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

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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 genome resemblance and resistance mechanisms of MDR clinical isolates of A. baumannii in region of south-eastern Europe.

Introduction

Material 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 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 blaOXA-51-like, blaOXA-40-like, blaOXA-23-like, blaOXA-58-like and blaOXA-143-like genes was performed to investigate carbapenem resistance. All obtained amplicons of blaOXA genes were sequenced on both strands (commercial service Macrogen Europe, The Netherlands). Raw nucleotide sequences were assembled and manually edited using AfullView software (http://afmolbunkar.se/afview/). 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.

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 blaOXA-40-like genes in half (6/12) of the collected isolates from neighbouring countries, besides the presence of blaOXA-23 gene. Sequencing (both strands) of the blaOXA-40-like amplicons revealed the presence of a gene encoding OXA-72 oxacillinase.

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 harboured OXA-72 oxacillinases confirming long time (more than a decade) of this mechanism of resistance to carbapenems in south-eastern Europe.
- The blaOXA-72 gene sequence determined in this study has been marked as Cro1 and deposited in GenBank under number MN366238.

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