



IX. HRVATSKI SIMPOZIJ O REZISTENCIJI
BAKTERIJA NA ANTIBIOTIKE
s međunarodnim sudjelovanjem

*9th Croatian Symposium on
Antibiotic Resistance
with international participation*

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Resistant *Acinetobacter baumannii* – what's new?

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Resistant *Acinetobacter baumannii* – what's new?



[US National Library of Medicine National Institutes of Health](http://pubmed.ncbi.nlm.nih.gov/)

- Search: resistant *A. baumannii*
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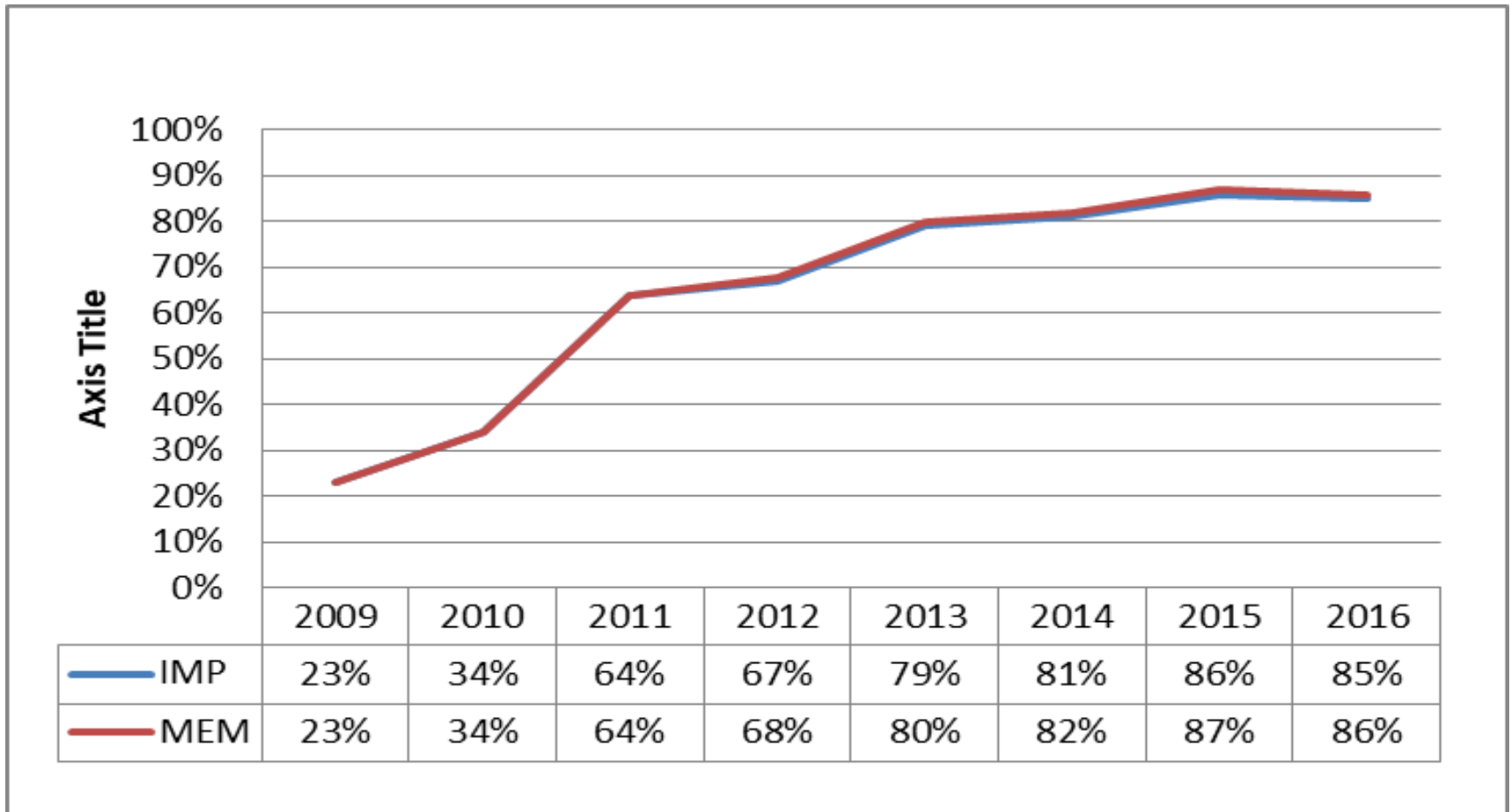
Resistant *Acinetobacter baumannii* – what's new?

- Resistance to antimicrobials including colistin
- Epidemiology of microorganism including survival of *A. baumannii* in natural seawater
- Pandrug resistant isolate, unusually resistance phenotype, new ST inside CC 2

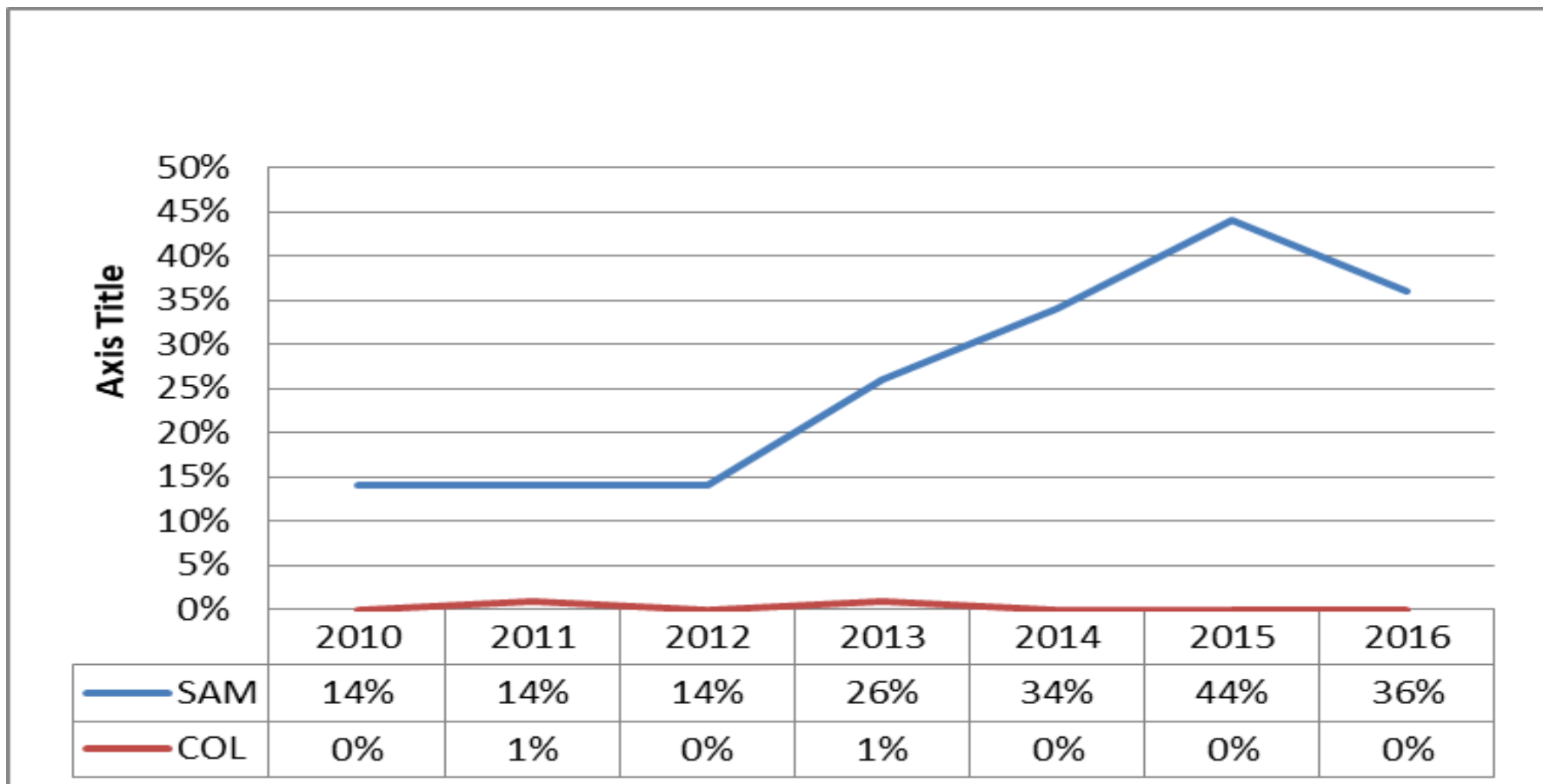
Figure 3.19. *Acinetobacter* spp. Percentage (%) of invasive isolates with resistance to carbapenems, by country, EU/EEA countries, 2014



Resistance to carbapenems in Croatia



Resistance to ampicillin/sulbactam and colistin



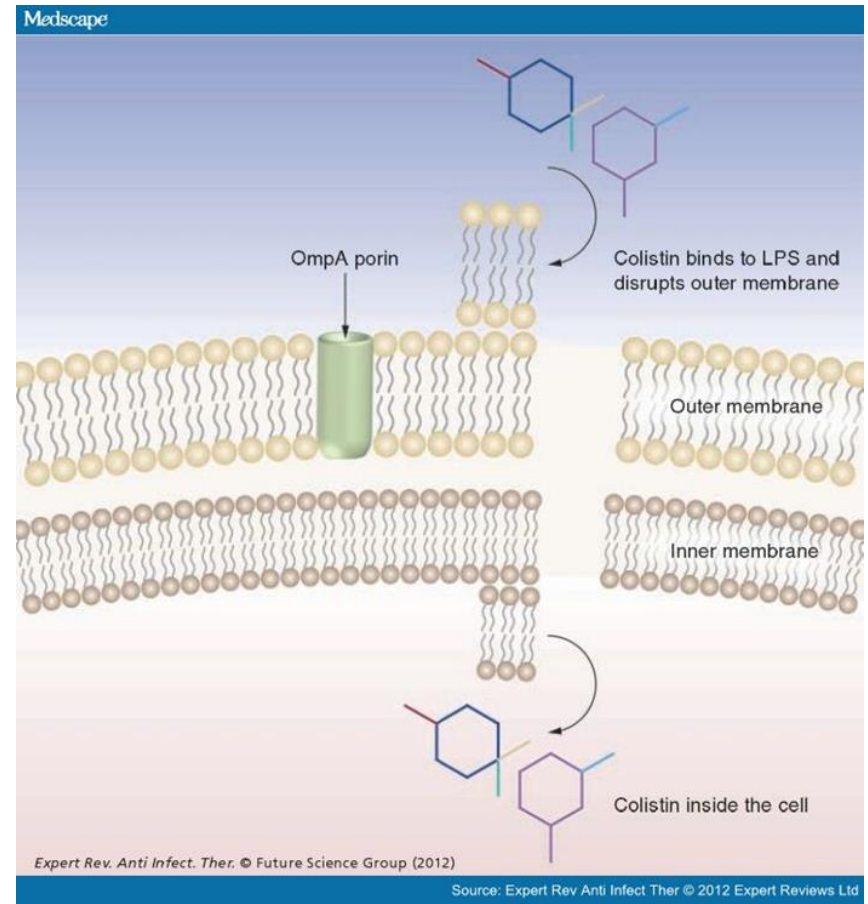
Croatian Committee for Antibiotic Resistance Surveillance

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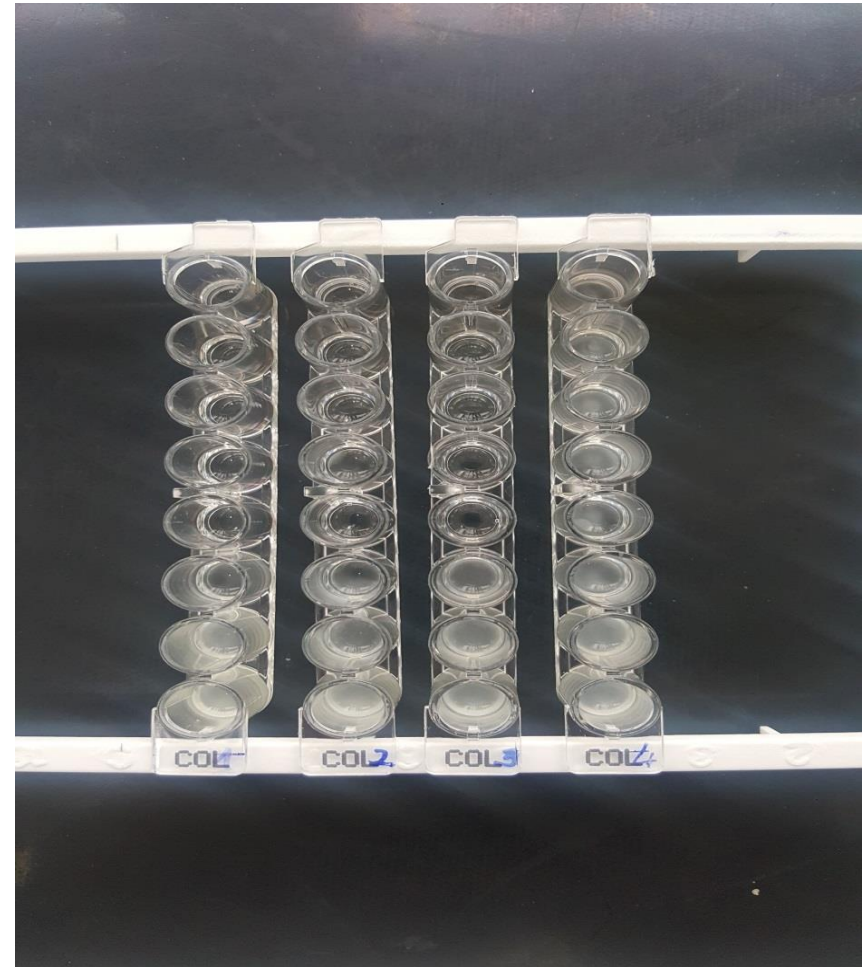
Resistance to colistin

- heteroresistance -possible plasmid-encoded mobile colistin resistance-1 (MCR-1) protein
- mutation in *lpxA*, *lpxC* and *lpxD* genes – high level of resistance
- loss of the initial binding target of polymyxin
- lipid A component of lipopolysaccharide (LPS)




Antimicrobial susceptibility testing of colistin

- broth microdilution method [www. eucast.org](http://www.eucast.org)
22 March 2016
- in the last four months several colistin resistant isolates of *A. baumannii* in UHS
- MIC >16 mg/L
- mechanism of resistance under investigation



High level of MIC to colistin

27th **ECCMID** Vienna, Austria
22 – 25 April 2017

The congress of  ESCMID

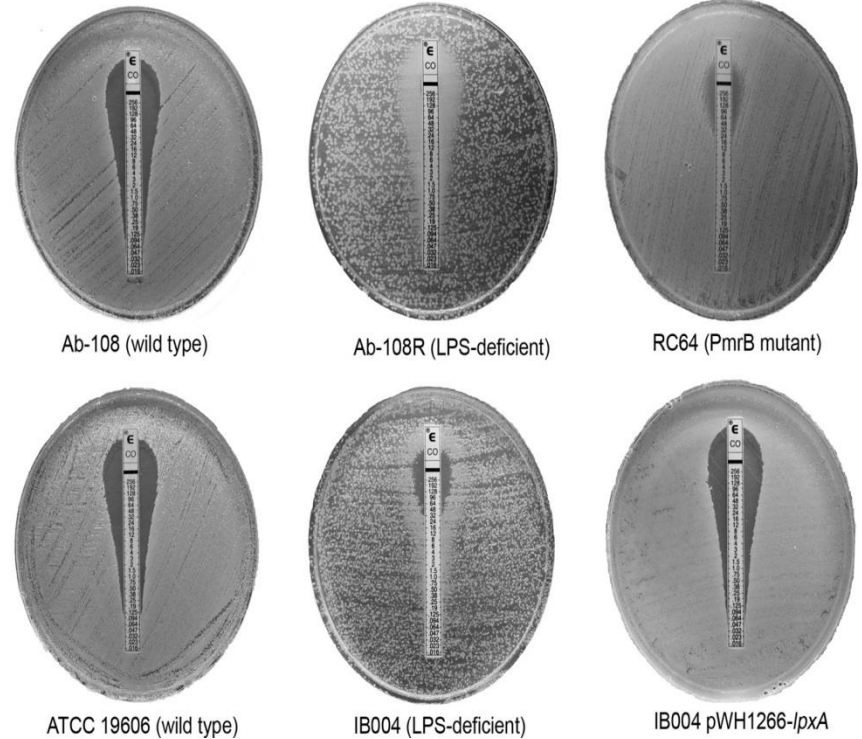
High level colistin resistance in *Acinetobacter baumannii* isolate from patient with mediastinitis after coronary artery bypass graft and aortic valve replacement

Zana Rubic, Zlatko Marovic, Martina Seruga Music, Dijana Skoric, Anita Novak, Marija Tonkic, Jasna Hrenovic, Ivana Goic-Barisic

We tested a total of 1430 *A. baumannii* isolates and 4 showed resistance to colistin. While in three of them minimal inhibitory concentrations (MICs) were slightly above the limit value displaying heteroresistance, **one isolate had a high-level colistin resistance with MIC >256 mg/L**. Only this isolate showed a presence of mutations in *lpxC* gene resulting in amino-acid changes (alterations of valine to alanine at position 95 and glutamine to glutamic acid at position 184), that possibly introduced modifications in the lipid A component of lipopolysaccharide (LPS) and the initial binding target of polymixin antibiotics. Beside mutations in *lpxC*, there were two mutations in *lpxA* gene (positions 290 and 411), within the first one caused alteration of leucine to serine at position 97, and the second one was silent.

Heteroresistance to colistin?

- As of 1 September 2016, the **mcr-1 gene** was detected in 35 countries worldwide in livestock/retail meat and in human sources
- This plasmid is already characterized and can be mobilized by conjugation



Deciphering *Acinetobacter* resistance

- Efflux and colistin resistance?
- Novel pmrAB mutation
- First report of detection of the plasmid-mediated mcr-1 gene in human isolates (Turkey)



28th **ECCMID**

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Epidemiology of MRAB

Hospital wastewater as a route for transmission carbapenem-resistant *Acinetobacter baumannii* outside hospital setting

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³Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia

eP 2254

Background

Acinetobacter baumannii origin and epidemiology is under a great concern worldwide since this microorganism has become a leading nosocomial pathogen of the 21st century among the "ESCAPE" group of microorganisms. Since 2009 University Hospital Centre Split (UHCS) in Croatia has a growing problem in the number of infections caused by carbapenem-resistant isolates of *A. baumannii* which is now almost endemically present in most of the intensive care units inside the hospital. The recent literature confirmed the appearance of carbapenem resistant isolates of *A. baumannii* in nature that correlated with clinical isolates. Therefore, in order to explore epidemiology and surveillance control of this important hospital pathogen in Croatia we investigated presence of *A. baumannii* in hospital wastewater as a route for possible transmission outside of hospital setting.

Fig.1 Multiplex PCR results from wastewater isolates (2-12) and clinical isolates (13-16). 1-neg control. K 1-3 pos control



Materials/methods

For prospective investigation UHCS wastewater was sampled for five times, on two different locations, in the period from October 2015 to April 2016. Samples of hospital wastewater were taken in 500 ml sterile bottles and inoculated within two hours on solid media. The isolation of *A. baumannii* was performed on CHROMagar *Acinetobacter* supplemented with CR102 (CHROMagar) and 15mg/L of cefsulodin sodium salt hydrate (Sigma-Aldrich). The plates were incubated at 42°C/48h. Identification of *A. baumannii* was performed by routine bacteriological techniques and confirmed by MALDI-TOF MS (Bruker Daltonics) on cell extracts. Antibiotic susceptibility was assessed by disk diffusion method. The MICs values were confirmed by Vitek2 system or E-tests (AB Biodisk), and interpreted according to the EUCAST criteria, except for ampicillin/sulbactam and tigecycline that were interpreted according to CLSI criteria. The presence of *blaOXA* genes encoding OXA-type carbapenemases (OXA-51-like, OXA-23like, OXA-40-like, OXA-58-like, and OXA-143) was investigated by multiplex PCR and sequencing. Genotyping was performed using PFGE analysis and the results were compared with unpublished data of previously typed four clinical isolates (c.i.) from the same monitoring period.

Results

During the examination period fourteen both carbapenem and multi-resistant isolates of *A. baumannii* were isolated from hospital wastewater. According to the PFGE analysis and resistance phenotype (profile) 9 isolates (2-4, 6-9 and 11-12) were selected for further molecular characterization and comparison with four clinical isolates. The clinical isolates were collected in the same period of time, during routine surveillance of patient's samples (tracheal aspirates).

Multiplex PCR confirmed that wastewater isolates 2,4,7 and 9 harbored *blaOXA*-23-like while wastewater isolates 3,6,8,11 and 12 harbored *blaOXA*-40-like genes (Fig 1). Phylogenetic analyses of all amplified and sequenced *blaOXA* fragments clearly supported the affiliation of detected *blaOXA* genes to two different clusters identical as those from clinical isolates (13-16) and available in GenBank (Fig 2). Clinical isolates 13-15 shared 100% sequence identity with *blaOXA*-72 sequence described in the same hospital in 2009, confirming the presence of endemic cluster. Since *OXA*-72 within *OXA*-40-like group was described as dominant mechanism of resistance in clinical isolates of *A. baumannii* in 2009 inside UHCS, this investigation revealed also a new oxacillinase belonging to *OXA*-23-like group (c.i.16) which contributed to the resistance rate to carbapenems of 90% in the last two years.

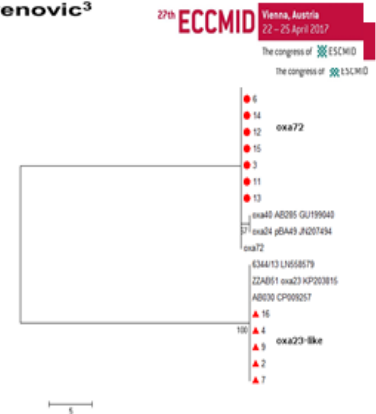


Fig.2 Phylogenetic tree constructed on the basis of *blaOXA* genes encoding OXA-type carbapenemases for wastewater isolates (2-12) and clinical isolates from UHCS (13-16). GenBank accession numbers are given next to the name of each strain.

Conclusion

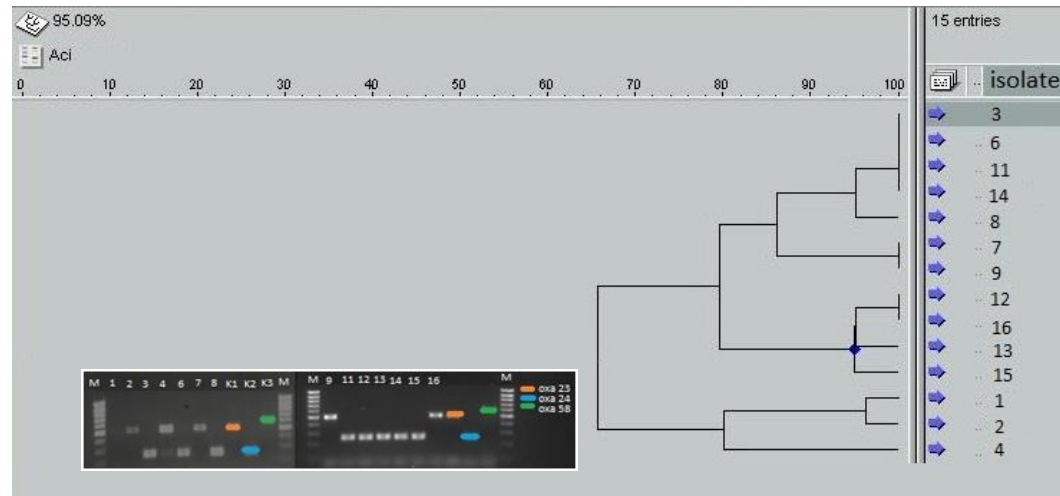
This study confirmed the possible spread of multi-resistant *A. baumannii* through hospital wastewater in nature. The possible impact on the horizontal transfer of *blaOXA* genes, surviving in selected condition or occurrence of infection outside the hospital setting should be further investigated.

This research was supported by University of Split School of Medicine and a part by the Croatian Science Foundation (grant no. IP-2014-09-5656).



Transmission and survival of carbapenem resistant *Acinetobacter baumannii* outside hospital setting

Ana Kovacic, Martina Seruga Music, Svjetlana Dekic, Marija Tonkic, Anita Novak, Zana Rubic, Jasna Hrenovic, Ivana Goic-Barisic*



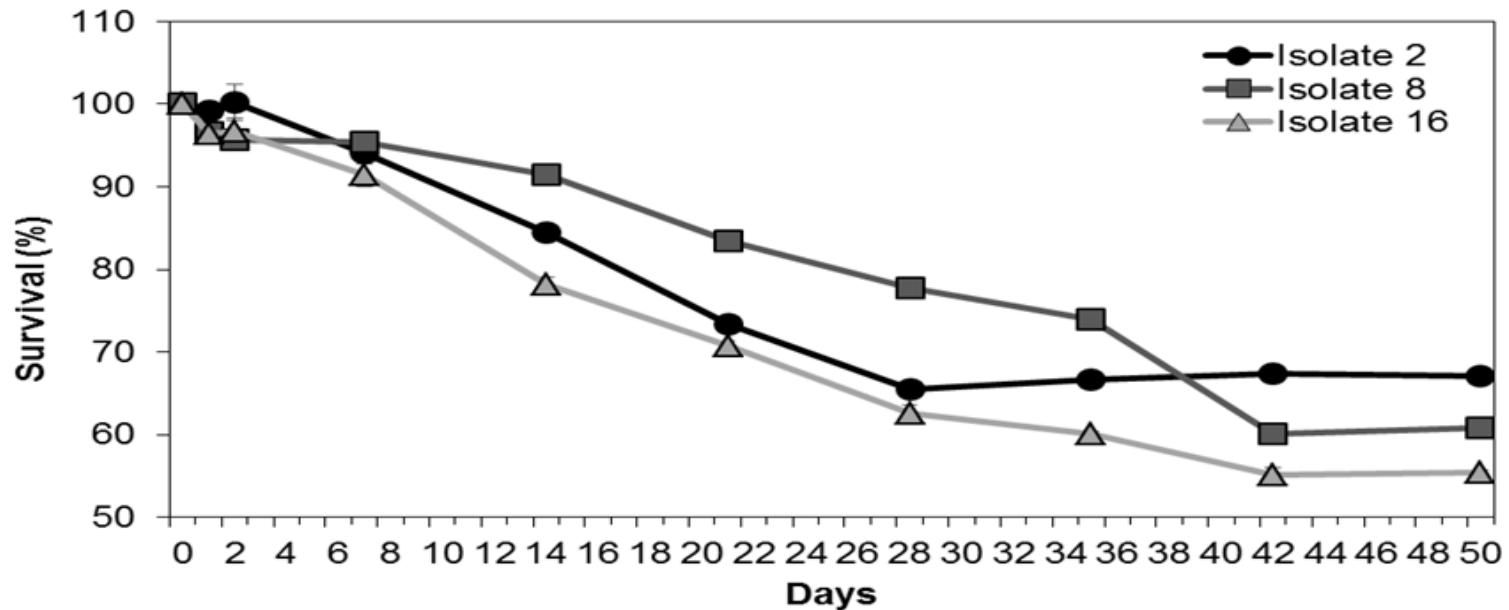
First prospective study in Croatia

Wastewater was sampled for five times, in the period from October 2014 until April 2015. 10 isolates of *A. baumannii* were recovered from hospital wastewater and compared with 4 isolates from hospitalized patients.



Accepted paper, 2018

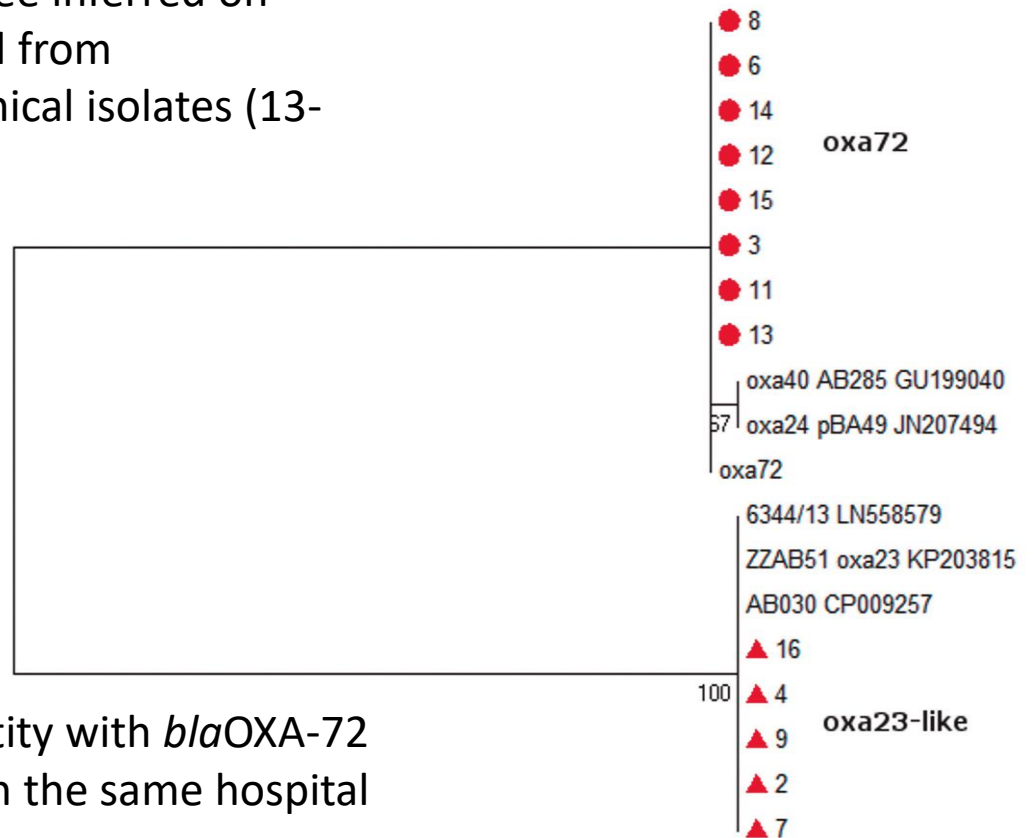
Survival of *A. baumannii*



Survival of wastewater isolates 2 and 8, and clinical isolate 16 in seawater during **50 days** of monitoring

Neighbour-joining phylogenetic tree of wastewater and clinical isolates

Neighbour-joining phylogenetic tree inferred on *bla*OXA genes fragments amplified from wastewater isolates (2-12) and clinical isolates (13-16) of *Acinetobacter baumannii*.



100% sequence identity with *bla*OXA-72
sequence described in the same hospital

5



**Multidrug resistant *Acinetobacter baumannii*
inside and outside hospital setting**



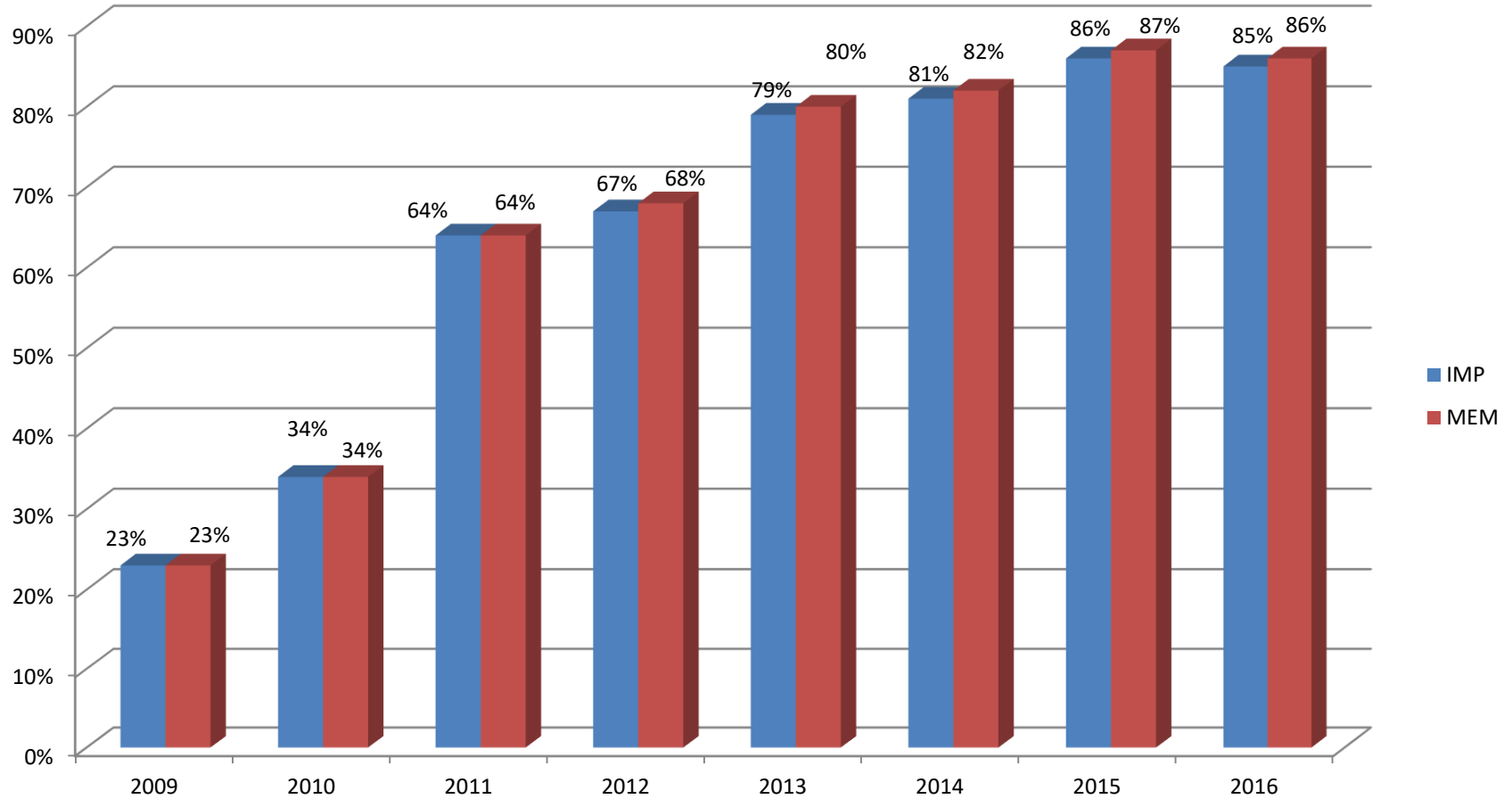
Epidemiology of MRAB

- Survival in the environmental conditions, including seawater especially in the warm period of the year up to 50 days, may also pose a **potential epidemiological reservoir** of carbapenem resistant genes.
- Our results suggest that the nosocomial pathogen *A. baumannii* is well adapted to **different environments**, not only to the hospital setting.

Resistant *Acinetobacter baumannii* – what's new?

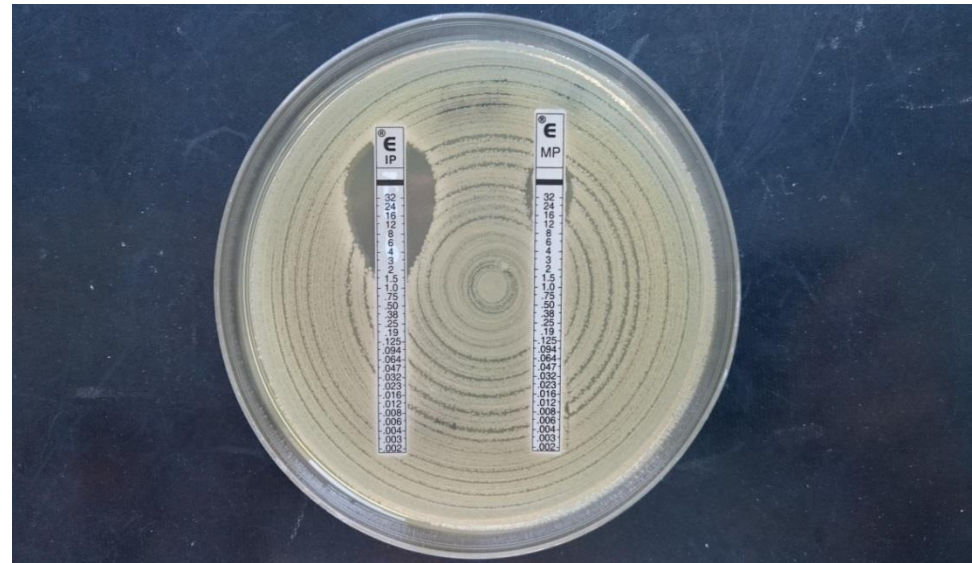
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Small differences in resistance?



New outbreak in UHS with ST 502

- Neurology Intensive Care Unit (NICU), started in March 2017
- 10 new isolates with same resistant pattern from NICU and Pulmonary Department inside UHS
- transfer of patient from a hospital in a neighboring state

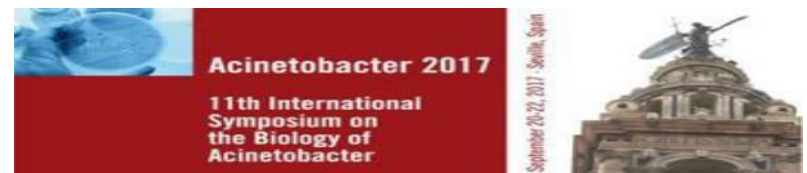


New ST 502 with OXA-72

- According to the MLST analysis by using Oxford scheme fragments of seven housekeeping genes (*gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi* and *rpoD*) were amplified by PCR
- Previously ST-195, ST-451, ST-425 ST-1421 inside clonal complex 92 (CC92)

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>			
2777	1	12	3	2	2	100	3	502	92	2
3058	1	12	3	2	2	100	3	502	92	2
3084	1	12	3	2	2	100	3	502	92	2

MLST results have revealed that all isolates belong to the ST (sequence type) 502, within the clonal complex 92 and IC 2



Thank you

- Croatian science foundation – project 252556
**Natural habitat of clinically important
Acinetobacter baumannii (NATURACI)**

Jasna Hrenović

Martina Šeruga Musić

Blaženka Hunjak

Svjetlana Dekić

Ana Kovačić

Marija Tonkić

