

# ANTIBIOTIC SUSCEPTIBILITY AND CARBAPENEMASE PRODUCTION OF INVASIVE ISOLATES OF ACINETOBACTER BAUMANNII FROM CROATIA

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## Introduction and purpose

*Acinetobacter baumannii* has become one of the most challenging agents of nosocomial infections in recent years; its relevance largely depends on a wide antimicrobial resistance, arising from a variety of intrinsic and acquired mechanisms. Of particular concern is resistance to  $\beta$ -lactam antibiotics, particularly carbapenems, which is observed more and more frequently all over the world and which is often associated with few international clones, namely international clonal lineages I, II and III. The first carbapenem-resistant isolates in Croatia were found in University Hospital Split in 2002. The carbapenem-resistance was mediated by hyperproduction of OXA-51 due the IS*Aba1* located upstream of the genes. Later, the studies of carbapenem-resistance in Croatia reported OXA-72  $\beta$ -lactamase in University Hospital Center Zagreb and University Hospital Split. These observations gave rise to a multicenter study conducted in last three months of 2009. In Northern Croatia and Istria. The aim of the study was to characterize the carbapenem resistance and to compare the genotypes of the isolates.

## Methods

Out of 185 isolates collected from 13 centers in Northern Croatia and Istria 26 isolates from invasive infections (bloodstream or cerebrospinal fluid infections) were selected for this study.

Antibiotic susceptibilities were determined by broth microdilution.

Genes encoding OXA-23-like, OXA-24/40-like, OXA-51-like, OXA-58-like, OXA-143-like carbapenem-hydrolyzing oxacillinases in addition to metallo-beta-lactamases (MBLs) of VIM, IMP and SIM series, and PER-1 and TEM-1 beta-lactamases were detected by PCR and when needed identified by sequencing.

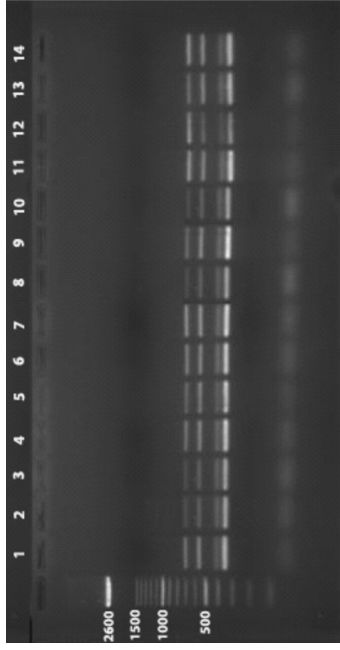
Presence of IS*Aba1* upstream of *bla*<sub>OXA-51</sub> gene was detected by PCR mapping. Sequence groups (SG) corresponding to international clonal lineages (ICL I - III) were determined by multiplex PCR and genotyping of the strains was performed by multilocus-sequence typing (MLST), pulsed-field gel electrophoresis (PFGE) and random amplification of polymorphic DNA (RAPD).

## Results

Nine strains were found to possess acquired oxacillinase; five belonging to OXA-24/40 group, three to OXA-23 and one to OXA-58 group. These strains were uniformly resistant to imipenem, meropenem, piperacillin, cefotaxime, ceftriaxone, gentamicin and ciprofloxacin. The strains were in high percentage resistant to ceftazidime, cefepime and ampicillin/subactam but no resistance to colistin was observed. (Table 1, Table 2)

The strains originated from University Hospital Center Zagreb (mostly from surgery ward), belonged to sequence group 1 (International clonal Lineage I) (Figure 1) and according to PFGE belonged to three major clones with > 85% similarity of their PFGE profiles, which contained subclusters with > 90% of similarity. (Figure 2) The sequencing of *bla*<sub>OXA-51-like</sub> gene revealed the presence of *bla*<sub>OXA-66</sub> gene.

The remaining 17 isolates possessed only the naturally occurring OXA-51  $\beta$ -lactamase which was upregulated by IS*Aba1* located upstream of *bla*<sub>OXA-51</sub> gene in 14 strains. MBLs were not found. All but three of these strains were susceptible or intermediate susceptible to imipenem and meropenem. The majority of these strains originated from surgical unit of General Hospital in Pula and belonged to SG 2 (ICL I) while PFGE identified two major clones which showed more than 85% similarity of their banding patterns. The sequencing of *bla*<sub>OXA-51-like</sub> gene revealed the presence of *bla*<sub>OXA-69</sub> and *bla*<sub>OXA-107</sub> genes.



**Figure 1. Multiplex PCR for determination of sequence group 1**

1. A. baumannii 191 (+), 2. A. baumannii 195 (+), 3. A. baumannii 198 (+), 4. A. baumannii 204 (+), 5. A. baumannii 206 (+), 6. A. baumannii 213 (+), 7. A. baumannii 217 (+), 8. A. baumannii 218 (+), 9. A. baumannii 229 (+), 10. A. baumannii 232 (+), 11. A. baumannii 236 (+), 12. A. baumannii 251 (+), 13. baumannii 11 (positive control), 14. A. baumannii 13 (positive control)

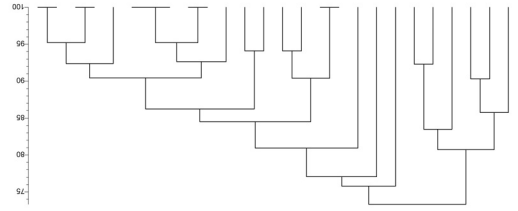
**Table 1. Susceptibility to carbapenems, sequence group and OXA content of invasive Acinetobacter baumannii isolates.**

ISOLATE	MIC	IMP	SG	ICL	OXA CONTENT	OXA-51 TYPE	IS <i>Aba1</i> /OXA-51	CENTER	WARD	SPECIMEN
6	64	32	1	2	51,23	66	+	UHC ZAGREB	SURG/ICU	Blood culture
40	64	16	1	2	51,23	66	+	UHC ZAGREB	SURG/ICU	Blood culture
57	≥128	128	1	2	51,23	66	+	UHC ZAGREB	SURG/ICU	Blood culture
19	32	32	1	2	51,24	66	+	UHC ZAGREB	MED/DIAB	Blood culture
32	128	128	1	2	51,24	66	+	UHC ZAGREB	SURG/ICU	Cerebrospinal fluid
56	≥128	≥128	1	2	51,24	66	+	UHC ZAGREB	SURG/ABD	Blood culture
99	≥128	128	1	2	51,24	66	+	UHC ZAGREB	ICU/PED	Cerebrospinal fluid
128	≥128	128	1	2	51,24	66	+	UHC ZAGREB	SURG/ICU	Blood culture
135	128	64	1	2	51,58	66	+	UHC ZAGREB	SURG/ICU	Blood culture
14	16	16	1	2	51	107	+	GH VARAŽDIN	INFECT	Blood culture
52	2	4	2	1	51	107	+	UHC ZAGREB	SURG/ICU	Blood culture
97	8	4	2	1	51	107	+	UHC ZAGREB	NEURL	Cerebrospinal fluid
111	0.5	1	2	1	51	107	-	UHC ZAGREB	SURG/ICU	Blood culture
141	32	32	2	1	51	107	+	UHC ZAGREB	SURG/ICU	Blood culture
191	8	4	2	1	51	69	+	GH PULA	SURG/ICU	Cerebrospinal fluid
195	8	8	2	1	51	69	+	GH PULA	SURG/ICU	Blood culture
198	8	8	2	1	51	69	+	GH PULA	SURG/ICU	Cerebrospinal fluid
204	8	8	2	1	51	69	+	GH PULA	SURG/ICU	Cerebrospinal fluid
206	8	8	2	1	51	69	+	GH PULA	SURG/ICU	Cerebrospinal fluid
213	8	8	2	1	51	69	+	GH PULA	SURG/ICU	Blood culture
217	≤0.06	≤0.06	2	1	51	69	-	GH PULA	SURG/ICU	CSF
218	8	4	2	1	51	69	+	GH PULA	SURG/ICU	Cerebrospinal fluid
229	8	4	2	1	51	69	+	GH PULA	SURG/ICU	Cerebrospinal fluid
232	4	8	2	1	51	69	+	GH PULA	SURG/ICU	Cerebrospinal fluid
236	8	16	2	1	51	69	+	GH PULA	SURG/ICU	Cerebrospinal fluid
251	8	8	2	1	51	69	+	GH PULA	SURG/ICU	Cerebrospinal fluid

**ABBREVIATIONS:** UHC-University Hospital Center Zagreb, GH-Pula-General Hospital Pula, GH-Varaždin-General Hospital Varaždin, MIC-minimum inhibitory concentration, SG-sequence group, ICL-international clonal lineage

**Table 2. Antibiotic susceptibility of invasive A. baumannii isolates.**

Antibiotic and resistance breakpoint according to CLSI (mg/L)	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>	Number and percentage of resistant isolates (%)
Ceftriaxone (≥ 64)	256 - ≥256	256	>256	26/26 (100)
Cefotaxime (≥ 64)	32 - ≥2256	64	256	21/26 (81)
Ceftazidime (≥ 32)	1 - ≥256	32	256	23/26 (88)
Piperacillin (≥ 128)	128 - ≥256	256	≥256	26/26 (100)
Piperacillin/tazobactam (≥ 128/4)	8 - ≥256	≥128	256	26/26 (100)
Gentamicin (≥ 16)	≤0.5 - ≥256	128	256	25/26 (96)
Meropenem (≥ 16)	≤0.06 - ≥128	4	128	11/26 (42)
Imipenem (≥ 16)	≤0.06 - ≥128	4	64	12/26 (46)
Colistin (≥ 4)	0.5 - 2	1	1	0/26 (0)
Ampicillin/sulbactam (≥ 32/16)	4 - ≥256	16	256	14/26 (54)
Ciprofloxacin (≥ 4)	0.25 - ≥256	64	256	25/26 (96)
Cefepime (≥ 32)	≤0.5 - ≥256	32	128	19/26 (73)
Amikacin (≥ 64)	1 - ≥256	64	≥128	16/26 (62)



**Figure 2. PFGE dendrogram of invasive Acinetobacter baumannii isolates.**

## Conclusions

- The study revealed high prevalence of acquired oxacillinases associated with high level of resistance to carbapenems among invasive isolates of *A. baumannii* from Croatia.
- High diversity of acquired oxacillinases was noticed.
- IS*Aba1* driven overexpression of OXA-51-like beta-lactamase was associated with slightly elevated MICs of carbapenems whereas the presence of acquired oxacillinases correlated with high level of resistance to carbapenems.
- In conclusion, this study highlights the multiple strategies of carbapenemase-mediated resistance among invasive isolates of *A. baumannii* from Croatia.