OXA Carbapenemase Genes in Multidrug-Resistant A. baumannii Strains in Southeast of Turkey

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Abstract

Increasing carbapenem resistance led to difficulties in treatment of multidrug-resistant (MDR) A. baumannii infections. We aimed to investigate the carbapenemase genes in MDR A. baumannii isolates. A total of 80 MDR A. baumannii strains were studied by multiplex PCR with the hyplex® CarbOxa ID test system. It involves amplification of blaOXA-51-like, blaOXA-23-like, blaOXA-40-like and blaOXA-58-like gene families and hybridisation of the PCR products to specific oligonucleotide probes in an ELISA-based system.

All isolates were resistant against imipenem and meropenem while susceptible to colistin. The the intrinsic gene of A. baumannii was detected from 77 (96.25%) strains. The presence of
blaOXA-23-like, blaOXA-58-like and blaOXA-40-like genes were demonstrated in 48 (60%), 12 (15%), and 8 (10%) isolates, respectively. The blaOXA-23-like was a common gene and colistin was a rare option for treatment of MDR A. baumannii infections in southeast of Turkey.

Key words: MDR A. baumannii, hyplex® CarbOxa ID, blaOXA-23-like, blaOXA-40-like and blaOXA-58-like

Introduction

Acinetobacter baumannii is an opportunistic pathogen causing nosocomial infections and outbreaks worldwide, especially in the intensive care units (ICUs) [1,2]. Carbapenems have been frequently used for the treatment of infections due to multidrug-resistant (MDR) A. baumannii isolates. However, currently the increasing resistance to these antimicrobial agents led to difficulty in treatment of MDR A.baumannii infections [3,4].

The resistance mechanisms for these antibiotics are efflux pump overexpression, decreased permeability, and carbapenemase production. Many studies showed that among carbapenemases carbapenem-hydrolyzing class D beta-lactamases (CHDLs) are the most common cause of carbapenem resistance in A. baumannii [4-6]. Carbapenem-hydrolyzing class D beta-lactamases were grouped into six subclasses including OXA-51-like, OXA-23-like, OXA-24/40-like, OXA-58-like, OXA-143-like, and OXA-235-like beta-lactamases (7,8). OXA-51-like beta-lactamases are known as chromosom al beta-lactamases while the others are known as acquired beta lactamases. Worldwide, OXA-23-like enzymes are the most common CHDLs [1–9].

In Turkey, studies regarding CHDLs and their epidemiology in A.baumannii were limited. Especially in Southeast of Turkey, there was no available data about the distribution of CHDLs in nosocomial MDR A.baumannii isolates belonging to recent years. In current study we aimed to investigate the types of CHDL genes and their epidemiology in MDR A.baumannii. In addition with this study we aimed to determine antibiotic susceptibilities of these strains.
Materials and methods

Patients and study design:

A total of 80 MDR A. baumannii strains isolated from variable clinical samples of hospitalized patients with the diagnosis of nosocomial infection were included in this study. Non MDR A.baumannii isolated samples and the patients colonized with A. baumannii were excluded. In the study, the microorganism was defined as multidrug resistant if it was resistant to one of the carbapenem group of antibiotics and resistant to more than three of the following antimicrobial agents: amikacin, ceftazidime, ciprofloxacin, piperacillin/tazobactam, and tetracycline. The diagnosis of hospital acquired infection was determined on the basis of diagnostic criteria of “Center for Disease Control and Prevention” for hospital-acquired infection [10]. According to this criteria, hospital-acquired infection was defined as “one that was neither present, nor incubating at the time of the patient’s admission but had its onset during hospitalization.” [10]

Identification of strains and antibiotic susceptibility testing:

Microbiological analyses were performed in clinical microbiology laboratories of Dicle University Medicine Faculty Hospital. The samples were inoculated in Eosin Metilen Blue Agar and Blood Agar in the laboratory and incubated for a period of 18 to 24 hours at 37°C in an incubator (WTB Binder, Tuttlingen, Germany). At the end of this period, identification of microorganisms and antibiotics-susceptibility testing were performed with PHOENIX 100 (Becton Dickinson, Franklin Lakes, NJ). Antimicrobial susceptibilities of Acinetobacter baumannii isolates against tigecycline and netilmicin were performed by the disk diffusion test (Oxoid, Hampshire, England). Susceptibility tests were performed following recommendations of the Clinical Laboratory Standards Institute [11]. Antimicrobial susceptibility for Tigecycline was performed according to the criterias for A.baumannii of Jones RN et al [12].

Detection of carbapenem-hydrolyzing class D beta-lactamases (CHDLs) by molecular method

In this study, the hyplex® CarbOxa ID test system - a qualitative in vitro diagnostic tool- was used. The hyplex®CarbOxa ID method involves amplification of genes for the identification
of *A. baumannii* and the bla*OXA-23*, bla*OXA-40* and bla*OXA-58* gene families, by multiplex PCR and hybridisation of the PCR products to specific oligonucleotide probes in an ELISA-based system.

Pure bacterial cells were used as sample material. A single bacterial colony was suspended in 300 µl hyplex® Lysis Buffer and incubated in a thermal block (99°C) for 10 minutes. After a centrifugation step of 2 minutes at 10,000 rpm, 5 µl supernatant was used for amplification. It was added to the mixture comprising primers, nucleotides, buffer and DNA polymerase enzyme provided by the manufacturer. After initial denaturation of DNA at 94°C for five minutes, amplification continued with 35 cycles consisting 25 seconds at 94°C, 25 seconds at 52°C for binding of primers and 45 sec at 72°C. PCR was completed with final extension of 3 minutes at 72°C.

Using a hybridisation buffer, hybridisation of complementary sequences were detected using the ELISA principle. After several stringent wash steps, a peroxidase (POD) conjugate was added. This conjugate binds highly specifically to the labelling of the single strand of the PCR product bounded to the oligonucleotide probe. After further wash steps, tetramethylbenzidin (TMB) substrate solution, which produces a blue colour when converted by POD, was added. This reaction was stopped by adding a stop solution. A colour change to yellow was observed. Extinction of the various wells was then measured in a photometer at a wavelength of 450 nm. Positive signals indicated specific amplification of certain DNA sequences, thus the presence of the corresponding genes in the sample.

**Results and discussions**

Of the 80 strains included in this study, 46 (57%) strains belonged to males and 34 (23%) to females. Average age of the patients was 46.4375 ± 28.78899. A total of 32 (40%) strains were isolated from sputum samples, 21 (26%) blood samples, 11 (14%) wound swabs, 10 (12%) the catheter swabs, 5 (6%) urine samples. One strain was isolated from the drain fluid. All isolates (100%) were found to be susceptible to colistin. A total of 22 (27.5%) strains were susceptible to netilmicin. Among the isolated strains, 18 (22.5%) strains were susceptible, 62 (77.5%) were resistant to tigecycline. Thirteen (16.25%) strains were
susceptible to amikacin while ten (12.5%) were susceptible to trimethoprim-sulfamethoxazole. All of the strains were resistant against any remaining tested antibiotics (ampicillin-sulbactam, piperacillin-tazobactam, ceftazidime, cefepime, cefotaxime, ceftriaxone, imipenem, meropenem, tobramycin, ciprofloxacin).

Among the strains, 77 (96.25%) were positive for \( \text{bla}_{\text{OXA-51-like}} \), the intrinsic gene for the identification of \( A. \text{baumannii} \). The \( \text{bla}_{\text{OXA-23-like}} \) genes were detected in 48 (60%) strain, while \( \text{bla}_{\text{OXA-58-like}} \) and \( \text{bla}_{\text{OXA-40-like}} \) genes in 12 (15%) and 8(10%) strains, respectively. Among the strains, 65 (81.25%) of them had at least one gene group of \( \text{bla}_{\text{OXA-23-like}} \), \( \text{bla}_{\text{OXA-58-like}} \) or \( \text{bla}_{\text{OXA-40-like}} \) genes. Distribution of OXA carbapenemase genes among intensive care units is shown in Table 1.

**Table 1: Distribution of oxacillinase genes among intensive care units**

<table>
<thead>
<tr>
<th>Intensive care units</th>
<th>( \text{bla}_{\text{OXA-23-like}} )</th>
<th>( \text{bla}_{\text{OXA-58-like}} )</th>
<th>( \text{bla}_{\text{OXA-40-like}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal medicine</td>
<td>12</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Neurology</td>
<td>8</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Pulmonary diseases</td>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pediatrics</td>
<td>5</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>General surgery</td>
<td>3</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Anaesthesiology and reanimation</td>
<td>1</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Cardiology</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Burn unit</td>
<td>1</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Neurosurgery</td>
<td>2</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Other clinics</td>
<td>6</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>12</td>
<td>8</td>
</tr>
</tbody>
</table>
Treatment of nosocomial infections caused by MDR *A. baumannii* has become increasingly difficult. Carbapenems, sulbactam-cefoperazone, colistin and tigecycline are effective antibiotics against *A. baumannii* strains showing multi-drug resistance. However, currently many strains have become resistant to these antibiotics [13-15]. In our study all of the strains were resistant to carbapenems, 77.5% of strains were resistant to tigecycline. All of our strains were susceptible to colistin. According to our data we suggested that treatment of MDR *A. baumannii* infections was a significant problem in our hospital. Consistent with the studies from Turkey and other countries, we observed that colistin was one of rare options for the treatment of MDR *A. baumannii* infections [16,17]. In some countries tigecycline was found to be an effective agent for MDR *A. baumannii* strains in vitro. In a Pakistan study [18] 80% of MDR *A. baumannii* strains showed resistance for tigecycline while in a Turkey study [17] 94% of MDR *A. baumannii* strains were resistant for tigecycline. In our study we found that tigecycline was not an effective antimicrobial agent for MDR *A. baumannii* strains in our hospital.

Among the carbapenemases, carbapenem-hydrolyzing class D beta-lactamases are considered the most prevalent cause of carbapenem resistance in *A. baumannii* [4-6]. The CHDLs in *A. baumannii* can be grouped into six subclasses: chromosomal OXA-51-like, acquired OXA-23-like, OXA-24/40-like, OXA-58-like, OXA-143-like, and OXA-235-like beta lactamases [7,8]. OXA-23-like enzymes are the most common CHDLs and have been reported worldwide [1-9].

However, the distribution of CHDLs are changing according to the countries by the years. In a study conducted in Croatia, the molecular epidemiology and the genetic basis of carbapenem resistance was investigated in 185 MDR *A. baumannii* isolates during 2009–2010. In this study 35% of the strains were resistant to both imipenem and meropenem. ISAba1-driven over expression of the intrinsic blaOXA-51-like gene was observed in all carbapenem resistant isolates, and 69% of these also produced acquired OXA-type carbapenemases. The presence of blaOXA-58-like, blaOXA-24/40-like, and blaOXA-23-like genes was demonstrated in 33%, 27% and 9% of carbapenem-resistant isolates, respectively. None of the isolates harbored the bla IMP, bla VIM, bla SIM, bla NDM or bla PER β-lactamase genes,
while bla TEM-1 was detected in five carbapenem-and ampicillin/sublactam-resistant isolates. Sequence group determination showed a high prevalence (81 %) of isolates belonging to the International clonal lineage (ICL)-I, although the majority (80 %) of isolates carrying acquired carbapenemase genes belonged to the ICL-II. Random amplified polymorphic DNA analysis and multilocus-sequence typing of a subset of carbapenem-resistant isolates revealed a low degree of genetic variability within both ICL-I and ICL-II populations, irrespective of the genetic basis of carbapenem resistance. The authors suggested that an increasing trend toward carbapenem resistance was observed for *A. baumannii* in Croatia, and the emergence of ICL-II strains producing a variety of acquired carbapenemases [19].

In a Pakistan study, the researchers collected 90 Acinetobacter isolates from patients with secondary or nosocomial infections. Of the 90 isolates, 59 were found resistant to carbapenem. Among oxacillinases (OXA) genes, blaOXA-51-like was common in all isolates including combination with blaOXA-23-like in fourteen isolates; however, blaOXA-24-like and blaOXA-58-like were completely absent. Among metallo-β-lactamase (MBL) genes, only blaNDM-1 was found in one isolate while the other three genes; blaIMP, blaVIM, and blaSIM were completely absent. None of the isolates were found to harbour blaCTX-M gene [18].

In a China study, 57 carbapenem resistant *A. baumannii* strains and 20 non carbapenem resistant *A. baumannii* strains were studied. The ISAba1- blaOXA-23-like gene was detected in all 57 CRAB isolates but was detected in none of the non-CRAB isolates. Pulsed-field gel electrophoresis (PFGE) revealed that clones A and B were the dominant genotypes, and all blaOXA-23-like gene positive strains were classified as either clone A or B strains. ST75 and ST137 were the most prominent sequence types (STs). *A. baumannii* isolates of clone A, C and F were all demonstrated to be genetically similar to the previously identified European clone II. It was concluded that ST75- and ST137-type CRAB isolates that produced the blaOXA-23-like gene with an upstream ISAba1 contributed to the nosocomial outbreaks [20].

In another China study, the researchers characterized the molecular epidemiology of 174 non-repetitive clinical isolates of *A. baumannii* collected in 2009. These isolates harbored *A. baumannii* intrinsic gene blaOXA-51-like. 74 out of 174 isolates were identified as carbapenemase-producing strains, among which blaOXA-23 gene was found in 71 isolates. These 74 carbapenemase expression strains could be divided into four genotypes by
enterobacterial repetitive intergenic consensus (ERIC)-PCR, with 19, 17, 33 and 5 clones in each group. They also found that four imipenem resistant isolates carrying bla\textsubscript{OXA-23-like} gene without showing carbapenemase phenotype. They suggested that the bla\textsubscript{OXA-23-like} gene is the common carbapenemase gene among carbapenem-resistant \textit{Acinetobacter spp.} isolates and clonal spread of carbapenemase-producing isolates may be one important factor which results in the high carbapenem resistance rate [21].

In a Taiwan study, during 2007, researchers collected 291 nonrepetitive \textit{A baumannii} isolates. Among 142 imipenem-resistant isolates, 30 harbored the bla\textsubscript{OXA-23-like}. These imipenem-resistant isolates with bla\textsubscript{OXA-23-like} were also resistant to other antimicrobial agents, except colistin. The PCR methods showed the presence of bla\textsubscript{OXA-51-like} in all isolates. The bla\textsubscript{OXA-23-like} gene was detected in the plasmids of 6 isolates. Tn2006 was present in 22 (73.3\%) isolates, and Tn2008, in 6 other isolates (26.7\%). Two strains had bla\textsubscript{OXA-23–ΔATPase} but lacked upstream ISA\textsubscript{ba1}. They concluded that the high prevalence of bla\textsubscript{OXA-23-harboring imipenem-resistant A baumannii} might be attributed to the transposition event of Tn2006 [22].

In a Poland study, OXA encoding genes and presence of ISA\textsubscript{ba1} were investigated. PCR analysis showed the presence of bla\textsubscript{OXA-51-like} gene and ISA\textsubscript{ba1} in all isolates. 46 strains carried bla\textsubscript{OXA-51-like} and bla\textsubscript{OXA-23-like} genes while 48 bla\textsubscript{OXA-51-like} and bla\textsubscript{OXA-40-like} genes. Three isolates carried: bla\textsubscript{OXA-51-like}, bla\textsubscript{OXA-23-like} and bla\textsubscript{OXA-40-like} genes. Seven strains encoded a bla\textsubscript{OXA-51} carbapenemase but were negative for enzymes belonging to the other families tested [23].

In India, Tiwari et al. established the emergence of OXA-51 in clinical strains of \textit{A. baumannii} in India which suggests its role in carbapenem resistance [24].

In an Iran study, among imipenem resistant isolates with bla\textsubscript{OXA-51-like} gene, 88.7\% carried bla\textsubscript{OXA-23-like}, 1.6\% carried bla\textsubscript{OXA-40-like}, and 3.2\% had bla\textsubscript{OXA-58-like} resistance genes. Ninety percent of isolates contained ISA\textsubscript{ba1} element and in 74.2\% of imipenem resistant isolates, ISA\textsubscript{ba1} was located in upstream of bla\textsubscript{OXA-23-like} [25].

In Turkey, the first report of OXA-24/40 carbapenemases in \textit{A. baumannii} were presented in Izmir [26]. Çiçek AÇ et al. collected 101 clinical strains. By multiplex PCR, all strains were
positive for bla\textit{OXA}-51-like, 79 strains carried bla\textit{OXA}-23-like and one strain carried bla\textit{OXA}-40-like. In 79 strains, bla\textit{OXA}-51-like and bla\textit{OXA}-23-like were found together. ISA\textit{ba1} element was detected in 81 strains, and in all cases it was found upstream of bla\textit{OXA}-51. GES-type carbapenemases were found in 24 strains while bla\textit{PER}-2, bla\textit{VEB}-1, bla\textit{NDM}-1, bla\textit{IMP}- and bla\textit{VIM}-type carbapenemases were not observed [16]. A study conducted in Ankara, Turkey reported that carbapenem-resistant invasive \textit{A. baumannii} isolates carrying the bla\textit{OXA}-23-like gene became more prevalent and replaced isolates carrying the bla\textit{OXA}-58-like carbapenemase gene through the 7 years [17].

Conclusions

In our presented study, 90% of strains were positive for bla\textit{OXA}-51-like, the intrinsic gene for \textit{A. baumannii}. Among the isolates, 70% was positive for bla\textit{OXA}-23-like, 27% for bla\textit{OXA}-58-like and 26% for bla\textit{OXA}-40-like. In the light of our data we can suggest that bla\textit{OXA}-23-like was common in \textit{MDR A.baumannii} isolates of southeast of Turkey but the rate of bla\textit{OXA}-58-like and bla\textit{OXA}-40-like were in substantial levels.

In conclusion, treatment of MDR \textit{A.baumannii} infections still remains as a significant problem in our hospital. Colistin was one of rare options for the treatment of MDR \textit{A.baumannii} infections. For the development of new treatment strategies for MDR \textit{A.baumannii} infections, antibiotic resistance patterns of these microorganisms and antibiotic resistance mechanisms must be determined.

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References


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