ESKAPE pathogens in Croatian soil

Jasna Hrenovic[1], Goran Durn[2], Martina Seruga Music[1], Svjetlana Dekic[2]

[1] University of Zagreb, Faculty of Science, Division of Biology, Zagreb, Croatia.
[2] University of Zagreb, Faculty of Mining, Geology and Petroleum Engineering, Zagreb, Croatia.

Introduction

The Infectious Diseases Society of America in 2009 summarized the highly problematic bacteria by the "ESKAPE" mnemonic: ***E****nterococcus faecium,* ***S****taphylococcus aureus,* ***K****lebsiella pneumoniae,* ***A****cinetobacter baumannii,* ***P****seudomonas aeruginosa*, ***E****nterobacter* spp. [1]. The ESKAPE pathogens developed mechanisms to escape the biocidal action of available antibiotics, and cause emerging human infections worldwide.

Bacterial resistance to carbapenems has received special attention, because carbapenems are used as a last-resort antibiotics to treat infections caused by antibiotic-resistant bacteria. Among the ESKAPE pathogens, carbapenem-resistant *Acinetobacter baumannii* has been positioned in 2017 at the top of the World Health Organization priority list, for which new antibiotics are urgently needed.

Nowadays, *A. baumannii* is a leading cause of hospital outbreaks, but community-acquired infections outside hospital environment also have been recorded [2]. Little is known about the presence of this ESKAPE pathogen outside hospital settings, and the role of environmental isolates in the epidemiology of *A. baumannii* is not elucidated. *A. baumannii* has been unsuccessfully searched in soils as a source of infection among US service members injured during Operation Iraqi Freedom, probable due to the long storage duration of archived soils [3].

Here we report the finding of three carbapenem-resistant isolates of *A. baumannii* in technosol at an illegal dumpsite in Croatia.

Materials and Methods

Examined dump site is situated in a karst pit above City of Rijeka in Croatia (Fig. 1). At this dump site the hazardous industrial waste was continuously disposed during 20th century, and later is used as an illegal dump site. Developed technosol at the edge of the dump was aseptically collected in October 2016. Three *A. baumannii* were recovered from inoculated plates of CHROMagar Acinetobacter supplemented with 15 mg/L of cefsulodin sodium salt hydrate, after incubation at 42°C/48h (Fig. 2).

The identification of isolates was performed by MALDI-TOF mass spectrometry on cell extracts. The relationship of three environmental and known clinical isolates was determined by multilocus sequence typing (MLST) of seven housekeeping genes (*gltA, gyrB, gdhB, recA, cpn60, gpi, rpoD*) according to MLST Oxford scheme. The antibiotic susceptibility profile was determined by minimum inhibitory concentration (MIC) values, and interpreted according to the official criteria for clinical isolates [4,5]. Genes encoding the acquired OXA-type carbapenemases (oxacillinases) were amplified by multiplex PCR.

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Fig. 1. Dumpsite containing 250,000 m^3 of waste.

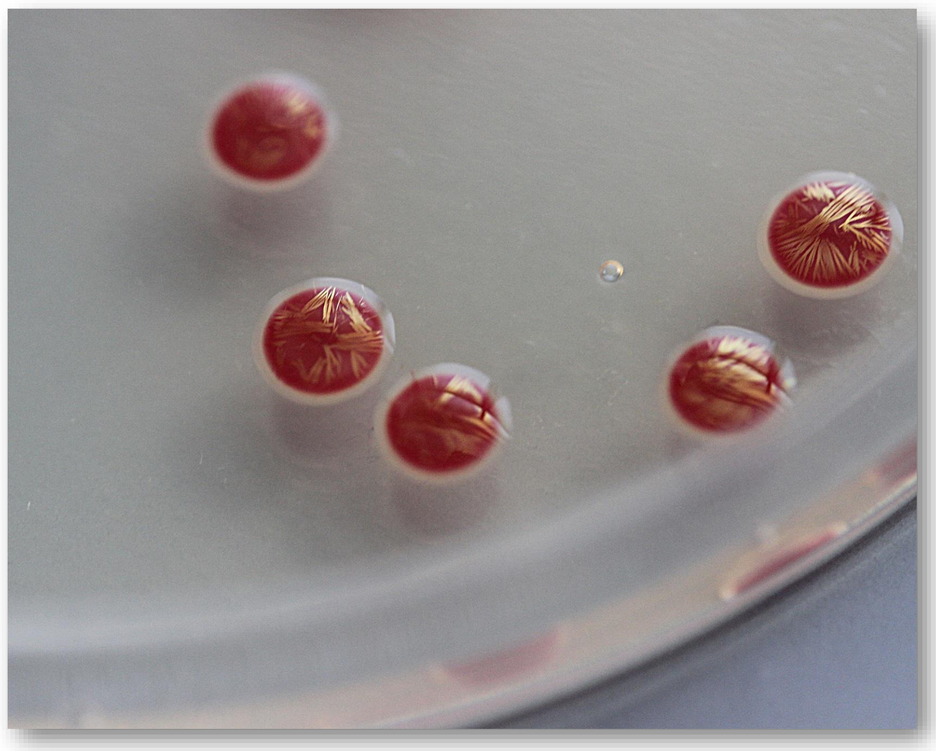

Fig. 2. Colonies of *A. baumannii* grown on CHROMagar Acinetobacter.

Results

Three isolates of *A. baumannii* shared features with the widespread human clinical isolates (Table 1):

- affiliation to the international clonal lineage IC1 (sequence type ST-231) or IC2 (ST-195);
- multi-drug resistance (non-susceptible to ≥1 agent in ≥3 antimicrobial categories);
- carbapenem-resistance mediated by acquired bla_{OXA-72} and bla_{OXA-23} genes.

These features classified three isolates of *A. baumannii* as ESKAPE pathogens. Close relatedness of environmental and clinical isolates suggest the illegally disposed hospital waste as the most probable source of *A. baumannii* in technosol.

Table 1. MLST results (international clonal lineage-IC and sequence type-ST), MIC values of tested antibiotics[a], and the presence of acquired *bla*OXA genes in three *A. baumannii* isolates originating from technosol.

[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), folate pathway inhibitors (SXT- trimethoprim/sulfamethoxazole), polymyxins (CST-colistin).

[R] - resistant, [I] - intermediate according to EUCAST and CLSI criteria.

Isolate name	IC lineage	ST	MIC values of antibiotics (mg/L)													*bla*OXA gene
			MEM	IPM	CIP	LVX	TOB	GEN	AMK	MIN	SAM	TIM	TZP	SXT	CST	
Sovjak 1	1	231	≥16[R]	≥16[R]	≥4[R]	4[R]	≤1	≤1	32[R]	≤1	16[I]	≥128[R]	≥128[R]	≤20	≤0.5	OXA-72
Sovjak 2	1	231	≥16[R]	≥16[R]	≥4[R]	4[R]	≤1	≤1	16[I]	≤1	16[I]	≥128[R]	≥128[R]	≤20	≤0.5	OXA-72
Sovjak 3	2	195	≥16[R]	≥16[R]	≥4[R]	4[R]	≤1	≤1	>64[R]	8[I]	16[I]	≥128[R]	≥128[R]	≥320[R]	≤0.5	OXA-23

Conclusions

- ESKAPE pathogens are present in soil influenced by illegally disposed human waste in Croatia.
- Proper management and disposal of human waste is mandatory to prevent the spread of ESKAPE pathogens in nature.

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References

1. Gilmore Boucher HW, Talbot GH, Bradley JS, *et al.* Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis. 2009;48:1-12.
2. Dexter C, Murray GL, Paulsen IT, *et al.* Community-acquired *Acinetobacter baumannii*: clinical characteristics, epidemiology and pathogenesis. Expert Rev Anti Infect Ther. 2015;13:567-573.Scott P, Deye G, Srinivasan A, *et al.* An outbreak of multidrug-resistant *Acinetobacter baumannii-calcoaceticus* complex infection in the US military health care system associated with military operations in Iraq. Clin Infect Dis. 2007;44:1577-1584.
3. European Committee on Antimicrobial Susceptibility Testing, 2017. EUCAST Reading guide. Version 7.1.
4. Clinical and Laboratory Standards Institute, 2015. Performance standards for antimicrobial susceptibility testing; Twenty-third informational supplement. CLSI Document M100-S25.