Evaluation of Polymyxin B Susceptibility Profile and Detection of Drug Resistance Genes among Acinetobacter Baumannii Clinical Isolates in Tehran, Iran during 2015-2016

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Abstract. Acinetobacter baumannii is an important opportunistic pathogen, responsible for approximately 10% of all gram-negative nosocomial infection. The aim of this study was to determine aminoglycoside and quinolone resistance genes and their antimicrobial susceptibility profile in the clinically A. baumannii. In this cross-sectional study, a total of 100 nonduplicative A. baumannii isolates were collected from different clinical samples. Antimicrobial susceptibility test was performed by disk diffusion method. QnrA, anrB, qnrS, aac(3)–IIa, and aac(6′)–Ib genes were identified using PCR method. The results of antibiotic susceptibility test showed that polymyxin B was the most effective antimicrobial against A. baumannii. 97%, 95% and 82% of isolates were resistant to cefepime, ceftriaxone, and amikacin, respectively. The molecular distribution of aac(3)–IIa, aac(6′)–Ib, and qnrA genes were 45%, 50%, and 50% of isolates, respectively. However, qnrB and qnrS genes could not be detected in any strain. This study showed that polymyxin B was the best drug against A. baumannii clinical isolates. This data is also valid for polymyxin E (colistin), which is mostly used in clinics. There is a high level of resistance genes among clinical A. baumannii isolates. This high prevalence rate highlights the necessity for the development of rapid diagnostic assays and continuous monitoring of antibiotic resistance.

Keywords: Acinetobacter baumannii, Aminoglycoside, Quinolone, Iran.

Introduction. Acinetobacter baumannii is a lactose non-fermenting gram-negative bacillus (NF-GNB) that has emerged as a highly troublesome pathogen particularly in critically ill patients.¹ Clinical isolates of A. baumannii are responsible for pulmonary, device-related, bloodstream, and urinary tract infections and are frequently isolated from hospitalized ICU patients.² These isolates were associated with multiple antibiotic resistance, and the spread of drug-resistant A. baumannii strains among hospitalized patients have become an increasing
public health threat.\(^3\)\(^4\) Furthermore, due to the intrinsic resistance mechanisms in this opportunistic nosocomial pathogen, it is quicker to become multidrug-resistant (MDR).\(^5\)

Polymyxin B and polymyxin E (colistin), are an increasingly significant part of the antimicrobial agents against MDR gram-negative bacteria. These two drugs have the same spectrum and are appropriate for use in the clinical settings.\(^6\)\(^7\) At present in Europe in the patients with MDR A. baumannii the clinician utilize the colistin, and in the future, it is possible using the new derivatives.\(^8\)

Aminoglycosides are used most commonly in the treatment of life-threatening infections caused by A. baumannii strains.\(^9\) The efflux pumps, decreased outer membrane permeability, amino acid substitutions and enzymatic modification, are the main mechanisms of aminoglycoside resistance in these bacteria.\(^10\) Enzymatic modification is the most common type of aminoglycoside resistance in A. baumannii clinical isolates and usually results in high-level drug resistance.\(^11\) Most enzyme-mediated resistance in A. baumannii is due to the genes encoding for aminoglycoside-modifying enzymes (AMEs) which found on plasmids and transposons. Three types of AMEs include N-acetyltransferases (AAC), O-acyltransferases (ANT), and O-phosphotransferases (APH).\(^12\)\(^13\)

The plasmid-mediated quinolone resistance (PMQR) genes, such as qnrA, qnrB, and qnrS, are responsible for quinolone resistance in A. baumannii isolates. PMQRs were first detected in the 1990s as a plasmid gene in Klebsiella pneumoniae clinical isolates. Subsequent studies have shown that qnr genes have a worldwide distribution in a range of Gram-negative opportunistic pathogens. Although the qnr expression mechanism which confers clinical quinolone resistance is the least understood, the DNA topoisomerase protection protein Qnr protects DNA from quinolone binding and causes resistance to quinolones.\(^14\)\(^15\)\(^16\) The prevalence of quinolone- and/or aminoglycoside-resistant A. baumannii was increased during the past decade. The present study was carried out to investigate antibiotic resistance pattern and resistance-related genes such as qnrA, qnrB, qnrS, aac(3)-Ia, and aac(6’)-Ib in A. baumannii clinical isolates by polymerase chain reaction (PCR) assay.

Materials and Methods. The current study was a cross-sectional descriptive research which conducted from February 2015 to April 2016, at two teaching hospitals (Bagiyatallah and Moheb mehr hospitals) in Tehran, Iran. One hundred non-repetitive strains of A. baumannii were obtained from different clinical specimens, including tracheal secretion, blood, wound, urine, and other samples. The isolates were identified using well-recognized biochemical tests such as Gram staining, oxidative/fermentative glucose test, catalase test, motility, oxidase test, citrate utilization, and capability to grow at 42–44°C.\(^17\) Species identification was confirmed by detection of blaOXA-51-like genes, as described previously.\(^18\) All strains were preserved in Luria–Bertani broth (Merck Co., Germany) containing 20% glycerol (v/v) at −80°C for further use.

Antimicrobial susceptibility was carried out on the Mueller-Hinton agar plates (Merck Co., Germany) using the Kirby–Bauer (KB) method as suggested by the Clinical and Laboratory Standards Institute guideline (CLSI document M100-S14).

The antimicrobial agents were as follows: meropenem (10 μg), gentamicin (10 μg), amikacin (30 μg), imipenem (10 μg), tobramycin (10 μg), tetracycline (30 μg), piperacillin–tazobactam (100–10 μg), cefepime (30 μg), ceftriaxone (30 μg), ampicillin–sulbactam (10–10 μg), and polymyxin B (300 μg) (MAST Diagnostics, Merseyside, UK). Multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) isolates were detected according to the instruction suggested by the Centers for Disease Control and Prevention (CDC). Escherichia coli ATCC 25922 and Acinetobacter baumannii ATCC 19606 were used as negative and positive controls, respectively.

Genomic DNA was extracted from A. baumannii colonies grown overnight on blood agar by Bioneer Co., Korea Kit and used as a template for PCR assay. PCR amplification was done to detect aminoglycoside-(aac(3)-Ia and aac(6’)-Ib) and quinolone-(qnrA, qnrB, and qnrS) related resistance genes. Amplification of AME and PMQR genes was carried out using a thermal gradient cycler (Eppendorf Co., Germany) with the following protocol: 5 minutes at 94°C for the initial denaturation and 36 cycles of amplification consisting of 45 seconds at 94°C, 45 seconds at 52–58°C, and 45 seconds at 72°C, with 5 minutes
Table 1. PCR primers and annealing temperatures used in this study.

| Target genes | Forward | Reverse | Annealing (°C) | Amplicon size (bp) |
|--------------|---------|---------|----------------|-------------------|
| qnrA         | ATTTCTCACGCCAGGATTG | GATCGGCAAAGGTTAGGTCA | 58              | 649               |
| qnrB         | GGCTCGAAATTGCCACTG  | TTTGCTTGTCGCCAGTGCAA  | 52              | 469               |
| qnrS         | GCAAGTTCATAGAACGAGGT | TCTAACCAGTGTTGCACGCAG | 50              | 428               |
| aac(3)-IIa   | CGGAAGGCAATAACGAGGAG | TCGAACCAGTGACTGAG     | 58              | 740               |
| aac(6’)-Ib   | TTGCAGTCTATGAGTGGCTA | CTCGAATGCCGGCGTGTTT   | 55              | 611               |

at 72°C for the final extension. The specific primers, temperatures of annealing, and amplicons size used for PCR are detailed in Table 1.

The current survey was a descriptive research. The MINITAB16 software was used for statistical analyses. The P value and confidence intervals were ≤0.05 and 95%, respectively.

Results. One hundred isolates of A. baumannii were obtained from different clinical specimens. The samples included blood (n=40, 40%), tracheal secretion (n=27, 27%), wound (n=12, 12%), urine (n=8, 8%), and unknown (n=13, 13%) specimens isolated from hospitalized patients in ICU (n=40, 40%), emergency department (n=20, 20%), and infectious disease department (n=30, 30%), and other departments (n=10, 10%).

The resistance percentage of meropenem, gentamicin, amikacin, imipenem, tobramycin, tetracycline, piperacillin-tazobactam, cefepime, ceftiraxone, ampicillin-sulbactam and polymyxin B was 69%, 82%, 63%, 74%, 56%, 51%, 70%, 97%, 95%, 49%, 3% (Table 2).

All 100 isolates of the main outbreak strains of A. baumannii were PCR positive for blaOXA-51-like genes.

Molecular distribution of aminoglycoside resistance profile among A. baumannii isolates.

| Antibiotic       | Resistant No (%) | Intermediate No (%) | Sensitive No (%) |
|------------------|------------------|---------------------|-----------------|
| Meropenem        | 69(69%)          | 16(16%)             | 15(15%)         |
| Gentamicin       | 82(80.4%)        | 6(5.9%)             | 14(13.7%)       |
| Amikacin         | 63(63%)          | 10(10%)             | 27(27%)         |
| Imipenem         | 74(76%)          | 14(14%)             | 10(10%)         |
| Tobramycin       | 56(56%)          | 7(7%)               | 37(37%)         |
| Tetracycline     | 51(51%)          | 14(14%)             | 35(35%)         |
| Piperacillin-    | 70(70%)          | 0(0%)               | 30(30%)         |
| Tazobactam       |                  |                     |                 |
| Cefepime         | 97(97%)          | 1(1%)               | 2(2%)           |
| Ceftiraxone      | 95(95%)          | 5(5%)               | 0(0%)           |
| Ampicillin-      | 49(49%)          | 17(17%)             | 34(34%)         |
| Sulbactam        |                  |                     |                 |
| Polymyxin B      | 3(3%)            | 0(0%)               | 97(97%)         |

Figure 1. A) PCR amplification of the aac(3)-IIa gene. Lane M: Ladder (100 bp), lane Cont-: negative control, lane Cont+: positive control (740bp); lane 1, 6, and 7: negative results and lane 2, 3, 4, and 5: positive results. B) PCR amplification of the aac(6’)-Ib gene. Lane M: Ladder (100 bp), lane Cont-: negative control, lane Cont+: positive control (482bp); lane 1-7: positive results. C) PCR amplification of the qnrA gene. Lane M: Ladder (100 bp), lane Cont-: negative control, lane Cont+: positive control (649bp); lane 1-4, 5, and 7: positive results, lane 5: negative result.
resistance genes including aac(3)-IIa and aac(6’)-Ib were 45% and 50%, respectively is shown in the figure 1 A and B. Half of the isolates (50%) contained the qnrA (Figure 1C). QnrB and qnrS were not found in any strains. Sequencing of PCR products for AME and PMQR genes were confirmed by BLAST at NCBI.

Discussion. Drug resistance in A. baumannii has become a global problem for the severely infected patients who critically rely on Antimicrobial therapy. The emergence of clinical A. baumannii strains with different antibiotic resistance phenotypes causes difficulties in treating infections caused by this organism.19,20

Multidrug-resistant A. baumannii (MDR-Ab) is a subject of profound anxiety as it not only causes severe and fatal infections but also increases the length of hospital stay, resulting in augmented treatment charges.21

In this study, the most antibiotic resistance in A. baumannii isolates were related to cefepime (97%), ceftriaxone (95%), and amikacin (82%), and the most effective drug against these isolates was polymyxin B. This data is also valid for colistin, which is mostly used in clinics worldwide.

Henwood et al.,22 showed that more than 75% of A. baumannii strains were resistant to cefotaxime and ceftazidime. In another study, Karlowsky et al.,23 showed that >90% of A. baumannii isolates were susceptible to imipenem and meropenem; fewer strains were susceptible to amikacin, and <60% were susceptible to ceftazidime and gentamicin.

In agreement with the current study, polymyxins, are active agents against the overwhelming majority of A. baumannii throughout the world. In a systematic review study directed by Razavi Nikoo et al.,24 polymyxins presented adequate activity against A. baumannii collected. The frequencies of MDR and XDR isolates were 70% and 19% respectively. No PDR isolates were identified in this study.

Hujer et al.,25 in their study reported that 89% of A. baumannii were resistant to at least three different classes of antibiotics, and 15% were resistant to all antibiotics tested.

Aminoglycosides are used most commonly in the treatment of A. baumannii infections. Most enzyme-mediated resistance in A. baumannii is due to AMEs encoded genes which found on the mobile genetic elements.

PMQR genes including qnrA, qnrB, and qnrS are responsible for quinolone resistance in A. baumannii which the prevalence of quinolone-resistant A. baumannii was increased in recent years. In our study, the prevalence rate of PMQR genes including qnrA, qnrB, and qnrS was 50%, 0%, and 0%, respectively. In contrast with our data, Chagas et al.,26 showed that the prevalence of qnrA gene was 37.5% (n=15). The differences mentioned above can result from the geographical distance, surveillance strategies, and restraint in antibiotic prescriptions in other regions.

Conclusions. This study showed that the most effective antibiotic against clinical strains of A. baumannii was polymyxin B and we recommend clinicians to use polymyxins (B or E) in patients infected with MDR A. baumannii. However, overusing can lead to polymyxin resistance, and the drug’s toxicity problems should be considered. There is a high level of aminoglycoside resistance genes among A. baumannii isolates circulating in hospitals in Iran. This trend of MDR profiles associated with the presence of aac(6’)-Ib and aac(3)-IIa genes are worrying. The high prevalence rate of these resistance genes highlights the necessity for establishing more rapid diagnostic assays, more antimicrobial susceptibility tests, more clinician-laboratory correlation, and continuous monitoring of antibiotic resistance due to A. baumannii.

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