Molecular epidemiology of carbapenem-resistant Acinetobacter baumannii in Serbia: a review (2010-2023)

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Abstract: Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is endemic in Serbia. CRAB initially emerged around the 2000s, and since then, carbapenemases have played a crucial role in its appearance. Oxacillinases (OXAs) are the most prevalent carbapenemases detected in CRAB isolates collected from patients admitted to Serbian hospitals. Among acquired OXA enzymes, OXA-23 and OXA-24 are the most commonly found. NDM-1-producing CRAB was also detected. The predominant OXA-23 and OXA-24 producers are associated with multilocus sequence typing (MLST) Pasteur scheme sequence type (ST) 2 and ST492 clonal strains of the international clone (IC) II. Isolated CRABs are typically multidrug-resistant (MDR) strains which complicates the treatment options. This review aims to go through the molecular epidemiology of CRAB clinical isolates in Serbia, as one of the most important aspects for implementing infection control measures and adjusted antimicrobial treatment strategies in hospital settings that could confine clonal CRAB spread.

Keywords*:* CRAB, OXA, MBL, clonal lineages, molecular epidemiology

1. Introduction

In the last two decades, *Acinetobacter baumannii* has emerged as a major nosocomial pathogen, able to cause significant morbidity and mortality in healthcare settings and almost impossible to eradicate in the hospital environment (Roy *et al*, 2022). The increasing resistance of *A. baumannii* to the last-line treatment options is a global problem. Carbapenems used to be the mainstay for the treatment of nosocomial infections caused by *A. baumannii* (Maragakis & Perl, 2008). Unfortunately, the overuse of carbapenems, especially in the treatment of extended spectrum beta-lactamases producing *Enterobacteriaceae,* has rapidly resulted in the worldwide dissemination of carbapenem-resistant nonfermentors (Towner *et al*., 2009). Carbapenem-resistant *A. baumannii* (CRAB) isolates are, as a rule, multidrug-resistant (MDR) strains and represent a severe epidemiological and therapeutical challenge (Perez *et al.*, 2007). That's why CRAB is one of the critical-priority pathogens on the World Health Organization's priority list of antibiotic-resistant bacteria for effective drug development (Tacconelli *et al.*, 2018).

The most prevalent mechanism responsible for carbapenem resistance in *A. baumannii* is the production of carbapenem-hydrolyzing enzymes, Ambler class D β-lactamases or oxacillinases (OXAs): the intrinsic OXA-51 and the most common acquired OXAs (OXA-23, OXA-24/40, OXA-58 and OXA-143) (Poirel *et al*., 2010). Chromosomally located carbapenemases can confer higher resistance with the IS*Aba*1- an enhanced expression of the intrinsic chromosomal $bla_{\text{OXA-51}}$ gene (Turton *et al*., 2006). Less frequent, but much more powerful carbapenem-hydrolyzing enzymes in *A. baumannii* (VIM, SIM, IMP, NDM) belong to class B β-lactamases or metallo-β-lactamases (MBLs) (Bonin *et al*., 2012; Poirel & Nordmann, 2006).

In the attempt to monitor the epidemic evolution of CRAB, several genotypic methods exist. The most commonly used typing techniques include PCR-based and sequencing methods [repetitive sequence-based PCR (rep-PCR), multilocus sequence typing (MLST), sequence-based typing and their allele-specific multiplex PCRs], DNA-based fingerprinting methods [pulsed-field gel electrophoresis (PFGE)] and whole genome sequencing (WGS) (Kamolvit *et al*., 2015). Molecular typing of the CRAB strains from various European hospitals has shown the emergence of three successful clones originally named European clones I to III, which were renamed as international clones or clonal complexes (ICs/CCs) I to III, after being identified worldwide (Zarrilli *et al*., 2013).

Although Serbia is a country with a significant trend of rising prevalence of carbapenem-resistant *Acinetobacter* spp., from 95.1% in 2017 to 98% in 2021 (ECDC, 2023), there is no systematic data or continuous monitoring of the occurrence of CRAB isolates. The present review aims to go through the molecular epidemiology of CRAB clinical isolates in Serbia, according to current published data, and attempt to identify future challenges and trends.

2. Carbapenem-resistant *A. baumannii* in Serbia

2.1. Resistance rates

CRAB isolates were first recorded in our hospitals in the early 2000s. The resistance to imipenem rose remarkably from 6.9% in 2002 to 67.4% in 2010. Since resistance to third and fourth-generation cephalosporins as well as ciprofloxacin reached 100% in 2010, strains were characterized as MDR or 'nonsusceptible to at least one agent in ≥3 antimicrobial categories', according to proposed criteria (Medić *et al*., 2011). From the early beginnings, the prevalence of carbapenem-resistant *Acinetobacter* spp. in Serbian hospitals reached 98% in 2021 (ECDC, 2023).

2.2. Molecular epidemiology

Despite the increasing prevalence of carbapenem resistance, data about the molecular epidemiology of CRAB in Serbia is lacking. However, there were reports of OXA and MBL-positive CRAB strains, recovered from patients migrating from Serbia to Western Europe.

German authors reported the molecular characterization of MDR *A.baumannii* collected from a patient with a femorocrural dacron bypass infection admitted to the intensive care unit at the Frankfurt University Hospital in 2007, after hospitalization in Serbia. The strain was resistant to β-lactams (including carbapenems), fluoroquinolones, aminoglycosides, tigecycline and aztreonam. It remained susceptible to colistin. Isolate harboured $bla_{\text{NDM-1}}$ and intrinsic $bla_{\text{OXA-64}}$ gene, without the insertion sequence IS*Aba*1 located upstream. PCR-based *A. baumannii* typing in combination with ApaI PFGE analysis confirmed that it was not related to European clonal lineages 1–3 (Göttig *et al*., 2010; Pfeifer *et al*., 2011).

The following year brought another record on MDR *A. baumannii* isolate recovered from a patient hospitalized in Geneva University Hospitals, Switzerland, from 2009 to 2010, after a transfer from Serbia. *A. baumannii* was recovered from rectal swabs. It was resistant to all β-lactams (including carbapenems), gentamicin, amikacin, chloramphenicol, tetracycline, and fluoroquinolones and remained susceptible to tobramycin and netilmicin, with minimum inhibitory concentrations (MICs) of colistin, rifampin, and tigecycline being at 0.5, 1, and 1 μg/ml, respectively. PCR and sequencing revealed that *A. baumannii* coharboured $bla_{\text{OXA-23}}$ and $bla_{\text{NDM-1}}$ genes. MLST was performed, following the Institut Pasteur scheme, and showed that the isolate belonged to the sequence type 1 (ST1) (Poirel *et al*., 2012).

Three years later, the first study from Serbia reported the distribution of carbapenemases among clinical isolates of *A. baumannii* from a single pediatric hospital. Twenty-eight consecutive, non-duplicate MDR and carbapenem-resistant *A.baumannii* clinical isolates were collected from June 2012 to February 2014 at the Institute for Mother and Child Health Care "Dr. Vukan Čupić", a tertiary care paediatric hospital in Belgrade. The majority of isolates (67.68%) belonged to the ST1, European CC II. All isolates harbored intrinsic OXA-51 carbapenemase, while OXA-24, OXA-23, and OXA-58 were detected in 82.14%, 57.14%, and 39.29%, respectively. This study did not

detect β-lactamases OXA-143, OXA-235, NDM-1, VIM and IMP. IS*Aba*1 was present upstream of OXA-51 in one isolate, and upstream of OXA-23 in 10 isolates (Novovic *et al*., 2015).

Molecular epidemiology of 222 CRABs recovered from inpatients with confirmed bacterial infections admitted at nine hospitals throughout Serbia (General hospitals in Subotica, Pančevo and Sombor, Institute for Pulmonary Diseases of Vojvodina, University Hospital Medical Center Bežanijska kosa,

University Hospital Center dr Dragiša Mišović, Institute for Cardiovascular Diseases Dedinje, Clinical Center Kragujevac, Clinical Center Niš) during the period January–June 2018 was published as a part of the prospective, observational, multicenter study in 2020. All isolates carried the naturally occurring $bla_{\text{OXA-51}}$ gene, while $bla_{\text{OXA-24}}$ and $bla_{\text{OXA-23}}$ were detected in 44.2%, and 34.5% CRABs, respectively. However, $bla_{\text{OXA-58}}$ and $bla_{\text{OXA-143}}$ genes were not discovered. Overall, IS*Aba*1 was present in 71.8% of CRABs. The $bla_{\text{NDM-1}}$ was the only MBL gene detected in the study. None of the isolates carried bla_{IMP}, bla_{VIM}, bla_{GIM}, bla_{SPM} and bla_{SIM} genes. There were no substantial differences between the hospitals and regions regarding the proportion of isolates carrying different acquired bla_{OXA} genes. However, $bla_{\text{NDM-1}}$ positive isolates were detected only in Belgrade and Niš. Sequencing of the bla_{OXA} genes revealed the presence of the $bla_{\text{OXA-66,}}$ $bla_{\text{OXA-72,}}$ and $bla_{\text{OXA-23}}$ variants. MLST assigned the analyzed CRABs to 3 STs: ST2, ST492 (a single locus variant of ST2), and ST636 (a triple locus variant of ST2). ST2 and ST492 belonged to ICII, while ST636 was a singleton, not categorized in any IC (Lukovic *et al*., 2020).

The following year, Serbian authors reported an *A. baumannii* outbreak among preterm neonates in a neonatal intensive care unit at the Institute of Neonatology in Belgrade. During the outbreak period (May-July 2018), there were 13 cases of *A. baumannii* bloodstream infection among 82 hospitalized neonates. All *A. baumannii* strains were carbapenem-resistant and susceptible to colistin. Molecular characterization of the isolates revealed that they harboured OXA-66 and OXA-72 β-lactamases and belonged to ST636 (ICII), while the PFGE pattern indicated clonal spread. Lower gestational age, lower Apgar score, vaginal delivery and mechanical ventilation were risk factors for *A. baumannii* infection. Four patients died, eight patients were treated successfully with colistin, and one patient with sepsis and meningitis on dual ampicillin-sulbactam and colistin therapy recovered with sequelae. The outbreak was eventually controlled by reinforcement of the infection control measures based on a multi-tiered interventional approach (Gajic *et al*., 2021).

Kabic *et al.* (2022) analyzed comparative genomics and molecular epidemiology of 30 colistin-resistant *A. baumannii* isolated from clinical specimens collected from patients admitted to the hospitals in nine cities throughout Serbia between August 2018 and August 2021. All isolates were CRAB and were resistant or had reduced susceptibility to amoxicillin-clavulanic acid, piperacillin-tazobactam, ceftazidime, cefepime, cefoxitin, meropenem, imipenem, amikacin, gentamicin, tobramycin, levofloxacin, ciprofloxacin, and tetracycline. Three STs were identified based on the Pasteur MLST scheme. Among them, ST2 was the most prevalent (76.66 %), followed by ST492 (20 %), and ST636 (3.33 %). Several β-lactamase-encoding genes were detected. Overall, 76.66 % of isolates carried the $bla_{\text{OXA-23}}$ gene, while 23.33 % and 10 % of isolates harboured the $bla_{\text{OXA-72}}$ and $bla_{\text{NDM-1}}$ as single-copy genes, respectively. Additionally, ISAba125 was located upstream of the $bla_{\text{NDM-1}}$ gene.

Since the World Health Organization declared the COVID-19 pandemic in March 2020, the disease has spread rapidly, leading to an overload of the health system, and many of the patients infected with SARS-CoV-2 needed to be admitted to the intensive care units (ICUs). The 64 *A. baumannii* isolates were recovered from COVID-19 patients admitted to ICU at General Hospital "Dr Laza K. Lazarevic" in Šabac, during the period from December 2020 to February 2021. All patients required mechanical ventilation, and the mortality rate was 100%.

A. baumannii isolates were sensitive to colistin, while resistant to meropenem, imipenem, gentamicin, tobramycin, and levofloxacin according to the broth microdilution method and MDR phenotype was confirmed. Typically for *A. baumannii*, intrinsic genes encoding for oxacillinase OXA-51 were detected in all tested isolates by PCR method. Additionally, all isolates gave a positive PCR signal for the $bla_{\text{OXA-23}}$ gene and IS*Aba*1 upstream of this gene. The gene encoding for OXA-24 oxacillinase was identified in 28.12% of isolates, while the $bla_{\text{OXA-58}}$ gene was found in 6.25% of isolates. IS*Aba*1 was not detected upstream of $bla_{\text{OXA-24}}$, $bla_{\text{OXA-51}}$, and $bla_{\text{OXA-58}}$ genes in analyzed isolates. The $bla_{\text{NDM-1}}$ gene was not present in the genomes of tested isolates. MLST analyses revealed that all isolates obtained from Šabac hospital were identified as ST2 (IC II) according to Pasteur nomenclature (Novović *et al*., 2023).

Carbapenem-hydrolyzing enzymes, IS, ST and IC connected to CRAB strains isolated in Serbia are shown in Table 1.

Table 1. Carbapenem-hydrolyzing enzymes isolated in carbapenem-resistant *Acinetobacter baumannii* strains in Serbia.

Intrinsic OXA	Acquired OXA	MBL	IS	ST	IC/CC	Year of isolation	City of isolation	Ref.
OXA-51	OXA-24 OXA-23 OXA-58	-	IS*Aba*1	ST1 ST2 ST3	CC 1 CC 2 CC 3	June 2012- February 2014	Belgrade	(11)
OXA-66	OXA-72 OXA-23	NDM-1	IS*Aba*1	ST2 ST492 ST636	IC II	January-June 2018	Subotica Sombor S.Kamenica Pančevo Belgra de Kragujevac Niš	(7)
OXA-66	OXA-72	No data	No data	ST636	IC II	May-July 2018	Belgrade	(3)
OXA-51 OXA-66	OXA-72 OXA-23	NDM-1	IS*Aba*125	ST2 ST492 ST636	IC II	August 2018-August 2021	Subotica Sombor S.Kamenica Belgrade Kragujevac Čačak Užice Požarevac Kraljevo	(5)
OXA-51	OXA-24 OXA-23 OXA-58	-	IS*Aba*1	ST2	IC II	December 2020-February 2021	Šabac	(10)

OXA-oxacillinase; MBL- metallo-β-lactamase; IS-insertion sequence; ST-sequence type; IC-international clone; CC-clonal complex

3. Conclusion

In summary, this study highlights the molecular epidemiology of the CRAB clinical isolates. Since their first occurrence around 2000, CRAB strains have become endemic in Serbia. Their emergence was associated with carbapenem consumption, while the percentage for carbapenem resistance reached 98% in 2021. OXA carbapenemases constitute the most prevalent mechanism of resistance. CRABs harboring $bla_{\text{OXA-51}}$, $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-24}}$ genes are dominant. The prevalence of OXA-23 and OXA-24 producing CRAB may have occurred due to their higher hydrolytic activity, compared with OXA-58 producers. The higher MICs to carbapenems possibly provided them a comparative advantage to survive and prevail in the nosocomial setting.

This review states that Serbia might be an endemic region for CRAB isolates carrying $bla_{\text{NDM-1}}$ genes. NDM-1-producing CRABs were recovered in our hospitals and also from patients migrating from Serbia to Western Europe.

There are several major epidemic lineages, including ST1 (CC II), ST2 (IC II), ST492 (IC II), and ST636, with various acquired carbapenemase genes, usually associated with a plasmid and transposable mobile genetic elements in these strains, which could explain the horizontal transfer of bla_{OXA} and bla_{MBL} across different lineages.

The extremely high antimicrobial resistance rates and the emergence of specific CRAB clones point out the critical need for the implementation of continuous surveillance of infections caused by *A. baumannii* and the development of accurate prevention strategies in Serbia.

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