

OCCURRENCE OF ACINETOBACTER CALCOACETICUS-BAUMANNII COMPLEX IN MUNICIPAL WASTEWATER

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Background: *Acinetobacter calcoaceticus-baumannii* complex (ABC) includes six genetically closely related but phenotypically undistinguishable species: *A. calcoaceticus*, *A. baumannii*, *A. pittii*, *A. nosocomialis*, genomic species “close to 13TU”, and genomic species “between 1 and 3”. *A. baumannii* is most often studied as an emerging hospital pathogen. Reports on the occurrence of ABC in wastewater treatment plants are scarce.

The **objective** of this study was to screen the municipal wastewater of Zagreb, the capital of Croatia, for the presence of viable ABC and for its possible discharge into a natural recipient after the wastewater passage through the treatment process.

Material/methods: The sampling was done at the secondary type of wastewater treatment plant of the City of Zagreb (capacity 1,200,000 population equivalents, Fig. 1) where the municipal wastewater treated consists of domestic, industrial, hospital and storm wastewaters. The composite 24h samples of the influent and effluent wastewater were collected during 6 months in 2014.

The isolation of ABC from wastewater was performed at 42°C/48h on CHROMagar Acinetobacter with or without the addition of commercial supplement CR102 (CHROMagar) allowing the growth of carbapenem-resistant isolates.



Figure 1. Wastewater treatment plant of the City of Zagreb.

Presumptive ABC colonies (Fig. 2) were characterized phenotypically by routine bacteriological techniques and by using Vitek 2 system. Further identification was carried out by MALDI-TOF MS and RNA polymerase β -subunit (*rpoB*) gene analyses. Antibiotic resistance profiles were determined for carbapenems meropenem and imipenem by Vitek 2 system and interpreted according to EUCAST criteria.

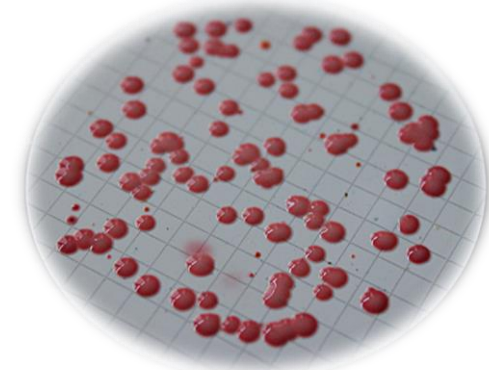


Figure 2. Presumptive ABC colonies grown on CHROMagar Acinetobacter. Colonies of ABC were large, circular, convex, smooth, red with a paler central area.

Results: On 6 sampling occasions 34 isolates belonging to ABC, as determined by Vitek 2 system, were recovered: 28 from influent and 6 from effluent wastewater (Table 1). The comparison of ribosomal proteins by MALDI-TOF MS with strains of bacteria in MALDI Biotyper database gave the score values from 2.013 - 2.409 identifying the ABC isolates as *A. baumannii* or *A. pittii*. Phylogenetic analysis of the *rpoB* gene fragment confirmed the identity of isolates as *A. baumannii* or *A. pittii* and showed their close relatedness to the clinical isolates with 100% sequence ID (Fig. 3). Molecular identification of ABC confirmed that 22 and 6 isolates of *A. baumannii* were recovered from influent and effluent wastewater, respectively, while 6 *A. pittii* were recovered only from influent wastewater. The 28/34 isolates (27 *A. baumannii* and 1 *A. pittii*) were resistant to carbapenems (Table 1).

Table 1. Date of sampling of influent or effluent wastewater, MALDI-TOF MS score values of ABC isolates, and MIC values of carbapenems. Isolates named as IN were isolated from influent wastewater and isolates named as EF were isolated from effluent wastewater. All isolates were determined by Vitek 2 system as *A. calcoaceticus-baumannii* complex. ^R resistant; ^I intermediate according to EUCAST criteria.

Sampling date	Isolate name	MALDI TOF identification (score value)	MIC values of antibiotics (mg/L)	
			meropenem	imipenem
16.4.2014	EF1	<i>A. baumannii</i> (2.262)	>16 ^R	>16 ^R
	EF2	<i>A. baumannii</i> (2.352)	>16 ^R	>16 ^R
	EF3	<i>A. baumannii</i> (2.329)	>16 ^R	>16 ^R
7.5.2014	IN1	<i>A. pittii</i> (2.177)	0.25	<0.25
	IN2	<i>A. pittii</i> (2.156)	0.25	0.25
	IN3	<i>A. pittii</i> (2.222)	0.25	<0.25
11.6.2014	IN4	<i>A. baumannii</i> (2.231)	>16 ^R	>16 ^R
	IN5	<i>A. baumannii</i> (2.085)	>16 ^R	>16 ^R
	IN6	<i>A. baumannii</i> (2.157)	>16 ^R	>16 ^R
	IN8	<i>A. baumannii</i> (2.168)	>16 ^R	>16 ^R
	IN9	<i>A. baumannii</i> (2.167)	>16 ^R	>16 ^R
	IN10	<i>A. baumannii</i> (2.193)	>16 ^R	>16 ^R
	IN11	<i>A. baumannii</i> (2.409)	>16 ^R	>16 ^R
29.10.2014	EF4	<i>A. baumannii</i> (2.191)	>32 ^R	>32 ^R
	EF5	<i>A. baumannii</i> (2.161)	>16 ^R	>16 ^R
	EF6	<i>A. baumannii</i> (2.219)	>16 ^R	>16 ^R
	IN12	<i>A. baumannii</i> (2.190)	>16 ^R	>16 ^R
	IN13	<i>A. baumannii</i> (2.118)	>32 ^R	>32 ^R
	IN14	<i>A. baumannii</i> (2.213)	>16 ^R	>16 ^R
	IN15	<i>A. baumannii</i> (2.121)	>16 ^R	>16 ^R
	IN16	<i>A. baumannii</i> (2.244)	>16 ^R	>16 ^R
	IN17	<i>A. baumannii</i> (2.163)	32 ^R	>32 ^R
IN18	<i>A. baumannii</i> (2.048)	>16 ^R	>16 ^R	
IN19	<i>A. baumannii</i> (2.090)	>16 ^R	>16 ^R	
5.11.2014	IN20	<i>A. pittii</i> (2.291)	1.5	0.25
	IN21	<i>A. baumannii</i> (2.328)	0.25	0.25
3.12.2014	IN22	<i>A. baumannii</i> (2.118)	>16 ^R	>16 ^R
	IN23	<i>A. pittii</i> (2.013)	4 ^I	0.50
	IN24	<i>A. baumannii</i> (2.168)	>16 ^R	>16 ^R
	IN25	<i>A. baumannii</i> (2.041)	>16 ^R	>16 ^R
	IN26	<i>A. baumannii</i> (2.223)	32 ^R	8 ^R
	IN27	<i>A. baumannii</i> (2.199)	8 ^R	2
	IN28	<i>A. baumannii</i> (2.085)	16 ^R	8 ^R
	IN29	<i>A. pittii</i> (2.094)	12 ^R	8 ^R

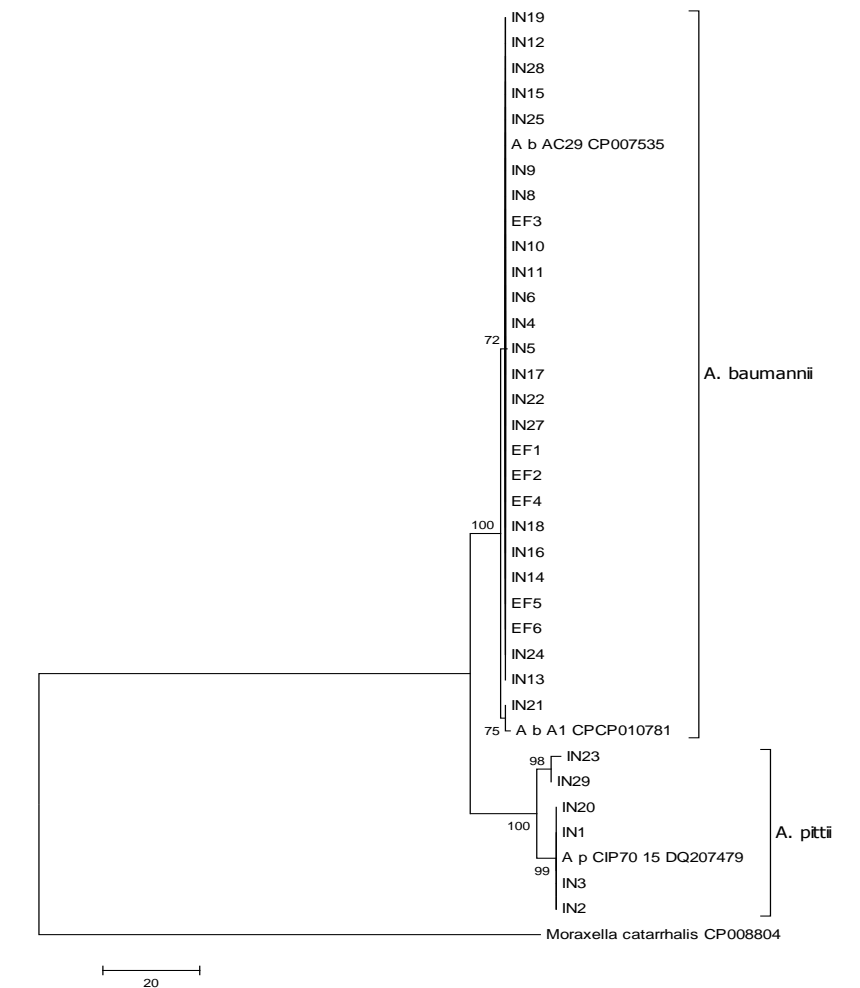


Figure 3. Phylogenetic tree (NJ method, number of differences) constructed on the basis of *rpoB* gene fragment sequence analysis representing the molecular identification of ABC isolates. *Moraxella catarrhalis rpoB* gene fragment was used as an outgroup to root the tree. GenBank accession numbers are given next to the names of reference strains.

Conclusions: Municipal wastewaters of Zagreb are continuously polluted with ABC probably due to the input of untreated hospital wastewaters. Among the ABC, only *A. baumannii* and *A. pittii* species are present with *A. baumannii* as a predominant species. More frequent isolation of *A. baumannii* from influent than from effluent suggests its moderate elimination, but also its persistence in the secondary type wastewater treatment system. The absence of *A. pittii* isolation from effluent suggests its complete elimination in the secondary type of wastewater treatment plant.