PRESENCE OF *ACINETOBACTER BAUMANNII* IN NATURAL ENVIRONMENT IN CROATIA

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Hrvatska zaklada za znanost

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Genus *Acinetobacter* includes 53 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.

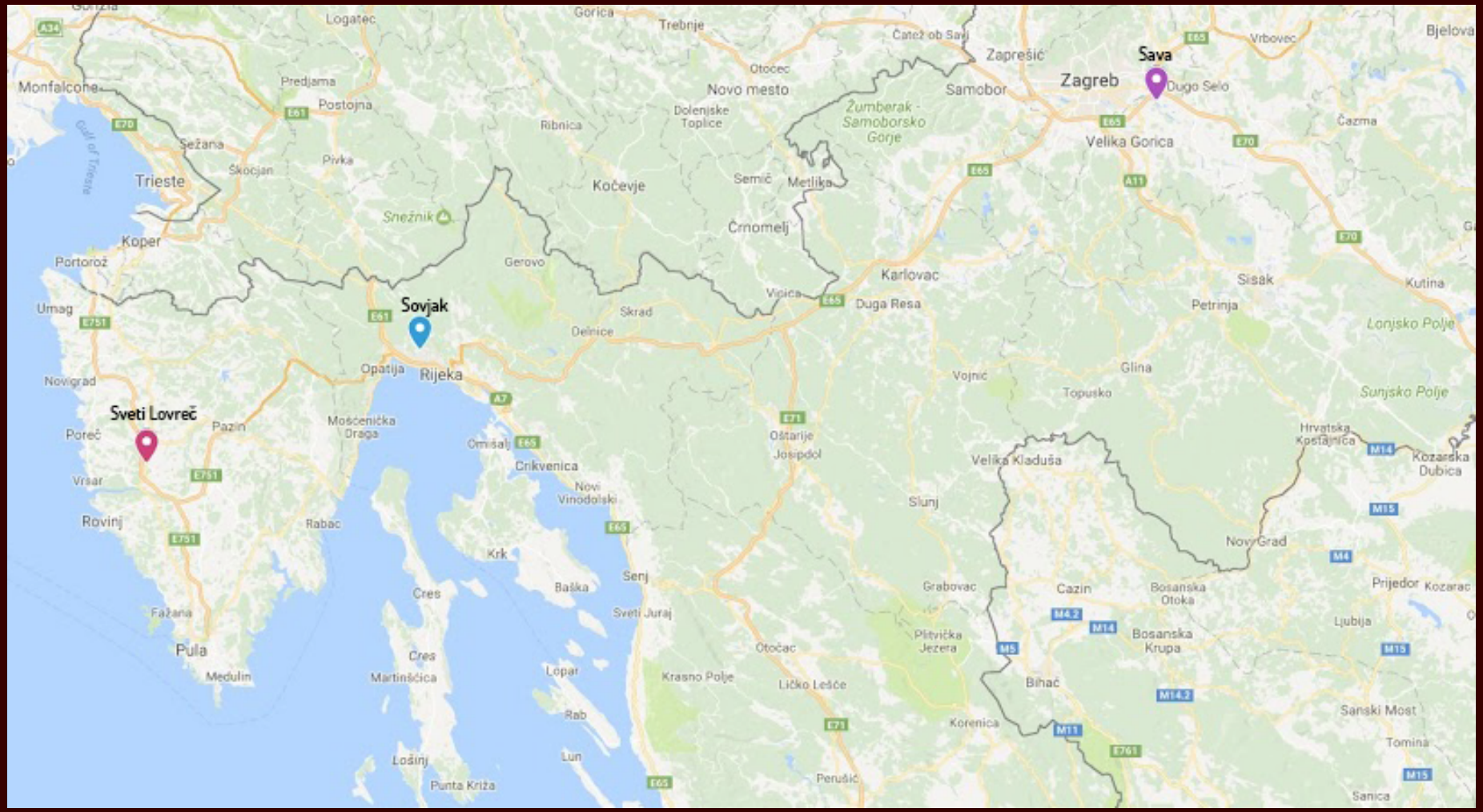
The significance of environmental isolates in the epidemiology of *A. baumannii* is under a great concern worldwide.

There is no clear evidence about:

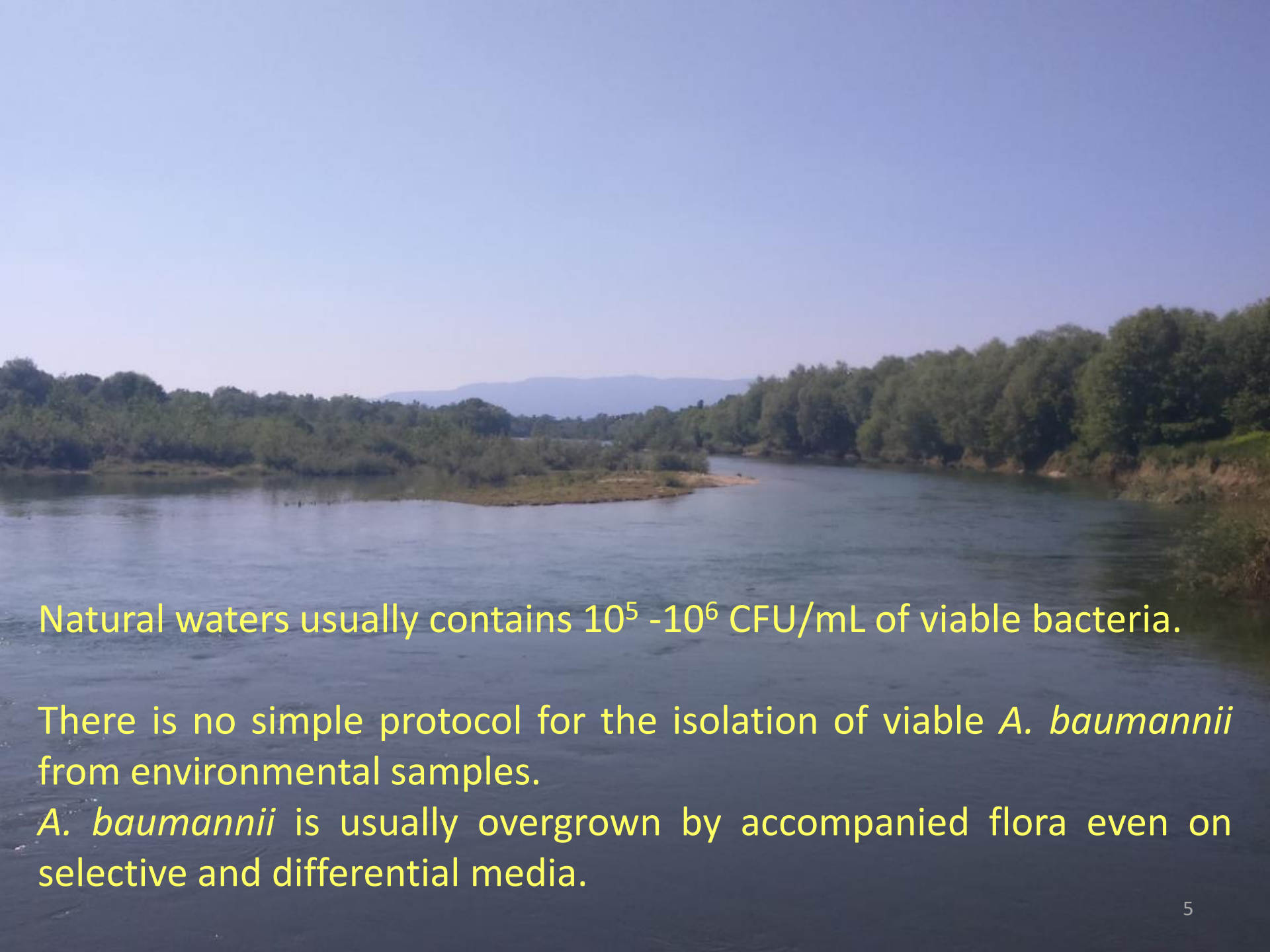
- the way of introduction of *A. baumannii* into hospital environment,
- its propagation from hospital settings to the natural environment,
- its natural habitat outside hospitals.

Aim:

In this study, the overview of the presence of *A. baumannii* in natural environment in Croatia is given.



Position of locations in Croatia from which *A. baumannii* isolates were recovered.



Natural waters usually contains 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 media.

CHROMagar™ Acinetobacter

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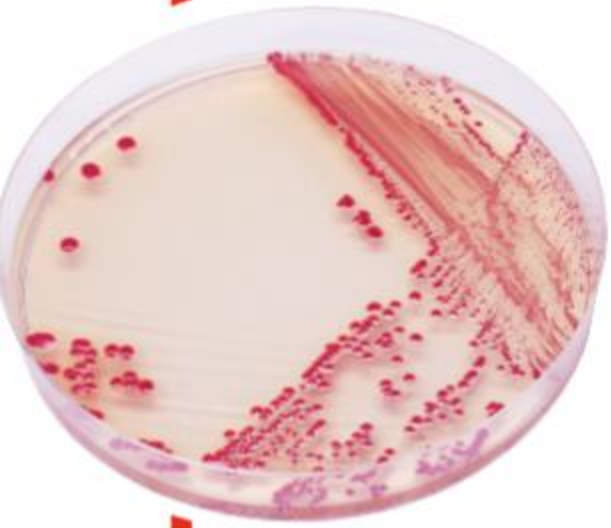


For detection of *Acinetobacter* and MDR *Acinetobacter* sp.

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.:

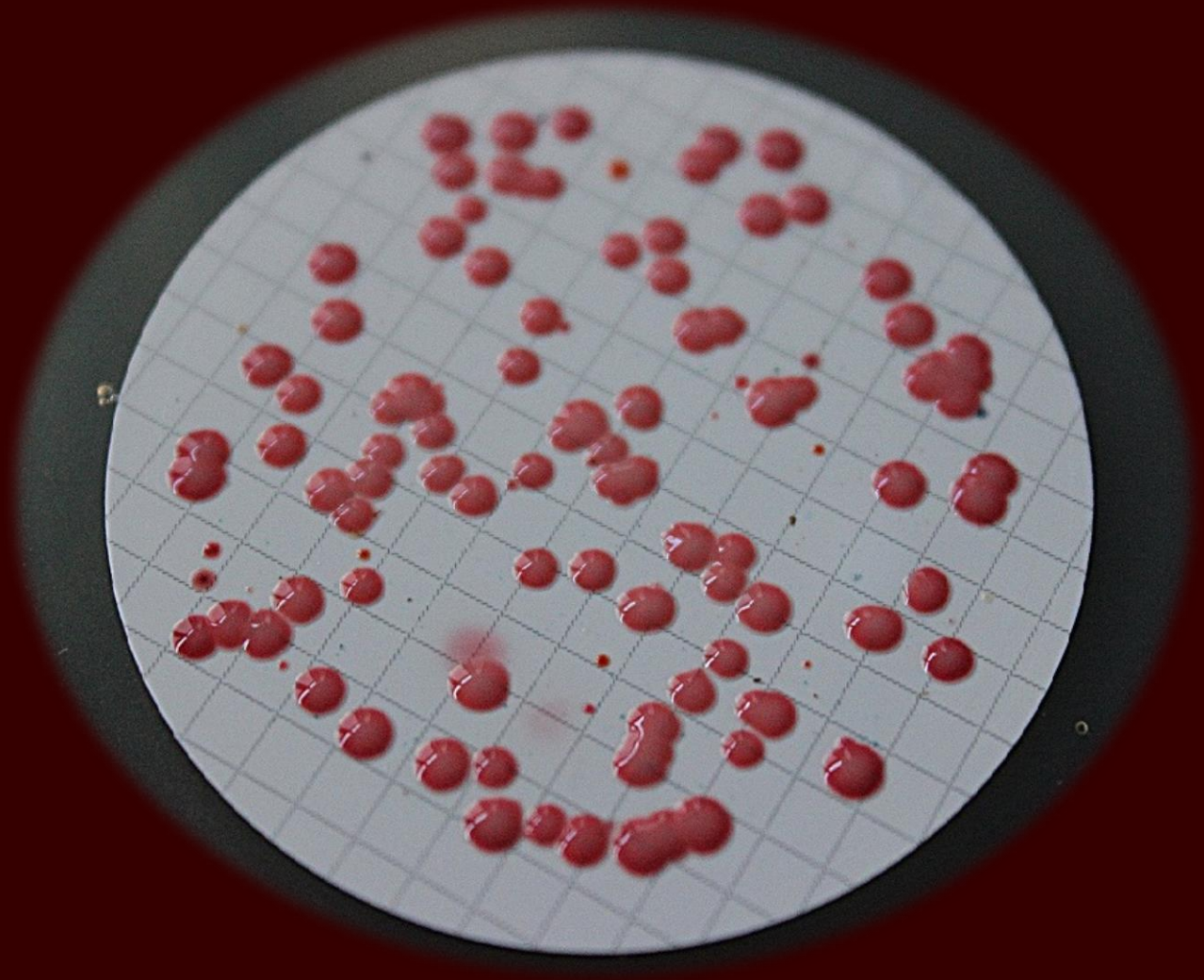
- *Acinetobacter* sp.
→ red
- Other gram (-)
→ blue or mostly inhibited
- Gram(+) bacteria and yeasts
→ inhibited

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

Medium Performance

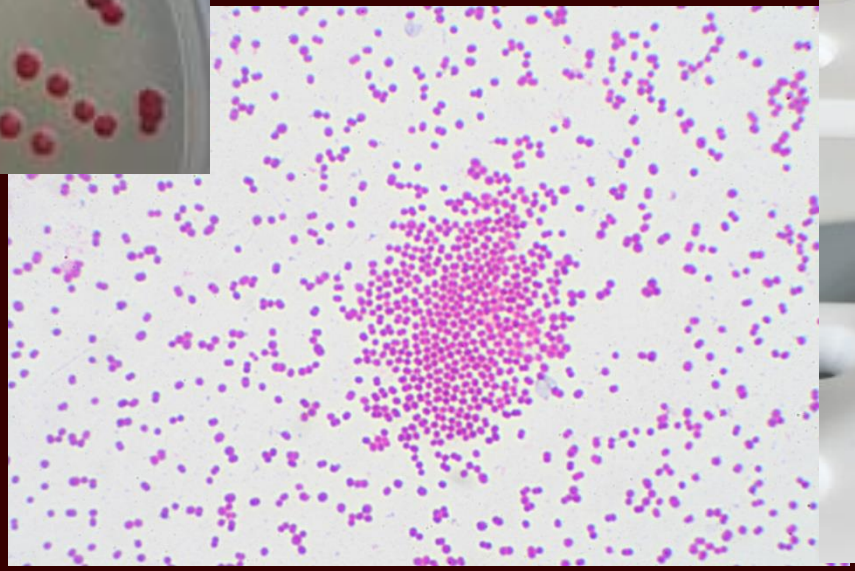
1. **One unique Red colour:** Detection of *A. baumanii* from traditional culture media might be a difficult and tedious task due to the abundance of background flora found in collected specimens, especially when using media based on differentiation by the lactose/non-lactose fermentation ability. To 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.

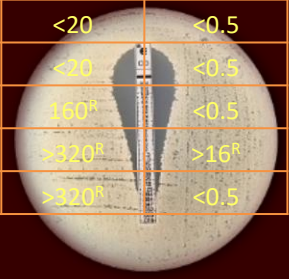
AnalyteName	AnalyteID	Organism(best match)	ScoreValue	Organism(second best match)	ScoreValue
B1(++) (A)	Š 2/6	Acinetobacter baumannii	2.232	Acinetobacter baumannii	2.195
B2(++) (A)	Š 2/5	Acinetobacter baumannii	2.067	Acinetobacter baumannii	2.046
B3(++) (A)	OB 3929	Acinetobacter baumannii	2	Acinetobacter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

Antibiotic resistance profile

- Vitek2 system
- 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	<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



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

Greenish-gray paleosol in the Tri Jezerca quarry near town of Sveti Lovreč, City of Pula. A clean profile was prepared for sampling.

MALDI-TOF MS analysis gave the reliable score value of 2.354, identifying them as *A. baumannii*.

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

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

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

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

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

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

[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). ^{R} – resistant according to EUCAST and CLSI criteria; n.m. - not measured.

Isolate	**MIC values of antibiotics (mg/L)**											
	MEM	IPM	CIP	LVX	TOB	GEN	AMK	MIN	SAM	TIM	SXT	CST
Paleosol	≤0.5	≤0.5	≥4^{R}	4^{R}	≤1	>16^{R}	2	n.m.	4	n.m.	160^{R}	≤0.5

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

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

Dump site Sovjak situated near the settlement Marinići above City of Rijeka.

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

MALDI-TOF MS score values above 2.000 identified them as *A. baumannii*. All three isolates were MDR and shared the complete or intermediate resistance to carbapenems, fluoroquinolones, amikacin, and penicillins/β-lactamase inhibitors.

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

Sava River downstream of the City of Zagreb.

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.

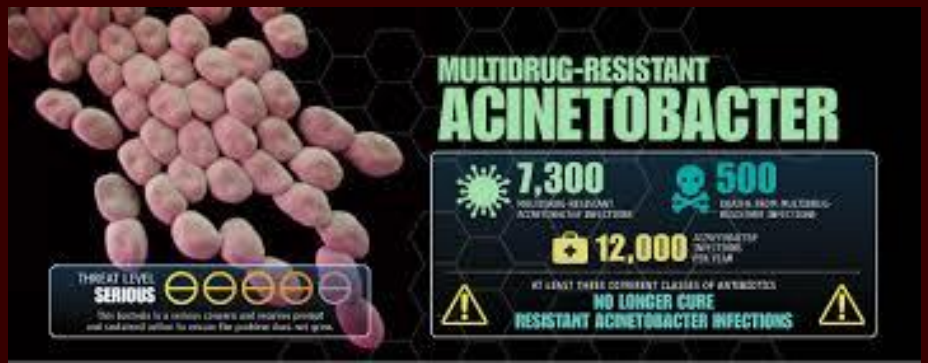
[a] carbapenems (MEM-meropenem, IMI-imipenem), fluoroquinolones (CIP-ciprofloxacin, LVX-levofloxacin), aminoglycosides (TOB-tobramycin, GEN-gentamicin, AMK-amikacin), tetracyclines (MIN-minocycline), penicillins/β-lactamase inhibitors (SAM-ampicillin/sulbactam, TIM-ticarcillin/clavulanic acid), folate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

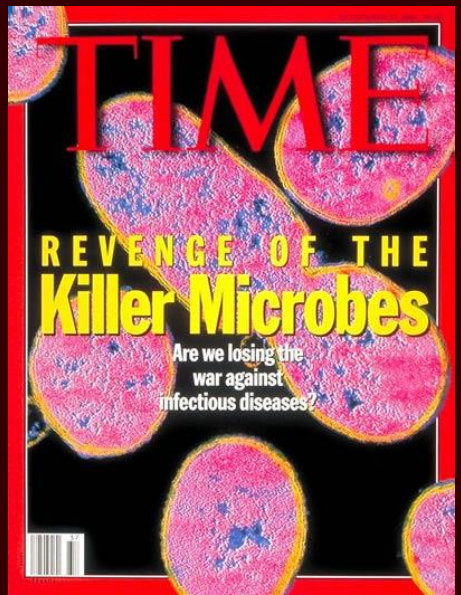
Isolate	MALDI TOF score value	MIC values of antibiotics (mg/L)											
		MEM	IPM	CIP	LVX	TOB	GEN	AMK	MIN	SAM	TIM	SXT	CST
Sava3	2.075	>16[R]	>16[R]	>4[R]	>8[R]	>16[R]	>16[R]	>64[R]	4	16[I]	>128[R]	>320[R]	<0.5
Sava4	2.081	>16[R]	>16[R]	>4[R]	>8[R]	<1	8[R]	16[I]	8[I]	8	>128[R]	>320[R]	<0.5
Sava5	2.149	>16[R]	>16[R]	>4[R]	>8[R]	>16[R]	>16[R]	>64[R]	8[I]	8	>128[R]	<20	<0.5
Sava6	2.052	>16[R]	>16[R]	>4[R]	>8[R]	>16[R]	>16[R]	>64[R]	4	16[I]	>128[R]	>320[R]	<0.5

MALDI-TOF MS score values ranged from 2.052 to 2.149 for *A. baumannii*. All four isolates were MDR and shared the resistance to carbapenems, fluoroquinolones, aminoglycosides and ticarcillin-clavulanic acid.

Conclusion:

- MDR *A. baumannii* were present in natural environment influenced by human solid and liquid waste.
- The proper management and disposal of human waste is mandatory to prevent the spread of MDR *A. baumannii* in nature.





Thank you for attention!

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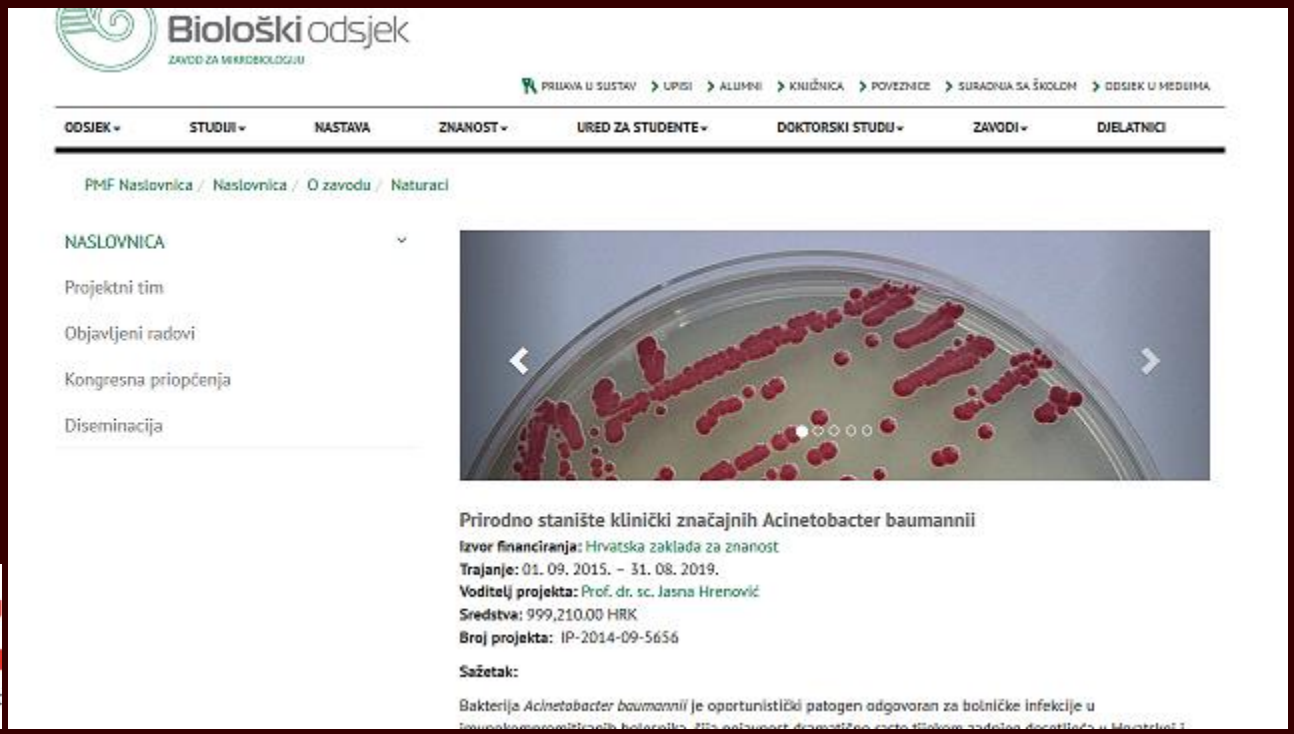

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