THE NATURAL HABITATS OF CLINICALLY IMPORTANT *ACINETOBACTER BAUMANNII*

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Genus *Acinetobacter* includes 57 species:

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)

Emergent hospital pathogen of 21st century

Uncommon organisms in clinical infections

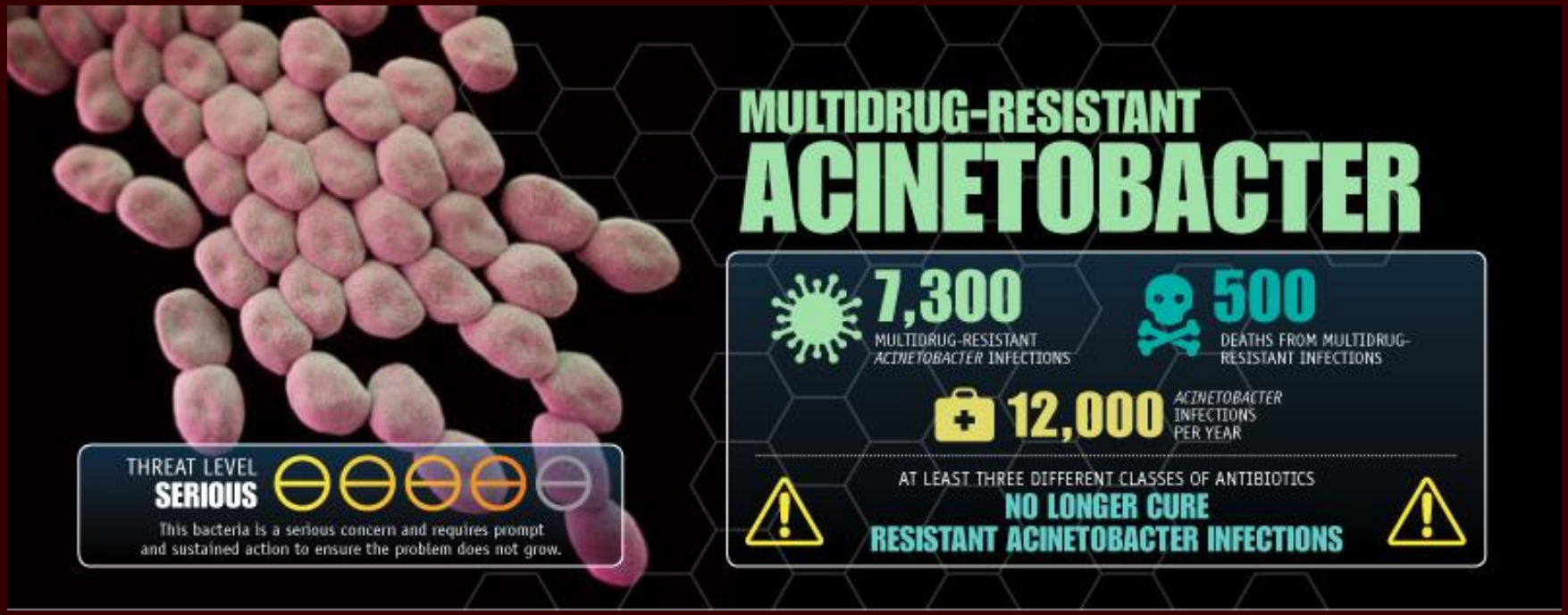
A. baylyi	*A. guillouiae*	*A. lwoffii*	*A. soli*
A. beijerinckii	*A. gyllenbergii*	*A. nectaris*	*A. tandoii*
A. bereziniae	*A. haemolyticus*	*A. parvus*	*A. tjernbergiae*
A. boissieri	*A. harbinensis*	*A. puyangensis*	*A. towneri*
A. bouvetii	*A. indicus*	*A. qingfengensis*	*A. ursingii*
A. brisouii	*A. johnsonii*	*A. radioresistens*	*A. venetianus*
A. gerneri	*A. junii*	*A. rudis*	
A. grimontii[a]	*A. kookii*	*A. schindleri*	

[a]Synonym of *A. junii*.

http://apps.szu.cz/anemec/Classification.pdf

Acinetobacter baumannii is a leading emerging pathogen of the 21st century, which is frequently recovered from patients during hospital outbreaks.

Acute community-acquired human infections suggest a source of this pathogen outside of the hospital settings.



Up to 2010 *A. baumannii* was considered as an exclusively hospital pathogen.

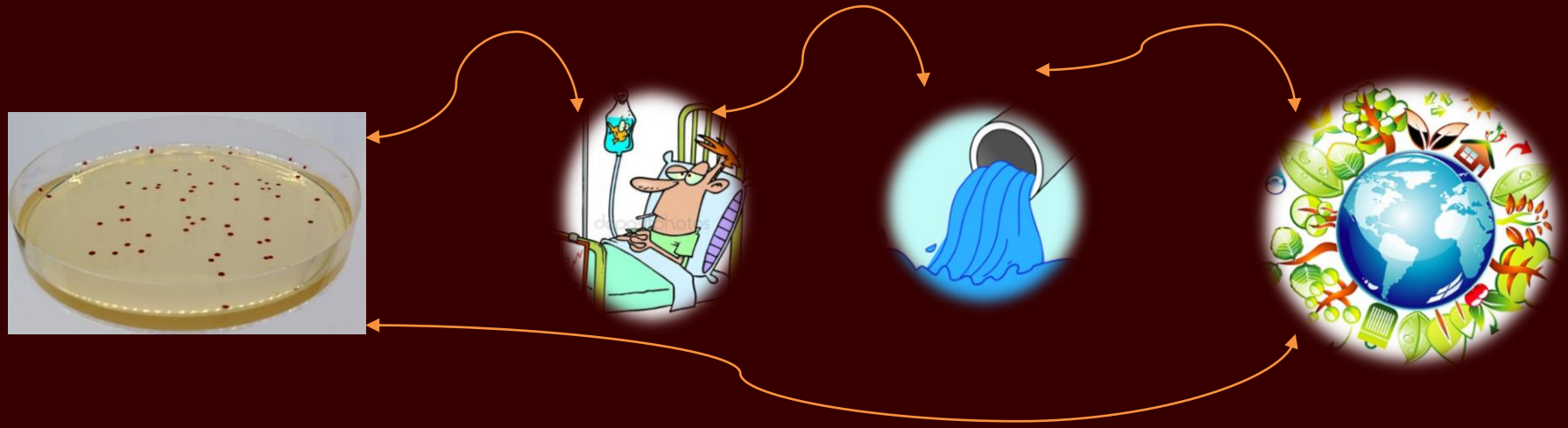
After 2010 onwards, there are reports on its occurrence outside hospital settings:

- Water of Seine River (2010) – 1 isolate
- Untreated hospital wastewater in Brazil (2011) – 3 isolates
- Untreated and chlorinated hospital wastewater in China (2013) – 9 and 1 isolate
- Natural environment in Croatia (2014 onwards)

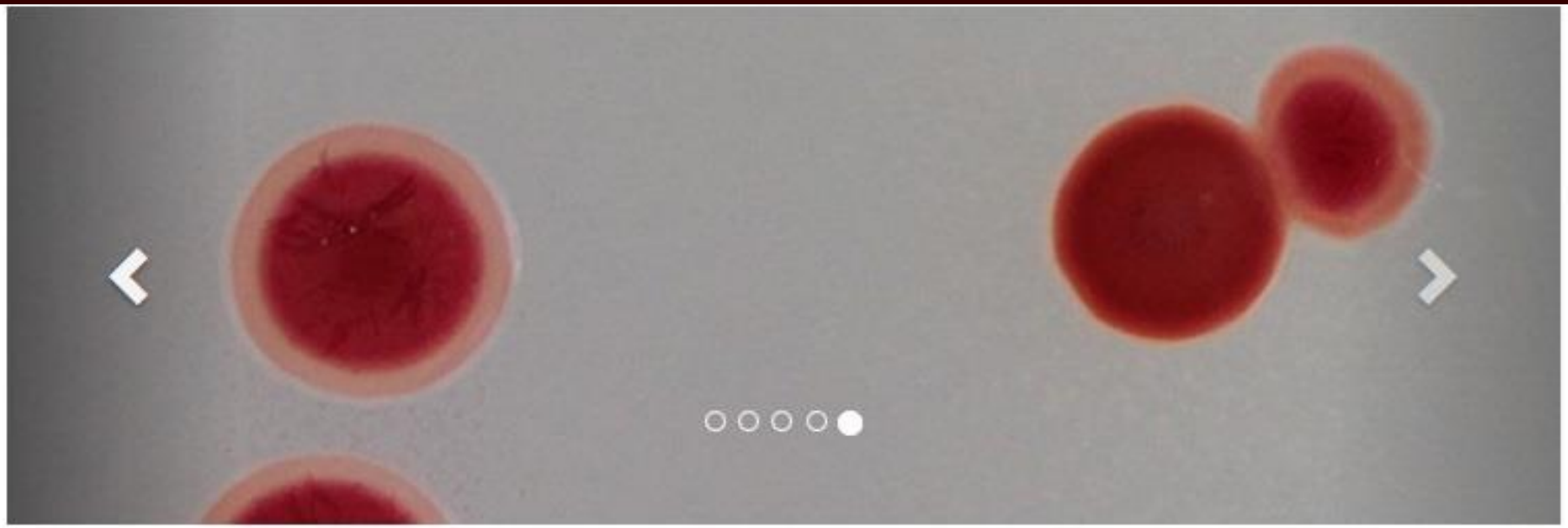
The significance of environmental isolates in the epidemiology of *A. baumannii* is under a great concern worldwide.

There is no clear evidence about:

- the way of introduction of *A. baumannii* into hospital environment,
- its propagation from hospital settings to the natural environment,
- its natural habitat outside hospitals.



Aim: the overview of the presence of *A. baumannii* in natural environment in Croatia.



Natural habitat of clinically important Acinetobacter baumannii

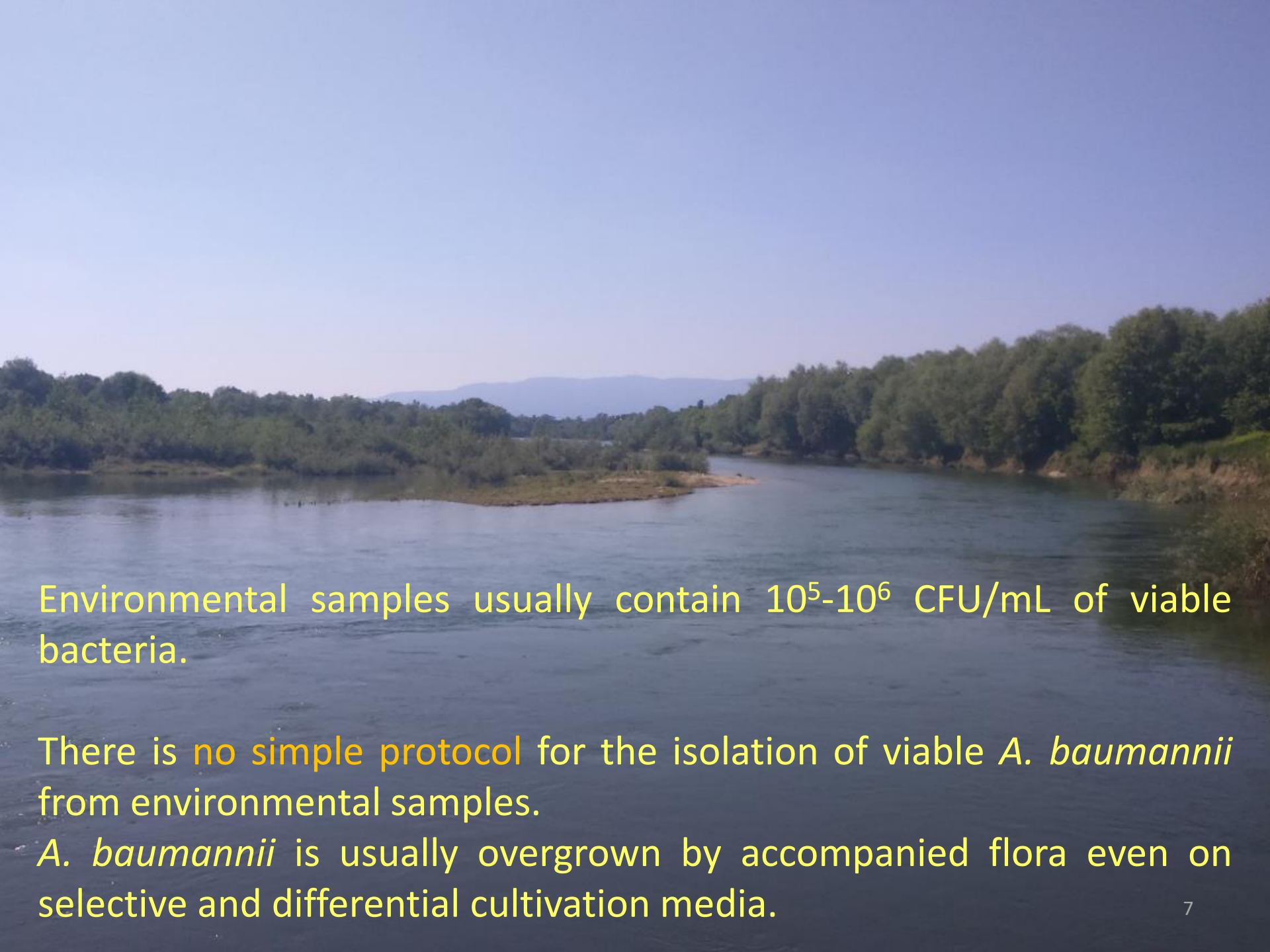
Funding source: Croatian Science Foundation

Duration: 01. 09. 2015 – 31. 08. 2019

Principal investigator: **Prof. Dr. Jasna Hrenović**

Budget: 999,210.00 HRK

Project no.: IP-2014-09-5656



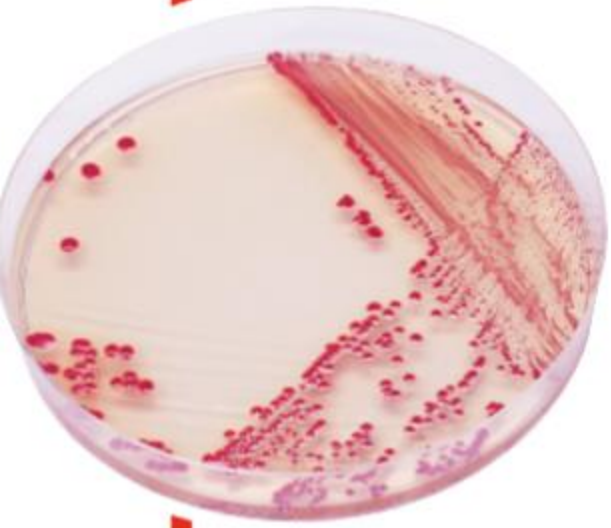
Environmental samples usually contain 10^5-10^6 CFU/mL of viable bacteria.

There is no simple protocol for the isolation of viable *A. baumannii* from environmental samples.

A. baumannii is usually overgrown by accompanied flora even on selective and differential cultivation media.

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.:

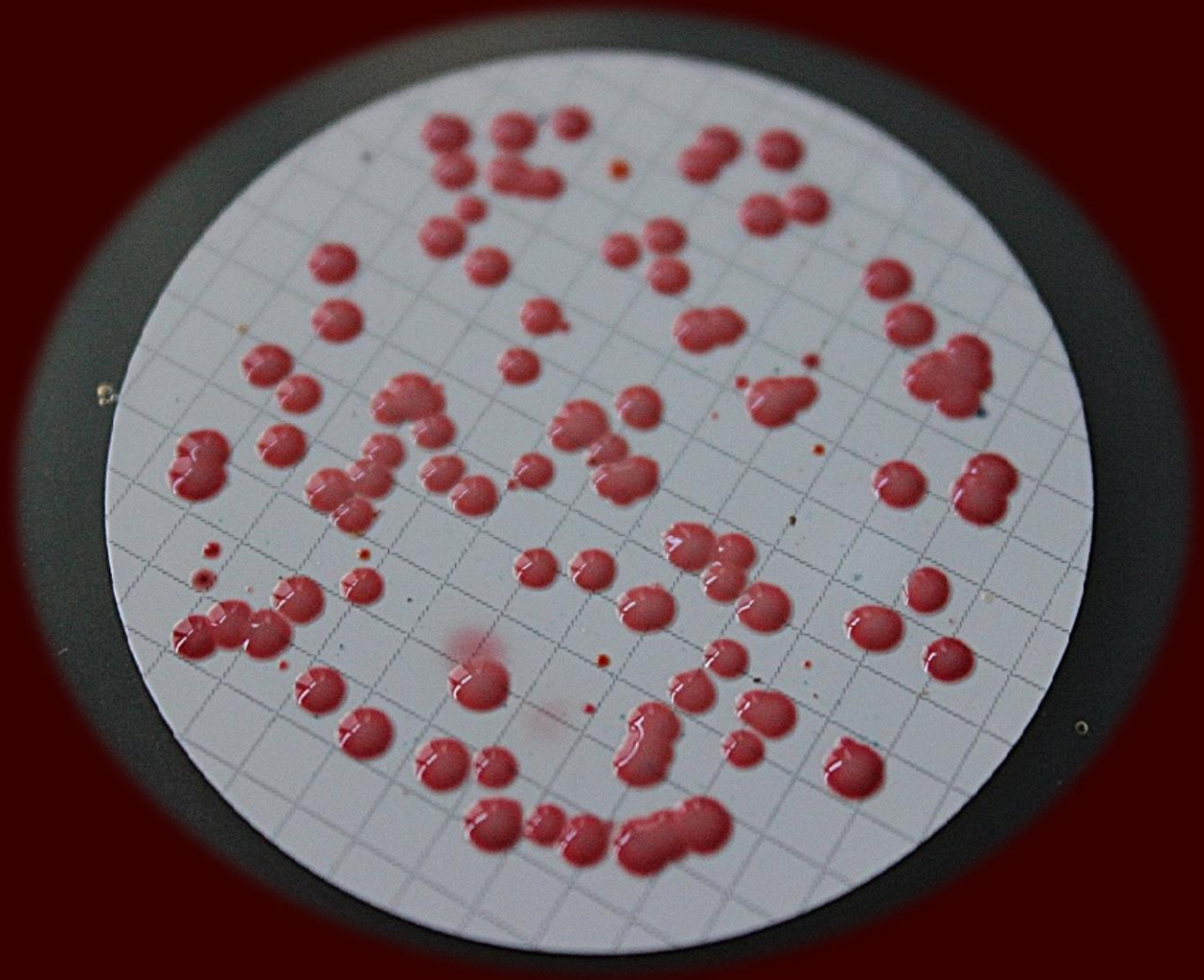
- *Acinetobacter* sp.
→ red
- Other gram (-)
→ blue or mostly inhibited
- Gram(+) bacteria and yeasts
→ inhibited

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

Medium Performance

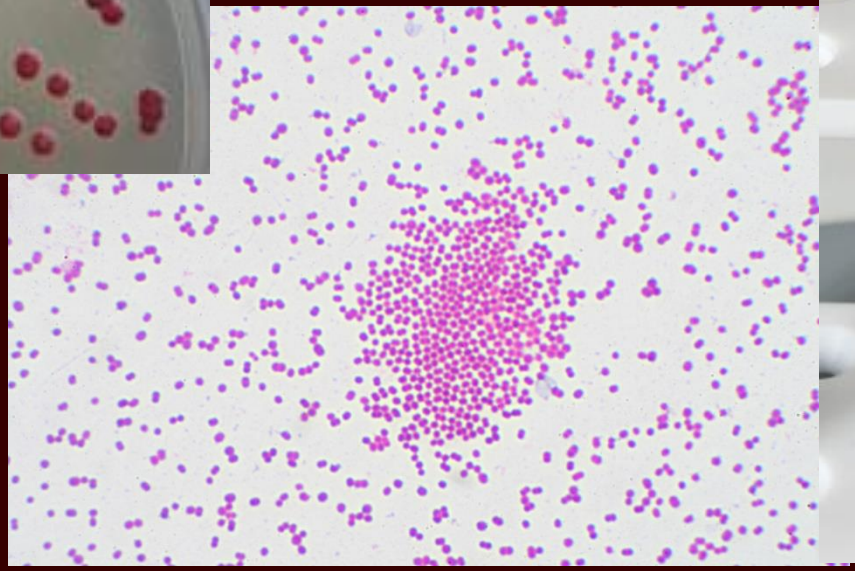
1. **One unique Red colour:** Detection of *A. baumanii* from traditional culture media might be a difficult and tedious task due to the abundance of background flora found in collected specimens, especially when using media based on differentiation by the lactose/non-lactose fermentation ability. To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

The recovery of *A. baumannii* was performed on commercial CHROMagar Acinetobacter supplemented with 15 mg/L of cefsulodin sodium salt hydrate after incubation at 42°C/48h.



Identification of environmental isolates I

Phenotypically by routine bacteriological techniques: Gram negative coccobacilli, with typical negative reaction on the Kligler Iron Agar, oxidase negative, catalase positive.



Identification of environmental isolates II

- ✓ Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) on cell extracts

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

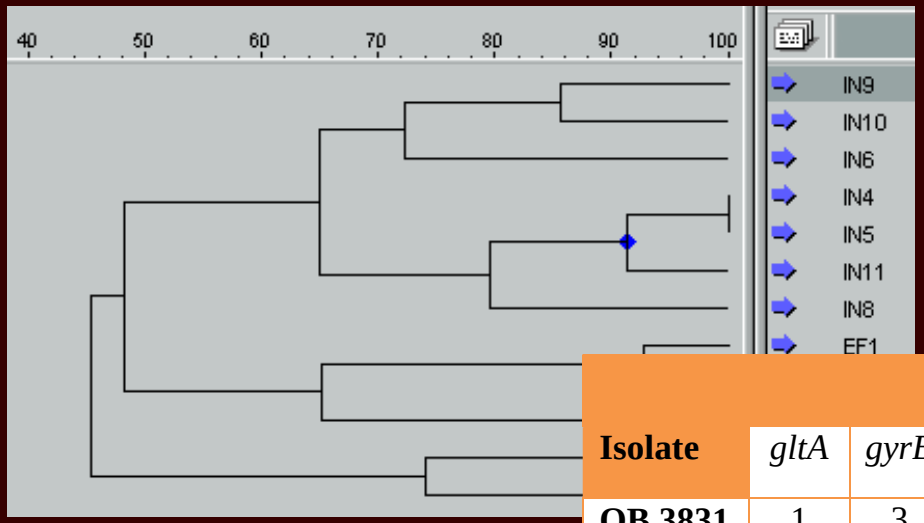
Range	Description	Symbols	Color
2.300 ... 3.000	highly probable species identification	(+++)	green
2.000 ... 2.299	secure genus identification, probable species identification	(++)	green
1.700 ... 1.999	probable genus identification	(+)	yellow
0.000 ... 1.699	not reliable identification	(-)	red

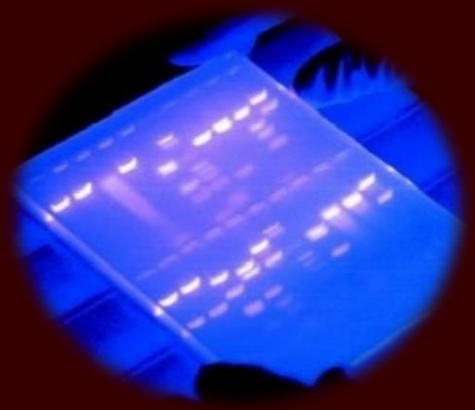
Identification of environmental isolates III

- ✓ amplification and sequencing of *rpoB* gene

Genetic relationship of environmental and clinical isolates

- ✓ PFGE (Pulsed field gel electrophoresis)
- ✓ MLST (Multilocus sequence typing) analysis of seven housekeeping genes (*cpn60, fusA, gltA, pyrG, recA, rplB,* and *rpoB*)



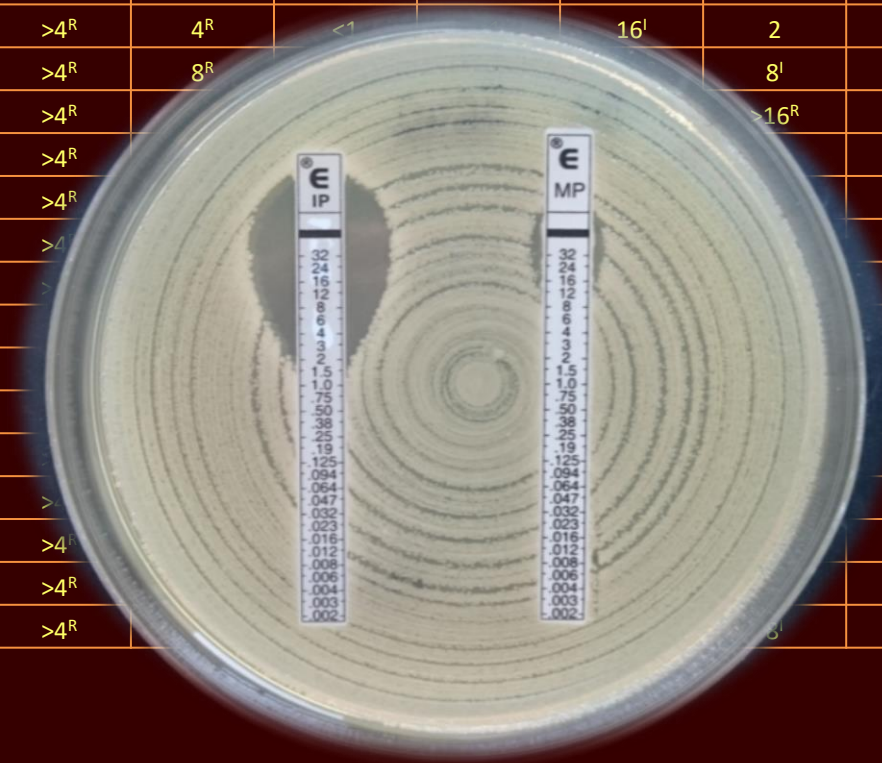


Isolate	Gene locus/allele							Sequence type	Clonal complex	IC type
	gltA	*gyrB*	*gdhB*	*recA*	*cpn60*	*gpi*	*rpoD*			
OB 3831	1	3	3	2	2	96	119 [a]	1421 [a]	92	2
OB 3929	1	3	3	2	2	96	3	195	92	2
OB 3930	1	3	3	2	2	100	3	425	92	2
OB 4027	1	3	3	2	2	96	119 [a]	1421 [a]	92	2
OB 4138	1	3	3	2	2	96	3	195	92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

Antibiotic resistance profile I

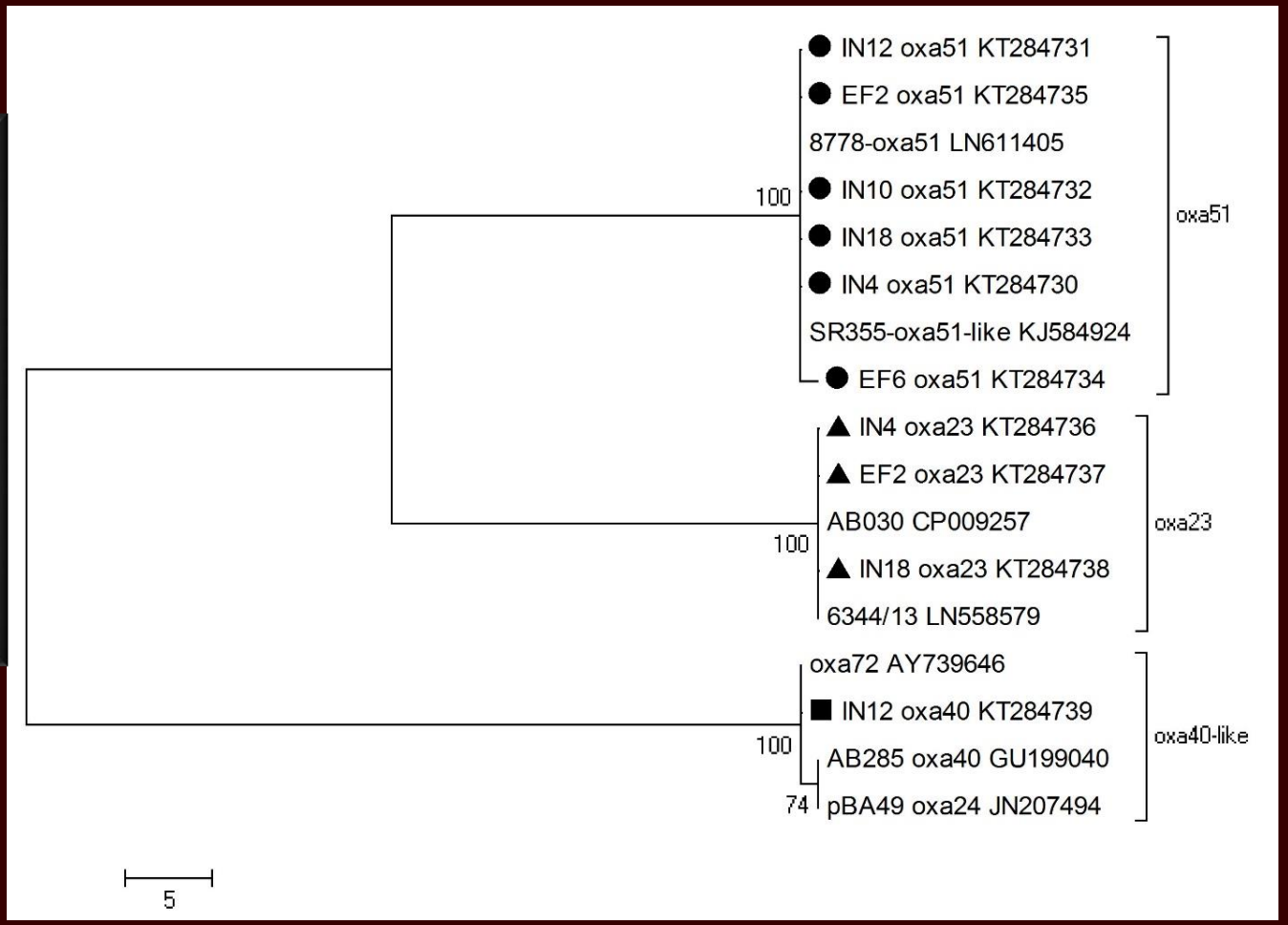
- ✓ Vitek2 system, E-test and broth microdilution
- ✓ interpretation according to EUCAST and CLSI criteria for clinical isolates of *A. baumannii*

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		16^{I}	2	16^{I}	128^{R}	>320^{R}	<0.5
OB 4027	>16^{R}	>16^{R}	>4^{R}	8^{R}				8^{I}	4	>128^{R}	>320^{R}	<0.5
OB 4138	>16^{R}	>16^{R}	>4^{R}					>16^{R}	16^{I}	128^{R}	<20	<0.5
S2/1	>16^{R}	>16^{R}	>4^{R}						8	128^{R}	>320^{R}	<0.5
S2/2	>16^{R}	8^{I}	>4^{R}						<2	128^{R}	>320^{R}	>16^{R}
S2/3	>16^{R}	>16^{R}	>4						8	>128^{R}	>320^{R}	<0.5
S2/4	8^{I}	>16^{R}							4	64^{I}	>320^{R}	>16^{R}
S1/1	>16^{R}	>16^{R}							<2	>128^{R}	>320^{R}	<0.5
S2/5	>16^{R}	>16^{R}							>32^{R}	>128^{R}	<20	<0.5
S2/6	>16^{R}	>16^{R}							>32^{R}	>128^{R}	<20	<0.5
S2/7	>16^{R}	>16^{R}							>32^{R}	>128^{R}	<20	<0.5
S2/8	>16^{R}	>16^{R}	>4						>32^{R}	>128^{R}	<20	<0.5
S2/9	>16^{R}	>16^{R}	>4^{R}						16^{I}	>128^{R}	160^{R}	<0.5
S2/10	8^{I}	>16^{R}	>4^{R}						4	64^{I}	>320^{R}	>16^{R}
IN32	>16^{R}	>16^{R}	>4^{R}					8^{I}	16^{I}	128^{R}	>320^{R}	<0.5



Antibiotic resistance profile II

- ✓ In carbapenem-resistant isolates the acquired oxacillinases: $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}40\text{-}like}$, $bla_{OXA\text{-}58\text{-}like}$, $bla_{OXA\text{-}143\text{-}like}$ are searched by multiplex PCR



A single isolate of *A. baumannii* was incidentally recovered in the abandoned quarry near City of Pula, from 0.1g of acid paleosol (pH=2.55) influenced by illegally disposed solid waste.

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Journals.ASM.org

Occurrence of an Environmental *Acinetobacter baumannii* Strain Similar to a Clinical Isolate in Paleo

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
Croatia[b]; Department of Clinical Microbiology, University Hospital Centre Split and Univ
and Dalmatia County, Split, Croatia[d]

**Over the past decade, bacteria of the genus *Acinetobacter* have eme
breaks of *Acinetobacter* infections are considered to be caused excl
ronments. The natural habitats of clinically important multiresist
report an incidental finding of a viable multidrug-resistant strain
paleosol from Croatia. The environmental isolate of *A. baumannii*
hospital in this geographic area and was resistant to gentamicin, t
cin. In paleosol, the isolate was able to survive a low pH (3.37), des
of *A. baumannii* in paleosol is illegally disposed waste of external
The bacteria could have been leached from waste by storm water a**

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*

Minimum inhibitory concentration (MIC) values of tested antibiotics[a] against environmental isolate of *A. baumannii*.

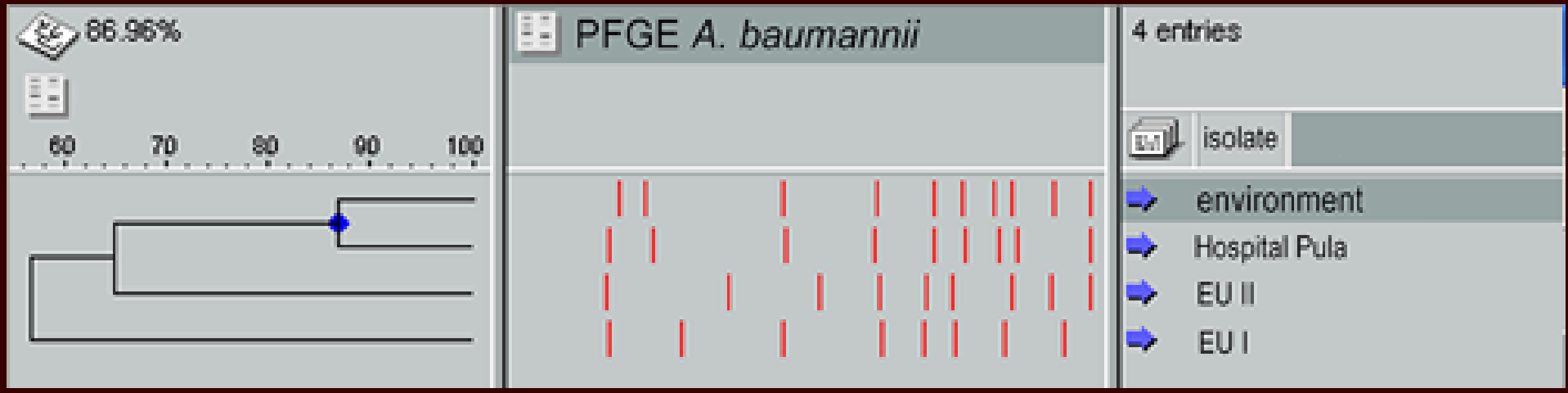
[a] carbapenems (MEM-meropenem, IPM-imipenem), fluoroquinolones (CIP-ciprofloxacin, LVX-levofloxacin), aminoglycosides (TOB-tobramycin, GEN-gentamicin, AMK-amikacin), penicillins/β-lactamase inhibitors (SAM-ampicillin/sulbactam), folate pathway inhibitors (SXT- trimethoprim/sulfamethoxazole), polymyxins (CST-colistin). [R] – resistant according to EUCAST and CLSI criteria.

Isolate	MIC values of antibiotics (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

Multidrug-resistance (MDR) to fluoroquinolones, gentamicin and trimethoprim-sulfamethoxazole

MDR *A. baumannii* from paleosol is related to a clinical isolate from hospital in Pula.

Probable source: illegally disposed solid waste.



Three isolates of *A. baumannii* were recovered from 0.01-1g of technosol at a dump site Sovjak situated above City of Rijeka in a karst pit.

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 J[1], Durn G[2], Music MS[1], Dekic S[1], Troskot-Corbic T[3],

⊕ Author information

Abstract

In a karst pit above City of Rijeka in Croatia the hazard
was periodically used as an illegal dump site. The surfa
geochemically and bacteriologically. From the technos
Acinetobacter baumannii were recovered. Isolates from
isolates: the affiliation to IC1 and 2, multi-drug resistan
carbapenem resistance mediated by bla_{OXA72} and bla
isolates were able to survive in contact with technosol
technosol was the illegally disposed hospital waste. Pr
the spread of clinically important A. baumannii in natur

KEYWORDS: Bacteria; Environment; Hydrocarbons; Tar; Waste

PMID: 28724243 DOI: 10.1016/j.scitotenv.2017.07.108

Minimum inhibitory concentration (MIC) values of tested antibiotics[a] against environmental isolates of *A. baumannii*. [R] - resistant, [I] - intermediate according to EUCAST and CLSI criteria.

[a] carbapenems (MEM-meropenem, IMI-imipenem), fluoroquinolones (CIP-ciprofloxacin, LVX-levofloxacin), aminoglycosides (TOB-tobramycin, GEN-gentamicin, AMK-amikacin), tetracyclines (MIN-minocycline), penicillins/β-lactamase inhibitors (SAM-ampicillin/sulbactam, TIM-ticarcillin/clavulanic acid), folate pathway inhibitors (SXT- trimethoprim/sulfamethoxazole), polymyxins (CST-colistin).

Isolate	MALDI TOF score value	MIC values of antibiotics (mg/L)											
		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

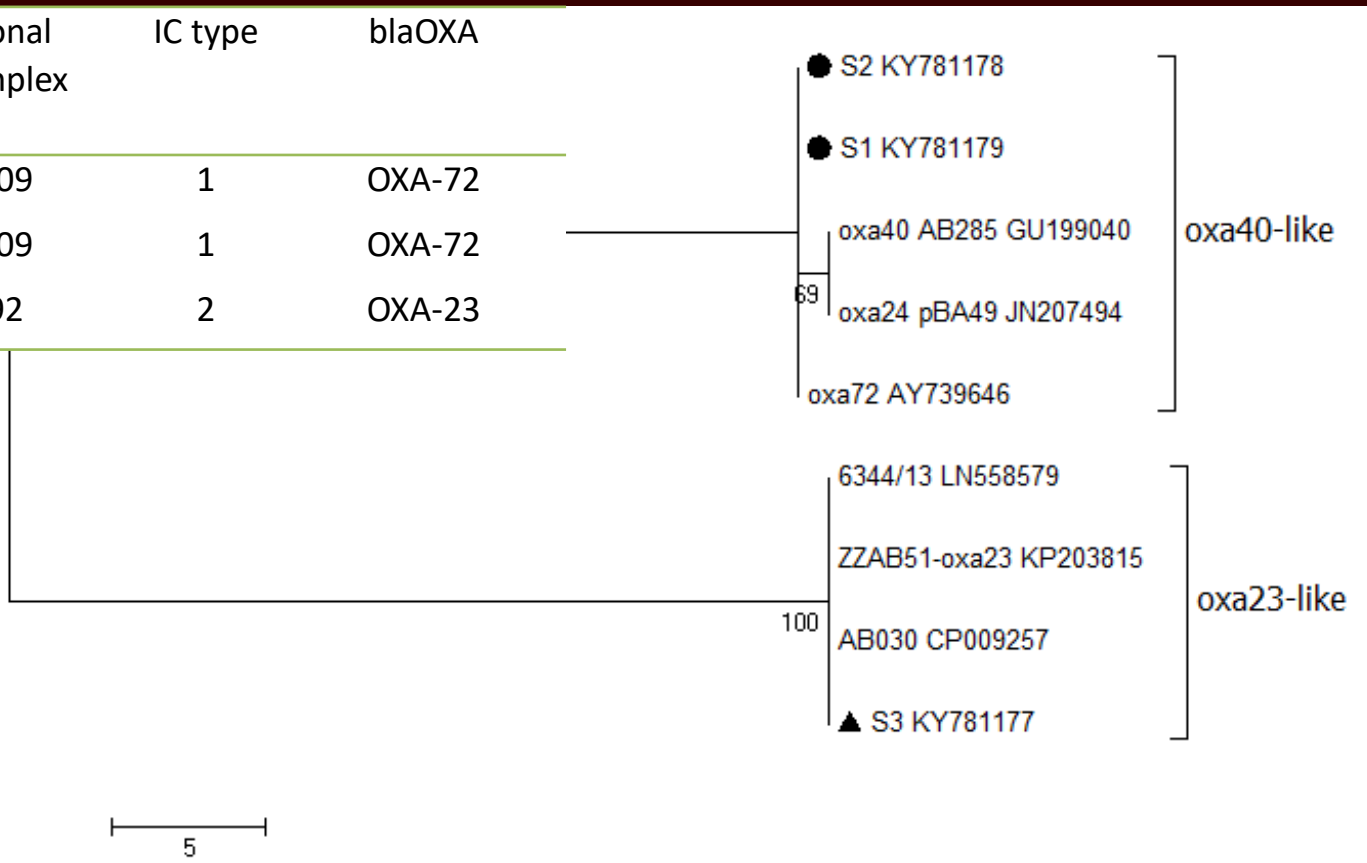
Two isolates (Sovjak 1, 2) multidrug-resistant (MDR)
One isolate (Sovjak 3) extensively drug-resistant (XDR)

A. baumannii from technosol share features characteristic for clinical isolates:

- MDR/XDR antibiotic resistance profile
- Affiliation to IC1 and 2
- Resistance to carbapenems mediated by acquired bla_{OXA72} and bla_{OXA23} genes

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

Probable source: illegally disposed hospital solid waste.



Hospital wastewater was collected at the central manhole of one hospital in Zagreb.

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 isolates were recovered from 0.001 - 0.01 mL hospital wastewater.

Isolate	Origin	Date	Sequence type	International clonal lineage
OB 3929	Tracheal aspirate	18. 9. 2015	195	2
OB 4138	Bronchial aspirate	2. 10. 2015	195	2
S2/1	Hospital wastewater	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 from wastewater and clinical isolates belong to the same ST.

	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}	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

All isolates extensively drug-resistant (XDR)

A. baumannii in urban wastewaters

Urban wastewaters in Zagreb are consisted of: domestic, hospital, industrial and storm waters.

Monitoring was performed at the central wastewater treatment plant.

RESEARCH ARTICLE

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

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Article submitted on 05 February 2...

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 in... [2,9].

The most im... ance in *A.* ... mases, whic... main phylog...

Isolate	Sequence type	International clonal lineage
Influent	ST-195	IC2
	ST-195	IC2
	ST-1604	IC1
	ST-1523	unclustered
Activated sludge	ST-195	IC2
	ST-195	IC2
	ST-1524	IC5
Digested sludge	ST-195	IC2
	ST-195	IC2
	ST-231	IC1
	ST-1525	unclustered
Effluent	ST-195	IC2
	ST-195	IC2
	ST-195	IC2
	ST-195	IC2
	ST-1523	unclustered

A. baumannii recovered from each stage of wastewater treatment, except alkaline lime-treated stabilised sludge (pH 12).

Clonal lineage	Acquired bla_{OXA}	Intrinsic bla_{OXA}
IC2	bla_{OXA-23}	bla_{OXA-66}
	bla_{OXA-23}	bla_{OXA-66}
	bla_{OXA-23}	bla_{OXA-66}
	bla_{OXA-23}	bla_{OXA-66}
	bla_{OXA-23}	bla_{OXA-66}
	bla_{OXA-23}	bla_{OXA-66}
	bla_{OXA-23}	bla_{OXA-66}
	bla_{OXA-23}	bla_{OXA-66}
	bla_{OXA-23}	bla_{OXA-66}
	bla_{OXA-23}	bla_{OXA-66}
IC1	bla_{OXA-72}	bla_{OXA-69}
	bla_{OXA-72}	bla_{OXA-69}
IC5	-	bla_{OXA-65}
unclustered	-	bla_{OXA-51}
	-	$bla_{OXA-208-like}$
	-	$bla_{OXA-117-like}$

Carbapenem-resistant isolates belonged to IC2 carrying the acquired OXA-23 (dominant) or IC1 carrying OXA-72.

Susceptible isolates belonged to IC5 or were unclustered.

Oxacillinases from carbapenem-resistant environmental isolates are highly related to those from clinical isolates.

MICROBIAL DRUG RESISTANCE
Volume 22, Number 7, 2016

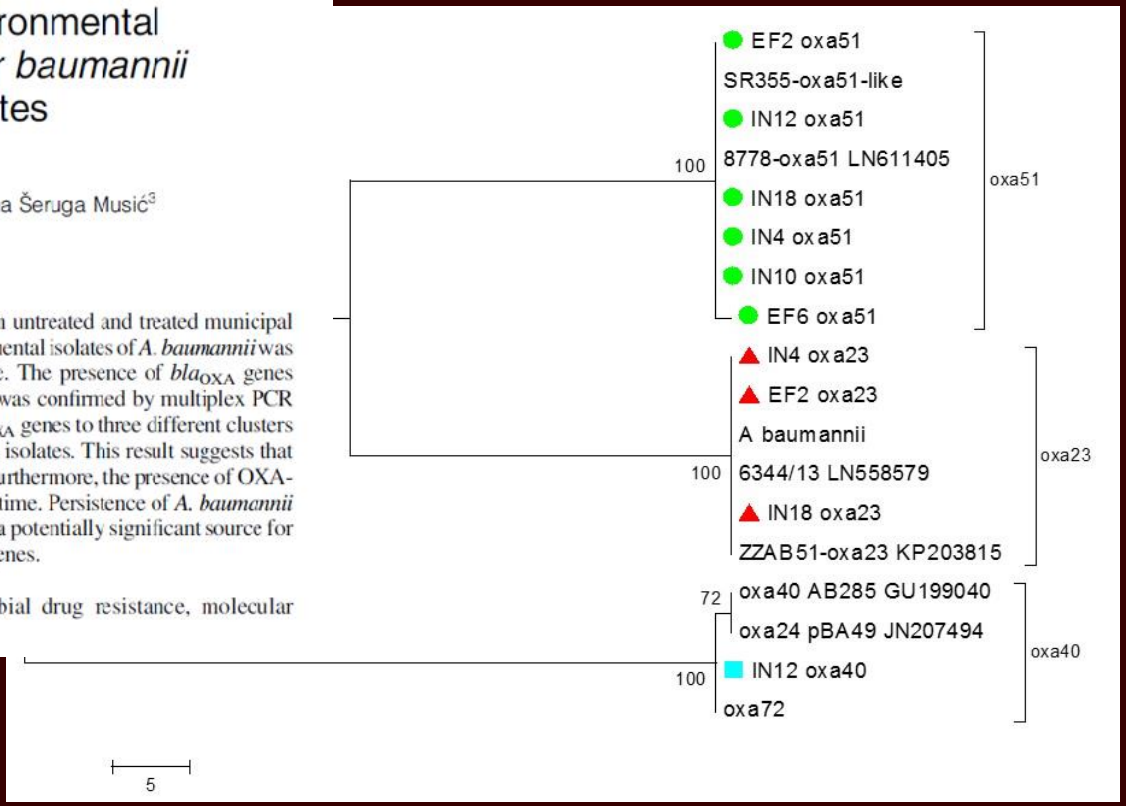
DOI: 10.1089/mdr.2015.0275

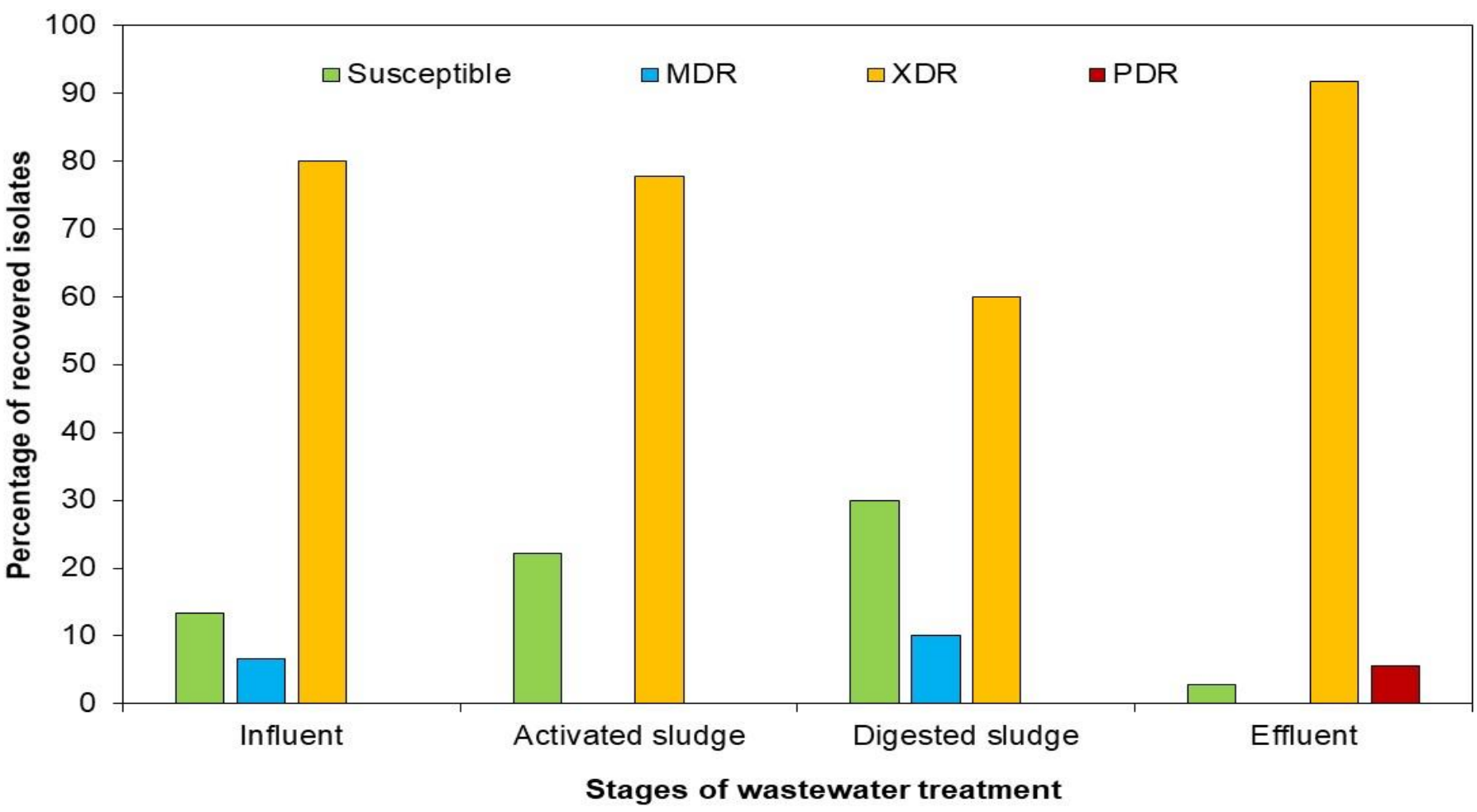
Emergence of Oxacillinases in Environmental Carbapenem-Resistant *Acinetobacter baumannii* Associated with Clinical Isolates

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





Antibiotic susceptibility profile of *A. baumannii* isolates recovered from different stages of the wastewater treatment process. Number of isolates: influent 45; activated sludge 18; digested sludge 20; effluent 36; all stages (total) 119.

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].

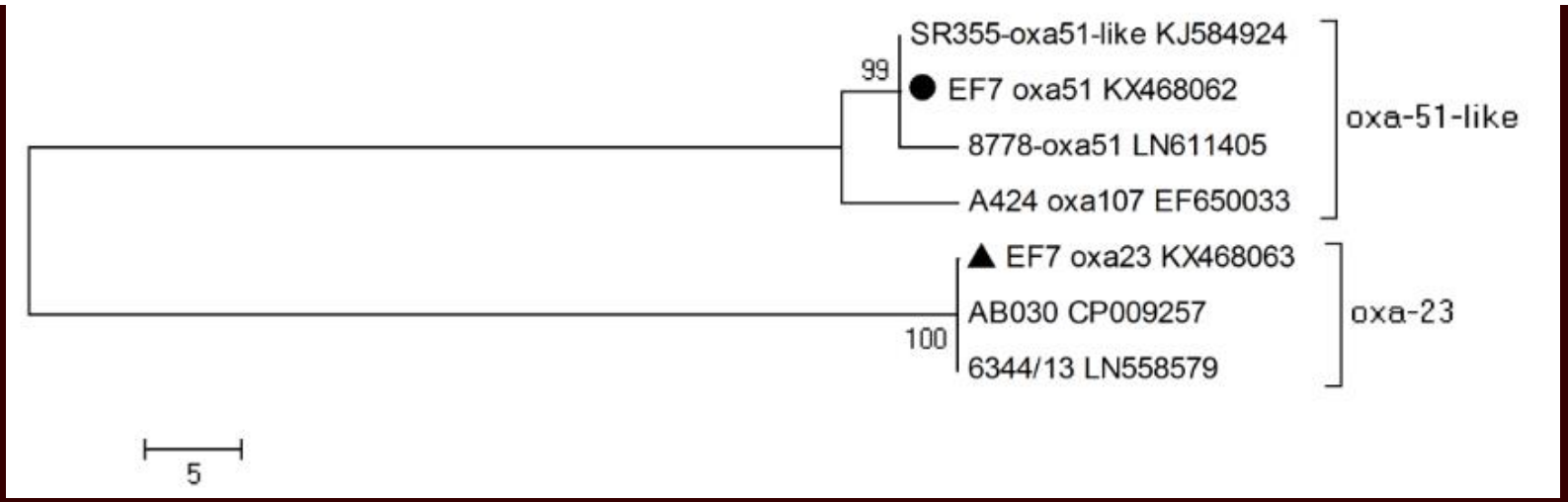
⊕ Author information

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



87% of isolates carbapenem-resistant, IC 1 i 2 = clinically important

13% of antibiotics-sensitive isolates, unclustered = native strains in natural habitat?

Four isolates of *A. baumannii* were recovered from 10mL of water from Sava River downstream the City of Zagreb, after discharge of the urban wastewaters into the natural recipient.

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]

Show more

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

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.

Minimum inhibitory concentration (MIC) values of tested antibiotics[a] against environmental isolates of *A. baumannii*. [R] - resistant, [I] - intermediate according to EUCAST and CLSI criteria.

[a] carbapenems (MEM-meropenem, IMI-imipenem), fluoroquinolones (CIP-ciprofloxacin, LVX-levofloxacin), aminoglycosides (TOB-tobramycin, GEN-gentamicin, AMK-amikacin), tetracyclines (MIN-minocycline), penicillins/β-lactamase inhibitors (SAM-ampicillin/sulbactam, TIM-ticarcillin/clavulanic acid), folate pathway inhibitors (SXT- trimethoprim/sulfamethoxazole), polymyxins (CST-colistin).

Isolte	MIC values of antibiotics (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

All isolates extensively drug-resistant (XDR)

A. baumannii in river

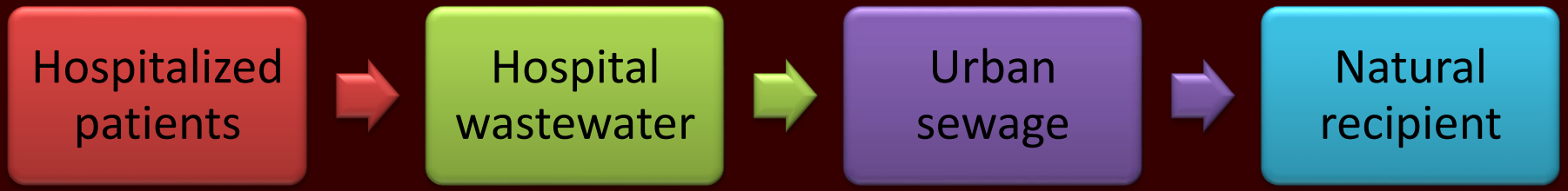
Isolate	Origin	Date	Sequence type	International clonal lineage
OB 3831	Sputum	11. 9. 2015	1421 [a]	2
OB 3929	Tracheal aspirate	18. 9. 2015	195	2
OB 4027	Sputum	24. 9. 2015	1421 [a]	2
OB 4138	Bronchial aspirate	2. 10. 2015	195	2
S2/1	Hospital wastewater	27. 8. 2015	195	2
S2/2			195	2
S2/3			195	2
S2/4			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	Urban sewage	23. 9. 2015	195	2
Sava3	Sava River	11. 10. 2015	1421 [a]	2
Sava4			195	2
Sava5			1421 [a]	2
Sava6			1421 [a]	2

A. baumannii from Sava, urban sewage, hospital wastewaters, and clinical isolates belong to the same ST.

[a] new ST

Hospital wastewaters are discharged into the urban sewage system without pre-treatment.
Urban wastewater, treated or not, is discharged into the Sava River.

Probable source of *A. baumannii* in Sava River: hospital and consequently urban wastewater.



Conclusion:

- Human solid and liquid waste is a source of clinically relevant *A. baumannii* in environment

- Natural environment could represent a secondary habitat of *A. baumannii*

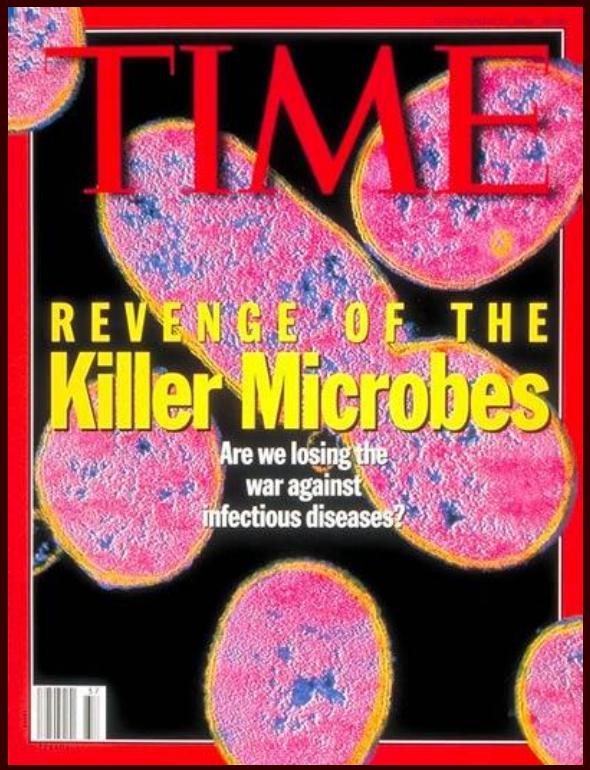
- Measures for prevention of spread of *A. baumannii* in environment:

- proper management and disposal of human solid waste
- novel technologies of disinfection of hospital wastewater.

Thank you for attention!

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

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



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Hrvatska zaklada za znanost