Evaluating the antimicrobial resistance patterns and molecular frequency of blaoxa-48 and blages-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 blages-2 and blaoxa-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 blages-2 and blaoxa-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 blages-2 gene among P. aeruginosa and A. baumannii was 87.5% and 58%, respectively. Moreover, blaoxa-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, blaoxa-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...
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 blaOXA-48 gene is widespread in K. pneumoniae and plays a number of critical roles such as biofilm formation and resistance to carbapenems [21,22]. The blaOXA-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 blaOXA-48 gene has not been detected in P. aeruginosa isolates [24,25]. Screening for blaOXA-48 in patients is essential to prevent the nosocomial outbreak before hospital admission, and identifying blaOXA-48 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 blaOXA-48 and blaGES-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 ≥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 blaOXA-48 and blaGES-2 genes was screened by PCR. The PCR was performed on a 25-μL reaction mixture containing 3 μL of 10 × PCR buffer without MgCl₂, 2.5 mM MgCl₂, 0.5 μL of 10 mM of each...
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 GES-2-F and GES-2-R primers.

### Statistical Analysis

The results of this study were assessed using the statistical package SPSS v23.0 (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 ceftazidime 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%), pipercillin/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 cefazidime (96%), imipenem (94%), meropenem (94%), cefepime (94%), amikacin (94%), ciprofloxacin (94%), pipercillin/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 cefazidime plus clavulanic acid was ≥ 5 mm larger than that for cefazidime 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 blaoxa-48 and blaGES-2 genes

The PCR was conducted to detect blaoxa-48 and blagas genes in *P. aeruginosa* and *A. baumannii* isolates using specific primers. PCR and sequencing showed that 87.5% (42/48) of *P. aeruginosa* strains and 58% (29/50) of *A. baumannii* were positive for blaGES-2 genes. In contrast, the frequencies of blaoxa-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 blaoxa-48 and blagas 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
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,
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 P. aeruginosa of tance rate was reported by Shariati et al., who claimed that 95% aztreonam and piperacillin/tazobactam. Similarly, a high resis- nmNITarafdar P. aeruginosa level resistance [35]. According to the results of a published (>29 days) and (b) the existence of study, it can be concluded that two independent risk factors that 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 rec- ommended 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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