Original Article

Investigation of the Clonal Associations in *Acinetobacter baumannii* Strains Isolated from the Respiratory Samples of Patients in a Tertiary Research Hospital

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ABSTRACT

Objective: The *blaOXA* resistance genes and ISAba1 were examined in 70 samples from lower respiratory tract of hospitalized patients. **Materials and Methods:** Of the 67 isolates obtained, almost half (46.3%) of them were from endotracheal aspirate, and most were collected from the intensive care units of the reanimation (37.3%) and internal medicine (32.8%) units. **Results:** Three samples from the internal medicine intensive care unit had positive cultures. Of the multidrug resistant (MDR) samples, 70 isolates (>50%) were moderately sensitive, while fewer (10%) were resistant to tigecycline. In contrast, 100% were sensitive to colistin. All strains were found to be positive for *blaOXA*-23-like and *blaOXA*-51-like genes, whereas no *blaOXA*-40-like and *blaOXA*-58-like genes were detected. The ISAba1 positivity rate was 90.0%. Pattern 5 was mainly identified among the 22 different patterns. Of note, 50% of Pattern 5 was found in the patients of the internal medicine intensive care unit, and a third was associated with ventilator-associated pneumonia. Importantly, the internal medicine unit's equipment was found to be culture positive. **Conclusion:** Findings obtained from this study suggest that isolates can easily spread through the hospital via isolate cross-contamination caused by health personnel. These contaminating isolates may be able to maintain their presence within the hospital for a long time.

Keywords: *Acinetobacter baumannii, clonal relation, Diversi-Lab, rep-PCR*

Introduction

Lower respiratory tract (LRT) infections are a major source of morbidity and mortality in hospitalized patients.[1] Gram-negative bacterial pathogens, particularly *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, are responsible for a significant portion of nosocomial LRT infections.[2] The most important factor affecting the treatment response is the type of causative agent for the antibiotic susceptibility and the patient.[1,2] *A. baumannii*, the most common type of the *A. baumannii* complex group, is a gram-negative, non-fermentative bacteria causing severe hospital-originated infections such as pneumonia, sepsis, meningitis, urinary tract, and wound infections.[3] Mutations and acquired resistance determinants result in an increased number of multidrug-resistant (MDR), extensively drug-resistant (XDR) or pan drug-resistant (PDR) *A. baumannii* isolates worldwide. Carbapenem-resistant strains have caused life-threating problems in recent years.[4] The resistance mechanism in *A. baumannii* stems mainly from the presence of carbapenem hydrolyzing class-D oxacillinases (CHDLs; CHDL/OXA enzymes). OXA-type carbapenemases are mainly encoded by *blaOXA*-23-like, *blaOXA*-40-like, *blaOXA*-58-like, *blaOXA*-143-like, and *blaOXA*-235-like

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genes and are chromosomally encoded by intrinsic *blaOXA*-51-like and acquired *blaOXA*-23-like genes. CHDLs exhibit weak carbapenem hydrolysis but can trigger resistance when overexpressed. This resistance is mediated through a combination of naturally low permeability to beta-lactams, efflux pumps, and ISAba elements located upstream of the gene, providing strong promoter activity.[5]

The ISAba-1 segment located in the upstream region of the *blaOXA*-51 gene induces the expression of the beta-lactamase gene as a transcriptional promoter. Determining the relationship between genetic and epidemiological factors requires the genomic fingerprinting of clinical isolates. For molecular typing, the repetitive extragenic palindromic sequence-based polymerase chain reaction (rep-PCR) is one of the most effective methods that has been made commercially available (DiversiLab microbial typing system; BioMe'rieux, Marcy L'Etoile, France).[6]

In this study, we aimed to determine the *OXA* resistance genes and ISAba-1 by using rep-PCR. Their clonal relationship in *A. baumannii* isolates obtained from LTR samples of hospitalized patients was then determined.

Materials and Methods

Bacterial isolates and antibiotic susceptibility testing

A total of 70 (67 nonduplicates and 3 ambient) specimens from the LRT of inpatients hospitalized in a training and research hospital of a medical school between June 2014 and February 2015 with positive cultures of *A. baumannii* were included in this study.

The study, funded by the Scientific Research Fund of the University (Project code: 2016.53007.106.03.02), was approved by the Ethics Committee (decision #2016/23).

Our hospital is located in the Black Sea region of Turkey and serves around half a million people. In 1.5 years, 52828 patients were hospitalized. Of these, 1379 were admitted to intensive care units (ICU) (surgical [SICU, 9 beds]; medical [MICU, 8 beds]; anesthesia-reanimation [ARICU, 7 beds]; cardiovascular surgery [CVSICU, 6 beds]; coronary [CICU, 16 beds]). Mechanical ventilation is provided at all ICUs, and two of them have isolation rooms.

Gram staining, oxidase testing, conventional biochemical tests (triple sugar, urease, citrate, motility), and microbial identification via the Vitek 2 (BioMeriéux, France) automated system were used to determine the type of microorganisms. The antibiotic susceptibilities of the isolates were determined by a disk diffusion test (DDT) and evaluated according to the Clinical and Laboratory Standards Institute (CLSI) criteria. Susceptibilities of strains to tigecycline and colistin were interpreted according to the British Society for Antimicrobial Chemotherapy criteria.[7] *P. aeruginosa* ATCC 27853 was used as the quality control strain. The isolates were stocked in 10% glycerol buoyant at -20°C before the molecular studies.

DNA isolation and reproduction of resistance genes

Bacterial stocks were plated on 5% sheep blood agar, and a single colony was inoculated in 3 mL Luria-Bertani liquid medium (1% tryptone, 0.5% yeast extract, 0.5% NaCl, pH: 7.4) and incubated in a shaking incubator for 16-h at 37°C. The culture was taken in a 1.5 mL microcentrifuge tube for DNA isolation and settled for 5 min at 13,000 rpm. The top of the precipitate was obtained and washed sequentially with 1 mL of Tris buffer and sterile distilled water. The final precipitate was dissolved in 500 µL of deionized water, disintegrated by heating at 95°C for 10 min, and precipitated by centrifugation at 13,000 rpm for 5 min. The upper part of the liquid was transferred to a tube and used as template DNA in 5 µL PCR.

Multiplex PCR for the detection of blaOXA

Multiplex PCR was used to detect *blaOXA*-23-24-51 and 58-like genes using the primers listed in Table 1. Polymerase chain reactions were performed in a final volume of 50 µL and included 5 µL of genomic DNA, 20 pM of each primer, 10 µL of 10 × polymerase activity buffer, 3 µL of 25 mM $MgCl_2$, 200 µM of each Dntp, and 1.5 U of *Taq* Polymerase (Fermentas Thermo Fisher Scientific Inc., Waltham, USA). PCR amplification was performed using initial denaturation at 94°C for 3 min followed by 30 cycles of 25 s at 94°C, 40 s at 52°C and 50 s at 72°C for *blaOXA* genes. All polymerase chain reaction results were analyzed on 1% agarose containing 0.5 mg ethidium bromide. Samples were subsequently visualized under UV light and evaluated according to their molecular size.

Clonal relationship determination

The clonal relationship of the *A. baumannii* isolates was determined by the rep-PCR DiversiLab system (bioMeriéux, France). The DNA extraction was performed according to the manufacturer's recommendations, taking 1 µL of the wells per day of the bloody agar-passaged isolates with the UltraClean ™ Microbial DNA Isolation Kit (MoBio Laboratories, USA). The DNA quantities were measured by a NanoDrop instrument and diluted to 35 ng/µL. Bacterial DNAs were amplified using the Acinetobacter DNA fingerprinting kit DiversiLab ™ DNA Fingerprinting Kit (BioMeriéux, France). The thermal cycling

parameters were initial denaturation at 94°C for 2 min, denaturation at 35°C for 30 s at 94°C, primer binding at 50°C for 30 s, chain extension at 70°C for 90 s, and final elongation at 70°C for 3 min. Microfluidic electrophoresis was performed on the Agilent 2100 Bioanalyzer (Agilent Technologies Inc, USA) with the Diversilab ™ DNA LabChip Kit (BioMerieux, France). Rep-PCR fingerprint results were obtained with the internet-based DiversiLab analysis program (BioMerieux, France).

For determining the similarities of the samples and to generate dendrograms, the Pearson correlation coefficient and the unweighted pair group method with arithmetic mean method were used. The isolates showing similarity of more than 95% were considered to be the main clone while the isolates showing over 97% similarity as the main clone was considered to be subtype (indistinguishable). The isolates with similarity rates below 95% (>2 band differences) were evaluated as different clones.

Results

Patient isolates were obtained from 67 specimens of endotracheal aspirates (ETA) (n = 31, 46.3%), bronchoalveolar lavage (n = 7, 10.4%), sputum (n = 21, 31.3%), bronchial washing fluid or brushing (n = 3, 4.5%), lung aspirates or abscesses (n = 1, 1.5%), and pleural fluid (n = 4, 6.0%). Among the 47 medium-culture samples obtained at three different times from 47 different mediums in all ICUs, three *A. baumannii* were isolated from the MICU equipment such as the connection tubes of the aspiration unit and patient beds or from the nurses' hands. Of all of the isolates, 9/67 (13.4%) were from patients hospitalized in the SICU, 22/67 (32.8%) in the MICU, 25/67 (37.3%) in the ARICU, 8/67 (12.0%) in the pulmonary diseases department, and 3/67 (4.5%) in the infectious diseases department.

Three isolates were from ambient cultures from the isolation chamber of IMICU. Disk diffusion tests revealed that 67 of the study isolates and three peripheral isolates were MDR strains. Seventy isolates showed 100% resistance to piperacillin-tazobactam, ampicillin-sulbactam, trimethoprim/sulfamethoxazole, levofloxacin, ciprofloxacin, ceftazidime, imipenem, and meropenem, while the rate was 52.9% and 55.7% for amikacin and gentamicin, respectively. The isolates showed 52.9% sensitivity to tigecycline. Nearly 35.7% were moderately susceptible and 11.4% were resistant. All isolates were sensitive to colistin 100% [see Table 2].

Table 1: The primers used for PCR

Primers	5'-3'	Amplicon size	T	Reference
$bla_{\text{OXA-51-like}}$	F: TAATGCTTTGATCGGCCTTG	353	52°C	15
	R: TGGATTGCACTTCATCTTGG			
$bla_{\text{OXA-24/40-like}}$	F: GGTTAGTTGGCCCCCTTAAA	246	52°C	15
	R: AGTTGAGCGAAAAGGGGATT			
$bla_{\text{OXA-23-like}}$	F: GATCGGATTGGAGAACCAGA	501	52°C	15
	R: ATTTCTGACCGCATTTCCAT			
$bla_{\text{OXA-58-like}}$	F: AAGTAT TGGGGCTTGTGCTG	599	52°C	15
	R: CCCCTCTGCGCTCTACATAC			
ISAba1	F: CACGAATGCAGAAGTTG	549	56°C	15
	R: CGACGAATACTATGACAC			

T: temperature

Table 2: Antibiogram results of the isolates (n=70)

Antibiotics	Sensitive (S)	Intermediate (I)	Resistant (R)
	n (%)	n (%)	n (%)
Amicasin (AK)	30 (42.8)	3 (4.3)	37 (52.9)
Gentamicin (GN)	20 (28.6)	11 (15.7)	39 (55.7)
Ciprofloxacin (CIP)	0	0	70 (100.0)
Levofloxacin (LEV)	0	0	70 (100.0)
Ceftazidime (CAZ)	0	0	70 (100.0)
Imipenem (IPM)	0	0	70 (100.0)
Meropenem (MEM)	0	0	70 (100.0)
Ampicillin-sulbactam (SAM)	0	0	70 (100.0)
Piperacillin/tazobactam (TZP)	0	0	70 (100.0)
Trimethoprim/sulfamethoxazole (SXT)	0	0	70 (100.0)
Colistin (COL)	70 (100.0)	0	0
Tigecycline (TIG)	37 (52.9)	25 (35.7)	8 (11.4)

All strains were positive for *blaOXA*-23-like and *blaOXA*-51-like genes. No *blaOXA*-40-like and *blaOXA*-58-like genes were detected, and the ISAba-1 positivity rate was 90%.

Twenty-two different (P1-P22) patterns of 70 *A. baumannii* isolates were typed by rep-PCR, P5 was identified as the main pattern, and the similarity rate among all was 97%. Twenty-two (97.3%) of the P5 formed the largest cluster of isolates, and 3 different types (P5a-P5c) were separated. The subtypes in the P5 pattern were clustered as P5a (n = 2), P5b (n = 15), and P5c (n = 5) [Figure 1].

Other patterns were P3 with 6 (97.1%) isolates. The P6 and P8 patterns also contained 6 isolates, with fewer isolates comprised of three isolates for P1, P2, P7, and P11. The P9 and P10 were comprised of 2 isolates, with one isolate clustered independently of the other patterns for P12, P13, P14, P15, P16, P17, P18, P19, P20, P21, and P22. The first isolates in P5 were from the ETA samples of two patients hospitalized in the MICU on June 12, 2014, and in the CICU on June 12, 2014, while the last isolate was isolated from the ETA culture of a patient in the MICU on December 7, 2014. It was noted that 50% of the isolates constituting P5 were isolated from patients hospitalized in the MICU and 31.8% were ETA isolates of patients with ventilator-associated pneumonia (VAP). In the MICU's isolation chamber, patient beds, nurses' hands, and aspiration units all served as breeding grounds for the connection hose, and all were placed in P5 in these isolates.

During the study period, 1379 patients were hospitalized in adult ICUs, and 3790 patients underwent mechanical ventilation or reintubation. The VAP rate was 15/1000 ventilator days among all adult ICU patients, with an average mechanical ventilator utilization rate of 53%. The mean duration of the ICU stay was 36 ± 19 (12–89) days.

The VAP rate in the ICUs was the highest at 19.26%. Surveillance data in the adult ICUs, VAP rates, numbers of patients, and length of hospitalization are shown in Tables 3-5.

All *Acinetobacter spp*. isolates were carbapenem-resistant. There was no resistance to colistin, and all patients were given double or triple combination

Table 3: The infection rate of the hospital (in general)

Infectious disease	Number of infection	Pace	Density
Surgical field infection (SFI)	1	0	0.01
Bloodstream infection	71	0.26	0.66
Ventilator-related pneumonia	40	0.15	0.37
Urinary system infection	16	0.06	0.15
Hospital infections	128	0.47	1.18

Table 4: VIP numbers and pace of in intensive care units (ICU)

Unit	Inpatiens number	Day of stay	Ventilator number	VR-pneumonia	Gadget-use rate	VIP infection pace
Anesthesia ICU	272	1790	1291	10	0.72	7.75
Surgery ICU	287	2032	1237	12	0.61	9.7
Internal med ICU	392	1944	727	14	0.37	19.26
Cardiovascular ICU	428	1250	540	4	0.43	7.41
Total adult ICU	1379	7016	3795	40	0.53	0.15

VR: ventilation-related, ICU: intensive care unit

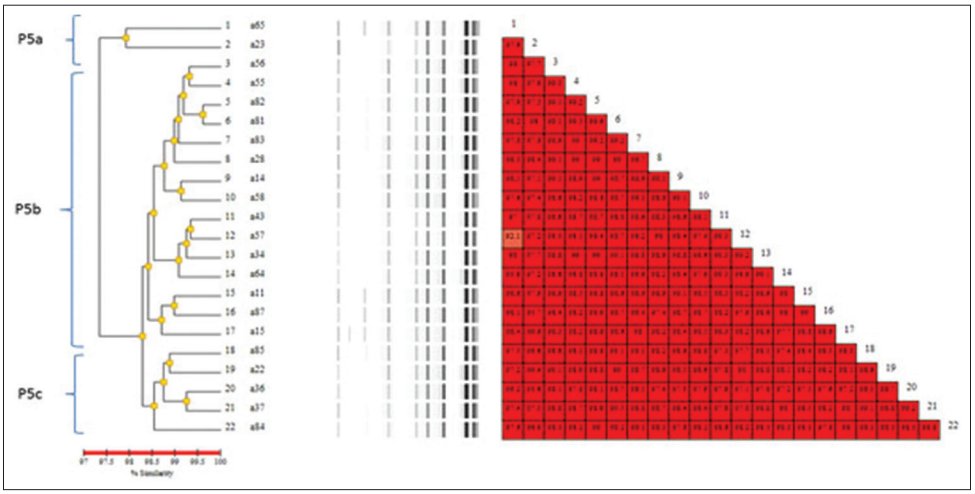

Figure 1: The pattern of P5' subtypes including P5a (n = 2), P5b (n = 15), and P5c (n = 5)

Table 5: Antibiotics preferred for and demographics of VAP cases infected by Acinetobacter spp (*n*=22)

Characteristics	Mean±SD (minimum-maximum) or *n* (%)
Age (years)	67±19 (25–95)
Male	15 (68)
Diagnosis	
Respiratory failure	7 (31.8)
Neurological	6 (27.3)
Trauma	3 (13.6)
Cardiovascular	3 (13.6)
Cardiac arrest	2 (9.2)
Other	1 (4.5)
Underlying disease presence	17 (77.3)
Mortality	13 (59.1)
ICU staying time (days)	36±19 (12-89)
Colistin usage	16 (77.7)
Colistin inhaler usage	12 (54.5)
Meropenem usage	3 (13.6)
Imipenem usage	11 (50.0)
Tigecycline usage	8 (36.3)
Rifampicin usage	6 (27.3)
B-lactam usage	6 (27.3)
Carbapenem-resistant Acinetobacter baumanii	22 (100.0)
Colistin-resistant Acinetobacter baumanii	0
Combined-treatment rate	22 (100.0)
Colistin plus Colistin inhaler combined usage	15 (68.2)

antibiotic treatments. The most preferred antibiotic was intravenous colistin (77.7%), and 15 (68.2%) of the patients were given intravenous colistin plus a colistin inhaler. The mortality rate among the active VAP cases was 13/22 (59.1%) [Table 5].

Discussion

A. baumannii infections are one of the most important causes of hospital-acquired infections worldwide. Given its resistance to dryness and disinfectants, *A. baumannii* could easily persist on hospital surfaces and medical equipment with long-term viability. It is also on the agenda since it displays MDR-including carbapenem at an increasing level and could lead to outbreaks, especially in ICUs.

The most important problem in nosocomial *Acinetobacter* infections is the emergence of antibiotic-resistant strains and the increase in resistance rates.[8,9] It was reported that the epidemiologically dominant types of MDR strains tend to increase the level of resistance if cloning is not performed with genotyping methods of MDR strains obtained from susceptibility tests in local regions.[10] In our study, 97% of the *Acinetobacter* strains were composed of MDR isolates. The immunosuppression of patients in the ICU and the overuse of broad-spectrum antibiotics generally increases the problem of resistance.

Multidrug-resistant (MDR) agents have been reported in the last decade and other *Acinetobacter* species, especially *A. baumannii*, have been among the first to be identified as "re-emerging infectious agents".[11] When the distribution of *A. baumannii*-isolated clinical specimens is examined, respiratory specimens appear to be the most common. While *A. baumannii* isolates show different resistance rates to antibiotics, resistance to beta-lactam antibiotics, aminoglycosides, and fluoroquinolones are often observed.[12] In our study, a total of 70 isolate DDT results revealed that ampicillin-sulbactam, trimethoprim, and meropenem resistance ratios were 100%; for amikacin and gentamycin, they were 52.9% and 55.7%, respectively. The isolates were sensitive to tigecycline; 52.9% and 35.7% were moderately susceptible, and 11.4% were resistant while all of them were sensitive to colistin. MDR in *Acinetobacter* species has led to the intensive use of carbapenems in this treatment. In our study, 100% resistance was determined against the tested carbapenems, and similar ratios were also noted in studies conducted in different countries.

Treatment options of *A. baumannii* infections include fluoroquinolones (e.g. levofloxacin), cefepime, polymyxin E and B, minocycline, doxycycline, and tigecycline.[13] The treatment of non-MDR *A. baumannii* infections usually includes aminoglycosides in combination with a beta-lactam such as piperacillin or imipenem. However, the incidence and high prevalence of all antibiotic-resistant *A. baumannii* strains, including carbapenems and polymyxins, especially colistin, are considered. Colistin or tigecycline is the only remaining effective treatment options in the very low MDR *Acinetobacter* strains. However, increased colistin resistance has been reported along with increased resistance to carbapenems in later surveillance studies in various European countries.[14]

Since colistin should be given at higher doses to achieve effective bactericidal concentrations at infectious tissues, the risk of adverse events is high.[15] To reduce systemic toxicity, colistin sulfate and colistin methanesulfonate formulations have been developed. Inhaled colistin was used as monotherapy or in addition to other systemic, less toxic antibiotics.[16] In the Infectious Diseases of America guidelines which include a meta-analysis of randomized controlled trials, the use of inhaled colistin as adjunctive therapy to intravenous colistin against colistin-sensitive *A. baumannii* was proposed to reduce the mortality.[17] A recently reported systematic review and meta-analysis showed that breast cholestasis monotherapy may have deserved further consideration as

a mode for the administration of colistin in the treatment of respiratory tract infections due to MDR *A. baumannii* and *P. aeruginosa*.[18] Combination therapies are of the utmost importance in hospital/ventilator-acquired pneumonia (HAP/VAP), partly because of the recent history of colistin resistance, toxicity and increased mortality and morbidity.[19] Previous *in vitro* and *in vivo* studies have evaluated various synergistic combinations of carbapenem, colistin, rifampin, piperacillin/tazobactam, and ampicillin/sulbactam. Recently, Tucker *et al*. (2017) have shown results from 14 Phase-III and Phase-IV clinical trials investigating the efficacy of intravenous tigecycline in patients with *Acinetobacter* complex infections. Other antibiotics available in the treatment of patients with *Acinetobacter* infection may be considered as an option, either alone or in combination, but the mortality is lower in patients receiving monotherapy compared with combination therapies, particularly for MDR and XDR *A. baumannii pinomonas*.[20]

According to a study by Karaiskos *et al*.,[21] there is moderate evidence to support monotherapy for carbapenemase-producing *A. baumannii* infections; nonetheless, combination therapy for septic shock patients with an isolate with a MIC at the upper limit of sensitivity is recommended. The antibiotics used at the time of our study were evaluated retrospectively. Considering local surveillance data, *Acinetobacter spp*. combination therapy was used for all respiratory tract infections in which the causative agent was active, and in 68.2% of infections, it was given via an intravenous and inhaler colistin combination.

The OXA23-like plasmid is the most common reason for carbapenem resistance and can be transferred chromosomally. In this study, we determined that all strains produced OXA23-like enzymes. Resistance rates vary according to centers. However, in studies in Turkey, high rates of OXA23-like enzyme-producing strains have been reported. The fact that these studies were conducted in MDR *A. baumannii* strains can be interpreted as evidence that the presence of OXA23-like genes correlates with carbapenem resistance. In Turkey, the presence of *blaOXA*-58 (0-23%), *blaOXA*-23 (31-78%), and *blaOXA*-24 (low rates) have been reported similar to other studies.[8,12,22,23] However, in this study, the *blaOXA*-51 gene was detected in all isolates. In addition, the presence of the *blaOXA*-23 gene was reported to be 46.7%, 78%, 91.5% and 94.5% of *blaOXA*-24 as 2% and of *blaOXA*-58 as 7%, and 53.3% in several other studies.[8,12,22,23] In our study, the *blaOXA*-23 gene positivity rate was 100.0%. All these results emphasize that *A. baumannii* isolates of *blaOXA*-51 and the *blaOXA*-23 gene regions constitute the dominant mechanism of imipenem resistance. However, it has been reported that blaOXA genes in *A. baumannii* strains differ between regions while gene changes are observed in the same region over several years.[23] The ISAba1 gene region was reported to be important in OXA carbapenemase gene expression in *A. baumannii*. It predicted that in this group, carbapenemase resistance encoded on chromosomes and plasmids is accompanied by this gene region, as well as the ISAba-2 and ISAba-3 gene regions.[24] The IsaBaI positivity rate in our study was 90.0%. The ISAba1 genetic element has been shown to increase expression by coming to the *blaOXA*-51 genome.[9,25] Although antibiotyping results warn that MDR *Acinetobacter* isolates may cause hospital outbreaks, it is not quite enough to distinguish between strains. Therefore, nosocomial *A. baumannii* epidemics should be investigated with genotyping methods.[26] It was reported that the rep-PCR DiversiLab system identifies 56 and 15 *A. baumannii* isolates from the patients and the environmental samples of the ICU which were found to be clonally related.[27] Studies comparing rep-PCR results with fluorescent amplified fragment length polymorphism method demonstrated that the reporter power of rep-PCR is comparable to the gold standard pulsed-field gel electrophoresis method.[28] In this study, rep-PCR was used to reveal the clonal relationship between *Acinetobacter* isolates isolated from the respiratory tract specimens in our hospital.

A total of 22 different (P1-P22) patterns were identified in our study; the main pattern was P5. It was found that 22 (97.3%) of the P5 formed the largest cluster of isolates and 3 different types (P5a-P5c) were separated. The subtypes in the P5 were clustered as P5a (n = 2), P5b (n = 15), and P5c (n = 5). The first isolates in P5 were isolated from the ETA sample of two patients on June 06, 2014, and at IMICU on June 12, 2014, and the last isolate was isolated from the ETA culture of a patient in the MICU on December 12, 2014. It was noted that 50.0% of the isolates constituting P5 were isolated from patients in the MICU, and 31.8% were isolates of ETA belonging to a patient with VAP. In addition, in the MICU isolation chamber of ambient cultures, patient beds, nurses' hand, and the aspiration unit were breeding sites, as well as the connection hose, and all were included in P5 in these isolates. With the data obtained, it has been concluded that with patients using multiple services, cross-contamination through the patients and health personnel cause the isolates to spread easily within the hospital and to continue to exist for a long time. Resistance rates of *Acinetobacter* isolates of our hospital were monitored during approximately 9 months, and the increase in the resistance rates was

found to be parallel to the spread of epidemiologically related isolates of the same clone. Frequent patient transfers between different wards or wards and ICUs have also likely facilitated the spread of *Acinetobacter* strains.

In conclusion, the nosocomial MDR *A. baumannii* isolates were highly resistant to antibiotics and carry the *blaOXA*-23 resistance gene. The analysis of these isolates by rep-PCR revealed that they were very closely related strains of the same origin, recently separated from each other. This situation highlights that the treatment options for patients in our hospital are very limited and that infection control measures should be increased.

Ethical approval

Approval of the ethics committee numbered 2016/23 was obtained from the local ethical board.

Informed constant

Written constants were obtained from all participants.

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Conflicts of interest

There are no conflicts of interest.

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