Molecular characteristics and antibiotic resistance of *Acinetobacter baumannii* beta-lactamase-producing isolates, a predominance of intrinsic *bla*<sub>OXA-51</sub> and detection of TEM and CTX-M genes

Amir IBRAHIMAGIC<sup>1,4</sup>, Farah KAMBEROVIĆ<sup>2</sup>, Selma UZUNOVIĆ<sup>1</sup>, Branka BEDENIĆ<sup>3,4</sup>, Emina IDRIZOVIĆ<sup>1</sup>

<sup>1</sup>Department for Laboratory Diagnostics, Cantonal Public Health Institute Zenica, Zenica, Bosnia and Herzegovina
<sup>2</sup>Faculty of Biology, University of Barcelona, Barcelona, Spain
<sup>3</sup>School of Medicine, University of Zagreb, Zagreb, Croatia
<sup>4</sup>Department for Clinical and Molecular Microbiology, Clinical Hospital Center Zagreb, Zagreb, Croatia

**Background/aim:** The aim of this study was to determine the molecular characteristics and antibiotic resistance of 13 (10 inpatient and three outpatient) *Acinetobacter baumannii* beta-lactamase-producing isolates collected in Bosnia and Herzegovina between December 2009 and May 2010.

**Materials and methods:** Susceptibility testing was performed by disk diffusion and broth microdilution methods. The modified Hodge and combined disk test with EDTA/phenylboronic acid was used to screen for carbapenemase production. Production of extended-spectrum beta-lactamases (ESBLs) was determined by double-disk synergy test. PCR was used to detect *bla*<sub>ESBL</sub>/*bla*<sub>carb</sub> genes.

**Results:** Ten (22.2%) inpatient and three (13.6%) outpatient isolates produced beta-lactamases, ESBLs, or oxacillinases. More than 50% of the isolates showed multidrug resistance. Resistance rates to gentamicin and ciprofloxacin of the inpatients and outpatients were 80.0%, 60.0%, 75.0%, and 25.0%, respectively. MICs of carbapenems for resistant isolates ranged from 32 to >256 µg/mL. All imipenem-resistant *Acinetobacter baumannii* strains contained *bla*<sub>OXA-51</sub>. Three of the 10 inpatient isolates and one outpatient isolate containing *bla*<sub>OXA-51</sub> additionally produced other beta-lactamases (TEM/CTX-M/OXA-1). None of the inpatient or outpatient isolates were positive for other carbapenemases, especially acquired oxacillinases (*bla*<sub>OXA-23</sub>/*bla*<sub>OXA-24</sub>/*bla*<sub>OXA-58</sub>/*bla*<sub>OXA-143</sub>).

**Conclusion:** Production of *bla*<sub>OXA-51</sub> presents an emerging threat in imipenem-resistant *Acinetobacter* spp. from Bosnia and Herzegovina.

**Key words:** Narrow-spectrum beta-lactamases, extended-spectrum beta-lactamases, oxacillinases, antibiotic resistance

1. Introduction

Among the different *Acinetobacter* species, *Acinetobacter baumannii* is an important nosocomial pathogen with the ability to cause infection outbreaks (1–3). Potential risk factors associated with the development of colonization or infection of hospitalized patients with *Acinetobacter* spp. include prolonged length of hospital stay, severity of underlying disease, invasive procedures and treatment (mechanical ventilation, urinary catheterization, parenteral nutrition), exposure to broad-spectrum antimicrobial agents such as carbapenems or third-generation cephalosporins, primary and acquired immunodeficiency, and age (4,5).

A wide range of class A beta-lactamas, including the narrow-spectrum, extended-spectrum, and carbapenem-hydrolyzing variants, have been identified in all species of *Acinetobacter* such as *A. calcoaceticus, A. baumannii, A. gerner*, and others (6).

OXA enzymes encoded by *bla*<sub>OXA</sub> genes can be subclassified into four distinct subgroups, of which *bla*<sub>OXA-23</sub>, *bla*<sub>OXA-24</sub>, *bla*<sub>OXA-51</sub>, and *bla*<sub>OXA-58</sub> have been identified in *Acinetobacter* spp. (6). In some countries *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-24</sub> are the most prevalent oxacillinases, like in Poland (1), Iran (7), and Ireland (8), while in some countries *bla*<sub>OXA-58</sub> and *bla*<sub>OXA-23</sub> are the most prevalent, as in Saudi Arabia (9), Croatia (10), and Italy (11).

Antibiotic resistance in *Acinetobacter* spp. has been previously reported from Bosnia and Herzegovina in 4.5% of isolates causing blood infections with multidrug resistance rates of 45%–85% (12), but the molecular characteristics have not been described.

The aim of this study was to investigate the molecular characteristics and antibiotic resistance of infections caused by *Acinetobacter baumannii* producing beta-lactamases/extended-spectrum beta-lactamases (ESBLs) or oxacillinases.
2. Materials and methods

2.1. Setting, bacterial isolates, and study design
All consecutive, nonduplicate Acinetobacter spp. isolates resistant to expanded-spectrum cephalosporins included in the analysis were collected from various specimens and various hospital departments, including the outpatient department, in the period between December 2009 and May 2010.

Information records for study patients consisted of age, sex, occupation, place of residence at time of admission to the hospital (e.g., at home, other hospital, nursing home), contact with person(s) with history of hospitalization in the past 12 months, hospital department, surgery during a stay in the hospital, antibiotics usage in the past 4 months, and glucocorticoid therapy.

Institutional review board approval of the Ethics Committee of the Cantonal Hospital Zenica was obtained prior to the initiation of the study.

2.2. Susceptibility testing
Antimicrobial susceptibility testing was performed by a twofold microdilution technique according to the standard Clinical and Laboratory Standards Institute (CLSI) procedure (13) with amoxicillin + clavulanic acid, ceftazidime, cefotaxime, ceftriaxone, imipenem, meropenem, gentamicin, and ciprofloxacin. The following MIC resistance breakpoints were used: ≥32 for amoxicillin/clavulanic, ceftazidime, and cefotaxime; ≥64 for cefotaxime and ceftazidime; ≥16 for imipenem and meropenem; ≥8 for gentamicin; and ≥4 for ciprofloxacin. Escherichia coli ATCC 25922 (ESBL-negative) and Klebsiella pneumoniae 700603 (ESBL-positive) were used as quality control strains.

2.3. Detection of ESBLs
A double-disk synergy test using the combination of amoxicillin/clavulanate with cefotaxime, ceftazidime, ceftriaxone, and cefepime was performed in order to detect the production of ESBLs. Distortion of the inhibition zones around cephalosporin disks towards the central disk was considered as a positive result (14). Production of ESBLs was confirmed by CLSI combined disk test.

2.4. Phenotypic detection of carbapenemases
Production of group A or group B carbapenemases was confirmed by combined disk test using EDTA and PBA. The augmentation of the inhibition zones around disks with PBA for at least 5 mm was suggestive of KPC production while the increase of the inhibition zones around disks with EDTA indicated MBL production (15).

2.5. Molecular characterization of ESBL
Polymerase chain reaction (PCR) was used to detect alleles encoding ESBL enzymes. The presence of blaCTX_M, blaSHV, and blaTEM genes was investigated by PCR using primers and conditions as described previously (16).

Template DNA was extracted by the boiling method. PCR mix (50 µL) contained 25 µL of master mix (Roche), 20 µL of ultrapure water, 1 µL of each primer (10 pmol), and 3 µL of the template DNA. Strains positive for CTX-M beta-lactamases were further tested by multiplex PCR with primers specific for CTX-M groups 1, 2, 8, 9, and 25 (16). Amplicons were column-purified (QIAGEN DNA purification kit) and sequenced directly using the ABI PRISM 377 Genetic Analyzer (Applied Biosystems). Sequences were analyzed using BioEdit v.7.0.9 (Ibis Biosciences). Designation of bla genes based on the identified mutations was done according to a previous study based on amino acid sequences for TEM, SHV, and OXA extended-spectrum and inhibitor-resistant beta-lactamases (17).

2.6. Molecular detection of carbapenemases
Genes encoding carbapenemases of group A (KPC), group B (MBLs belonging to the VIM, IMP, and NDM family), and OXA-48 were detected by PCR as described previously (1,18).

2.7. Molecular detection of oxacillinases
All isolates were subjected to multiplex PCR to detect the blaOXA-51, blaOXA-23, blaOXA-24, blaOXA-58, and blaOXA-14 genes (19).

3. Results

3.1. Clinical specimens
In the period between December 2009 and May 2010, of a total of 1254 inpatient gram-negative bacteria samples and 2857 outpatient, 45 (3.9%) and 22 (0.8%) were identified as Acinetobacter spp., respectively.

Among inpatient samples, ten (22.2%) A. baumannii produced beta-lactamases or ESBLs or oxacillinases, of which 30.0% were from skin and soft tissue infections, 20.0% from surgical wounds, 20.0% from cannulae, and 10.0% from each sample of burn, stoma, and ear.

Among outpatient samples, three (13.6%) were beta-lactamases or ESBLs or oxacillinases, of which 66.7% were from surgical wounds and 33.3% from genital swabs.

More than half of both the inpatients and outpatients with infections caused by A. baumannii were male (n = 10; 76.9%) (Table 1). Mean patient age was 46 years (minimum: <1, maximum: 78). Duration of hospitalization (median) was 12 days (range: 8–23), excluding one case where the duration of hospitalization was 23 days (Table 1).

3.2. Antibiotic susceptibility
Table 2 shows the susceptibility of 13 A. baumannii isolates tested with twofold microdilution. Thirteen isolates were resistant to imipenem and four (out of 13; 30.8%) isolates were resistant to meropenem. The resistance rate to gentamicin was 84.6%. Prevalence of resistance to ceftazidime, cefotaxime, ceftriaxone, and cefepime was in the range of 75% to 92%.
3.3. Detection and characterization of β-lactamases

Seven *A. baumannii* isolates carried *bla*<sub>TEM</sub>, of which four isolates coproduced *bla*<sub>OXA-51</sub>, while the other isolates produced TEM along with CTX-M, OXA-1, or OXA-51. Three (23.1%) isolates carried a *bla*<sub>CTX-M</sub> group 1 beta-lactamase (Table 1).

<table>
<thead>
<tr>
<th>Isolate origin</th>
<th>Age</th>
<th>Sex</th>
<th>Duration of hospitalization</th>
<th>Hospital department</th>
<th>Type and sequences of beta-lactamases</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSTI</td>
<td>&lt;1</td>
<td>Female</td>
<td>8</td>
<td>Pediatrics</td>
<td>TEM-1, OXA-51</td>
</tr>
<tr>
<td>Wound</td>
<td>62</td>
<td>Male</td>
<td>10</td>
<td>Surgery</td>
<td>TEM-1, CTX-M-1, OXA-1, OXA-51</td>
</tr>
<tr>
<td>Burn</td>
<td>05</td>
<td>Male</td>
<td>15</td>
<td>Surgery</td>
<td>OXA-51</td>
</tr>
<tr>
<td>Wound</td>
<td>46</td>
<td>Male</td>
<td>12</td>
<td>Surgery</td>
<td>OXA-51</td>
</tr>
<tr>
<td>Stoma</td>
<td>54</td>
<td>Male</td>
<td>23</td>
<td>Surgery</td>
<td>TEM-1, OXA-51</td>
</tr>
<tr>
<td>Cannula</td>
<td>69</td>
<td>Male</td>
<td>15</td>
<td>ICU</td>
<td>OXA-51</td>
</tr>
<tr>
<td>Cannula</td>
<td>78</td>
<td>Male</td>
<td>9</td>
<td>ICU</td>
<td>TEM-1, CTX-M-1, OXA-1, OXA-51</td>
</tr>
<tr>
<td>SSTI</td>
<td>53</td>
<td>Male</td>
<td>†</td>
<td>Oncology</td>
<td>OXA-51</td>
</tr>
<tr>
<td>SSTI</td>
<td>69</td>
<td>Female</td>
<td>13</td>
<td>Dermatology</td>
<td>OXA-51</td>
</tr>
<tr>
<td>Ear</td>
<td>49</td>
<td>Male</td>
<td>†</td>
<td>ORL</td>
<td>TEM-1, OXA-51</td>
</tr>
<tr>
<td>Genital swabs</td>
<td>58</td>
<td>Female</td>
<td></td>
<td>Outpatient</td>
<td>TEM-1, OXA-51</td>
</tr>
<tr>
<td>Wound</td>
<td>46</td>
<td>Male</td>
<td></td>
<td>Outpatient</td>
<td>TEM-1, CTX-M-1, OXA-51</td>
</tr>
<tr>
<td>Wound</td>
<td>28</td>
<td>Male</td>
<td></td>
<td>Outpatient</td>
<td>OXA-51</td>
</tr>
</tbody>
</table>

SSTI: Skin and soft tissue infections, ICU: intensive care unit, †: no data.

### Table 2. Minimum inhibitory concentration (MICs) of various antibiotics for 13 *A. baumannii* beta-lactamase/ESBL/oxacillinase-producing isolates.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>No. (%) of resistant isolates</th>
<th>MIC&lt;sub&gt;S&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;S&lt;/sub&gt;</th>
<th>MIC range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin/clavulanate</td>
<td>8 (61.5)</td>
<td>16</td>
<td>≥128</td>
<td>2 to ≥128</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>12 (92.3)</td>
<td>≥256</td>
<td>≥256</td>
<td>1 to ≥256</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>10 (76.9)</td>
<td>128</td>
<td>≥256</td>
<td>1 to ≥256</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>11 (84.6)</td>
<td>256</td>
<td>≥256</td>
<td>4 to ≥256</td>
</tr>
<tr>
<td>Ceferpime</td>
<td>10 (76.9)</td>
<td>64</td>
<td>64</td>
<td>2–64</td>
</tr>
<tr>
<td>Imipenem</td>
<td>13 (100.0)</td>
<td>≥128</td>
<td>≥128</td>
<td>32 to ≥128</td>
</tr>
<tr>
<td>Meropenem</td>
<td>4 (30.8)</td>
<td>0.25</td>
<td>≥128</td>
<td>≤0.06 to ≥128</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>11 (84.6)</td>
<td>64</td>
<td>≥256</td>
<td>≤0.12 to ≥256</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>7 (53.8)</td>
<td>4</td>
<td>256</td>
<td>≤0.12 to ≥256</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>7 (53.8)</td>
<td>128</td>
<td>≥256</td>
<td>2 to ≥256</td>
</tr>
<tr>
<td>Tazobactam</td>
<td>3 (23.1)</td>
<td>16</td>
<td>128</td>
<td>2–128</td>
</tr>
</tbody>
</table>

4. Discussion

*Acinetobacter* spp. has emerged as one of the most important pathogens involved in healthcare-associated infections in the recent decades (3). Production of β-lactamases is one of the most important resistance mechanisms of many bacterial species (20). In the present study, the prevalence of beta-lactamases/ESBLs or oxacillinases in *Acinetobacter* spp. isolates of 22.2% of inpatients and 14% of outpatients, respectively, was found. The prevalence of β-lactamases in *Acinetobacter* isolates found in this study is lower than that
reported in other countries, such as India with 70% (20) or Hungary with 68% (21). Imipenem-resistant *A. baumannii* strains in this study originated from various specimens including surgical wound infections, skin and soft tissue infections, burns, catheters, ear, and stoma, which is in concordance with reports from Ireland and Norway (8,18).

The resistance rate of *A. baumannii* isolates to different antibiotics in this study was high. This is similar to a report from Turkey (22) as a piperacillin-tazobactam and meropenem susceptibility rate of 50% and 70%, respectively, was found in this study. These agents are the most effective β-lactams against *Acinetobacter* isolates, and this finding is similar to a report from Tehran (7). According to the results of this study, the first-line therapy drugs used in hospital departments were amoxicillin/clavulanic and imipenem, β-lactam/β-lactamase-inhibitor combinations. Similarly, Boo et al. reported administration of amoxicillin/clavulanic and meropenem as the first-line therapy for the treatment of infections caused by *Acinetobacter* spp. (8).

In recent years, the number of *Acinetobacter* isolates showing resistance to imipenem has been increasing (7). One of the possible reasons for the high prevalence of imipenem resistance of *A. baumannii* in this study is the presence of OXA-carbapenemases (intrinsic blaOXA-51 in 100% of isolates, or deficiency in porin or reduced expression of outer membrane proteins that was found). The present results support previous ones, suggesting that *bla*OXA-51 is species-specific to *A. baumannii* (23). This study has not detected any other acquired oxacillinas, e.g., *bla*OXA-23, *bla*OXA-24, *bla*OXA-58 or *bla*OXA-143 except intrinsic *bla*OXA-51. This is in contrast to reports from Poland with *bla*OXA-23 and *bla*OXA-24 in 45% of cases (1); Iran with *bla*OXA-23 and *bla*OXA-24 in 20% (7); Ireland with *bla*OXA-23 in 10% (8); Saudi Arabia with *bla*OXA-23 and *bla*OXA-58 in 100% and 2% (9); and Croatia with *bla*OXA-58, *bla*OXA-24 and *bla*OXA-23 in 33%, 27%, and 9% (10). Some authors reported that ISAba1 (insertion sequence, *A. baumannii*), which is adjacent to *bla*OXA-51, plays a major role in the development of resistance to carbapenems (24). In previous studies, imipenem resistance was associated only with isolates in which ISAba1 was upstream of *bla*OXA-51, suggesting that ISAba1 provides the promoter for this gene (24). One isolate in this study was positive for ISAba1, resulting in a low prevalence, and this is similar to a Norwegian study (18). The relationship between *bla*OXA-51 and the resistance of *A. baumannii* isolates to imipenem still needs to be investigated. This study found a high prevalence of multidrug resistance of 54% in *A. baumannii*, limiting the appropriate treatment of infections caused by *Acinetobacter* spp. isolates. Seven isolates coproduced TEM, CTX-M, OXA-1, or OXA-51 β-lactamas, which is in concordance with other reports (3).

Almost all patients were immunocompromised, predisposing for an infection with *Acinetobacter* spp. (25). Similarly to other reports, carbapenemase-producing gram-negative bacteria could cause a wide spectrum of infections including bacteremia, nosocomial pneumonia, wound infections, endocarditis, and urinary tract infections (25). Those infections are often associated with treatment failure, long hospital stay, and high mortality rates (25).

The main mode of transmission of genes is between patients via the hands of healthcare workers, although ESBL outbreak has been attributed to contaminated medical devices, too (e.g., ultrasound gel) (26). Thus, hand hygiene should be the most effective preventive measure (26). Contact precautions in addition to other infection prevention measures, e.g., environmental cleaning, use of gloves and aprons/gowns, or restriction of antibiotics, have been shown to be effective in preventing transmission in outbreak situations (26).

A surveillance of antimicrobial resistance in Bosnia and Herzegovina, which should be the first step toward appropriate control of antibiotic usage, does not exist. There are still debates about optimizing possible treatment approaches in infections caused by carbapenem-producing strains (27). It is strongly suggested that combination therapy, including colistin, tigecycline, aminoglycosides, aztreonam, and carbapenems in different combination schemes, is still superior to monotherapy and that carbapenem-containing regimens are superior to others when an appropriate dose is applied (25). Active surveillance, hand hygiene, contact precautions, and appropriate antibiotic usage should be a part of the effective approach to reducing the incidence of colonization and infections caused by these life-threatening microorganisms.

Our study has several limitations. The first is the small number of *A. baumannii* isolates collected in a period of 6 months, but, on the other hand, we have a low prevalence of these isolates causing hospital and community infections. One of the limitations is that pulsed-field gel electrophoresis was not done for the isolates, which creates a problem in the interpretation of the mode of gene transfer. In the future, it would be good to conduct research about the mechanisms of resistance in *Acinetobacter* spp. isolates collected from hospital and community settings.

In conclusion, the production of *bla*OXA-51 presents an emerging threat in imipenem-resistant *Acinetobacter* spp. from Bosnia and Herzegovina. Results regarding molecular characteristics and antimicrobial resistance among *Acinetobacter* strains are limited while reduced susceptibility to these agents is a matter of increasing clinical concern worldwide. Molecular characterization and strain typing of such epidemic bacteria are important.
for the detection of the sources and mode of spread, which is the main step in order to design targeted infection control strategies. Further studies are required in order to monitor mechanisms of resistance levels among A. baumannii strains.

References


Acknowledgment

We wish to thank Advija Hedzić, head of the Department of Microbiology, Cantonal Hospital Zenica.


