CROCMID 2019

Multidrug resistant *Acinetobacter baumannii* – a decade of the successful clone in Croatia

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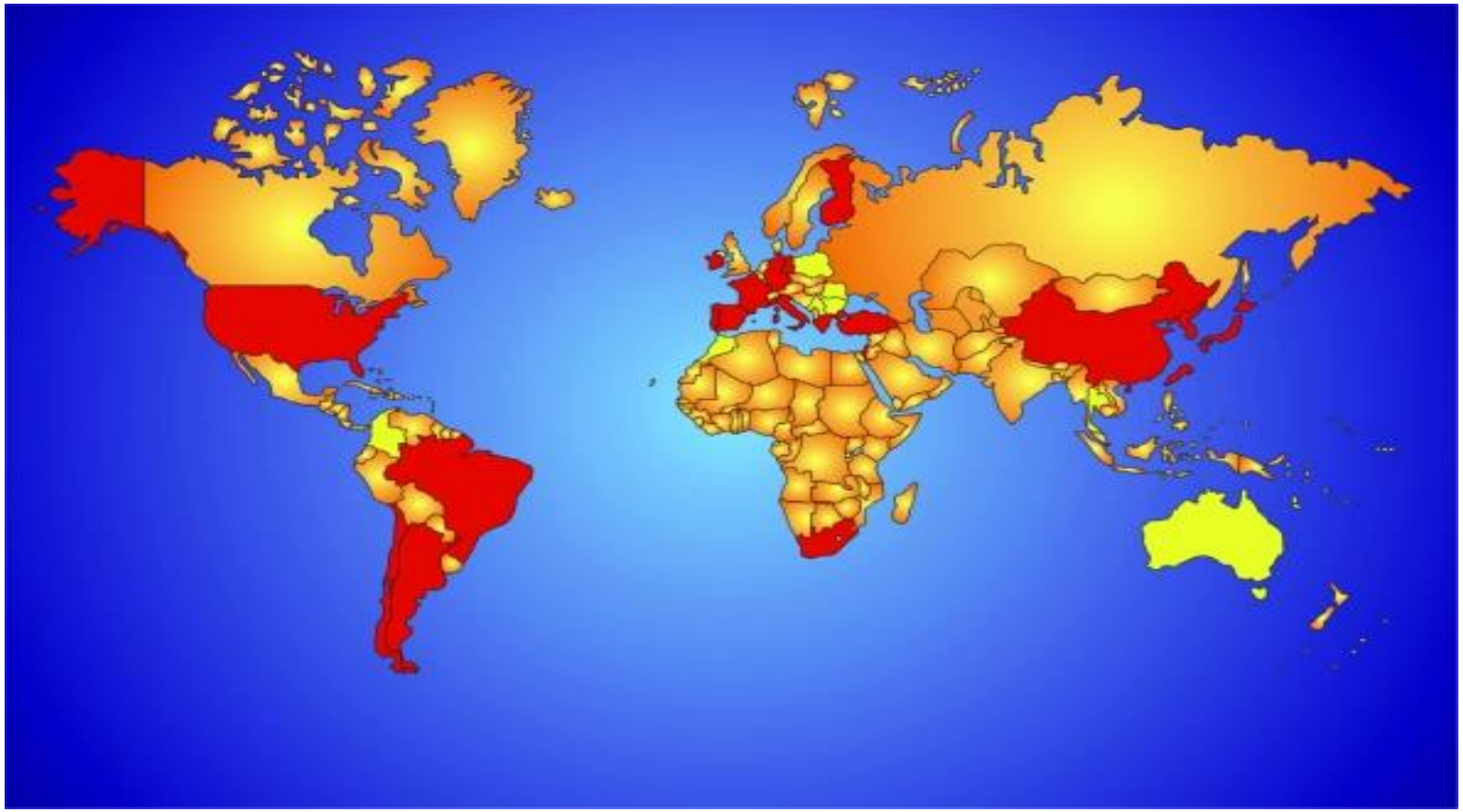
SVEUČILIŠTE U SPLITU MEDICINSKI FAKULTET

KBC SPLIT
Klinički bolnički centar Split

Bacteria (WHO category)	WHO	CDC	ESKAPE
Acinetobacter baumannii, carbapenem-R	Critical	Serious (MDR)	Yes
Pseudomonas aeruginosa, carbapenem-R	Critical	Serious (MDR)	Yes
Enterobacteriaceae, carbapenem-R, 3rd-gen ceph-R (ESBL+)	Critical	Urgent (carbapenem-R) Serious (ESBL+)	Yes
Enterococcus faecium, vancomycin-R	High	Serious (VRE)	Yes
Staphylococcus aureus, methicillin-R, vancomycin-I/R	High	Serious (MRSA) Concerning (VRSA)	Yes
Helicobacter pylori, clarithromycin-R	High		
Campylobacter spp., fluoroquinolone-R	High	Serious (drug-R)	
Salmonellae spp., fluoroquinolone-R	High	Serious (drug-R)	
Neisseria gonorrhoeae, 3rd-gen ceph-R, fluoroquinolone-R	High	Urgent (drug-R)	
Streptococcus pneumoniae, penicillin-NS	Medium	Serious (drug-R)	
Haemophilus influenzae, ampicillin-R	Medium		
Shigella spp., fluoroquinolone-R	Medium	Serious	
Clostridium difficile		Urgent	
Candida spp. fluconazole-R		Serious (Flu-R)	
M. tuberculosis		Serious (drug-R)	
Group A *Streptococcus*		Concerning (erythro-R)	
Group B *Streptococcus*		Concerning (clinda-R)	

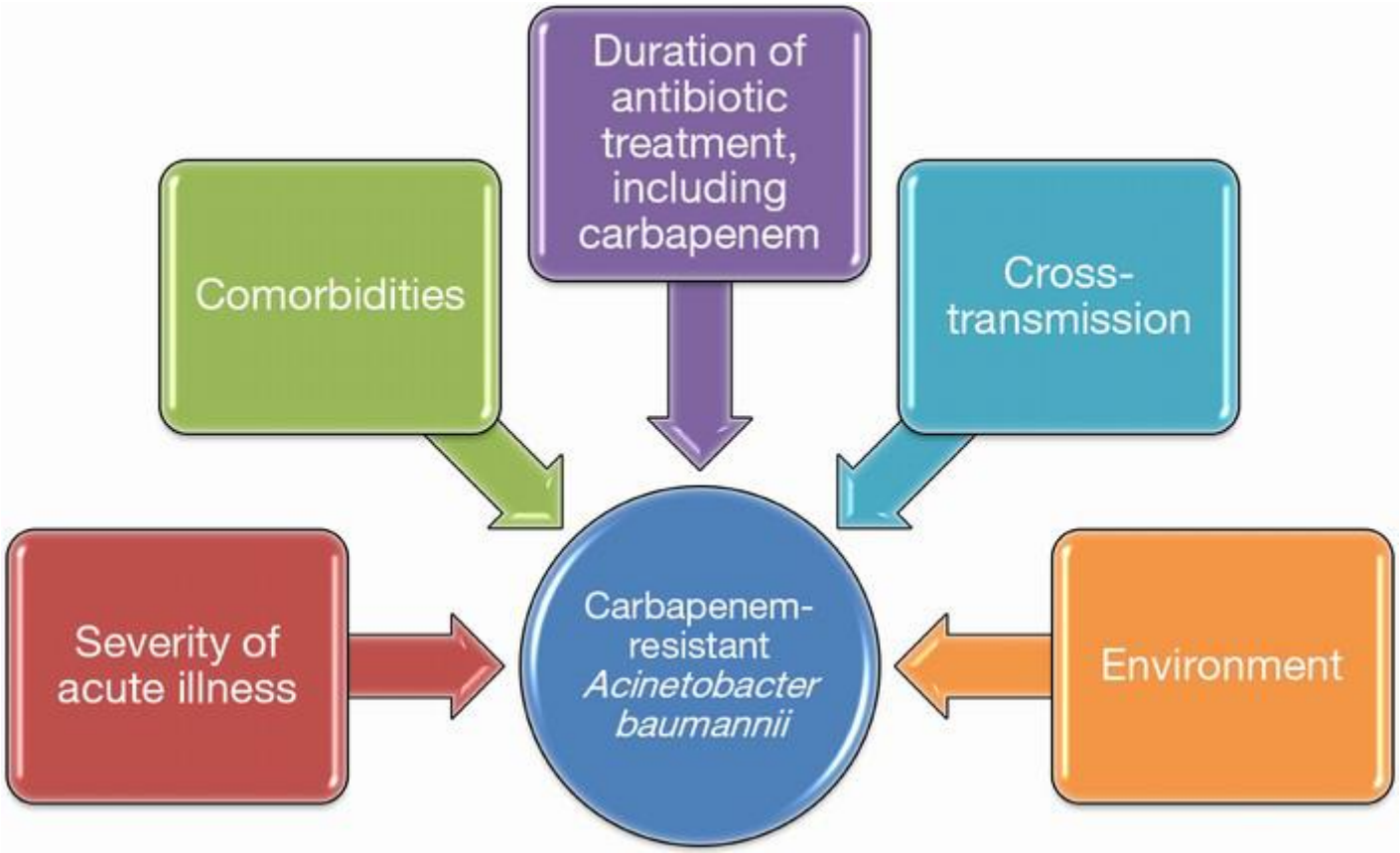
Acinetobacter baumannii: Emergence of a Successful Pathogen



Countries that have reported an outbreak of carbapenem-resistant *Acinetobacter baumannii*. Red signifies outbreaks reported before 2006, and yellow signifies outbreaks reported since 2006.

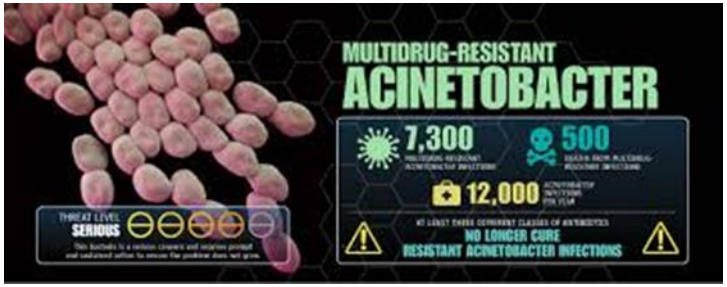
Peleg et al., Clin Microbiol Rev., 2008

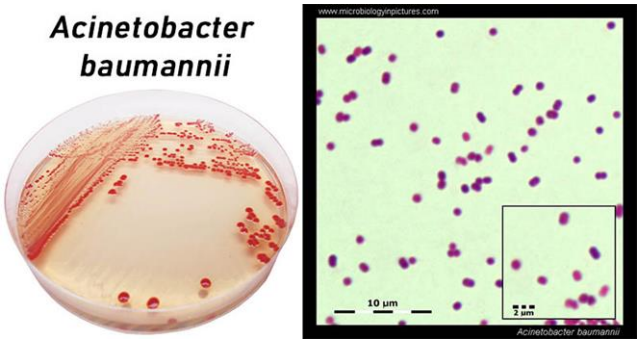
MDR *Acinetobacter baumannii*



MDR *Acinetobacter baumannii*

- crude mortality rates in patients with *A. baumannii* bacteremia varied between 30 and 76%
- factors associated with worse prognosis include immunosuppression, drug resistance, severity of underlying illness, inappropriate antimicrobial therapy, septicemia, and prior antibiotic exposure

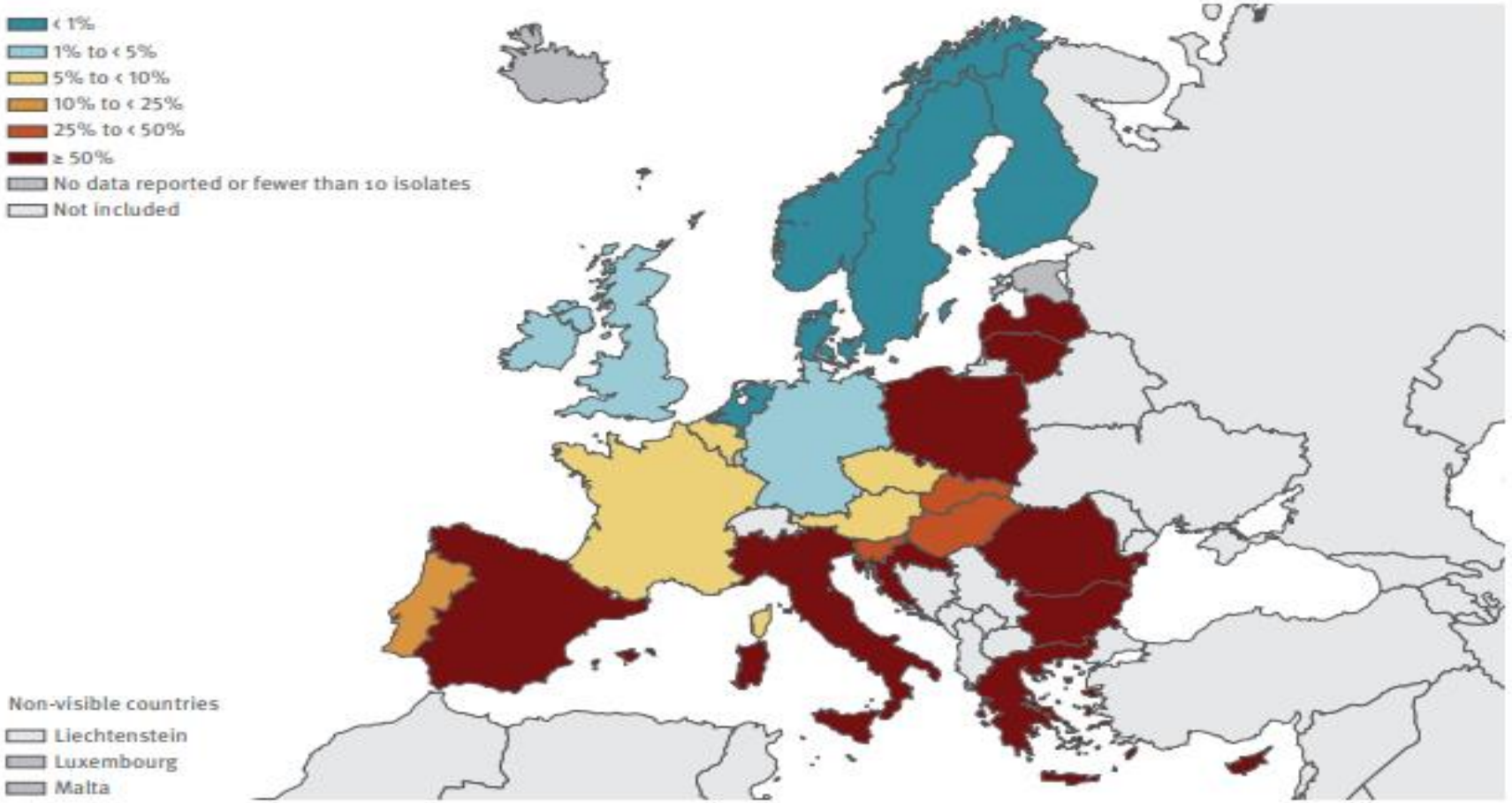




Front Cell Infect Microbiol, 2017

ECDC

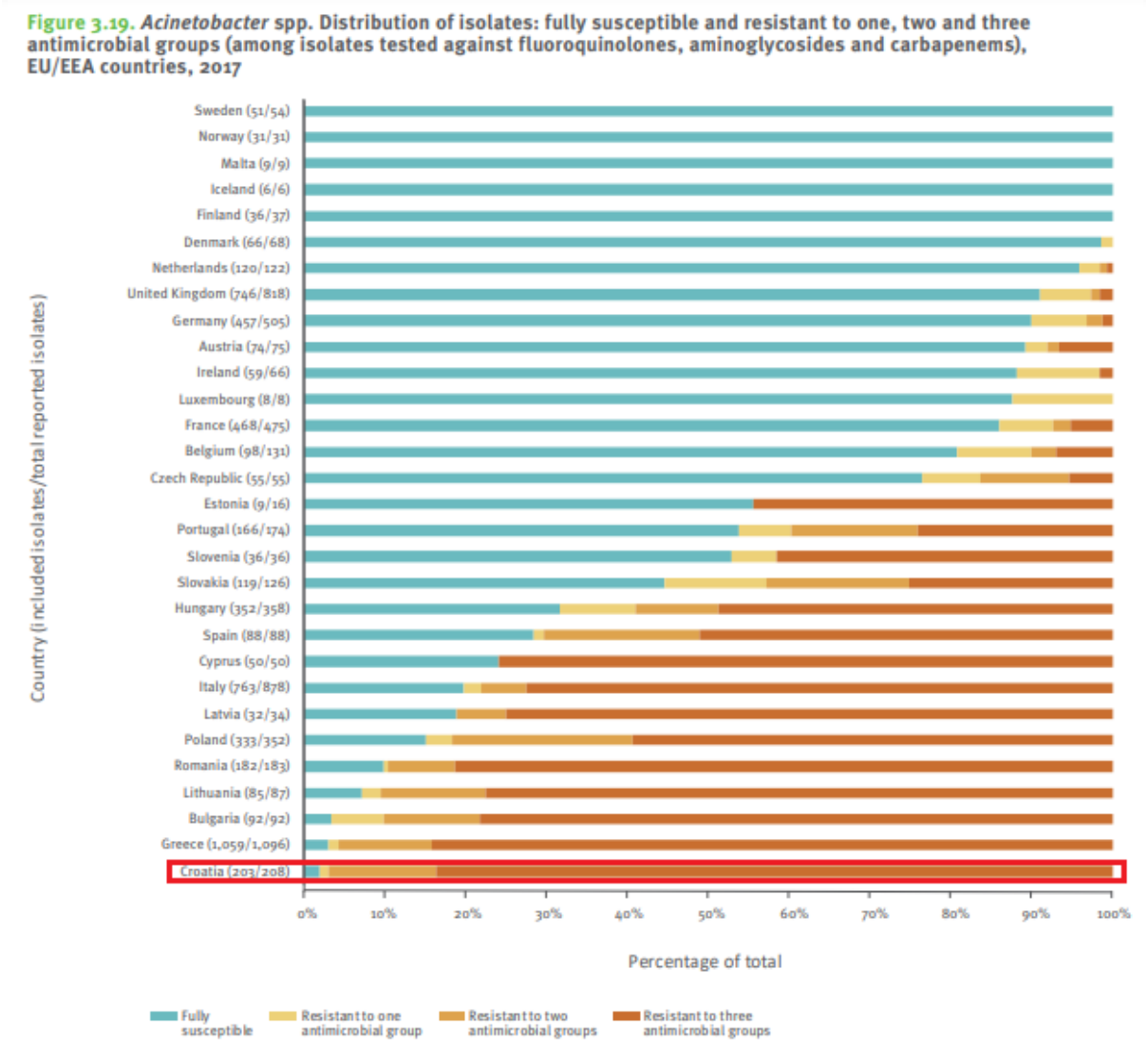
Figure 3.23. *Acinetobacter* spp. Percentage (%) of invasive isolates with combined resistance to fluoroquinolones, aminoglycosides and carbapenems, by country, EU/EEA countries, 2017



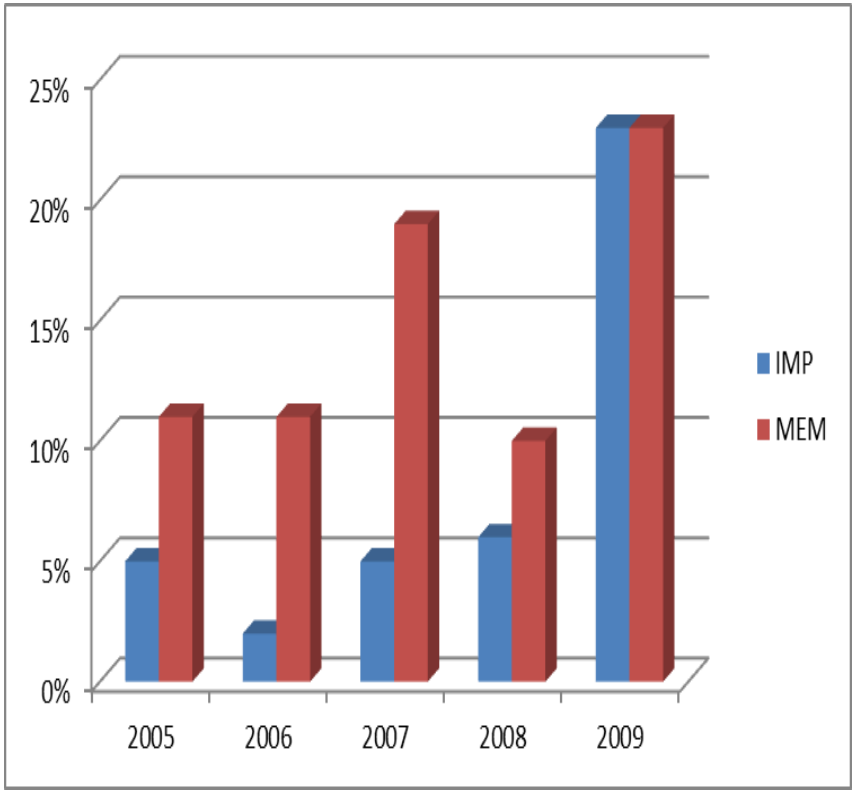
CROCMID 2016 – CROCMID 2019

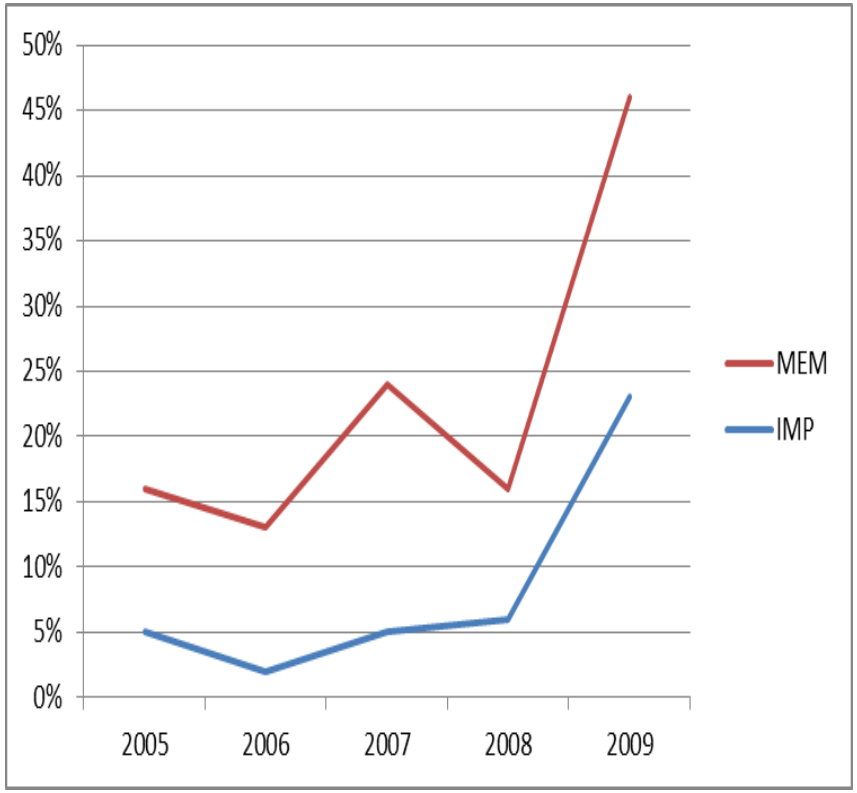
Figure 3.19. ***Acinetobacter spp.*** **Distribution of isolates: fully susceptible and resistant to one, two and three antimicrobial groups (among isolates tested against fluoroquinolone, aminoglycoside and carbapenems), EU/EEA countries, 2016**

CROCMID 2016 – CROCMID 2019



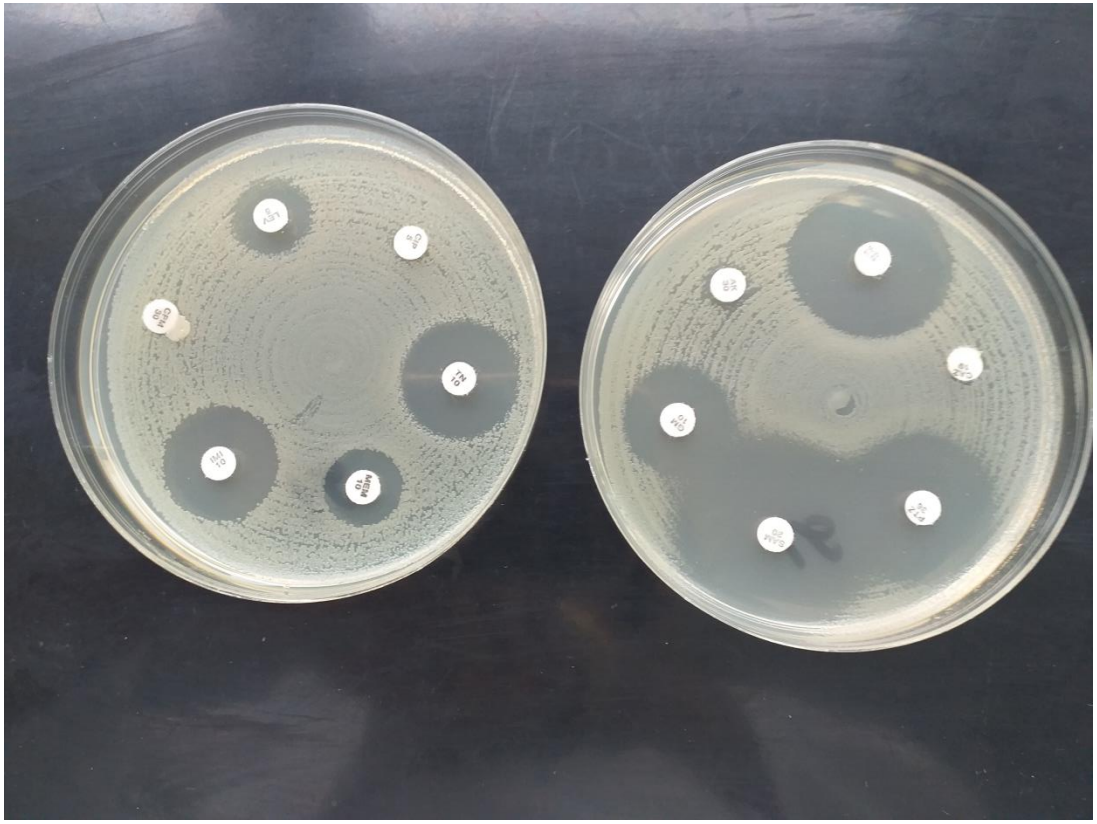
Resistance to carbapenems in Croatia 2005-2009





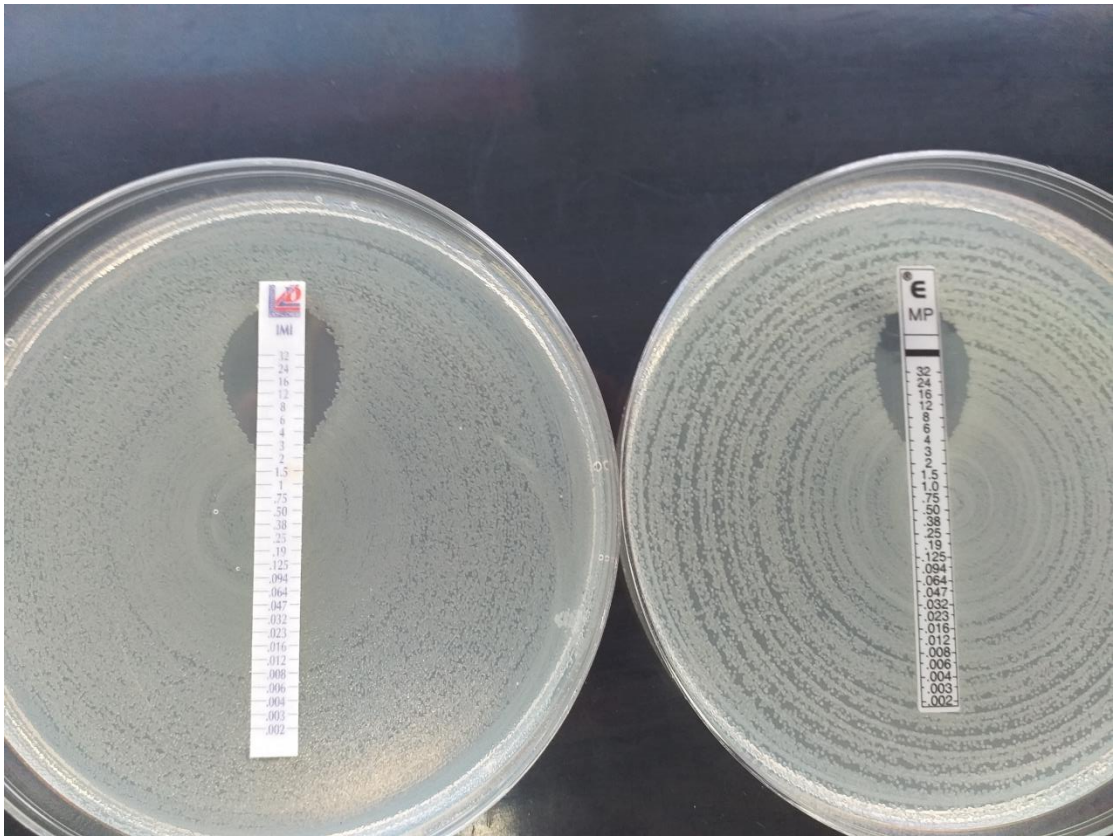
Croatian Committee for Antibiotic Resistance Surveillance

Clinical isolate from UHS in 2004



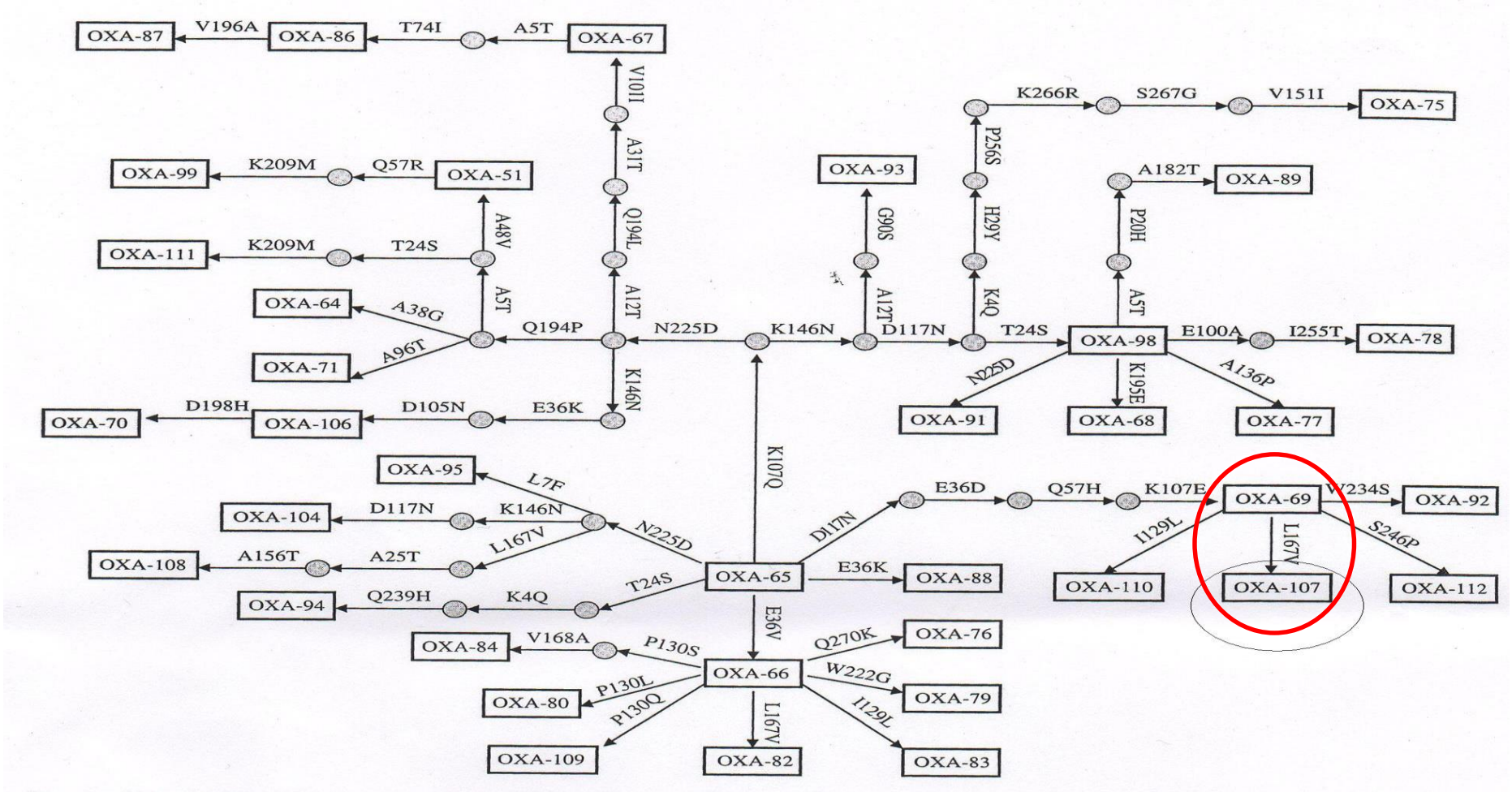
Goić-Barišić I., PhD, 2009

Clinical isolate from UHS in 2004



Goić-Barišić I., PhD, 2009

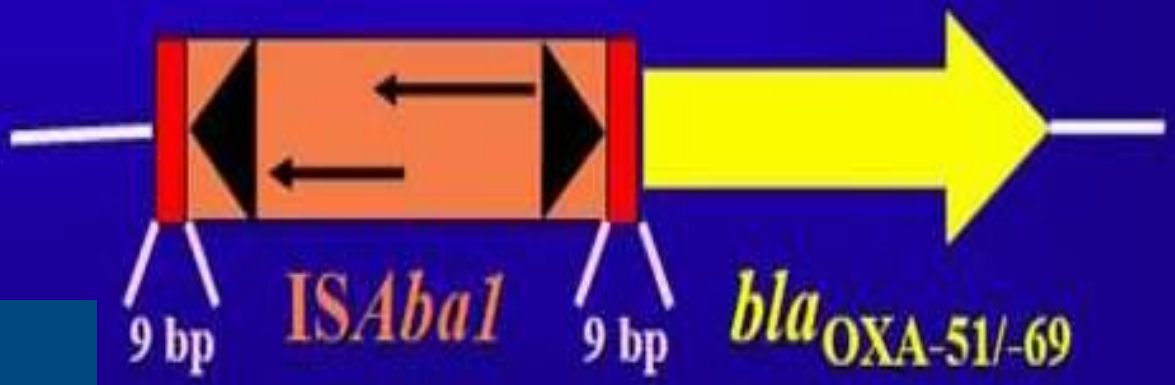
Mechanism of resistance – hyperproduction of OXA-107 due to the IS*Aba*1 location upstream of the gene



Evans et al., CMI, 2008

The role of IS*Aba1* in expression of OXA carbapenemase genes in *Acinetobacter baumannii*

Jane F. Turton[1], M. Elaina Ward[2], Neil Woodford[2], Mary E. Kaufmann[1], Rachel Pike[2], David M. Livermore[2] & Tyrone L. Pitt[1]

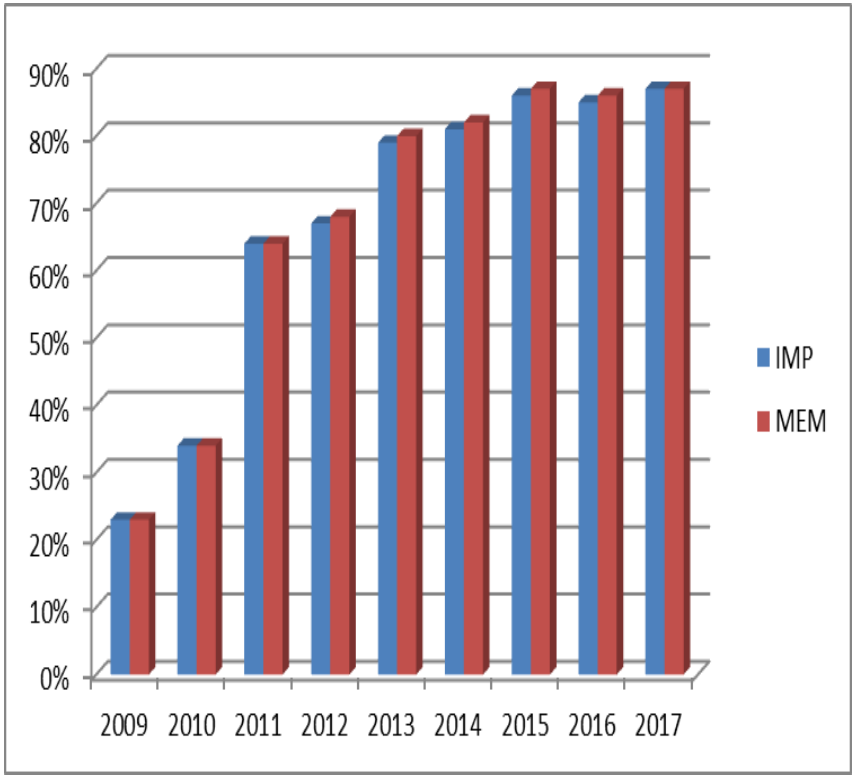


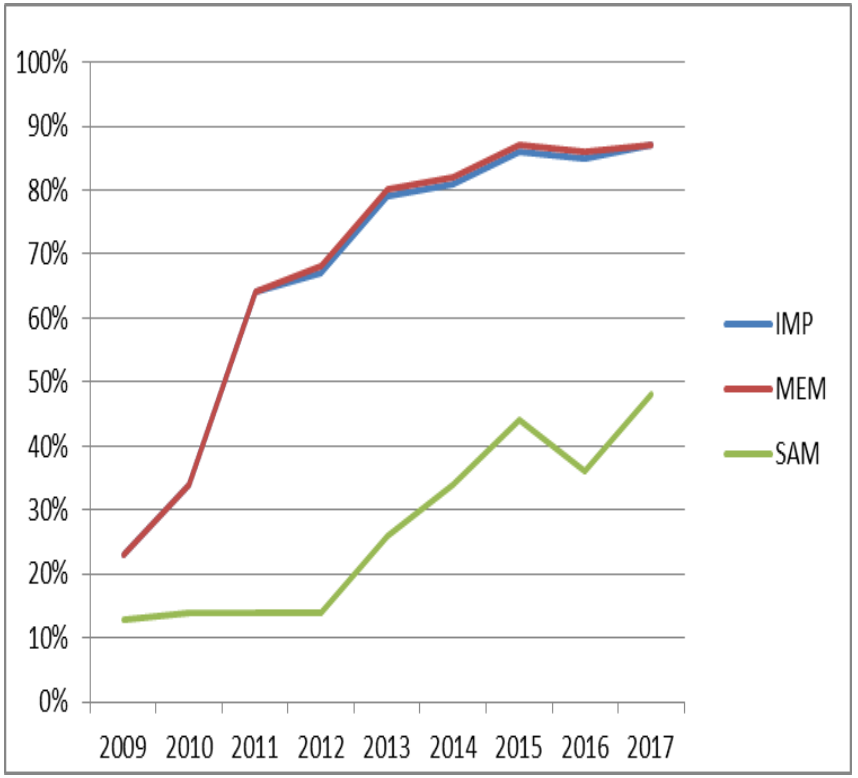
JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2009, p. 3348–3349 Vol. 47, No. 10
0095-1137/09/$08.000 doi:10.1128/JCM.02394-08

Occurrence of OXA-107 and IS*Aba*1 in Carbapenem-Resistant Isolates of *Acinetobacter baumannii* from Croatia

Ivana Goic-Barisic,1* Branka Bedenic,2 Marija Tonkic,1 Anita Novak,1 Stjepan Katic,2 Smilja Kalenic,2 Volga Punda-Polic,1 and Kevin J. Towner3

Resistance to carbapenems in Croatia 2009-2017





Croatian Committee for Antibiotic Resistance Surveillance

2009 - 2019

ARTICLE IN PRESS YJHIN3608_proof ■ 7 January 2011 ■ 1/2

Journal of Hospital Infection xxx (2011) 1–2

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevierhealth.com/journals/jhin

Letter to the Editor

Outbreak in Croatia caused by a new carbapenem-resistant clone of *Acinetobacter baumannii* producing OXA-72 carbapenemase

Madam,

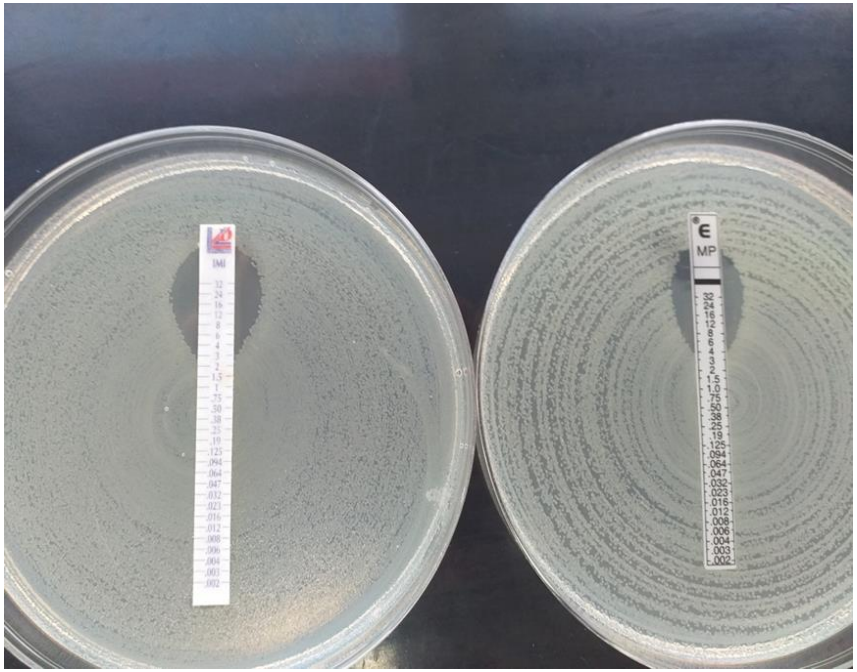
Acinetobacter baumannii is a multidrug-resistant opportunistic pathogen that causes nosocomial infections and outbreaks, particularly in the intensive care unit (ICU) setting.[1] Many outbreak strains belong to one of three worldwide lineages, known originally as European clones I, II and III. These correspond to sequence groups 2, 1 and 3, respectively, each of which includes a number of different genotypes defined by pulsed-field gel electrophoresis (PFGE).[2] Only

During the next 6 months (January to July 2009), 32 similar consecutive isolates were obtained from blood cultures, urine samples, catheter tip specimens, cerebrospinal fluid, throat and nasal swabs, and bronchial secretions collected from 23 different patients hospitalised in two ICUs and three different departments at Split University Hospital. PFGE following macrorestriction of genomic DNA with *Apa*I revealed that all isolates belonged to the European clone 2 lineage. All isolates also displayed the same multidrug resistance pattern (with no inhibition zone around imipenem or meropenem discs), but susceptibility to sulbactam and colistin (Table I).

Bacterial DNA was extracted using a DNAze kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The presence of genes encoding class D carbapenemases was detected by multiplex polymerase chain reaction using primers specific for the

Goic-Barisic I, et al., Journal of Hospital Infection (2011), doi:10.1016/j.jhin.2010.12.003

2003/04

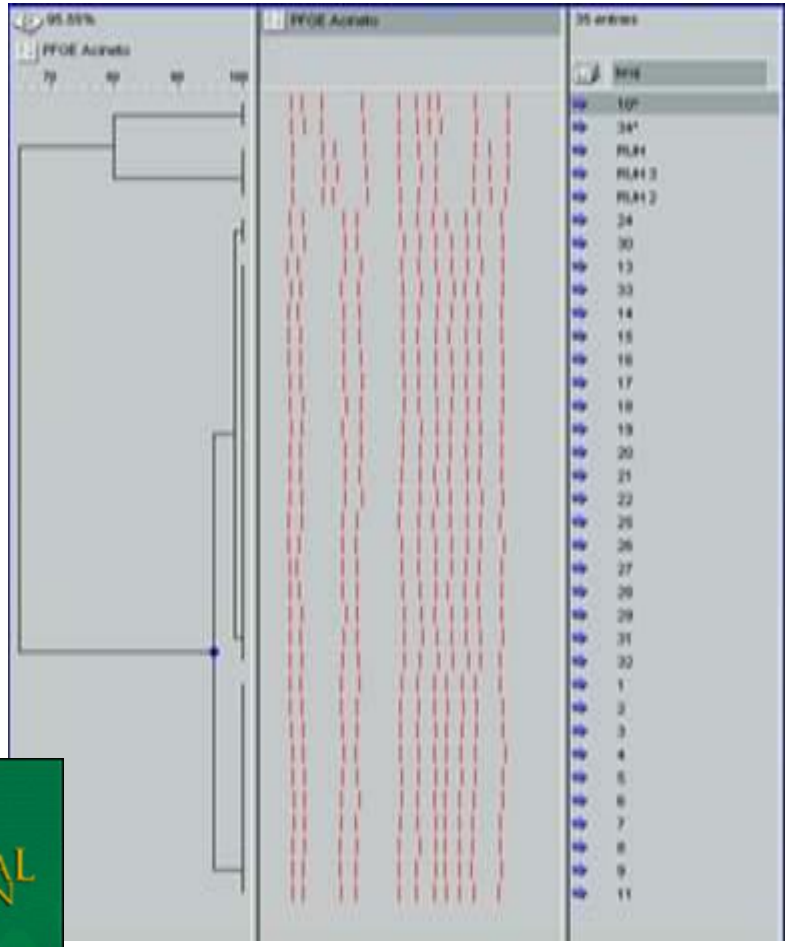


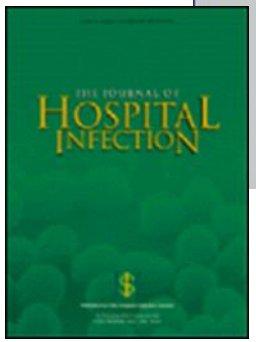
2009/10

Goić-Barišić I.

Molecular investigation - PFGE

- PFGE typing of new clone in UHC Split
- similarity in PFGE profile
- similarity in antibiotic resistance
- OXA-40 (OXA-72) carbapenemase (Macrogen, Europe)





Carbapenem-resistant isolates (2012-2016)

	CC Ox	CC Pas	OXA-51 variant
IC1	CC109	CC1	OXA-69
IC2	CC92	CC2	OXA-66
IC3	CC929	CC124	OXA-71
IC4	CC103	CC15	OXA-51
IC5	CC227	CC79	OXA-65
IC6	CC944	CC78	OXA-90
IC7	CC110	CC25	OXA-64
IC8	CC447	CC10	OXA-68
IC9	CC1078	CC464	OXA-94

Croatia

Each IC has an OXA-51 variant

courtesy Paul Higgins, 2019

Carina Müller manuscript in preparation

MLST typing IC2 from 2009-2016

- 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 most common ST-195 inside CC 2
- ST – 231 in Pula and Zagreb

Ladavac R et al., J Glob Antimicrob Resist 2017

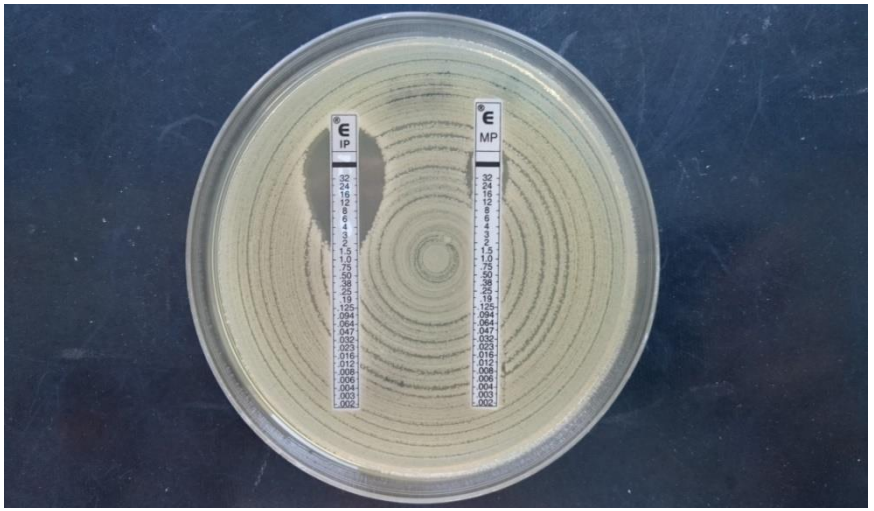
Isolate	Origin	Date of isolation	Sequence type	Clonal complex	IC type
OB 3831	Sputum	11.09.2015	1421[a]	92	2
OB 3929	Tracheal aspirate	18.09.2015	195	92	2
OB 4027	Sputum	24.09.2015	1421[a]	92	2
OB 4138	Bronchial aspirate	02.10.2015	195	92	2
S2/1	Hospital wastewater	27.08.2015	195	92	2
S2/2			195	92	2
S2/3			195	92	2
S2/4			195	92	2
S1/1		06.10.2015	195	92	2
S2/5			195	92	2
S2/6			195	92	2
S2/7			195	92	2
S2/8			195	92	2
S2/9			195	92	2
IN32	Urban sewage	23.09.2015	195	92	2
Sava3	River water	11.10.2015	1421[a]	92	2
Sava4			195	92	2
Sava5			1421[a]	92	2
Sava6			1421[a]	92	2

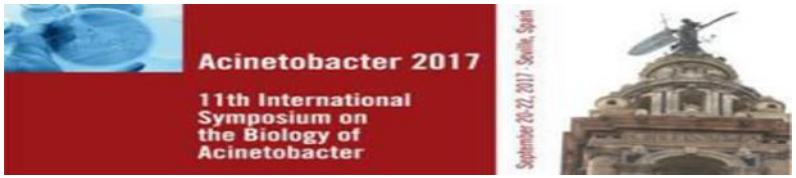
Seruga Music et al., J Hosp Infect, 2017

MLST typing IC2 in 2017

- New resistotype and new ST 502 in UHS in 2017
- OXA-72 carbapenemase
- Unusual resistance pattern with MIC for imipenem inside susceptible range according EUCAST rules and high level of resistance to meropenem

Isolate	Gene locus/allele							Sequence type	Clonal complex	IC type
	gltA	*gyrB*	*gdhB*	*recA*	*cpn60*	*gpi*	*rpoD*			
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





CC 92 inside IC 2

- dominant clone in hospitals in Croatia
- biofilm formation
- survival in the environmental conditions, including seawater
- reduced susceptibility to disinfectants of *A. baumannii* biofilms

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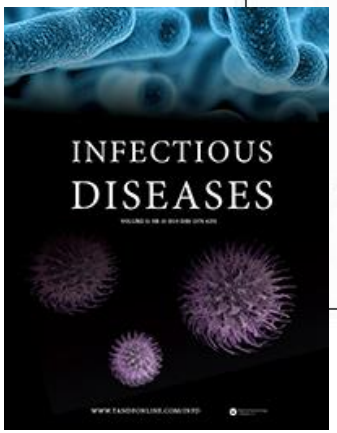


NZJZ RIJEKA

KBC RIJEKA

ZZJZ PULA

ZZJZ ZADAR

ZZJZ ŠIBENIK

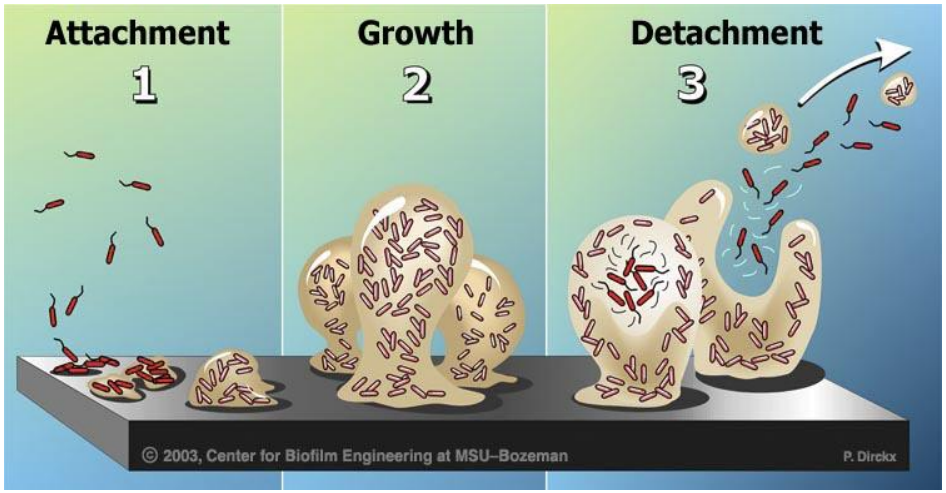
NZJZ SPLIT

KBC SPLIT

ZZJZ DUBROVNIK

Kaliterna V. et al., 2015

Multicenter investigation in Croatia



- more than 100 clinical isolates of *A. baumannii* (2009)

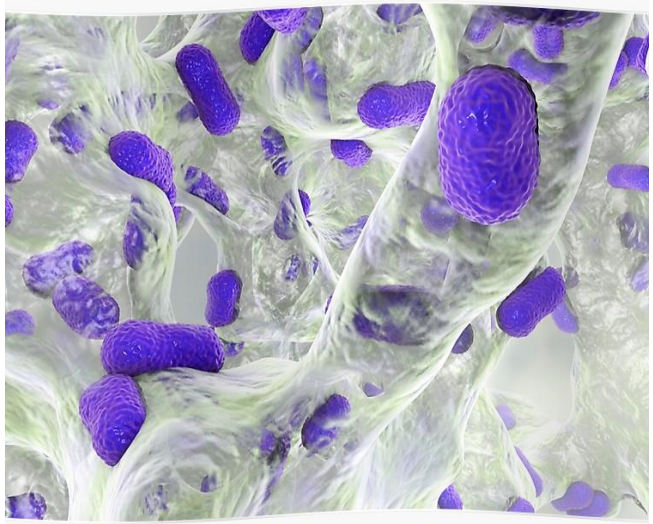
- focused on ability to form biofilm in correlation to genotypes (clones), origin of tested isolates and resistance to antibiotics

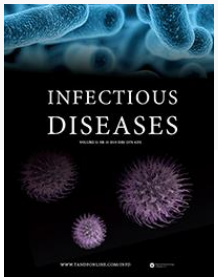
Kaliterna V., Goić-Barišić I.,
Croatian Committee for Antibiotic Resistance Surveillance, 2014

Ability to form biofilm

Stronger ability to form biofilm

- from respiratory specimens
- in ICUs
- in susceptible and intermediate susceptible isolates to imipenem, meropenem and amikacin





- Kaliterna V. et al., 2015

CC 92 inside IC 2

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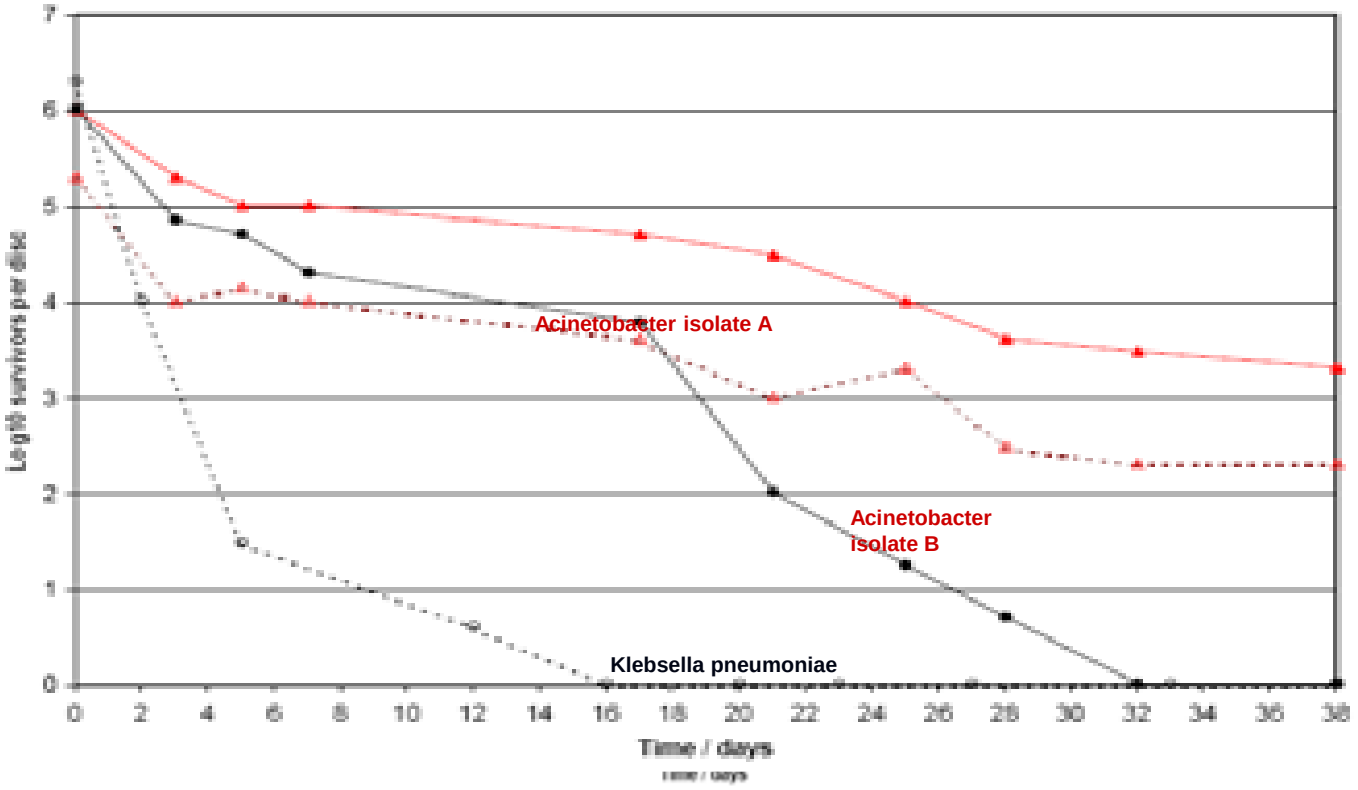
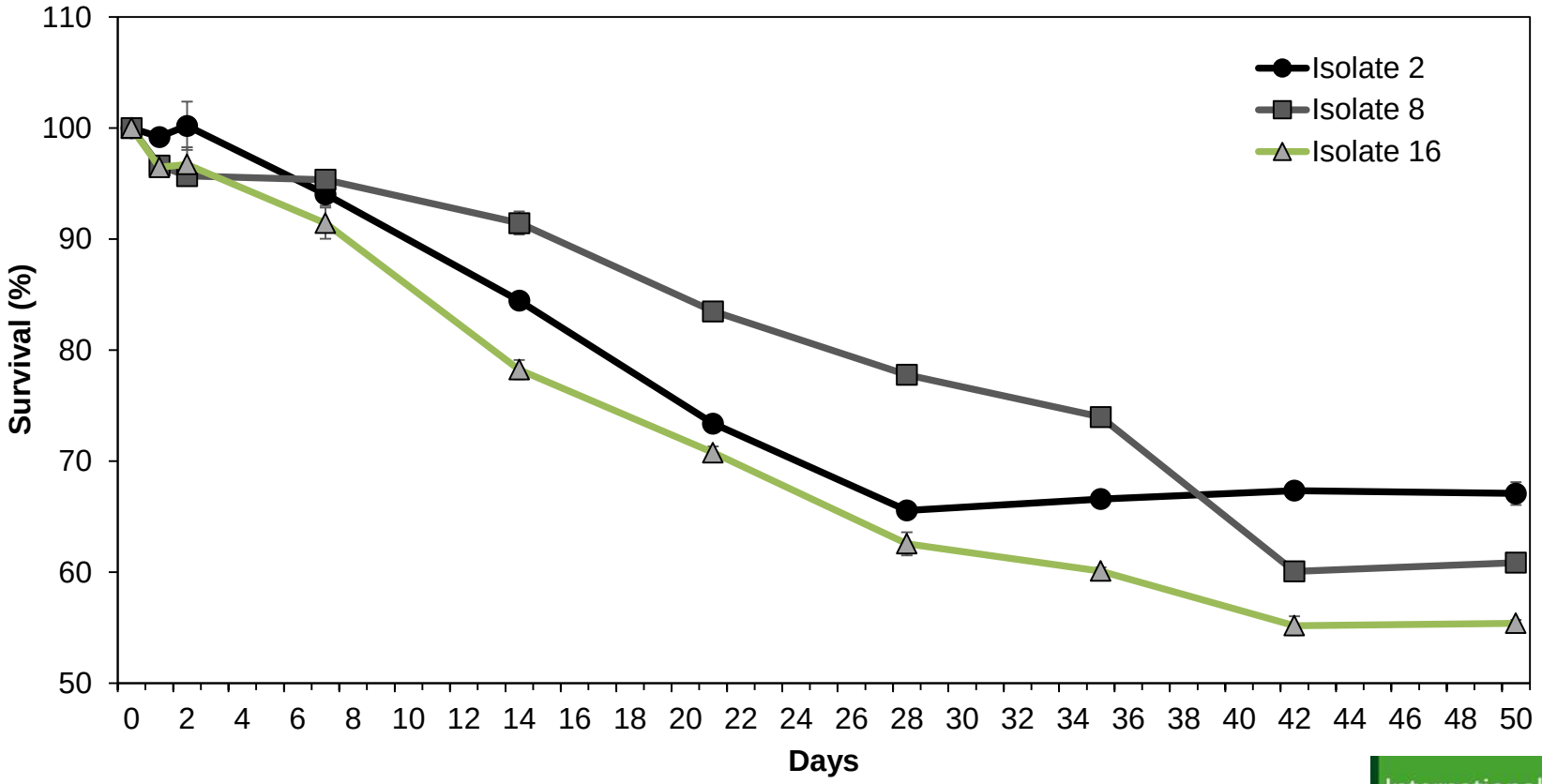
Acinetobacter baumannii - long survival among Gram-negatives



Hrenović J. et al., Eurosurveillance, 2016

Acinetobacter baumannii - long survival among Gram-negatives

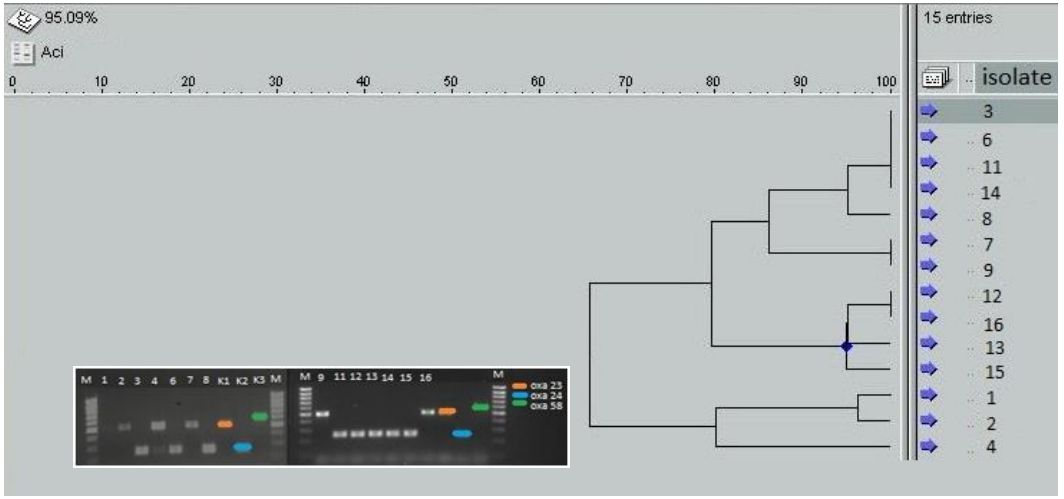
survival in seawater during 50 days days of monitoring



Kovačić A. et al., 2017

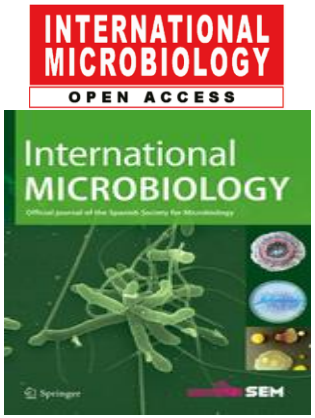
Transmission and survival of carbapenem resistant *Acinetobacter baumannii* outside hospital setting

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.



CC 92 inside IC 2

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Susceptibility to disinfectants

- benzalkonium chloride and chlorhexidine

Table 1 A. baumannii *isolates used in the experiments*

Designation	Origin
ATCC	ATCC 19606 strain
EU1	Hospital isolate, UHCS, 2004
EU2	Hospital isolate, UHCS, 2009
ST4	Hospital isolate, UHC, 2009
ST10	Hospital isolate, UHC, 2009
IN12	Environmental isolate, WWTP of Zagreb, 2014, influent
IN21	Environmental isolate, WWTP of Zagreb, 2014, influent
EF4	Environmental isolate, WWTP of Zagreb, 2014, effluent

UHCS - University Hospital Centre Split, Croatia; WWTP Zagreb – Central wastewater treatment plant of the city of Za

EU2 and ST4 showed the highest resistance to both disinfectants

Ivanković T., Goić-Barišić I., Hrenović J., 2017

Susceptibility to disinfectants

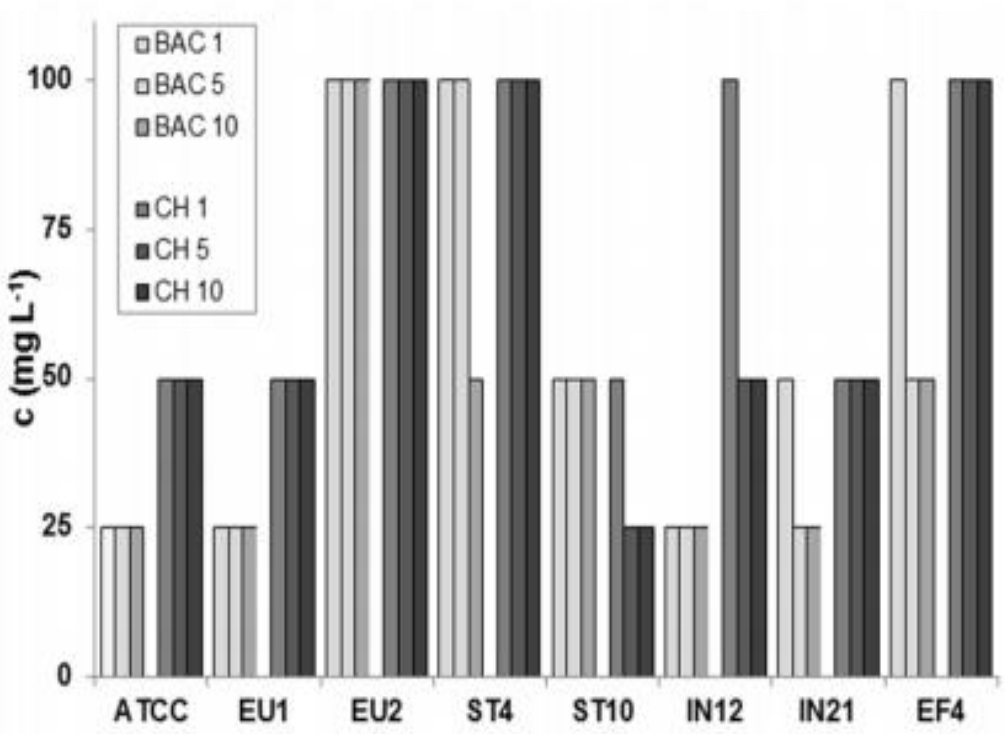


Figure 3 *Minimal bactericidal concentrations of benzalkonium chloride (BAC) and chlorhexidine digluconate (CH) against environmental and hospital isolates of* A. baumannii *after 1, 5, and 10 min of contact*

Ivanković T., Goić-Barišić I., Hrenović J., 2017

Susceptibility to disinfectants

- The biofilm bacteria were more resistant to disinfectants than the planktonic populations, as more than 50 % of the biofilm population and none of the planktonic population survived 5-minute exposure to disinfectans tested in this study
- The biofilm populations on ceramic tiles were significantly more resistant than those on glass coverslips, even though the amount of biofilm was practically the same on ceramics and glass

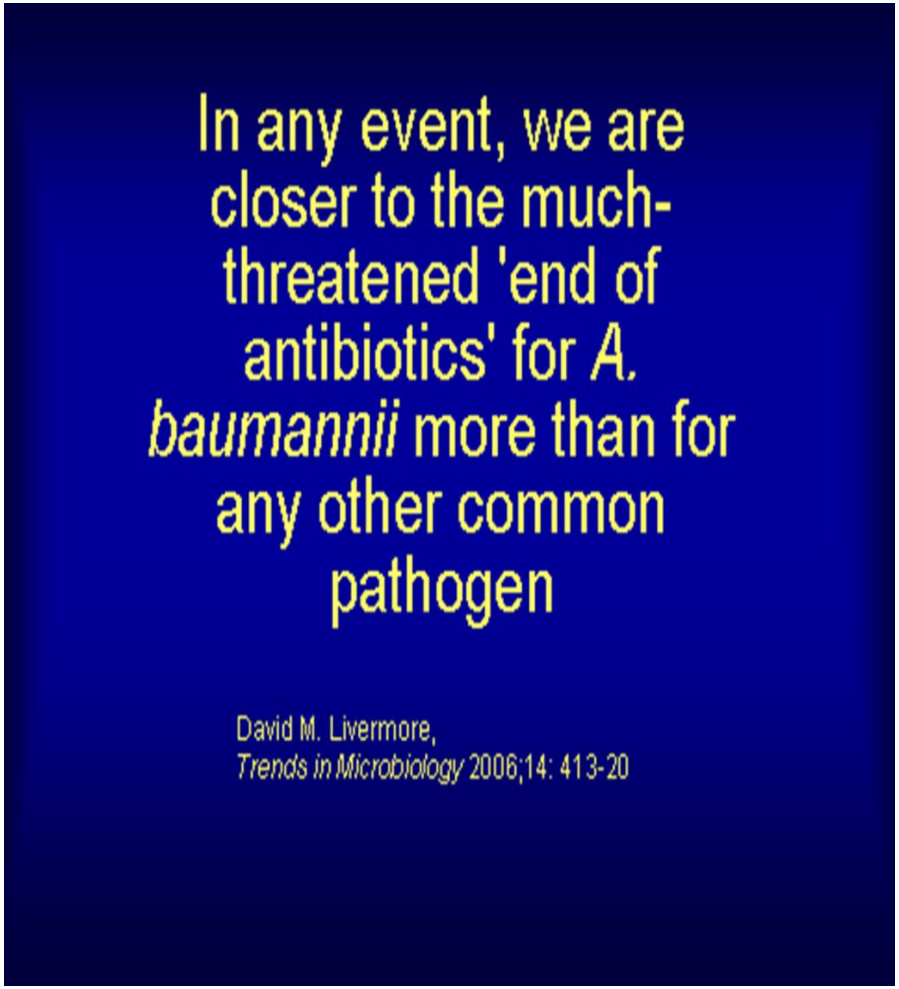
Ivanković T., Goić-Barišić I., Hrenović J., 2017

Conclusion

- decade of persistence CC 92 in Croatia
- similar results from Iran, China, Brazil, Colombia, India
- OXA-72 and OXA-23 most common oxacillinases in CRAB
- ability to form biofilm and reduced susceptibility to disinfectans
- endemic in hospitals in Croatia

Conclusion

- Once endemic in a healthcare unit, *A. baumannii* is extremely difficult to eradicate.
- Nevertheless, it is still possible to eradicate these organisms from a unit when an uncompromising approach is taken to infection control.



Towner K. J., Hospit Infect, 2009

Thank you

Kevin Towner, UK
Ana Kovačić
Jasna Hrenović
Vanja Kaliterna

All collaborators on project
Croatian science foundation
Natural habitat of clinically important *Acinetobacter baumannii* (project 252556)