

Molecular characterization of MDR clinical isolates of *A. baumannii* from three neighbouring countries in south-eastern Europe

Ivana Goic-Barisic^{1,2}, Ana Kovacic³, Deana Medic⁴, Sanja Jakovac⁵, Tanja Petrovic⁵, Marija Tonkic^{1,2}, Anita Novak^{1,2}, Zana Rubic^{1,2}, Marina Radic^{1,2}, Mario Pirija², Jasna Hrenovic⁶

¹University of Split School of Medicine, Split, Croatia, ²University Hospital of Split, Split, Croatia, ³Institute of Public Health of Split and Dalmatia Country, Split, Croatia, ⁴University of Novi Sad, Faculty of Medicine and Institute of Public Health of Vojvodina, Novi Sad, Serbia, ⁵University Hospital Mostar and University of Mostar School of Medicine, Mostar, Bosnia and Herzegovina, ⁶Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia

Introduction

In the last decade, *Acinetobacter baumannii* has emerged as a major relevant nosocomial pathogen in the most European countries. Multidrug-resistant (MDR) clinical isolates represent a major problem in the epidemiological and therapeutic sense and challenge for clinicians.

Carbapenem- and MDR resistant isolates of *A. baumannii* in Croatia have been continuously monitored since the last decade when percentage of carbapenem-resistant isolates reached 97%. Along Croatia, neighbouring countries Bosnia and Herzegovina and Serbia also recorded a significant increase in the incidence and prevalence of carbapenem-resistant isolates of *A. baumannii*.

The aim of this pilot study was to compare the genotype resemblance and resistance mechanisms of MDR clinical isolates of *A. baumannii* in region of south-eastern Europe.

Results

All collected isolates shared high level of resistance to carbapenems with MIC >32mg/L to both imipenem and meropenem. Beside the carbapenem resistance, isolates were uniformly resistant to gentamicin and ciprofloxacin, but susceptible to colistin. The relatedness of collected *A. baumannii* isolates was assessed by using pulsed-field gel electrophoresis (PFGE) and displayed diversity of genotyping profiles (data not shown). The multiplex PCR confirmed the presence of *bla*OXA-40-like genes in half (6/12) of the collected isolates from neighbouring countries, besides the presence of *bla*OXA-23 gene. Sequencing (both strands) of the *bla*OXA-40-like amplicons revealed the presence of a gene encoding OXA-72 oxacillinase.

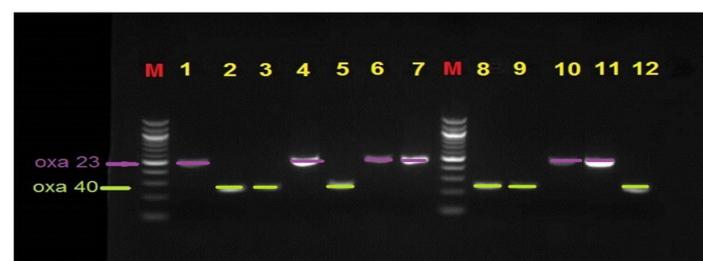


Figure 1. Multiplex PCR results from clinical isolates of *A. baumannii* from Novi Sad, Serbia (1-6), Mostar, Bosnia and Herzegovina (7-8) and Split, Croatia (9-12) with positive controls (M, OXA 23 and OXA 40). Isolates of *A. baumannii* from Novi Sad, Serbia (1-3 from 2017, and 4-6 from 2018) were characterized for the first time. Isolates of *A. baumannii* from UHS Split, Croatia belong to different outbreaks periods from 2009-2018.

Materials and methods

In total, 12 clinical isolates of carbapenem-resistant *A. baumannii* were collected from three different hospitals in neighbouring countries: Croatia, Bosnia and Herzegovina, and Serbia. Four isolates originated from University Hospital of Split, Croatia and were isolated from the tracheal and bronchoalveolar aspirates of patients from adult and paediatric Intensive Care Units, in different outbreaks periods from 2009-2018. Two isolates were collected from University Hospital of Mostar, Bosnia and Herzegovina at the beginning of 2018. Six isolates came from different wards of Clinical Centre of Vojvodina, a hospital in university-affiliated medical centre Novi Sad, Serbia and were collected from blood cultures during 2017 and 2018.

Identification of collected isolates was confirmed by MALDI-TOF MS on cell extracts. The antibiotic susceptibility profile was determined according to MICs values obtained by Vitek2 system and gradient E-test for carbapenems. The broth microdilution method proposed by EUCAST was performed for testing susceptibility to colistin. Multiplex polymerase chain reaction (PCR) using specific primers for *bla*OXA-51-like, *bla*OXA-40-like, *bla*OXA-23-like, *bla*OXA-58-like and *bla*OXA-143-like genes was performed to investigate carbapenem resistance. All obtained amplicons of *bla*OXA genes were sequenced on both strands (commercial service Macrogen Europe, The Netherlands). Raw nucleotide sequences were assembled and manually edited using AliView software (<http://ormbunker.se/aliview/>). Subsequent phylogenetic analyses were performed by using MEGA X software, with neighbour-joining method and number of differences model. In order to estimate the stability of nodes and to support the inferred clades, bootstrap analyses of 500 replicates were performed.

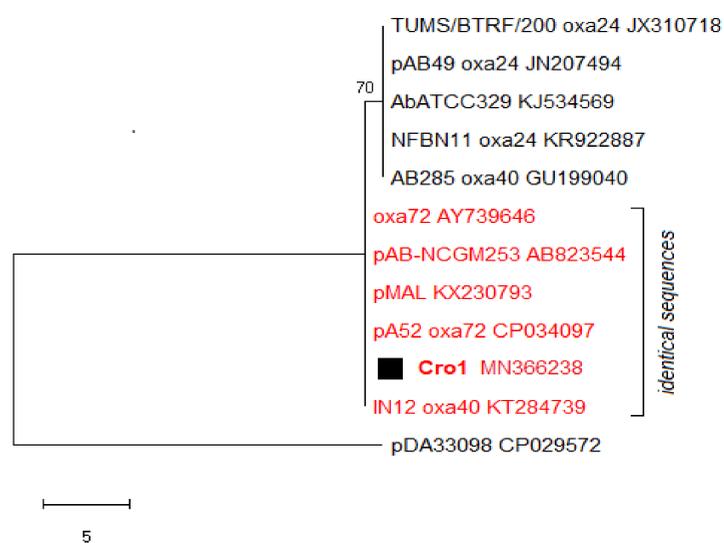


Figure 2. Neighbour-joining phylogenetic tree inferred on *bla*OXA-72 gene fragments amplified from clinical isolates from Serbia, Bosnia and Herzegovina and Croatia (Cro1). Identical sequences were obtained from all clinical isolates harbored OXA-72 oxacillinases. GenBank accession numbers are given next to the name of each strain.

Conclusion

- These are the first results of a pilot study on MDR clinical isolates of *A. baumannii* originating from three neighbouring countries in south-eastern Europe.
- Identical sequences were obtained from all clinical isolates and harboured OXA-72 oxacillinases confirming long time (more than a decade) of this mechanism of resistance to carbapenems in south-eastern Europe.
- The *bla*OXA-72 gene sequence determined in this study has been marked as Cro1 and deposited in GenBank under number MN366238.

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Contact to:
igoic@kbsplit.hr or
igoicbarisic@mefst.hr