ANTIBIOTIC SUSCEPTIBILITY AND CARBAPENEMASE PRODUCTION OF INVASIVE ISOLATES OF ACINETOBACTER BAUMANNII FROM CROATIA

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Introduction and purpose

Acinetobacter baumannii has become one of the most challenging agents of nosocomial infections in recent years; its relevance largely depends on a wide antimicrobial resistance, arising from a variety of intrinsic and acquired mechanisms. Of particular concern is resistance to β-lactam antibiotics, particularly carbapenems, which is observed and more frequently all over the world and which is often associated with few international clones, namely international clonal lineages I, II and III. The first carbapenem-resistant isolates in Croatia were found in University Hospital Split in 2002. The carbapenem-resistance was mediated by hyperproduction of OXA-51 due the ISAbA1 isolated upstream of the gene. Later, the studies of carbapenem-resistance in Croatia showed OXA-23 β-lactamase in University Hospital Center Zagreb and University Hospital Split. These observations gave rise to a multicenter study conducted in last three months of 2009, in Northern Croatia and Istria. The aim of the study was to characterize the carbapenem resistance and to compare the genotypes of the isolates.

Methods

Out of 185 isolates collected from 13 centers in Northern Croatia and Istria 26 isolates from invasive infections (bacteremia or cerebrospinal fluid infections) were selected for this study.

Antibiotic susceptibilities were determined by broth microdilution.

Genes encoding OXA-23-like, OXA-24/40-like, OXA-51-like, OXA-58-like, OXA-54-like carbapenem-hydrolyzing β-lactamases in addition to metallo-β-lactamases (MBLs) of VIM, IMP and SIM series, and PER-1 and TEM-1 β-lactamases were detected by PCR and when needed identified by sequencing.

Presence of ISAbA1 upstream of blaOXA-23 gene was detected by PCR mapping. Sequence groups (SGs) corresponding to international clonal lineages (ICL I - III) were determined by multiplex PCR and genotyping of the strains was performed by multilocus-sequencing typing (MLST), pulsed-field gel electrophoresis (PFGE) and random amplification of polymorphic DNA (RAPD).

Results

Nine strains were found to possess acquired oxacillinase; five belonging to OXA-24/40 group, three to OXA-23 and one to OXA-58 group. These strains were uniform resistant to imipenem, meropenem, piperacillin, cefotaxime, ceftriaxone, gentamicin and cefoxitin. The strains were in high percentage resistant to cefazidime, ceftizoxime and ampicillin/sulbactam but no resistance to colistin was observed. (Table 1, Table 2)

The strains originated from University Hospital Center Zagreb (mostly from surgery wards, belonged to sequence group I (International clonal lineages I) (Figure 1) and according to PFGE belonged to three major clones with > 85% similarity of their PFGE profiles, which contained subclusters with > 90% of similarity. (Figure 2) The sequencing of blaOXA-23-like gene revealed the presence of blaOXA-23-like gene.

The remaining 17 isolates possessed only the naturally occurring OXA-51-like β-lactamase which was upregulated by ISAbA1 located upstream of blaOXA-51-like gene in 14 strains. MBLs were not found. All but three of these strains were carbapenem-resistant or intermediate sensitive to carbapenems and meropenem. The majority of these strains originated from surgical unit of General Hospital in Pula and belonged to ICL I (IIP) while PFGE identified two major clones which showed more than 85% similarity of their banding patterns. The sequencing of blaOXA-51-like gene revealed the presence of blaOXA-51-like and blaOXA-58-like genes.

Conclusions

1. The study revealed high prevalence of acquired oxacillinases associated with high level of resistance to carbapenems among invasive isolates of A. baumannii from Croatia.

2. High diversity of acquired oxacillinases was noticed.

3. ISAbA1 driven overexpression of OXA-51-like beta-lactamase was associated with slightly elevated MICs of carbapenems whereas the presence of acquired oxacillinases correlated with high level of resistance to carbapenems.

4. In conclusion, this study highlights the multiple strategies of carbapenemase-mediated resistance among invasive isolates of A. baumannii from Croatia.

Table 1. Susceptibility to carbapenems, sequence group and OXA content of invasive Acinetobacter baumannii isolates.

Table 2. Antibiotic susceptibility of invasive A. baumannii isolates.

Figure 1. Multiplex PCR for determination of sequence group 1

Figure 2. PFGE dendrogram of invasive Acinetobacter baumannii isolates.