ORIGINAL ARTICLE

Evaluating the antimicrobial resistance patterns and molecular frequency of *bla*$_{oxa-48}$ and *bla*$_{GES-2}$ genes in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains isolated from burn wound infection in Tehran, Iran

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Abstract

The aim of this study is to evaluate the antimicrobial resistance patterns and molecular frequency of bla$_{GES-2}$ and bla$_{oxa-48}$ genes in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains isolated from burn wound infection in Tehran, Iran. In this study, 50 isolates of *A. baumannii* and 48 isolates of *P. aeruginosa* were collected from the Burn Unit of Shahid Motahari Hospital at Tehran, Iran. Antibiotic susceptibility tests of all isolates were carried out using the disc diffusion method, and the production of extended-spectrum β-lactamases (ESBLs) in isolates was surveyed by the double disc synergy method and based on CLSI (2019 AST M100) criteria. Finally, the frequency of bla$_{GES-2}$ and bla$_{oxa-48}$ genes was surveyed by PCR. Antibiotic susceptibility tests showed that 48/48 (100%) of *P. aeruginosa* isolates and 49/50 (98%) of *A. baumannii* isolates were resistant to ceftriaxone and cefotaxime, respectively. Ceftazidime exhibited the lowest (26/48; 54.1%) resistance rates against *P. aeruginosa* isolates. The production of ESBLs was seen in 8/48 (16.6%) and 3/50 (6%) of *P. aeruginosa* and *A. baumannii* isolates, respectively. On the basis of conventional PCR and sequencing, the frequencies of the bla$_{GES-2}$ gene among *P. aeruginosa* and *A. baumannii* was 87.5% and 58%, respectively. Moreover, bla$_{oxa-48}$ gene was detected in 70.83% and 92% of *P. aeruginosa* and *A. baumannii* isolates, respectively. Results suggest that antibiotic-resistant *A. baumannii* and *P. aeruginosa* strains isolated from burn patients are frequently found; therefore, it is absolutely necessary to implement continuous screening and follow-up programmes for detecting antimicrobial resistance.

Keywords: *Acinetobacter baumannii*, antibiotic resistance, *bla*, *bla*$_{oxa-48}$, *Pseudomonas aeruginosa*
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Introduction

Hospital infections are known as one of the most critical problems in health and treatment systems [1]. The factors causing such infections lead to a pervasive spread of such diseases in different hospital wards, and to a high mortality rate. Moreover, these factors are the reasons for the limited treatment efficacy and high costs [2]. Burning is one of the common and destructive injuries that requires immediate care to prevent its side effects. One of the most important concerns about burn patients is bacterial infections [3]. *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and methicillin-resistant *Staphylococcus aureus* are the most common pathogens found in infections in burn patients [4–7]. Some of the most noticeable infections induced by these agents are bacteraemia, ventilator-associated pneumonia, urinary tract infections, meningitis and wound infection among hospitalized patients, especially in the intensive care unit [8]. The intrinsic resistance of *P. aeruginosa* and *A. baumannii* against different groups of antibiotics and their ability to apply novel resistance mechanisms are the main problems when controlling infections at health-care centres [9,10]. Extended-spectrum β-lactamases (ESBLs) of GES type were detected for the first time in a clinical isolate of *Klebsiella*

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pneumoniae [11], and then in *Enterobacteriaceae*, *P. aeruginosa* and A. baumannii isolates [12,13]. Carbapenems are widely used to treat infections caused by *P. aeruginosa* and *A. baumannii* [14,15]. The ESBL genes stimulate resistance against extended-spectrum cephalosporins and cause many problems in and obstacles to the treatment of infections caused by *P. aeruginosa* and *A. baumannii* [16,17]. Currently, several GES-type β-lactamases, including GES-1, GES-2, GES-4, GES-5, GES-6, GES-8, GES-9, GES-11 and GES-19, have been identified in Enterobacteriaceae and among *P. aeruginosa* and *A. baumannii* around the world [18,19]. Changes in the amino acid position 170 in the case of GES-1, GES-2 and GES-5 genes can induce resistance against carbapenems [20]. The emergence of the OXA (oxacillinase) group of β-lactamases (Class D) resulted in several problems in controlling and treating opportunistic infections. The bla_{oxa48} gene is widespread in *K. pneumoniae* and plays a number of critical roles such as biofilm formation and resistance to carbapenems [21,22]. The bla_{OXA-48} gene is frequently recognized in Escherichia coli and K. pneumoniae [23]. *However,* only two studies have reported OXA-48 in A. baumannii *and, so far,* the bla_{OXA-48} gene has not been detected in *P. aeruginosa* isolates [24,25]. Screening for bla_{oxa48} in patients is essential to prevent the nosocomial outbreak before hospital admission, and identifying bla_{oxa48} and its variant with a short turnaround time promotes the time to active treatment [26]. Therefore, the objective of the present research is to evaluate the antimicrobial resistance patterns and molecular frequency of bla_{oxa-48} and bla_{GES-2} genes among *P. aeruginosa* and *A. baumannii* strains isolated from burn wound infection in Tehran, Iran.

Materials and methods

Ethics statement

The study protocol was approved by the Ethics Committee of Islamic Azad University, Ahar Branch (IR.IAU-AHAR.REC.1398.105).

Bacterial isolates and species identification

In the current study, from May 2018 until the end of July 2019, 98 clinical isolates comprising 50 isolates of *A. baumannii* and 48 isolates of *P. aeruginosa* were collected from those patients hospitalized at the Burn Ward of Shahid Motahari Hospital in Tehran, Iran. Briefly, the surface layer of the burn wound was cleaned and washed with normal saline, and swab samples were collected. Samples were transferred to the medical laboratory using Stuart transport medium. In the next step, swab samples were inoculated into several *bacterial* growth media including blood agar, MacConkey agar and Tryptic Soy Broth, then were incubated at 37°C for 24 hours. Strains were identified as *A. baumannii* and *P. aeruginosa* using standard biochemical tests including Gram stain, pigment production on Mueller–Hinton agar (Merck, Darmstadt, Germany), catalase and oxidase test, growth on triple sugar iron agar and Kligler iron agar, oxidation–fermentation, citrate test, sulphide indole motility, Methyl Red, Voges–Proskauer tests, motility and growth at 42° C. Following a definitive diagnosis, *A. baumannii* and *P. aeruginosa* isolates were inoculated into trypticase soy broth (Merck) supplemented with 20% glycerol and were preserved at −70°C until further processing [27].

Antibiotic susceptibility testing

The susceptibility of *A. baumannii* and *P. aeruginosa* to piperacillin/tazobactam (10/100 μg), imipenem (10 μg), meropenem (10 μg), ceftazidime (30 μg), ceftriaxone (30 μg), cefotaxime (30 μg), cefepime (30 μg), aztreonam (30 μg), amikacin (30 μg), gentamicin (10 μg) and ciprofloxacin (5 μg) was determined using a Kirby–Bauer disc diffusion method (DDM) on Mueller–Hinton agar. *Pseudomonas aeruginosa* (ATCC 27853) was used as a control for DDM. The finding of the DDM method was then interpreted based on the CLSI (CLSI 2019 AST M100) criteria.

Based on the US Centers for Disease Control and Prevention and the European Centre for Disease Prevention and Control, multidrug-resistant (MDR) isolates were identified, and *P. aeruginosa* and *A. baumannii* isolates were selected as MDR, which were resistant to at least one antimicrobial among at least three or more antibiotic groups.

Phenotypic detection of ESBL production

To evaluate the production of ESBL by isolates, this study used the double-disc synergy test (DDST) according to the CLSI (2019 M100) criteria (with either 30 μg cefotaxime or 30 μg ceftazidime alone, or with either 30 μg cefotaxime or 30 μg ceftazidime plus 10 μg clavulanic acid), and the test was performed on Mueller–Hinton agar plates. If the inhibition zone produced by the combined effects of either cefotaxime or ceftazidime plus clavulanic acid was $\geq$5 mm larger than that produced by either cefotaxime or ceftazidime alone, the test would be determined positive.

DNA extraction and PCR surveying

Genomic DNA of *A. baumannii* and *P. aeruginosa* isolates was extracted using a High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) in line with the manufacturer's guidelines and was preserved at −80°C. The presence of bla_{oxa-48} and bla_{GES-2} genes was screened by PCR. The PCR was performed on a 25-μL reaction mixture containing 3 μL of 10 × PCR buffer without $MgCl_2$, 2.5 mM $MgCl_2$, 0.5 μL of 10 mM of each

TABLE 1. Primers used for detection of bla_{oxa-48} and bla_{GES-2} genes

Primer	Gene sequence	Size (bp)
GES 2-F	GTTTTGCAATGTGCTCAACG	371
GES 2-R	TGCCATAGCAATAGGCGTAG	
OXA 48-F	TTGGTGGCATCGATTATCGG	744
OXA 48-R	GAGCACTTCTTTTGTGATGGC	

deoxynucleoside triphosphate (dNTPs), 1 μL of forward primer (10 pmol) and 1 μL of forward primer (10 pmol), 1 unit of *Taq* polymerase (Cinnagene, Tehran, Iran), 4 μL of template DNA, and sterile distilled water up to 25 μL.

The primer sequences used for PCR are listed in Table 1. Amplification reactions were carried out on a 9700 Gene Amp thermocycler (Applied Biosystems, Foster City, CA, USA). PCR conditions were as follows: one cycle of 95°C for 4 min, 35 cycles of denaturation at 95°C for 45 s, annealing at 52°C to 55°C (according to the primers) for each gene for 1 min and elongation at 72°C for 45 s with a final extension at 72°C for 10 min following the last cycle. PCR products were transferred to 1.5% agarose gel, stained with DNA safe stain (SinaClon Co., Tehran, Iran), visualized by a UV transilluminator, and screened in the presence of bla_{oxa-48} and bla_{GES-2} genes. Finally, amplicons representing each studied gene were confirmed based on sequencing analysis by ABI 3730X capillary sequencer (Pishgam; Macrogen, Seoul, Korea). *Acinetobacter baumannii* ATCC 19606 and distilled water were used as positive and negative controls, respectively.

Statistical analysis

The results of this study were analysed using the statistical package SPSS v.23.0 (SPSS Inc., Chicago, IL, USA) and descriptive statistic tests.

Results

Number of specimens and distribution of bacteria

In this cross-sectional study, a total of 220 swab samples were collected from May 2018 until the end of July 2019. Of these samples, 98 cultures (44.5%) (50 (22.7%) *A. baumannii* and 48 (21.8%) *P. aeruginosa*) were determined to be positive for *A. baumannii* and *P. aeruginosa*. Of the isolates, 36/48 (75%) and 28/50 (56%) of the *P. aeruginosa* and *A. baumannii* isolates were male, respectively (Fig. 1) and the mean age was 44 years (range 15 days to 90 years). *Pseudomonas aeruginosa* (38/48; 79.2%) had the highest proportion in the 31–45-year age group. In contrast, *A. baumannii* (20/50; 40%) had the highest proportion in the 46–60-year age group. The frequency of *P. aeruginosa* and *A. baumannii* isolates is shown in Fig. 2 by different age groups.

Antimicrobial susceptibility profile

The susceptibility profiles of *A. baumannii* and *P. aeruginosa* isolates to commonly used antimicrobials are shown in Table 2. The DDM results showed that 100% and 98% of *P. aeruginosa* and *A. baumannii* isolates exhibited resistance to ceftriaxone and cefotaxime, respectively. The *P. aeruginosa* strains showed a high degree of resistance to imipenem (95.83%), meropenem (93.75%), amikacin (93.75%), gentamicin (93.75%), ciprofloxacin (93.75%), cefepime (89.58%), aztreonam (81.25%) and piperacillin/tazobactam (81.25%), respectively. However, *P. aeruginosa* was found to have low levels of resistance to ceftazidime (54.17%). The *A. baumannii* strains showed a high level of resistance to ceftazidime (96%), imipenem (94%), meropenem (94%), cefepime (94%), amikacin (94%), ciprofloxacin (94%), piperacillin/tazobactam (90%), aztreonam (86%) and gentamicin (86%), respectively. In total, 98% (49/50) of *A. baumannii* and 100% (48/48) of *P. aeruginosa* were MDR.

DDST test result

The results of DDST showed that 16.6% (8/48) of *P. aeruginosa* strains and 6% (3/50) of *A. baumannii* strains for which the zone of inhibition for ceftazidime plus clavulanic acid was $\geq$5 mm larger than that for ceftazidime alone were positive in the DDST, respectively. Therefore, eight isolates of *P. aeruginosa* and three isolates of *A. baumannii* were classified as ESBL producers.

Screening for bla_{oxa-48} and bla_{GES-2} genes

The PCR was conducted to detect bla_{oxa-48} and bla_{GES-2} genes in *P. aeruginosa* and *A. baumannii* isolates using specific primers. PCR and sequencing showed that 87.5% (42/48) of *P. aeruginosa* and 58% (29/50) of *A. baumannii* were positive for bla_{GES-2} genes. In contrast, the frequencies of bla_{oxa-48} gene in *P. aeruginosa* and *A. baumannii* isolates were 70.8% (34/48) and 92% (46/50), respectively.

Discussion

This study evaluates the antimicrobial resistance patterns and molecular frequency of bla_{oxa-48} and bla_{GES-2} genes in a large number of *P. aeruginosa* and *A. baumannii* strains isolated from burn wound infections in Tehran, Iran. Both *P. aeruginosa* and *A. baumannii* are opportunistic and nosocomial pathogens that can induce several infections including otitis media, and respiratory tract, burn and wound infections with high mortality in patients, especially in immunocompromised individuals hospitalized in various wards of a hospital [28–30]. Antibiotic resistance among *P. aeruginosa* and *A. baumannii* has been accepted as a global public health problem around the world [31]. Based on a

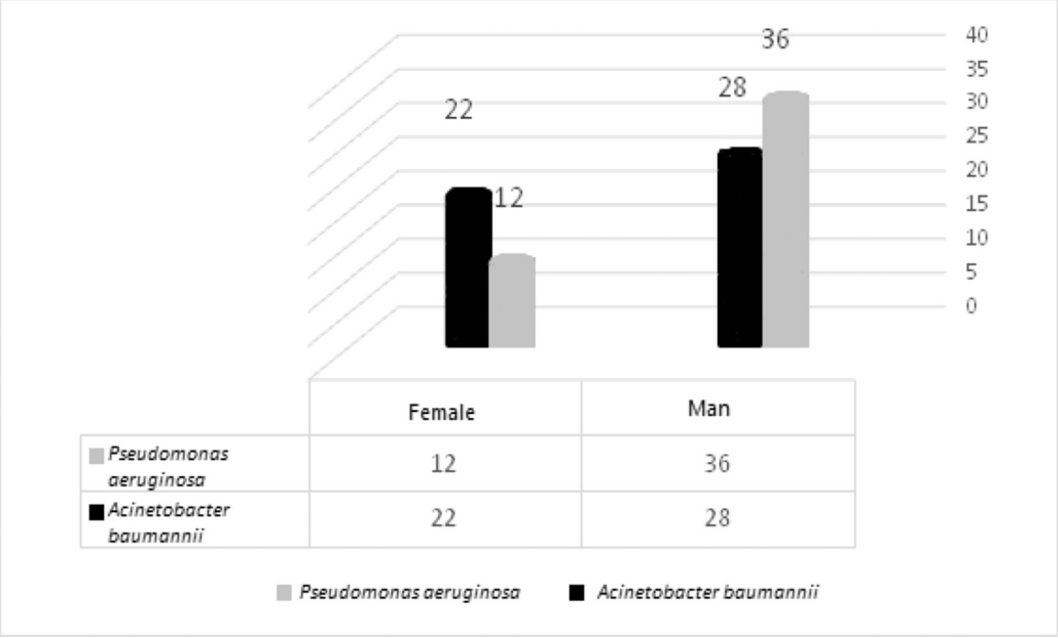

FIG. 1. Frequency and distribution of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates among the male and female gender.

report by the WHO in 2017, carbapenem-resistant *P. aeruginosa*, carbapenem-resistant *A. baumannii* complex and carbapenem-resistant or ESBL-producing *Enterobacteriaceae* are critical priority pathogens [32,33]. Recently, the emergence of carbapenem (imipenem and meropenem) resistance among these bacteria has become a severe clinical problem, mainly in low- and middle-income countries [34]. Moreover, it is predictable that the unavailability of organized antibiotic resistance surveillance programmes in these countries will lead to unsuitable use between patients and health-care staff [1]. Among the antibiotics that were tested against *A. baumannii* and *P. aeruginosa*, ceftriaxone and cefotaxime had the highest resistance rate. Moreover, *P. aeruginosa* strains showed a high level of resistance to imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, cefepime,

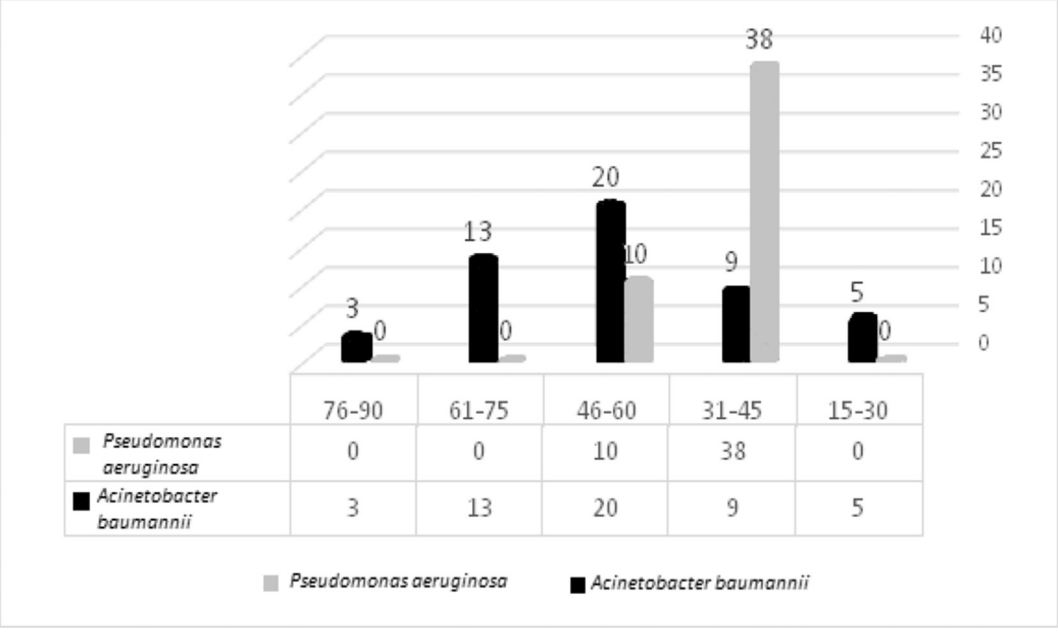

FIG. 2. The frequency of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates by different age groups.

TABLE 2. Antibiotic susceptibility of the *Acinetobacter baumannii* and *Pseudomonas aeruginosa* by disc diffusion method

	Pseudomonas aeruginosa (n = 48)		*Acinetobacter baumannii* (n = 50)		Total (n = 98)	
Antibiotics	**Resistance**	**%**	**Resistance**	**%**	**Resistance**	**%**
Piperacillin + Tazobactam	39	81.25	45	90.00	84	85.71
Imipenem	46	95.83	47	94.00	93	94.90
Meropenem	45	93.75	47	94.00	92	93.88
Ceftazidime	26	54.17	48	96.00	74	75.51
Ceftriaxone	48	100.00	49	98.00	97	98.98
Cefotaxime	48	100.00	49	98.00	97	98.98
Cefepime	43	89.58	47	94.00	90	91.84
Aztreonam	39	81.25	43	86.00	82	83.67
Amikacin	45	93.75	47	94.00	92	93.88
Gentamicin	45	93.75	43	86.00	88	89.80
Ciprofloxacin	45	93.75	47	94.00	92	93.88

aztreonam and piperacillin/tazobactam. Similarly, a high resistance rate was reported by Shariati et al., who claimed that 95% of *P. aeruginosa* strains were resistant to imipenem, meropenem and gentamicin [3]. Results of a previously conducted study in Mofid Children's Hospital revealed that the resistance rates of *P. aeruginosa* isolates, collected from 2013 to 2018, to imipenem, meropenem, gentamicin, amikacin, ciprofloxacin and piperacillin/tazobactam were 50.4%, 70.3%, 58.1%, 43.2%, 16.7% and 40.5%, respectively [1]. On the other hand, Farhan et al. reported low-level resistance [35]. According to the results of a published study, it can be concluded that two independent risk factors that include (a) prolonged hospitalization at the intensive care unit (>29 days) and (b) the existence of *P. aeruginosa* in the bacteriological specimens taken before treatment can lead to the acquisition of carbapenem resistance [31]. The present study also revealed that ceftazidime, in comparison with other antibiotics, showed the lowest resistance rate against *P. aeruginosa* isolates. *Acinetobacter baumannii* strains had a high level of resistance to all tested antibiotics, the results of which are in agreement with those obtained by Boral et al. [36], Azimi et al. [1], Romanin et al. [37], Rossi et al. [14], Kumar et al. [38] and Ardehali et al. [39]. In the current study, 98% of *A. baumannii* and 100% of *P. aeruginosa* were MDR. Similar findings were shown by Shariati et al. [3], Farhan et al. [35] and Ahmad and Ali [40]. Moreover, these results were in contrast with the results of Dutta et al. [41]. On the other hand, ESBL production was seen in 8/48 (16.6%) and 3/50 (6%) of *P. aeruginosa* and *A. baumannii* isolates, respectively. In recent years, the increase of MDR strains of *A. baumannii* and *P. aeruginosa* isolates has led to widespread and extensive use of carbapenems. Consequently, the degree of carbapenem resistance in *A. baumannii* and *P. aeruginosa* is now increasing around the world [42,43]. An increase in the health-care costs, prolonged hospitalization, reduced success rate of infection treatments, and an increase in morbidity and mortality rates are a number of the unfortunate consequences that result from MDR bacterial infections [44,45]. On the basis of conventional PCR and sequencing, the frequencies of bla_{GES-2} and bla_{oxa-48} genes among *P. aeruginosa* isolates were 87.5% and 70.83%, respectively. Moreover, bla_{GES-2} and bla_{oxa-48} genes were detected in 58% and 92% of *A. baumannii* isolates, respectively. These results were in contrast with those of Boral et al. [36] and Cheikh et al. [42] who reported that none of the *A. baumannii* isolates contained the bla_{oxa-48} gene. Romanin et al. reported that none of the *A. baumannii* isolates contained bla_{oxa-48} and bla_{GES-2} genes [37]. These results revealed that the frequency of bla_{oxa-48} could vary from country to country.

In conclusion, the results of the study showed that the prevalence of *P. aeruginosa* and *A. baumannii* resistant to multiple antibiotics dramatically increased, and the finding suggests that antibiotic-resistant *A. baumannii* and *P. aeruginosa* strains are frequently isolated from burn patients. Moreover, the results suggest that the use of antibiotics, especially carbapenems, must be carefully controlled in patients who are colonized by or infected with *A. baumannii* and *P. aeruginosa*. Finally, it is recommended that combination therapy including imipenem plus meropenem, aztreonam plus aminoglycosides, aztreonam plus colistin, ceftolozane plus tazobactam, ceftazidime/avibactam, piperacillin/tazobactam plus amikacin or piperacillin/tazobactam plus colistin, or meropenem/ceftazidime plus colistin could exert the highest synergistic effect against MDR and carbapenem-resistant *A. baumannii* and *P. aeruginosa* isolates, compared with each separate isolate.

Author contributions

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data. They played an active role in drafting the article or revising it critically to achieve important intellectual content, gave the final approval of the version to be published, and agreed to be accountable for all aspects of the work.

Conflict of interest

All of the authors declare that there are no commercial, personal, political, nor any other potentially conflicting interests related to the submitted manuscript.

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