Original Article

Detection of Carbapenems and Colistin Resistance Genes in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: A Single-center Study in Iran

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Abstract

Background: This study aimed to determine carbapenems, colistin resistance genes, and antimicrobial susceptibility profiles of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates.

Materials and Methods: In this cross-sectional study, specimens of patients with bloodstream, urinary tract, and surgical site nosocomial infections were enrolled. *P. aeruginosa* and *A. baumannii* isolates were identified using conventional methods. Antimicrobial susceptibility testing (AST) on isolates was performed using the disk diffusion method and minimum inhibitory concentration (MIC) for colistin as recommended by the Clinical and Laboratory Standards Institute (CLSI). The combination meropenem disk method was used to detect metallo-β-lactamases (MBLs). The bla_{NDM}, bla_{VIM}, bla_{IMP}, and *mcr-1* genes were identified using the polymerase chain reaction (PCR) method and Sequencing.

Results: Forty strains of *P. aeruginosa* and forty strains of *A. baumannii* were isolated from hospitalized patients. The overall prevalence of multidrug-resistance (MDR) was 50% and 95% in *P. aeruginosa* and *A. baumannii* isolates, respectively. Almost all the MDR isolates were resistant to cefepime and piperacillin. Colistin had significant inhibitory activity against the isolates. MBL was detected in 25.0% and 15.0% of clinical isolates of *P. aeruginosa* and *A. baumannii*, respectively. We detected no bla_{NDM}, bla_{VIM}, bla_{IMP}, and *mcr-1* genes in our *A. baumannii* isolates. Moreover, only three *P. aeruginosa* isolates were positive for bla_{IMP} gene.

Conclusion: The alarming proportion of MDR *P. aeruginosa* and *A. baumannii* isolates was reported in the current study. Effective infection prevention practices are required and AST should guide patients' treatment.

Keywords: *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, Multidrug-resistance, Metallo-β-lactamase

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Introduction

Due to the limited antimicrobial treatment options, the emergence and increase of multidrug-resistant gram-negative bacteria (MDR-GNB) seriously threaten public health. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are being reported worldwide as the major cause of MDR-GNB infections[1,2]. The most common infections attributable to these pathogens include lower respiratory tract

infection, pneumonia, bloodstream infection, and urinary tract infection[3]. MDR strains of *P. aeruginosa* and *A. baumannii* remain susceptible to only one or two antibiotics[1,4,5]. Carbapenems (i.e. imipenem, meropenem, or doripenem), polymyxins (i.e. colistin), are extremely potent drugs in treating infections caused by pathogens mentioned above[6,7]. However, MDR-GNB organisms have a remarkable ability to develop resistance to several antimicrobial drugs [8,9]. Likewise, recent studies have reported increasing resistance to carbapenems and colistin among *P. aeruginosa* and *A. baumannii*[10-12]. There are various types of mechanisms leading to carbapenems-resistant strains. One of the main mechanisms includes the production of metallo-β-lactamases (MBLs) such as IMP, VIM, SIM, and NDM[13]. The most common strategies for resistance to colistin are modifications of the bacterial outer membrane through alteration of the LPS, the overexpression of efflux-pump systems, and overproduction of capsule polysaccharides. A plasmid-mediated colistin resistance gene, *mcr-1*, suggests the acquisition of resistance in MDR-GNB[14,15]. In Iran, some studies reported a high prevalence of drug resistance in clinical isolates of *P. aeruginosa*, and *A. baumannii*[12]. This study aimed to determine carbapenems, colistin resistance genes, and antimicrobial susceptibility profiles of *P. aeruginosa* and *A. baumannii* isolates.

Methods

Setting and samples: This cross-sectional study was performed at Labbafinejad hospital (Tehran, Iran) from January 2019 to January 2020. All clinical specimens (bronchoalveolar lavage, swab specimen's sputum, pus, etc) were collected from the hospitalized patients with bloodstream, urinary tract, and surgical site nosocomial infections. They were processed according to standard microbiological methods for isolating and identifying *P. aeruginosa* and *A. baumannii*[16]. Also, specimens from patients with a history of antibiotic consumption were excluded.

Antibiotic Susceptibility Testing (AST): The antibiotic sensitivity profiles of *P. aeruginosa* and *A. baumannii* isolates were determined by the Kirby–Bauer disk diffusion method against the commercially available discs (Mast Diagnostics Ltd, Merseyside, UK); ceftazidime (30 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), cefepime (30 µg), aztreonam (30 µg), meropenem (10 µg), imipenem (10 µg), piperacillin (100 µg) and Piperacillin/Tazobactam (PTZ, 100/10µg) following standard procedures recommended by the Clinical and Laboratory Standards Institute (CLSI)[17]. The CLSI broth microdilution reference method[17] also determined the MIC of colistin. *A. baumannii* ATCC 19606 and *Escherichia coli* ATCC 25922 were used as positive and negative controls, respectively.

Identification of MDR isolates and detection of MBL: Clinical isolates of *P. aeruginosa* and *A. baumannii* were identified as MDR if they were resistant to at least one antimicrobial agent in three or more antimicrobial classes[18]. The combination meropenem disk method was applied to detect MBL[19]. In this test, two disks were used; a meropenem disk (10 µg), and a meropenem disk (10 µg) with EDTA, as an inhibitor of MBL. Production of MBL was considered when the zone of inhibition diameter around the meropenem+EDTA disks was increased ≥7 mm than the zone of inhibition diameter around the meropenem disk alone.

PCR and Sequencing: The DNA was extracted by the commercial DNA purification kit for bacteria according to the manufacturers' instructions (Roche Diagnostics GmbH, Mannheim, Germany). PCR amplification was performed to detect carbapenems (*bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$) and colistin (*mcr-1*) related resistance genes using specific primers as previously described[20,21]. DNA sequencing was done on the purified PCR products (Macrogen Company, Korea) and the obtained sequences for each isolate were aligned and compared with the existing sequences via nucleotide Basic Local Alignment Search Tool (BLAST) search (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Data analysis: Frequency and percentage were reported by descriptive analysis using SPSS software (version 22, SPSS Inc., Chicago, IL, USA).

Ethical considerations: This study was approved by the ethics committee of the School of Medicine, Shahid Beheshti University of Medical Sciences (ID: IR.SBMU.MSP.REC.1397.598).

Results

Antimicrobial susceptibility testing: Eighty non-duplicate non-consecutive isolates of *P. aeruginosa* and *A. baumannii* were isolated from hospitalized patients using conventional methods.

Metallo-β-lactamases production: The combination meropenem disk detected MBL in 25.0% and 15.0% of *P. aeruginosa* and *A. baumannii* isolates.

Carbapenems and colistin resistance genes: Out of 40 *P. aeruginosa* isolates, 3 (7.5%) had bla_{IMP} gene. The bla_{NDM}, bla_{VIM}, and *mcr-1* genes were not detected in *P. aeruginosa* isolates. No bla_{IMP}, bla_{NDM}, bla_{VIM}, and *mcr-1* genes were detected in any of the *A. baumannii* isolates.

Discussion

The emergence and spread of MDR-GNB is an important public health problem worldwide, especially in developing countries. In Iran, there were increasing reports of MDR-GNB in several health-care settings[12]. The current study indicates a high rate of drug resistance in the two frequently isolated GNB in our hospital, where 20 (50%) of *P. aeruginosa* and 38 (95%) of *A. baumannii* isolates were found to be MDR. This rate of resistance was similar to the previous studies from Iran. During 2012-2013, Saderi *et al.* reported that more than 50.0% of *P. aeruginosa* isolates were characterized as MDR[22]. According to a study conducted by Sarhaddi *et al.* MDR phenotype was reported in all isolates of *A. baumannii*[23]. Likewise, subsequent investigations observed nearly the same percentages for MDR isolates: 100% and 58.0% for *A. baumannii* and *P. aeruginosa*, respectively[24,25]. A high prevalence of nosocomial MDR-GNB was also observed by authors in Iraq, Turkey, and Pakistan[26-29]. The high MDR proportion is mainly attributed to the fact that there are poor infrastructures, frequent use of broad-spectrum antibiotics, cross-transmission of these bacteria, the ability of environmental persistence, and poor infection control strategies in the clinical area in Iran. In this study, almost all the MDR isolates were resistant to cefepime and piperacillin. This was

Table 1: Antibiotic Resistance Profiles of *Pseudomonas aeruginosa*.

Antibiotics	Resistant	Intermediate	Sensitive
Aztreonam	30(75.0)	4(10.0)	4(10.0)
Gentamicin	20(50.0)	8(20.0)	12(30.0)
Ciprofloxacin	30(75.0)	4(10.0)	6(15.0)
Amikacin	21(52.5)	7(17.5)	12(30.0)
Ceftazidime	32(80.0)	4(10.0)	4(10.0)
Cefepime	34(85.0)	2(5.0)	4(10.0)
Piperacillin	35(87.5)	2(5.0)	3(7.5)
Imipenem	21(52.5)	8(20.0)	11(27.5)
Meropenem	20(50.0)	9(22.5)	11(27.5)
Piperacillin/Tazobactam	34(85.0)	2(5.0)	4(10.0)

Values are expressed as No (%)

Table 2: Antibiotic Resistance Profiles of *Acinetobacter baumannii*.

Antibiotics	Resistant	Intermediate	Sensitive
Aztreonam	39(97.5)	0(0.0)	1(2.5)
Gentamicin	38(95.0)	0(0.0)	2(5.0)
Ciprofloxacin	40(100)	0(0.0)	0(0.0)
Amikacin	39(97.5)	0(0.0)	1(2.5)
Ceftazidime	38(95.0)	0(0.0)	2(5.0)
Cefepime	38(95.0)	1(2.5)	1(2.5)
Piperacillin	40(100)	0(0.0)	0(0.0)
Imipenem	38(95.0)	0(0.0)	2(5.0)
Meropenem	37(92.5)	1(2.5)	2(5.0)
Piperacillin/Tazobactam	40(100)	0(0.0)	0(0.0)

Values are expressed as No (%)

significantly higher than studies from Turkey (28.7%), China (12%), and Italy (25%)[30-32]. The observed difference might be due to the number of investigated isolates, the nature of study patients, and infection control practices.

Moreover, 75.0% and 100% of *P. aeruginosa*, and *A. baumannii* isolates were resistant to ciprofloxacin, respectively. The resistance level to ciprofloxacin in the present study was comparable to studies from China (89.6%) and Nigeria (100%)[31,33]. In recent years, the wide use of fluoroquinolones as broad-spectrum antimicrobial agents in the hospital setting has often led to increased resistance to ciprofloxacin[34]. In this study, colistin had significant inhibitory activity against the investigated isolates.

In contrast, in the studies by Rossi *et al.* and Joseph *et al.*, 6.3% and 20% of *P. aeruginosa* and *A. baumannii* were resistant to colistin, respectively[35,36]. The increase of resistance to colistin among GNB is a therapeutic challenge due to restricted treatment options. Thus, effective infection-control initiatives are of paramount importance. Two *P. aeruginosa* strains carried the *mcr-1* gene. The screening for this gene can provide a rapid and useful description of colistin resistance in MDR-GNB. In the current study, bla_{NDM}, bla_{VIM}, and bla_{IMP} genes were not detected in our *A. baumannii* isolates. Ramadan *et al*. indicated a similar observation, in which metallo-β-lactamases genes were not present in the *A. baumannii* isolates [20]. According to the recent meta-analysis, the presence of *ISAba1*, bla_{OXA23}, and bla_{OXA40} genes was reported [12]. Also, the major resistant mechanism to carbapenems is the efflux pump.

Moreover, three *P. aeruginosa* isolates were positive for the gene. Based on Lin *et al*., more than 18% of *P. aeruginosa* isolates were found to carry bla_{VIM} genes[37]. MBLs producing *P. aeruginosa* have been reported by many authors, suggesting that these enzymes are an important mechanism of carbapenem resistance among *P. aeruginosa*[37]. Our study had some limitations. The effect of different variables such as length of hospitalization on drug resistance status was

not investigated due to the unavailability of sufficient data from the patients. Although we detected the MDR isolates and carbapenems resistance genes, their association with outcome was not analyzed. Finally, our study was conducted in Tehran, the capital of Iran, and the situation may differ in other provinces.

Conclusion

In conclusion, the alarming proportion of MDR *P. aeruginosa* and *A. baumannii* isolates was reported in the current study. Effective infection prevention practices are required and patients' treatment should be guided by AST.

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