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Original Article

β-lactamase genes in carbapenem resistance *Acinetobacter baumannii* isolates from a Turkish university hospital

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Abstract

Introduction: The spread of *Acinetobacter baumannii*, resistant to most of the available antimicrobial agents, is a serious health problem. The high rate of carbapenem resistance among *Acinetobacter baumannii* isolates is considered as a threat to public health. In this study, we aimed to determine the antibiotic resistance and related genes in carbapenem-resistant *Acinetobacter baumannii* isolates.

Methodology: Ninety six isolates of *A. baumannii* were included. Antimicrobial susceptibility was performed by Phoenix Automated System and disk diffusion method. Carbapenem resistane was characterized by scrneeing of resistance genes such as *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M1-2}$, *bla*$_{PER}$, *bla*$_{VEB}$, *bla*$_{KPC}$, *bla*$_{GES}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$ and *bla*$_{OXA23-24-51-58}$ using multiplex polymerase chain reaction.

Results: Resistance for the levofloxacin, gentamicin, amikacin, and tigecycline were determined as 96.9%, 93.7%, 72.9% and 45.8% respectively. Colistin was the only susceptible antibiotic against all clinical isolates. All isolates were defined as multidrug resistance and of these, 31.2% were extensively drug-resistant (sensitive only to colistin). *Bla*$_{OXA-51}$ and *bla*$_{OXA-23}$ genes were detected in 100% strains while *bla*$_{TEM}$ was found in only 2% strains. There was no amplification for the *bla*$_{SHV}$, *bla*$_{CTX-M1-2}$, *bla*$_{PER}$, *bla*$_{VEB}$, *bla*$_{KPC}$, *bla*$_{GES}$ *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$ and *bla*$_{OXA24-58}$ genes.

Conclusions: The high frequency of *bla*$_{OXA-23}$ and low frequency of *bla*$_{TEM}$ gene was observed that indicate prevalence of a variety of *A. baumannii* strains. The rates of resistance genes vary from region to region. Studies are required for the prevention and control of *A. baumannii* infection and to formulate the strategies of antibiotic usage.

Key words: *Acinetobacter baumannii;* multi drug resistance; resistance genes; *bla*$_{OXA-23}$.

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Introduction

Acinetobacter baumannii (*A. baumannii*) is the opportunistic pathogen that causes nosocomial infection such as urinary tract infection, wound infection, pneumonia and sepsis [1]. *A. baumannii* is resistant to stressful environmental conditions. In addition, presence of multiple resistance mechanisms and its ability to gain new resistance characteristics against available antibiotics help to cause hospital-acquired infection more easily. *A. baumannii* with a variety of resistance mechanisms causes difficulties in treatment by aminoglycosides, cephalosporins, carbapenems and ciprofloxacin. Its involvment in clinical infections is increased day by day [2].

Prevelance of β-lactamase enzymes has reduced the susceptibility to carbapenems. Class D β-lactamases (OXA-type) and Ambler class B metallo-β-lactamase (MBL) provide the most significant contribution to the carbapenem resistance. Another resistance mechanism is due to presence of clavulanic acid-inhibited extended-spectrum β-lactamases (ESBLs) that comprise of PER_1, PER_2, VEB_1, MBL_s, VIM_{1-4}, VIM_2 and $IMP_{1-2-4-5-6}$ type genes [3,4].

It is of great concern that if multidrug resistant (MDR) *A. baumannii* infections are not controlled, they may cause epidemics in the hospital and may spread intercities and even cross-countries [1,2]. Therefore, the investigation for the prevalence of MDR *A. Baumannii* is an important step in combating this infection. The aim of this study was to characterize the susceptibility profiles and genetic mechanisms of resistance of clinical strains of *A.baumannii* in Turkey.

Methodology

This study was approved by the Scientific and Ethical Committee of Tokat Gaziosmanpasa University Clinical Research Ethics Committee (Tokat, Turkey), (16-KAEK- 013/19.01.2015).

Bacterial strains and antimicrobial susceptibility testing

Clinical isolates of *A. baumannii* (n = 96) were collected from several units of Duzce University Hospital in Turkey between January 2014 and July 2015. The isolates were identified by Phoenix Automated System (BD Diagnostic Systems, Sparks, MD, USA) according to the manufacturer's instructions. Antimicrobial susceptibility testing was performed by Phoenix Automated System and disc diffusion method. The results were interpreted according to the guidelines by Clinical and Laboratory Standards Institute [5].

Multiplex PCR for detection of bla_{OXA} genes

Genomic DNA was obtained from bacterial culture grown overnight in Luria Broth [6] and used in all PCR amplification. Multiplex PCR was used for detecting $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-40-like}}$ and $bla_{\text{OXA-58-like}}$ genes. Primers used for the detection of resistance genes are shown in Table 1. PCRs were performed in a final volume of 50 µL that included 5 µL of genomic DNA, 20 pM of each primer, 10 µL reaction buffer (Promega), 3 µL 25 mM $MgCl_2$, 200 µM of each dNTPs and 1.5 U of *Taq* Polymerase (Promega, Madison,WI, USA). PCR amplification conditions were as follows: initial denaturation at 94°C for 3 minutes followed by 30 cycles of 25 seconds at 94°C, 40 seconds at 52°C and 50 seconds at 72°C with a final extension 5 minutes at 72°C.

Multiplex PCR for detection $bla_{CTX\text{-}M1\text{-}2}$ genes

Multiplex PCR was used for detecting $bla_{\text{CTX-M1}}$ and $bla_{\text{CTX-M2}}$ group β-lactamase genes. Primers used for detection $bla_{\text{CTX-M}}$ genes are shown in Table 1. PCRs were performed in a final volume of 50 µL and included 5 µL of genomic DNA, 20 pM of each primer, 10 µL reaction buffer (Promega, Madison, WI, USA), 3 mL 25 mM $MgCl_2$, 200 mM of each dNTPs and 1.5 U of Taq Polymerase (Promega, Madison, WI, USA). PCR

Table 1. Primers used in the amplification of selected genes.

Primer	5′-3′ Sequence	Amplicon Size (bp)	Tm (°C)	Reference
GES	F:ATGCGCTTCATTCACGCAC R:CTATTTGTCCGTGCTCAGGA	863	56	[29]
VEB	F:ATTTCCCGATGCAAAGCGT R:TTATTCCGGAAGTCCCTGT	542	55	
PER-2	F:ATGAATGTCATCACAAAAT R:TCAATCCGGACTCACT	927	50	[30]
IMP	F:CATGGTTTGGTGGTTCTTGT R:ATAATTTGGCGGACTTTGGC	488	56	[31]
VIM	F:ATTGGTCTATTTGACCGCGTC R:TGCTACTCAACGACTGAGCG	780	58	
NDM	F:TGGAATTGCCCAATATTATGC R:TCAGCGCAGCTTGTCGGCCATGC	813	54	[18]
CTX-M-1group	F:GCGTGATACCACTTCACCTC R:TGAAGTAAGTGACCAGAATC	260	50	[32]
CTX-M-2 group	F:TGATACCACCACGCCGCTC R:TATTGCATCAGAAACCGTGGG	341	50	
TEM	F:AGTATTCAACATTTYCGTGT R:TAATCAGTGAGGCACCTATCTC	860	49	[33]
SHV	F:ATGCGTTATATTCGCCTGTG R:TTAGCGTTGCCAGTGCTC	843	55	[34]
KPC	A: CGTTCTTGTCTCTCATGGCC B: CCTCGCTGTGCTTGTCATCC	796	52	[35]
OXA-51	F: TAATGCTTTGATCGGCCTTG R:TGGATTGCACTTCATCTTGG	353	52	[36]
OXA-23	F:GATCGGATTGGAGAACCAGA R: ATTTCTGACCGCATTTCCAT	501		
OXA-40	F:GGTTAGTTGGCCCCCTTAAA R:AGTTGAGCGAAAAGGGGATT	246		
OXA-58	F:AAGTATTGGGGCTTGTGCTG R:CCCCTCTGCGCTCTACATAC	599		

amplification condition was as follows: initial denaturation at 95°C for 2 minutes followed by 30 cycles of 1 minute at 95°C, 1 minute at 55°C and 1 minute at 72°C, with a final extension of 10 minutes at 72°C.

PCR amplifications of the ESBLs and MBLs genes

Simplex PCR was used to amplify ESBL and MBL genes and the primers listed in Table 1 were used. PCRs were performed in a final volume of 50 µL and included 5 µL of genomic DNA, 20 pM of each primer, 10 µL reaction buffer (Promega, Madison, WI, USA), 3 µL 25 mM $MgCl_2$, 200 of µL dNTPs and 1.5 U Go *Taq* Flexi Polymerase (Promega, Madison,WI, USA) in a final volume of 50 µL. PCR amplification conditions was performed according to references listed in Table 1. All PCR results were analyzed on 1% agarose containing 0.5 µg/mL ethidium bromide, and subsequently visualized under UV light.

Results

A total of 96 clinical isolates of *A. baumannii* were collected from Duzce University hospital in Turkey over a period of 18 months. All patients were hospitalized into several units such as sixty one patients (63.5%) in intensive care unit, 24 patients (25%) in the internal units (cardiology, pulmonology, etc.) and 14 patients (14.6%) in surgery clinics. Most of the isolates were obtained from respiratory specimens (tracheal aspirates 54.2%, sputum 12.5%, bronchoalveolar lavage 5.2%) followed by wound (8.3%), urine (8.3%), blood (8.3%) and cerebrospinal fluid (3.1%). All strains were identified as *A. baumannii* by Phoenix Automated System and $bla_{\text{OXA-51}}$ PCR for specify the *A. baumannii* species.

All of the *A. baumannii* strains were resistant to imipenem, meropenem, ampicillin-sulbactam, ceftazidime, cefepime, piperacillin-tazobactam and ciprofloxacin. Resistance for the levofloxacin, gentamicin, amikacin and tigecycline were 96.9%, 93.7%, 72.92% and 45.8% respectively. However colistin resistance was not observed in any strain. All strains were defined as MDR based on resistance to more than two antibiotic groups. The resistance rates of *A. baumannii* against antibiotics are shown in Table 2.

The molecular analysis revealed that all strains (100%) carried the $bla_{\text{OXA-23-like}}$ gene and $bla_{\text{OXA-51-like}}$. Two strain (2%) were positive for bla_{TEM} and there were no positive results for the bla_{SHV}, $bla_{\text{CTX-M1-2}}$, bla_{PER}, bla_{VEB}, bla_{KPC}, bla_{GES} bla_{NDM}, bla_{VIM}, bla_{IMP} and $bla_{\text{OXA24-58}}$ genes.

Table 2. Resistance rates of *A. baumannii* isolates.

Antibiotic	Resistance Rate (%)
Ampicillin-sulbactam	100
Ceftazidime	100
Cefepime	100
Piperacillin-tazobactam	100
Ciprofloxacin	100
Levofloxacin	96.9
Gentamicin	93.7
Amikacin	72.9
Tigecycline	45.8
Imipenem	100
Meropenem	100
Colistin	0

Discussion

A. baumannii often develops resistance against carbapenems. Since carbapenems are broad-spectrum antimicrobial and hydrolyze β-lactamases, they play a crucial role in the treatment of nosocomial infections caused by Gram-negative bacteria [7]. The high genome plasticity of *A. baumannii* contributes to its virulence and high adaptation on inanimate surfaces particularly in hospital environment. This reduces the response to long term treatment and generates the multidrug resistant (MDR) strains that show resistance to last three groups of antibiotics [8]. MDR strains are often resistant to carbapenems [9,10]. In this study, all strains were defined as MDR and of these, 31.2% were extensively drug-resistant (sensitive only to colistin).

High rates of resistance against cephalosporins are seen all over the world [11-15]. The most frequently used treatment regime for *A. baumannii* infections include carbapenems and aminoglycosides. Carbapenems produce synergistic bactericidal activity in combination with aminoglycosides; therefore, carbapenems are often used in combination therapy with aminoglycosides [11]. Although several studies have reported different rates of resistance for aminoglycoside and quinolone, their resistance rates are still high in the world [11,13,14]. According to the annual report of the European Antimicrobial Resistance Surveillance Network, MDR *A. baunannii* is very common in Europe and combined resistance to fluoroquinolones, aminoglycosides and carbapenems are the most frequently reported resistance phenotype and accounted for almost half of the reported isolates in 2015 [16]. In this study, the resistance rate of *A. baumannii* strains to ciprofloxacin, levofloxacin,

gentamicine, amikacin were 100%, 96.9%, 93.7% and 72.9% respectively. In our study, the rates of resistance to the indicated antibiotics were consistent with the literature.

Although, yearly tigecycline resistance rates ranged from 0 to 42%, tigecycline and colistin are used in combination or alone as the last option for the treatment of MDR *A. baumannii* strains [10-12,14,15]. In our study, we found 45.8% resistance against tigecycline. Given that the history of tigecycline is not very old, rapidly increased resistance propose that MDR *A. baumannii* strains may not be cured by tigecycline in near future. This situation poses a serious threat to infections whose treatment options are very limited.

Resistance rates for colistin around the world are between 0 and 21.3% [10-12,14,15]. However, Ciftci *et al.* [17] and Cicek *et al.* [18] did not determine resistance in Turkey. Mengeloglu *et al.* [19], Ergin *et al*. [20] and Keskin *et al.* [21] identified 3.9%, 2%, 6% resistance respectively. Colistine resistance has not been detected in this study. The low resistance rates to colistin is seen as the best option in the treatment of MDR *A. baumannii*.

$OXA_{23\text{-}24\text{-}51\text{-}58\text{-}like}$ Class D β-lactamases produced by *A. baumannii* are investigated under 4 phylogenetic groups. The $bla_{\text{OXA-51-like}}$ genes naturally present in the genome of *A. baumannii and* were found as an intrinsic gene in all *A. baumannii* strains in this study. $Bla_{\text{OXA-23-like}}$ is the most common source which causes plasmid or chromosomal transferable carbapenem resistance. $Bla_{\text{OXA-23}}$ carraige has been reported all over the world for instance; China 46.31% [13], USA 58.3% [22], Kuwait 85% [12], Poland 27.9% [11]. The $Bla_{\text{OXA-23}}$ positive *A. baumannii* strains have been involved in nosocomial outbreaks. It was studied that a horizontal gene transfer within various isolates of the species constitutes a primary factor in the continued increase of carbapenem resistance over the years [23]. In Turkey, the prevalence rate of $bla_{\text{OXA-23}}$ were between 31 and 91.5% [18,20,21]. In this study, all strain had the $bla_{\text{OXA-23}}$ genes as $bla_{\text{OXA-51}}$.

In current study, any strain that contain $bla_{\text{OXA58/40-like}}$ are not detected. According to the centers, variation of the prevalence of $bla_{\text{OXA58/40-like}}$ has been drawn attention. Based on literature, strains which have this variant, are mostly reported from Asia and Middle East countries. It suggests that $bla_{\text{OXA58/40-like}}$ are not very common in Turkey. It was confirmed that one strain had $bla_{\text{OXA40-like}}$ gene in clinical *A. baumannii* isolate [18].

Extended-Spectrum β-lactamases (ESBLs) are mostly transferred by plasmids and they are enzyme family comprised of bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M}}$ [24] and bla_{GES}, bla_{PER} [25]. In a research performed in Saudi Arabia, *A. baumannii* strains had bla_{TEM} 71%, $bla_{\text{CTX-M}}$ (81%) [26]. In Iran, it was recorded that $bla_{\text{CTX-M}}$ rate were 25% [27]. In another study from Iran in 2015, $bla_{\text{CTX-M}}$ were not found but bla_{TEM}, bla_{SHV} and bla_{VIM} were found in 20%, 58% and 30% strains respectively [28].

Carbapenemase genes from class A, bla_{KPC} and bla_{GES} types were detected in *A. baumannii* [28]. It was reported that the prevalence of bla_{GES} in America [22] and Kuwait [12] were 75% and 18% respectively. The prevalence of bla_{KPC} in *A. baumannii* is rarely obseved. In Turkey, according to Cicek *et al.* $bla_{\text{GES-like}}$ genes were detected in 24 strains (GES-11 in 16 strains, GES-22 in eight strains) [18] while Keskin *et al.* indicated that 21% bla_{PER} positive [21]. In this study bla_{TEM} was detected in 2% strains but bla_{SHV}, $bla_{\text{CTX-M1-2}}$, bla_{KPC}, bla_{PER}, bla_{VEB}, bla_{GES} genes were not detected.

Conclusions

In conclusion, MDR *A. baumannii* poses a significant threat to patients and healthcare systems. A number of β-lactamase coding genes have been identified in Mediterranean, Middle East countries, Asia and Europe. Even though $bla_{\text{OXA-23}}$ was present in all our isolates, it is noteworthy that frequency of bla_{TEM} was and other resitance genes were not detected low in our study. Our results suggest that the prevalence of resistance genes vary from region to region. Therefore, studies for genotypic fingerprinting of MDR *A. baumannii* should be encouraged.

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