Clonal spread of carbapenem-resistant OXA-72-positive Acinetobacter baumannii in a Croatian university hospital

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SUMMARY

Background: From July to October 2008, 34 Acinetobacter baumannii isolates were involved in an outbreak at the Clinical Hospital Center, Zagreb. The aim of this study was to characterize the mechanisms of carbapenem resistance in our A. baumannii isolates and determine their epidemiology.

Methods: Antibiotic susceptibilities were determined by broth microdilution. PCR was used to detect the presence of carbapenemases. Genotyping of the isolates was performed by random amplification of polymorphic DNA (RAPD), pulsed-field gel electrophoresis (PFGE), and repetitive sequence-based PCR (rep-PCR).

Results: Thirty-three carbapenem-resistant isolates were positive for the acquired blaOXA-72 and one unrelated isolate was positive for blaOXA-58. The blaOXA-72-positive isolates were shown to be clonally related by RAPD, rep-PCR, and PFGE.

Conclusions: On the basis of susceptibility testing, β-lactamase characterization, and genotyping of the isolates we can conclude that clonal spread of endemic isolates was responsible for the high frequency of OXA-72-positive multidrug-resistant A. baumannii in this setting. Most of the isolates originated from the intensive care unit indicating local dissemination within the hospital and pointing to the potential source of isolates.

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1. Introduction

Carbapenems have a potent activity against Acinetobacter and are often used as a last resort for the treatment of infections due to multi-resistant Acinetobacter baumannii isolates.1 However, A. baumannii may develop resistance to carbapenems through various combined mechanisms, including decreased permeability, altered penicillin binding proteins (PBPs) and, rarely, efflux pump overexpression.2 The most frequent mechanism of resistance is through the production of class D oxacillinases (OXA). The intrinsic blaOXA-51-like and the acquired blaOXA genes (23-like, 40-like, 58-like, and 143) are often associated with insertion elements that are thought to upregulate expression.3 Less common are class B IMP- and VIM-type metallo-β-lactamases (MBLs).4 Recently an increase in the prevalence of carbapenem-resistant A. baumannii isolates has been observed at the Clinical Hospital Center Zagreb. The aim of this study was to characterize the mechanisms of carbapenem resistance in our A. baumannii isolates and their molecular epidemiology.

2. Materials and methods

2.1. Bacterial isolates

Thirty-four non-duplicate A. baumannii isolates (one isolate per patient) with reduced susceptibility to imipenem and meropenem by disk diffusion (zone diameter ≤16 mm) were collected between July and October 2008 from various clinical specimens and hospital units (Table 1).

2.2. Susceptibility testing

The antimicrobial susceptibility to a wide range of antibiotics was determined by broth microdilution in accordance with the
like, metallo-
carbapenemases
baumannii
Table 1
Minimum inhibitory concentrations (µg/ml) of various antibiotics and genotyping of oxacillinase-producing Acinetobacter baumannii strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Specimen</th>
<th>Hospital ward</th>
<th>Date of isolation</th>
<th>OXA-51-like</th>
<th>Acquired oxacillinase</th>
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</thead>
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<tr>
<td>1^b</td>
<td>Gastric fluid</td>
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<td>11/08/08</td>
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<tr>
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<td>OXA-66/76</td>
<td>OXA-72</td>
</tr>
<tr>
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<td>Gastric fluid</td>
<td>ICU</td>
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<td>OXA-66/76</td>
<td>OXA-72</td>
</tr>
<tr>
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<td>Urine</td>
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<td>OXA-66/76</td>
<td>OXA-58</td>
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<tr>
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<td>OXA-66/76</td>
<td>OXA-72</td>
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<td>Tr. aspirate</td>
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<td>16/07/08</td>
<td>OXA-66/76</td>
<td>OXA-72</td>
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<tr>
<td>7</td>
<td>CSF</td>
<td>ICU</td>
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<td>OXA-72</td>
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<td>OXA-72</td>
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<td>OXA-66/76</td>
<td>OXA-72</td>
</tr>
<tr>
<td>12</td>
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<tr>
<td>13^b</td>
<td>Canula swab</td>
<td>Ped ICU</td>
<td>01/08/08</td>
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<td>OXA-72</td>
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<tr>
<td>14</td>
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<td>01/08/08</td>
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<td>OXA-72</td>
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<td>OXA-72</td>
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<td>OXA-72</td>
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<td>27/10/08</td>
<td>OXA-66/76</td>
<td>OXA-72</td>
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</tbody>
</table>

FEP, cephalosporin; TIZP, piperacillin/tazobactam; SAM, sulbactam/ampicillin; IPM, imipenem; MEM, meropenem; GEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin; COL, colistin; ICU, neurosurgical intensive care unit; Ped ICU, pediatric intensive care unit; Tr. aspirate, tracheal aspirate; CSF, cerebrospinal fluid.

^a Day/month/year.
^b Isolates on which rep-PCR was performed.

Clinical and Laboratories Standards Institute (CLSI) guidelines. Minimum inhibitory concentrations (MICs) of imipenem and meropenem were also determined in the presence of sodium chloride (200 mM) to inhibit OXA-58 β-lactamase and cloxacillin (200 mg/l) to inhibit chromosomal AmpC β-lactamase. Pseudomonas aeruginosa ATCC 27853 was used as the quality control strain.

2.3. Detection of metallo-β-lactamases

Etest MBL strips were used for the detection of metallo-carbapenemases following the manufacturer’s instructions (AB Biodisk, Solna, Sweden). Furthermore, the isolates were tested by synergy test using imipenem and ethylenediaminetetraacetic acid (EDTA)-containing disks to screen for metallo-β-lactamase production.7

2.4. Characterization of β-lactamases

PCR was used to detect the presence of the genes encoding metallo-β-lactamases, OXA-encoding genes, and blab_{TEM} genes as previously described.8–10 The genetic context of blaoXA-40-like and blaoXA-58-like genes was determined by PCR mapping using ISbaal and ISAb_77 primers.11,12 Partial sequences of selected blaoXA-51-like, blaoX-blaoX-40-like, and blaoX-58-like genes were determined.

To determine if acquired oxacillinase genes were plasmid-encoded, plasmid DNA was extracted using the QIAprep Spin Miniprep Kit (Qiagen, Hilden, Germany) and transferred to A. baumannii ATCC 19606 recipient isolate by electroporation. Transformsants were selected on 100 µg/ml ticarcillin. In addition, plasmid DNA was used as template for PCR for the detection of blaoX-blaoX-40 and blaoX-58 genes.

2.5. Genotyping of the isolates

All A. baumannii isolates were initially genotyped by random amplification of polymorphic DNA (RAPD) using M13 primer.13 Sequence groups (1–3) corresponding to EU clones I–III were determined by multiplex PCR as described previously.14 Representative isolates were further investigated by repetitive sequence-based PCR (rep-PCR) (DiversiLab System; bioMérieux, Nürtingen, Germany) following the manufacturer’s instructions. Isolates that clustered above 95% were considered related.15 Pulsed-field gel electrophoresis (PFGE) genotyping of XbaI-digested genomic DNA was performed with a CHEF-DRIII system (Bio-Rad, Zagreb, Croatia),16 the images were processed using Gel-Compar software, and a dendrogram was computed after band intensity correlation using global alignment with 1.5% optimization and UPGMA (unweighted pair group method with arithmetic mean) clustering.17,18

3. Results

3.1. Antimicrobial susceptibility

MIC data are summarized in Table 1. All isolates were uniformly resistant to cefazidime, cefotaxime, ceftriaxone, piperacillin/penicillin-gastrointestinal diseases. However, few studies have focused on the prevalence of these enzymes in various hospital wards and the impact on antimicrobial susceptibility.
tazobactam, gentamicin, and ciprofloxacin, and to meropenem and imipenem. All isolates remained susceptible to colistin. Addition of sodium chloride lowered the carbapenem MICs of the OXA-58 β-lactamase-producing strain by two dilutions. Addition of cloroxillin did not have any effect on MICs of imipenem or meropenem.

3.2. Characterization of β-lactamases

Thirty-three isolates were positive for the gene encoding OXA-40-like β-lactamase and one OXA-58-like. ISAball was found upstream of the blaOXA-58-like gene and ISAba1 was associated with the blaOXA-40-like genes. ISAba1 was not associated with blaOXA-51-like. Only OXA-58-like β-lactamase was inhibited by sodium chloride, lowering the imipenem MIC from 16 mg/l to 4 mg/l. No MBLs were found. All isolates were positive for blatem. Sequencing of the acquired OXA genes revealed blaOXA-72 and blaOXA-58 (Table 1). Electroploration experiments using plasmid extracts from blaOXA-72- and blaOXA-58-positive isolates were unsuccessful in transferring carbapenem resistance to electrocompetent A. baumannii ATCC 19606. Plasmid bands were not visible after electrophoresis of plasmid DNA and these plasmid preparations were negative for blaOXA-72. Partial sequencing of the blaOXA-51-like genes revealed that the blaOXA-72 isolates possessed blaOXA-66/76. These genes differ at nucleotide 808, which was not covered by the sequencing reaction. The blaOXA-58 isolate possessed blaOXA-68 (Table 1).

3.3. Genotyping of the isolates

The OXA-72 producers belonged to EU clone 2, which is in agreement with possession of blaOXA-66/76 (sequence group 1). The OXA-58 strain did not cluster with any of the European clones. RAPD analysis grouped the OXA-72 isolates together, and these isolates showed identical banding patterns in rep-PCR (Figure 1). The OXA-58 isolate was distinct. PFGE confirmed the clonality of the predominant A. baumannii blaOXA-72-positive isolates.

4. Discussion

Previously in Croatia, carbapenem resistance was associated with upregulation of the blaOXA-51 gene by ISAbal.19 This study found OXA-72, a member of the OXA-40 subclass, to be the most prevalent carbapenem resistance determinant among our A. baumannii isolates, and this represents the first report of OXA-72 in Croatia. Outbreaks of OXA-40-like are generally rare except in Spain,29 as they are the least prevalent of OXAs and when tested have been found more often in EU complex II.21–23 Our isolates emerged in the summer of 2008. In January 2009, OXA-72-producing A. baumannii isolates were reported from University Hospital Split in Croatia. In contrast to our isolates, these isolates were shown to possess integron-associated metallo-β-lactamase in addition to OXA-72.

Clustering of A. baumannii isolates suggests either patient-to-patient transmission or a common source of acquisition (e.g., contaminated equipment for mechanical ventilation). The wide dissemination of EU clone II OXA-72 isolates has probably resulted from its selective advantage in the antibiotic-rich hospital environment and could further be facilitated by the lack of effective measures to prevent hospital transmission. Other European studies have already demonstrated the spread of EU clone II isolates and the association of carbapenem resistance with these isolates.21–23 OXA-72 β-lactamase with similar properties has previously been reported in France,2,3 South Korea,25 Taiwan,26 China,27 and Brazil.28 In contrast to our results, they proved the plasmid location of blaOXA-72 gene.

The isolates displayed high MICs for carbapenems, which could also be attributed to other resistance mechanisms, such as porin loss or upregulated efflux pumps,2 but clarification of these additional resistance mechanisms was beyond the scope of this study. Resistance to ampicillin/sulbactam is worrying and was most likely due to the production of TEM-β-lactamases.22 Colistin became the antibiotic of choice for the treatment of infections caused by our A. baumannii isolates, as this was the only antimicrobial to have activity. However, the risk of nephrotoxicity is of clinical concern. Tigecycline has been shown to be effective in vitro and in vivo against multidrug-resistant A. baumannii,29 but is not yet licensed in Croatia. The combination of tigecycline, colistin, and meropenem has been shown to yield a favorable clinical outcome against multi-resistant A. baumannii.30

In conclusion, this study highlights the propensity of clonal spread of multidrug-resistant A. baumannii, in particular carbapenem-resistant isolates. Furthermore, the factors responsible for dissemination of such isolates need to be identified, controlled, and prevented to avoid major outbreaks.

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Conflict of interest: No conflict of interest to declare.

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