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ACINETOBACTER BAUMANNII RECOVERED FROM TECHNOSOL AT A DUMP SITE

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Introduction

Acinetobacter baumannii is well known emerging hospital pathogen. However, data on the presence of *A. baumannii* in natural environment influenced by human solid waste are very scarce and the role of environmental isolates in the occurrence of human infections is not elucidated. There is only one literature report on the incidental finding of one multi-drug resistant (MDR) *A. baumannii* in acid paleosol influenced by illegally disposed solid waste (Hrenovic et al., 2014).

Here we report the finding of three isolates of *A. baumannii* recovered from technosol at a dump site (Hrenovic et al., 2017).

Materials and methods

The dump site is situated above City of Rijeka in Croatia in a karst pit (Figure 1). At this dump site the hazardous industrial waste was continuously disposed from 1956 to 1990. After that period the dumpsite was periodically used as an illegal dump site (Ribic, 2008). The surface part of a technosol at the edge of dump was collected in October 2016.

Triplicate of 1g of soil was suspended in and diluted with peptone water. The isolation of *A. baumannii* was performed on CHROMagar Acinetobacter supplemented with CR102 and 15mg/L of cefsulodin sodium salt hydrate after incubation at 42°C/48h. Identification of presumptive colonies was performed by routine bacteriological techniques and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) on cell extracts (Sousa et al., 2014).

The susceptibility profile for 13 antibiotics was determined according to MICs values obtained by Vitek2 system and E-tests for colistin. MICs were interpreted according to EUCAST criteria (2017) for all antibiotics with defined breakpoints for *Acinetobacter* spp., while for penicillins/β-lactamase inhibitors and minocycline CLSI breakpoints (2015) were used.

Figure 1. The dump site "Sovjak" is situated in a karst pit. The surface part of a technosol was sampled at the edge of a dump (geological hammer for scale).

Results

Three isolates of Gram-negative coccobacilli gave negative oxidase, positive catalase reaction, with typical orange-red reaction on Kligler Iron Agar (Figure 2). MALDI-TOF MS score values ranged from 2.000-2.086 for *A. baumannii* (Table 1). Single colonies of *A. baumannii* were isolated from plates inoculated with 0.01-1g of soil.

All three isolates of *A. baumannii* were MDR (Table 1). They shared the resistance to carbapenems, fluoroquinolones, ticarcillin/ clavulanic acid and piperacillin/ tazobactam, and resistance or intermediate resistance to amikacin, tigecycline and ampicillin/ sulbactam. Only one isolate was resistant to trimethoprim/ sulfamethoxazole and intermediate resistant to minocycline. All three isolates were sensitive to tobramycin, gentamicin and colistin.

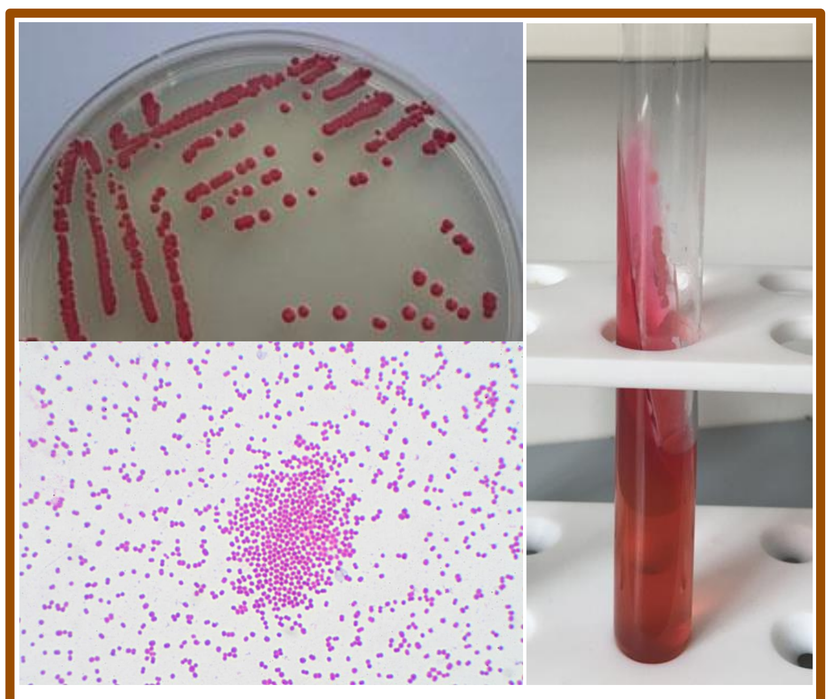

Figure 2. Pure cultures isolated on CHROMagar Acinetobacter were Gram-negative coccobacilli and gave typical orange-red reaction on Kligler Iron Agar.

Table 1. MALDI-TOF MS score and MIC values of tested antibiotics[a] against three isolates of *A. baumannii* isolated on 5th October 2016.

[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, TZP-piperacillin/tazobactam), SXT- trimethoprim/sulfamethoxazole, CST-colistin. R - resistant, I - intermediate according to EUCAST and CLSI criteria.

Isolate	MALDI TOF score value	MIC values of antibiotics (mg/L)												
		MEM	IPM	CIP	LVX	TOB	GEN	AMK	MIN	SAM	TIM	TZP	SXT	CST
Sovjak 1	2.036	≥16^{R}	≥16^{R}	≥4^{R}	4^{R}	≤1	≤1	32^{R}	≤1	16^{I}	≥128^{R}	≥128^{R}	≤20	≤0.5
Sovjak 2	2.086	≥16^{R}	≥16^{R}	≥4^{R}	4^{R}	≤1	≤1	16^{I}	≤1	16^{I}	≥128^{R}	≥128^{R}	≤20	≤0.5
Sovjak 3	2.000	≥16^{R}	≥16^{R}	≥4^{R}	4^{R}	≤1	≤1	>64^{R}	8^{I}	16^{I}	≥128^{R}	≥128^{R}	≥320^{R}	≤0.5

Conclusion

- This study confirmed the illegal dump sites of human solid waste as a source of MDR *A. baumannii*.
- Isolates of MDR *A. baumannii* are able to survive in anthropogenically influenced soil.
- The proper management and disposal of human solid waste is mandatory to prevent the spread of MDR *A. baumannii* in nature.

References

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