

MULTI-DRUG RESISTANT ACINETOBACTER BAUMANNII FROM HOSPITAL WASTEWATER OF ZAGREB, CROATIA

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Background

Acinetobacter baumannii is well known emerging hospital pathogen of the 21st century. Multi-drug resistant (MDR) *A. baumannii* are found in untreated as well as in chemically treated hospital wastewaters. However, there is no clear evidence about their origin.

Objectives

The aim of this study was to screen the hospital wastewater for the presence of viable *A. baumannii* and to find the correlation between recovered wastewater and clinical isolates.

Here we report the finding of MDR carbapenem-resistant *A. baumannii* in untreated hospital wastewater which is related to clinical isolates.

Materials and Methods

The sampling of the hospital wastewater was done at the central manhole of the Special Hospital for Pulmonary Diseases in Zagreb, Croatia. The clinical isolate was routinely isolated on the same date from bronchial aspirate of a patient hospitalized in the same hospital.

The isolation of *A. baumannii* was performed on CHROMagar Acinetobacter supplemented with CR102 and 15 mg/L of cefsulodin sodium salt hydrate after incubation at 42°C/48h (Figure 1). Identification of *A. baumannii* was performed by routine bacteriological techniques and Vitek2 system (BioMerieux). The identification was confirmed by MALDI-TOF MS on cell extracts. The molecular identification was performed by amplification of a fragment of *rpoB* gene. The antibiotic susceptibility profile was determined according to MICs values obtained by Vitek2 system.

The genetic relatedness of *A. baumannii* isolates from wastewater to clinical isolate was determined by multilocus sequence typing (MLST) of seven housekeeping gene fragments (*gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi* and *rpoD*) according to Oxford MLST scheme.



Figure 1. Pure culture of presumptive *A. baumannii* grown on CHROMagar Acinetobacter. Colonies were large, circular, convex, smooth, red with a paler central area.

Results

During the monitoring period, in total 7 isolates were collected: 1 clinical isolates and 6 isolates from hospital wastewater. By phenotypical analyses and Vitek2 system isolates were determined as *A. calcoaceticus-baumannii* complex. MALDI-TOF MS analysis gave the reliable score values above 2.0, identifying them as *A. baumannii* (Table 1), which was also confirmed by phylogenetic analysis of the *rpoB* gene (Figure 2). According to the MLST analysis by using Oxford scheme, all isolates showed the same MLST profile and were identified as ST-195, belonging to the CC 92 and IC 2 (Table 2).

The antibiotic resistance profile of clinical isolate was comparable to those of hospital wastewater isolates (Table 3). All isolates were MDR and shared the resistance to carbapenems and fluoroquinolones, but sensitivity to colistin.

Table 1. Origin, date of isolation, and MALDI-TOF MS score values of *A. baumannii* isolates. All isolates were determined by Vitek 2 system as *A. calcoaceticus-baumannii* complex.

Isolate	Origin	Date of isolation	MALDI TOF score value
OB 4138	Bronchial aspirate	2. 10. 2015	2.021
S1/1	Hospital wastewater	6. 10. 2015	2.271
S2/5	Hospital wastewater	6. 10. 2015	2.067
S2/6	Hospital wastewater	6. 10. 2015	2.232
S2/7	Hospital wastewater	6. 10. 2015	2.102
S2/8	Hospital wastewater	6. 10. 2015	2.077
S2/9	Hospital wastewater	6. 10. 2015	2.041

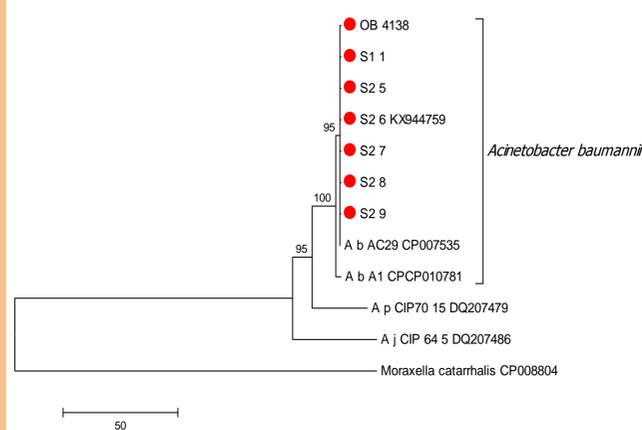


Figure 2. Phylogenetic tree (NJ method, number of differences) constructed on the basis of *rpoB* gene representing the molecular identification of *A. baumannii* isolates. GenBank accession numbers are given next to the name of each strain. *Moraxella catarrhalis rpoB* gene sequence was used as an outgroup to root the tree. Red dots represent the sequences of strains analyzed in this study.

Table 2. Multilocus sequence typing of *A. baumannii* clinical isolate and isolates originating from hospital wastewater according to MLST Oxford scheme (<http://pubmlst.org/abaumannii/>).

Isolate	Gene locus/allele							Sequence type	Clonal complex	IC type
	<i>gltA</i>	<i>gyrB</i>	<i>gdhB</i>	<i>recA</i>	<i>cpn60</i>	<i>gpi</i>	<i>rpoD</i>			
OB 4138	1	3	3	2	2	96	3	195	92	2
S1/1	1	3	3	2	2	96	3	195	92	2
S2/5	1	3	3	2	2	96	3	195	92	2
S2/6	1	3	3	2	2	96	3	195	92	2
S2/7	1	3	3	2	2	96	3	195	92	2
S2/8	1	3	3	2	2	96	3	195	92	2
S2/9	1	3	3	2	2	96	3	195	92	2

Table 3. MIC values of tested antibiotics against isolates of *A. baumannii*.

Carbapenems (MEM-meropenem, IMI-imipenem), fluoroquinolones (CIP-ciprofloxacin, LVX-levofloxacin), aminoglycosides (TOB-tobramycin, GEN-gentamicin, AMK-amikacin), tetracyclines (MIN-minocycline, TGC-tigecycline), 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	MIC values of antibiotics (mg/L)												
	MEM	IPM	CIP	LVX	TOB	GEN	AMK	MIN	TGC	SAM	TIM	SXT	CST
OB 4138	>16 ^R	>16 ^R	>4 ^R	8 ^R	>16 ^R	>16 ^R	>64 ^R	>16 ^R	4 ^R	16 ^I	128 ^R	<20	<0.5
S1/1	>16 ^R	>16 ^R	>4 ^R	8 ^R	>16 ^R	>16 ^R	>64 ^R	8 ^I	2 ^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	4 ^R	>32 ^R	>128 ^R	<20	<0.5
S2/6	>16 ^R	>16 ^R	>4 ^R	8 ^R	>16 ^R	>16 ^R	8	>16 ^R	4 ^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	4 ^R	>32 ^R	>128 ^R	<20	<0.5
S2/8	>16 ^R	>16 ^R	>4 ^R	8 ^R	>16 ^R	>16 ^R	8	8 ^I	4 ^R	>32 ^R	>128 ^R	<20	<0.5
S2/9	>16 ^R	>16 ^R	>4 ^R	>8 ^R	>16 ^R	>16 ^R	8	8 ^I	4 ^R	16 ^I	>128 ^R	160 ^R	<0.5

Conclusions

This study confirmed the occurrence of viable MDR *A. baumannii* in hospital wastewater in Zagreb. Close relatedness of isolates from hospital wastewater with the clinical isolate recovered from the same hospital in the period of monitoring, suggests that the isolates from wastewater originated from the hospitalized patient. This finding confirms the need for proper treatment and disposal of untreated hospital wastewaters in order to prevent the spread of MDR *A. baumannii* in nature.

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