



ACINETOBACTER BAUMANNII SURVIVE ANAEROBIC DIGESTION OF SEWAGE SLUDGE

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Introduction

From 2010 onwards (Girlich et al. 2010), continuous reports on the occurrence of *Acinetobacter baumannii* outside hospital environment can be found. Multi-drug resistant (MDR) *A. baumannii* were found in untreated as well as in biologically or chemically treated hospital and municipal wastewaters (Ferreira et al. 2011; Zhang et al. 2013, Seruga Music et al. 2017). However, there is no evidence about the fate of this emerging hospital pathogen in the wastewater treatment plants with sewage sludge treatment process.

The **aim** of this study was to screen the sewage sludge after its stabilization by anaerobic mesophilic digestion for the presence of *A. baumannii*.

Materials and methods

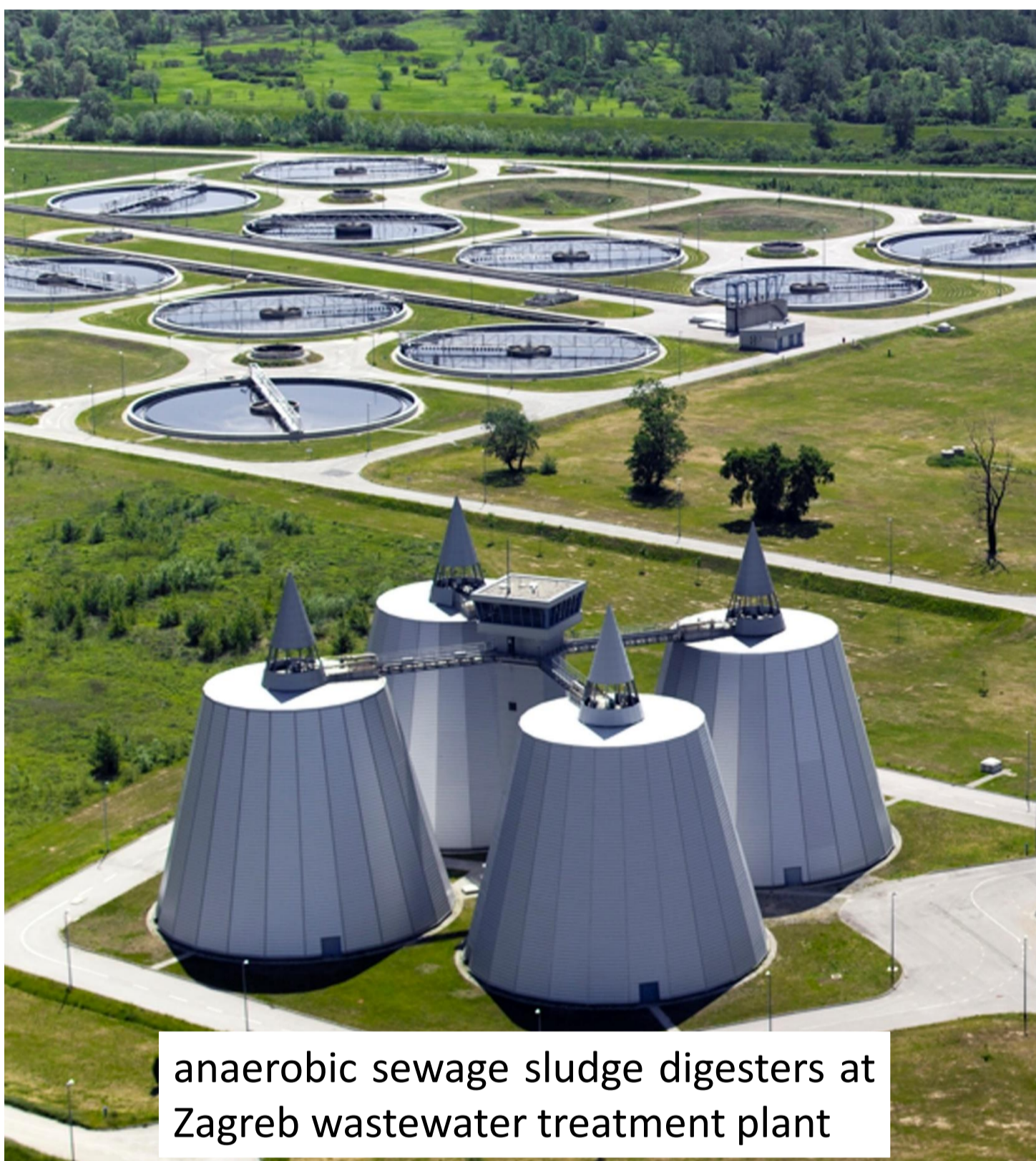
The sampling of digested sludge was done during 10 months (September 2015-June 2016) at the municipal wastewater treatment plant of the City of Zagreb, Croatia. The isolation of *A. baumannii* was performed on CHROMagar Acinetobacter supplemented with 15 mg/L of cefsulodin sodium salt hydrate after incubation at 42°C/48h (Seruga Music et al. 2017).

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 antibiotic susceptibility profile was determined according to MICs values obtained by Vitek2 system and E-test for colistin.

Results

On 9 sampling occasions 17 isolates of *A. baumannii* were recovered from digested sludge with MALDI-TOF MS score values ranging from 2.026-2.288. 3/17 isolates were sensitive to all 12 antibiotics tested, while 14 MDR isolates shared the resistance to carbapenems and fluoroquinolones but sensitivity to colistin.

Technology of anaerobic sludge digestion was performed at 36°C, neutral pH and digestion time of 21-36 days. The ability of isolates to survive or multiply in anaerobic atmosphere was checked in controlled laboratory conditions. Isolates were able to survive on Nutrient agar in anaerocult A system during 30 days, after which multiplied normally in aerobic conditions. However, isolates were not able to multiply directly in anaerocult A.



anaerobic sewage sludge digesters at Zagreb wastewater treatment plant

Table 1. Date of isolation, MALDI-TOF MS score values, and MIC values of tested antibiotics^a against isolates 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), SXT-trimethoprim/sulfamethoxazole, CST-colistin.

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

Isolate	Date of isolation	MALDI TOF score value	MIC values of antibiotics (mg/L)											
			MEM	IPM	CIP	LVX	TOB	GEN	AMK	MIN	SAM	TIM	SXT	CST
DIG10	23.9.2015	2.248	0.5	≤0.25	≤0.25	≤0.12	≤1	≤1	≤2	≤1	≤2	≤8	≤20	≤0.5
DIG11	14.10.2015	2.103	≥16 ^R	≥16 ^R	≥4 ^R	4 ^R	≤1	≤1	32 ^R	≤1	16 ^I	≥128 ^R	≤20	≤0.5
DIG12	14.10.2015	2.037	≥16 ^R	≥16 ^R	≥4 ^R	4 ^R	≤1	≤1	16 ^I	≤1	16 ^I	≥128 ^R	≤20	≤0.5
DIG13	18.11.2015	2.048	≤0.25	≤0.25	≤0.25	≤0.12	≤1	≤1	≤2	≤1	≤2	≤8	≤20	≤0.5
DIG15	13.1.2016	2.071	≥16 ^R	≥16 ^R	≥4 ^R	≥8 ^R	≥16 ^R	≥16 ^R	8	≥16 ^R	≥32 ^R	≥128 ^R	≤20	≤0.5
DIG16	26.1.2016	2.081	≤0.25	≤0.25	≤0.25	≤0.12	≤1	≤1	≤2	≤1	≤2	≤8	≤20	≤0.5
DIG17	10.2.2016	2.253	≥16 ^R	≥16 ^R	≥4 ^R	≥8 ^R	≥16 ^R	≥16 ^R	≥64 ^R	8 ^I	16 ^I	≥128 ^R	≥320 ^R	≤0.5
DIG18	10.2.2016	2.062	≥16 ^R	≥16 ^R	≥4 ^R	≥8 ^R	≥16 ^R	≥16 ^R	≥64 ^R	8 ^I	≥32 ^R	≥128 ^R	≥320 ^R	≤0.5
DIG19	10.2.2016	2.184	≥16 ^R	≥16 ^R	≥4 ^R	≥8 ^R	≥16 ^R	≥16 ^R	≥64 ^R	8 ^I	≥32 ^R	≥128 ^R	≥320 ^R	≤0.5
DIG20	10.2.2016	2.081	≥16 ^R	≥16 ^R	≥4 ^R	≥8 ^R	8 ^R	≥16 ^R	≥64 ^R	8 ^I	16 ^I	≥128 ^R	≥320 ^R	≤0.5
DIG21	10.2.2016	2.155	≥16 ^R	≥16 ^R	≥4 ^R	≥8 ^R	≥16 ^R	≥16 ^R	≥64 ^R	8 ^I	≥32 ^R	≥128 ^R	≥320 ^R	≤0.5
DIG22	10.2.2016	2.232	≥16 ^R	≥16 ^R	≥4 ^R	≥8 ^R	≥16 ^R	≥16 ^R	≥64 ^R	8 ^I	16 ^I	≥128 ^R	≥320 ^R	≤0.5
DIG23	10.2.2016	2.026	≥16 ^R	≥16 ^R	≥4 ^R	≥8 ^R	≥16 ^R	≥16 ^R	≥64 ^R	8 ^I	16 ^I	≥128 ^R	≥320 ^R	≤0.5
DIG24	10.2.2016	2.206	≥16 ^R	≥16 ^R	≥4 ^R	≥8 ^R	≥16 ^R	≥16 ^R	≥64 ^R	8 ^I	16 ^I	≥128 ^R	≥320 ^R	≤0.5
DIG25	24.2.2016	2.071	≥16 ^R	≥16 ^R	≥4 ^R	4 ^R	≤1	8 ^R	≥64 ^R	≤1	≤2	≥128 ^R	80 ^I	≤0.5
DIG26	23.3.2016	2.054	≥16 ^R	≥16 ^R	≥4 ^R	≥8 ^R	≤1	≥16 ^R	≥64 ^R	8 ^I	16 ^I	≥128 ^R	≤20	≤0.5
DIG27	8.6.2016	2.288	≥16 ^R	≥16 ^R	≥4 ^R	≥8 ^R	≥16 ^R	≥16 ^R	≥64 ^R	8 ^I	16 ^I	≥128 ^R	≤20	≤0.5



30 days in anaerobic atmosphere



normal growth in aerobic conditions

A. baumannii isolates were able to survive on Nutrient agar in anaerocult A during 30 days, after which multiplied normally in aerobic conditions



Aerobic cultivation

Anaerobic cultivation

A. baumannii isolates were not able to multiply directly in anaerocult A

Conclusion

The study confirmed the ability of *A. baumannii* to survive the technological process of anaerobic mesophilic digestion of sewage sludge.

The finding confirms the need of proper management and disposal of sewage sludge generated at wastewater treatment plants in order to prevent the spread of MDR *A. baumannii* in nature.

Moreover, it indicates the anaerobic environments as possible ecological niches that enable the survival of this emerging human pathogen.

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