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IDENTIFICATION OF OXA-GENE IN ACINETOBACTER BAUMANNII ISOLATES OBTAINED FROM CLINICAL SPECIMENS AND THE CLONALITY BETWEEN THESE ISOLATES

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ABSTRACT

Objective: *Our study aimed to reveal the OXA-like carbapenemase genes and molecular epidemiological relationship in A. baumannii isolates and compare antibiotic susceptibility status-resistance genes-Pulsed Field Gel Electrophoresis (PFGE) patterns.*

Material and Method: *A total of 100 A. baumannii isolates obtained from several clinical specimens were examined by VITEK2 automated identification system (Biomerieux, France) for the antibiotic susceptibility profile, by multiplex polymerase chain reaction (PCR) method for OXA-type β-lactamases and by PFGE method for the clonal relationship.*

Results: *57% of the isolates were obtained from respiratory system specimens. Among all the antibiotics, colistin was the most effective agent with 100% sensitivity, followed by tigecycline with 93%. All of the isolates were resistant to piperacillin, piperacillin/tazobactam and ciprofloxacin. Resistance against both imipenem and meropenem were detected as 95%. OXA-51 gene was found in all and OXA-23 gene was found in 92 (92%) of the isolates. None of the isolates had OXA-24 or OXA-58 genes. We identified 19 different clonal clusters among 100 isolates by PFGE method. We revealed that some of the clones were clustered in a certain period of time, and this was supported by the antibiogram results and OXA gene profiling.*

Conclusion: *Our study identified high rates OXA-23 gene locus positivity, presented the current clonal similarity and time relationship among the clonal clusters. These results emphasize the importance of molecular epidemiological methods as well as standard infection control programs to prevent spreading of A. baumannii.*

Keywords: *Acinetobacter baumannii, antibiotic resistance, oxa-type carbapenemases, polymerase chain reaction (PCR), pulsed field gel electrophoresis (PFGE).*

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KLİNİK ÖRNEKLERDEN ELDE EDİLEN ACİNETOBACTER BAUMANNİİ İZOLATLARINDA OXA-GENİ ARAŞTIRILMASI VE BU İZOLATLAR ARASINDAKİ KLONAL İLİŞKİ

ÖZET

Amaç: Çalışmamızda A. baumannii izolatlarında; OXA-benzeri genlerin varlığının ortaya çıkarılması, moleküler ilişkinin araştırılması ve antibiyotik duyarlılık durumlarının-direnç genlerinin (PFGE) paternlerinin karşılaştırılması amaçlanmıştır.

Materyal ve Metot: Çeşitli klinik örneklerden elde edilen toplam 100 A. baumannii izolatı VITEK2 otomatize identifikasyon sistemi (Biomerieux, Fransa) ile antibiyotik duyarlılık profillerini tespit için, multipleks polimeraz zincir reaksiyonu ile OXA tipi β-laktamazları bulmak için ve PFGE yöntemiyle de klonal ilişkinin gösterilmesi için incelendi.

Bulgular: İzolatların %57'si solunum sistemi örneklerinden elde edildi. Tüm antibiyotikler içinde kolistin %100 duyarlılık oranıyla en etkili ajan olarak bulundu. Tigesiklin %92 duyarlılık oranıyla ikinci en etkin ajandı. Tüm izolatlar piperasilin, piperasilin/tazobaktam ve siprofloksasine dirençliydi. İmipenem ve meropenemin her ikisine karşı %95 direnç tespit edildi. OXA-51 geni tüm izolatlarda, OXA-23 geni ise 92 (%92) izolatta bulundu. İzolatların hiçbirisinde OXA-24 veya OXA-58 genine rastlanmadı. PFGE yöntemiyle 100 izolat arasında 19 farklı klonal küme tanımlandı. Bazı klonların belirli zaman aralıklarında kümelendiği ortaya çıkarıldı ve bu bulgu antibiyogram sonuçları ve OXA gen profile ile desteklendi.

Sonuç: Bizim çalışmamız yüksek oranda OXA-23 gen lokusu pozitifliği tanımlamış, mevcut klonal benzerliği ve klonal kümeler arasındaki ilişkiyi ortaya çıkarmıştır. Bu sonuçlar A. baumannii yayılımın önlenmesinde standart enfeksiyon kontrol programlarının yanında moleküler epidemiyolojik yöntemlerin önemini vurgulamaktadır.

Anahtar kelimeler: Acinetobacter baumannii, antibiyotik direnci, oxa-tip karbapenemazlar, polimeraz zincir reaksiyonu, pulsed field jel elektroforezis.

INTRODUCTION

Healthcare-associated infections (HAI) are one of the most common cause of morbidity and mortality, especially in Intensive Care Units (ICU), and despite all the developments in health systems the rates are still high worldwide.[1] Acinetobacter baumannii is one of the most challenging pathogens to treat and it is often encountered as a common cause of HAI. Carbapenem resistant A. baumannii infection reports are increasing day by day from all over the world. It's an opportunistic, obligate aerobic, non-fermentative, oxidase negative, gram negative coccobacillus.[2,3]

Ambler class D carbapenemases (Oxacillinases, OXA), summarized in five groups as OXA-23-type, OXA-40/24-type, OXA-58-type, OXA-51/69-type and OXA-143-type enzymes are the main carbapenemase-encoding genes in A. baumannii.[3-6] OXA-51-like enzymes are known to be ubiquitous and intrinsic enzymes in A. baumannii isolates, which are assumed as diagnostic findings for A. baumannii, however presence of other resistance genes may vary within A. baumannii isolates.[7,8]

Epidemiologic surveys are very important for the management of healthcare associated infections, and the genetic investigations are one of the most important part of these surveys.[9] Although there are many classification methods, Pulsed-Field Gel Electrophoresis (PFGE) is still the gold standart method for revealing clonal relationship within the isolates due to its high repeatability rates.[9,10]

In our study A. baumannii isolates obtained from clinical specimens were investigated with polymerase chain reaction (PCR) for OXA-like carbapenemase genes. PFGE method was used to describe molecular epidemiological relation and compare antibiotic susceptibility status-resistance genes-PFGE patterns.

MATERIAL AND METHOD

This study was carried out after approval by the Non-invasive Clinical Studies Ethics Committee, GATA Haydarpaşa Training Hospital, İstanbul, Turkey (Project number: 2014-63, Session number: 26, Session date: 17.07.2014).

A total number of 100 A. baumannii isolates, isolated from various clinical samples (Deep tracheal aspirate [DTA], bronchoalveolar lavage fluid [BAL], blood, tissue, sterile body fluid- pleural effusion, peritoneal fluid [SBF], urine specimens) sent from Anesthesiology and Reanimation Intensive Care Unit (ARIC) and Internal Medicine Intensive Care Unit (IMICU) at Sultan Abdülhamid Han Training Hospital (with 1000 beds and 30 intensive care unit beds) during January 2012 and December 2014 were included in our study.

Table 1. Primers used for PCR applications and expected band sizes

Primers	Sequence (5'- 3')	Expected Band Sizes
OXA-23-	GAT CGG ATT GGA GAA CCA GA	501bp
OXA-23-	ATT TCT GAC CGC ATT TCC AT	
OXA-24-	GGT TAG TTG GCC CCC TTA AA	246bp
OXA-24-	AGT TGA GCG AAA AGG GGA TT	
OXA-51-	TAA TGC TTT GAT CGG CCT TG	353bp
OXA-51-	TGG ATT GCA CTT CAT CTT GG	
OXA-58-	AAG TAT TGG GGC TTG TGC TG	599bp
OXA-58-	CCC CTC TGC GCT CTA CAT AC	

Clinical specimens were inoculated on 5% sheep blood agar and EMB (Eosin Methylene Blue) agar plates and incubated at 37°C for 18-24 hours in a 5% CO_2 atmosphere. Identification process of isolates was carried out with conventional methods (oxidase, catalase, sugar fermentation, motility, citrate, reproduction at 41°C and 44°C) and VITEK2 automated identification system GN ID card (Biomerieux, France) was used for confirmation.[3,11] In case of presence of more than one A. baumannii isolates from the same patient, the first isolate was taken into the study during January 2012 and December 2014.

The VITEK2 automated identification system AST-N262 card (Biomerieux, France) was used to determine the antibiotic (amikacin, ampicillin/sulbactam, cefepime, cefaperazone/sulbactam, ceftazidime, ciprofloxacin, colistin C, gentamycin, imipenem, levofloxacin, meropenem, netilmicin, piperacillin, piperacillin/tazobactam, tetracycline, tigecycline, trimethoprim/sulfamethoxazole) susceptibility profile of the isolates. For antimicrobials except tigecycline Clinical and Laboratory Standards Institute (CLSI) criteria for A. baumannii were used for the assesment of the results.[12] For tigecycline Food and Drug Administration (FDA) approved MIC breakpoints (for susceptibility and resistance are≤2mg/L and≥8mg/L, respectively) were used in VITEK2 automated identification system (Biomerieux, France) as it is similar researches in literature.[13-16]

The MagCore® HF16 automated DNA extraction system and genomic DNA bacterial kit (RBCBioscience, Taiwan) were used in accordance with the manufacturer's instructions for the extraction of the template DNA to be used in the PCR applications. In the study, the multiplex PCR method of Woodford *et al*. and Keskin *et al*. was used to investigate the OXA-type carbapenemase genes.[17,18] The primers used for PCR applications and the expected band sizes are shown in Table 1.

Clonal analysis of isolates was performed by using the ApaI enzyme according to the PFGE protocol standardized by Durmaz *et al*. that is based on the protocol of Turabelidze *et al*.[19,20] In the analysis of the band profiles, the open-source PyElph software system (version 1.4) was used and the band tolerance was taken as 2% in calculating the similarity coefficient. Using the 'Unweighted pair group method with mathematical averaging' (UPGMA) calculation system, dendograms of PFGE profiles were generated and clustering analysis was performed.

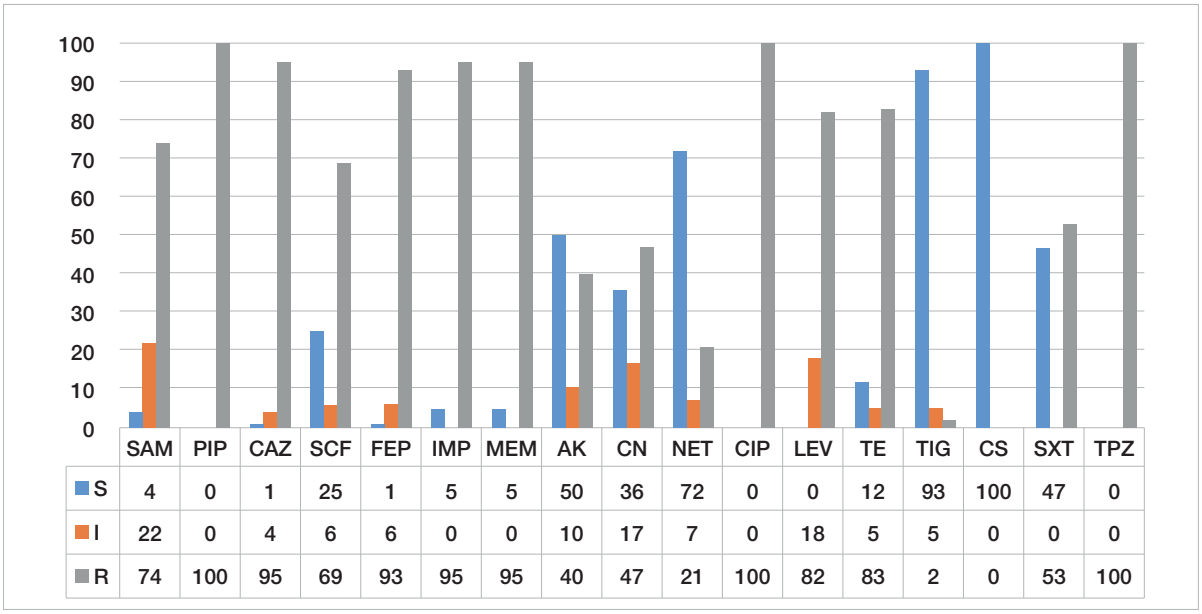
RESULTS

All of the 100 isolates were first identified as A. baumannii by conventional methods. After that all isolates were identified as Acinetobacter baumannii complex confirmed by the VITEK2 automated identification system (Biomerieux, France). Most of the isolates were obtained from respiratory system with the rate of 57% (DTA: 35%, BAL: 22%). The rest of the clinical specimens were obtained from blood (20%), tissue (10%), urine (10%) and SBF (3%).

Antibiotic susceptibility rates are shown in Figure 1. All of the isolates were resistant to piperacillin, piperacillin/tazobactam and ciprofloxacin. Among all the antibiotics tested, the most effective agents were colistin with 100% sensitivity (MIC value from VITEK is≤0.5mg/L); and tigecycline with a sensitivity rate of 93% (MIC values from VITEK: 12 isolates≤0.5mg/L Sensitive, 29 isolates 1mg/L Sensitive, 52 isolates 2mg/L Sensitive, five isolates 4mg/L Intermediate, two isolates≥8mg/L Resistant).

OXA-51 gene region was presented in all isolates and OXA-23 gene region was revealed in 92 (92%) of the isolates (Figure 2). Neither of the OXA-24 and OXA-58 gene locations were detected in any of the isolates.

Five of the eight isolates containing OXA-51 alone (without other OXA genes) were sensitive to imipenem and meropenem phenotypically (MIC values from VITEK for both imipenem and meropenem: One isolate 2mg/L Sensitive, two isolates 0.5mg/L Sensitive, two isolates 1mg/L Sensitive) and three were found to be resistant (MIC values from VITEK for both imipenem and meropenem: 8mg/L Resistant). All the eight isolates were found to be susceptible to gentamicin, clarithromycin, netilmicin, tigecycline and colistin. All of the 92 isolates containing both of the OXA-23 and OXA-51 genes were resistant to imipenem and meropenem (MIC values from VITEK for both imipenem and meropenem: ≥16mg/L Resistant).



	SAM	PIP	CAZ	SCF	FEP	IMP	MEM	AK	CN	NET	CIP	LEV	TE	TIG	CS	SXT	TPZ
S	4	0	1	25	1	5	5	50	36	72	0	0	12	93	100	47	0
I	22	0	4	6	6	0	0	10	17	7	0	18	5	5	0	0	0
R	74	100	95	69	93	95	95	40	47	21	100	82	83	2	0	53	100

Figure 1. Antibiotic susceptibility rates of isolates

S: Sensitive; **I:** Intermediate; **R:** Resistant
SAM: Ampicillin/sulbactam; **PIP:** Piperacillin; **CAZ:** Ceftazidime; **SCF:** Cefoperazone/sulbactam; **FEP:** Cefepime; **IMP:** Imipenem; **MEM:** Meropenem; **AK:** Amikacin; **CN:** Gentamycin; **NET:** Netilmicin; **CIP:** Ciprofloxacin; **LEV:** Levofloxacin; **TE:** Tetracycline,; **TIG:** Tigecycline; **CS:** Colistin **C; SXT:** Trimethoprim/sulfamethoxazole; **TPZ:** Piperacillin/tazobactam

As a result of molecular typing, the similarity coefficients of all isolates varied between 67% and 100%. To determine the PFGE genotypes, the limit value of the similarity coefficient was determined as 80% and total of 100 isolates were in 19 different clusters. In order to determine the number of subtypes, the limit value of the similarity coefficient was determined as 100%, and the isolates in 19 different clusters were in 92 subtypes (Figure 3).

Seven dominant clones [A (n: 25), B (n: 18), C (n: 12), D (n: 9), E (n: 8), F (n: 5), G (n: 4)] were found among 19 different clusters. Clone A was dominant in 2012 and in 2013 its replaced by clone B. Clone C and E were seen during the three years that the study was conducted. Clone D was observed in 2013 and 2014, Clone F and G were observed only in 2014. Clones A, B, C, D and E were isolated from various samples from both ICU and except one isolate in Clone E, all isolates were OXA-23 and OXA-51 positive. Clone F was isolated from various clinical samples obtained from IMICU and all isolates were OXA-23 negative. Clone G was isolated from respiratory system specimens (two DTA, two BAL) sent from IMICU and all of them were found positive for both OXA-23 and OXA-51. A, D and G clones were all resistant to beta-lactams (MIC values from VITEK for both imipenem and meropenem: ≥16mg/L) and fluoroquinolones (MIC values from VITEK for ciprofloxacin and levofloxacin ≥4mg/L and ≥8mg/L, respectively) and all sensitive for tigecycline (MIC values from VITEK: ≤0.5mg/L, 8 isolates; 1mg/L, 9 isolates and 2mg/L, 8 isolates) and colistin (MIC value from VITEK: ≤0.5mg/L). B and C clones were all resistant to beta-lactams (MIC values from VITEK for both imipenem and meropenem: ≥16mg/L) and all sensitive to colistin (MIC value from VITEK: ≤0.5mg/L).

DISCUSSION

A. baumannii, that frequently colonizes respiratory equipments in health care settings, causes a wide variety of infections, primarily respiratory infections and then bloodstream infections.[16,21] As in the literature, most of the isolates were obtained from respiratory tract samples at a rate of 57% (DTA: 35%, BAL: 22%) in our study. This is followed by blood samples with an isolation rate of 20%.

Although antimicrobial susceptibility rates of A. baumannii can vary, resistance against beta-lactams, aminoglycosides and fluoroquinolones is common.[8,16,17,22] In this study all of the isolates were resistant to piperacillin (≥128mg/L), piperacillin/tazobactam (≥128mg/L) and ciprofloxacin (≥4mg/L) as it is in literature. But, resistance against amikacin 40% (≥32mg/L), gentamicin 47% (≥16mg/L) and netilmicin 21% (≥32mg/L) were found lower than literature. This may be due to restricted antimicrobial reporting policy of our hospital. Amikacin resistance (≥32mg/L) rate was also reported as 33% in carbapenem resistant A. baumannii isolates in a study conducted by Maciel *et al.*, similar to our findings.[23]

It has been reported in different studies (from Turkey and abroad) that the carbapenem resistance in A.

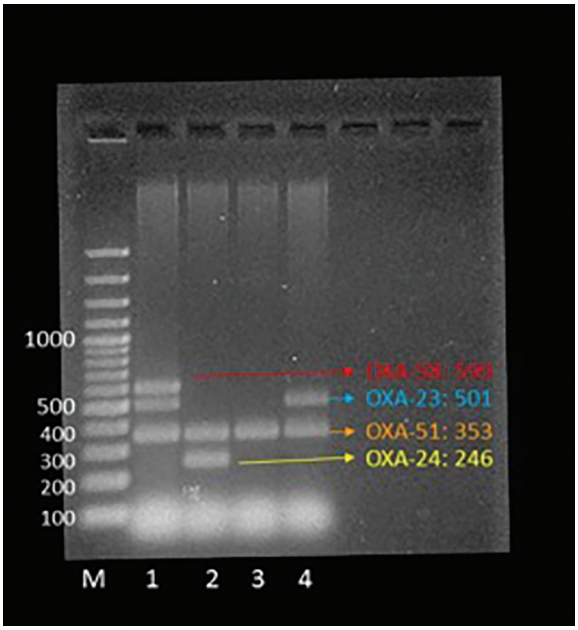


Figure 2. Detection of OXA-carbapenemase encoding genes by multiplex PCR

M: 100 bp DNA Marker
1. Line: Positive control isolate containing OXA-23,OXA-51 and OXA-58 genes.
2. Line: Positive control isolate containing OXA-24 and OXA-51 genes.
3. Line: Representation of eight isolates containing only OXA-51 (not including other OXA genes).
4. Line: Representation of 92 isolates containing both OXA-23 and OXA-51 genes.

baumannii can rise up to 90% due to rapid transmission of the genes.[8,16,17,22,24] In our study, it was observed that this ratio was 95% (≥16mg/L, 92 isolates; 8mg/L, 3 isolates) which is consistent with literature. The most effective antimicrobial agent in our study was colistin with 100% sensitivity (≤0.5mg/L). In the second row, tigecycline was found to have a sensitivity rate of 93% (≤0.5mg/L, 12 isolates; 1mg/L, 29 isolates; 2mg/L, 52 isolates). In literature, A. baumannii was reported with a sensitivity of 96.8-100% for colistin and 91.3-99% for tigecycline.[15,16,25-27]

Multiplex PCR is a method that can be easily used in the detection of OXA-type carbapenemases, allowing the investigation of multiple gene regions by a single reaction.[4,9,25] With the multiplex PCR method applied in the study, OXA-51 gene region was found in all isolates and OXA-23 gene region was found in 92 (92%) of isolates. In different studies it was emphasized that regional differences may occur in the distribution patterns and it has been emphasized that enzymes like OXA-23 take the place of enzymes like OXA-58.[16,25-28] The possible cause of this is explained by the fact that the carbapenemase activity of OXA-23 is higher than that of OXA-58.[16,27]

It is known that OXA-23 carbapenemase producing isolates are associated with high levels of imipenem and meropenem hydrolysis.[29,30] In our study, imipenem and meropenem resistance were detected in all of the 92 isolates (≥16mg/L) with OXA-23 gene positivity. Five (62,5%) of the eight isolates harboring only OXA-51 (other OXA genes were not present) were found sensitive for imipenem and meropenem (2mg/L, one isolate; 1mg/L, two isolates; 0.5mg/L, two isolates), three of these eight isolates were found resistant (8mg/L) againts two carbapenems tested. In a study conducted by Raible *et al*. MIC values of imipenem and meropenem were found varying between 32-64µg/mL for OXA-23 carbapenemase gene positive isolates.[8] In a study conducted by Ambrosi *et al*. carbapenem resistant isolates harboring only OXA-51 (other OXA genes were not present) had lower MIC values (8µg/mL) than carbapenem resistant isolates with other OXA genes. The reason for this may be other enzyme variants with weaker carbapenemase activity according to researchers.[27] In this study, like in literature, carbapenem resistant OXA-23 positive 92 isolates had MIC values (≥16mg/L) higher than carbapenem resistant, OXA-23 negative three isolates (8mg/L).

We determined 19 different types of PFGE clusters with a 80% similarity coefficient limit between our study isolates. In other studies like our study, it is accepted with a 80% similarity coefficient as a limit for PFGE analysis.[16,19,29] In this 19 different types of clusters 7 clones are [A (n: 25), B (n: 18), C (n: 12), D (n: 9), E (n: 8), F (n: 5), G (n: 4)] dominant. In literature from our country, Keskin *et al*. reported that; 201 Acinetobacter spp. isolates are clustered under four dominant genotypes and genotype A had 29 (14,4%), genotype B had 23 (11,4%), genotype C had 18 (8,9%) and genotype D had 7 (3,4%) isolates.[17] Çetinkol *et al*. showed all 50 multidrug resistant A. baumannii isolates, except one, were grouped in same cluster (A) by PFGE and considered as epidemic isolates.[31] In a study conducted by Karagöz *et al*. a total number of 54 A. baumannii isolates, 47 isolates isolated from blood cultures and seven isolates from environment, were found originated from a single clone and considered responsible for an outbreak.[32] In global literature; Ambrosi *et al*. had found 31 A. baumannii isolates grouped under three subtypes (A, B, C).[27] Guo-Xin *et al*. had found 62 A. baumannii isolates forming nine different clones and named the most common clones as A, B and C.[15]

In our study, we observed that among 19 different clusters, the biggest cluster was clone A and it was dominant in 2012 and it was replaced with clone B in 2013. C and E clones were observed in every years during study conducted. Clone D was observed in 2013 and 2014, clone F and G were observed only in 2014. In a study conducted by Keskin *et al*. isolates of genotype C and D were dominant in 2010 and they were replaced by genotype A and B in 2011.[17] Clone A and B were found as dominant clones among 10 clusters formed by 79 A. baumannii isolates evaluated

with PFGE in a study by Direkel *et al*.[14] A total number of 66 A. baumannii isolates were dispersed into 5 dominant and 6 single clones with PFGE in a study by Ulu-Kılıç *et al*. Clone 1 was dominant with its 39 members among dominant clones.[33]

In our study, we found all isolates of A, B, C, D and G clones and some isolates of E and F clones resistant against beta-lactams (8mg/L-≥16mg/L) and sensitive to colistin (≤0.5mg/L). Similar to ours, Guo-Xin *et al*. reported all isolates of clone A and some isolates of clone B and C were carbapenem resistant.[15]

In our study, all of the isolates in A, B, C, D, G clones and all of the isolates except one isolate in clone E were OXA-23 positive and F clone was OXA-23 negative. One isolate without the OXA-23 gene in clone E was carbapenem susceptible (0.5mg/L). Two isolates in clone F were also carbapenem susceptible (0.5mg/L) but three isolates were resistant (8mg/L). Uwingabiye at al. reported that 83 A. baumannii isolates formed nine clones (0001-0009) being two clones dominant (0008,0007) and all isolates carried bla OXA-23-like gene and carbapenem resistance was associated with bla OXA-23-like gene.[34] Keyik *et al*. reported 49 (46.7%) isolates out of 105 imipenem and meropenem resistant A.baumannii isolates had bla OXA 23-like gene and found these isolates widely dispersed into 32 different clones.[35]

While, overall tigecycline susceptibility was 93%, it was 83.3% (n:18) and tigecycline MIC was 4mg/L (Intermediate Susceptibility) in clone B. Common features of these isolates were being resistant against all tested amtimicrobials, except colistin (≤0.5mg/L) and trimethoprim/sulfamethoxazole (≤0.5mg/L) and 100% similarity in PFGE profiles. Resistance against tigecycline (≥16mg/L) was observed in one isolate in cluster C and it was found as being resistant against all antimicrobials except colistin (≤0.5mg/L). In a study by Direkel *et al*., high level of resistance was also observed in some clones similar to our findings.[14]

A, B, C, D and E clones were isolated from both ICU, but F and G clones were isolated only from IMICU. Clones isolated from both ICU proves cross contamination problem. Likewise, reports by Direkel *et al*., Karagöz at al., Ulu-Kılıç *et al*. and Ertürk at al. show, low compliance to contact isolation precautions and hygiene practiceslead to cross contamination and hospital infections.[14,32,33,36] Uwingabiye *et al*. suggest that A. baumannii isolates are in constant exchange between environment and patients and transmission can occur mutually among three points: patients, health care personel and environment.[34]

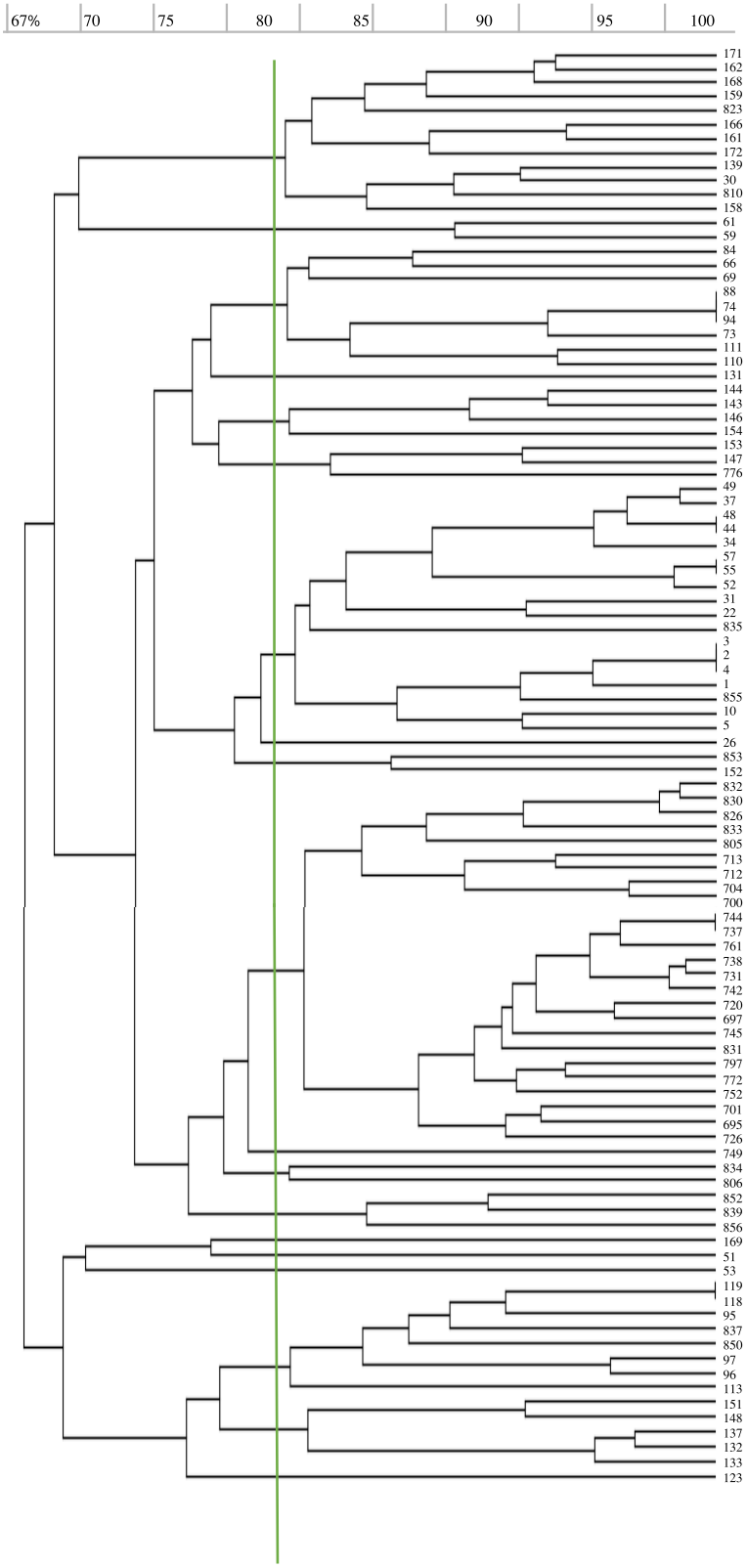


Figure 3. Results of phylogenetic analysis of isolates

F and G clones were limited to IMICU and seen for a short period, this means they were not transferred between units. In a multicentered study conducted by Ahmed *et al*. in 11 different hospitals from Turkey and Azerbaijan, a total number of 112 Acinetobacter spp. isolates were included and 15 clones and eight unique isolates were identified. The largest clone

was cluster D, with six subtypes. D and I clones were scattered widely to various geographic regions of Turkey, but clone F was restricted to Northern-East Turkey and Azerbaijan. It was found that, 16% of the isolates were belonging to European clones I and II. European clone I was found in Antalya, Istanbul, and Erzurum, which are touristic destinations. European clone II was grouped in cluster U and found only in Kayseri and Diyarbakır; however, these two regions are geographically far from each other. But these cities were relatively close to Syrian border and travel and migration can be factors for the transfer of the clones.[37]

The isolation of a clone (F clone) from a patient who was transferred to the IMICU from another hospital for the first time suggests that this clone is also a source for other patients.[29] This emphasizes the importance of taking surveillance cultures for the detection of colonization prior to acceptance of the patient to ICU and importance of cohorting the cases with colonization or infection.[21]

Isolates of clones A, B, C, D, E and F were isolated from various samples but it is remarkable that isolates of clone G was isolated only from respiratory samples (two DTA and two BAL). This finding suggests that there may be a role of respiratory system equipment in transferring these clones between different ICU and different patients, and emphasizes the importance and necessity of monitoring the spread of A. baumannii isolates by molecular epidemiological methods.[14,21,27,28]

In a study that 109 A. baumannii isolates were dispersed into nine main clones, conducted by Ertürk *et al*.; blood gas analysis device was found responsible for transmission.[36] Karagöz *et al*. have emphasised once again the importance of disinfectant use and cleaning of the environment and the medical devices even proper infection countermeasures were taken.[32]

Some isolates have different aminoglycoside susceptibility patterns, although the band profile similarity rate between the isolates is 100%. In relation to aminoglycoside resistance of A. baumannii, aminoglycoside modifying enzymes such as phosphotransferase, acetyltransferase and nucleotidyltransferase are important and the genes encoding these enzymes are transmitted between bacteria by plasmids and transposons.[22,38] We think that phenotypic and genotypic incompatibility which was detected in our study is due to the fact that plasmids can not be detected from chromosomal DNA by routine PFGE protocols.[39]

Our study identifying high rate of OXA-23 gene locus positivity and presenting the current clonal similiarity, reveals the importance of molecular epidemiological methods as well as standard infection control programs to prevent spreading of A. baumanii infections.

*The authors declare that there are no conflicts of interest.

REFERENCES

1. Parajuli NP, Acharya SP, Dahal S, et al. Epidemiology of device-associated infections in an intensive care unit of a teaching hospital in Nepal: a prospective surveillance study from a developing country. Am J Infect Control 2017 1; 45: 1024-1029.
2. Custovic A, Smajlovic J, Tihic N, et al. Epidemiological monitoring of nosocomial infections caused by Acinetobacter baumannii. Med Arch 2014; 68: 402-406.
3. Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: emergence of a successful pathogen. Clin Microbiol Rev 2008; 21: 538-582.
4. Pogue JM, Mann T, Barber KE, et al. Carbapenem-resistant Acinetobacter baumannii: epidemiology, surveillance and management. Expert Rev Anti Infect Ther 2013; 11: 383-393.
5. Ambler RP. The structure of beta-lactamases. Philos Trans R Soc Lond B Biol Sci 1980; 289: 321-331.
6. Higgins P, Dammhayn C, Hackel M, et al. Global spread of carbapenem resistant Acinetobacter baumannii. J Antimicrob Chemother 2010; 65: 233-238.
7. Gerischer U. Acinetobacter Molecular Microbiology. Norfolk, UK: Caister Academic Press; 2008.
8. Raible KM, Sen B, Law N, et al. Molecular characterization of β-lactamase genes in clinical isolates of carbapenem-resistant Acinetobacter baumannii. Ann Clin Microbiol Antimicrob 2017 16; 16: 75.
9. Durmaz R. Hastane infeksiyonu salgınında moleküler biyolojik yöntemler. Hastane İnfeksiyonları Derg 2005; 9: 196-202.
10. Goering RV. Pulsed field gel electrophoresis: a review of application and interpretation in the molecular epidemiology of infectious disease. Infect Genet Evol 2010; 10: 866-875.
11. Brisou J and Prevot AR. Family II: Moraxellaceae, Genus II: Acinetobacter. In: Garrity G, Brenner DJ, Krieg NR, Staley JR, editors. Bergey's Manual Of Systematic Bacteriology Volume 2: The proteobacteria, Part B: The Gammaproteobacteria. 2th ed. New York, USA: Springer-Verlag New York Inc; 2005. pp. 425-435.
12. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty-Fourth Informational Supplement, M100-S24. CLSI, Wayne, PA: 2014.
13. Marchaim D, Pogue JM, Tzuman O, et al. Major variation in MICs of tigecycline in Gram-negative bacilli as a function of testing method. J Clin Microbiol 2014; 52: 1617-1621.
14. Direkel Ş, Çopur Çiçek AÇ, Karagöz A, et al. Antimicrobial susceptibility and molecular characterization of multidrug-resistant Acinetobacter baumannii isolated in an university hospital. Mikrobiyol Bul 2016; 50: 522-534.

15. Guo-Xin M, Dan-Yang S, Xi-Zhou G, et al. Laboratory to clinical investigation of carbapenem resistant Acinetobacter baumannii outbreak in a general hospital. Jundishapur J Microbiol 2014; 7(1): e13120.

16. Mezzatesta ML, Caio C, Gona F, et al. Carbapenem and multidrug resistance in gram-negative bacteria in a single centre in Italy: considerations on in vitro assay of active drugs. Int J Antimicrob Agents 2014; 44: 112-116.

17. Keskin H, Tekeli A, Dolapcı İ, et al. Molecular characterization of beta lactamase-associated in Acinetobacter baumannii strains isolated from clinical samples. Mikrobiyol Bul 2014; 48: 365-376.

18. Woodford N, Ellington MJ, Coelho JM. Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. Int J Antimicrob Agents 2006; 27: 351-353.

19. Durmaz R, Otlu B, Koksal F, et al. The optimization of a rapid pulsed-field gel electrophoresis protocol for the typing of Acinetobacter baumannii, Escherichia coli and Klebsiella spp. Jpn J Infect Dis 2009; 62: 372-377.

20. Turabelidze D, Kotetıshvili M, Kreger A, et al. Improved pulsed-field gel electrophoresis for typing vancomycin-resistant enterococci. J Clin Microbiol 2000; 38: 4242-4245.

21. Weber DJ, Rutala WA, Miller MB, et al. Role of hospital surfaces in the transmission of emerging health care-associated pathogens: Norovirus, Clostridium difficile, and Acinetobacter species. Am J Infect Control 2010; 38(5 Suppl 1): 25-33.

22. Jaidane N, Naas T, Mansour W, et al. Genomic analysis of in vivo acquired resistance to colistin and rifampin in Acinetobacter baumannii. Int J Antimicrob Agents 2018; 51: 266-269.

23. Maciel WG, da Silva KE, Croda J, et al. Clonal spread of carbapenem-resistant Acinetobacter baumannii in a neonatal intensive care unit. J Hosp Infect 2018; 98: 300-304.

24. Leblebicioglu H, Cakir N, Celen M, et al. Comparative activity of carbapenem testing (the COMPACT study) in Turkey. BMC Infect Dis 2012; 12: 42.

25. Ergin A, Hascelik G, Eser OK. Molecular characterization of oxacillinases and genotyping of invasive Acinetobacter baumannii isolates using repetitive extragenic palindromic sequence-based polymerase chain reaction in Ankara between 2004 and 2010. Scand J Infect Dis 2013; 45: 26-31.

26. Gur D, Korten V, Unal S, et al. Increasing carbapenem resistance due to the clonal dissemination of oxacillinase (OXA-23 and OXA-58)-producing Acinetobacter baumannii: report from the Turkish SENTRY Program sites. J Med Microbiol 2008; 57: 1529-1532.

27. Ambrosi C, Aleandri M, Giordano A, et al. Molecular characterisation of extensively drug-resistant Acinetobacter baumannii: first report of a new sequence type in Italy. J Glob Antimicrob Resist 2016; 7: 154-156.

28. D'Arezzo S, Principe L, Capone A, et al. Changing carbapenemase gene pattern in an epidemic multidrug-resistant Acinetobacter baumannii lineage causing multiple outbreaks in central Italy. J Antimicrob Chemother 2011; 66: 54-61.

29. Park YS, Lee H, Lee KS, et al. Extensively drug-resistant Acinetobacter baumannii: risk factors for acquisition and prevalent OXA-type carbapenemases-a multicentre study. Int J Antimicrob Agents 2010; 36: 430-435.

30. Abdallah M, Olafisoye O, Cortes C, et al. Reduction in the prevalence of carbapenem-resistant Acinetobacter baumannii and Pseudomonas aeruginosa in New York City. Am J Infect Control 2015; 43: 650-652.

31. Çetinkol Y, Telli M, Altunçekiç Yıldırım A, et al. Evaluation of the efficacy of colistin/sulbactam combination on carbapenem-resistant Acinetobacter baumannii strains. Mikrobiyol Bul 2016; 50: 460-465.

32. Karagöz A, Baran I, Aksu N, et al. Characterization and determination of antibiotic resistance profiles of a single clone Acinetobacter baumannii strains isolated from blood cultures. Mikrobiyol Bul 2014; 48: 566-576.

33. Ulu-Kilic A, Gundogdu A, Cevahir F, et al. An outbreak of bloodstream infection due to extensively resistant Acinetobacter baumannii among neonates. Am J Infect Control 2018; 46: 154-158.

34. Uwingabiye J, Lemnouer A, Roca I, et al. Clonal diversity and detection of carbapenem resistance encoding genes among multidrug-resistant Acinetobacter baumannii isolates recovered from patients and environment in two intensive care units in a Moroccan hospital. Antimicrob Resist Infect Control 2017; 6: 99.

35. Keyik S, Arslan U, Türk Dağı H, et al. Investigation of OXA type beta-lactamases and PFGE patterns in Acinetobacter baumannii strains resistant to carbapenems. Mikrobiyol Bul 2014; 48: 556-565.

36. Ertürk A, Çiçek AÇ, Gümüş A, et al. Molecular characterisation and control of Acinetobacter baumannii isolates resistant to multi-drugs emerging in inter-intensive care units. Ann Clin Microbiol Antimicrob 2014; 13: 36.

37. Ahmed SS, Alp E, Ulu-Kilic A, et al. Spread of carbapenem-resistant international clones of Acinetobacter baumannii in Turkey and Azerbaijan: a collaborative study. Eur J Clin Microbiol Infect Dis 2016; 35: 1463-1468.

38. Dal T, Dal MS, Ağır İ. Acinetobacter baumannii'de antibiyotik direnci ve AdeABC aktif pompa sistemleri: literatürün gözden geçirilmesi. Van Tıp Derg 2012; 19: 137-148.

39. Barton BM, Harding GP, Zuccarelli AJ. A general method for detecting and sizing large plasmids. Anal Biochem 1995; 226: 235-240.