ACINETOBACTER BAUMANNII U OKOLIŠU

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poslijediplomski tečaj stalnog medicinskog usavršavanja, 10.03.2017., Zagreb

Rod *Acinetobacter* broji 34 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. gerneri	*A. junii*	*A. rudis*	
A. grimontii[a]	*A. kookii*	*A. schindleri*	

[a]Synonym of *A. junii*.

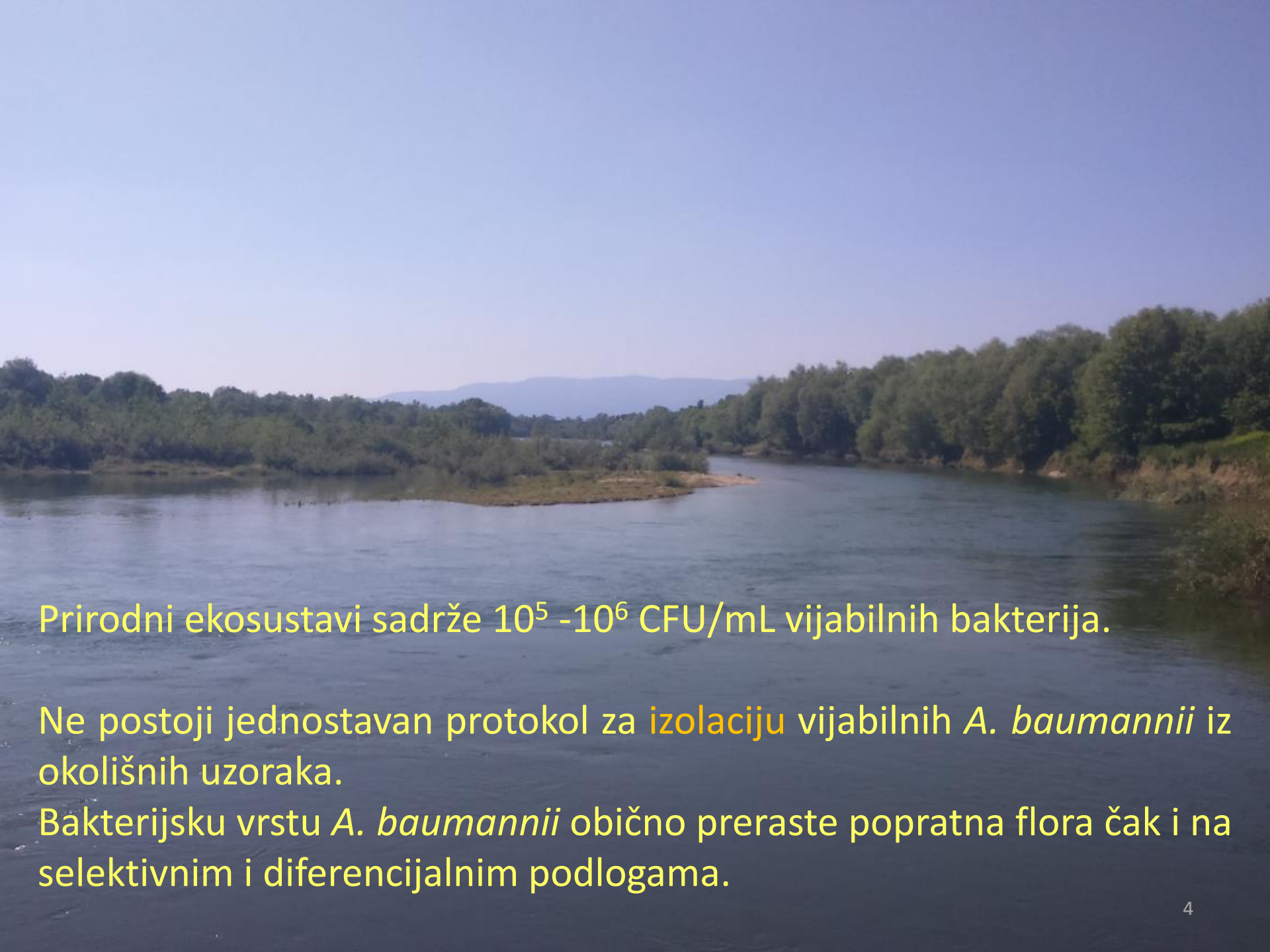
Kim UJ et al. Update on the Epidemiology, Treatment, and Outcomes of Carbapenem-resistant *Acinetobacter infections.* Chonnam Med J. 2014; 50(2): 37–44.

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

Akutne sporadične infekcije ukazuju na mogući izvor ovog patogena izvan bolničkog okruženja.

Za *A. baumannii* nisu razriješeni:

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

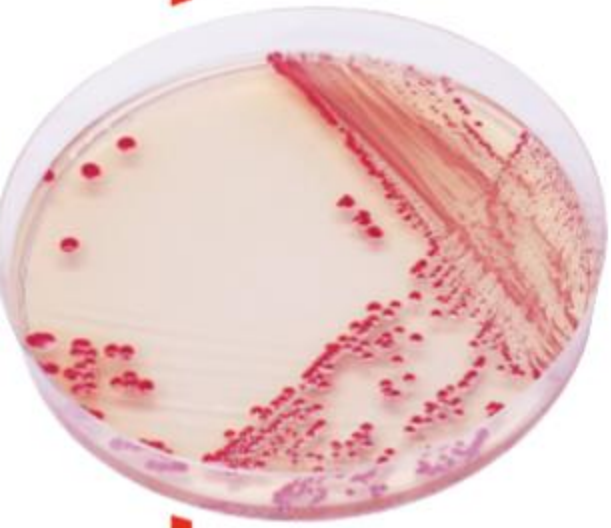


Prirodni ekosustavi sadrže 10^5 -10^6 CFU/mL vijabilnih bakterija.

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



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.

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

Medium Performance

1. **One unique Red colour:** Detection of *A. baumanii* 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 conspiciously red colonies, after overnight incubation.

CHROMagar Acinetobacter, inkubacija 24-72h/37°C

Izolirane vrste:

Stenotrophomonas maltophillia,

Pseudomonas sp. *(P. aeruginosa, P. fluorescens),*

Chryseobacterium sp.*,*

Elizabethkinga meningoseptica,

Enterobacteriaceae…

Bezuspješna izolacija

PREPARATION (Calculation for 1L)

Step 1
Preparation

- Disperse slowly 32.8 g of powder base in 1L of purified water.
- Add 4.0 ml of the liquid supplement AC092(S) into slurry.
- Stir until agar is well thickened.
- Heat and bring to boil (100°C) while swirling or stirring regularly.

DO NOT HEAT TO MORE THAN 100°C. DO NOT AUTOCLAVE AT 121°C.

Warning 1: If using an autoclave, do so without pressure.

Advice: in case of product samples containing a high load of Pseudomonas and/or Aeromonas, Cefsulodin can be added at 5 mg/L.

- Cool in a water bath to 45-50°C, swirling or stirring gently.

Bez promjena - bezuspješna izolacija

OPTION: If screening is focused on MDR Acinetobacter, add the MDR Selective supplement ref CR102 as following:

HELPING CALCULATION

1 L final media --> Use 1 vial

5 L final media --> Use 5 vials

Step 2
OPTIONAL

- Rehydrate one vial with 5ml of purified water.
- Add 5ml of this solution to the melted mix (step 1) at 45-50°C.
- Stir well for homogenization.

Step 3
Pouring

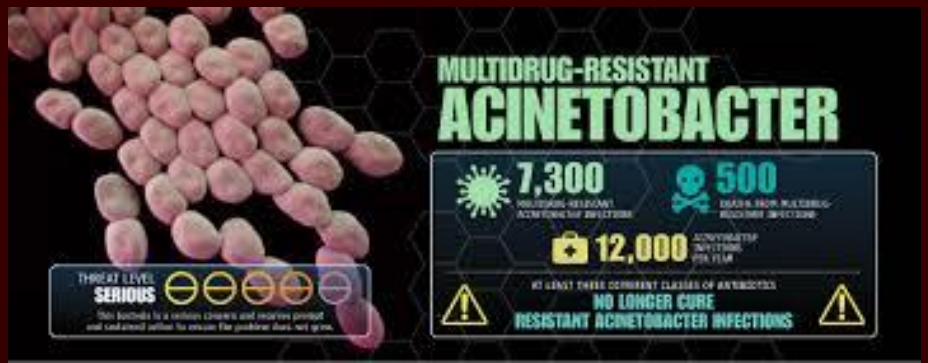
- Pour into sterile Petri dishes.
- Let it solidify and dry.

Warning 2: Slight variation of the media colouration after solidification can be observed, from yellowish to light orange.

Storage

- Store in the dark before use.
- Prepared media plates can be kept for one day at room temperature.
- Plates can be stored for up to one month under refrigeration (2/8°C) if properly prepared and protected from light and dehydration.

Advice: MDR supplement solution can be re-used up to 2 weeks if properly stored at 2/8°C



Bez promjena - bezuspješna izolacija

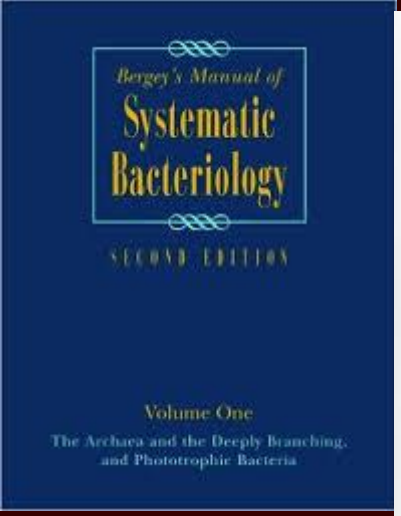


Table 4.12 Differentiation of the species of the genus *Acinetobacter*[a,b]

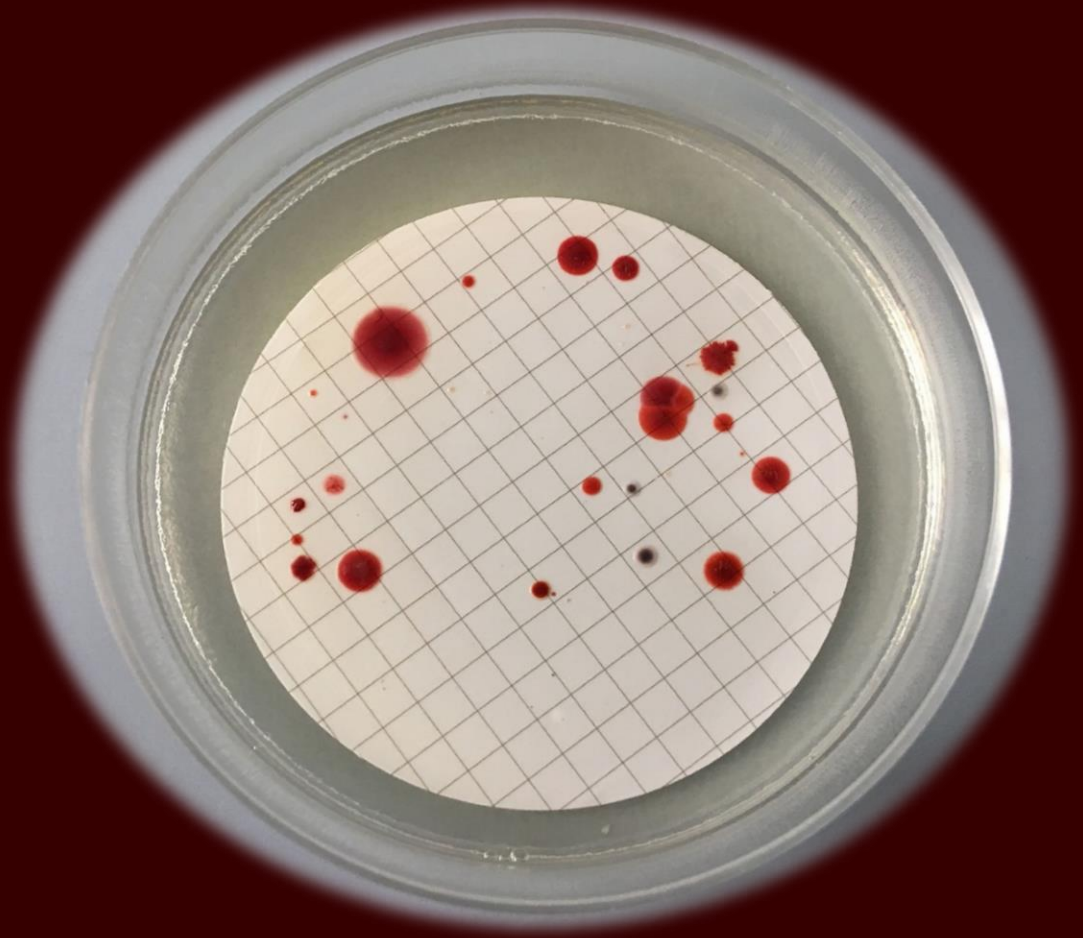
Characteristic	*A. baumanni*	*A. calcoaceticus*	*A. haemolyticus*	*A. johnsonii*	*A. junii*	*A. lwoffii*	Unnamed species "3"	"6"	"10"	"11"	"12"
Growth at:											
44°C	+	–	–	–	–	–	–	–	–	–	–
41°C	+	–	–	–	90	–	+	–	–	–	–
37°C	+	+	+	–	+	+	+	+	+	+	+
Gelatin hydrolysis	–	–	96	–	–	–	–	+	–	–	–
Hemolysis	–	–	+	–	–	–	–	+	–	–	–
Glutamyltransferase	99	+	4	–	–	–	+	66	–	–	–
Citrate (Simmons)	+[c]	+	91	+	82	–	+	+[c]	+	+	–
Acid from glucose	95	+	52	–	–	6	+	66	+	–	33
β-Xylosidase	95	–	52	–	–	6	+	66	–	–	–
Utilization of:											
DL-Lactate	+	+	–	+	+	+	+	–	+	+	+
Glutarate	+	+	–	–	–	–	+	–	+	+	+
L-Phenylalanine	87	+	–	–	–	–	+	–	–	–	+
Phenylacetate	87	+	–	–	–	94	66	–	25	50	+
Malonate	98	+	–	13	–	–	87	–	–	–	+
L-Histidine	98	+	96	–	+	–	94	+	+	+	–
Azelate	90	+	–	–	–	+	+	–	50	25	+
D-Malate	98	–	96	22	+	76	+	66	+	+	–
L-Aspartate	+	+	64	61	40	–	+	66	+	75	–
L-Leucine	97	38	96	–	11	–	94	+	–	–	+
Histamine	–	–	–	–	–	–	–	–	75	+	–
L-Tyrosine	+	+	5	70	60	3	+	66	+	75	+
β-Alanine	95	+	–	–	–	–	94	–	+	+	–
Ethanol	+[c]	+	96	+	+	97	+	+	+	+	+
2,3-Butanediol	+	+	–	35	–	–	+	–	+	+	+
trans-Aconitate	99	+	52	–	–	–	+	–	–	–	–
L-Arginine	98	+	96	35	95	–	+	+	–	–	+
L-Ornithine	93	+	–	4	–	2	+	–	–	–	
DL-4-Amino-butyrate	+	+	+	35	88	40	+	–	+	+	+

[a] Data from Bouvet and Grimont, Int. J. Syst. Bacteriol. *36*: 228-240, 1986.

[b] Symbols: +, all strains positive; –, all strains negative. The numbers are percentages of positive strains.

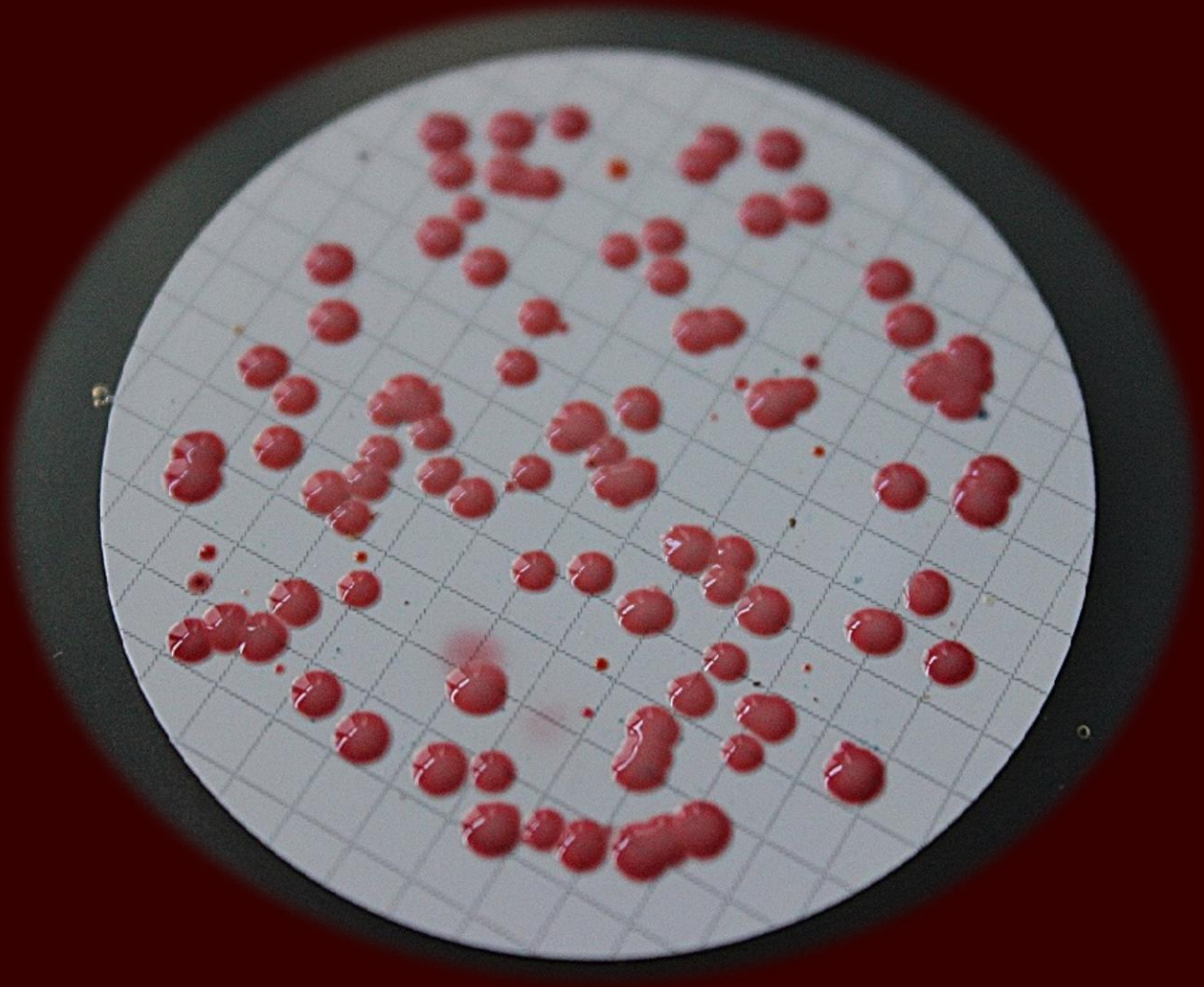
[c] All strains except one or two auxotrophic strains.

CHROMagar Acinetobacter + 5 mg/L cefsulodina, inkubacija 24-72h/42°C



Bezuspješna izolacija

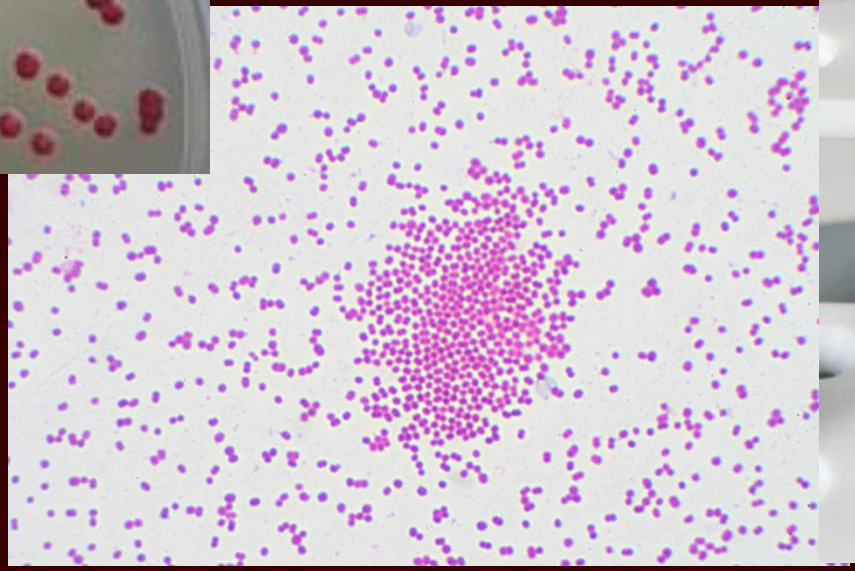
CHROMagar Acinetobacter + 15 mg/L cefsulodina, sa ili bez dodatka suplementa CR102, inkubacija 18-26h/42°C



Izoliran *Acinetobacter baumannii*!

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

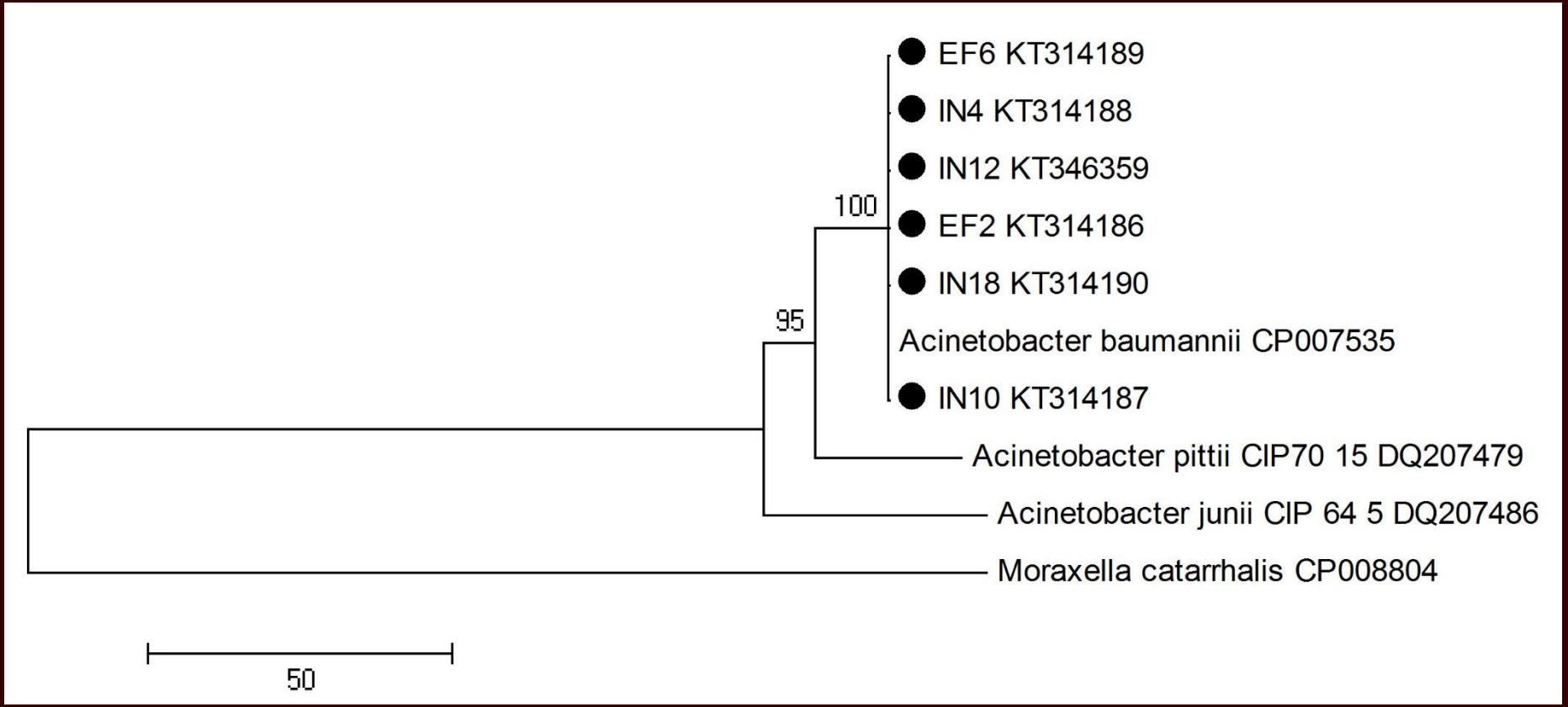
AnalyteName	AnalyteID	Organism(best match)	ScoreValue	Organism(second best match)	ScoreValue
B1(++) (A)	Š 2/6	Acinetobacter baumannii	2.232	Acinetobacter baumannii	2.195
B2(++) (A)	Š 2/5	Acinetobacter baumannii	2.067	Acinetobacter baumannii	2.046
B3(++) (A)	OB 3929	Acinetobacter baumannii	2	Acinetobacter 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

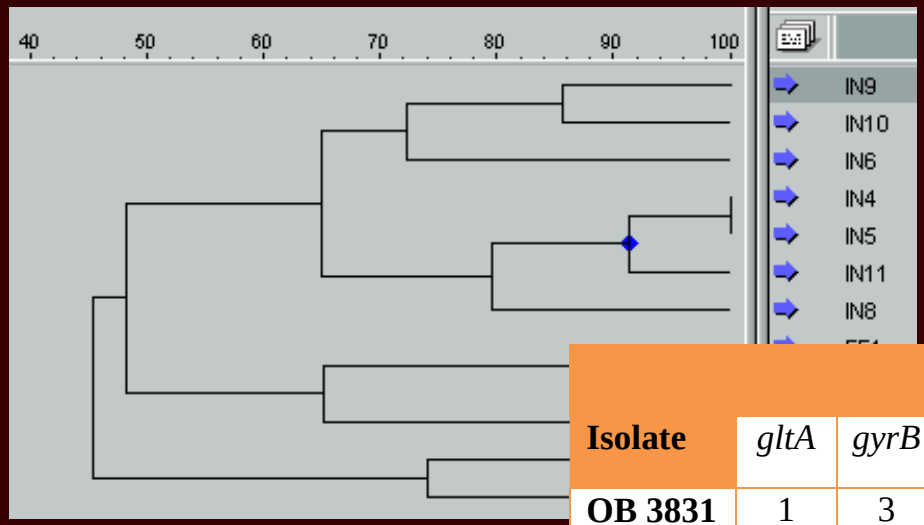
Potvrda identifikacije:

✓ 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

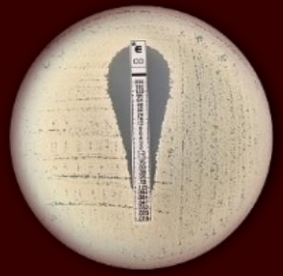


Isolate	Gene locus/allele							Sequence type	Clonal complex	IC type
	gltA	*gyrB*	*gdhB*	*recA*	*cpn60*	*gpi*	*rpoD*			
OB 3831	1	3	3	2	2	96	119 [a]	1421 [a]	92	2
OB 3929	1	3	3	2	2	96	3	195	92	2
OB 3930	1	3	3	2	2	100	3	425	92	2
OB 4027	1	3	3	2	2	96	119 [a]	1421 [a]	92	2
OB 4138	1	3	3	2	2	96	3	195	92	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

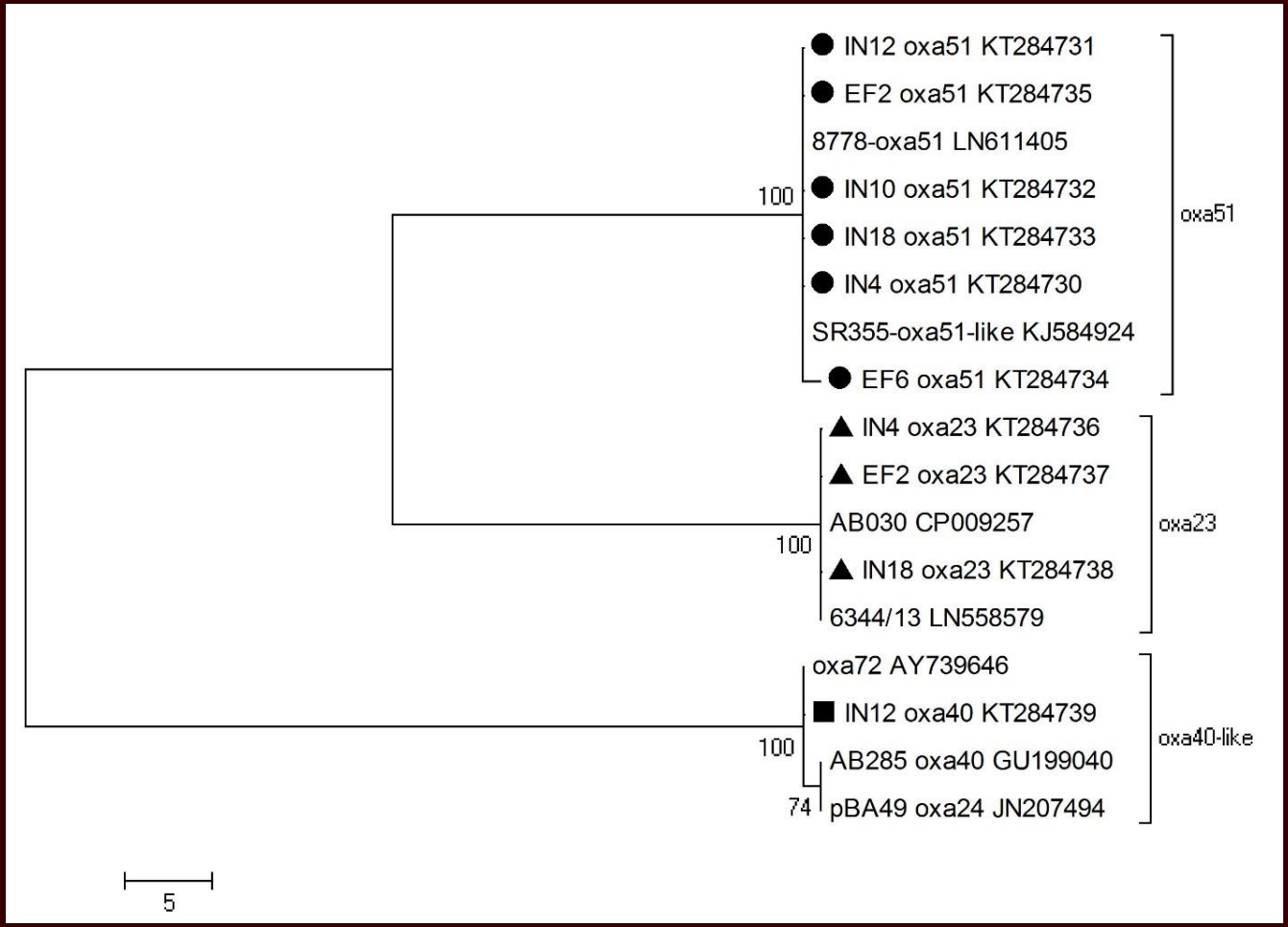
- ✓ Vitek 2 sistem te E-test, razrjeđenje u bujonu
- ✓ tumačenje prema EUCAST i CLSI kriterijima za kliničke izolate

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}	>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 antibiotske rezistencije II

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



A. baumannii u tlu

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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.

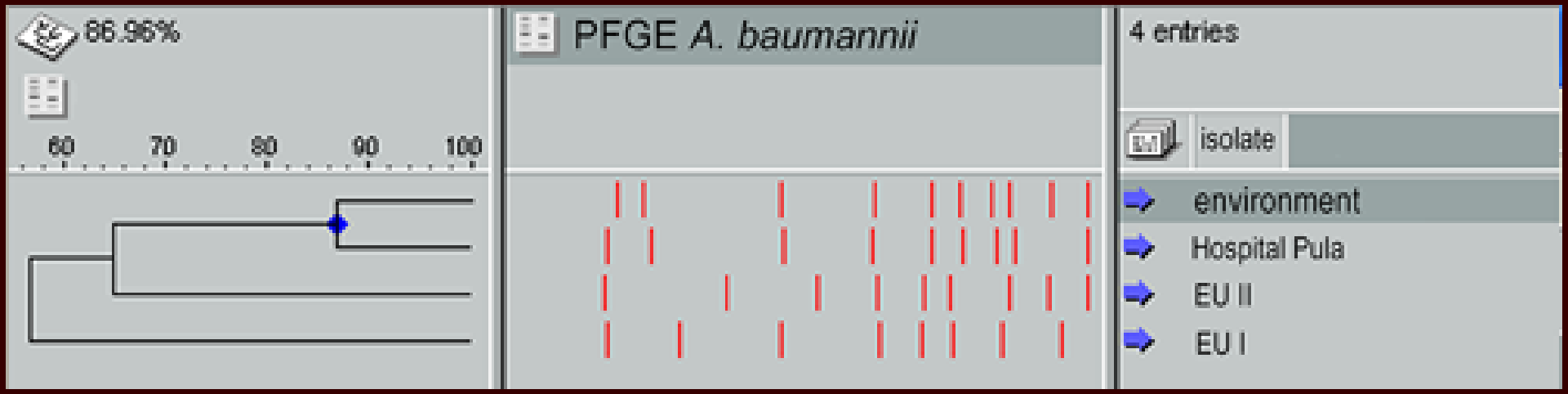
Bacteria of the genus *Acinetobacter* have been recognized as significant hospital pathogens since the late 1970s, but at that time they were easily treated, because they were susceptible to commonly used antimicrobials. *Acinetobacter* spp. have an increasing ability to develop resistance to commonly 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*

MDR *A. baumannii* izoliran iz kiselog (pH 2.55) paleotla u Istri.

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 kruti otpad u okolici napuštenog kamenoloma.



A. baumannii u bolničkim vodama

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

Uzorkovanje izvršeno u glavnom revizionom oknu jedne Zagrebačke bolnice, te u skupnom kanalizacijskom sustavu.

Izolati iz otpadnih voda su uspoređeni s kliničkim izolatima prikupljenim iz iste bolnice u istom vremenskom periodu praćenja.

	MIC values of antibiotics (mg/L)											
Isolate	**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}	$>320^{R}$	$>16^{R}$
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
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

Isolate	Gene locus/allele							Sequence type	Clonal complex	IC type
	gltA	*gyrB*	*gdhB*	*recA*	*cpn60*	*gpi*	*rpoD*			
OB 3929	1	3	3	2	2	96	3	195	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
S2/3	1	3	3	2	2	96	3	195	92	2
S2/4	1	3	3	2	2	96	3	195	92	2
S1/1	1	3	3	2	2	96	3	195	92	2
S2/5	1	3	3	2	2	96	3	195	92	2
S2/6	1	3	3	2	2	96	3	195	92	2
S2/7	1	3	3	2	2	96	3	195	92	2
S2/8	1	3	3	2	2	96	3	195	92	2
S2/9	1	3	3	2	2	96	3	195	92	2
IN32	1	3	3	2	2	96	3	195	92	2

MDR *A. baumannii* iz bolničkih voda i klinički izolati, te izolat iz skupnog kanalizacijskog sustava pripadaju istom ST.

A. baumannii u komunalnim vodama

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 [2], S Kazazic [3], A Kovacic [4], M Ganjto [5], M Tonkic [2]
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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 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 Apr

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 mechanis
ance in *A. baumannii* involv
mases, which are encoded by
main phylogenetic subgroup

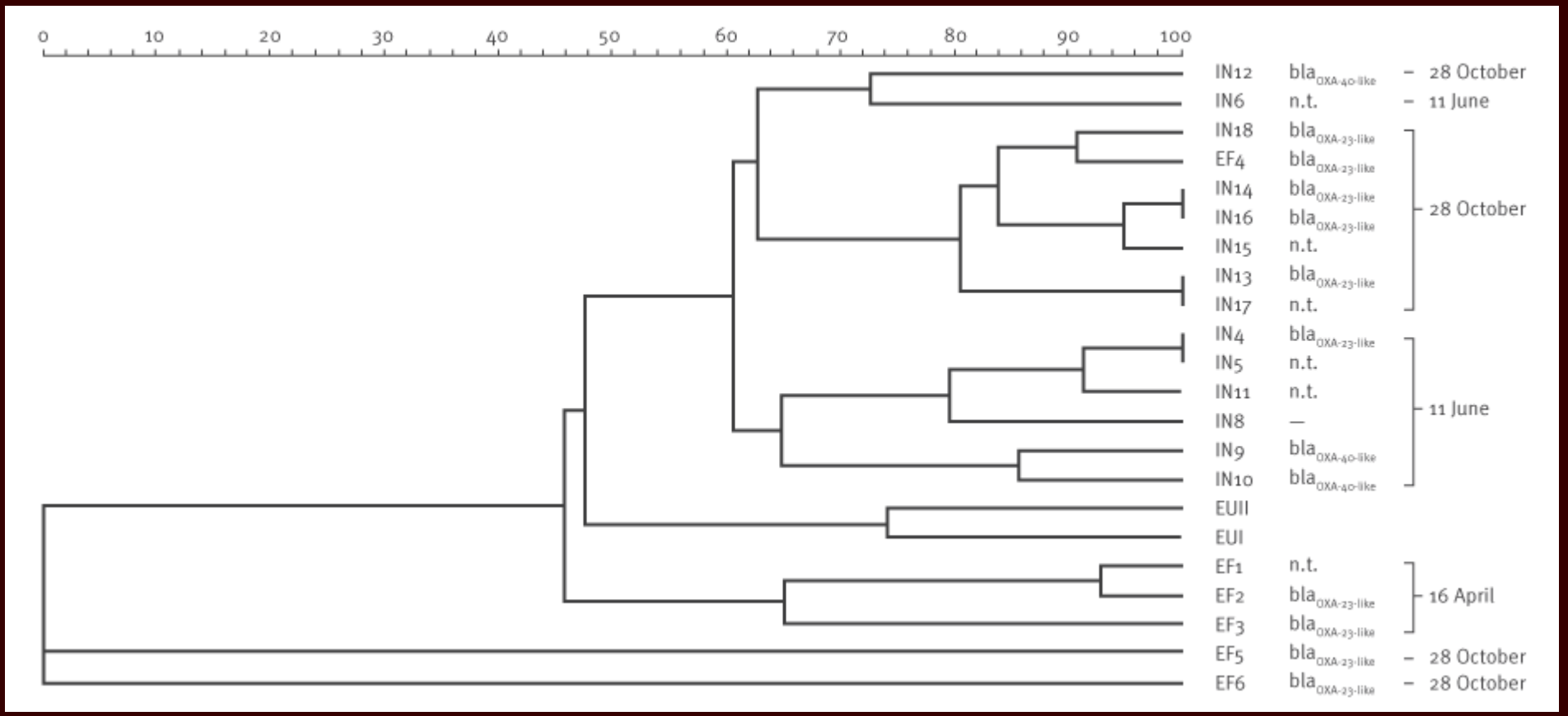
Sampling date	Isolate name	MALDI TOF score value	Antibiotic profile								
			MEM	IPM	CIP	LVX	TOB	GEN	AMK	SXT	CST
16.4.2014	EF1	2.262	R	R	R	R	R	R	S	S	S
	EF2	2.352	R	R	R	R	R	R	S	S	S
	EF3	2.329	R	R	R	R	R	R	S	S	S
11.6.2014	IN4	2.231	R	R	R	R	R	R	S	S	S
	IN5	2.085	R	R	R	R	R	R	S	S	S
	IN6	2.157	R	R	R	R	S	R	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

90% izolata rezistentno na karbapeneme i većinu ostalih antibiotika = antropogeni sojevi

10% izolata senzitivno na sve antibiotike = nativni sojevi?

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

Svi izolati posjeduju konstitutivni gen $bla_{\text{OXA-51-like}}$, 71% izolata posjeduje stečeni gen $bla_{\text{OXA-23-like}}$, a 21% izolata posjeduje stečeni gen $bla_{\text{OXA-40-like}}$.



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

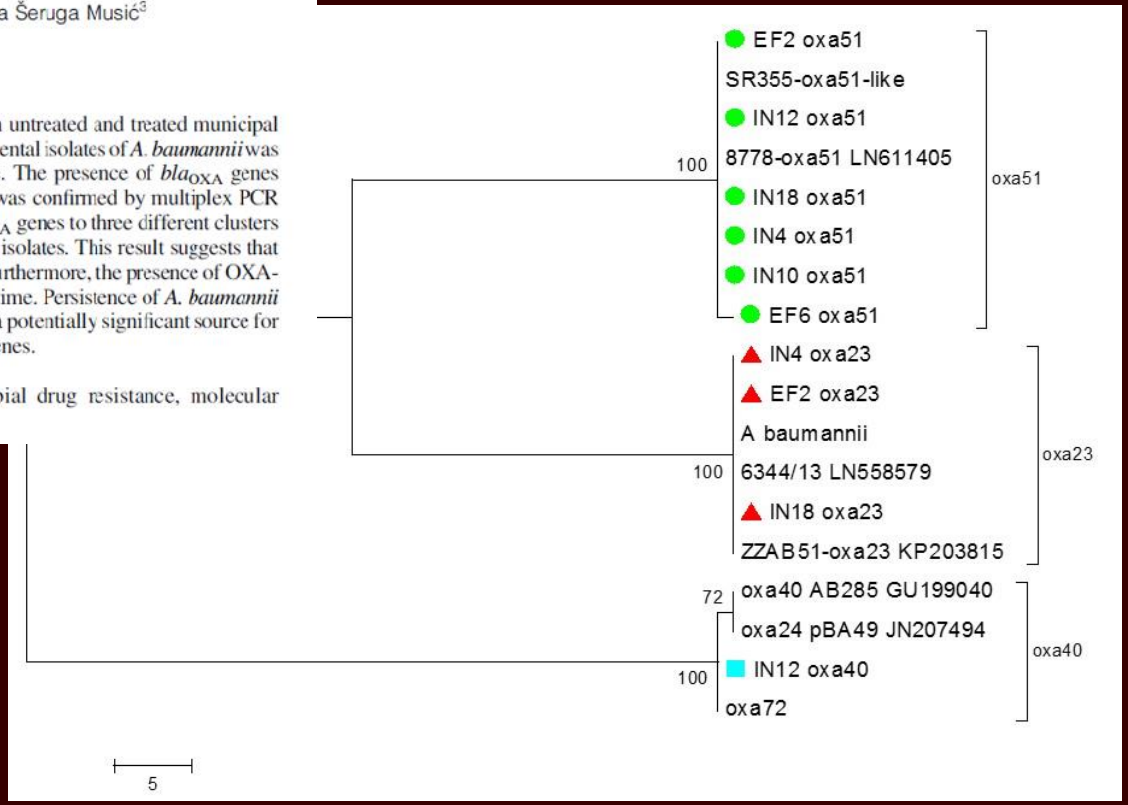
DOI: 10.1089/mdr.2015.0275

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 Musić[3]

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. 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, oxacillinanase, 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 M[3], Kovacic A[4], Tonkic M[1,2], Hrenovic J[3].

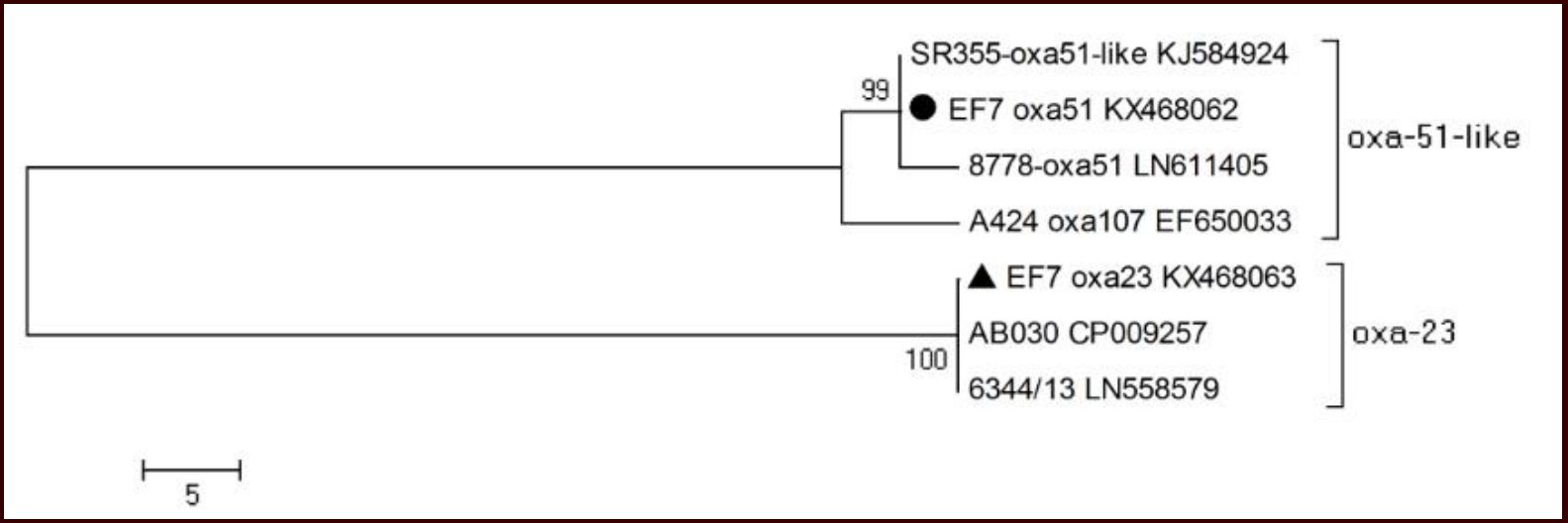
⊕ Author information

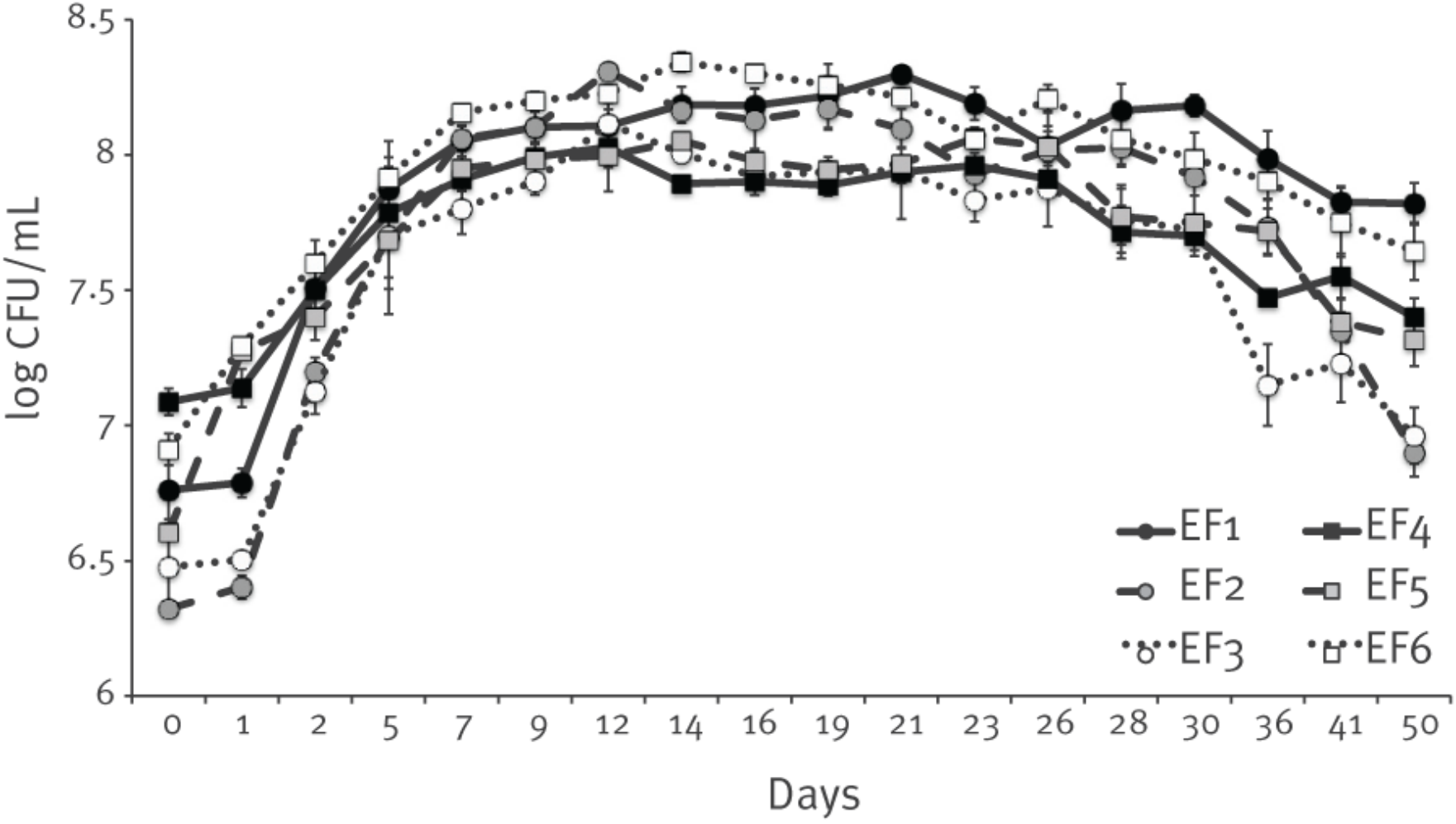
Abstract

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 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

Taylor & Francis
Taylor & Francis Group

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]

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

Abstract

Background: Acinetobacter baumannii is one of the most preva
sible for the dramatic increase in carbapenem resistance in C
research focused on the organism's ability to form biofilm
Methods: Biofilm formation in 109 unrelated clinical isolat
was investigated. Genotyping was performed by pulsed-fiel
ing the disc diffusion method and confirmed by determinir
biofilm in vitro was determined from overnight cultures of
crystal violet, and quantified at 570 nm after solubilizatio
appropriate program with level of statistical confidence. *Re*

6th Croatian-Slovenian-Serbian Symposium on Zeolites

NATURAL ZEOLITE INFLUENCE ON THE SURFACE MOTILITY OF ACINETOBACTER BAUMANNII

Jasna Hrenović[1], Darko Tibljaš[1], Marin Ganjto[2], Goran Durn[3], Ivana Goić-Barišić[4]
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[2]Zagreb Wastewater - Management and Operation Ltd., Zagreb, Croatia
[3]University of Zagreb, Faculty of Mining, Geology and Petroleum Engineering, Zagreb, 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. infectants, and adverse conditions lead to its long
varming surface motility together with biofilm
ute to its pathogenesis. Inhibition of twitching
n is promising tool to suppress the virulence of *A.*
zeolitized tuff (NZ) on twitching and swarming
nnii was tested. The NZ at concentration 1-3%
motility of *A. baumannii* on polystyrene due to
particles. The swarming motility on the surface
nized NZ could find application in control of the
of pathogenic bacteria on abiotic surfaces.

ng; swarming; zeolitized tuff.

EXCELLENT PUBLISHERS

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

Svjetlana Dekic[1], Jasna Hrenovic[1]*, Blazenka Hunjak[2], Snjezana Kazazic[3], Darko Tibljas[1] and Tomislav Ivankovic[1]

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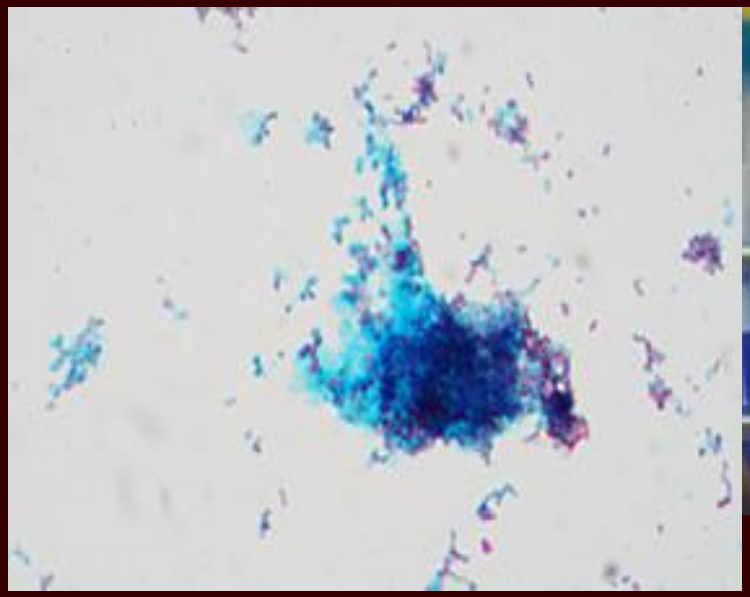
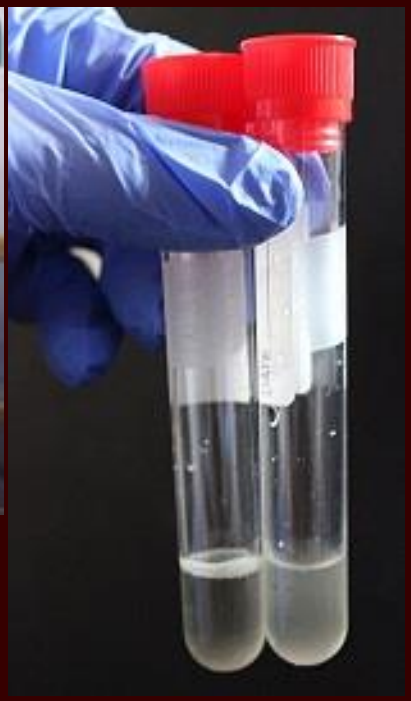
[2]Croatian Institute of Public Health, Zagreb, Croatia
[3]Ruđer Boskovic Institute, Zagreb, Croatia
**Corresponding author: jasna.hrenovic@biol.pmf.hr*

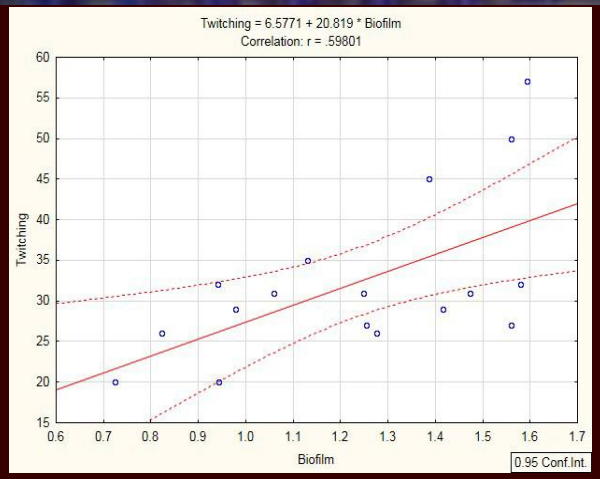
ABSTRACT

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

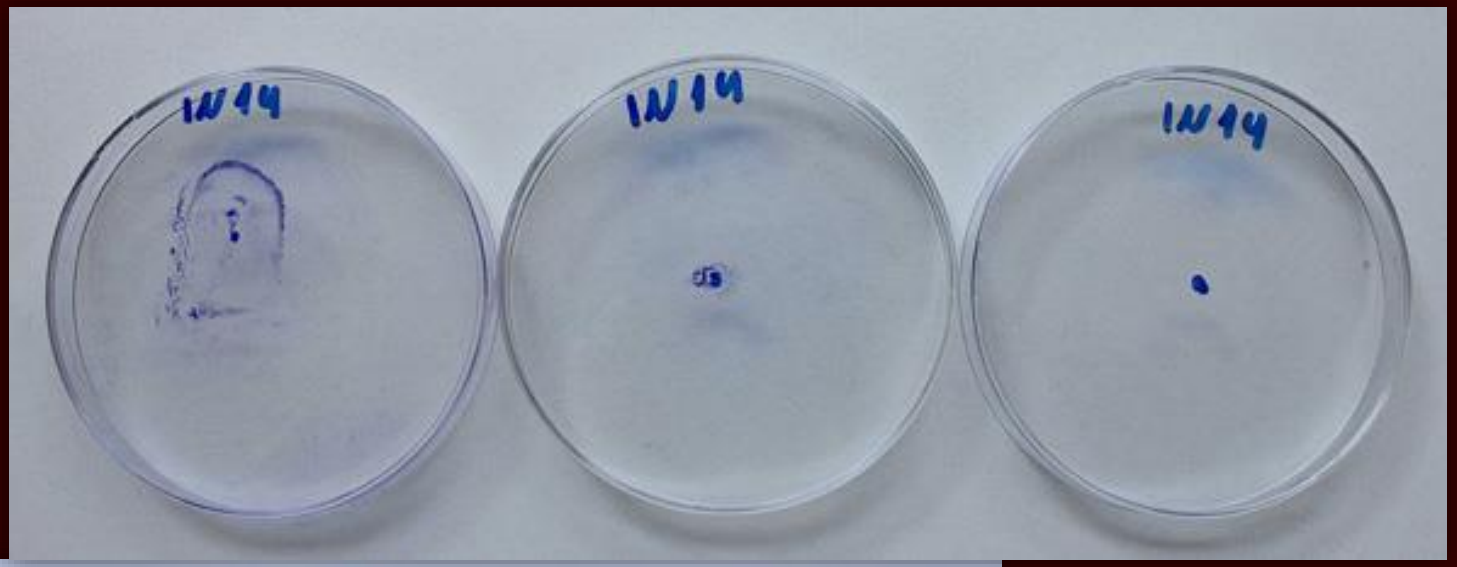






Okolišni izolati *A. baumannii* usporedivo kliničkim izolatima:

- 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.

Zaključci:

- *A. baumannii* se nalazi u okolišu utjecajnom ljudskim krutim ili tekućim 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 okoliš.
- MDR *A. baumannii* mogu preživjeti u okolišu.
- Nove tehnologije dezinfekcije bolničkih otpadnih voda prije ispuštanja u skupni kanalizacijski sustav, su obećavajuća strategija za sprečavanje širenja ovog emergentnog patogena u okoliš.

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