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ANTIBIOTIC RESISTANCE AND INTEGRON GENE CASSETTES IN ACINETOBACTER BAUMANNII ISOLATES PRODUCED IN LOWER RESPIRATORY TRACT SAMPLES TAKEN FROM THE INTENSIVE CARE UNIT

Yoğun Bakım Ünitesinden Alınan Alt Solunum Yolu Örneklerinde Üretilen Acinetobacter baumannii İzolatlarında Antibiyotik Direnci ve İntegron Gen Kasetleri

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ABSTRACT

Objective: The spread of antibiotic resistance genes among bacteria causes serious problems in the treatment of infectious diseases. Recently, it has been shown that these resistance genes are also found in integrons. This study investigated the class 1, 2 integrons and the antibiotic-resistant genes in the infection agent *Acinetobacter baumannii* produced in respiratory tract samples.

Material and Methods: The study involved 86 *A. baumannii* strains isolated from lower respiratory tract samples collected between March 2014 and March 2015 in the Intensive Care Units. Identification and antibiograms of the isolates were made using conventional methods and Vitek 2 Compact systems. The presence of integrons in strains was investigated by polymerase chain reaction method using specific primer pairs for class 1and class 2 integrase regions. All samples in which integron amplification was carried out were subjected to DNA sequence analysis both by cloning and as a PCR product. The resistance genes were screened by polymerase chain reaction.

Results: Isolates were obtained from specimens including tracheal aspirates (33.7%), bronchoalveolar lavage (29.1%), sputum (22.1%), bronchial washing fluid/bronchial brushing (4.7%), transbronchial biopsy (4.7%), pleural fluid (3.4%) and lung aspirates/abscess (2.3%). While resistance to imipenem, meropenem, ampicillin-sulbactam, ceftazidime, and piperacillin-tazobactam was determined in all isolates, resistance rates to ciprofloxacin, levofloxacin, gentamicin, amikacin and tigecycline were 97.7%, 93.0%, 60.5%, 53.5%and 9.3%, respectively. Colistin was the only antibiotic to which all strains were susceptible. The positivity rates of integrase I and II genes were 45.4%and 9.3%, respectively. Class 1 and 2 integron positivity rates in all isolates were 31.4%and 8.1%, respectively. *blaTEM, blaSHV, blaCTX-M1, blaCTX-M2, blaOXA23, blaOXA40, blaOXA58* were 70.9%, 2.3%, 4.7%, 7.0%, 95.3%, 5.8%and 3.5%, respectively.

Conclusion: Increasing association of multi drug resistant strains and integron resistant genes in *A. baumannii* infections will lead to the failure of antibiotic treatment strategies.

***Keywords**: Acinetobacter baumannii; Lower respiratory tract specimens; Integron; Antibiotic-resistant genes*

ÖZ

Amaç: Antibiyotik direnç genlerinin bakteriler arasındaki yayılımı, enfeksiyon hastalıklarının tedavisinde ciddi sorunlara yol açmaktadır. Son zamanlarda bu direnç genlerin integronlarda da bulunduğu gösterilmiştir. Bu çalışmada alt solunum yollarından alınan kültürlerde üretilen infeksiyon etkeni olan *Acinetobacter baumannii* suşlarında sınıf 1, sınıf 2 integron varlığı ve antibiyotik direnç genleri araştırıldı.

Gereç ve Yöntemler: Çalışmaya, Mart 2014- Mart 2015 tarihleri arasında alt solunum yolu örneklerinden izole edilen toplam 86 A. baumannii suşu dahil edildi. İzolatların tanımlanması ve antibiyogramları konvansiyonel metodlar ve Vitek 2 Compact sistemleriyle yapıldı. Suşlarda integron varlığı, sınıf 1 (intI1) ve sınıf 2 (intI2) integraz bölgeleri için özgül primer çiftleri kullanılarak PCR yöntemiyle araştırıldı. İntegron amplifikasyonunun gerçekleştirildiği tüm örnekler hem klonlanarak hem de PCR ürünü olarak DNA dizi analizine tabi tutuldu. Direnç genleri polimeraz zincir reaksiyonu ile tarandı.

Bulgular: İzolatlar trakeal aspiratlar (%33.7), bronkoalveoler lavaj (%29,1), balgam (%22.1), bronşiyal yıkama sıvısı / bronşiyal fırçalama (%4.7), transbronşiyal biyopsi (%4.7), plevral sıvı (%3.4) ve akciğer aspirasyonları / apse (%2.3) örneklerinden elde edildi. Tüm izolatlarda imipenem, meropenem, seftazidim, piperasillin-tazobaktam, ampisilin-sulbaktama karşı direnç saptanırken, siprofloksasin, levofloksasin, gentamisin, amikasin ve tigesikline karşı direnç sırasıyla %97.7, %93.0, %60.5, %53.5 ve %9.3 olarak tespit edildi. Kolistin, tüm suşlara karşı tek etkin antibiyotikti. Integraz I ve II gen pozitiflik oranı sırasıyla %45.4 ve %9.3 iken, sınıf 1 ve 2 integron pozitiflik oranı tüm izolatlarda sırasıyla %31.4 ve %8.1 olarak bulundu. *blaTEM, blaSHV, blaCTX-M1, blaCTX-M2, blaOXA23, blaOXA40, blaOXA58* sırasıyla %70.9, %2.3, %4.7, %7.0, %95.3, %5.8 ve %3.5 bulundu.

Sonuç: *A. baumannii* infeksiyonlarında çoklu ilaç direnci olan suşların ve integron direnç genlerinin birlikte artışı, antibiyotik tedavi stratejilerinin başarısızlığına yol açacaktır.

***Anahtar Kelimeler**: Acinetobacter baumannii; Alt solunum yolu örnekleri; İntegron; Antibiyotik direnç genleri*

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INTRODUCTION

Lower respiratory tract infections are a major cause of morbidity and mortality in hospitalized patients. *Acinetobacter baumannii* (*A. baumannii*) causes serious healthcare-related infections and its incidence has increased with many strains that have become resistant to many antibiotic classes over the past decades (1).

Mutations and transfer of resistance determinants in multi drug resistant (MDR) isolates via plasmids, transposons and integrons have caused an increase in *A. baumannii* infections. This has now become a major problem worldwide (2,3).

In recent years, the presence of mobile DNA elements has been implicated in the transfer of bacteria-resistant genes from bacteria, and these may be integrated in the bacteria genome with a 'site-specific' recombination mechanism causing resistance. These mobile DNA elements are known as integrons and are found in plasmids and transposons. Integrons have been implicated in the epidemiological spread of resistance to antibiotics between isolates (4). The increasing prevalence of β-lactamase enzymes has reduced susceptibility to carbapenems. Class D β-lactamases (OXA-type) and Ambler class B metallo-β-lactamase provide the most significant contributions to carbapenem resistance. Another resistance mechanism is clavulanic-acid-inhibited extended-spectrum β-lactamases (ESBLs), consisting of PER-1, PER-2, VEB-1, MBLs, VIM-1/4, VIM-2 and IMP-1,2,4,5,6 type genes. If MDR *A. baumannii* infections are not brought under control, they may cause nosocomial epidemics, and these may spread among hospitals, between cities and even across countries. Investigation of the resistance mechanisms of MDR *A. baumannii* will therefore represent an important step in combating such infections (5,6).

The purpose of this study was to investigate class 1 and 2 integrons of the infection agent in *A. baumannii* strains isolated from lower respiratory tract clinical samples and to characterize the antibiotic-resistance genes carried by these.

MATERIALS AND METHODS

Bacterial Isolates and Antimicrobial Sensitivity Tests

The study investigated 86 non-duplicate samples taken from patients in Recep Tayyip Erdoğan Training and Research Hospital (RTETRH) intensive care units (ICU) between March 2014 and March 2015. Over the one-year study period, 1698 patients were admitted to ICU of 36,840 hospitalized patients in the RTETRH. Respectively there are 9, 8, 6, 16 beds in the surgery intensive care unit, the medical intensive care unit, the cardiovascular surgery intensive care unit, the coronary care unit. All intensive care units have ventilator equipment.

Samples were sent to the microbiology laboratory and were confirmed as producing the infection agent. The clinical isolates were identified using conventional methods and the Vitek 2 Compact system (BioMerieux, France). Isolates were evaluated for sensitivity to amikacin, gentamicin, ciprofloxacin, levofloxacin, ceftazidime, piperacillin-tazobactam, trimethoprim/sulfamethoxazole, meropenem, imipenem, sulbactam/ampicillin, colistin, and tigecycline. *Pseudomonas aeruginosa* ATCC 27853 was used as the quality control strain. The antimicrobial sensitivity experiments were evaluated based on Clinical and Laboratory Standards Institute criteria (7). For tigecycline, the E test was applied and the MIC (Minimum Inhibitor Concentration) results determined were evaluated based on the criteria recommended by the Food and Drug Administration for Enterobacteriaceae (≤2 mg/L sensitive, 4 mg/L moderate sensitivity and ≥8 mg/L resistant) (8).

DNA Extraction and PCR Assays for Integrons

Genomic DNA was extracted from bacterial suspensions incubated overnight in Luria Broth with

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oscillations at a temperature of 37° C. These suspensions were then subjected to centrifugation at 13,000 rpm for 5 min. Pellets were subsequently placed into 500 uL distilled water and boiled for 10 min. In the final stage, debris was subjected to centrifugation at 13,000 rpm for 5 min. Five microliters were extracted from each supernatant for use as a template for PCR. Resistance genes and integron-specific PCR assays were applied according to the protocol previously described by Cicek et al (9).

The genes and primers used in PCR to determine the resistance of the genes are shown in Table 1. All PCR findings were evaluated on 1%agarose containing 0.5 mg/L ethidium bromide prior to visualization under ultraviolet light. A 100 bp DNA marker in the gel controls was used as a molecular marker (100 bp DNA Ladder, New England Biolabs, UK).

Base Sequence Analysis: Base sequence analysis was applied to all the samples by cloning after integron amplification (Macrogen, Holland). Competent cells of *Escherichia coli* JM101 root were prepared using the calcium chloride method. In order to determine the base sequence, ligation was achieved to the pGEM-T easy vector (Promega, USA) of the PCR products, following the manufacturer's protocol. The ligation products were transformed to the previously prepared *E. coli* JM101 competent cells. Cells containing plasmids carrying the PCR product were planted by smearing on petri dishes with LB agar with ampicillin (50µg/ml) containing 1mM IPTG and X-Gal. Cells were separated by examination of the formation of a blue-white color.

The plasmid was isolated by selecting a white colony from each petri dish (Wizard Plus SV Minipreps DNA Purification System, Promega, USA) and was maintained in agarose gel by cutting with EcoRI restriction endonuclease enzyme. PCR product was used as a control and for nucleotide sequence analysis. Plasmids giving the same fragment as the PCR product were regarded as positive and were sent to Macrogen Inc. (Amsterdam, Holland). Base sequence analysis of the DNA sequence in the clone plasmids was performed using T7 promoter and SP6 primers on pGEM-T. For Bioinformatic comparisons, BLAST analyses were applied on the NCBI and Expasy (http://blast.ncbi.nlm.nih.gov/Blast.cgi) websites. Amino acid sequences were used for comparisons.

The study was approved by the local ethics committee (Recep Tayyip Erdoğan University Ethics Committe of Non-interventional Research, date: 05.05.2017; number: 2017/74).

RESULTS

Eighty-six clinical *A. baumannii* strains were taken from patients in the ICU of the RTETRH, Turkey, over a 12-month period. The majority of non-duplicate isolates were from tracheal aspirates 29/86 (33.7%), bronchoalveolar lavage 25/86 (29.1%), sputum 19/86 (22.1%), bronchial washing fluid/bronchial brushing 4/86 (4.7%), transbronchial biopsy 4/86 (4.7%), pleural fluid 3/86 (3.4%) and lung aspirates/abscess 2/86 (2.3%).

Analysis of the antibiotic resistance rates of the *A. baumannii* strains revealed that all the strains were resistant to ampicillin-sulbactam, piperacillin-tazobactam, ceftazidime, imipenem, and meropenem, and all were sensitive to colistin. Forty-six (53.5%) samples with sensitivity to colistin only were defined as Extensively Drug-Resistant (XDR). The sensitivity rates of the isolates to the antibiotics tested are shown in Table 2.

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Table 1: Primers used in the study

Primers	5'-3'	Amplicon Size	Tm	References
bla$_{\text{OXA-51-like}}$	F: TAATGCTTTGATCGGCCTTG	353	52°C	10
	R: TGGATTGCACTTCATCTTGG			
bla$_{\text{OXA-40-like}}$	F: GGTTAGTTGGCCCCCTTAAA	246	52°C	10
	R: AGTTGAGCGAAAAGGGGATT			
bla$_{\text{OXA-23-like}}$	F: GATCGGATTGGAGAACCAGA	501	52°C	10
	R: ATTTCTGACCGCATTTCCAT			
bla$_{\text{OXA-58-like}}$	F: AAGTAT TGGGGCTTGTGCTG	599	52°C	10
	R: CCCCTCTGCGCTCTACATAC			
bla$_{\text{CTX-M1}}$	F: GCGTGATACCACTTCACCTC	260	55°C	11
	R: TGAAGTAAGTGACCAGAATC			
bla$_{\text{CTX-M2}}$	F: TGATACCACCACGCCGCTC	341	55°C	11
	R: TATTGCATCAGAAACCGTGGG			
bla$_{\text{SHV}}$	F: ATGCGTTATATTCGCCTGTG	843	55°C	11
	R: TTAGCGTTGCCAGTGCTC			
bla$_{\text{TEM}}$	F: AGTATTCAACATTTYCGTGT	847	56°C	11
	R: TAATCAGTGAGGCACCTATCTC			
bla$_{\text{NDM}}$	F: GAGATTGCCGAGCGACTTG	497	57°C	10
	R: CGAATGTCTGGCAGCACACTT			
bla$_{\text{VIM}}$	F: ATTGGTCTATTTGACCGCGTC	780	58°C	10
	R: TGCTACTCAACGACTGAGCG			
bla$_{\text{IMP}}$	F: CATGGTTTGGTGGTTCTTGT	488	56°C	10
	R: ATAATTTGGCGGACTTTGGC			
bla$_{\text{GES}}$	F: ATGCGCTTCATTCACGCAC	863	56°C	10
	R: CTATTTGTCCGTGCTCAGGA			
bla$_{\text{KPC}}$	F: CGTTCTTGTCTCTCATGGCC	796	52°C	12
	R: CCTCGCTGTGCTTGTCATCC			
bla$_{\text{VEB}}$	F: ATTTCCCGATGCAAAGCGT	542	55°C	10
	R: TTATTCCGGAAGTCCCTGT			
bla$_{\text{PER}}$	F: ATGAATGTCATCACAAAATG	927	50°C	10
	R: TCAATCCGGACTCACT			
bla$_{\text{OXA-48-like}}$	F: TTGGTGGCATCGATTATCGG	743	57°C	12
	R: GAGCACTTCTTTTGTGATGGC			

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Table 2: The antibiogram results of the isolates

Antibiotics	**S (n %)**	**I (n %)**	**R (n %)**
Amikacin (AK)	35 (40.7)	5(5.8)	46 (53.5)
Gentamicin (GN)	23 (26.7)	11 (12.8)	52 (60.5)
Levofloxacin (LEV)	2 (2.3)	4 (4.7)	80 (93.0)
Ciprofloxacin (CIP)	0	2 (2.3)	84 (97.7)
Ceftazidime (CAZ)	0	0	86 (100.0)
Ampicillin-sulbactam (SAM)	0	0	86 (100.0)
Piperacillin/tazobactam (TZP)	0	0	86 (100.0)
Imipenem (IPM)	0	0	86 (100.0)
Meropenem (MEM)	0	0	86 (100.0)
Colistin (COL)	86 (100.0)	0	0
Tigecycline (TIG)	44 (51.2)	34 (39.5)	8 (9.3)
Trimethoprim/sulfamethoxazole (SXT)	11(12.7)	12(14.0)	63(73.3)

Table 3: Distribution of integrase 1 and 2 genes, class 1 and 2 integron gene cassettes and resistant genes

Integrons, genes	**n**	**%**
Int I1	39	45.4
Class 1Integron	27	31.4
Int I2	8	9.3
Class 2 Integron	7	8.5
bla$_{\text{OXA-51-like}}$	86	100.0
bla$_{\text{OXA-23-like}}$	82	95.3
bla$_{\text{OXA-40-like}}$	5	5.8
bla$_{\text{OXA-58-like}}$	3	3.5
blaTEM	61	70.9
blaSHV	2	2.3
blaCTX-M1	4	4.7
blaCTX-M2	6	7.0
bla$_{\text{OXA-23-like}}$/*bla*$_{\text{OXA-51-like}}$	83	96.5
bla$_{\text{OXA-23-like}}$/*bla*$_{\text{OXA-51-like}}$ /*bla*$_{\text{TEM}}$	59	68.6
bla$_{\text{OXA-23-like}}$/*bla*$_{\text{OXA-51-like}}$/*bla*$_{\text{CTX-M2}}$	6	7.0
bla$_{\text{OXA-23-like}}$/*bla*$_{\text{OXA-51-like}}$/*bla*$_{\text{CTX-M1}}$	4	4.7
bla$_{\text{OXA-58-like}}$/*bla*$_{\text{OXA-51-like}}$	3	3.5
bla$_{\text{TEM}}$/ *bla*$_{\text{SHV}}$	1	1.7
bla$_{\text{OXA-40-like}}$/ *bla*$_{\text{CTX-M1}}$/ *bla*$_{\text{SHV}}$	1	1.7
bla$_{\text{TEM}}$/*bla*$_{\text{SHV}}$/ *bla*$_{\text{CTX-M1}}$/ *bla*$_{\text{CTX-M2}}$	1	1.7
bla$_{\text{TEM}}$/*bla*$_{\text{CTX-M2}}$	1	1.7

Of the 86 strains, integrase I gene positivity was determined in 39 (45.4%), and the class 1 integron gene cassette rate was 27 (31.4%). Integrase II gene positivity was positive in 8 (9.3%) and class 2 gene cassette was determined in 7(8.1%) isolates.

According to the resistance gene analysis, all of 86 (100%) isolates were shown to carry the blaOXA-51 gene. All strains were negative for *blaOXA-48, blaNDM, blaVIM, blaIMP, blaGES, blaKPC, blaPER* and *blaVEB*. The distribution of the blaOXA-23, blaOXA-40, blaOXA-58, suppressed beta-lactamase resistance genes (*blaTEM-1, blaCTX-M2, blaCTX-M1 blaSHV*) and the resistance genes combinations (*blaOXA-23-like/blaOXA-51-like, blaOXA-58-like/blaOXA-51-like, blaOXA-23-like/blaOXA-51-like/blaCTX-M1, blaOXA-23-like/blaOXA-51-like/blaCTX-M2, blaOXA-23-like/blaOXA-51-like/blaTEM, blaTEM/blaSHV, blaOXA-40-like/blaCTX-M1/blaSHV, blaTEM/blaSHV/blaCTX-M1/ blaCTX-M2*, and *blaTEM/blaCTX-M2)* are shown in Table 3.

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Diagnosis of ventilator-associated pneumonia (VAP) and other healthcare-related pneumonias was based on the criteria set out by the Centers for Disease Control and Prevention. Speed of infection and rates of use of invasive tools were determined as required by the National Hospital Infections Surveillance Web. VAP was defined as a pneumonia involving mechanical ventilation for >2 calendar days at the time of the event, the day of ventilator attachment being taken as day 1, and the ventilator being in place on the date of the event or the previous day.

The number of patients hospitalized at ICU during the study period was 1698, the number of hospital days was 8686, the ventilator use rate was 0.5%, number of VAPs was 52, the VAP rate was 0.14%, and the VAP infection density was 0.35%. Ventilators use and VAP rates were compatible with that reported rates in our country. Both rates were 50%to 75%according to Ministry of Health 2014 National Hospital Infections Surveillance Web reports.

The most common microorganism species in VAP infections were *A. baumannii* in 58.49%, pseudomonas in 20.75%, and Acinetobacter spp. in 1.89%(Table 4). We found that carbapenem resistance rate was more than 90%in our hospital and this rate was compatible the antibiotic resistance report of the microorganism species in the national surveillance reports of the Ministry of Health in our country.

Table 4: The microorganism species in VAP infections

Microorganism Specieses	**Number**	**Rate %**
Acinetobacter baumannii	31	58.49
Pseudomonas aeruginosa	11	20.75
Enterobacter aerogenes	2	3.77
Serratia marcescens	2	3.77
Stenotrophomonas maltophilia	2	3.77
Acinetobacter spp.	1	1.89
Escherichia coli	1	1.89
Coagulase-negative staphylococcus (S. epidermidis, S. haemolyticus, S. hyicus, S. lugdunensis, S. saprophyticus)	1	1.89
Pseudomonas spp.	1	1.89

DISCUSSION

MDR *A. baumannii* infections with severe and mortal courses are increasing throughout the world. The treatment and control of *A. baumannii*-related hospital infections is extremely difficult. *A. baumannii* strains have most commonly been isolated from lower respiratory tract samples in previous studies (13-14).

MDR Acineteobacter strains create selective suppression of commonly employed antibiotics, resulting in rapidly spreading and prolonged outbreaks in the hospital environment. In the current study, the most effective antibiotics were identified as colistin (100.0%) and tigecycline (97.3%), while all strains were resistant to ampicillin-sulbactam, piperacillin-tazobactam, ceftazidime, imipenem and meropenem. Rates of resistance to ciprofloxacin, levofloxacin, trimethoprim-sulfamethoxazole, gentamicin and amikacin were 97.7%, 93.0%, 73.3%, 60.5%and

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53.5%, respectively. The rates of resistance to antibiotics were consistent with those reported in previous studies (13-16).

Among the beta-lactam agents, carbapenems are regarded as the first line agents in the empirical treatment of several serious infections, since they exhibit a very broad-spectrum effect, antimicrobial activity, good clinical efficacy and a positive safety profile. However, in previous studies from Turkey and other countries, as in the current study, resistance to carbapenems in *A. baumannii* isolates has approached close to 100%(13-16). Carbapenem resistance has severely restricted the treatment options in Acinetobacter infections, and polymyxins, regarded as the last choice in treatment, have started to be frequently used. The mechanisms related to colistin resistance and the genetic transfer routes of resistance between bacteria have not yet been fully clarified. In some studies, colistin resistance has been determined at a high level of 0-21.3%(13,17-18). No colistin-resistant strains were identified in the present study. The differences in the resistance rates may be related to excessive use of these antibiotics against MDR *A. baumannii.* However, colistin still seems to be the best choice for the treatment of XDR *A. baumannii.*

One long-term study of tigecycline activity in China (19) reported protection of stability with MIC90 ≤4 mg/L, while another study from China reported tigecycline sensitivity at 76.4%in phlegm cultures of patients with MDR-AB and XDR-AB pulmonary infections (20). Previous studies from Turkey have reported resistance rates of 3.8%-27.6%(21-22). In the current study, while tigecycline resistance was determined at 9.3%, the intermediate sensitivity rate was particularly high at 39.5%. Ceftriaxone, cefoperazone-sulbactam, moxifloxacin, and carbapenems are mainly used to treat patients diagnosed with pulmonary infection in our ICU. Other antibiotics are rarely employed.

Since integrons are carried by plasmid or transposons, they are able to pass from one bacterium to another and even from one integron to another within the gene cassette. This strong antibiotic selective suppression causes the spread and transport of antibiotic resistance determinants (14, 23). The presence of class 1 integrons is extremely widespread in Acinetobacter species. In the current study, class 1 integron positivity was detected at a rate of 45.4%. High integron transport is important in terms of showing the potential for the spread of MDR *A. baumannii* isolates obtained from clinical samples in our hospital. The presence of integrons in Acinetobacter has been implicated in resistance to beta-lactam, aminoglycoside, chloramphenicol, trimethoprim and rifampicin (4). It is known that TMP-SMZ resistance genes (dhfr) are located in integrons (9). In this study, susceptibility rates to SXT were determined as 12.7%(11/86) in all isolates and 6.4%(3/47) in isolates containing integron. Class D OXA-type enzymes are recognized as the main contributor to carbapenem resistance in *A. baumannii*. blaOXA$_{51}$ is originally intrinsic to *A. baumannii* and was determined to be 100%positive in our study. It was also used for validation of *A. baumannii* identification. The OXA$_{23\text{-like}}$ gene is the best known source of carbapenemase resistance and is transferred via plasmids or chromosomally (6). The *bla*$_{\text{OXA-23-like}}$ resistance rate in our study was 95.3%. In other studies, *bla*$_{\text{OXA-23-like}}$ genes have been found in 91.03 - 100.0%of isolates, indicating their responsibility for the dominant carbapenem resistance gene in local *A. baumannii* isolates (3,13,24). OXA$_{58\text{-like}}$ producers have generally been reported from Asian and Middle Eastern countries. The highest OXA$_{58\text{-like}}$ enzyme production to date was detected in India, at 15%(25). The highest resistance rate reported from Turkey is 53.3%(26). In the current study, OXA$_{58\text{-like}}$ was determined at 3.5%. OXA$_{58\text{-like}}$ production varies significantly even within countries. ESBLs are mainly transferred by plasmids and are also part of the enzyme family consisting of the

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TEM, SHV, and CTX-M groups, and VEB, PER, GES, and KPC. In a study from Saudi Arabia, *A. baumannii* strains were determined with bla_{TEM} (71%), and bla_{CTX-M} (81%) (27). In another study from Iran in 2015, bla_{CTX-M} was not identified, but bla_{TEM} (20%), bla_{SHV} (58%) and bla_{VIM} (30%) were determined (28). In another study from Saudi Arabia, Aly MM et al., determined a lower rate of bla_{TEM}, at 14%(15). While a high rate of TEM (70.9%) was observed in the current study, this was followed by CTX-M2 at 6.97%, CTX-M1 at 4.6%, and SHV at 2.3%. Carbapenemase genes from class A, bla_{KPC} and bla_{GES} types were detected in *A. baumannii*. The prevalence of bla_{GES} in the USA and Kuwait has been reported as 95.2%and 0%, respectively (18, 29). bla_{KPC} is very rarely seen in *A. baumannii*. In a 12-centre study from Turkey by Beris et al., TEM was identified as the most widespread type in *A. baumannii* isolates at a rate of 55.7%, followed by CTX-M2 at 12.1%, CTX-M1 at 8.1%, SHV at 7.7%and GES at 1.5%. The DNA sequence analysis applied for GES positive isolates identified the GES-11 type (16). In another study from Turkey, by Çiçek et al, GES-11 was determined in 16 of 101 *A. baumannii* isolates and GES-22 in eight. That study was also the first in the literature to demonstrate GES-11 in *A. baumannii* in a Turkish population (10). Zeka et al., reported that GES-11 was detected in five out of 60 isolates in samples from Turkey (30). In addition to GES-11, an *A. baumannii* BM4674 strain was also identified in France by Moubareck et al (31). The bla_{PER-1} gene has also been identified in ceftazidime-resistant *A. baumannii* strains across the world (6). While PER positivity was determined in the current study, rates between 18%and 76.3%have been reported in various previous studies (13, 15, 32). bla_{VEB}, bla_{KPC}, bla_{PER} and bla_{GES} were not detected in *A. baumannii* isolates. In the current study, no $bla_{VIM,}$ bla_{IMP} and bla_{NDM} positivity was determined, while NDM-1 was reported for the first time in three clinical isolates in Greece in 2016 and in Ethiopia in 2017 (33, 34). NDM-1 positivity in Nepalwas reported at 13.6 %(25). Similarly, to the results of the current study, studies from Kuwait in 2015 and from Iran in 2016 determined nobla_{VIM} and bla_{IMP} genes any *A. baumannii* isolates (18, 35).

In conclusion, sensitivity to colistin persisted in *A. baumannii* isolates exhibiting high resistance to most antibiotics used in antimicrobial treatment, including carbapenems. A marked increase over time was observed in *bla*-$_{OXA-23-like}$ positive isolates in carbapenem-resistant *A. baumannii* clinical isolates. All centers should develop resistance profiles and systematic surveillance networks for the control and observation of resistant isolates. The present study may be regarded as a precursor in terms of revealing the molecular properties, and particularly the presence of integrons in *A. baumannii* isolates produced in lower respiratory tract samples from patients in our hospital. This study can be seen as a pioneer in terms of the molecular properties and especially the presence of integron in *A. baumannii* isolates produced in the lower respiratory tract samples from patients during the years when the causative microorganism was the most common agent. Further studies of these bacteria with epidemiological data will yield a better understanding of the mechanisms causing resistance and the risk factors involved and will thus permit more effective and productive application of infection control and treatment protocols, especially in ICU.

Declaration

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