

UTJECAJ KRUTOG I TEKUĆEG OTPADA NA ZAGAĐENJE PRIRODNOG OKOLIŠA U HRVATSKOJ

Prof. dr. sc. Jasna Hrenović

Prirodoslovno-matematički fakultet,
Biološki odsjek, Zavod za mikrobiologiju, Zagreb



1. STUDENTSKI KONGRES OKOLIŠNOG ZDRAVLJA,
5.6.2017., Rijeka



Ljudski je otpad opće poznat izvor različitih patogena koji se mogu širiti u prirodnom okolišu, predstavljajući javnozdravstveni rizik.



Rod *Acinetobacter* broji 53 imenovane vrste:

TABLE 1. Updated list of validated named species of *Acinetobacter*

Commonly found human pathogens

A. baumannii (genospecies 2)

A. nosocomialis (genospecies 13TU)

A. pittii (genospecies 3)

A. calcoaceticus (genospecies 1)

emergentni bolnički
patogen 21. stoljeća

Uncommon organisms in clinical infections

A. baylyi

A. guillouiae

A. lwoffii

A. soli

A. beijerinckii

A. gyllenbergii

A. nectaris

A. tandoii

A. bereziniae

A. haemolyticus

A. parvus

A. tjernbergiae

A. boissieri

A. harbinensis

A. puyangensis

A. towneri

A. bouvetii

A. indicus

A. qingfengensis

A. ursingii

A. brisouii

A. johnsonii

A. radioresistens

A. venetianus

A. gernerii

A. junii

A. rudis

A. grimontii^a

A. kookii

A. schindleri

^aSynonym of *A. junii*.

Akutne sporadične infekcije ukazuju na mogući izvor *Acinetobacter baumannii* izvan bolničkog okruženja.

Nakon 2010. javljaju se nalazi ovog patogena izvan bolničkog okruženja:

- voda rijeke Seine (2010)
- sirove bolničke vode u Brazilu (2011)
- sirove i klorirane bolničke vode u Kini (2013)
- sirove i pročišćene urbane vode u Zagrebu (2016).

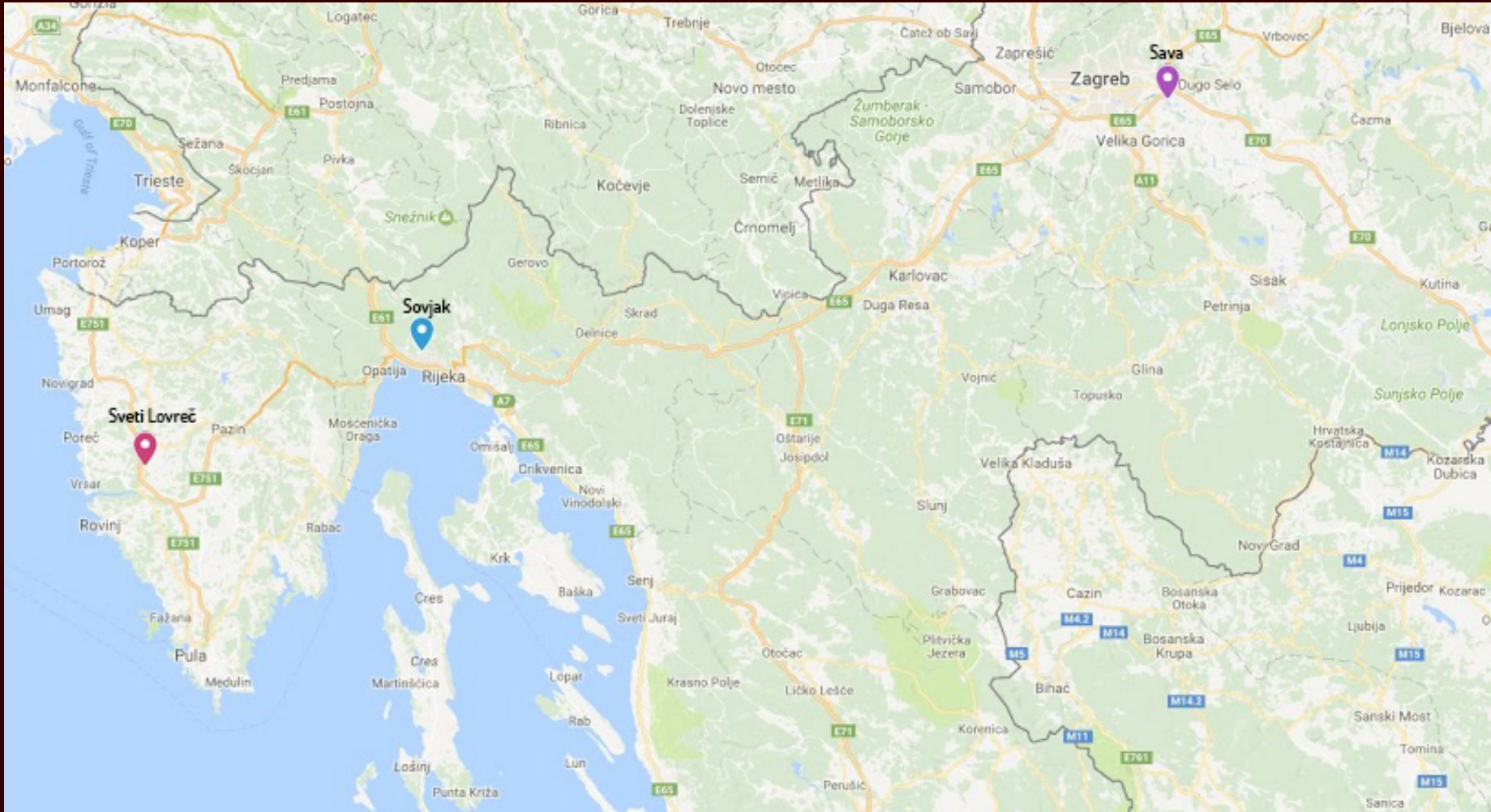
Prisutnost bakterije *A. baumannii* u prirodnom okolišu je od posebnog značaja, budući da nije razjašnjena veza između okolišnih izolata i pojave humanih infekcija.

Za *A. baumannii* nisu razriješeni:

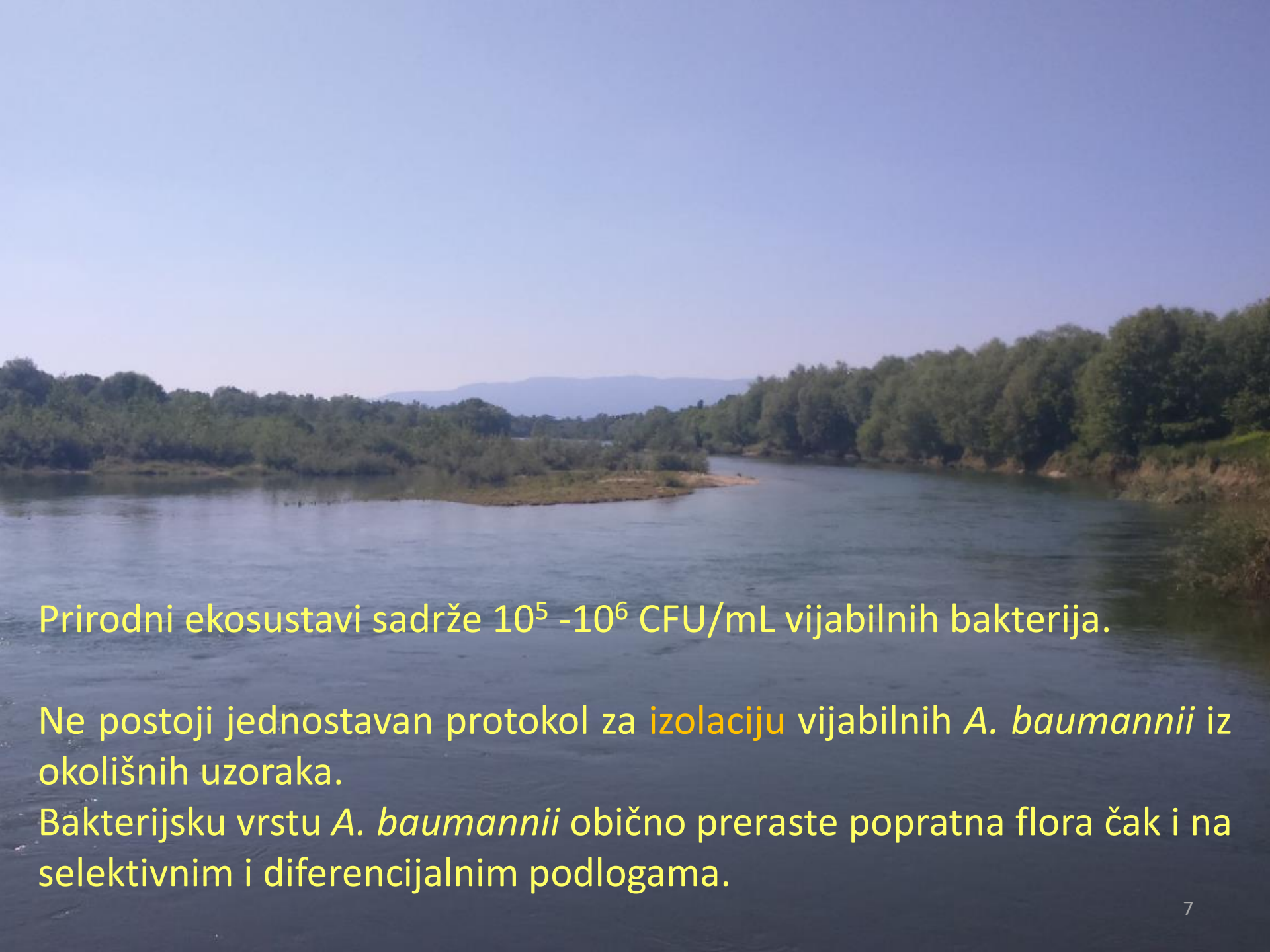
- način ulaska u bolničko okruženje,
- širenje iz bolničkog okruženja u prirodni okoliš,
- prirodno stanište.

Cilj:

U ovom radu dan je pregled prisutnosti multiplo-rezistentnih (MDR) *A. baumannii* u prirodnom okolišu pod utjecajem krutog i tekućeg otpada u Hrvatskoj.



Lokacije na kojima su izolirani *A. baumannii*.

A wide river flows through a lush green landscape under a clear blue sky. The river is surrounded by dense trees and vegetation on both banks. In the distance, there are low mountains or hills. The water is a deep blue-green color, and the sky is a clear, light blue.

Prirodni ekosustavi sadrže 10^5 - 10^6 CFU/mL vijabilnih bakterija.

Ne postoji jednostavan protokol za izolaciju vijabilnih *A. baumannii* iz okolišnih uzoraka.

Bakterijsku vrstu *A. baumannii* obično preraste popratna flora čak i na selektivnim i diferencijalnim podlogama.

● CHROMagar™ Acinetobacter

www.CHROMagar.com

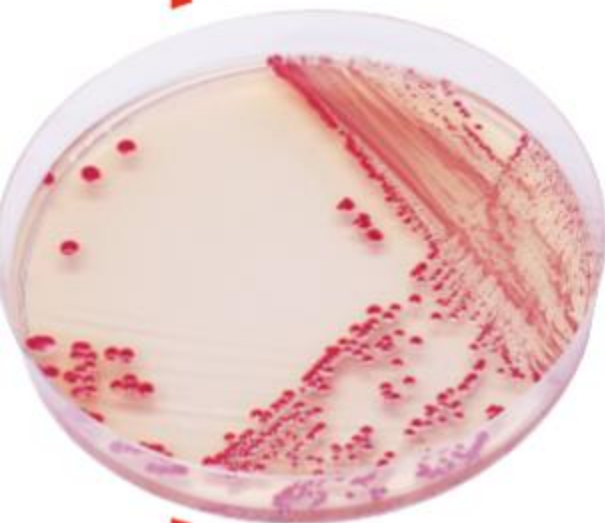


Plate Reading

For detection of *Acinetobacter* sp.:

- *Acinetobacter* sp.
→ red
- Other gram (-)
→ blue or mostly inhibited
- Gram(+) bacteria and yeasts
→ inhibited

For detection of MDR *Acinetobacter* sp.
(if using the optional supplement CR102):

- MDR *Acinetobacter*
→ red

For detection of *Acinetobacter* and MDR *Acinetobacter* sp.

Background

Common bacteria widely spread in the nature, *Acinetobacter* has the capacity to survive in dry as well as moist environments. It becomes a source of infection in hospital environment when colonizing medical equipments, human skin and sometimes foodstuff. *Acinetobacter* species are generally not pathogenic for healthy people but are life threatening in compromised patients. It is often isolated in nosocomial infections cases, intensive care units, and can for instance cause nosocomial pneumonia, bacteraemia, and meningitis.

Especially, *Acinetobacter baumannii* is becoming a major hospital-acquired infection issue because of its often multi-drug resistance (MDR : resistance to C3G, quinolones, carbapenem etc). This contributes to the increase of morbidity and mortality.

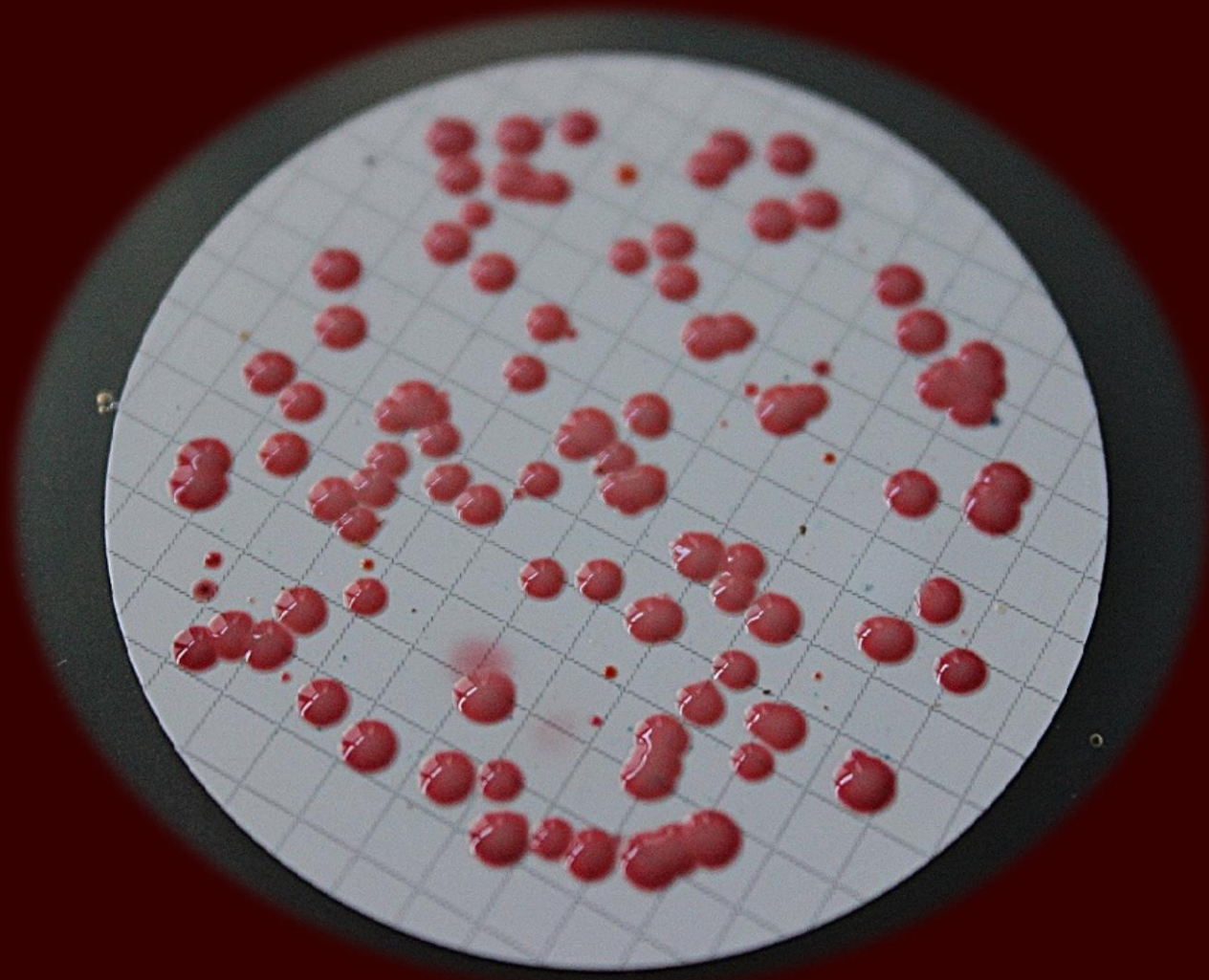
Active surveillance is necessary to control its spread in the facilities, to reduce the risk of cross-contamination, and to identify the carriers. Rapid identification of patients that are colonized with *Acinetobacter* would lead to infection control practices aimed at preventing spread of the organisms.

Medium Performance

1

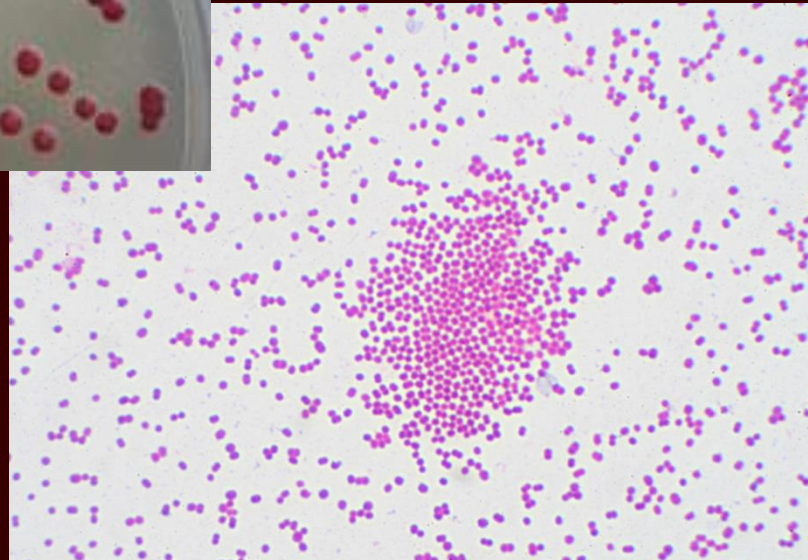
One unique Red colour: Detection of *A. baumannii* from traditional culture media might be a difficult and tedious task due to the abundance of background flora found in collected specimens, especially when using media based on differentiation by the lactose/non-lactose fermentation ability. To overcome these difficulties, CHROMagar Acinetobacter was designed as a highly selective medium, allowing the growth of *Acinetobacter* in conspicuously red colonies, after overnight incubation.

Izolacija *A. baumannii* provedena je na komercijalnom CHROMagar Acinetobacter oplemenjenom s 15 mg/L cefsulodina nakon inkubacije na 42°C/48h.



Identifikacija okolišnih izolata I

Čiste kulture suspektnih *A. baumannii* porasle na 42°C na hranjivom agaru se najprije karakteriziraju rutinskim bakteriološkim tehnikama: Gram negativni kokobacili, oksidaza negativni, katalaza pozitivni, tipične reakcije na Kliglerovom željeznom agaru.



Identifikacija okolišnih izolata II

Potvrda identifikacije:

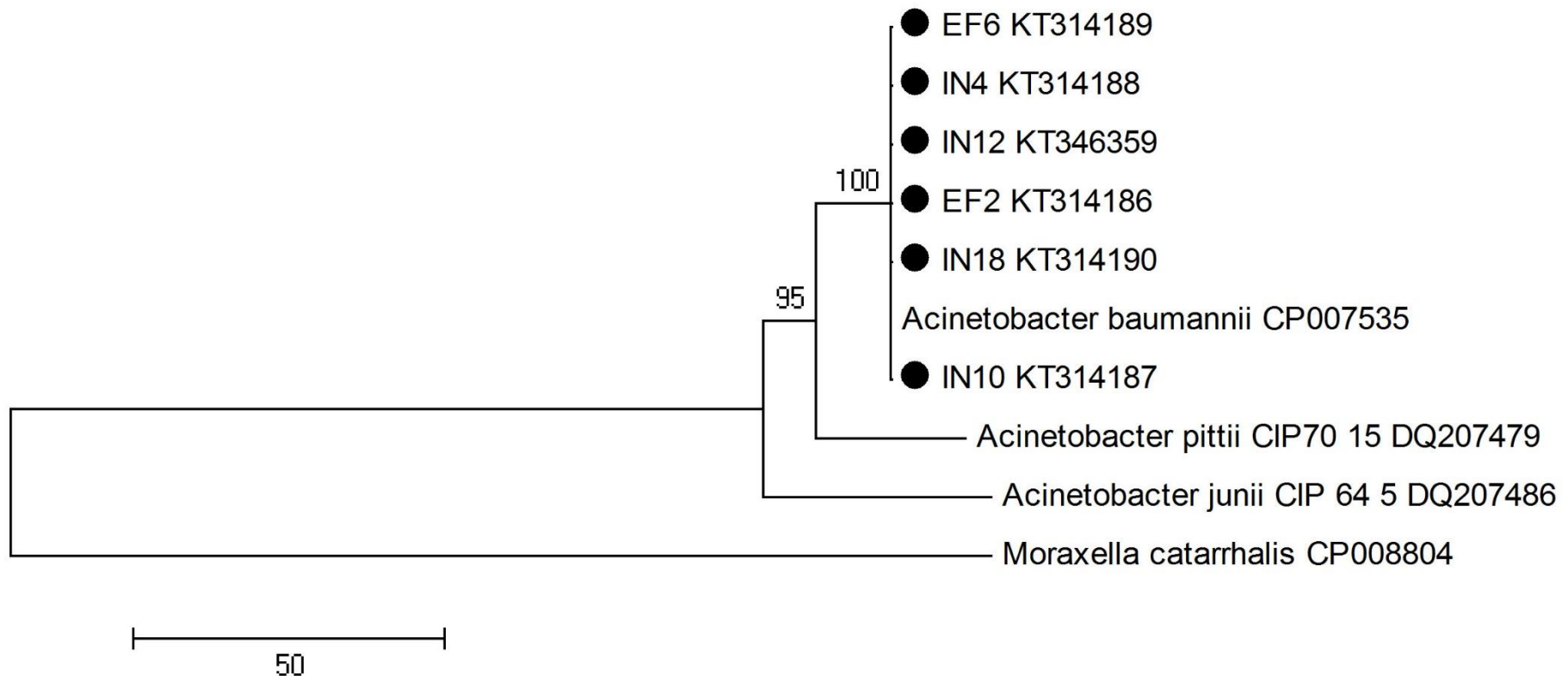
✓ matricom potpomognuta ionizacija laserskom desorpcijom spektrometrom masa s vremenom proleta - MALDI-TOF MS (Microflex LT, Bruker Daltonics) na staničnim ekstraktima

AnalyteName	AnalyteID	Organism(best match)	ScoreValue	Organism(second best match)	ScoreValue
B1 (++) (A)	Š 2/6	Acinetobacter baumannii	2.232	Acinetobacter baumannii	2.195
B2 (++) (A)	Š 2/5	Acinetobacter baumannii	2.067	Acinetobacter baumannii	2.046
B3 (++) (A)	OB 3929	Acinetobacter baumannii	2	Acinetobacter baumannii	1.978
B4 (++) (A)	Š 2/7	Acinetobacter baumannii	2.102	Acinetobacter baumannii	2.048
B5 (++) (A)	Š 2/10	Acinetobacter baumannii	2.231	Acinetobacter baumannii	2.191
Range	Description			Symbols	Color
2.300 ... 3.000	highly probable species identification			(+++)	green
2.000 ... 2.299	secure genus identification, probable species identification			(++)	green
1.700 ... 1.999	probable genus identification			(+)	yellow
0.000 ... 1.699	not reliable identification			(-)	red

Identifikacija okolišnih izolata III

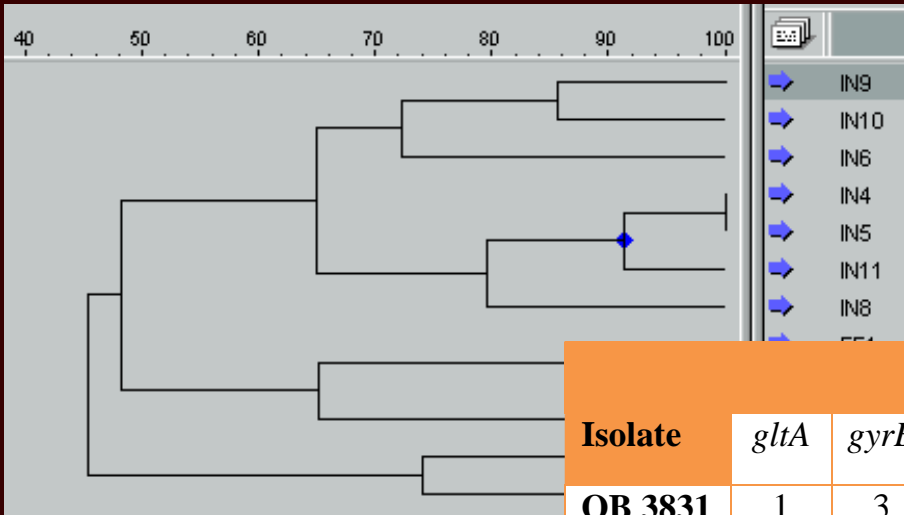
Potvrda identifikacije:

✓ amplifikacija i sekvenciranje gena za β -podjedinicu RNA-polimeraze (*rpoB*)



Genetička srodnost okolišnih i kliničkih izolata *A. baumannii*

- ✓ gel-elektroforeza u pulsirajućem polju (PFGE)
- ✓ MLST (multilocus sequence typing) analiza sedam konstitutivnih gena (*cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rplB*, and *rpoB*), Oxford shema

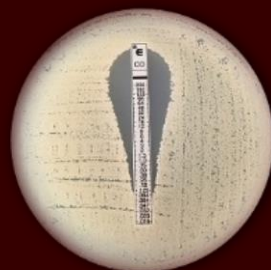


Isolate	Gene locus/allele							Sequence type	Clonal complex	IC type
	<i>gltA</i>	<i>gyrB</i>	<i>gdhB</i>	<i>recA</i>	<i>cpn60</i>	<i>gpi</i>	<i>rpoD</i>			
OB 3831	1	3	3	2	2	96	119 ^a	1421 ^a	92	2
OB 3929	1	3	3	2	2	96	3	195	92	2
OB 3930	1	3	3	2	2	100	3	425	92	2
OB 4027	1	3	3	2	2	96	119 ^a	1421 ^a	92	2
OB 4138	1	3	3	2	2	96	3	195	92	2
S2/1	1	3	3	2	2	96	3	195	92	2
S2/2	1	3	3	2	2	96	3	195	92	2
IN32	1	3	3	2	2	96	3	195	92	2

Profili antibiotske rezistencije I

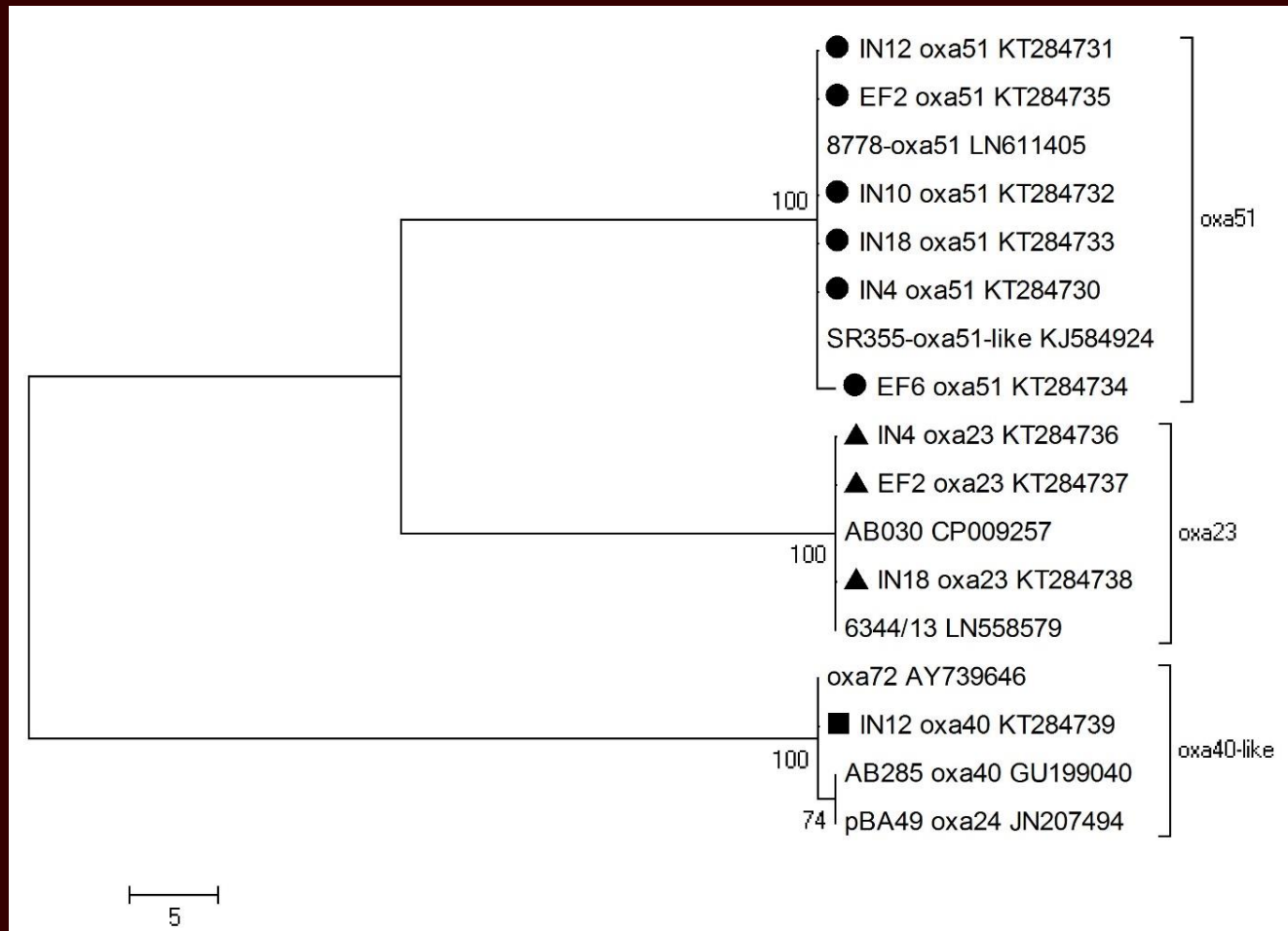
- ✓ Vitek 2 sistem te E-test, razrjeđenje u bujonu
- ✓ tumačenje prema EUCAST i CLSI kriterijima za kliničke izolate

MIC values of antibiotics (mg/L)												
Isolate	MEM	IPM	CIP	LVX	TOB	GEN	AMK	MIN	SAM	TIM	SXT	CST
OB 3831	>16 ^R	>16 ^R	>4 ^R	8 ^R	>16 ^R	>16 ^R	>64 ^R	8 ^I	16 ^I	128 ^R	>320 ^R	<0.5
OB 3929	>16 ^R	>16 ^R	>4 ^R	4 ^R	>16 ^R	>16 ^R	>64 ^R	2	16 ^I	128 ^R	>320 ^R	<0.5
OB 3930	>16 ^R	>16 ^R	>4 ^R	4 ^R	<1	<1	16 ^I	2	16 ^I	128 ^R	>320 ^R	<0.5
OB 4027	>16 ^R	>16 ^R	>4 ^R	8 ^R	>16 ^R	>16 ^R	>64 ^R	8 ^I	4	>128 ^R	>320 ^R	<0.5
OB 4138	>16 ^R	>16 ^R	>4 ^R	8 ^R	>16 ^R	>16 ^R	>64 ^R	>16 ^R	16 ^I	128 ^R	<20	<0.5
S2/1	>16 ^R	>16 ^R	>4 ^R	8 ^R	>16 ^R	>16 ^R	>64 ^R	4	8	128 ^R	>320 ^R	<0.5
S2/2	>16 ^R	8 ^I	>4 ^R	>8 ^R	>16 ^R	8 ^R	>64 ^R	2	<2	128 ^R	>320 ^R	>16 ^R
S2/3	>16 ^R	>16 ^R	>4 ^R	8 ^R	>16 ^R	>16 ^R	>64 ^R	4	8	>128 ^R	>320 ^R	<0.5
S2/4	8 ^I	>16 ^R	>4 ^R	>8 ^R	8 ^R	>16 ^R	>64 ^R	4	4	64 ^I	>320 ^R	>16 ^R
S1/1	>16 ^R	>16 ^R	>4 ^R	8 ^R	>16 ^R	>16 ^R	>64 ^R	8 ^I	<2	>128 ^R	>320 ^R	<0.5
S2/5	>16 ^R	>16 ^R	>4 ^R	8 ^R	>16 ^R	>16 ^R	8	8 ^I	>32 ^R	>128 ^R	<20	<0.5
S2/6	>16 ^R	>16 ^R	>4 ^R	8 ^R	>16 ^R	>16 ^R	8	>16 ^R	>32 ^R	>128 ^R	<20	<0.5
S2/7	>16 ^R	>16 ^R	>4 ^R	8 ^R	>16 ^R	>16 ^R	8	8 ^I	>32 ^R	>128 ^R	<20	<0.5
S2/8	>16 ^R	>16 ^R	>4 ^R	8 ^R	>16 ^R	>16 ^R	8	8 ^I	>32 ^R	>128 ^R	<20	<0.5
S2/9	>16 ^R	>16 ^R	>4 ^R	>8 ^R	>16 ^R	>16 ^R	8	8 ^I	16 ^I	>128 ^R	160 ^R	<0.5
S2/10	8 ^I	>16 ^R	>4 ^R	4 ^R	4	8 ^R	>64 ^R	2	4	64 ^I	>320 ^R	>16 ^R
IN32	>16 ^R	>16 ^R	>4 ^R	8 ^R	>16 ^R	>16 ^R	>64 ^R	8 ^I	16 ^I	128 ^R	>320 ^R	<0.5



Profili antibiotijske rezistencije II

✓ U karbapenem rezistentnih izolata utvrđuje se prisutnost gena *bla*_{OXA-51-like}, *bla*_{OXA-23-like}, *bla*_{OXA-58-like}, *bla*_{OXA-40-like} i *bla*_{OXA-143-like} metodom višestruke lančane reakcije polimerazom (*multiplex PCR*)



Jedan izolat *A. baumannii* je slučajno izoliran iz 0.1g kiselog (pH=2.55) paleotla u napuštenom kamenolomu, Sveti Lovreć pokraj Pule.



Zeleno-sivo paleotlo u kamenolomu Tri Jezerca pokraj Svetog Lovreća, Pula.

MALDI-TOF MS analizom dobivena je pouzdana vrijednost od 2.354, identificirajući izolat kao *A. baumannii*.



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*

Acinetobacter spp. Such reports in the literature of the ubiquity of clinically important *Acinetobacter* spp. in natural environments, such as soil and water, are now recognized as misconceptions (1).

The prevalence of clinically important *Acinetobacter* spp. in nature and their potential to migrate into and/or out of the hospital environments are undefined to date. The natural habitats of clinically important multiresistant *Acinetobacter* spp. remain to be defined. Colonization of the digestive tracts of patients with multidrug-resistant *Acinetobacter* spp. in hospitals occurs at high rates

Minimalne inhibitorne koncentracije (MIC) testiranih antibiotika^a prema okolišnom izolatu *A. baumannii*. ^R – rezistentni prema EUCAST i CLSI kriterijima; n.m. - nije mjereno.

^a karbapenemi (MEM-meropenem, IMI-imipenem), fluorokinoloni (CIP-ciprofloksacin, LVX-levofloksacin), aminoglikozidi (TOB-tobramycin, GEN-gentamicin, AMK-amikacin), tetraciklini (MIN-minocycline), penicilin/ β -laktamazni inhibitori (SAM-ampicilin/sulbactam, TIM-ticarcillin/clavulanic acid), inhibitori folatnog puta (SXT-trimethoprim/sulfamethoxazole), polimiksini (CST-colistin).

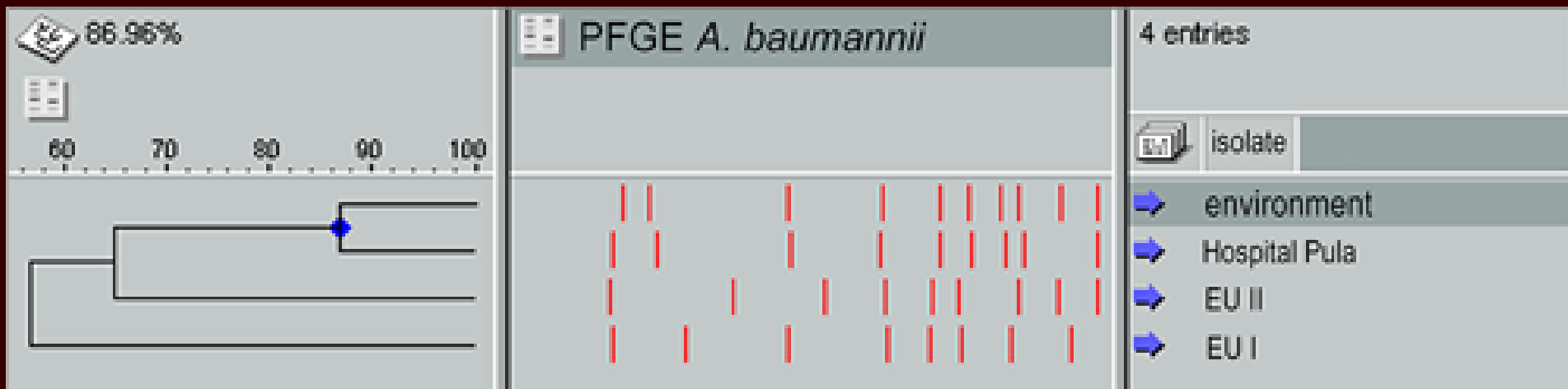
Isolat	MIC vrijednosti antibiotika (mg/L)											
	MEM	IPM	CIP	LVX	TOB	GEN	AMK	MIN	SAM	TIM	SXT	CST
Paleosol	≤0.5	≤0.5	≥4 ^R	4 ^R	≤1	>16 ^R	2	n.m.	4	n.m.	160 ^R	≤0.5

Multiple rezistentan (MDR) prema fluorokinolonima, gentamicinu i trimetoprim-sulfametoksazolu

MDR *A. baumannii* iz paleotla je srodan kliničkom izolatu iz bolnice u Puli.

Može preživjeti kiseli pH tla tijekom 2 dana, sušenje i visoku temperaturu od 50°C.

Vjerojatan **izvor** *A. baumannii* u paleotlu je ilegalno odbačen bolnički kruti otpad u okolici napuštenog kamenoloma.



Tri izolata *A. baumannii* izdvojena su iz 0.01-1g tla na odlagalištu otpada Sovjak iznad Rijeke.



Smetlište Sovjak smješteno u vrtaci kraj sela Marinići iznad Rijeke.

Minimalne inhibitorne koncentracije (MIC) testiranih antibiotika^a prema okolišnim izolatima *A. baumannii*. ^R - resistantni, ^I – intermedijarni prema EUCAST i CLSI kriterijima.

^a karbapenemi (MEM-meropenem, IMP-imipenem), fluorokinoloni (CIP-ciprofloksacin, LVX-levofloksacin), aminoglikozidi (TOB-tobramicin, GEN-gentamicin, AMK-amikacin), tetraciklini (MIN-minociklin), penicilin/β-laktamazni inhibitori (SAM-ampicilin/sulbaktam, TIM-ticarcilin/clavulanična kiselina), inhibitori folatnog puta (SXT-trimetoprim/sulfametoksazol), polimiksini (CST-kolistin).

Isolat	MALDI	MIC vrijednosti antibiotika (mg/L)											
	TOF	MEM	IPM	CIP	LVX	TOB	GEN	AMK	MIN	SAM	TIM	SXT	CST
Sovjak1	2.036	≥16 ^R	≥16 ^R	≥4 ^R	4 ^R	≤1	≤1	32 ^R	≤1	16 ^I	≥128 ^R	≤20	≤0.5
Sovjak2	2.086	≥16 ^R	≥16 ^R	≥4 ^R	4 ^R	≤1	≤1	16 ^I	≤1	16 ^I	≥128 ^R	≤20	≤0.5
Sovjak3	2.000	≥16 ^R	≥16 ^R	≥4 ^R	4 ^R	≤1	≤1	>64 ^R	8 ^I	16 ^I	≥128 ^R	≥320 ^R	≤0.5

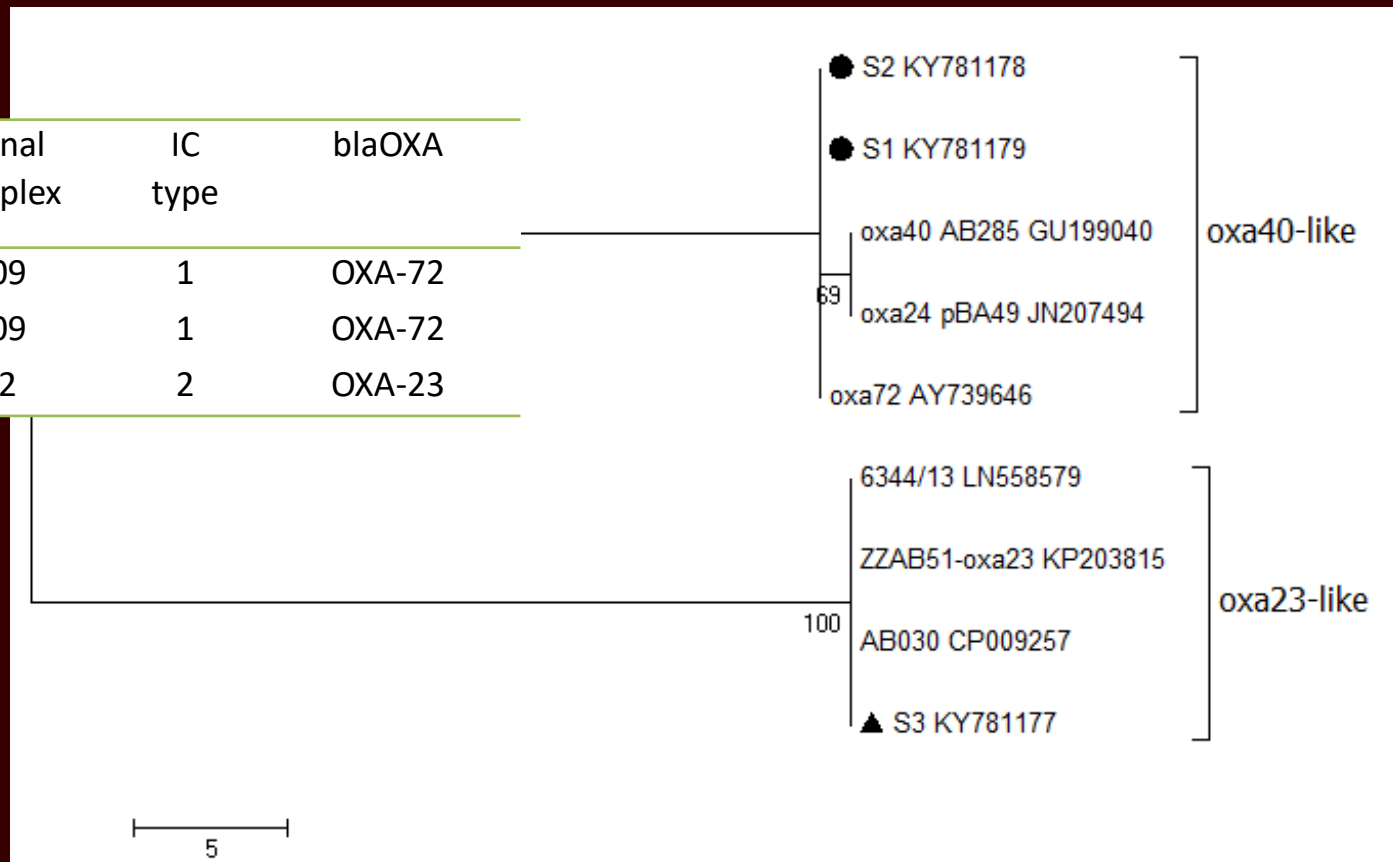
MALDI-TOF MS vrijednosti iznad 2.000 identificirala su izolate kao *A. baumannii*. Sva tri izolata bila su MDR i dijelila kompletnu ili intermedijarnu rezistenciju na karbapeneme, fluorokinolone, amikacin i penicilin/β-laktamazne inhibitore.

Izolati *A. baumannii* iz tla na Sovjaku dijele mnoge osobine opisane za kliničke izolate:

- pripadnost IC1 i 2 (u Hrvatskoj opisani od 2002. i 2009.)
- MDR profil antibiotske rezistencije
- rezistenciju na karbapeneme posredovanu genima *bla*_{OXA72} i

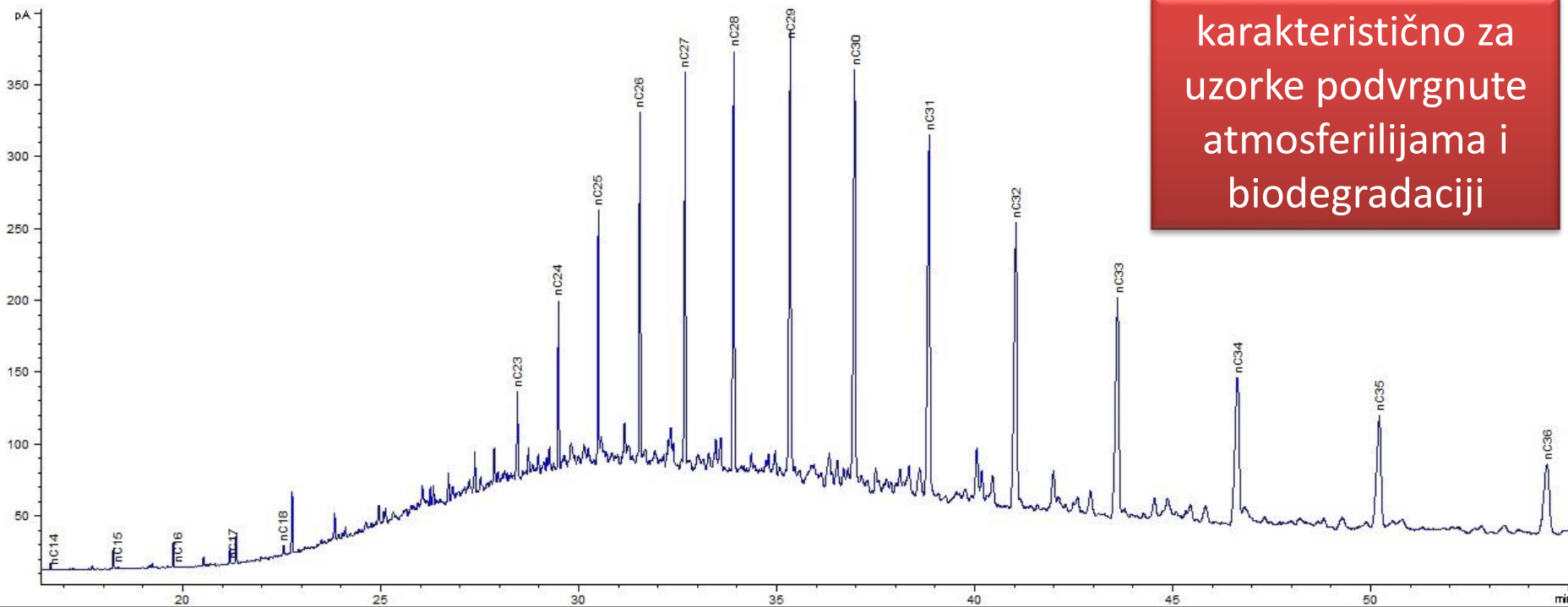
*bla*_{OXA23-like}

Izolat	Sequence type	Clonal complex	IC type	blaOXA
Sovjak 1	231	109	1	OXA-72
Sovjak 2	231	109	1	OXA-72
Sovjak 3	195	92	2	OXA-23



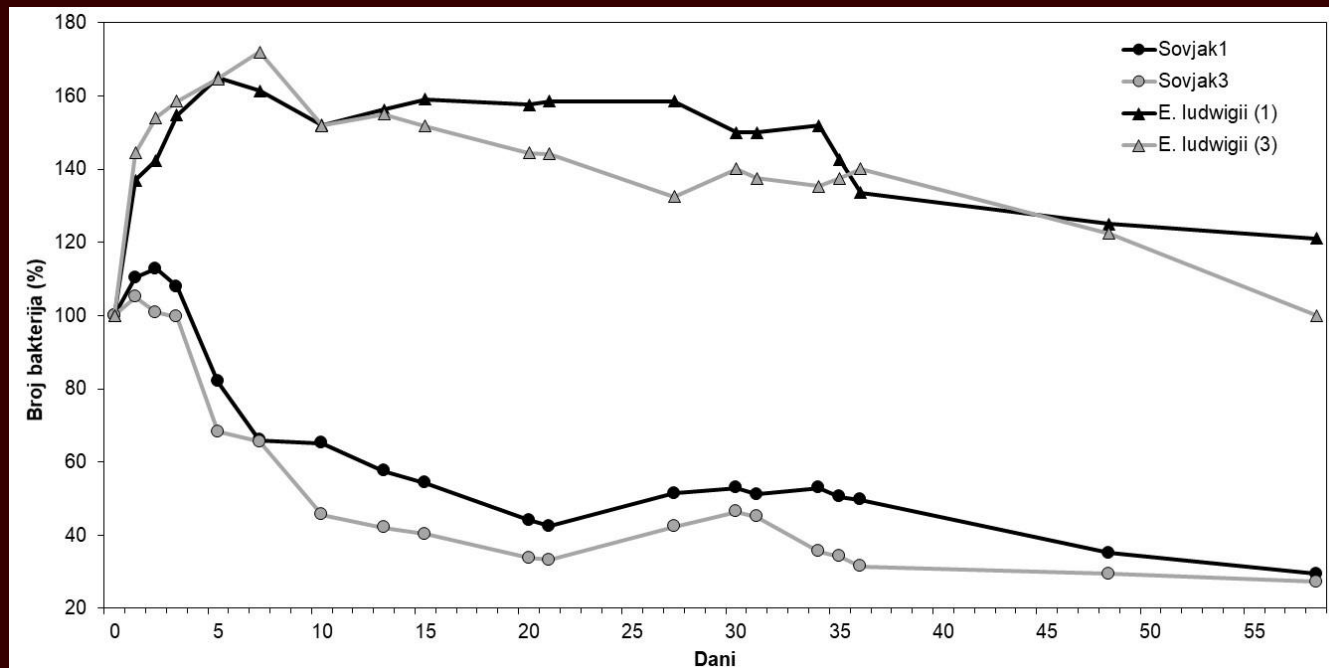
Vjerojatan izvor
A. baumannii je
ilegalno odbačen
bolnički kruti
otpad.

Tlo u vrtači na odlagalištu otpada Sovjak sadržavalo je 76 wt% naftnih ugljikovodika: zasićeni ugljikovodici 33; aromatski ugljikovodici 14; smole 8; asfalteni 46.



Plinski kromatogram alkanske frakcije: gubitak laganih ugljikovodika do n-C23 te nerazlučena grba u višem molekularnom rangu.

Simulacija daljnje bioremedijacije *in vitro* kroz 58 dana nije rezultirala smanjenjem ukupne koncentracije naftnih ugljikovodika u tlu (76 wt%). Razlog: rezistencija viših naftnih ugljikovodika na proces biodegradacije.



Iako naftno zagađenje pokazuje znakove prirodne bioremedijacije, daljnja bioremedijacija starog zagađenja bi vjerojatno bila vrlo slabo učinkovita.

Četiri izolata *A. baumannii* izdvojena su iz 10mL vode rijeke Save ispod Zagreba nakon ulijevanja urbanih otpadnih voda u rijeku kao prirodni prijemnik.



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In Press, Corrected Proof— Note to users



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

M. Seruga Music^a, J. Hrenovic^a, I. Goic-Barisic^b, B. Hunjak^c, D. Skoric^a, T. Ivankovic^a

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<https://doi.org/10.1016/j.jhin.2017.04.005>

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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.

Minimalne inhibitorne koncentracije (MIC) testiranih antibiotika^a prema okolišnim izolatima *A. baumannii*. ^R - resistantni, ^I – intermedijarni prema EUCAST i CLSI kriterijima.

^a karbapenemi (MEM-meropenem, IMI-imipenem), fluorokinoloni (CIP-ciprofloxacin, LVX-levofloxacin), aminoglikozidi (TOB-tobramycin, GEN-gentamicin, AMK-amikacin), tetraciklini (MIN-minocycline), penicilin/β-laktamazni inhibitori (SAM-ampicillin/sulbactam, TIM-ticarcillin/clavulanic acid), inhibitori folatnog puta (SXT-trimethoprim/sulfamethoxazole), polimiksini (CST-colistin).

Isolat	MALDI	MIC vrijednosti antibiotika (mg/L)											
	TOF	MEM	IPM	CIP	LVX	TOB	GEN	AMK	MIN	SAM	TIM	SXT	CST
Sava3	2.075	>16 ^R	>16 ^R	>4 ^R	>8 ^R	>16 ^R	>16 ^R	>64 ^R	4	16 ^I	>128 ^R	>320 ^R	<0.5
Sava4	2.081	>16 ^R	>16 ^R	>4 ^R	>8 ^R	<1	8 ^R	16 ^I	8 ^I	8	>128 ^R	>320 ^R	<0.5
Sava5	2.149	>16 ^R	>16 ^R	>4 ^R	>8 ^R	>16 ^R	>16 ^R	>64 ^R	8 ^I	8	>128 ^R	<20	<0.5
Sava6	2.052	>16 ^R	>16 ^R	>4 ^R	>8 ^R	>16 ^R	>16 ^R	>64 ^R	4	16 ^I	>128 ^R	>320 ^R	<0.5

MALDI-TOF MS vrijednosti iznosile su 2.052 - 2.149 za *A. baumannii*. Sva četiri izolata bila su MDR i dijelila rezistenciju na karbapeneme, fluorokinolone, aminoglikozide i tikarcilin-klavulansku kiselinu.

Izolat	Porijeklo	Sequence type	Clonal complex	IC type
OB 3831	Klinički izolati	1421 ^a	92	2
OB 3929		195	92	2
OB 4027		1421 ^a	92	2
OB 4138		195	92	2
S2/1		195	92	2
S2/2		195	92	2
S2/3		195	92	2
S2/4		195	92	2
S1/1	Bolnička otpadna voda	195	92	2
S2/5		195	92	2
S2/6		195	92	2
S2/7		195	92	2
S2/8		195	92	2
S2/9	195	92	2	
Sava3	Sava	1421 ^a	92	2
Sava4		195	92	2
Sava5		1421 ^a	92	2
Sava6		1421 ^a	92	2

Izolati su pokazali visoku srodnost s kliničkim izolatima iz jedne zagrebačke bolnice i izolatima iz bolničke otpadne vode.

Bolničke vode u Zagrebu se bez pred-tretmana ispuštaju u zajednički kanalizacijski sustav.

Otpadne vode iz skupnog kanalizacijskog sustava se s ili bez pročišćavanja ispuštaju u prirodni prijemnik, rijeku Savu.

Najvjerojatniji izvor *A. baumannii* u Savi su bolničke i posljedično urbane otpadne vode.

RESEARCH ARTICLE

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

J Hrenovic¹, I Goic-Barisic², S Kazazic³, A Kovacic⁴, M Ganjto⁵, M Tonkic⁶



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Pan Drug-Resistant Environmental Isolate of *Acinetobacter baumannii* from Croatia.

Goic-Barisic^{1,2}, Šeruga Music³, Kovacic⁴, Tonkic M^{1,2}, Hrenovic J³.

Author information

Abstract

Acinetobacter baumannii is an emerging nosocomial pathogen with also emerging resistance to different antibiotics. Multidrug and pan drug-resistant clinical isolates were reported worldwide. Here we report the first evidence of pan drug-resistant environmental isolate of *A. baumannii*. The isolate was recovered from the effluent of secondary treated municipal wastewater of the City of Zagreb, Croatia. The isolate was resistant to penicillins/ β -lactamase inhibitors, carbapenems, fluoroquinolones, aminoglycosides, folate pathway inhibitors, and polymyxins, except intermediately susceptible to minocycline and tigecycline. Intrinsic chromosomally located bla_{OXA-51}-like gene and acquired plasmid-located bla_{OXA-23}-like gene were related to clinical isolates. Pan drug-resistant *A. baumannii* can occur in natural environments outside of the hospital. Secondary treated municipal wastewater represents a potential epidemiological pan drug-resistant *A. baumannii* and carbapenem resistance gene.

Acinetobacter baumannii; antibiotics; microbial drug resistance; public health; wastewater

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

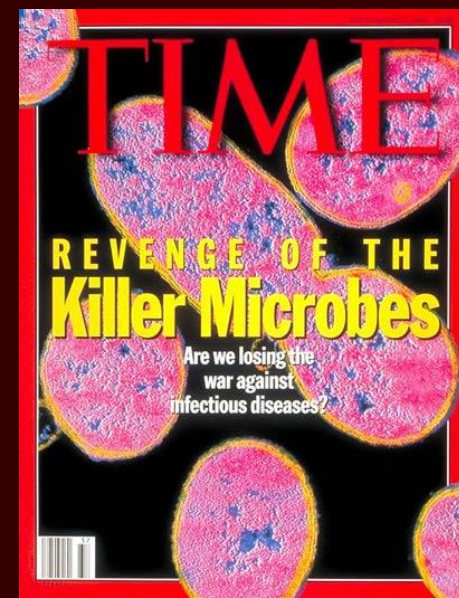
Ivana Goic-Barisic^{1,2}, Jasna Hrenovic³, Ana Kovacic⁴, and Martina Šeruga Music³

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 *proB* 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. Furthermore, the presence of OXA-40-like (OXA-72) in an environmental *A. baumannii* isolate is reported for the first time. Persistence of *A. baumannii* harboring the clinically important OXAs in the wastewater treatment process poses a potentially significant source for horizontal gene transfer and implications for wider spread of antibiotic resistance genes.

Keywords: *Acinetobacter baumannii*, carbapenemase, oxacillinase, microbial drug resistance, molecular characterization, public health

Zaključci:

- MDR *A. baumannii* prisutni su u prirodnom okolišu pod utjecajem ljudskog krutog i tekućeg otpada u Hrvatskoj.
- Nužno je potrebno pravilno zbrinjavanje ljudskog otpada kako bi se spriječilo širenje MDR *A. baumannii* u prirodu.



Ovaj je rad financirala-sufinancirala Hrvatska zaklada za znanost projektom IP-2014-09-5656 „Prirodno stanište klinički značajnih *Acinetobacter baumannii*”.

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



The screenshot shows the website of the Biological Department (Biološki odsjek) at the University of Zagreb. The page features a navigation menu with categories like 'ODSJEK', 'STUDIJI', 'NASTAVA', 'Znanost', 'UREĐ ZA STUDENTE', 'DOKTORSKI STUDIJI', 'ZAVODI', and 'DIJELATNICI'. Below the navigation, there is a breadcrumb trail: 'PMF Naslovnica / Naslovnica / O zavodu / Naturaci'. The main content area is titled 'NASLOVNICA' and includes a list of links: 'Projektni tim', 'Objavljeni radovi', 'Kongresna priopćenja', and 'Diseminacija'. A large image of a petri dish with red bacterial colonies is displayed. Below the image, the project title is 'Prirodno stanište klinički značajnih *Acinetobacter baumannii*'. The project details are as follows: 'Izvor financiranja: Hrvatska zaklada za znanost', 'Trajanje: 01. 09. 2015. – 31. 08. 2019.', 'Voditelj projekta: Prof. dr. sc. Jasna Hrenović', 'Sredstva: 999,210,00 HRK', and 'Broj projekta: IP-2014-09-5656'. A 'Sažetak:' section follows, with the beginning of the text: 'Bakterija *Acinetobacter baumannii* je oportunistički patogen odgovoran za bolničke infekcije u'.





Hvala na pažnji!