



The outbreak of *Acinetobacter baumannii* producing OXA-23 and OXA-51 type carbapenemases in a state hospital

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ABSTRACT

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Acinetobacter baumannii is non-fermentative gram-negative bacilli which plays an important pathogen especially in intensive care units causing infections and epidemics. Carbapenem resistance often is consisted of OXA-type carbapenemase. In this study, we aimed to determine carbapenem resistance and clonal relationships of *A. baumannii* isolated from patient and environmental samples by phenotypic and genotypic methods in 10-bed intensive care unit. Multiplex-PCR method was used to determine the genes of OXA type beta-lactamases (blaOXA) and clonal relations between strains were investigated by pulsed-field gel electrophoresis (PFGE) method. All of the isolates were found to be carbapenem resistant and had the blaOXA-51-like and blaOXA-23-like gene. Also, all of isolates were seen to be 100 % related by PFGE method. As a result, isolates of patients with ventilator-associated pneumonia and isolates survived on ventilator of Intensive Care Unit were found to be 100% clonal associated with PFGE and had same MIC values for imipenem and meropenem. blaOXA-23 and blaOXA-51 genes has been determined all of the isolates. It can be accepted a short-term and small outbreak.

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1. Introduction

A. baumannii, have been increasingly reported as significant microorganisms involved in various nosocomial infections and several hospital outbreaks especially in intensive care units (ICU) (Bergogne-Bérézin and Towner, 1996). Because of its ability to rapidly acquire antimicrobial resistance and its propensity to persist in the environment, *A. baumannii* is difficult to control in the hospital setting (Kohlenberg et al., 2009). Health-associated *A. baumannii* infections

are difficult to treat due to the presence of multidrug-resistant (MDR) organisms, which includes resistance to β -lactams, aminoglycosides, fluoroquinolones and more recently, carbapenems (Moniri et al., 2010).

Carbapenem resistance often is consisted of OXA type carbapenemase. OXA-type enzymes are collected in four subgroups (OXA-51, OXA-58, OXA-23 and OXA-24). The beta-lactamases (blaOXA) genes encoding enzymes are determined by PCR method using specific primers. While the blaOXA-51 is

intrinsic to *A. baumannii*, blaOXA-58, blaOXA-23 and blaOXA-24 may be part of different mobile elements (Naas et al., 2006). PCR products are confirmed by applying the sequence analysis. Also, Multiplex-PCR methods have been used for the determination of these genes rapidly.

Molecular epidemiology of nosocomial *A. baumannii* infections is essential to develop effective strategies to control their spread (Nasr and Attalah, 2012). The pulsed-field gel electrophoresis (PFGE) has shown to be suitable for the investigation of hospital outbreaks. PFGE has been validated as a useful epidemiologic tool to study *A. baumannii* outbreak and is considered as the gold standard of epidemiological typing (Sabat et al., 2013).

Antimicrobial susceptibility test

Antibiotic susceptibility was determined by the Kirby-Bauer disc diffusion method in accordance with EUCAST guidelines. The antibiotics tested were amikacin, gentamicin, ampicillin/sulbactam, piperacillin/tazobactam, ceftazidim, cefepime, imipenem, meropenem, ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxazole, colistin and tetracycline. Also, minimal inhibitory concentration values (MIC) of imipenem and meropenem were determined by agar dilution method ranged from 4 to 256 µg/ml according to EUCAST 2014 guideline. The phenotypic detection of carbapenamases was done by the modified Hodge Test. *E.coli* ATCC 25922 was used as control strain.

Table 1. Antibiotic resistance profile of patients and environmental *A. baumannii* isolates

	Patient 1 Tracheal aspirate	Patient 2 Tracheal aspirate	Patient 3 Tracheal aspirate	Patient 1 Out of ventilator hoses	Patient 1 Inside of ventilator hoses	Patient 1 Ventilator Surface	Patient 1 Ventilator Keyboard
Amikacin	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Ciprofloxacin	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Tetracycline	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Sulbactam/Ampicillin	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Trimethoprim Sulfamethoxazole	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Ceftazidime	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Gentamicin	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Levofloxacin	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Imipenem	Resistant MIC:16 µg/ml	Resistant MIC:16 µg/ml	Resistant MIC:16 µg/ml	Resistant MIC:16 µg/ml	Resistant MIC:16 µg/ml	Resistant MIC:16 µg/ml	Resistant MIC:16 µg/ml
Meropenem	Resistant MIC:32 µg/ml	Resistant MIC:32 µg/ml	Resistant MIC:32 µg/ml	Resistant MIC:32 µg/ml	Resistant MIC:32 µg/ml	Resistant MIC:32 µg/ml	Resistant MIC:32 µg/ml
Piperacillin/Tazobactam	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Cefepime	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Colistin	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive

In this study, we aimed to determine carbapenem resistance and clonal relationships of *A. baumannii* isolated from patients and environmental samples by phenotypic and genotypic methods in 10-bed ICU.

2. Materials and methods

Bacterial isolates

In March-2014, three tracheal aspirates and 30 environmental samples were collected from a 10-bed ICU admitted to our department with suspected outbreak in Adana Kadirli State Hospital. Patient cabinets, patient tables, hospital beds, ventilator surface, ventilator hoses inside-out, monitor surfaces were taken with swap and incubated Brain-Heart Infusion Broth (Merck, Germany) overnight at 37°C. Incubated samples were inoculated Blood and Endo agar (Merck, Germany). Gram-negative, oxidase-negative coccobacillus was identified by the commercial identification kit which BBL Crystal System (Becton Dickinson Microbiology Systems, USA).

Detection of carbapenem resistance genes

Multiplex PCR was done for the detection of the four families of OXA-type carbapenamases found in *A. baumannii* (Woodford et al., 2006). The PCR conditions were as follows: Initial denaturation at 94°C for 5 min, 33 cycles of 94°C for 25 s, 53°C for 40 s and 72°C for 50 s, followed by an elongation step at 72°C for 6 min. The PCR products were visualized by agarose gel electrophoresis (blaOXA-23-like: 501 bp, blaOXA-51-like: 353 bp, blaOXA-24-like: 246 bp and blaOXA-58-like: 599 bp). After that, products were purified by using the PCR DNA purification kit (QIA Quick Gel Extraction Kit; Qiagen, Valencia, CA, USA) and subjected to automated DNA sequencing (ABI 310, Genetic Analyser; Applied Biosystems, USA). Analysis results were performed with the BLAST program (<http://www.ncbi.nlm.nih.gov>).

PCR was done for the detection of the ISAbal segment (548 bp) located in the upstream of bla_{oxa} genes (Turton et al., 2006a).

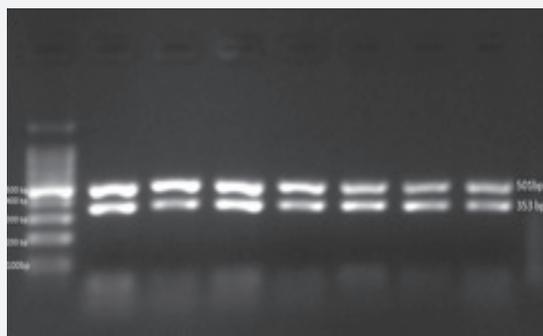


Fig. 1. PCR products obtained using bla_{OXA-51}, bla_{OXA-58}, bla_{OXA-24} and bla_{OXA-23} primers in patients and environmental *A.baumannii* isolates

Pulsed field gel electrophoresis

PFGE method detected total genome polymorphism is accepted as a “gold standard” for genotyping (Ertürk et al., 2014). Macrorestriction analysis of chromosomal DNA with ApaI (New England Biolabs, Boston, Mass.) was done by PFGE method (Durmaz et al., 2009). PFGE was run in a CHEF-DR II apparatus (Bio-Rad, USA), with pulses ranging from 5 to 30s at a voltage of 6 V/cm at 12°C for 20 h. Products were detected after staining with ethidium bromide (50 µg/ml) and photographed. Gel images were exported to Gelcompar II software (version 3.0; Applied Maths, Sint-Martens-Latem, Belgium) for analysis. Comparisons were made by using the band-based Dice coefficient. Dendrograms were generated by using the unweighted pair group method by arithmetic averaging method with 1% position tolerance. Isolates were considered to be genetically related if the Dice coefficient correlation was 80% or greater.

3. Results

Strain identification and characterization

A total of seven carbapenem-resistant isolates were studied. The isolates were cultured from three tracheal aspirate and four environmental samples (ventilator surface, inside and out of ventilator hoses, ventilator keys of one patient). All were identified as *A.baumannii* by the BBL Crystal System (Becton Dickinson Microbiology Systems, USA).

Antimicrobial susceptibility test

The MICs of all antimicrobial agents tested exceeded the EUCAST resistance breakpoints. Imipenem ve meropenem MIC value of all isolates was determined 16 and 32 µg/ml, respectively, by the agar dilution method according to EUCAST guidelines (Table 1). Presence of carbapenamases was corrected by the modified Hodge Test.

Detection of β-lactamase genes and ISAbal

PCR products of the appropriate size were obtained

from seven isolates using primers for blaOXA-23-like (501 bp), blaOXA-51-like (353 bp), but no amplicons were obtained with primers targeting blaOXA-24-like or blaOXA-58-like genes. DNA sequencing was applied to genes encoding the OXA-23 and OXA-51 carbapenamase. Also, ISAbal element was found in seven *A.baumannii* isolates.

Determination of the clonal relationship by PFGE

The strains of samples from clinical patients and environmental samples which were isolated from ICU, showed similarity of 100% in PFGE patterns (Fig. 2, Fig. 3).

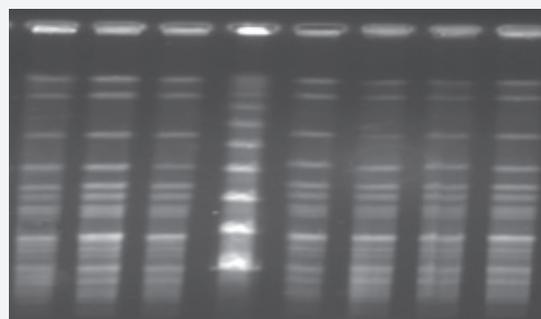


Fig. 2. PFGE profile of patients and environmental *A.baumannii* isolates

4. Discussion

In recent years, the carbapenem-resistant *A. baumannii* isolates has become one of the most important agents, particularly ventilator-associated pneumonia (VAP) cases, in intensive care units (Nhu et al., 2014). Also, some studies showed that the same strain has been responsible for infection and contamination in ICU (Ertürk et al., 2014).

Dice (Opt 1.00%) (Thr 1.5%-1.5%) (H+0.0% S+0.0%) (D 0.0%-100.0%)
A.baumannii

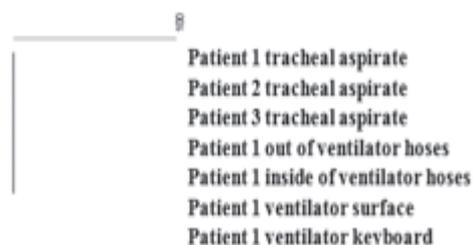


Fig. 3. The dendrogram of patients and environmental *A.baumannii* isolates

Carbapenem is often used as “last-line agents” or “antibiotics of last resort” when patients with infections become gravely ill or are suspected of harboring resistant bacteria. Unfortunately, the recent emergence of multidrug-resistant (MDR) pathogens seriously threatens this class of lifesaving drugs. Several recent studies clearly show that resistance to carbapenems is increasing throughout the world (Papp-Wallace et al., 2011). The various studies were performed for carbapenem resistance rate of *A. baumannii*. A study conducted in our region shows that imipenem resistance rate of *A. baumannii* was 15.1% (Taşova et al., 1999). In Ankara, imipenem resistance of *Acinetobacter* species was found to be 53.6% (Arıkan, 2003). In 2010, imipenem and meropenem resistance was determined as 49% and 63% respectively (Balıcı et al., 2010). In the 2011 report of national hospital infections surveillance network, carbapenem resistance have been reported as 74% *A. baumannii* strains on hospital infection (Gözütok et al., 2013). Carbapenem resistance rates shows a rising curve over the years. In our study, all of the *A. baumannii* isolates were resistant to imipenem and meropenem.

Carbapenem resistance mechanisms of *A. baumannii* are hydrolysis of beta-lactam antibiotics by beta-lactamase enzymes, changes on outer membrane proteins and penicillin-binding proteins and efflux pump (Peleg et al., 2008). The most common mechanism of resistance are the OXA-enzymes (Queenan and Bush, 2007).

OXA-type beta-lactamases continue to spread rapidly moving to dangerous levels carbapenem resistance among *A. baumannii* strains. OXA-type carbapenemases show a global distribution. The movement of blaOXA genes are performed by the presence of the insertion sequences and transposons in some cases, and therefore, they have the potential to spread very rapidly. OXA-type carbapenemases are responsible for a significant rate of the carbapenem resistance from all over the world. Especially, OXA-23 and OXA-51 are widely all over the world. In our country, the carbapenemases epidemiology of *Acinetobacter* studies have been performed and OXA-23 and OXA-58 outbreaks have been reported (Arslan, 2014).

In our region, in *A. baumannii* strains isolated a burn unit, blaOXA-24 and blaOXA-51 have been identified

(Gökmen et al., 2012). In addition to, the OXA-24/40 has been reported in our country (Sarı et al., 2013).

In various studies, the insertion sequence ISAbal has been determined to located upstream of the blaOXA-23, blaOXA-51, blaOXA-58 carbapenemase genes and cephalosporinase blaampC genes in many *A. baumannii* isolates and increased expression of these genes (Heritier et al., 2006; Poirel and Nordmann, 2006; Turton et al., 2006). In our study, isolates containing blaOXA-23 and blaOXA-51 have carried ISAbal segment. Consequently, they were resistant to imipenem and meropenem.

The mechanical ventilation is one of major risk factor in outbreaks of carbapenem resistant *A. baumannii* (Karabay et al., 2012). ICU mortality rate of VAP ranged from 45.6% to 60.9% and has been found to be as high as 84.3% when VAP was caused by Extreme Drug Resistant *A. baumannii* (Inchai et al., 2014).

Our study showed that three ventilator-associated pneumoniae (VAP) cases were caused by carbapenem resistant *A. baumannii* survived on ventilator of ICU. Isolates were found to be 100% clonal associated with PFGE and had same MIC values for imipenem and meropenem. blaOXA-23 and blaOXA-51 genes has been determined all of the isolates. Similarly, in patients with VAP of ICU, one pulsotype (similarity coefficient 95%) has contained 21 out of 23 isolates harbouring both the blaOXA-51 and blaOXA-23 genes (Nhu et al., 2014).

The limitation of our study is that, the number of isolates inadequate to say the outbreak. However, this outbreak occurred in two week and ten-bed ICU. In addition, the detection of the infection source and clonal relationship of the isolates was evidence that a small-scale outbreak.

As a result, resistance profile and DNA band profile were same in seven strains. Thus, it was considered that there was a small and short-term outbreak in our study.

After this study, effective disinfection applications were applied to ICU and environmental samples were taken. *A. baumannii* could not growth on culture. It showed that molecular epidemiology studies and effective disinfection process ended outbreak of carbapenem resistant *A. baumannii* strains in ICU.

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