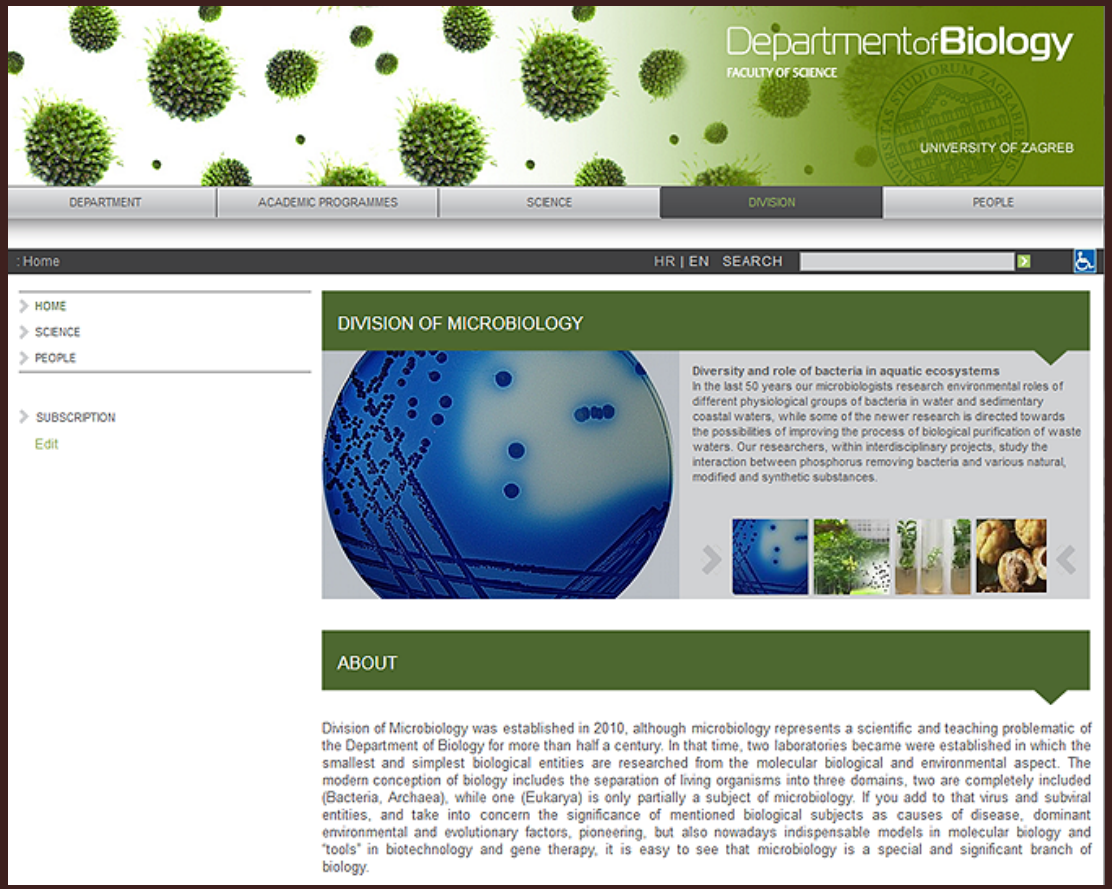


Project title:

Natural habitat of clinically important Acinetobacter baumannii

Leader:

Jasna Hrenović

Proposal type:

Research projects

Call:	Code:	Acronym:	Duration:	Status:	Total value:
2014-09	5656	NATURACI	01.09.2015. - 31.08.2019.		999.210,00 Kn

Scientific areas:

Interdisciplinary, Biomedicine and health sciences, Biomedicine and health sciences, Biotechnical sciences, Technological sciences

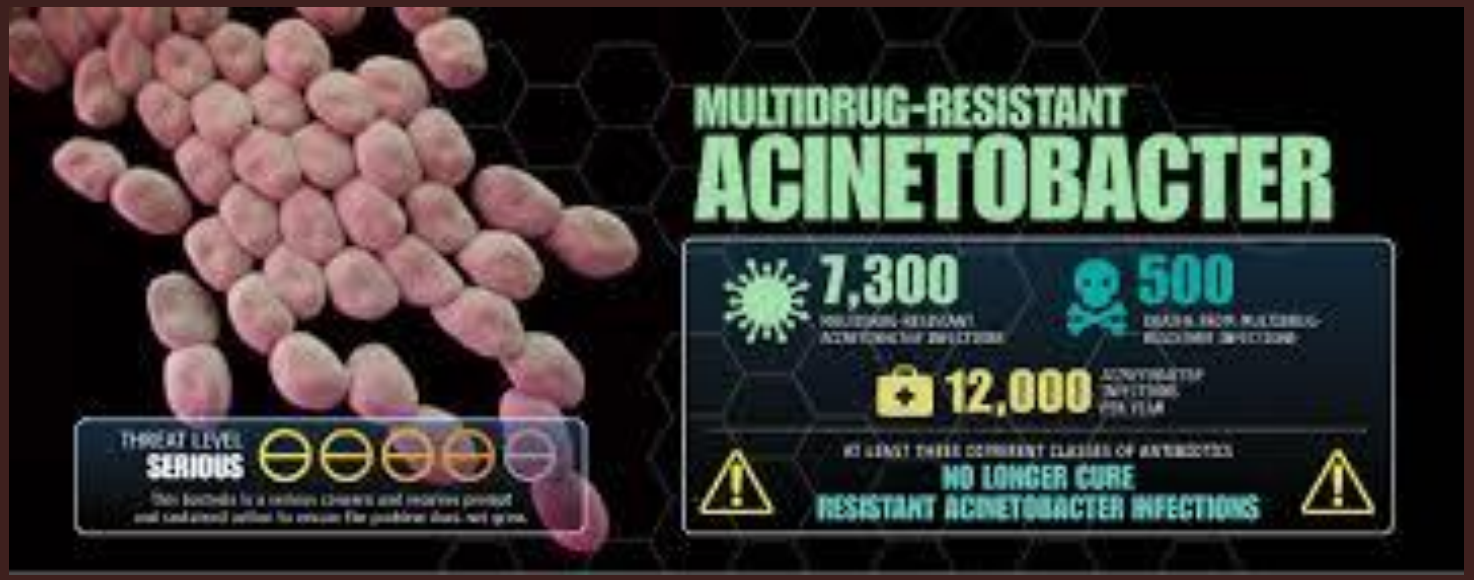
Scientific fields:

Public health and health services

- epidemiology of emerging hospital pathogen *A. baumannii*
- methods to reduce their numbers in contaminated environment

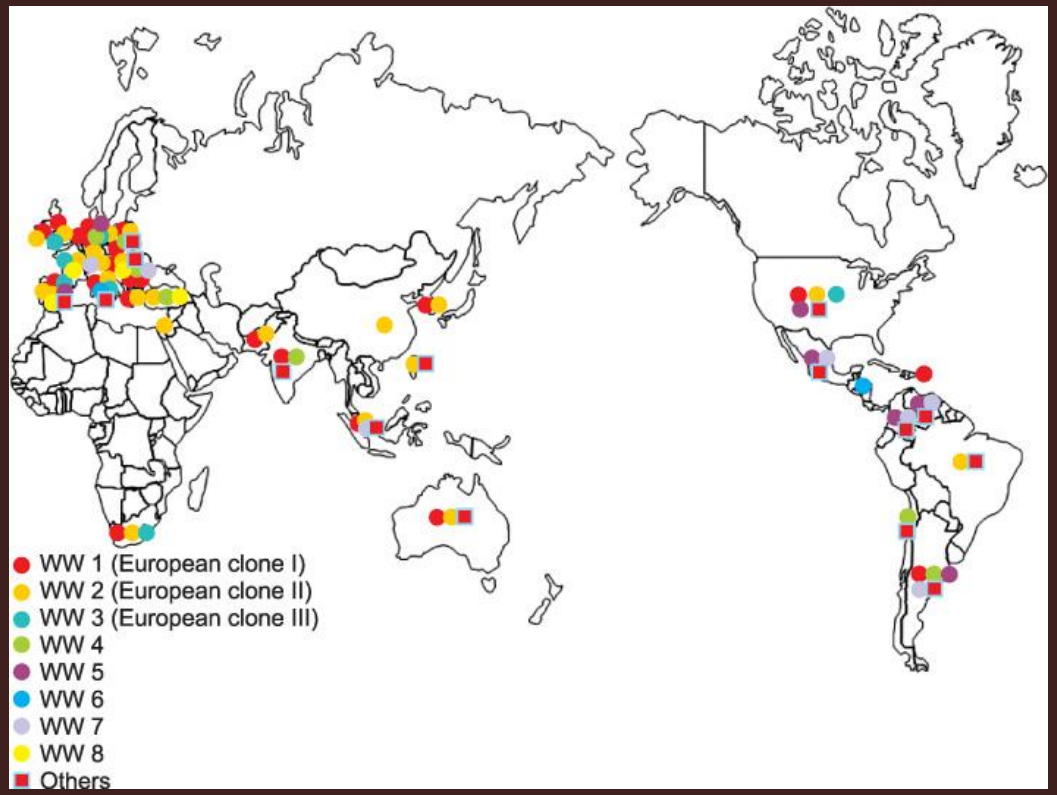
Acinetobacter baumannii is an emerging pathogen causing outbreaks in hospitals. Over the last decade, hospital-acquired infections due to *A. baumannii* have increased dramatically worldwide including in Croatia.

Acute community-acquired human infections have been reported mainly from tropical and subtropical areas. Even if such infections represent a minor proportion of all *A. baumannii* infections worldwide, they suggest a source of this pathogen outside of the hospital.



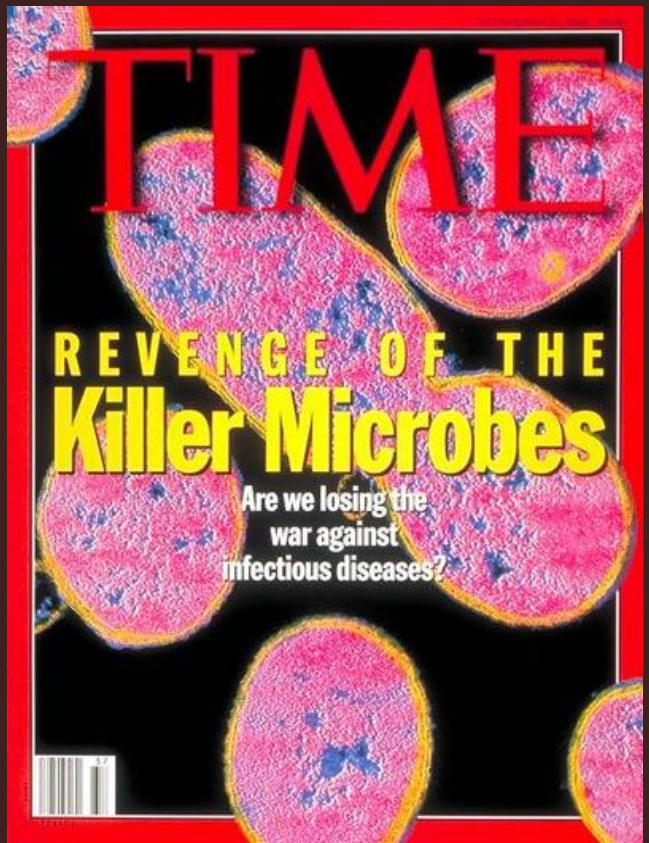
Globally distributed isolates of *A. baumannii* are designated as international clones (IC) 1-3, while some isolates still remain nonclonally related.

In Croatian hospitals isolates of *A. baumannii*:
- belonging to IC1 evidenced from 2002,
- belonging to IC2 evidenced from 2009,
- nonclonal isolates also persist.



A. baumannii express the:
- resistance to multiple antibiotics (MDR) as well as disinfectants,
- survives in adverse conditions,
- leading to long-term persistence in the hospital environment.

Additionally, virulence factors such as ability to form biofilm on abiotic or biotic surfaces influence the success of *A. baumannii*.

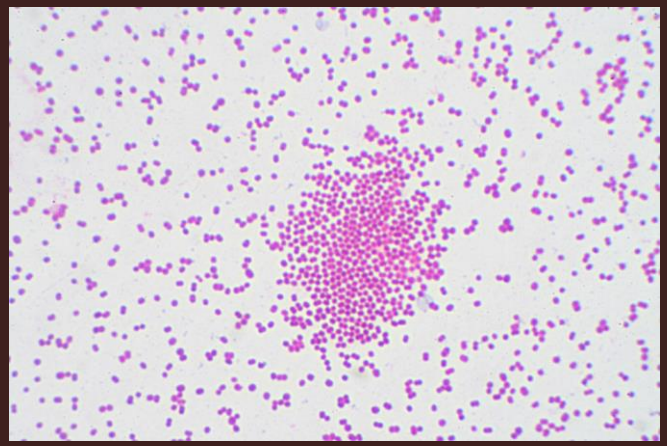


Crucial questions regarding the epidemiology of *A. baumannii* remains incompletely understood:

- 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.

Current work:

- screen the hospital wastewater
- municipal wastewater
- soil for the presence of viable *A. baumannii*.



Sampling of hospital wastewater was performed at the central manhole of one Zagreb's hospital from which the clinical isolates of *A. baumannii* were recovered.

Hospital wastewaters are released into the sewage system without pre-treatment, and reach the central wastewater treatment plant.

Sampling of municipal wastewater was done at the influent and effluent of the largest Croatian wastewater treatment plant of the City of Zagreb (capacity 1,200.000 population equivalents).
Municipal wastewater consisted of domestic, industrial, hospital and storm wastewaters pasages the secondary type of treatment.

Wastewater treatment plant of the City of Zagreb

The isolation of *A. baumannii* was performed at 42°C/48h on CHROMagar Acinetobacter either without or with the addition of commercial supplement CR102 (CHROMagar) 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.

Pure culture of *A. baumannii* grown on CHROMagar Acinetobacter

Date of sampling, origin, MALDI-TOF MS score values, and antibiotic[a] profile of *A. baumannii* clinical isolates. All isolates were determined by Vitek 2 system as *A. calcoaceticus-baumannii* complex.

R - resistant; I - intermediate; S - sensitive according to EUCAST criteria.

[a] 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	S	R	S
18.9.2015	OB 3929	Tracheal aspirate	2.000	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	S	R	S
2.10.2015	OB 4138	Bronchial aspirate	2.021	R	R	R	R	R	R	S	S	S
20.10.2015	OB 4358	Bronchial aspirate	2.194	R	R	R	R	S	R	S	R	S
22.10.2015	OB 4402	Swab of decubitus	2.019	R	R	R	R	S	R	S	R	S

Resistant to carbapenems and majority of tested antibiotics except colistin, MDR.

Resistance to carbapenems in Croatia is drastically increasing from 10% in 2008 to 82% in 2014.

A. baumannii isolates from hospital wastewater.

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	S
	Š2/3	2.101	R	R	R	R	R	R	R	R	S
6.10.2015	Š1/1	2.271	R	R	R	R	R	R	R	R	S
	Š2/5	2.067	R	R	R	R	R	R	S	S	S
	Š2/6	2.232	R	R	R	R	R	R	S	S	S
	Š2/7	2.102	R	R	R	R	R	R	S	S	S
	Š2/8	2.077	R	R	R	R	R	R	S	S	S
	Š2/9	2.041	R	R	R	R	R	R	S	R	S

Resistant to carbapenems and majority of tested antibiotics except colistin, MDR.

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	S	S	S
	EF2	2.352	R	R	R	R	R	R	S	S	S
	EF3	2.329	R	R	R	R	R	R	S	S	S
11.6.2014	IN4	2.231	R	R	R	R	R	R	S	S	S
	IN5	2.085	R	R	R	R	R	R	S	S	S
	IN6	2.157	R	R	R	R	S	R	S	S	S
	IN8	2.168	R	R	R	R	S	R	S	S	S
	IN9	2.167	R	R	R	R	R	S	S	S	S
	IN10	2.193	R	R	R	R	R	R	S	S	S
	IN11	2.409	R	R	R	R	R	R	S	S	S
29.10.2014	EF4	2.191	R	R	R	R	R	R	R	S	S
	EF5	2.161	R	R	R	R	R	R	R	S	S
	EF6	2.219	R	R	R	R	R	R	R	S	S
	IN12	2.190	R	R	R	R	R	R	S	S	S
	IN13	2.118	R	R	R	R	R	R	S	S	S
	IN14	2.213	R	R	R	R	R	R	S	S	S
	IN15	2.121	R	R	R	R	S	R	S	S	S
	IN16	2.244	R	R	R	R	R	R	S	S	S
	IN17	2.163	R	R	R	R	R	R	S	S	S
	IN18	2.048	R	R	R	R	R	R	S	S	S
	IN19	2.090	R	R	R	R	R	R	R	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	S	S
	IN24	2.168	R	R	R	R	R	R	R	S	S
	IN25	2.041	R	R	R	R	R	R	R	S	S
	IN26	2.223	R	I	S	S	S	S	S	S	S
	IN27	2.199	I	S	S	S	S	S	S	S	S
	IN28	2.085	R	I	S	S	S	S	S	S	S
23.9.2015	IN31	2.119	S	S	S	S	S	S	S	S	S
	IN32	2.104	R	R	R	R	R	R	R	R	S
	IN33	2.180	R	R	R	R	R	R	R	R	S
	IN34	2.066	R	R	R	R	R	R	R	S	S
	IN35	2.164	R	R	R	R	R	S	S	R	S
	IN36	2.184	S	S	S	S	S	S	S	S	S
	IN37	2.038	R	R	R	R	R	R	R	S	S
	IN38	2.075	R	R	R	R	R	R	R	S	S
	EF9	2.174	R	R	R	R	R	S	S	R	S

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.

90% of isolates resistant to carbapenems and majority of tested antibiotics except colistin, MDR

10% of isolates sensitive to all antibiotics

RESEARCH ARTICLE

Carbapenem-resistant isolates of *Acinetobacter baumannii* in a municipal wastewater treatment plant, Croatia, 2014

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Hrenovic J, Goic-Barisic I, Kazazic S, Kovacic A, Ganjto M, Tonkic M. Carbapenem-resistant isolates of Acinetobacter baumannii in a municipal wastewater treatment plant, Croatia, 2014. Euro Surveill. 2016;21(15):pii=30195. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2016.21.15.30195

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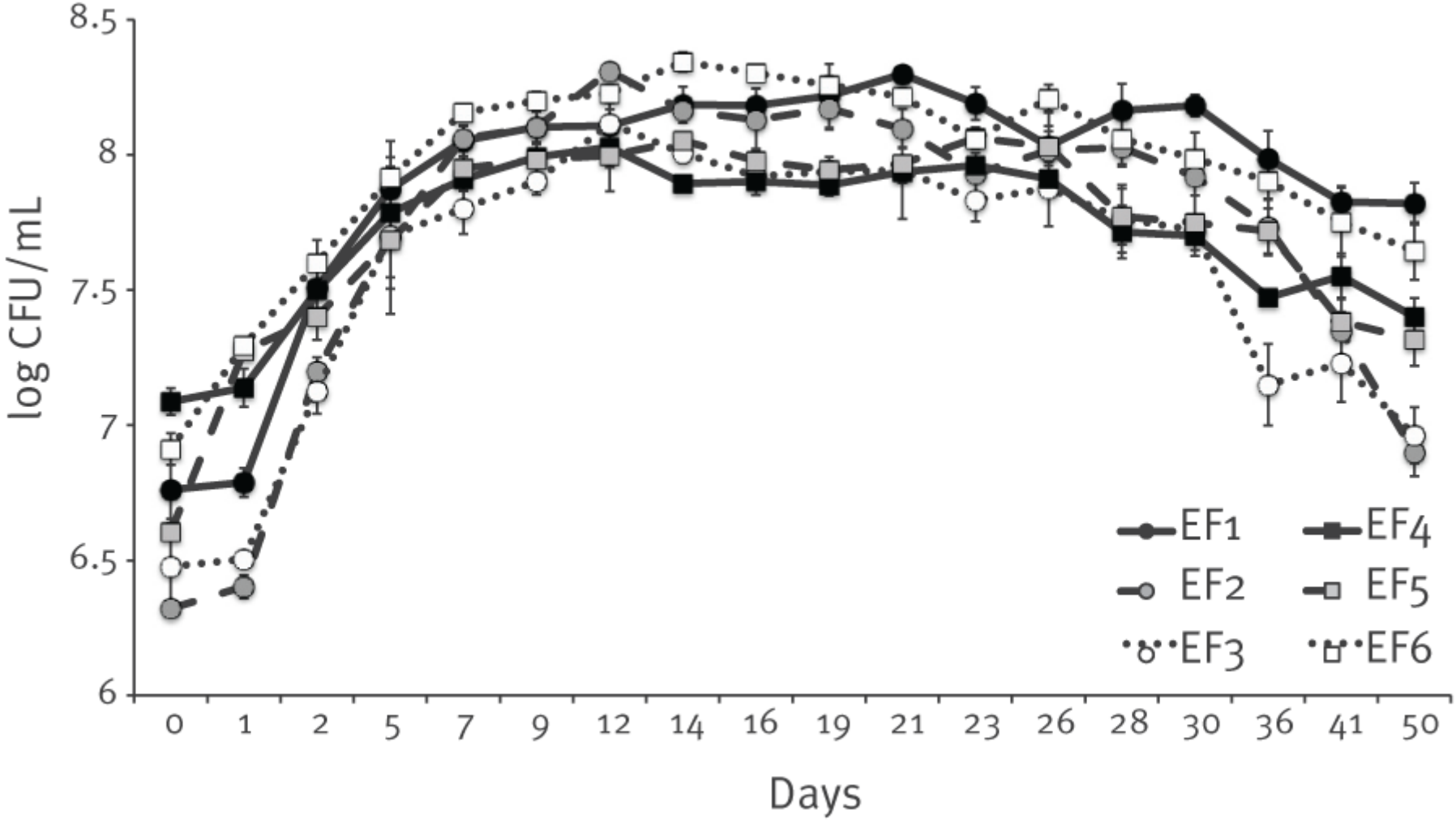
Acinetobacter baumannii is an emerging hospital pathogen. Whereas *A. baumannii* isolated from patients or hospitals has been reported, there are few data regarding propagation of viable *A. baumannii* in the natural environment. This study investigates the occurrence and antimicrobial susceptibility of viable *A. baumannii* in municipal wastewater and its per-

with some individual hospitals recording a rate of 90% [2,9].

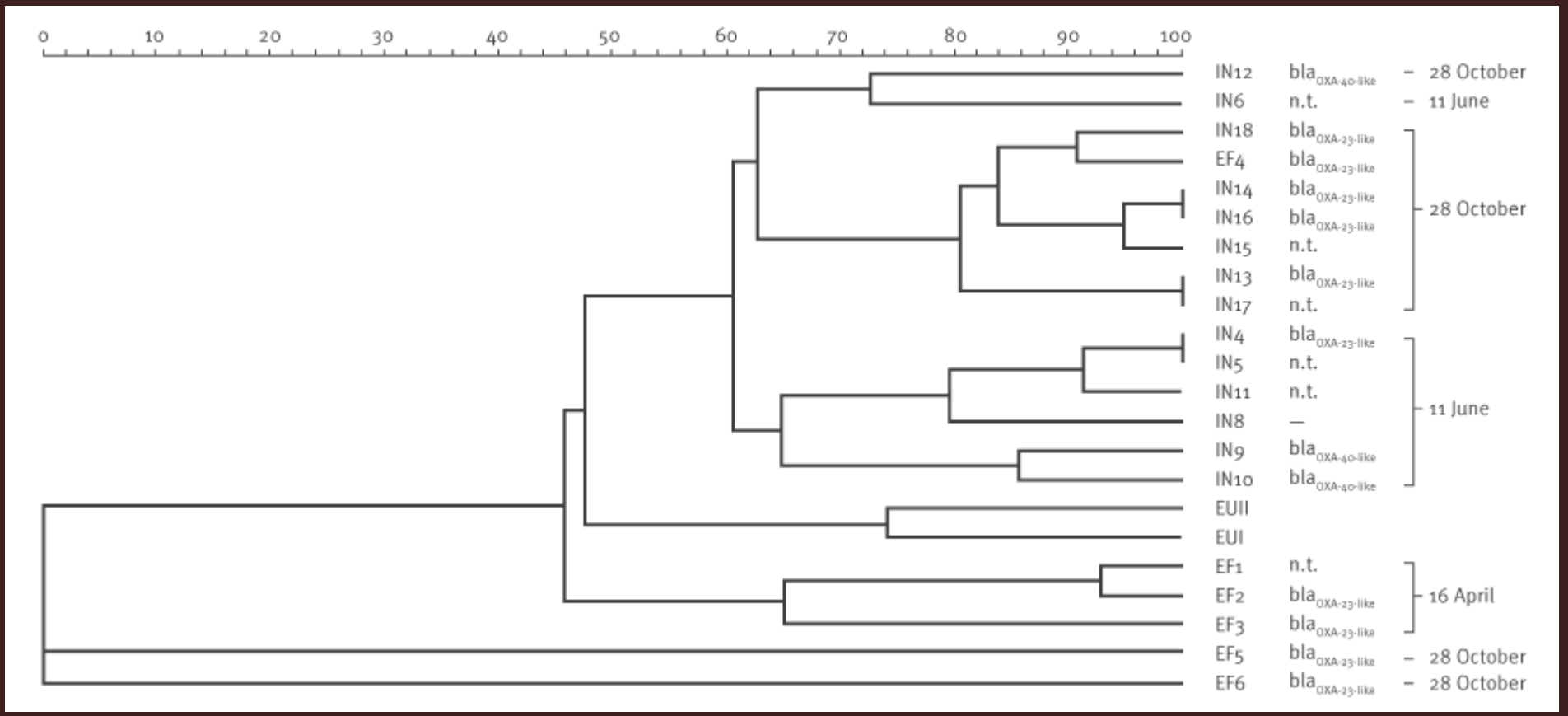
The most important mechanism of carbapenem resistance in *A. baumannii* involves OXA-type carbapenemases, which are encoded by bla_{OXA} lineage genes. Five main phylogenetic subgroups including OXA-23-like,

Six *A. baumannii* isolates recovered from effluent wastewater multiplied and survived in sterilised effluent wastewater up to 50 days.



The most important mechanism of carbapenem resistance in *A. baumannii* involves OXA-type carbapenemases, which are encoded by bla_{OXA} lineage genes.

Among 14 isolates tested, all harboured the constitutive $bla_{OXA\text{-}51\text{-}like}$ gene, while the acquired $bla_{OXA\text{-}23\text{-}like}$ and $bla_{OXA\text{-}40\text{-}like}$ genes were found in 10 and 3 isolates, respectively.



Microbial Drug Resistance

Emergence of Oxacillinases in Environmental Carbapenem-Resistant *Acinetobacter baumannii* Associated with Clinical Isolates

To cite this article:
Goic-Barisic Ivana, Hrenovic Jasna, Kovacic Ana, and Šeruga Musić Martina. Microbial Drug Resistance. February 2016, ahead of print. doi:10.1089/mdr.2015.0275.

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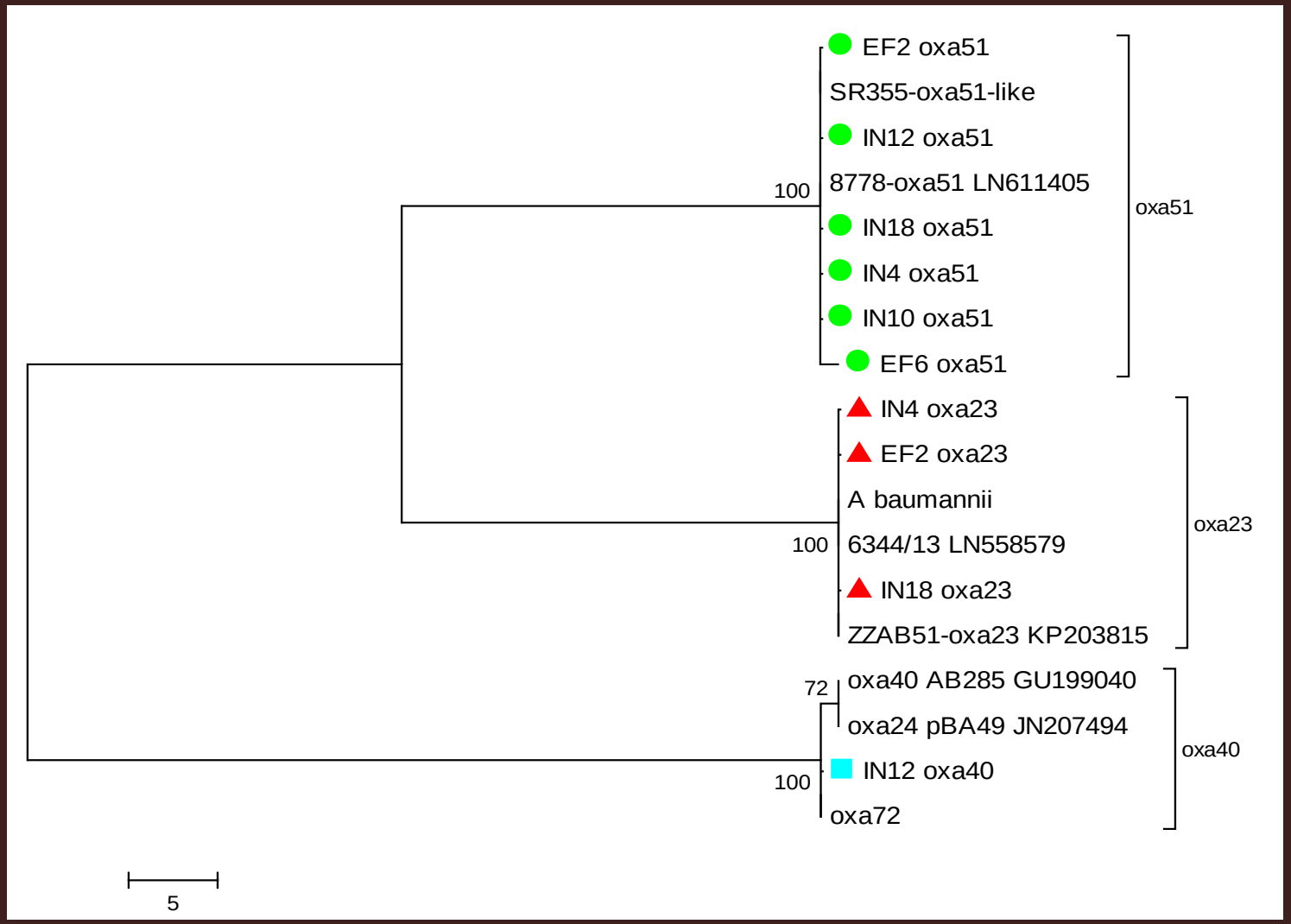
ABSTRACT

Six carbapenem-resistant isolates of *Acinetobacter baumannii* were recovered from untreated and treated municipal wastewater of the capital city of Zagreb, Croatia. Molecular identification of environmental isolates of *A. baumannii* was performed by amplification, sequencing, and phylogenetic analyses of *rpo*B gene. The presence of bla_{OXA} genes encoding OXA-type carbapenemases (OXA-51-like, OXA-23, and OXA-40-like) was confirmed by multiplex PCR and sequencing. Phylogenetic analyses corroborated the affiliation of detected bla_{OXA} genes to three different clusters and showed association of environmental OXAs with those described from clinical isolates. This result suggests that isolates recovered from municipal wastewater are most probably of clinical origin

The OXAs in carbapenem resistant isolates of *A. baumannii* recovered from untreated and treated municipal wastewater are closely related to clinical isolates.



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Occurrence of an Environmental *Acinetobacter baumannii* Strain Similar to a Clinical Isolate in Paleosol from Croatia

Jasna Hrenovic,[a] Goran Durn,[b] Ivana Goic-Barisic,[c] Ana Kovacic[d]

University of Zagreb, Faculty of Science, Division of Biology, Zagreb, Croatia[a]; University of Zagreb, Faculty of Mining, Geology and Petroleum Engineering, Zagreb, Croatia[b]; Department of Clinical Microbiology, University Hospital Centre Split and University of Split School of Medicine, Split, Croatia[c]; Institute of Public Health of Split and Dalmatia County, Split, Croatia[d]

Over the past decade, bacteria of the genus *Acinetobacter* have emerged as a leading cause of hospital-acquired infections. Outbreaks of *Acinetobacter* infections are considered to be caused exclusively by contamination and transmission in hospital environments. The natural habitats of clinically important multiresistant *Acinetobacter* spp. remain to be defined. In this paper, we report an incidental finding of a viable multidrug-resistant strain of *Acinetobacter baumannii*, related to clinical isolates, in acid paleosol from Croatia. The environmental isolate of *A. baumannii* showed 87% similarity to a clinical isolate originating from a hospital in this geographic area and was resistant to gentamicin, trimethoprim-sulfamethoxazole, ciprofloxacin, and levofloxacin. In paleosol, the isolate was able to survive a low pH (3.37), desiccation, and a high temperature (50°C). The probable source of *A. baumannii* in paleosol is illegally disposed waste of external origin situated in the abandoned quarry near the sampling site. The bacteria could have been leached from waste by storm water and thus infiltrated the paleosol.

Bacteria of the genus *Acinetobacter* have been recognized as significant hospital pathogens since the late 1970s, but at that time they were easily treated, because they were susceptible to commonly used antimicrobials. *Acinetobacter* spp. have an increasing ability to develop resistance to commonly used antimicrobial agents, leading to limited options for antibiotic treatment (1). Three major overlapping populations of bacteria of the genus *Acinetobacter* are known: multiresistant isolates from hospitals and hospitalized patients (*Acinetobacter baumannii*, *Acinetobacter*

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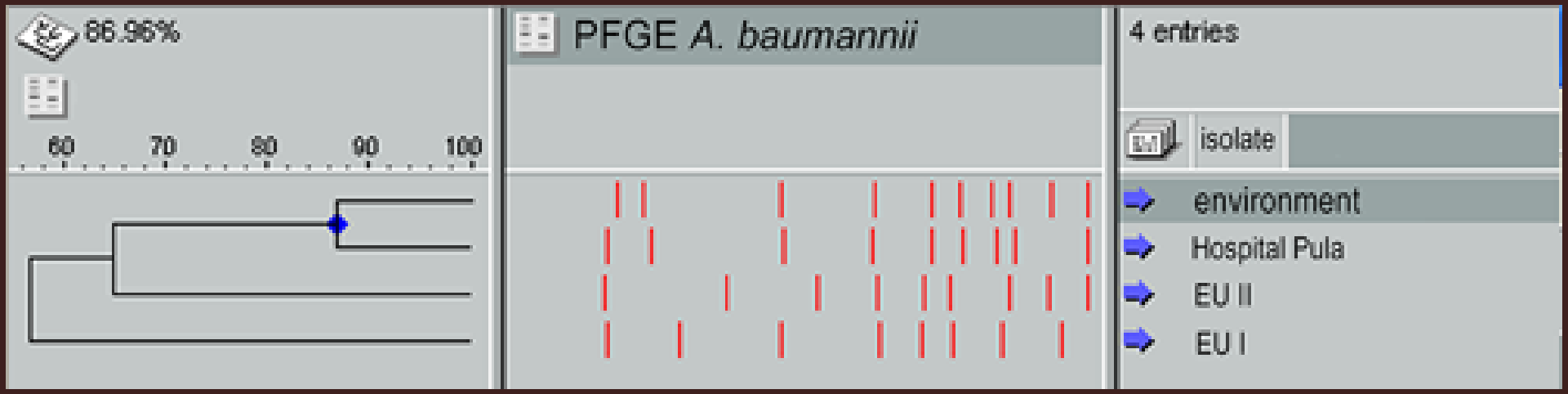
MDR *A. baumannii* was isolated from acid (pH 2.55) paleosol in Croatia.

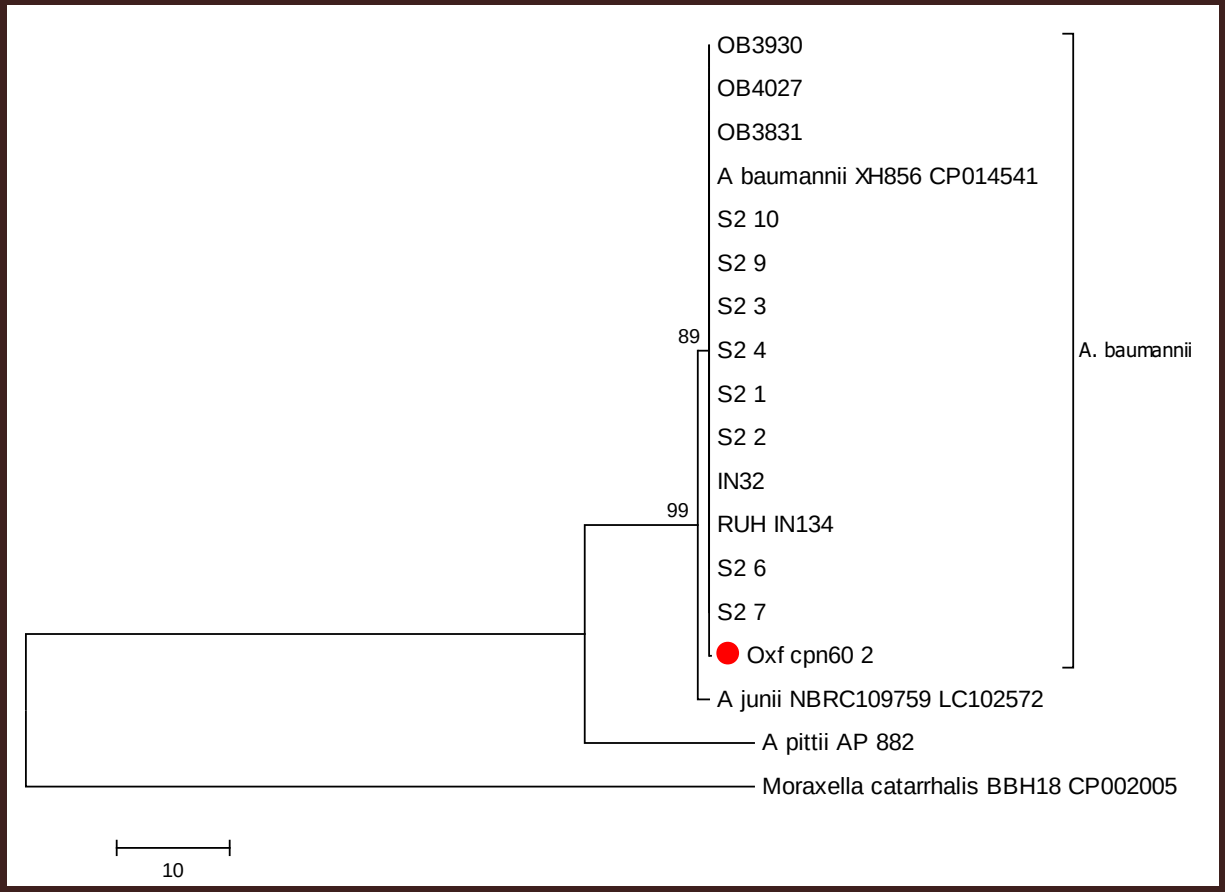
MDR *A. baumannii* was related to clinical isolate.
In paleosol, the isolate was able to survive a low pH, desiccation, and a high temperature (50°C).
The probable source of *A. baumannii* in paleosol is illegally disposed waste of external origin situated in the abandoned quarry near the sampling site.



Dendrogram based on ApaI-digested DNA from different isolates of *A. baumannii*

Investigation in progress:

The environmental isolates of *A. baumannii* should be compared with clinical isolates causing outbreaks and community-acquired isolates.



Multilocus sequence typing (MLST) of housekeeping gene *cpn60*

Infectious Diseases, 2015; 47: 902–907

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ORIGINAL ARTICLE

Acinetobacter baumannii in Southern Croatia: clonal lineages, biofilm formation, and resistance patterns

VANJA KALITERNA[1,4], MARIANO KALITERNA[2], JASNA HRENOVIĆ[3], ZVONIMIR BARIŠIĆ[1], MARIJA TONKIĆ[2,4] & IVANA GOIC-BARISIC[2,4]

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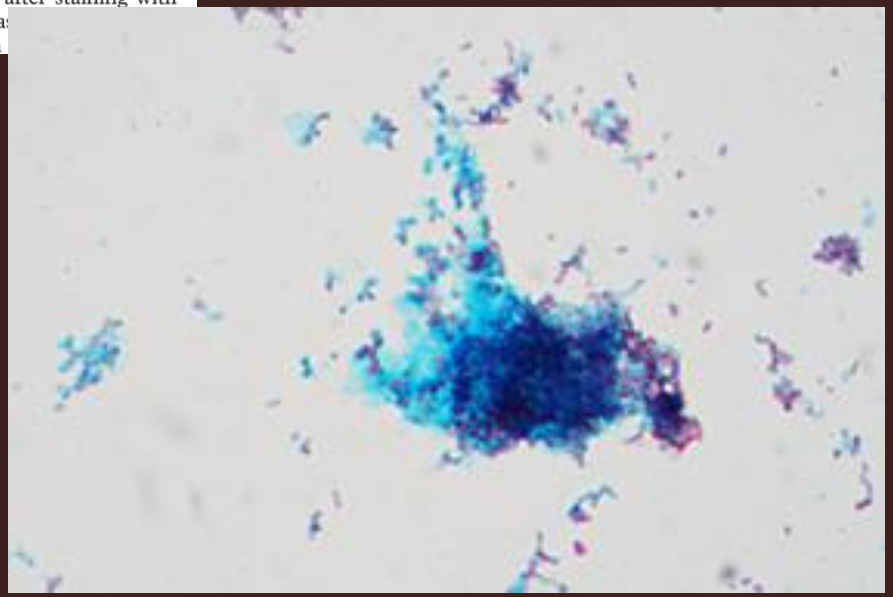
Abstract

Background: Acinetobacter baumannii is one of the most prevalent causes of severe hospital-acquired infections and is responsible for the dramatic increase in carbapenem resistance in Croatia in the last 5 years. Such data have encouraged multicenter research focused on the organism's ability to form biofilm, susceptibility to antibiotics, and particular genotype lineage. *Methods:* Biofilm formation in 109 unrelated clinical isolates of *A. baumannii* recovered in six cities of Southern Croatia was investigated. Genotyping was performed by pulsed-field gel electrophoresis and antibiotic profile was tested by applying the disc diffusion method and confirmed by determining the minimum inhibitory concentrations. The ability to form biofilm in vitro was determined from overnight cultures of the collected isolates on microtiter plates, after staining with crystal violet, and quantified at 570 nm after solubilization with ethanol. The statistical relevance wa
appropriate program with level of statistical confidence. *Results:* There was no significant difference in

Isolates of *A. baumannii* were able to produce biofilm



Biofouling, 2014
Vol. 30, No. 8, 965–973, http://dx.doi.org/10.1080/08927014.2014.959941

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Synergistic anti-biofouling effect of Ag-exchanged zeolite and D-Tyrosine on PVC composite against the clinical isolate of *Acinetobacter baumannii*

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(*Received 22 July 2014; accepted 26 August 2014*)

Due to their susceptibility to bacterial biofilm formation, commercial tubes for medical use are one of the main sources of hospital infections with *Acinetobacter baumannii*. The anti-biofouling activity of novel composites against the clinical isolate of the multi-drug resistant *A. baumannii* is reported here. The composites were prepared by addition of micronised silver-exchanged natural zeolite (Ag-NZ) into poly(vinyl chloride) (PVC), followed by coating of the composites with D-Tyrosine (D-Tyr). The Ag-NZ composites (containing 1–15 wt% of Ag-NZ) coated with D-Tyr (Ag-NZ-Tyr) showed a bactericidal effect (100% or a 6.9 log CFU reduction) towards immobilised bacterial cells. The uncoated Ag-NZ composites showed a reduction of up to 70% (4.4 log CFU) of immobilised bacteria in comparison with the original PVC. Rheological testing of the composites revealed that the addition of Ag-NZ slightly affected processability and formability of the PVC and increased the elasticity of the polymer matrix.

Keywords: antibacterial activity; bacteria; biofilm; endotracheal tube; public health

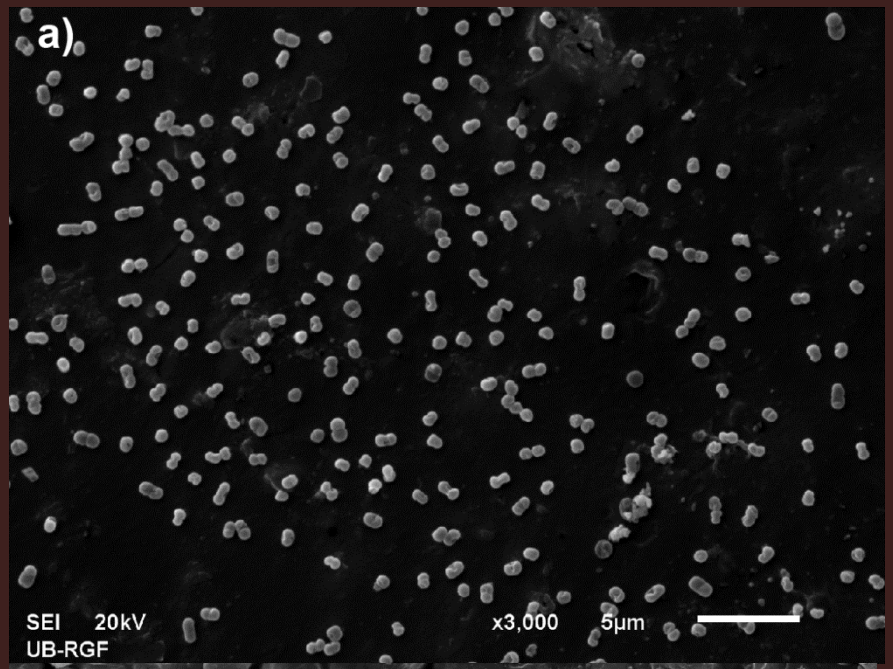
Introduction

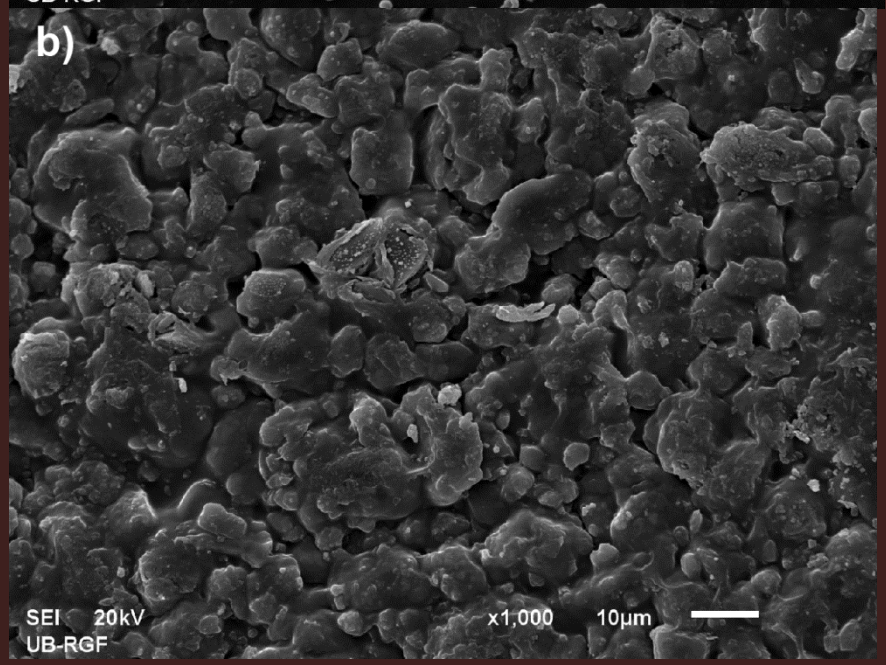
The use of biomedical devices is frequently connected with a risk of nosocomial infection. A high rate of infection of biomaterials is particularly encountered when such materials are brought into contact with human bodies. Thus, endotracheal tubes and catheters usually

ethylene, polyamide, poly(propylene), poly(vinyl chloride) (PVC) or polyurethane (Yeo et al. 2003; Balazs et al. 2004; Jeong et al. 2005; Kumar & Munstedt 2005). The second approach is based on coating the surface with different antibacterial agents using different techniques such as vapour or electrochemical deposition, sputtering or ion beam deposition. Silver (Ag) is most

The anti-biofouling activity of novel composites against the clinical isolate of MDR *A. baumannii*.

The composite material for ventilator tubes was prepared by addition of micronized silver-exchanged natural zeolite (Ag-NZ) into poly(vinyl chloride) (PVC), followed by coating of the composites with D-Tyrosine (D-Tyr).





SEM images after 24 h of contact
(a) immobilised cells of *A. baumannii* without any sign of biofilm formation on the surface of the Ag-NZ15 composite
(b) the absence of bacterial cells on the surface of the Ag-NZ15-Tyr composite.
The side of one surface of the composites was somewhat coarse due to the addition of NZ (imaged for Ag- NZ15-Tyr), while the side of the other surface was shiny (imaged for Ag-NZ15).

Conclusion:

- Municipal wastewaters of Zagreb are continuously polluted with MDR *A. baumannii* probably due to the input of untreated hospital wastewaters.
- More frequent isolation of *A. baumannii* from influent than from effluent suggests its moderate elimination, but also its persistence in the secondary type wastewater treatment system.
- MDR *A. baumannii* can occur and have the ability to survive in the environment.
- Finding of antibiotic-sensitive isolates in raw municipal wastewater opens the possibility that *A. baumannii* could have natural habitat in sewage system.
- Development of novel antibacterial materials for biomedical applications is promising in control of hospital acquired infections due to *A. baumannii*.


Thank you for attention!