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

Ivana Goić Barišić
University Hospital of Split
University of Split School of Medicine
<table>
<thead>
<tr>
<th>Bacteria (WHO category)</th>
<th>WHO</th>
<th>CDC</th>
<th>ESKAPE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acinetobacter baumannii</em>, carbapenem-R</td>
<td>Critical</td>
<td>Serious (MDR)</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em>, carbapenem-R</td>
<td>Critical</td>
<td>Serious (MDR)</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em>, carbapenem-R, 3rd-gen ceph-R (ESBL+)</td>
<td>Critical</td>
<td>Urgent (carbapenem-R)</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Enterococcus faecium</em>, vancomycin-R</td>
<td>High</td>
<td>Serious (VRE)</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em>, methicillin-R, vancomycin-I/R</td>
<td>High</td>
<td>Serious (MRSA)</td>
<td>Concerning (VRSA)</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em>, clarithromycin-R</td>
<td>High</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Campylobacter</em> spp., fluoroquinolone-R</td>
<td>High</td>
<td>Serious (drug-R)</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em> spp., fluoroquinolone-R</td>
<td>High</td>
<td>Serious (drug-R)</td>
<td></td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em>, 3rd-gen ceph-R, fluoroquinolone-R</td>
<td>High</td>
<td>Urgent (drug-R)</td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em>, penicillin-NS</td>
<td>Medium</td>
<td>Serious (drug-R)</td>
<td></td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em>, ampicillin-R</td>
<td>Medium</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Shigella</em> spp., fluoroquinolone-R</td>
<td>Medium</td>
<td>Serious</td>
<td></td>
</tr>
<tr>
<td><em>Clostridium difficile</em></td>
<td></td>
<td>Urgent</td>
<td></td>
</tr>
<tr>
<td><em>Candida</em> spp. fluconazole-R</td>
<td></td>
<td>Serious (Flu-R)</td>
<td></td>
</tr>
<tr>
<td><em>M. tuberculosis</em></td>
<td></td>
<td>Serious (drug-R)</td>
<td></td>
</tr>
<tr>
<td>Group A <em>Streptococcus</em></td>
<td></td>
<td>Concerning (erythro-R)</td>
<td></td>
</tr>
<tr>
<td>Group B <em>Streptococcus</em></td>
<td></td>
<td>Concerning (clinda-R)</td>
<td></td>
</tr>
</tbody>
</table>
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

- Comorbidities
- Duration of antibiotic treatment, including carbapenem
- Cross-transmission
- Severity of acute illness
- Environment

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

- **< 1%**
- **1% to < 5%**
- **5% to < 10%**
- **10% to < 25%**
- **25% to < 50%**
- **≥ 50%**

- **No data reported or fewer than 10 isolates**
- **Not included**

Non-visible countries:
- Liechtenstein
- Luxembourg
- Malta
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.
Resistance to carbapenemems 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 ISAbal location upstream of the gene

Evans et al., CMI, 2008
The role of ISAb1 in expression of OXA carbapenemase genes in Acinetobacter baumannii

Jane F. Turton¹, M. Elaina Ward², Neil Woodford², Mary E. Kaufmann¹, Rachel Pike², David M. Livermore² & Tyrone L. Pitt¹

Occurrence of OXA-107 and ISAb1 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
Outbreak in Croatia caused by a new carbapenem-resistant clone of *Acinetobacter baumannii* producing OXA-72 carbapenemase

**Method.**

*Acinetobacter baumannii* is a multi-drug resistant opportunistic pathogen that causes nosocomial infections and outbreaks, particularly in the intensive care unit (ICU) setting. Many outbreak strains belong to one of three worldwide lineages, known originally as European clones 1, 2, and 3. 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) method.

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 anal 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 ApaI revealed that all isolates belonged to the European clone 2 lineage. All isolates also displayed the same multi-drug resistant pattern (with no inhibition zone around imipenem or meropenem discs), but susceptibility to sulfacetam and colistin (Table 1).

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

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)
<table>
<thead>
<tr>
<th>IC</th>
<th>CC Ox</th>
<th>CC Pas</th>
<th>OXA-51 variant</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC1</td>
<td>CC109</td>
<td>CC1</td>
<td>OXA-69</td>
</tr>
<tr>
<td>IC2</td>
<td>CC92</td>
<td>CC2</td>
<td>OXA-66</td>
</tr>
<tr>
<td>IC3</td>
<td>CC929</td>
<td>CC124</td>
<td>OXA-71</td>
</tr>
<tr>
<td>IC4</td>
<td>CC103</td>
<td>CC15</td>
<td>OXA-51</td>
</tr>
<tr>
<td>IC5</td>
<td>CC227</td>
<td>CC79</td>
<td>OXA-65</td>
</tr>
<tr>
<td>IC6</td>
<td>CC944</td>
<td>CC78</td>
<td>OXA-90</td>
</tr>
<tr>
<td>IC7</td>
<td>CC110</td>
<td>CC25</td>
<td>OXA-64</td>
</tr>
<tr>
<td>IC8</td>
<td>CC447</td>
<td>CC10</td>
<td>OXA-68</td>
</tr>
<tr>
<td>IC9</td>
<td>CC1078</td>
<td>CC464</td>
<td>OXA-94</td>
</tr>
</tbody>
</table>

Each IC has an OXA-51 variant.

Croatia

courtesy Paul Higgins, 2019
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

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
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
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
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

• dominant clone in hospitals in Croatia

• biofilm formation

• survival in the environmental conditions, including seawater

• reduced susceptibility to disinfectants of \textit{A. baumannii} biofilms
Acinetobacter baumanii - long survival among Gram-negatives

Hrenović J. et al., Eurosurveillance, 2016
Acinetobacterbaumannii - long survival among Gram-negatives

survival in seawater during 50 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

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

- benzalkonium chloride and chlorhexidine

<table>
<thead>
<tr>
<th>Designation</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC</td>
<td>ATCC 19606 strain</td>
</tr>
<tr>
<td>EU1</td>
<td>Hospital isolate, UHCS, 2004</td>
</tr>
<tr>
<td>EU2</td>
<td>Hospital isolate, UHCS, 2009</td>
</tr>
<tr>
<td>ST4</td>
<td>Hospital isolate, UHC, 2009</td>
</tr>
<tr>
<td>ST10</td>
<td>Hospital isolate, UHC, 2009</td>
</tr>
<tr>
<td>IN12</td>
<td>Environmental isolate, WWTP of Zagreb, 2014, influent</td>
</tr>
<tr>
<td>IN21</td>
<td>Environmental isolate, WWTP of Zagreb, 2014, influent</td>
</tr>
<tr>
<td>EF4</td>
<td>Environmental isolate, WWTP of Zagreb, 2014, effluent</td>
</tr>
</tbody>
</table>

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 disinfectants 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 oxacillininases 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)