

KLINIČKI ZNAČAJNA BAKTERIJA *ACINETOBACTER BAUMANNII* IZVAN BOLNIČKOG OKRUŽENJA U HRVATSKOJ

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HAZU, 13. 11. 2017., Zagreb



Rod *Acinetobacter* broji 57 imenovanih vrsta:

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

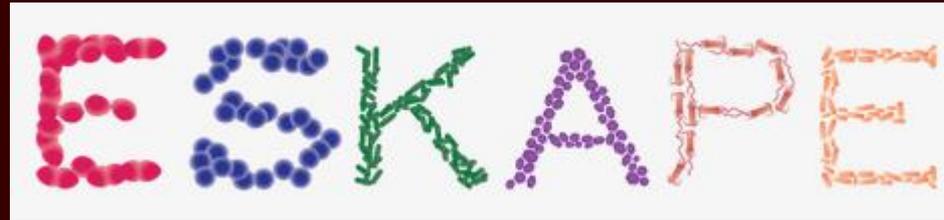
A. grimontii^a

A. kookii

A. schindleri

^aSynonym of *A. junii*.

Pojava bakterija višestruko rezistentnih na antibiotike globalni je problem 21. stoljeća.



E. faecium

S. aureus

K. pneumoniae

A. baumannii

P. aeruginosa

Enterobacter spp.

Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America FREE

Helen W. Boucher ✉, George H. Talbot, John S. Bradley, John E. Edwards, David Louis B. Rice, Michael Scheld, Brad Spellberg, John Bartlett

Clinical Infectious Diseases, Volume 48, Issue 1, 1 January 2009, Pages 1–12,

<https://doi.org/10.1086/595011>

Published: 01 January 2009 [Article history](#) ▼



InfectionControl.tips

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Join. Contribute. Make A Difference.
<https://infectioncontrol.tips>

ESKAPES: Emerging Pathogens of Concern

By: Nick Barsby, Pervinder Singh Johal
Edited by: Andrew Duong, Dr. Uyen Nguyen

Date : January 5, 2016

Osobito je zabrinjavajući razvoj rezistencije na karbapeneme.



Media centre

WHO publishes list of bacteria for which new antibiotics are urgently needed

News release

27 FEBRUARY 2017 | GENEVA - WHO today published its first ever list of antibiotic-resistant "priority pathogens" – a catalogue of 12 families of bacteria that pose the greatest threat to human health.

The WHO priority list

PRIORITY: CRITICAL

- ◆ **Acinetobacter baumannii** carbapenem-resistant
- ◆ **Pseudomonas aeruginosa** carbapenem-resistant
- ◆ **Enterobacteriaceae** carbapenem-resistant, ESBL-producing

PRIORITY 2: HIGH

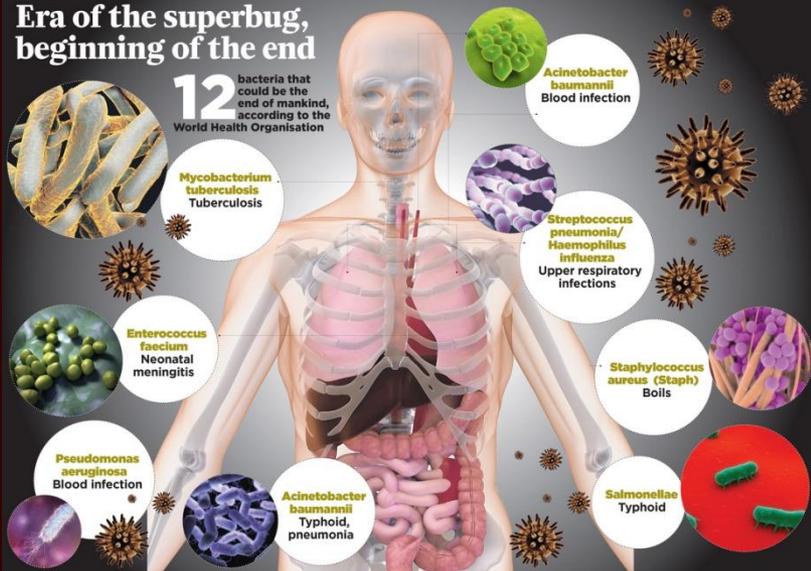
- ◆ **Enterococcus faecium** vancomycin-resistant
- ◆ **Staphylococcus aureus** methicillin-resistant vancomycin-intermediate and resistant
- ◆ **Helicobacter pylori** clarithromycin-resistant
- ◆ **Campylobacter spp.** fluoroquinolone-resistant
- ◆ **Salmonellae** fluoroquinolone-resistant
- ◆ **Neisseria gonorrhoeae** cephalosporin-resistant fluoroquinolone-resistant

PRIORITY 3: MEDIUM

- ◆ **Streptococcus pneumoniae** penicillin-non-susceptible
- ◆ **Haemophilus influenzae** ampicillin-resistant
- ◆ **Shigella spp.** fluoroquinolone-resistant

Era of the superbug, beginning of the end

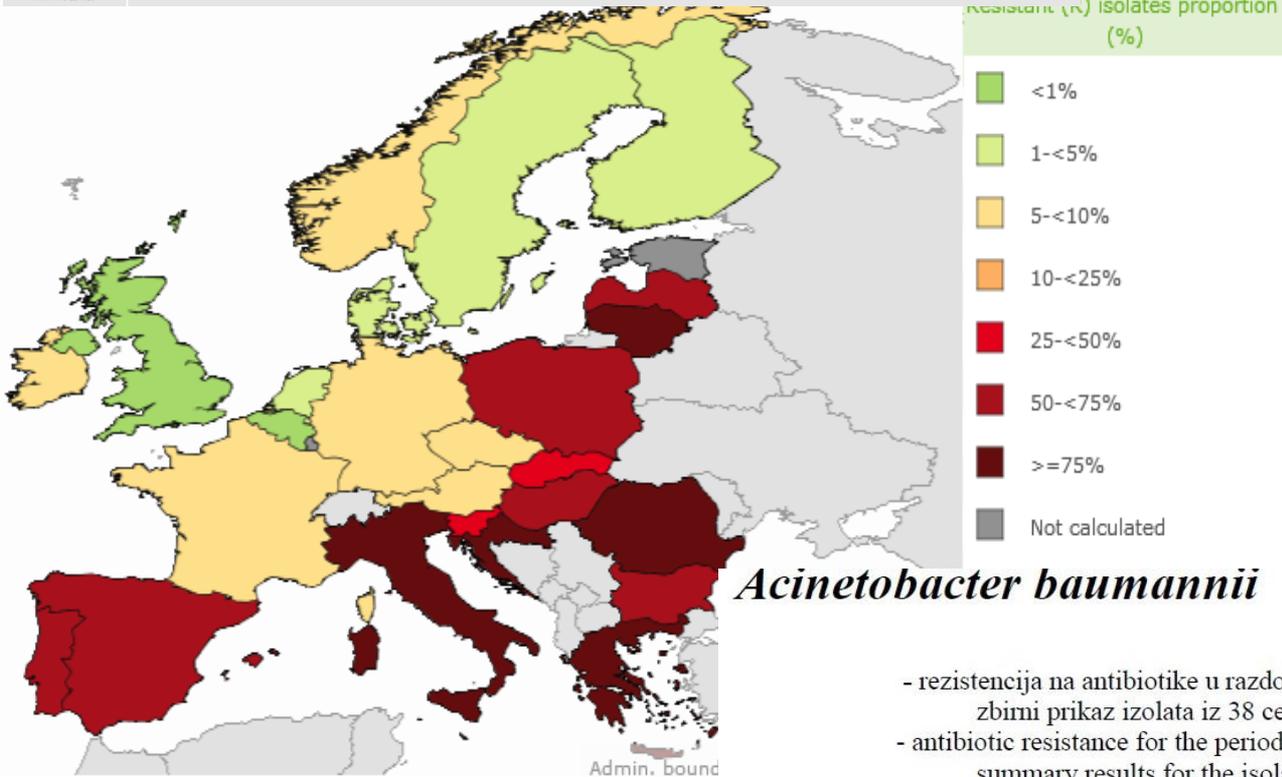
12 bacteria that could be the end of mankind, according to the World Health Organisation



ALARMANTNO UPOZORENJE

OVIH 12 BAKTERIJA PREDSTAVLJAJU NAJVEĆU OPASNOST ZA LJUDSKO ZDRAVLJE Smrtonosnije su od raka, godišnje bi mogle ubijati po deset milijuna ljudi

AUTOR: Jutarnji.hr OBJAVLJENO: 02.03.2017. u 10:55



A. baumannii
rezistentni na
karbapeneme
u Europi 2015.g.

- rezistencija na antibiotike u razdoblju od 1.10. - 31.12. 2015. zbirni prikaz izolata iz 38 centra u RH
- antibiotic resistance for the period 1.10. - 31.12. 2015. summary results for the isolates from 38 centers in Croatia

10% u 2008.g. →

ANTIBIOTIK ANTIBIOTIC	Broj izolata No. of isolates	% rezistentnih (% intermedijarnih) izolata % of resistant (% of intermediate) isolates	Raspon lokalnih rezultata* Range of local results*
Ampicillin + sulbactam	1 693	44 (11)	0 (8) - 85 (0)
Meropenem	1 724	87 (1)	66 (0) - 98 (0)
Imipenem	1 726	86 (1)	66 (0) - 98 (0)
Ciprofloxacin	1 681	91 (0)	77 (0) - 100 (0)
Gentamicin	1 726	84 (0)	65 (0) - 100 (0)
Netilmicin	1 301	83 (0)	56 (0) - 100 (0)
Amikacin	1 705	79 (2)	39 (0) - 93 (0)
Co-trimaxazole	1 633	76 (4)	52 (7) - 100 (0)
Colistin	1 342	0 (0)	0 (0) - 6 (0)

Rezistencija na karbapeneme u pravilu je povezana s rezistencijom na više klasa antibiotika. Razlikujemo:

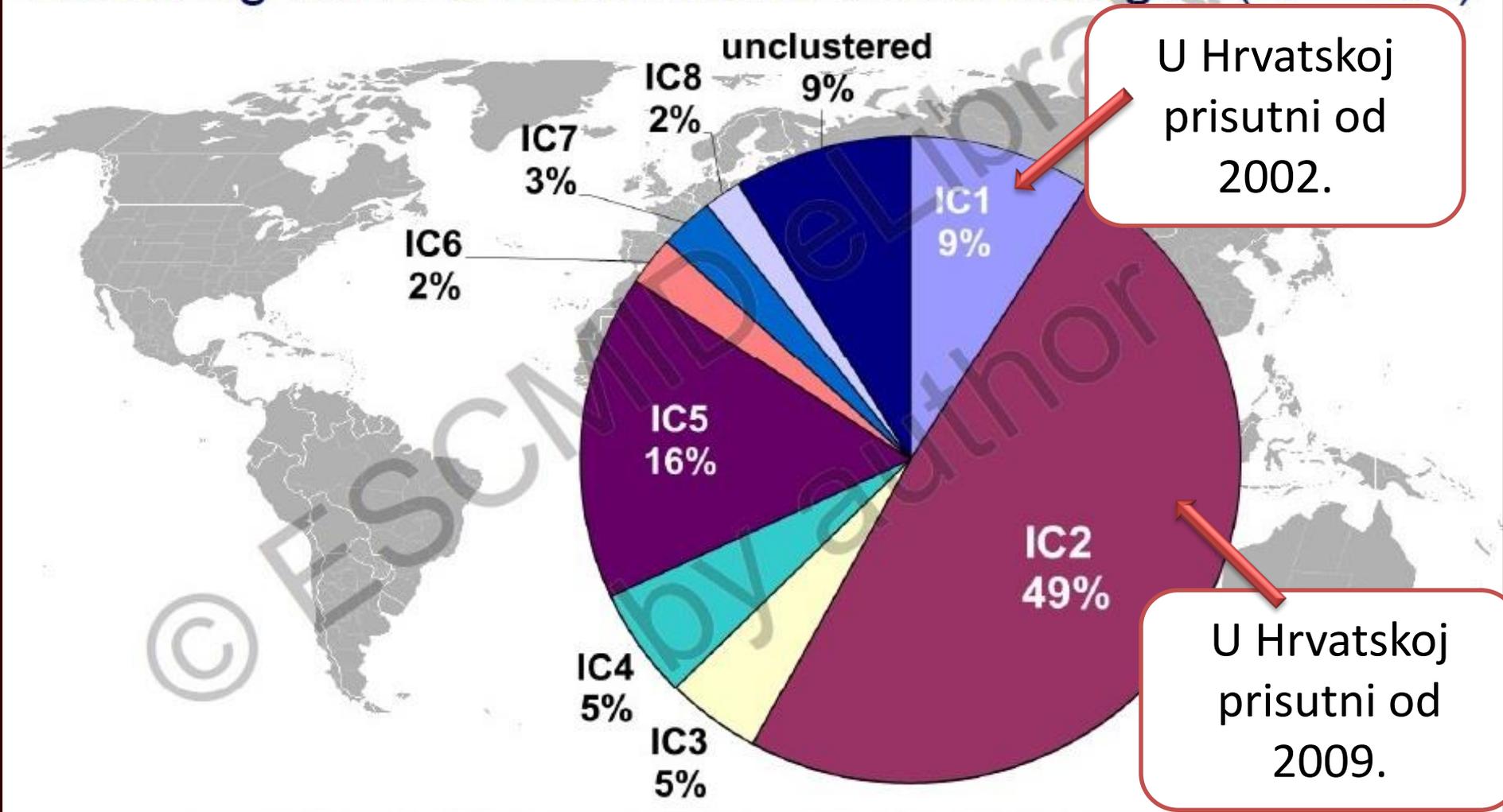
- **senzitivni** - osjetljivi na sve antibiotike;
- **višestruko rezistentni (MDR, multidrug-resistant)** - neosjetljivi na ≥ 1 agensa u ≥ 3 antimikrobne kategorije;
- **prošireno rezistentni (XDR, extensively drug-resistant)** - neosjetljivi na ≥ 1 agens u svim izuzev ≤ 2 antimikrobne kategorije;
- **sveopće rezistentni (PDR, pandrug-resistant)** - neosjetljivi na sve agense u svim antimikrobnim kategorijama.

[Clin Microbiol Infect.](#) 2012 Mar;18(3):268-81. doi: 10.1111/j.1469-0691.2011.03570.x. Epub 2011 Jul 27.

Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance.

[Magiorakos AP](#)¹, [Srinivasan A](#), [Carey RB](#), [Carmeli Y](#), [Falaqas ME](#), [Giske CG](#), [Harbarth S](#), [Hindler JF](#), [Kahlmeter G](#), [Olsson-Liljequist B](#), [Paterson DL](#), [Rice LB](#), [Stelling J](#), [Struelens MJ](#), [Vatopoulos A](#), [Weber JT](#), [Monnet DL](#).

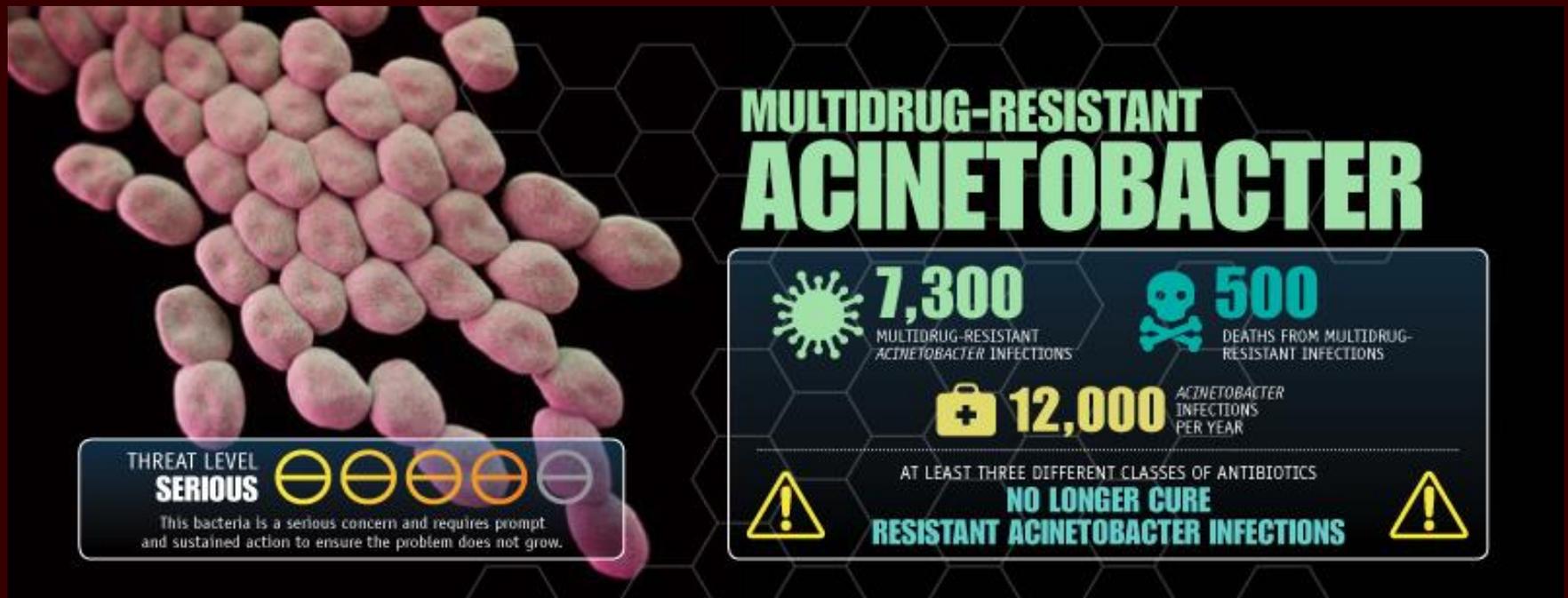
Proportion of carbapenem resistant *A. baumannii* clustering with the International clonal lineages (N = 492)



Higgins PG et al. 2010. Global spread of carbapenem-resistant *Acinetobacter baumannii*. JAC 65:233-8

Osim epidemija u bolnicama, *A. baumannii* se pojavljuje i kao uzročnik akutnih izvanbolničkih infekcija.

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



Do 2010. *A. baumannii* se smatrao ekskluzivnim bolničkim patogenom.

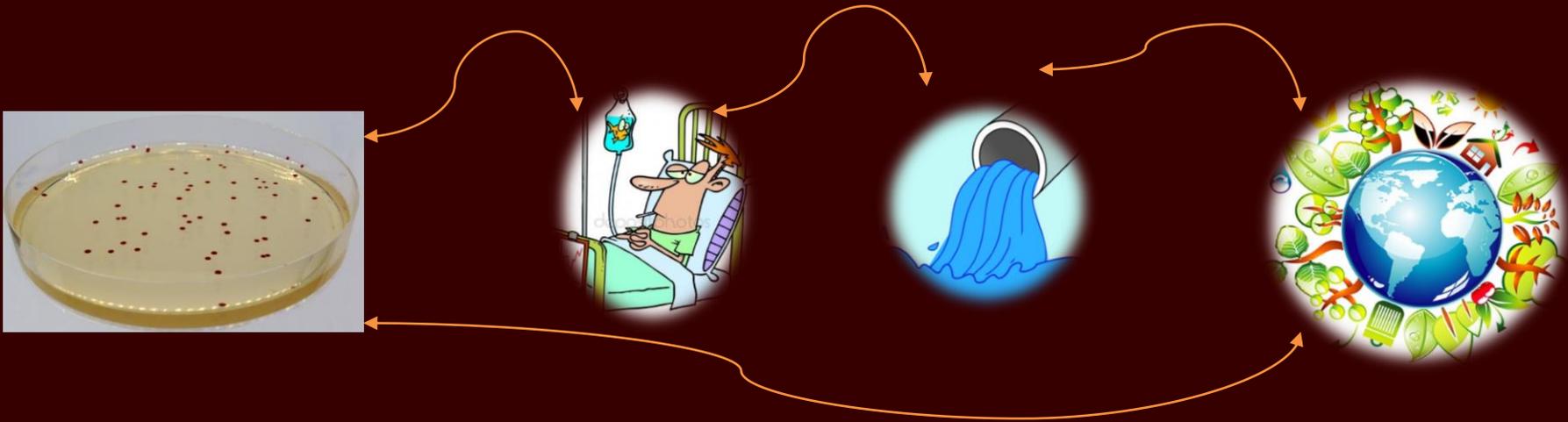
Nakon 2010. javljaju se nalazi ovog patogena izvan bolničkog okruženja:

- voda rijeke Seine (2010.) – 1 izolat
- sirove bolničke vode u Brazilu (2011.) – 3 izolata
- sirove i klorirane bolničke vode u Kini (2013.) – 9 i 1 izolat
- prirodni okoliš u Hrvatskoj (2014. ...)

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.



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



Cilj istraživanja:

pregled prisutnosti višestruko rezistentnih *A. baumannii* u prirodnom okolišu pod utjecajem krutog i tekućeg otpada u Hrvatskoj.

Nastavni zavod za
javno zdravstvo
Splitsko-
dalmatinske
županije

Prirodoslovno-
matematički
fakultet, Sveučilište
u Zagrebu

Rudarsko-
geološko-
naftni fakultet,
Sveučilište u
Zagrebu

Hrvatski
zavod za javno
zdravstvo

KBC Split i
Medicinski
fakultet,
Sveučilište u
Splitu

Zagrebačke
otpadne
vode d.o.o.

Tehnološko-
metalurški
fakultet,
Sveučilište u
Beogradu

Prirodno stanište klinički značajnih *Acinetobacter baumannii*

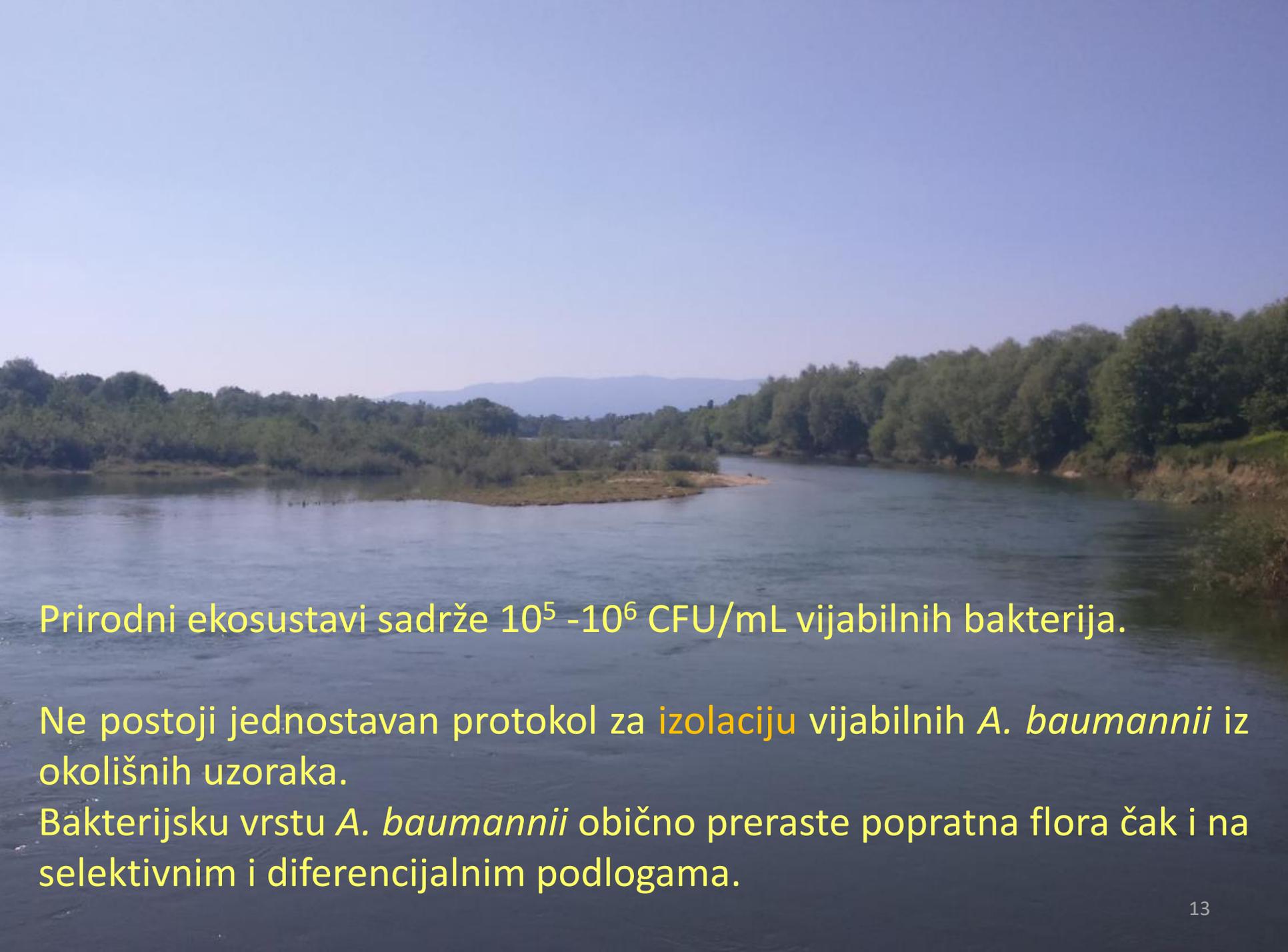
Izvor financiranja: Hrvatska zaklada za znanost

Trajanje: 01. 09. 2015. – 31. 08. 2019.

Voditelj projekta: **Prof. dr. sc. Jasna Hrenović**

Sredstva: 999,210.00 HRK

Broj projekta: IP-2014-09-5656

A wide river flows through a lush green landscape under a clear blue sky. The river is surrounded by dense green 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.

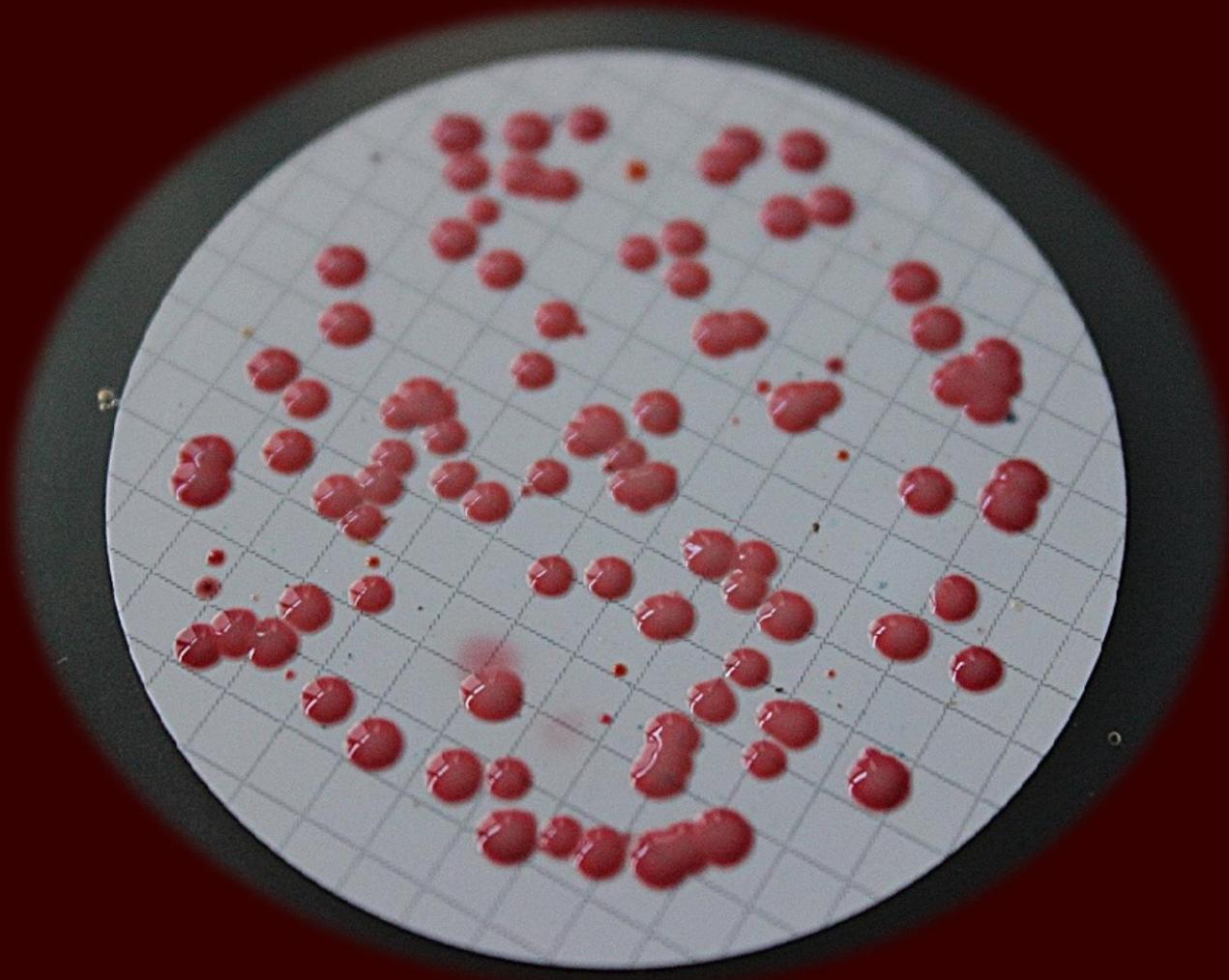
Active surveillance is necessary to control its spread in the facilities, to reduce the risk of cross-contamination, and to identify the carriers. Rapid identification of patients that are colonized with *Acinetobacter* would lead to infection control practices aimed at preventing spread of the organisms.

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:

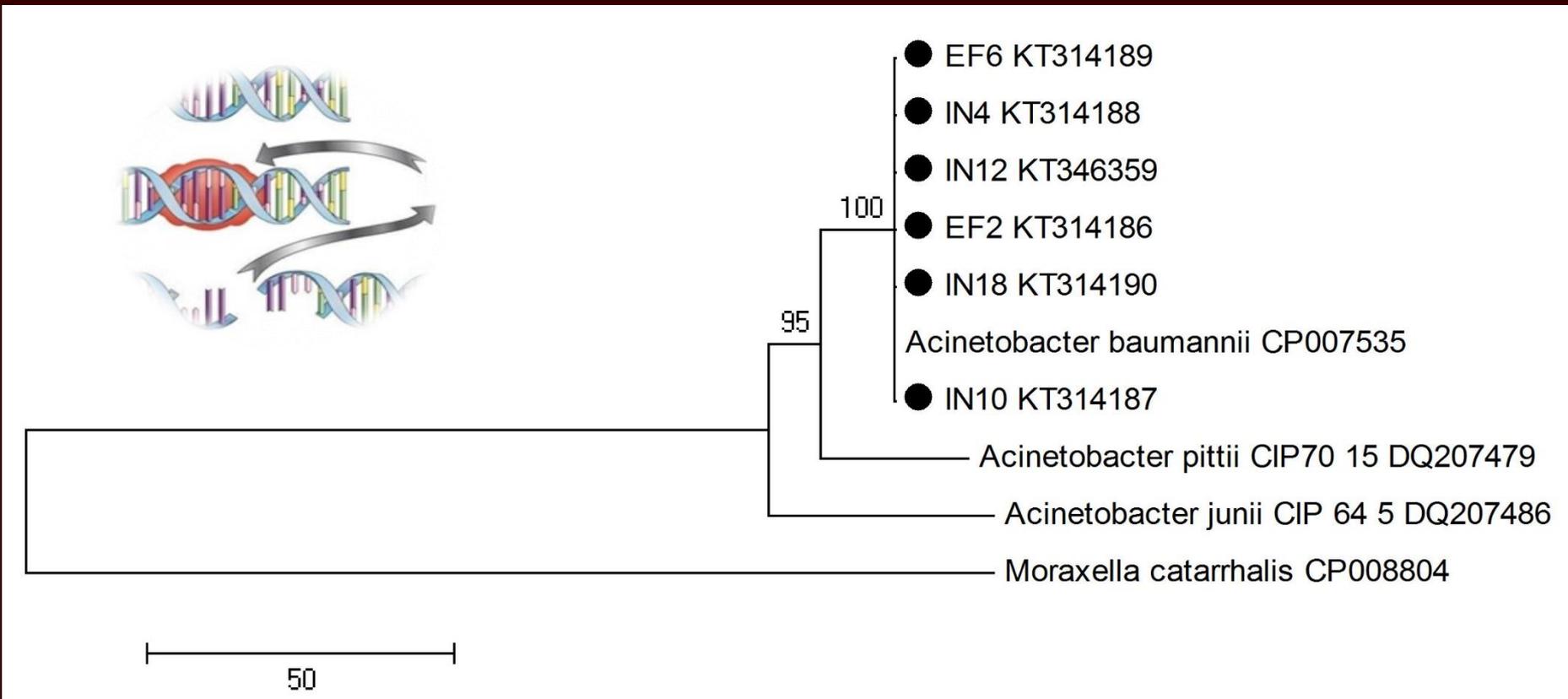
✓ matricom potpomognuta ionizacija laserskom desorpcijom spektrometrom masa s vremenom proleta - MALDI-TOF MS (Microflex LT, Bruker Daltonics) na staničnim ekstraktima

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

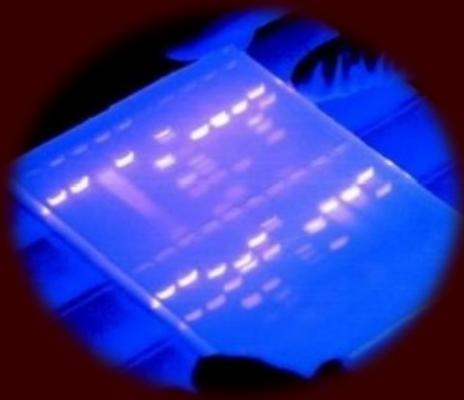
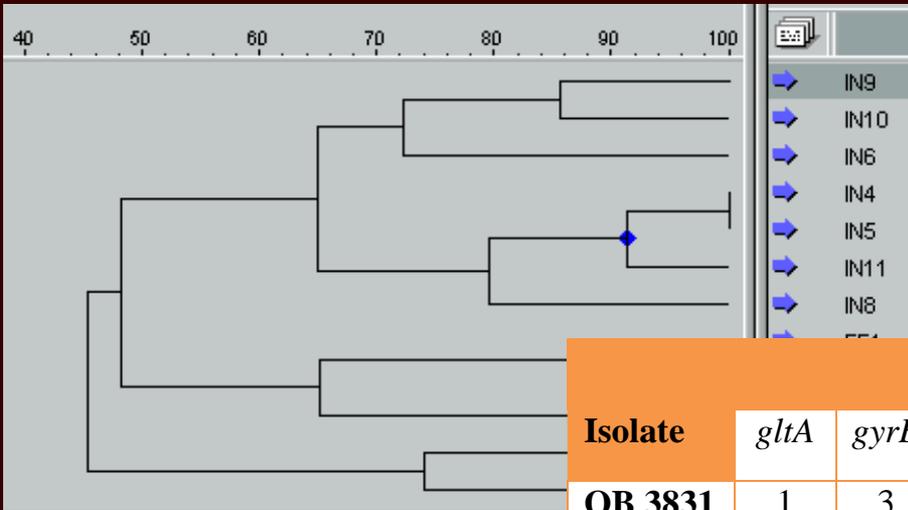
Potvrda identifikacije:

- ✓ 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

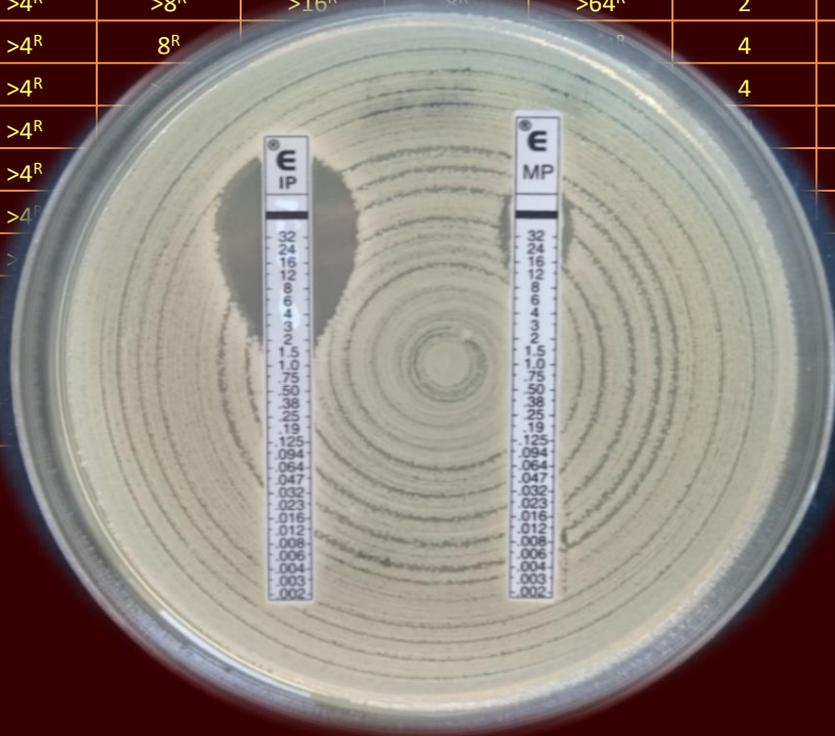


Isolate	Gene locus/allele							Sequence type	Clonal complex	IC type
	<i>gltA</i>	<i>gyrB</i>	<i>gdhB</i>	<i>recA</i>	<i>cpn60</i>	<i>gpi</i>	<i>rpoD</i>			
OB 3831	1	3	3	2	2	96	119 ^a	1421 ^a	92	2
OB 3929	1	3	3	2	2	96	3	195	92	2
OB 3930	1	3	3	2	2	100	3	425	92	2
OB 4027	1	3	3	2	2	96	119 ^a	1421 ^a	92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

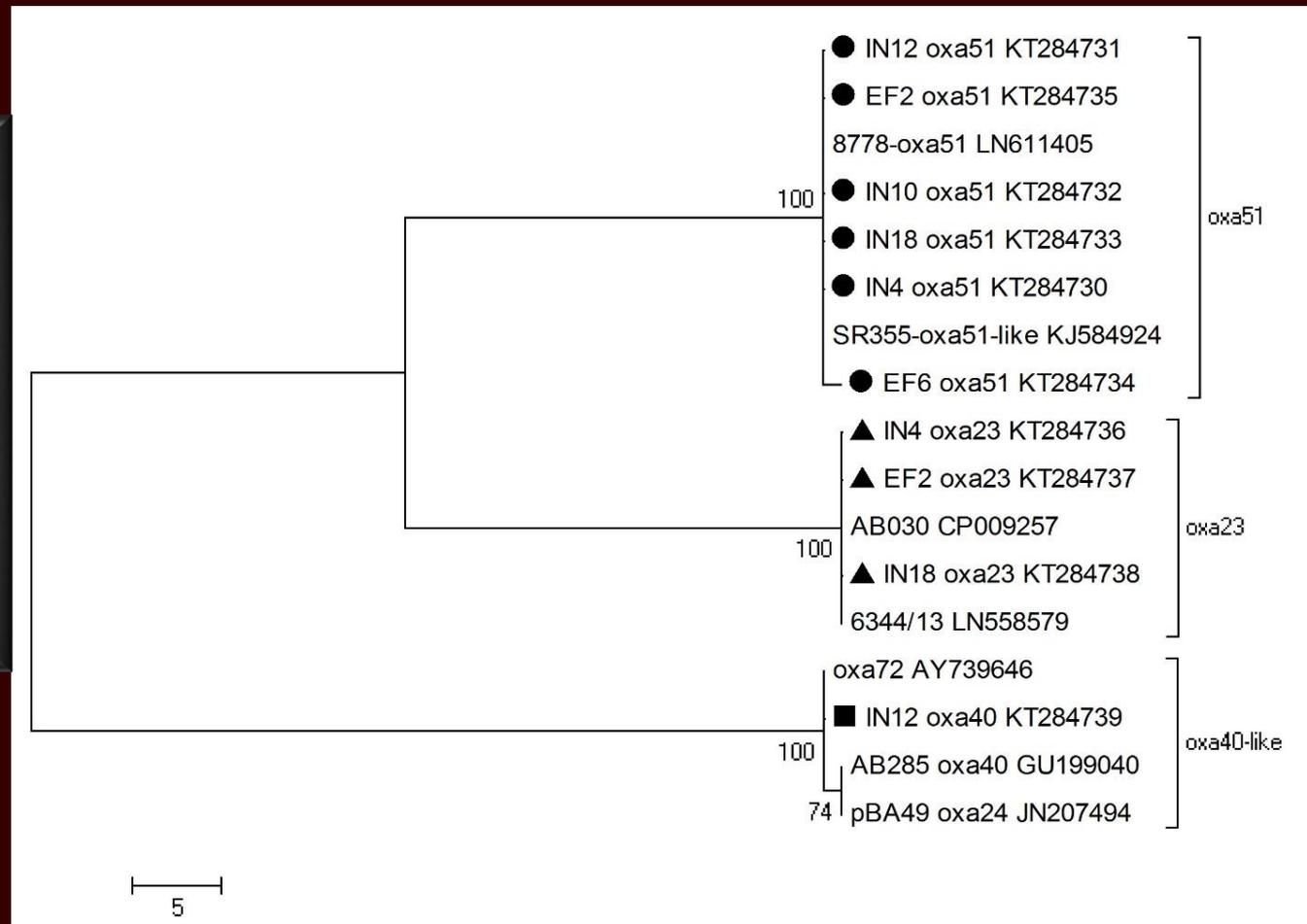
- ✓ Vitek 2 sistem te E-test, razrjeđenje u bujonu
- ✓ tumačenje prema EUCAST i CLSI kriterijima za kliničke izolate

Isolate	MIC values of antibiotics (mg/L)											
	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	>16 ^R	>16 ^R	>16 ^R	>64 ^R	4	4	64 ^I	>320 ^R	>16 ^R
S1/1	>16 ^R	>16 ^R	>4 ^R	>16 ^R	>16 ^R	>16 ^R	>64 ^R	4	<2	>128 ^R	>320 ^R	<0.5
S2/5	>16 ^R	>16 ^R	>4 ^R	>16 ^R	>16 ^R	>16 ^R	>64 ^R	4	>32 ^R	>128 ^R	<20	<0.5
S2/6	>16 ^R	>16 ^R	>4 ^R	>16 ^R	>16 ^R	>16 ^R	>64 ^R	4	>32 ^R	>128 ^R	<20	<0.5
S2/7	>16 ^R	>16 ^R	>4 ^R	>16 ^R	>16 ^R	>16 ^R	>64 ^R	4	>32 ^R	>128 ^R	<20	<0.5
S2/8	>16 ^R	>16 ^R	>4 ^R	>16 ^R	>16 ^R	>16 ^R	>64 ^R	4	>32 ^R	>128 ^R	<20	<0.5
S2/9	>16 ^R	>16 ^R	>4 ^R	>16 ^R	>16 ^R	>16 ^R	>64 ^R	16 ^I	>128 ^R	160 ^R	<0.5	
S2/10	8 ^I	>16 ^R	>4 ^R	>16 ^R	>16 ^R	>16 ^R	>64 ^R	4	64 ^I	>320 ^R	>16 ^R	
IN32	>16 ^R	>16 ^R	>4 ^R	>16 ^R	>16 ^R	>16 ^R	>64 ^R	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 Lovreč u Istri.



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 Science, Division of Biology, Zagreb, Croatia^b; Department of Clinical Microbiology, University Hospital Centre Split and University Hospital Centre Zadar, Split, Croatia^c; and Dalmatia County, Split, Croatia^d

Over the past decade, bacteria of the genus *Acinetobacter* have emerged as significant hospital pathogens. Breaks of *Acinetobacter* infections are considered to be caused exclusively in hospital environments. The natural habitats of clinically important multiresistant strains of *Acinetobacter* spp. are not well understood. We report an incidental finding of a viable multidrug-resistant strain of *Acinetobacter baumannii* in a paleosol from Croatia. The environmental isolate of *A. baumannii* was similar to a clinical isolate from a hospital in this geographic area and was resistant to gentamicin, trimethoprim-sulfamethoxazole, and tetracycline. In paleosol, the isolate was able to survive a low pH (3.37), demonstrating the ability of *A. baumannii* to survive in acidic environments. The presence of *A. baumannii* in paleosol is likely due to the illegal disposal of external waste. The bacteria could have been leached from waste by storm water.

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*



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

^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-ticarcilin/clavulanic acid), inhibitori folatnog puta (SXT-trimethoprim/sulfamethoxazole), polimiksini (CST-colistin).

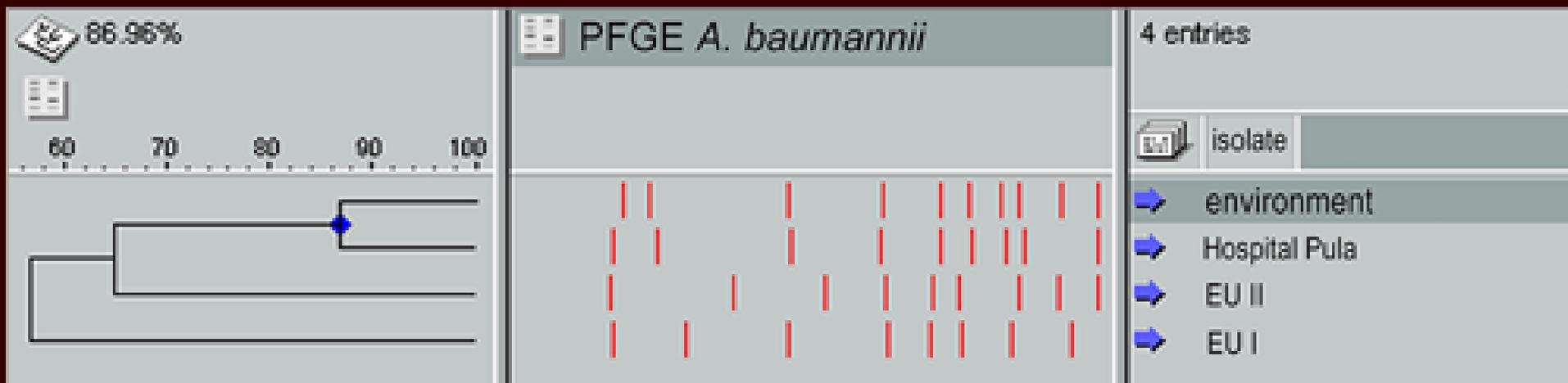
Izolat	MIC vrijednosti antibiotika (mg/L)									
	MEM	IPM	CIP	LVX	TOB	GEN	AMK	SAM	SXT	CST
Paleosol	≤0.5	≤0.5	≥4 ^R	4 ^R	≤1	>16 ^R	2	4	160 ^R	≤0.5

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

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

Ova nesporigena bakterija 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.

[Sci Total Environ.](#) 2017 Dec 31;607-608:1049-1055. doi: 10.1016/j.scitotenv.2017.07.108. Epub 2017 Jul 27.

Extensively and multi drug-resistant *Acinetobacter baumannii* recovered from technosol at a dump site in Croatia.

[Hrenovic J¹](#), [Durn G²](#), [Music MS¹](#), [Dekic S¹](#), [Troskot-Corbic T³](#), [Skoric D¹](#).

⊕ Author information

Abstract

In a karst pit above City of Rijeka in Croatia the hazardous waste was periodically used as an illegal dump site. The surface was geochemically and bacteriologically investigated. From the technosol *Acinetobacter baumannii* were recovered. Isolates from the dump site showed the affiliation to IC1 and 2, multi-drug resistant carbapenem resistance mediated by bla_{OXA72} and bla_{OX} isolates were able to survive in contact with technosol derived from the illegally disposed hospital waste. Proposed the spread of clinically important *A. baumannii* in nature.

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KEYWORDS: Bacteria; Environment; Hydrocarbons; Tar; Waste

PMID: 28724243 DOI: [10.1016/j.scitotenv.2017.07.108](#)



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-ciprofloksacin, LVX-levofloksacin), aminoglikozidi (TOB-tobramycin, GEN-gentamicin, AMK-amikacin), tetraciklini (MIN-minocycline), penicilin/ β -laktamazni inhibitori (SAM-ampicilin/sulbactam, TIM-ticarcilin/clavulanic acid), inhibitori folatnog puta (SXT-trimethoprim/sulfamethoxazole), polimiksini (CST-colistin).

Izolat	MIC vrijednosti antibiotika (mg/L)											
	MEM	IPM	CIP	LVX	TOB	GEN	AMK	MIN	SAM	TIM	SXT	CST
Sovjak1	$\geq 16^R$	$\geq 16^R$	$\geq 4^R$	4^R	≤ 1	≤ 1	32^R	≤ 1	16^I	$\geq 128^R$	≤ 20	≤ 0.5
Sovjak2	$\geq 16^R$	$\geq 16^R$	$\geq 4^R$	4^R	≤ 1	≤ 1	16^I	≤ 1	16^I	$\geq 128^R$	≤ 20	≤ 0.5
Sovjak3	$\geq 16^R$	$\geq 16^R$	$\geq 4^R$	4^R	≤ 1	≤ 1	$>64^R$	8^I	16^I	$\geq 128^R$	$\geq 320^R$	≤ 0.5

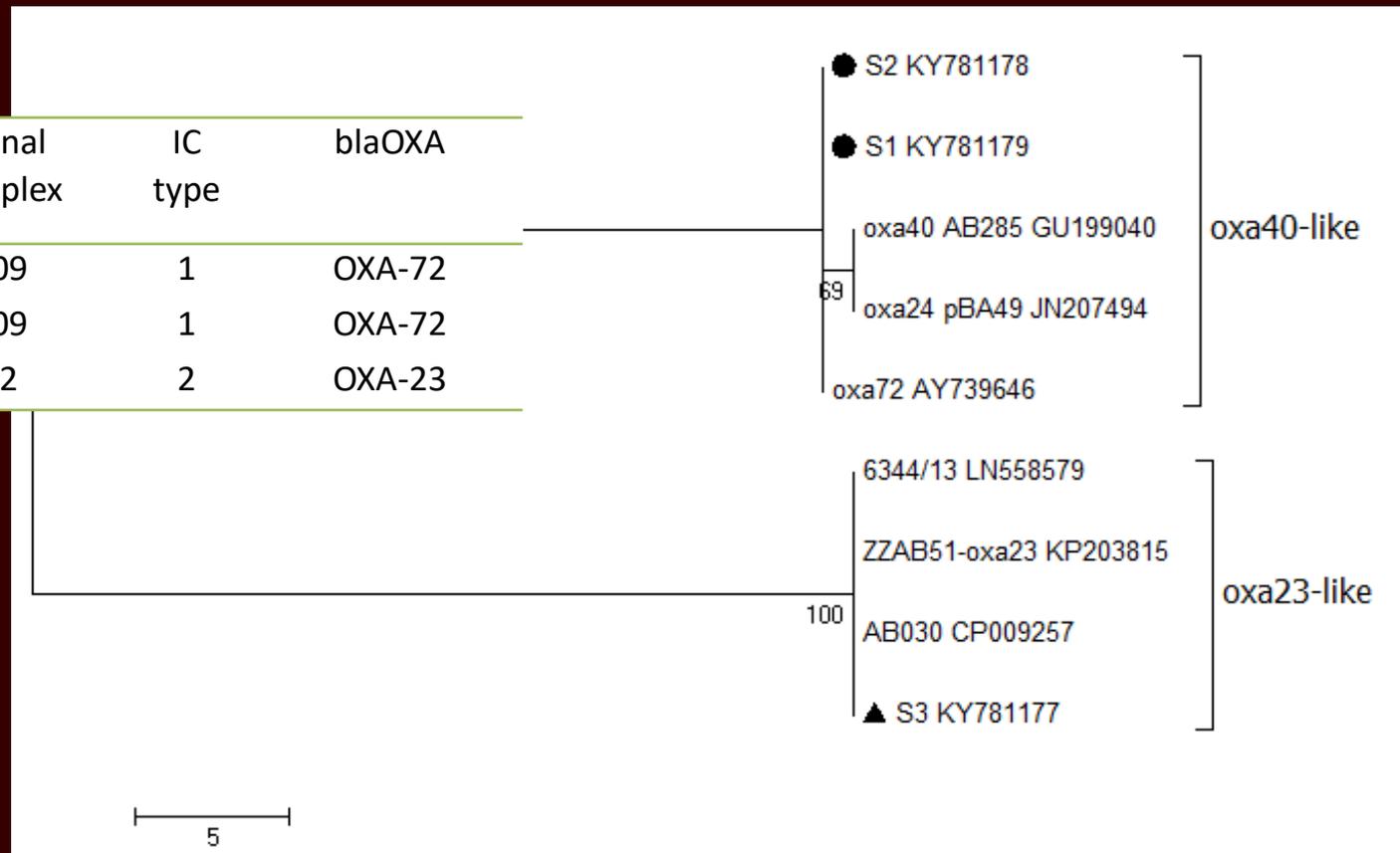
Dva izolata (Sovjak 1, 2) višestruko rezistentni (MDR), jedan izolat (Sovjak 3) prošireno rezistentan (XDR).

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/XDR profil antibiotske rezistencije
- rezistenciju na karbapeneme posredovanu genima *bla*_{OXA72} i

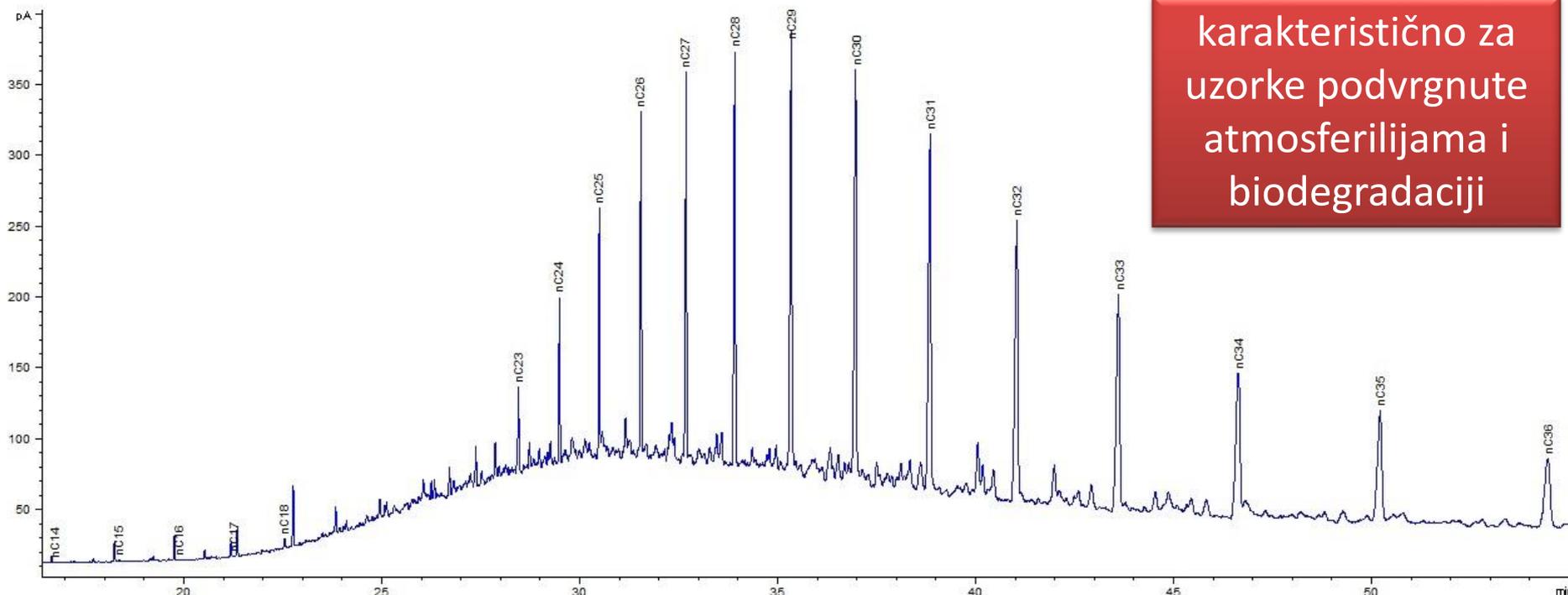
*bla*_{OXA23-like}

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



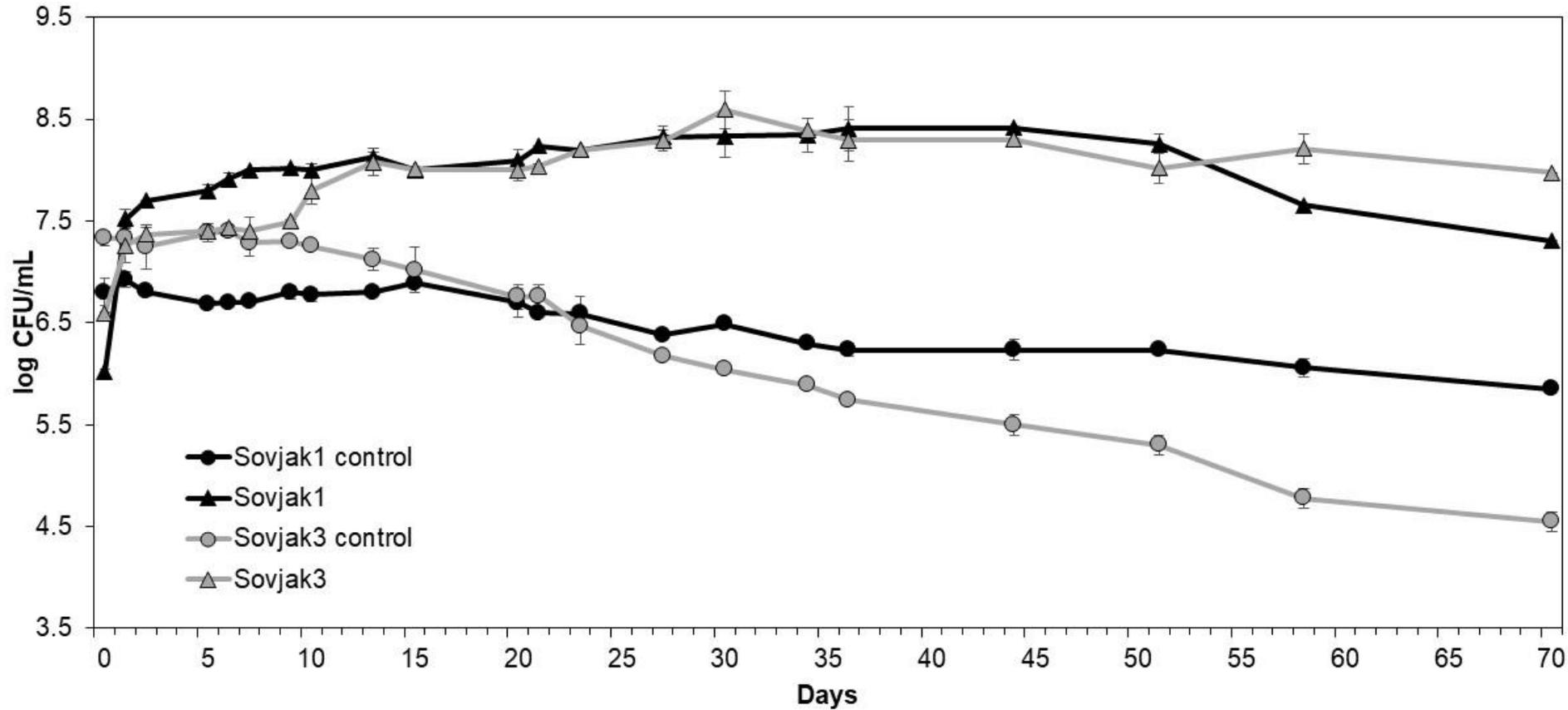
Vjerojatan izvor *A. baumannii* je ilegalno odbačen bolnički kruti otpad.

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.

Izolati se umnažaju i preživljavaju u tehnotlu 70 dana.



Preživljavanje izolata *A. baumannii* u prirodnoj izvorskoj vodi (kontrola) i prirodnoj izvorskoj vodi s 10% tehnotla.

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

Uzorkovanje izvršeno u glavnom revizionom oknu jedne Zagrebačke bolnice. Izolati iz otpadnih voda su uspoređeni s kliničkim izolatima prikupljenim iz iste bolnice u istom vremenskom periodu praćenja.

[J Hosp Infect.](#) 2017 Aug;96(4):323-327. doi: 10.1016/j.jhin.2017.04.005. Epub 2017 Apr 11.

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

[Seruga Music M](#)¹, [Hrenovic J](#)², [Goic-Barisic I](#)³, [Hunjak B](#)⁴, [Skoric D](#)¹, [Ivankovic T](#)¹.

⊕ Author information

Abstract

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.

METHODS: Clinical isolates from patients with hospital-acquired pneumonia and environmental isolates from water were collected during a two-month monitoring period. Recovery of *A. baumannii* was performed using CHROMagar *Acinetobacter* plates, incubated at 42°C for 48 h. Identification was performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry and analyses of *rpoB* gene. The antibiotic resistance profiles were interpreted according to criteria given for clinical isolates of *A. baumannii*. The sequence types (ST) were retrieved by multi-locus sequence typing.

RESULTS: Fourteen of 19 isolates recovered from patients, hospital wastewaters, urban sewage and river water belonged to ST-195. The remaining five isolates recovered from patients and river water were assigned to ST-1421. All isolates showed very strong relatedness and clustered into CC92, which corresponds to IC2. All isolates were non-susceptible to at least one agent in all but two or fewer antimicrobial categories, and thus were classified as 'extensively-drug-resistant' (XDR). Heteroresistance to colistin was found in two isolates from hospital wastewater.

CONCLUSION: Close relatedness of clinical and environmental isolates suggests the emission of XDR *A. baumannii* via the untreated hospital wastewater in the natural environment.

10 izolata izdvojeno je iz 0.001 - 0.01 mL bolničke vode.

Izolat	Porijeklo	Datum izolacije	Sekvencijski tip	Internacionalni klon
OB 3929	Trahealni aspirat	18. 9. 2015	195	2
OB 4138	Bronhialni aspirat	2. 10. 2015	195	2
S2/1	Bolnička otpadna voda	27. 8. 2015	195	2
S2/2			195	2
S2/3			195	2
S2/4			195	2
S1/1		6. 10. 2015	195	2
S2/5			195	2
S2/6			195	2
S2/7			195	2
S2/8			195	2
S2/9		195	2	

A. baumannii iz bolničkih voda i klinički izolati pripadaju istom ST.

MIC vrijednosti antibiotika (mg/L)

Izolant	MEM	IPM	CIP	LVX	TOB	GEN	AMK	MIN	SAM	TIM	SXT	CST
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 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	80 ^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	20 ^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

Svi izolati prošireno rezistentni (XDR).

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



Journal of Hospital Infection

Available online 11 April 2017

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.

A. baumannii u rijeci

A. baumannii iz Save pripadaju istom ST kao i klinički izolati, izolati iz bolničkih voda i skupne kanalizacije.

Izolat	Porijeklo	Datum izolacije	Sekvencijski tip	Internacionalni klon
OB 3831	Sputum	11. 9. 2015	1421 ^a	2
OB 3929	Trahealni aspirat	18. 9. 2015	195	2
OB 4027	Sputum	24. 9. 2015	1421 ^a	2
OB 4138	Bronhialni aspirat	2. 10. 2015	195	2
S2/1	Bolnička otpadna voda	27. 8. 2015	195	2
S2/2			195	2
S2/3			195	2
S2/4			195	2
S1/1		6. 10. 2015	195	2
S2/5			195	2
S2/6			195	2
S2/7			195	2
S2/8			195	2
S2/9		195	2	
IN32	Skupna kanalizacija	23. 9. 2015	195	2
Sava3	Voda rijeke Save	11. 10. 2015	1421 ^a	2
Sava4			195	2
Sava5			1421 ^a	2
Sava6			1421 ^a	2

^a novi ST

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

Svi izolati prošireno rezistentni (XDR).

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.



Komunalne vode u Zagrebu sastavljene su od kućanskih, bolničkih, industrijskih i oborinskih otpadnih voda.

Uzorkovanje izvršeno na centralnom uređaju za pročišćavanje otpadnih voda grada Zagreba.

RESEARCH ARTICLE

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

J Hrenovic ¹, I Goic-Barisic ¹, S Kazazic ³, A Kovacic ⁴, M Ganjto ⁵, M Tonkic ²

1. University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia
2. University Hospital Centre Split, Department of Clinical Microbiology and University of Split School of Medicine, Split, Croatia
3. Ruder Boskovic Institute, Division of Physical Chemistry, Zagreb, Croatia
4. Institute of Public Health of Split and Dalmatia County, Split, Croatia
5. Zagreb Wastewater - Management and Operation Ltd., Zagreb, Croatia

Correspondence: Jasna Hrenovic (jasna.hrenovic@biol.pmf.hr)

Citation style for this article:

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>

Article submitted on 05 February 2015 / accepted on 14 April 2015

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 hospital [2,9].

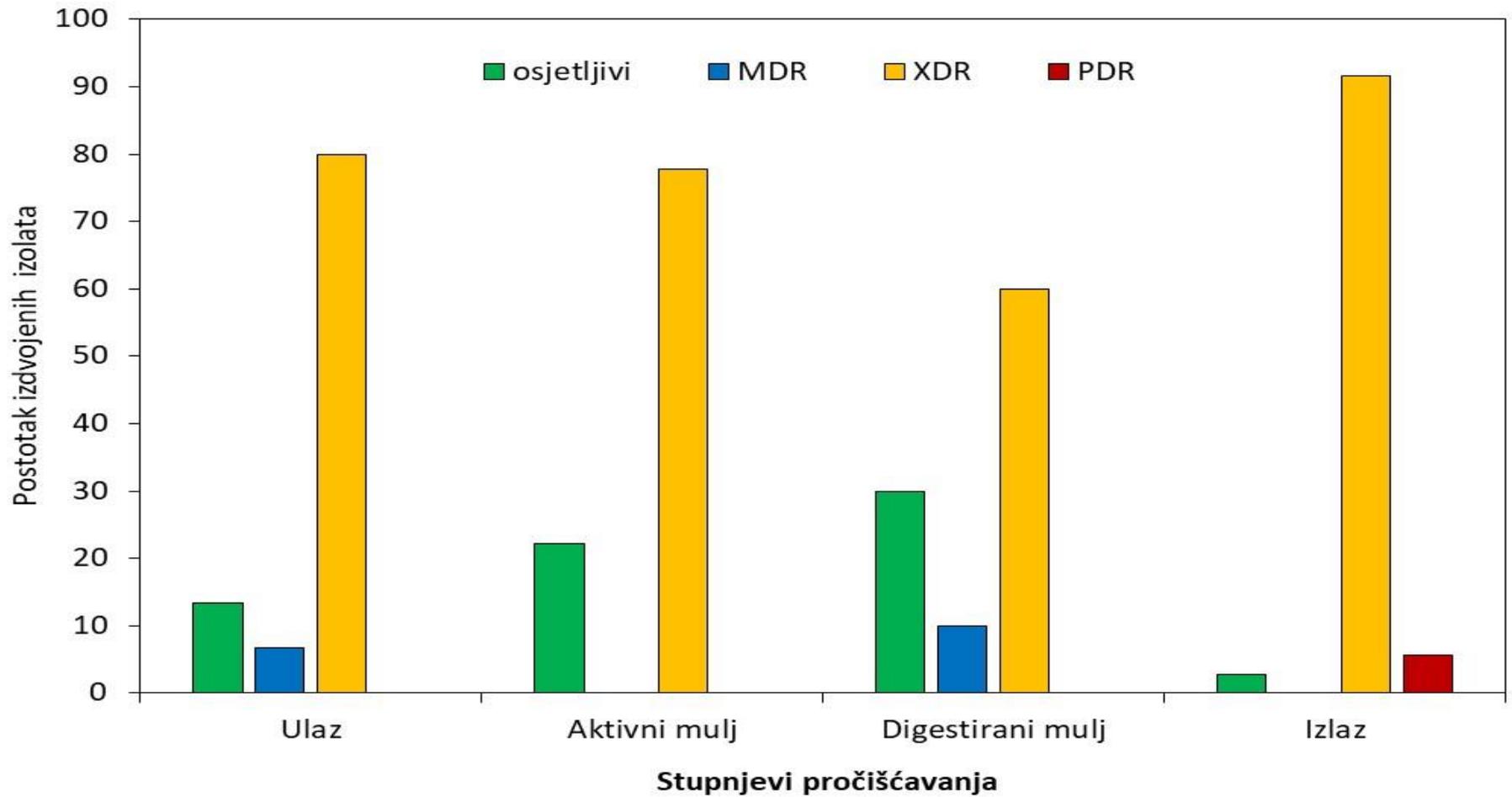
The most important mechanisms in *A. baumannii* involve plasmids, which are encoded by the main phylogenetic subgroup



A. baumannii u komunalnim vodama

Izolat	Sekvencijski tip	Internacionalni klon
Ulazna voda	ST-195	IC2
	ST-195	IC2
	ST-195	IC2
	ST-1526	neklonski
Aktivni mulj	ST-195	IC2
	ST-195	IC2
Digestirani mulj	ST-1525	neklonski
	ST-231	IC1
	ST-195	IC2
	ST-195	IC2
Izlazna voda	ST-195	IC2
	ST-195	IC2
	ST-1523	neklonski
	ST-231	IC1
	ST-195	IC2

A. baumannii izdvojeni iz svake faze pročišćavanja vode, osim stabiliziranog viška mulja (pH 12).



Profil antibiotske osjetljivosti 119 izolata *A. baumannii* izdvojenih iz različitih stupnjeva pročišćavanja otpadnih voda.

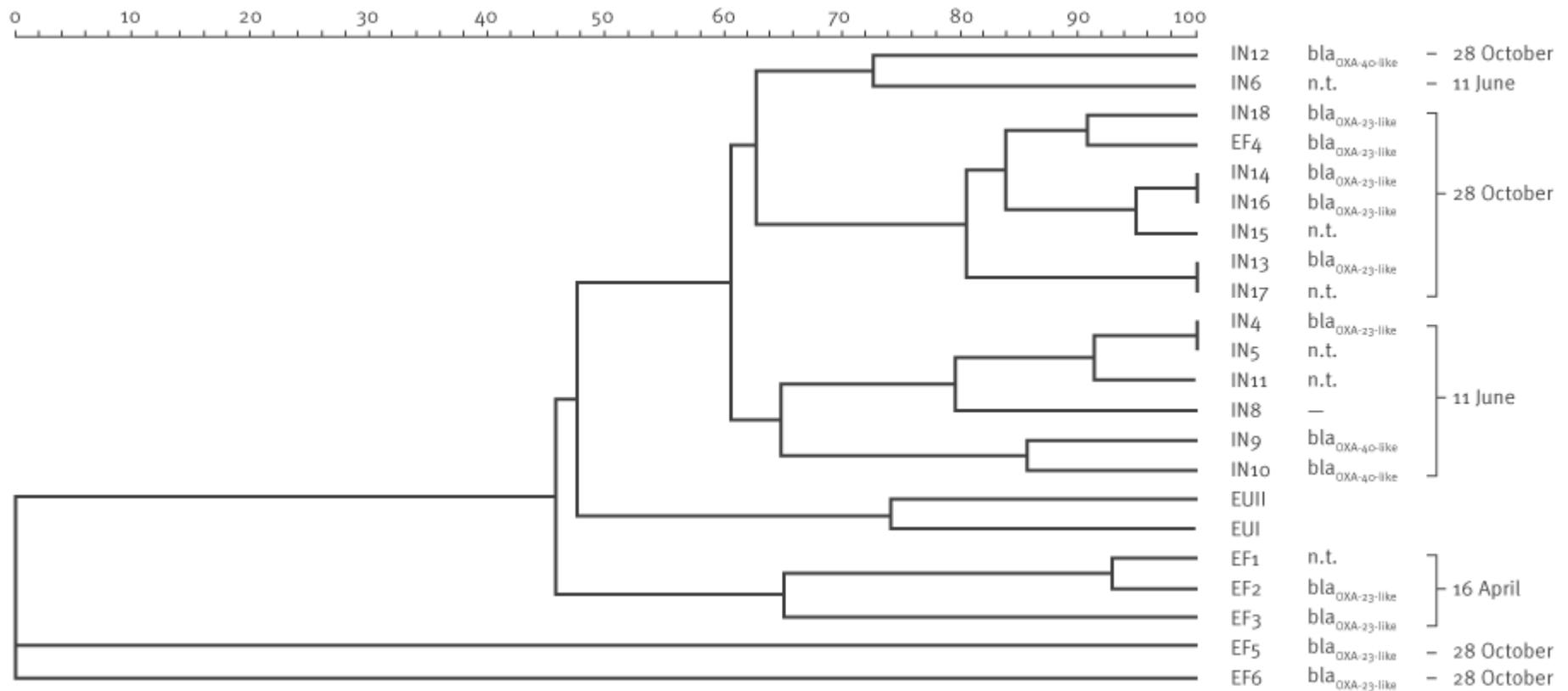
Broj izolata: ulaz 45; aktivni mulj 18; digestirani mulj 20; izlaz 36.

87% izolata rezistentno na karbapeneme i većinu ostalih antibiotika, IC 1 i 2 = klinički značajni

13% izolata senzitivno na sve antibiotike, neklonski = nativni sojevi, prirodno stanište?

Najvažniji mehanizam rezistencije na karbapeneme u *A. baumannii* uključuje OXA-tip karbapenemaza, koje su kodirane genima skupine *bla*_{OXA}.

Karbapenem-rezistentni izolati IC2 posjeduju stečeni gen OXA-23 (dominantni), a izolati IC1 posjeduju stečeni gen OXA-72.



Oksacilinaze u karbapenem rezistentnih izolata *A. baumannii* iz nepročišćenih i pročišćenih komunalnih voda su blisko srodne oksacilinazama u kliničkih izolata.

MICROBIAL DRUG RESISTANCE
Volume 22, Number 7, 2016
© Mary Ann Liebert, Inc.
DOI: 10.1089/mdr.2015.0275

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

Ivana Goic-Barisic,^{1,2} Jasna Hrenovic,³ Ana Kovacic,⁴ and Martina Šeruga Musić³

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 *rpoB* 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



Microb Drug Resist. 2016 Oct 28. [Epub ahead of print]

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

Goic-Barisic I^{1,2}, Seruga Music M³, Kovacic A⁴, Tonkic M^{1,2}, Hrenovic J³.

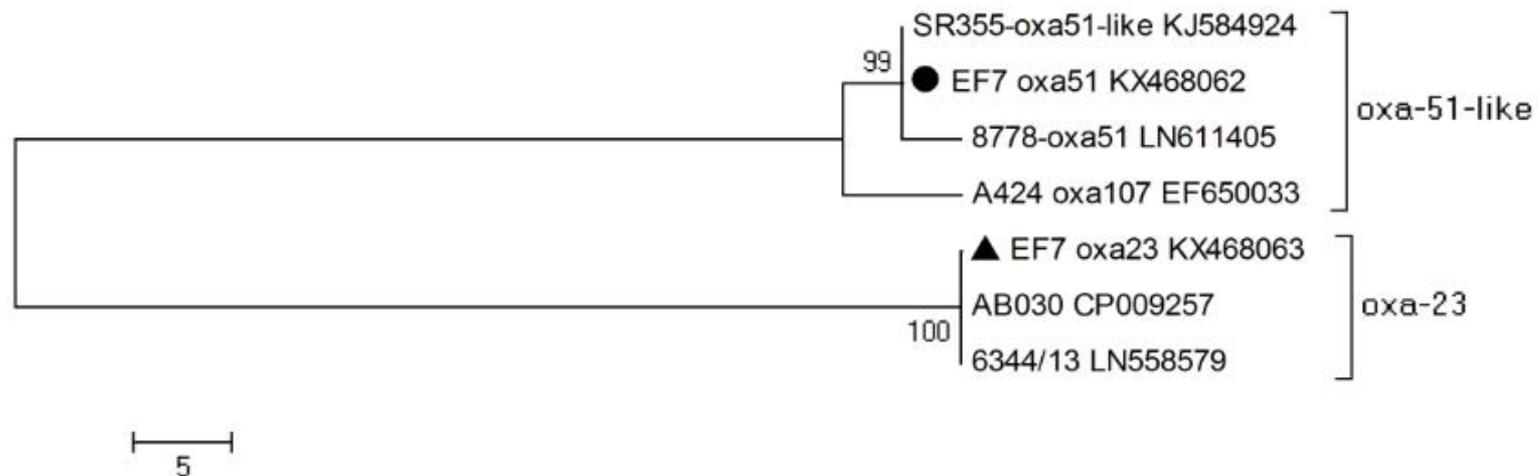
Author information

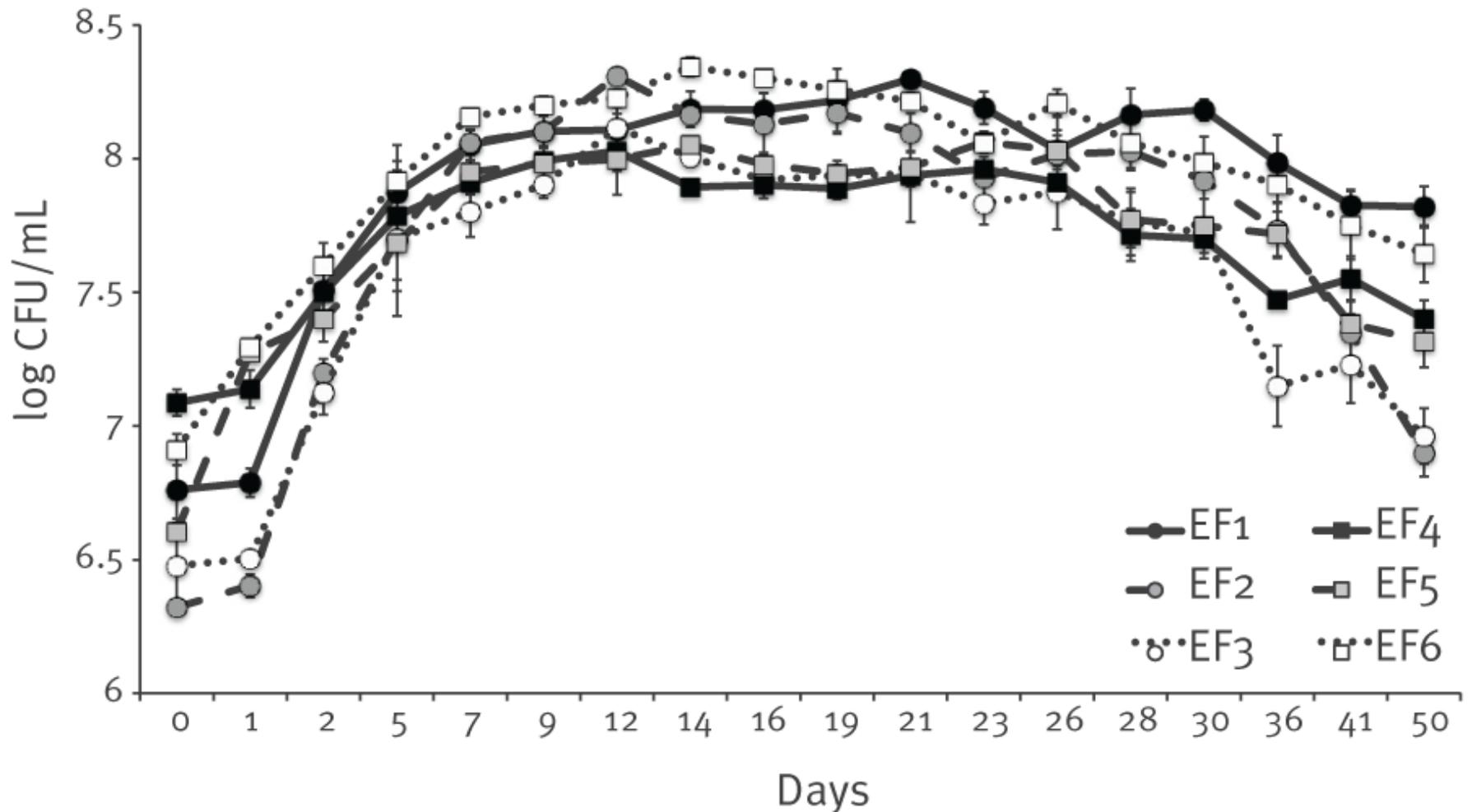
Abstract

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 reservoir of pan drug-resistant *A. baumannii* and carbapenem resistance gene.

KEYWORDS: *Acinetobacter baumannii*; antibiotics; microbial drug resistance; public health; wastewater

PMID: 27792476 DOI: 10.1089/mdr.2016.0229

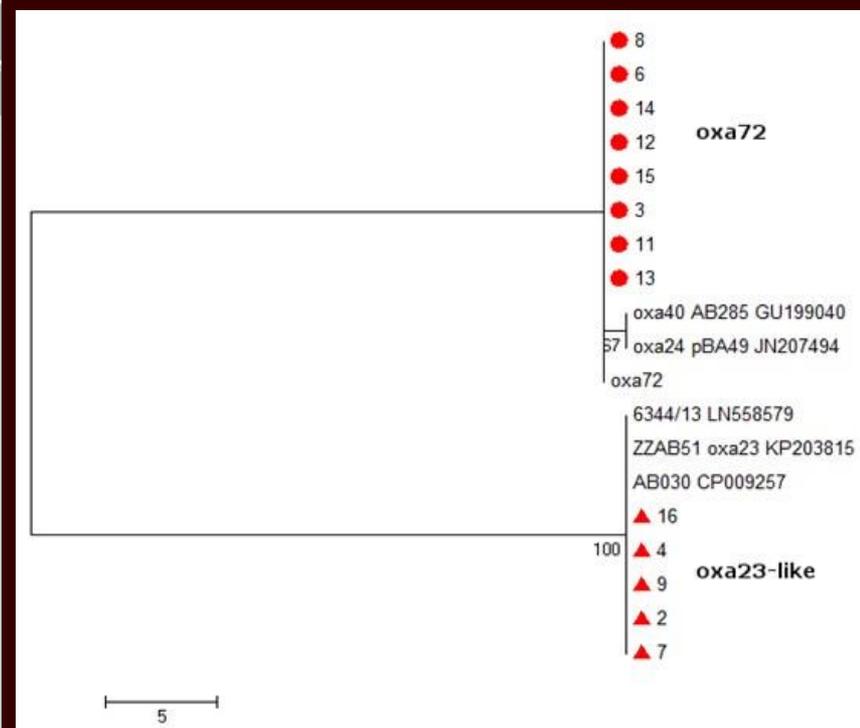
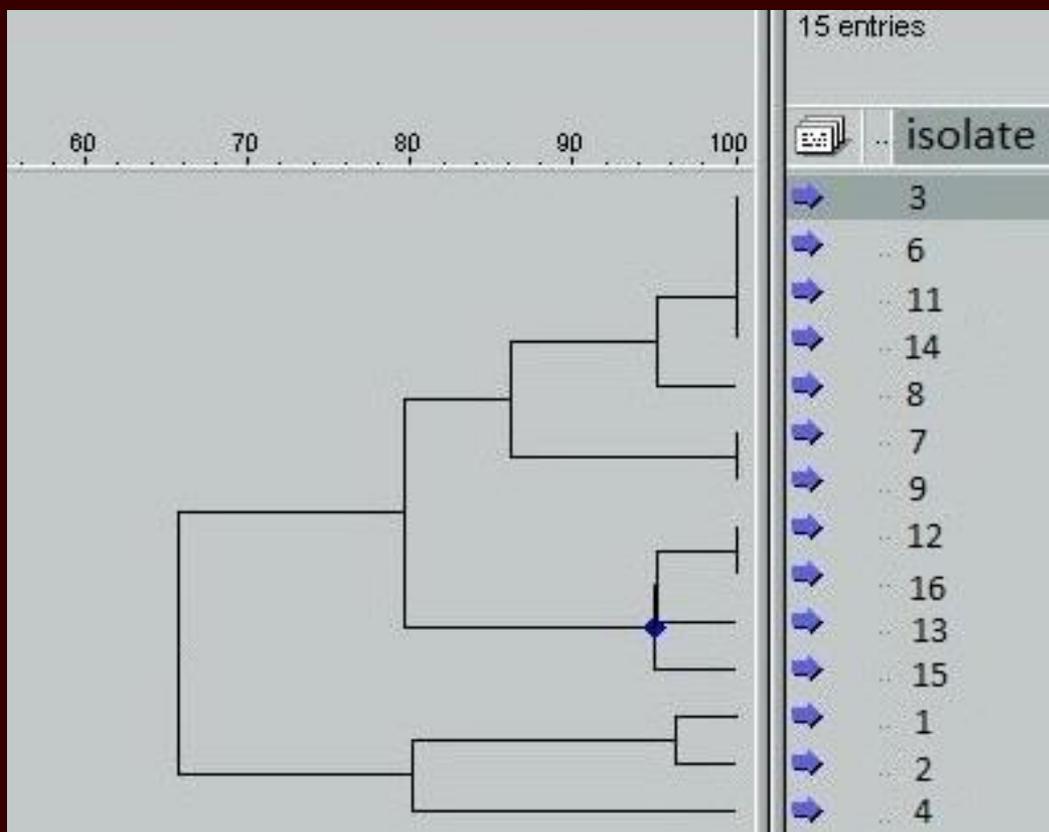


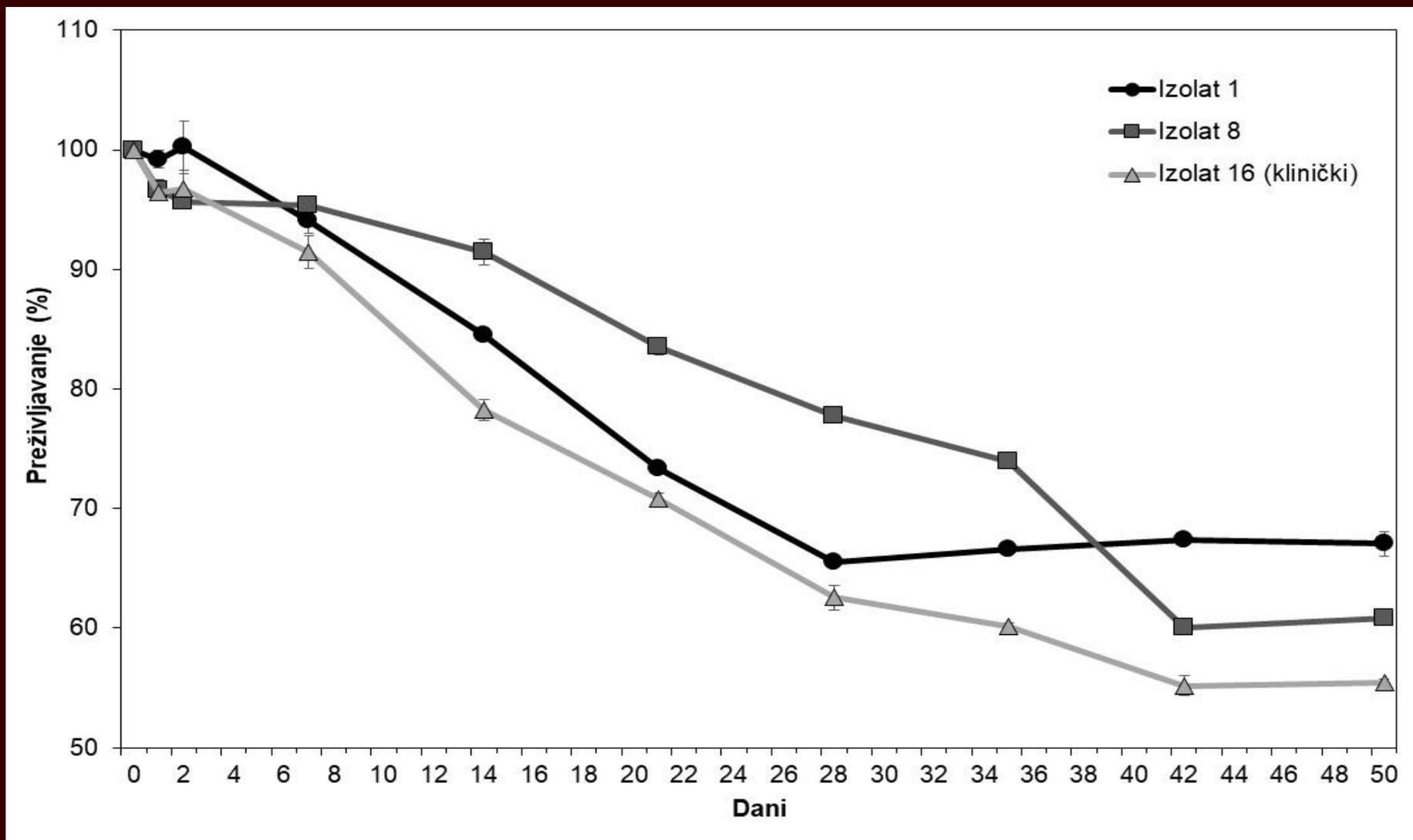


Šest izolata *A. baumannii* izdvojenih iz pročišćene komunalne vode se umnažalo i preživjelo u autoklaviranoj pročišćenoj vodi tijekom 50 dana praćenja.

U dva uzorkovanja iz nepročišćene bolničke vode KBC Split izdvojeno je 10 karbapenem-rezistentnih izolata *A. baumannii* (1-10) koji su genotipski slični kliničkim izolatima iz iste bolnice (13-16) i posjeduju identične oksacilinaze.

Nakon mehaničkog tretmana (rešetke) bolničke vode se ispuštaju u more.





Tri izolata *A. baumannii* preživjela su u autoklaviranoj morskoj vodi tijekom 50 dana praćenja.

U okolišnih izolata *A. baumannii* ispitani su čimbenici koji pridonose njihovoj virulenciji: stvaraje biofilma, pelikule, površinska pokretljivost trzanjem i rojenjem.

Infectious Diseases, 2015; 47: 902–907



ORIGINAL ARTICLE

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

VANJA KALITERNA^{1,4}, MARIANO KALITERNA², JASNA HRENOVIĆ³, ZVONIMIR BARIŠIĆ¹, MARIJA TONKIĆ^{2,4} & IVANA GOIC-BARISIC^{2,4}

From the ¹Public Health Institute of Split-Dalmatia County, Science, Division of Biology, University of Zagreb, Zagreb, a

Abstract

Background: *Acinetobacter baumannii* is one of the most prevalent for the dramatic increase in carbapenem resistance in Croatia. research focused on the organism's ability to form biofilm. **Methods:** Biofilm formation in 109 unrelated clinical isolates was investigated. Genotyping was performed by pulsed-field gel electrophoresis (PFGE) using the disc diffusion method and confirmed by determining biofilm in vitro was determined from overnight cultures of crystal violet, and quantified at 570 nm after solubilization with appropriate program with level of statistical confidence. Re



International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 Volume 6 Number 3 (2017) pp. xx-xx
Journal homepage: <http://www.ijcmas.com>



Original Research Article

<http://dx.doi.org/10.20546/ijcmas>

Virulence Factors of *Acinetobacter baumannii* Environmental Isolates and Their Inhibition by Natural Zeolite

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¹Faculty of Science, University of Zagreb, Zagreb, Croatia

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ABSTRACT

Acinetobacter baumannii is an emerging human pathogen causing great concern in hospitals. There are numerous studies regarding the virulence factors that contribute to the pathogenesis of *A. baumannii* clinical isolates, whereas data regarding environmental isolates are missing. The virulence factors (biofilm formation at the air-liquid/solid-liquid

Keywords

6th Croatian-Slovenian-Serbian Symposium on Zeolites

NATURAL ZEOLITE INFLUENCE ON THE SURFACE MOTILITY OF ACINETOBACTER BAUMANNII

Jasna Hrenović¹, Darko Tibljas¹, Marin Ganjto², Goran Durn³, Ivana Goić-Barišić⁴

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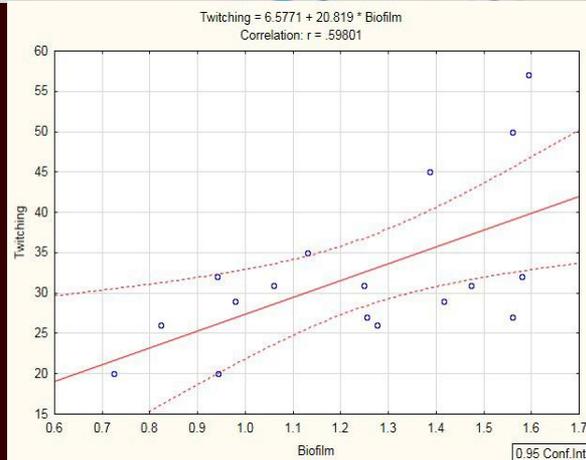
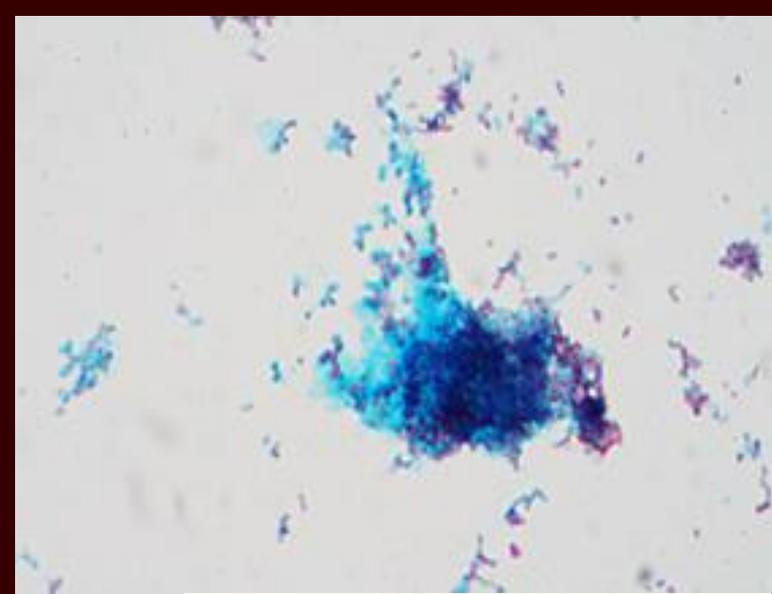
⁴University Hospital Centre Split, Department of Clinical Microbiology and University of Split, School of Medicine, Split, Croatia

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ABSTRACT

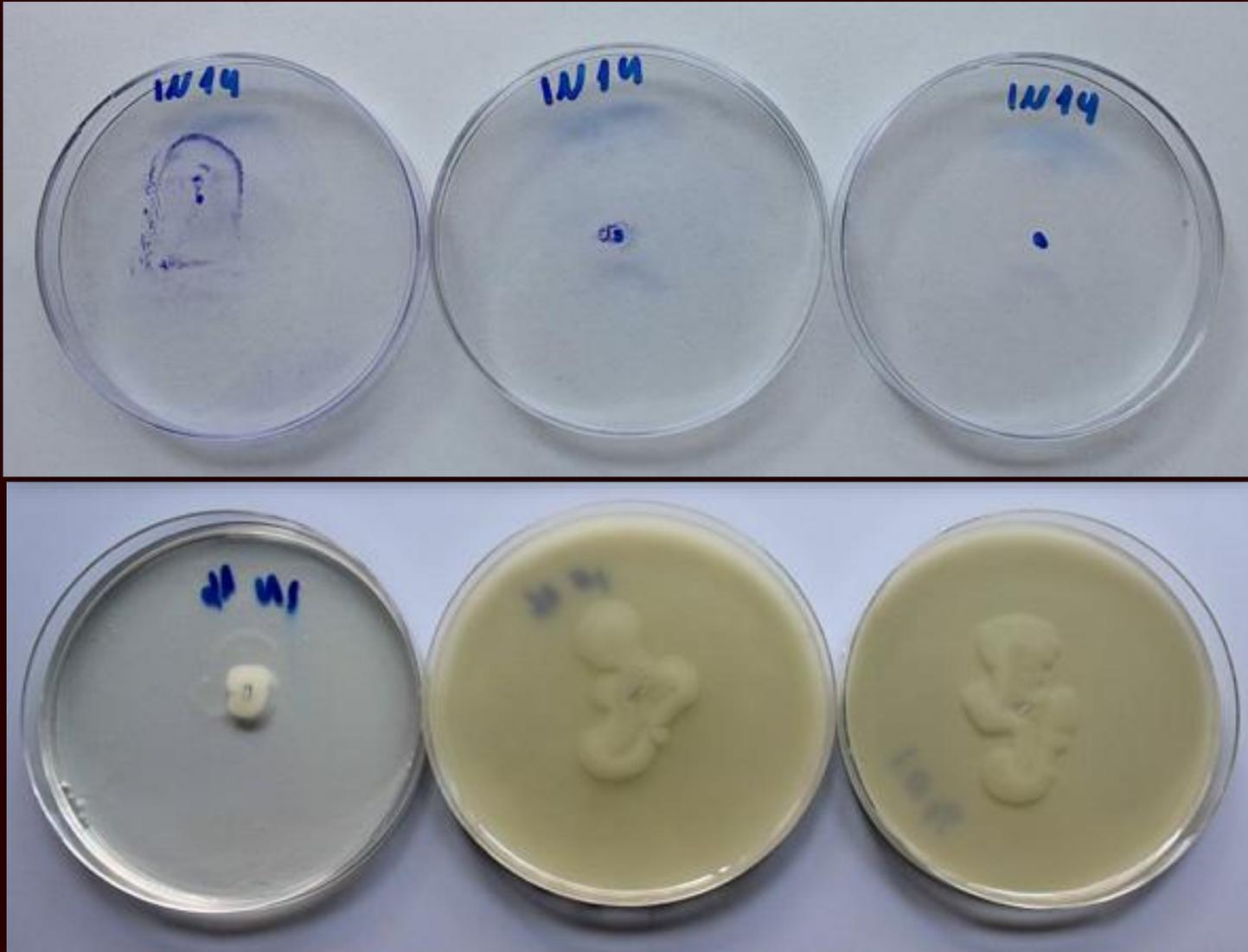
Acinetobacter baumannii is a human pathogen, emergence of which in hospital acquired infections increased dramatically over the last decade, both in Croatia and worldwide. Infection with *A. baumannii* is a major cause of nosocomial infections, and adverse conditions lead to its long-term survival. The ability of *A. baumannii* to form biofilms on various surfaces together with biofilm formation is promising tool to suppress the virulence of *A. baumannii*. Inhibition of twitching motility on zeolitized tuff (NZ) on twitching and swarming motility of *A. baumannii* on polystyrene due to NZ particles. The swarming motility on the surface of NZ could find application in control of the growth of pathogenic bacteria on abiotic surfaces.

Key words: swarming; zeolitized tuff.



Okolišni izolati *A. baumannii* usporedivo kliničkim izolatima:

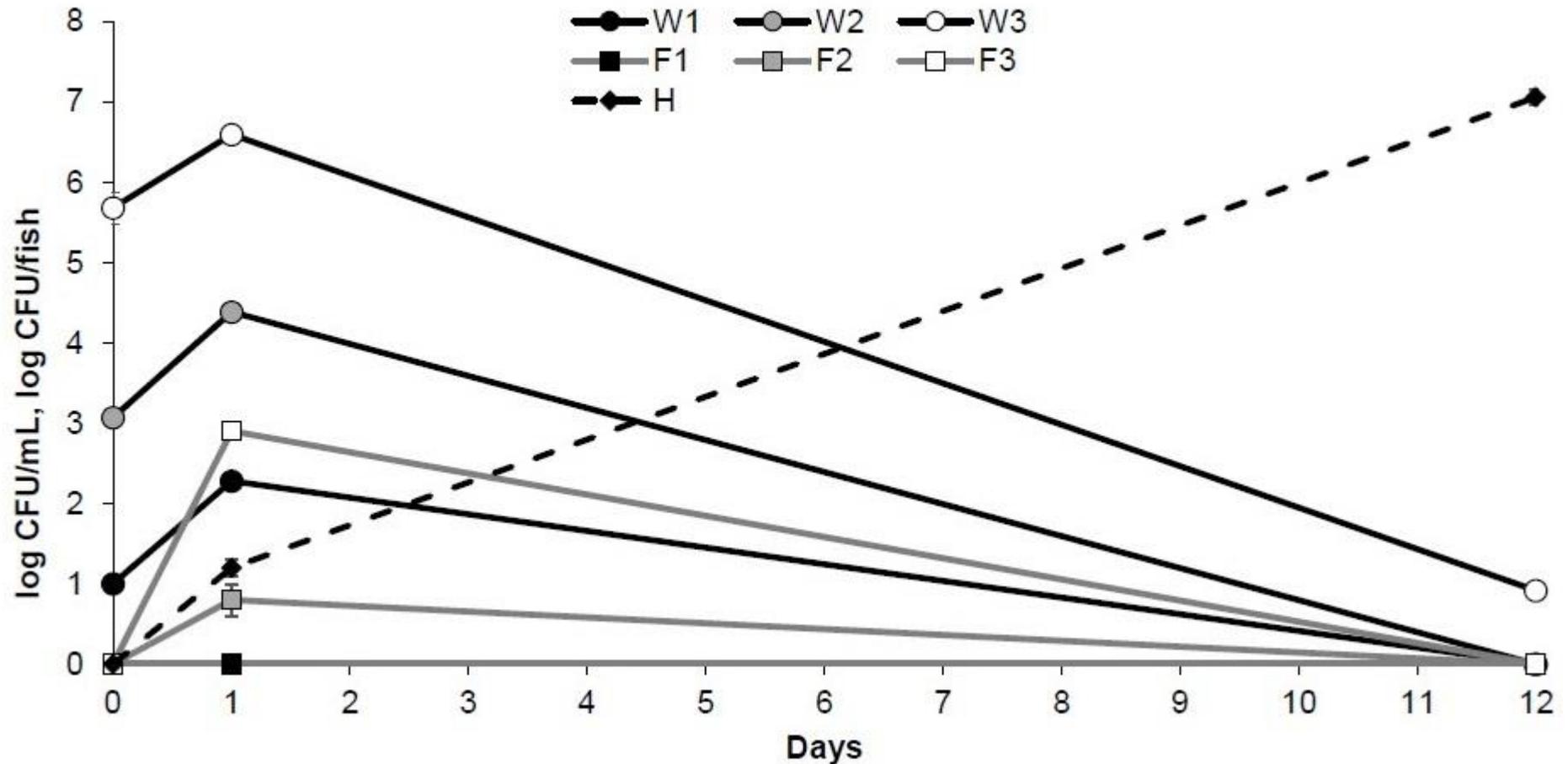
- produciraju ekstracelularne supstance
- tvore biofilm na čvrstim površinama
- tvore biofilm na kontaktu voda-zrak (pelikula)



Okolišni izolati *A. baumannii* usporedivo kliničkim izolatima pokazuju površinsku pokretljivost trzanjem i rojenjem.

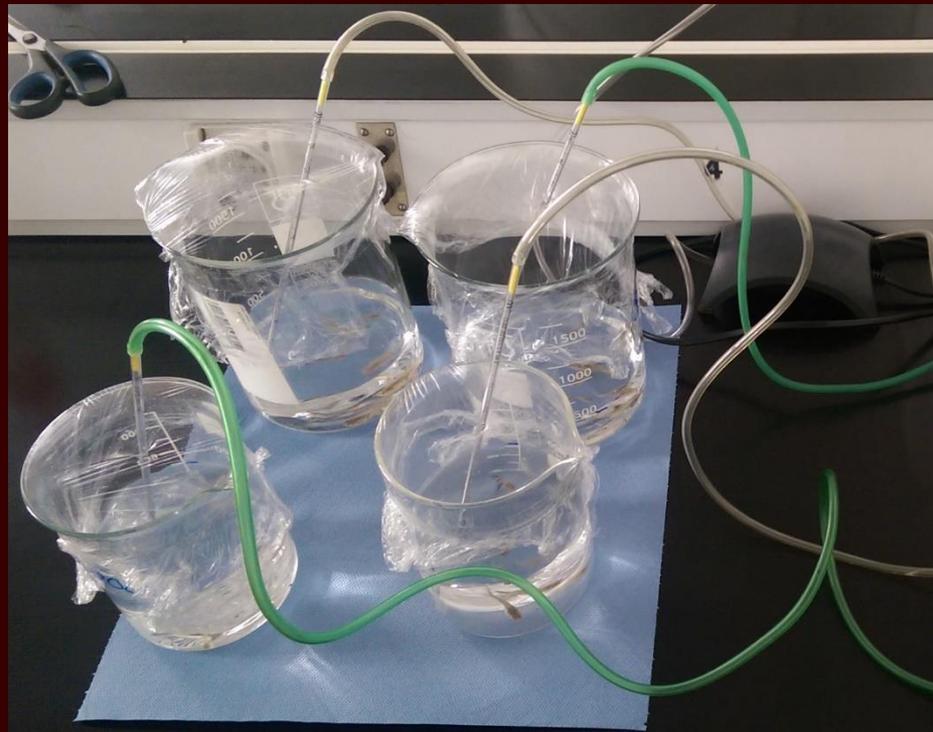
Ispitan je potencijal XDR *A. baumannii* izdvojenog iz Save (Sava4) da kolonizira slatkovodnu ribu iz laboratorijskog uzgoja *Poecilia reticulata* (gupi).





Broj *A. baumannii* u vodi (sustavi W 1-3) i ribama (sustavi F 1-3).
H - ukupne heterotrofne bakterije u vodi sva tri sustava.

- Potencijal kolonizacije *A. baumannii* ovisan je o koncentraciji bakterija u vodi
- Zdrave ribe mogu “odbiti” do 3.1 log CFU/mL *A. baumannii* prisutnih u okolnoj vodi
- *A. baumannii* pokazuje niski potencijal kolonizacije zdravih slatkovodnih riba



A. *baumannii* traženi, ali nisu nađeni:

- Vodama koje nisu pod direktnim utjecajem bolničkih otpadnih voda: izvori, potoci Medvednice, jezero Jarun, bunari na području Zagreba, Krapina uzvodno grada, Sava uzvodno Zagreba.
- Tlima koja nisu pod direktnim utjecajem infektivnog otpada: livade, poljoprivredne površine gnojene stajskim gnojem, koksare, građevinski otpad i drugi inertni otpad.



Zaključci:

- Klinički značajni *A. baumannii* prisutni su u prirodnom okolišu utjecajnom ljudskim infektivnim tekućim ili krutim otpadom.
- *A. baumannii* se nepročišćenim bolničkim vodama ispušta u skupni kanalizacijski sustav.
- U postupku pročišćavanja komunalnih voda *A. baumannii* se umjereno uklanja, ali se ispušta preko pročišćenih voda u prirodu.
- *A. baumannii* mogu preživjeti u prirodi.
- Nove tehnologije dezinfekcije bolničkih otpadnih voda prije ispuštanja u skupni kanalizacijski sustav obećavajuća su strategija za sprečavanje širenja *A. baumannii* u prirodu.
- Nužno je potrebno pravilno zbrinjavanje ljudskog krutog otpada kako bi se spriječilo širenje ovog emergentnog patogena u prirodu.

Hvala na pažnji!

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>

