

Molecular typing and investigation of carbapenemases in carbapenem resistant *Acinetobacter baumannii* isolates

Karbapenemlere dirençli *Acinetobacter baumannii* izolatlarında moleküler tiplendirme ve karbapenemazların araştırılması

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ABSTRACT

Objective: In this study, it was aimed to investigate the presence of carbapenemases in carbapenem-resistant *A. baumannii* strains and their chromosomal or plasmid origin.

Methods: Of the total 65 *A. baumannii* studied, 66% were isolated from patients in intensive care unit (ICU); most of strains were blood (37%) and lower respiratory tract samples (32%). VITEK2 automated system and disk diffusion tests were used to evaluate antibiotic susceptibilities. In addition, MIC values for carbapenems were determined using M.I.C. evaluator strips. Clonal relationship between the isolates was assessed by AP-PCR, using M13 and DAF4 primers. Carbapenemase genes were screened by multiplex-PCR, and presence of plasmid-borne carbapenemase was investigated.

Results: The The isolates were found to be susceptible to tobramycin, netilmicin, and colistin by 98.5%, 98.5% and 96.9%, respectively. The rates of resistance to other antibiotics were quite high, and resistance was found between 78.5% - 100%. The resistance rates were detected as 100% to ticarcillin, piperacillin, piperacillin+tazobactam, ceftazidime, ceftriaxone, sefepime, imipenem, meropenem, and

ÖZET

Amaç: Bu çalışmada karbapenem dirençli *A. baumannii*'lerde karbapenemazların varlığı ve bunların kromozomal veya plazmid kaynaklı olduğunun araştırılması amaçlanmıştır.

Yöntemler: Çalışılan 65 *A. baumannii*'nin %66'sı yoğun bakım ünitesi (YBÜ)'ndeki hastalardan, en çok kandan (%37) ve alt solunum yolu örneklerinden (%32) izole edilmiştir. Antibiyotik duyarlılıklarını değerlendirmede VITEK 2 otomatize sistemi ve disk difüzyon testi kullanılmıştır. Ayrıca, karbapenemler için MİK değerleri, MİK değerlendirici stripler kullanılarak belirlenmiştir. İzolatlar arasındaki klonal ilişki M13 ve DAF4 primerleri kullanılarak AP-PZR ile değerlendirilmiştir. Karbapenemaz genleri multipleks-PZR ile taranmış ve plazmit kaynaklı karbapenemaz araştırılmıştır.

Bulgular: İzolatlar tobramisin, netilmisin ve kolistine sırasıyla %98.5, %98.5 ve %96.9 oranında duyarlı bulunmuştur. Diğer antibiyotiklere direnç oranları oldukça yüksek olup %78.5-100.0 arasında saptanmıştır. Tikarsilin, piperasilin, piperasilin+tazobaktam, seftazidim, seftriakson, sefepim, imipenem, meropenem ve siprofloksasin için %100.0; tikarsilin+klavulanik asit, gentamisin,

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Geliş Tarihi / Received : 09.11.2015
Kabul Tarihi / Accepted : 27.05.2016

DOI ID : 10.5505/TurkHijyen.2016.91489

Özbeç N, Tatman-Ötkun M. Molecular typing and investigation of carbapenemases in carbapenem resistant *Acinetobacter baumannii* isolates. Turk Hij Den Biyol Derg, 2016; 73(4): 345-354

ciprofloxacin, 98.5% to ticarcillin+clavulanic acid, gentamicin, levofloxacin, tetracycline, doxycycline, and trimethoprim-sulfamethoxazole, 93.8% to minocycline, 92.3% to amikacin, and 78.5% to ampicillin+sulbactam. In the study, two different patterns were observed by AP-PCR. Chromosomal OXA-23 and OXA-51 carbapenemase enzyme genes positively were found in all isolates. Plasmids were isolated neither of isolates.

Conclusion: Isolates were detected more frequently from hemocultures in ICU. Isolates showed clonal similarity in AP-PCR where M13 and DAF4 primers were used. The presence of the single *Acinetobacter* clone in our hospital suggests that they all originated from the same source. For this reason, it is concluded that universal infection control measures should be taken in consideration to prevent cross-infection by carbapenem resistant isolates.

Key Words: *Acinetobacter baumannii*, oxacillinase, metallo-beta-lactamase, PCR

levofloksasin, tetrasiklin, doksisisiklin ve trimethoprim+sulfametoksazol için %98.5; minosiklin için %93.8; amikasin için %92.3 ve ampisilin+sulbaktam için %78.5 oranında direnç saptanmıştır. AP-PZR ile araştırmada iki farklı patern gözlenmiştir. Bütün izolatlarda kromozomal kaynaklı OXA-23 ve OXA-51 karbapenemaz enzim geni pozitif bulunmuştur. İzolatların hiçbirinde plazmit izole edilmemiştir.

Sonuç: İzolatlar en sık YBÜ'de yatmakta olan hastaların kan örneklerinde saptanmıştır. İzolatlar M13 ve DAF4 primerlerin kullanıldığı AP-PZR'de klonal benzerlik göstermiştir. Hastanemizde tek *Acinetobacter* kolonu bulunması, hepsinin aynı kaynaktan köken aldığını düşündürmektedir. Bu nedenle karbapenem dirençli izolatların çapraz enfeksiyonlarını önlemek için evrensel enfeksiyon kontrol önlemlerine dikkat etmek gerektiği sonucuna varılmıştır.

Anahtar Kelimeler: *Acinetobacter baumannii*, oksasilinaz, metallo-beta-laktamaz, PZR.

INTRODUCTION

Acinetobacter baumannii is one of the important causes of nosocomial infections. It is widely present in nature, soil and water, and an opportunistic pathogen that can cause nosocomial cross-transmission between patients, leading to colonization or serious infections (1).

As a result of misuse of broad-spectrum antibiotics, multiresistant *Acinetobacter* species have emerged. The most frequently isolated and antibiotic-resistant species in nosocomial infections is *A. baumannii* (1).

Acinetobacter species are gram-negative coccobacilli, they are immotile and do not have cytochrome oxidase activity. Currently there are at least 25 genomospecies described within the genus *Acinetobacter*. Because of problems in separating the saccharolytic strains belonging to DNA groups 1, 2, 3 and 13 using phenotypic tests, some laboratories have chosen to report members of this group as “*Acinetobacter calcoaceticus* - *A. baumannii* complex”, or saccharolytic *Acinetobacter*. *A. baumannii* acidifies most OF (oxidative-fermentative)

carbohydrates; in particular, definitive identification is made by demonstrating the rapid production of acid from lactose or glucose. Furthermore *A. baumannii* can grow at 42 °C, but other *Acinetobacter* species cannot grow. (1).

Although *Acinetobacter* species are currently less sensitive to carbanepems, they are still among the most effective antibiotics. However, changes in penicillin-binding proteins, reduced outer membrane permeability, and production of chromosomal or plasmid-induced beta-lactamases (carbapenemases) cause resistance to carbanepems (2). The most prevalent mechanism of carbapenem resistance in *A. baumannii* is the production of carbapenem-hydrolyzing β -lactamases, i.e., metallo- β -lactamases (ambler class B) and more usually oxacillinases (ambler class D). Carbapenem-hydrolyzing class D β -lactamases (CHDLs) are currently classified into six subgroups; acquired OXA-23-like, OXA-24/40-like, OXA-58-like, OXA-143-like and OXA-235-like, and the intrinsic OXA-51-like β -lactamases (3).

A. baumannii produces class D oxacillinase that belongs to the chromosomal OXA-51-like enzyme group showing weak carbapenemase activity. However, OXA-51-like enzyme is secreted in high quantities in the presence of ISAb1 gene, causing high-level carbapenem resistance (4).

The metallo-beta-lactamases (MBL), the other carbapenemase group, are named because of the metal ions they carry in their active regions. The described MBL groups include IMP, VIM, SIM, and SPM. *A. baumannii* carries various IMP variants (IMP-1, IMP-2, IMP-4, IMP-5, IMP-6, IMP-8, and IMP-11), but rarely carries the other enzymes (5).

The purpose of this study was to assess the presence of carbapenemases of chromosomal and plasmid origin in carbapenem-resistant *A. baumannii* strains isolated in our hospital and also to investigate the clonal relationship between isolates.

MATERIALS AND METHODS

The study included carbapenem- and multiple-resistant *A. baumannii* strains isolated in patients hospitalized in the School of Medicine Hospital between December 2009 and November 2011. In case of isolation of more than one strain of *A. baumannii* from the same patient, only one strain was included in the study if the antibiotic susceptibility patterns of all isolates were the same.

Strains isolated and identified as *A. calcoaceticus*-*A. baumannii* complex by a VITEK2 automated system (bioMérieux, France) were stored in liquid medium with beads (Pro-Lab, Canada) at -80 °C until the time of use. The stored strains and *A. baumannii* ATCC 19606 strain were subcultured twice on eosin-methylene blue agar (Salubris, Turkey) at 37 °C for 24 hours. Strains were confirmed as *A. baumannii*, with acid producing from glucose in OF medium and growth ability at 42 °C.

Disk diffusion tests with standard antibiotic disks (Oxoid, United Kingdom) were used for susceptibility tests except for carbapenems and colistin, in addition to VITEK2 automated system. M.I.C. evaluator strips (Oxoid, United Kingdom) were used for carbapenem susceptibility. The susceptibility to antibiotics other than netilmicin was evaluated according to the CLSI criteria (6). The susceptibility to netilmicin was evaluated according to the EUCAST (European Committee on Antimicrobial Susceptibility Testing) criteria (7). *Pseudomonas aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 served as the quality control strains (6).

The genomic DNA of *A. baumannii* isolates and *A. baumannii* ATCC 19606 strain was extracted by using GeneJET Genomic DNA Purification Kit (Fermentas, United Kingdom) according to the manufacturer's instructions.

In assessing the clonal relationship between the isolates, arbitrarily primed polymerase chain reaction (AP-PCR) was performed using the M13 and DAF4

primers (8). The PCR mixture was prepared to have a final volume of 25 µl. The final mixture contained 1X buffer solution, 25 pM M13 primer (5'-GAGGGTGGC-GGTTCT-3'), 1.5 mM MgCl₂; 400 µM from each dATP, dGTP, dCTP, and dTTP; 1 U Taq DNA polymerase, and sterile bi-distilled water; and finally 2 µl DNA was added to this mixture. The same mixture was prepared using 25pM of DAF4 primer (5'-CGGCAGC-GCC-3'). The amplification program for M13 primer in the thermal cycler (Eppendorf, Germany) was as follows: at 94°C for 2 minutes, 35 cycles at 94°C for 20 seconds, at 50°C for 1 minute, at 72°C for 20 seconds, and lastly at 72°C for 5 minutes. The amplification program for DAF4 primer was as follows: at 94°C for 2 minutes, 45 cycles at 94°C for 40 seconds, and at 45°C for 40 seconds, at 72°C for 40 seconds, and lastly at 72°C for 5 minutes. The obtained PCR product was analyzed with 1% agarose gel electrophoresis by applying 100 V for 45 minutes (Wealtec, USA), then studied under UV transilluminator and photographed. Different band patterns of the isolates were evaluated. To determine the size and quantity, 80-10.000 bp DNA ladder (Fermentas, United Kingdom) and 1 kb DNA ladder (Fermentas, United Kingdom) were used and *A. baumannii* ATCC 19606 was used as the positive control with sterile deionized water as the negative control.

To assess the presence of carbapenemase in the isolates, multiplex-PCR was carried with OXA-23, OXA-24, OXA-51 and OXA-58 as the first group and then with IMP, VIM, SIM and SPM as the second group, using the primer pairs shown in Table 1 (9, 10). The PCR mixture was prepared to have a final volume of 25 µl. The final mixture contained 1X buffer solution, 12.5 pM from each primer, 1.5 mM MgCl₂; 200 µM from each dATP, dGTP, dCTP, and dTTP; 1.5 U Taq DNA polymerase, and sterile bi-distilled water. Lastly, 3 µl DNA extract was added to the solution. The amplification program was as follows: at 94°C for 3 minutes, 35 cycles at 94°C for 45 seconds, at 57°C for 45 seconds, at 72°C for 1 minute, and lastly at 72°C for 5 minutes. The obtained PCR product was ana-

Table 1. Antimicrobial resistance rates of *A. baumannii* isolates (NEU Hospital 2010-2014).

OXA-23-likeF	5'-GAT CGG ATT GGA GAA CCA GA-3'
OXA-23-likeR	5'-ATT TCT GAC CGC ATT TCC AT-3'
OXA-24-likeF	5'-GGT TAG TTG GCC CCC TTA AA-3'
OXA-24-likeR	5'-AGT TGA GCG AAA AGG GGA TT-3'
OXA-51-likeF	5'-TAA TGC TTT GAT CGG CCT TG-3'
OXA-51-likeR	5'-TGG ATT GCA CTT CAT CTT GG-3'
OXA-58-likeF	5'-AAG TAT TGG GGC TTG TGC TG-3'
OXA-58-likeR	5'-CCC CTC TGC GCT CTA CAT AC-3'
IMP1-F	5'-CTA CCG CAG CAG AGT CTT TGC-3'
IMP1-R	5'-GAA CAA CCA GTT TTG CCT TAC C-3'
SPM-F	5'-CCT ACA ATC TAA CGG CGA CC-3'
SPM-R	5'-TCG CCG TGC CAG GTA TAA C-3'
VIM-F	5'-TCT ACA TGA CCG CGT CTG TC-3'
VIM-R	5'-TGT GCT TTG ACA ACG TTC GC-3'
SIM-F	5'-GTA CAA GGG ATT CGG CAT CG-3'
SIM-R	5'-TGG CCT GTT CCC ATG TGA G-3'

lyzed in 1% agarose gel electrophoresis by applying 100 V for 45 minutes (Wealtec, USA), then visualized under UV lamp and photographed. The presence of genes was assessed in the bands formed (10, 11). To determine the size and quantity, 80-10.000 bp DNA ladder and 1 kb DNA ladder were used. *A. baumannii* ATCC 19606 was used as the positive control with sterile deionized water as the negative control. Upstream presence of ISAb₁ was not studied.

In order to assess the presence of plasmid-borne carbapenemase, GeneJET Plasmid Miniprep Kit (Fermentas, United Kingdom) was used according to the manufacturer's instructions. To determine the plasmids, the obtained plasmid DNA was run in 1% agarose gel electrophoresis by applying 100 V for 45

minutes (Wealtec, USA), then studied under UV lamp and photographed, using 80-10.000 bp DNA ladder. As positive control *E. coli* strains isolated from patients were used with sterile deionized water as negative control.

RESULTS

The 65 clinical isolates of *A. baumannii* were isolated from 62 patients (33 male and 29 female). Different isolates were obtained from blood and respiratory secretion of one patient and from two cerebro-spinal fluid (CSF) specimens and blood of another patient. The age of the patients ranged from 4 to 91 with a mean age of 60.26 ± 18.20 years. Most of the isolates (43/65, 66.2%) were from the ICU, followed by other units ranging from 1.5 to 7.7%. The isolates were, in order of frequency, blood (24/65, 36.9%), endotracheal aspirate (16/65, 24.6%), wound (8/65, 12.3%), urine (8/65, 12.3%), sputum (5/65, 7.7%), CSF (2/65, 3.1%), and tissue (2/65, 3.1%) isolates.

The antibiotic susceptibility results of disk diffusion and VITEK2 automated system were identical. The isolates were most susceptible to tobramycin and netilmicin for 98.5% and to colistin for 96.9%. The isolates were highly resistant to other antibiotics tested. All of the isolates were found resistant to ticarcillin, piperacillin, piperacillin-tazobactam, ceftazidime, ceftriaxone, sefepime, imipenem, meropenem, and ciprofloxacin. Resistance rates to ticarcillin-clavulanic acid, gentamicin, levofloxacin, tetracycline, doxycycline, and trimethoprim-sulfamethoxazole were same and 98.5%. Resistance rates to minocycline, amikacin and to ampicillin-sulbactam were 93.8%, 92.3% and 78.5% respectively.

All isolates belonged to same clone except the 33rd isolate with M13 and DAF4 primers in AP-PCR test (Figures 1 and 2). The 33rd isolate was from a wound in a patient who had an operation for lumbar disc hernia in another city two months prior to

its isolation. This isolate was found to differ from other isolates by being susceptible to tetracycline, doxycycline, and minocycline; intermediately susceptible to levofloxacin, and resistant to tobramycin.

Three isolates from the same patient with different antibiotic susceptibility patterns were included in the study. This patient is a case who was hospitalized in the ICU with diagnosis of subarachnoid hemorrhage on December 3, 2010, was monitored by the Neurosurgery Clinic, and from whom *A. baumannii* had been isolated in the hemoculture on December 20, 2010 (35th isolate). This 35th isolate was the first isolate from this patient and its antibiotic susceptibility pattern was similar to those of other isolates from other patients and showed susceptibility to colistin. However, two isolates obtained from the CSF of the same patient on dates January 25 and February 17, 2011 (37th and 65th isolates, respectively) were found to be colistin-resistant. The AP-PCR study showed that all these three isolates were not different from other isolates.

According to the results of multiplex-PCR test to assess the presence of oxacillinase and MBL, all isolates had OXA-23 of size 501 bp and OXA-51 of size 353 bp (Figure 3), but no genes of OXA-24, OXA-58, IMP-1, SPM, VIM, and SIM were found.

In assessing plasmid-borne carbapenemases, no plasmids was obtained from the isolates.

DISCUSSION

Nosocomial infections (NIs), causing long hospitalization, increased morbidity and mortality, and economic loss due to extra treatment expenditures, are important. *Acinetobacter* species are increasingly isolated as agents of NIs, particularly of those in ICUs. However, the differentiation of *Acinetobacter* spp. needs molecular confirmation techniques (12). The presence of blaOXA-51 gene also continues to be an appropriate genetic marker for *A. baumannii* identification (13).

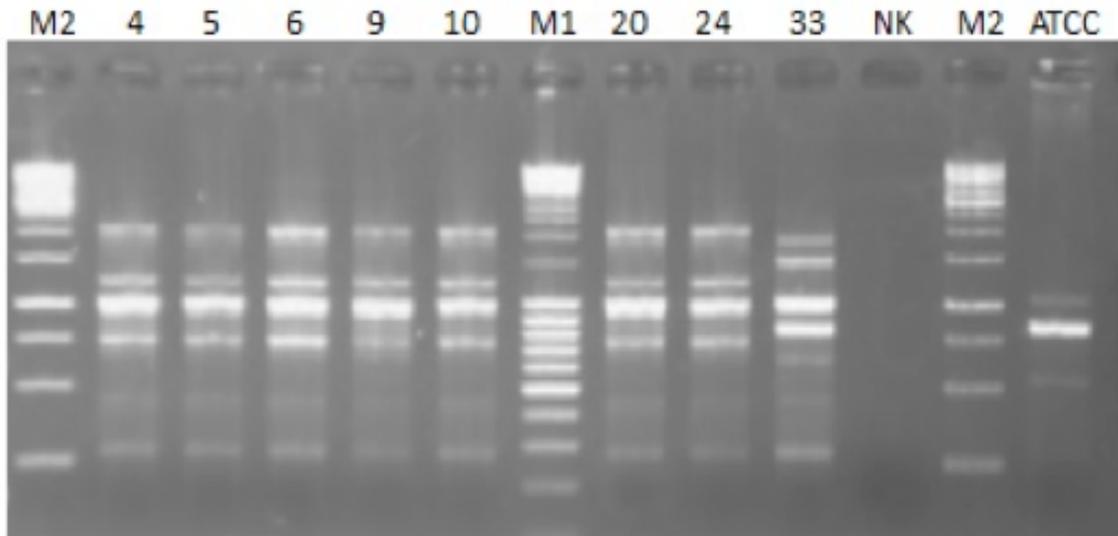


Figure 1. Different patterns by M13 primer. M1: 80-10.000 bp DNA ladder, M2: 1 kb DNA ladder, NK: Negative control, ATCC: *A. baumannii* 19606

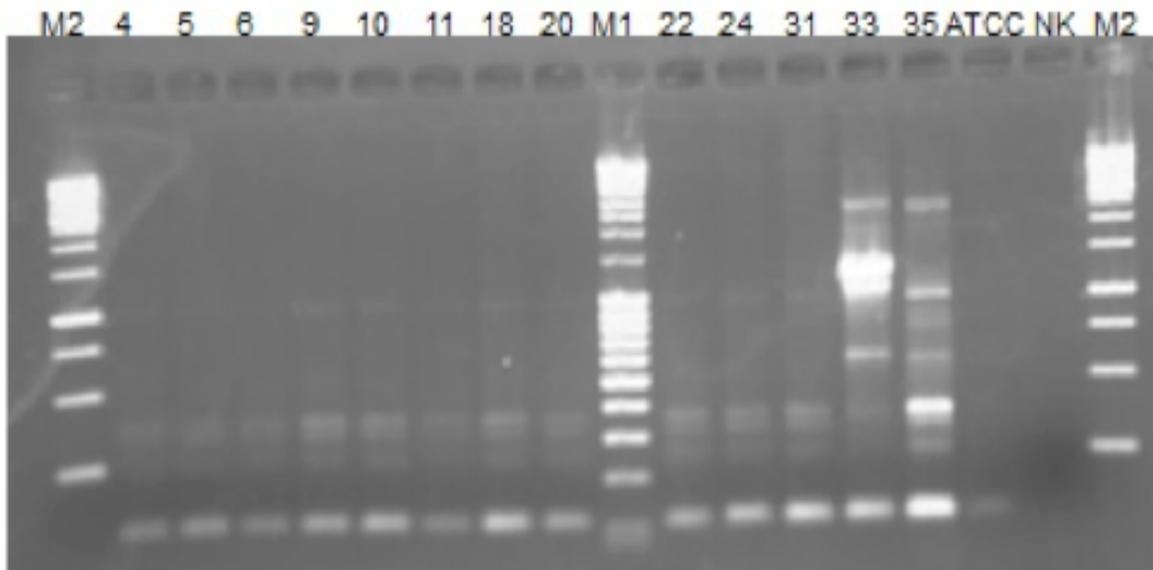


Figure 2. Different patterns by DAF4 primer. M1: 80-10.000 bp DNA ladder, M2: 1 kb DNA ladder, NK: Negative control, ATCC: *A.baumannii* 19606

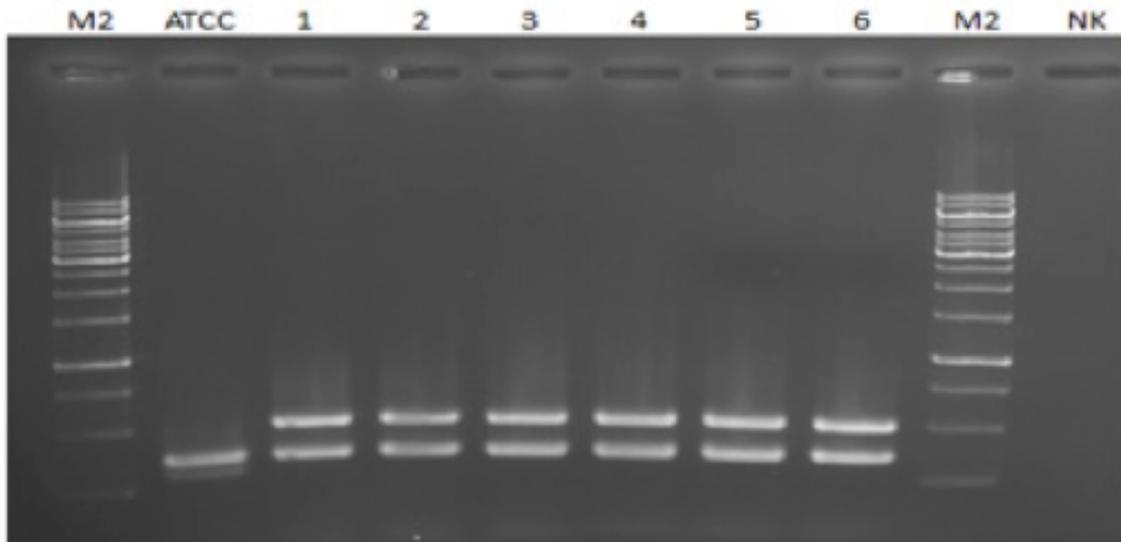


Figure 3. Multiplex PCR results. M2: 1 kb DNA ladder, NK: Negative control, ATCC: *A. baumannii* 19606

However, blaOXA-51 gene was also found recently in carbapenem-resistant non-baumannii species of *Acinetobacter*: *A. nosocomialis* (formerly *Acinetobacter* genomic species 13TU) and *Acinetobacter* genomic species “Close to 13U” (14). Members of the *A. calcoaceticus* - *A. baumannii* complex are *A. calcoaceticus*, *A. baumannii*, genomic species 3 and genomic species 13TU, so we accepted our isolates as *A. calcoaceticus* - *A. baumannii* complex. *A. baumannii* is the most frequently isolated agent among other species (12). It causes NIs such as pneumonia, sepsis, meningitis, and urinary infections in hospitalized patients, particularly among those treated in ICUs (15). Also in our study, we isolated *A. baumannii* most frequently from blood samples of patients in the ICU. The second most frequent body site for isolation was lower respiratory tract from which endotracheal aspirates and sputum were obtained.

Acinetobacter species are increasingly becoming multiple-antibiotic resistant, leading to difficulty in the treatment of infections they cause. Currently, NIs due to carbapenem-resistant *A. baumannii* strains

are increasing. According to the results of the MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) Antimicrobial Surveillance Program studies carried out by Hacettepe University, the most effective antibiotics against *A. baumannii* were meropenem by 53%, imipenem by 48%, and tobramycin by 44% (16). Iraz et al. in their study reported that their *Acinetobacter* isolates were susceptible to colistin and netilmicin at rates of 99% and 85%, respectively (17). In our study, we found that *A. baumannii* isolates were most susceptible to tobramycin, netilmicin and colistin, whereas they showed minimal susceptibility to other antibiotics which cannot be considered for therapy. Generally, many carbapenem resistant *A. baumannii* clinical isolates were found to be resistant to aminoglycosides in many studies. We did not study the mechanisms for aminoglycoside resistance. Different resistance genes located on plasmids, transposons or class I integrons may be responsible for this resistance (18). It is possible to find different resistance profiles in different locations; for example in a study from Australia the carbapenem resistant isolates have OXA-23 genes, however resistance to

netilmicin has not been found (19). The majority of our isolates belonged to a single clone, so the unusual aminoglycoside resistance profile (high resistance to amikacin compared to high susceptibility to netilmicin and tobramycin) may be associated with this matter.

The antibiotic resistance of *Acinetobacter* species has led investigators to seek other treatment alternatives. Infections caused by such resistant strains can be treated with colistin (20). In the last few years, infections caused by colistin-susceptible *Acinetobacter* have emerged and have been successfully treated with colistin. In our study, colistin was found to be the fourth most effective antibiotic against *A. baumannii*. In our hospital, colistin is used in the therapy of multiple-resistant *A. baumannii* infections since susceptibility to netilmicin cannot be assessed by VITEK2 automated system AST-N90 card and since providing tobramycin as a drug is difficult. However, colistin has some serious side-effects which render its use problematic. Also, colistin-resistant and heteroresistant strains which are difficult to determine have emerged in recent years, limiting the use of colistin in therapy (21). In our study, we found two isolates of *A. baumannii* were resistant to colistin, namely, isolates numbered (no.) 37 and 65, belonging to the same patient. Isolate no. 37 was the first isolate obtained a month prior to isolate no. 65. Following the isolation of colistin-resistant isolate no. 37, the patient had received colistin therapy which might have led the present hetero-colistin-resistance to develop into colistin-resistance.

Since it is not possible to identify *Acinetobacter* species with phenotypic methods, genotypic methods are required for identification (22). PCR-based methods are widely used for the epidemiologic typing of *A. baumannii*. When PCR-based typing is compared with other methods, it enables differentiation similar to that of ribotyping, but less than that of PFGE. Concordance between PFGE, rep-PCR and MLST for *A. baumannii* typing has been shown in various studies (23). In a multi-center study with participation of different countries, Grundmann et al. reported that PCRs carried out with isolated *A. calcoaceticus*-*A. baumannii* complex using M13, DAF4, ERIC2, and REP primer sets gave high rates of similar results. The authors also reported that similar

results were obtained when standard DNA extraction and amplification conditions were provided and that with M13 primer more marked band patterns and thus better differentiation were obtained when compared with other primers (8).

In our study, we used the M13 and DAF4 primers, and with each primer we obtained two different bands, more marked with M13 primer. Isolate no. 33 was accepted as an exogenous isolate since it was obtained from a patient who had undergone an operation in another medical center one month prior to its isolation and showed antibiogram patterns different from those of other isolates. The same patterns were shared by all 65 isolates included in this study except isolate no. 33 which indicates that there is one single *Acinetobacter* clone from the same source in our hospital. Since *A. baumannii* strains are occasionally isolated in our hospital since 2009, it would be more correct to describe this clonal strain as an endemic strain rather than an epidemic strain. It is suggested that more attention should be given to universal infection control measures to prevent the transmission of bacteria between patients. Studying environmental samples routinely is not recommended. However, during epidemics environmental sampling should definitely be completed to determine the source of the causative microorganism and to obtain more relevant data (24).

The resistance to carbapenems is frequently associated with acquirement of genes responsible for the synthesis of carbapenamases which include oxacillinase and MBLs. Besides these genes, resistance to carbapenems is also caused by excess production of OXA-51 type natural oxacillinase (5). Gür et al. (25) in their study found OXA-58 in 18 (40.9%) and OXA-23 in 26 (59.1%) isolates. A multicenter study in Turkey reported the presence of OXA-51 in 100%, OXA-23 in 74.4%, and OXA-58 in 17.3% of carbapenem-resistant *A. baumannii* isolates, but absence of OXA-24 in all isolates (26). Another study reported the presence of OXA-51 in 100% and OXA-23 in 78.2% of carbapenem-resistant *A. baumannii* isolates, but absence of OXA-58 in all isolates (27). A study in Greece found OXA-51 in 15 and OXA-58 in 14 of the 17 *A. baumannii* isolates, but no OXA-23 and OXA-24 (28). In the same study, the

enzymes IMP, VIM, SIM, and SPM were investigated by using Etest and molecular methods, but MBL enzymes were not found with either method (28). A study in Brazil reported the presence of OXA-23 in all of 172 *A. baumannii* isolates investigated, but absence of IMP, VIM, SIM, and SPM as MBL enzymes (10).

In our study, we investigated the presence of oxacillinase enzymes, namely, OXA-23, OXA-24, OXA-51, and OXA-58, and presence of MBL enzymes, namely, IMP, VIM, SIM, and SPM using multiplex-PCR. Since OXA-23 and OXA-51 were present and OXA-24, OXA-58, IMP, VIM, SIM, and SPM were absent in all isolates, we consider that carbapenemase resistance could be due to excess production of OXA-type natural oxacillinase and to OXA-23 enzyme gene. We did not study the existence of ISAb1 genes, though it is reported

that this gene provides the promoter for blaOXA-51-like and blaOXA-23-like genes upstream of these carbapenemases and the resistance to carbapenems is closely related to the ISAb1 promoter (4).

Antibiotics used in the therapy of nosocomial *A. baumannii* are limited in number and in the near future emerging multiple-antibiotic resistant *A. baumannii* strains may cause infections that cannot be effectively treated. In view of these facts, to prevent cross-transmission of *A. baumannii* among patients colonized or infected, contact isolation should be carefully performed, medical staff should be regularly and repeatedly trained, importance of hand washing should always be stressed, and disinfection of the surroundings should be more frequently repeated.

ACKNOWLEDGEMENTS

This investigation was supported by the Scientific Research Projects of University (Project No: 2011/114).

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