Original Article

Molecular Study of Colistin Resistance Genes in *Acinetobacter baumannii* Isolated from Patients in the Intensive Care Unit (ICU)

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Abstract

Backgrounds: Bacteria which form part of the genus *Acinetobacter* (*Acinetobacter* spp.) are widely distributed Gram-negative coccobacilli, which are found in nature and hospital wards and cause various nosocomial infections, including pneumonia, meningitis, endocarditis, skin and soft tissue infections, conjunctivitis, burn Wound infections, and bacteremia. These bacteria exhibit different resistance mechanisms. This study aimed to evaluate Acinetobacter baumannii strains' resistance against different antibiotics in the presence and absence of pmrA, pmrB, and pmrAB genes.

Methods: This descriptive-cross-sectional study was conducted on 100 isolates of *Acinetobacter baumannii* isolated from hospitalized patients in the intensive care unit (ICU) of Namazi, Ali Asghar, and Shahid Rajaei hospitals in Shiraz. Bacterial identification of the species was performed using standard biochemical tests, and Microgen kits with verified isolates. Antibiotic sensitivity was analyzed on 12 antibiotics according to the standard technique and CLSI criteria, and the *pmrA*, *pmrB*, and *pmrAB* genes were used to identify and multiply them.

Results: The isolates of *Acinetobacter baumannii* were resistant to most antibiotics and had less resistance to their latest treatment line, the antibiotic colistin. Most isolates were a carrier for *pmrA*, *pmrB*, and *pmrAB* genes.

Conclusions: This study has found that Acinetobacter baumannii resistance in Iran is increasing like in other parts of the world, indicating the necessity to adopt appropriate treatment strategies. [*Ethiop. J. Health Dev.* 2022; 36(1) 00-00]

Keywords: *Acinetobacter baumannii*, pmrAB, Polymyxin, Intensive Care Unit

Introduction

Acinetobacter spp. is an aerobic glucose non-fermentative, non-motile, non-troublesome, catalase-positive, oxidase-negative, and Gram-negative coccobacilli (1). Among *Acinetobacter* species, *A. baumannii* is the most critical isolate associated with nosocomial infections worldwide. This aerobic Gram-negative coccobacillus is considered a low-level pathogen. Its origins are essential, as it has a high capacity for compromise with a wide range of antibiotics, rapid transformation, and long-term sustainability in any media. It is a successful pathogen responsible for opportunistic infections such as pneumonia, meningitis, skin, blood circulation, urinary tract, and other soft tissue infections (2**)**. Since many *A. baumannii* infections have been reported suddenly among soldiers and veterans who served in Iraq and Afghanistan, it is sometimes called "*Iraqibacter*"(3). A multidrug-resistant *A. baumannii* also has an outbreak among civilian hospitals for wounded soldiers returning from war zones due to cross-infection (2). These infections occur in critically ill patients in the intensive care unit (ICU) (4) and account for more than 20% of ICU infections worldwide (5). *A. baumannii* virulence factors include the outer membrane proteins, phospholipases, proteases, lipopolysaccharides (LPS), capsule polysaccharides, protein secretion systems, and iron-chelating systems (6). Several reports indicated that *A. baumannii* is rapidly resistant to antimicrobial agents, and several drug-resistant isolates have successfully been isolated to date (7). The World Health Organization (WHO) has announced that *A. baumannii* is one of the most important ESKAPE organisms (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.), resistant to various antibiotics (8). Resistance mechanisms to enzymatically degradation of drugs, alteration of target sites, multidrug efflux pumps, and permeability defects have been identified in *A. baumannii* (9). Polymyxins are a group of multi-cationic peptide antibiotics discovered more than 60 years ago, exhibiting potent efficacy against most Gram-negative bacteria. Unfortunately, the emergence of colistin-resistant isolates across the globe has proven to be a serious problem in treating this bacterium today (10). Colistin exerts its bactericidal activity mainly by attaching to the bacterial cell membrane and disrupting its permeability, leading to the leakage of intracellular components. Polymyxins also have anti-endotoxin activity (11). The colistin resistance mechanism in *Acinetobacter* spp includes excluding lipopolysaccharide (LPS) and adding phosphoethanolamine (PEA) to LPS with mutations in the *PmrA/B* two-component system. Mutations in the *pmrA* and *pmrB* activate the *pmrC*, which cause the addition of PEA in the form of 7-Acyl-lipid (12). Colistin is the last treatment option for Acinetobacter baumannii. Unfortunately, over time, resistance to this antibiotic is increasing worldwide, which affects the health care system .This study examined the genes involved in the colistin resistance of *A. baumannii* due to the importance of treating *A. baumannii*.

Methods

This study was performed on patients admitted to the ICU ward of Namazi hospital in the various

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departments, including bacteriology, virology, mycology, hematology, immunology, diabetes, organ transplant (liver and kidney), the cancer ward, Infection and HIV/AIDS Control Department, Ali Asghar Hospital (Poison Hospital), and Shahid Rajaei Hospital who were admitted to the hospital in the past 12 months from April 2018 to April 2019 due to accidents and injuries.

***Sampling*:** This cross-sectional descriptive study was conducted on laboratory specimens suspected of being infected with *Acinetobacter baumannii* and who were referred to the Rajaei and Ali Asghar hospitals in Shiraz, Iran. Non-random sampling was performed following the sampling principles, and the samples were immediately transferred to the laboratory. A nurse extracted urine, wound, and blood samples from different patients in the ICU ward. After disinfecting the outer part of the genitourinary tract, urine was collected in a sterile container for urinary sampling. In lesion sampling, the site was first rinsed with sterile physiology serum by swap, and the lesion discharge was then harvested.

***Cultivation and isolation*:** Initially, the culture was first performed on a Blood agar enriched medium, MacConkey agar, EMB agar, and the culture was then conducted on a specific CHROM agar *Acinetobacter* medium. Subsequent standard biochemical tests such as growth at 42°C, oxidase, Catalase, TSI, motility tests, and the Microgen kit were utilized along with a review of the *blaOXA*-51-like gene by PCR, which confirmed the *A. baumannii*.

***Identification Acinetobacter Baumannii with Microgen kit GN-A*:** The Microgen Kit GN-A strip has 12-wells Included: well 1- Lysine, well 2- Ornithine, well 3- H_2S, well 4- Glucose, well 5- Mannitol, well 6- Xylose, well 7- ONPG, well 8- Indole, well 9- Urease, well 10- VP, well 11- Citrate and well 12- TDA, which was used to identify *A. baumannii* as follows:

- Select a single colony of the isolate
- Emulsify colony in 3ml saline
- Add 3-4 drops (100µL) of the suspension to each well of the strip(s)
- Overlay appropriate wells with mineral oil
- Incubate for 18-24 hours at 35-37°C
- Add the reagents in the next day
- Read and Record results using the color chart and Identification Software

***Antibiotic sensitivity test*:** An Antibiotic sensitivity test of the collected samples was performed using an antibiogram test, and the diffusion disc method was used on the Mueller-Hinton Agar media according to the CLSI instructions. The antibiotics used included Cefotaxime, Minocycline, Meropenem, Tigecycline, Ciprofloxacin, Piperacillin, Ceftazidime, Gentamicin, Amikacin, Cefepime, Ceftriaxone, and Colistin to accomplish this procedure. All antibiotics were purchased from ROSCO (Denmark). Standard *Pseudomonas aeruginosa* PTCC 17589 isolates were used as controls in this study, and the results were analyzed using Excel and IBM SPSS-20 software.

DNA extraction: The boiling method was used to extract the genomic *A. baumannii* samples. A 200 µl of sterile distilled water was poured into the microtubes, and 3 to 4 colonies from freshly grown colonies were dissolved in it, then mixed well and immersed in boiling water for 10 minutes. The above suspension was centrifuged at 10,000rpm for 5 minutes. The supernatant was then harvested and transferred to a new microtube. The supernatant contained DNA for PCR.

***PCR*:** PCR was performed to identify *pmrA* and *pmrB* genes using specific primers for these genes. *Pseudomonas aeruginosa* PTCC 17589 was used as a positive control. Each microtube contained 2µl of extracted DNA, and 1µl of each primer was added to the PCR MASTER kit with a final volume of 25µl. The samples were electrophoresed in 2% agarose gel to confirm the separation of DNA, and it was observed using a GELDOC (Bio Rad-USA) device following staining with ethidium bromide. A UV spectrophotometer was used to quantify the DNA value. The primers used to perform PCR were: pmrA-F 5´-ATGACAAAAATCTTGATGATTGAAGAT-3´ pmrA-R 5´TTATGATTGCCCCAAACGGTAG-3´, pmrB 5´GTGCATTATTCATTAAAAAAAC-3´ pmrB-R 5´ TCACGCTCTTGTTTCATGTA-3´and pmrAB-F 5´GCATCTAAAAGATTGTAGTCAC-3´ pmrAB-R 5´ GCGATTGTATTCATCGTTTGAG-3´which were related to accidents and injuries for 12 months from April 2018 to April 2019.

Statistical analysis: Statistical analysis was performed at the inferential level using Chi-square and Fisher's exact test using IBM SPSS ver24 software and with a 5% margin of error.

Results

Isolation and identification of *Acinetobacter baumannii:* Based on the tests conducted, including growth on media, biochemical tests, and the application of the Microgen kit,100 *Acinetobacter baumannii* strains were isolated and identified (Table 1).

Table 1: **The biochemical characteristics of 100 clinical isolates of *Acinetobacter Baumannii* strains**

Test, substrate	*Acinetobacter Baumannii*
Growth on	
MacConkey agar	+
EMB agar	+
CHROM agar	+
Oxidase	-
Catalase	+
Motility	**Nonmotile**
TSI acid:	
Slant	-
Butt	-
Lysine	-
Ornithine	-
H_2S	-
Glucose	+
Mannitol	-
Xylose	+
ONPG	-
Indole	-
Urease	-
VP	-
Citrate	+
TDA	-

***Distribution of samples*:** A hundred (100) non-recurring samples were collected from the ICU wards of three training centers of the Namazi Hospital (40), Ali Asghar Hospital (15), and Shahid Rajaei Accident Hospital in Shiraz (45). Most strains were isolated from patients hospitalized in the ICU ward for wound care, and the least strains were isolated from the blood (figure 1).

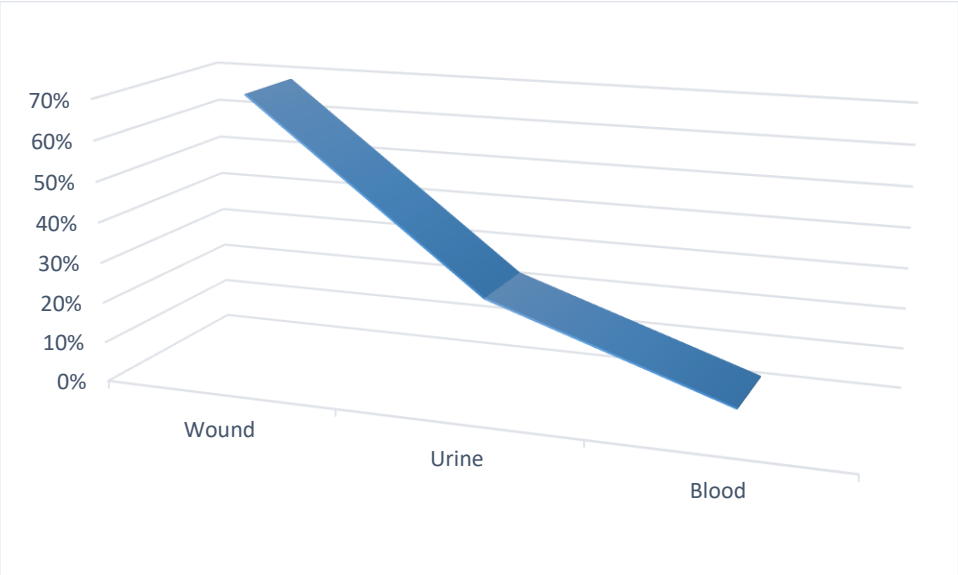

Figure 1: **distribution of *Acinetobacter baumannii* isolates in different samples**

***Antibiotic sensitivity test results*:** All isolates of *A. baumannii* were isolated from different infections of patients, who were treated and examined with the antibiotics Cefotaxime, Minocycline, Meropenem, Tigecycline, Ciprofloxacin, Piperacillin, Ceftazidime, Gentamicin, Amikacin, Cefepime, Ceftriaxone, and Colistin. Three hospitals performed this analysis on the isolates. The highest resistance of clinical samples of *A. baumannii* was observed in the following antibiotics: Ceftriaxone (100%), Meropenem (100%), Cefepime (100%), Cefotaxime (100%), Piperacillin (99%), Ciprofloxacin (98.1%), Ceftazidime (97.8%), Gentamicin (97.04), Minocycline (96%), and Amikacin (95%). The lowest resistance was related to two antibiotics, namely Tigecycline (48%) and Colistin (34%) (Table 2 and figure 2).

Table 2: **The table below indicates resistance to different antibiotics in men and women. Based on Fisher's exact test results, no significant difference was observed between men and women regarding antibiotic resistance (p <0.05).**

Antibiotic	Female (n=18)		Male (n=82)		p-value
	frequency	percent	frequency	Percent	
Colistin	9	50.0%	25	30.5%	.114
Cefipime	18	100.0%	82	100.0%	---
Meropenem	18	100.0%	82	100.0%	---
Ceftriaxone	18	100.0%	82	100.0%	---
Cefotaxime	18	100.0%	82	100.0%	---
Piperacilllin	18	100.0%	81	98.8%	1.00
Ciprofloxacin	18	100.0%	80	97.6%	1.00
Ceftazidime	16	88.9%	81	98.8%	.083
Gentamicin	16	88.9%	78	95.1%	.294
Minicycline	18	100.0%	78	95.1%	1.00
Amikacin	17	94.4%	79	96.3%	.554
Tigecycline	7	38.9%	41	50.0%	.393

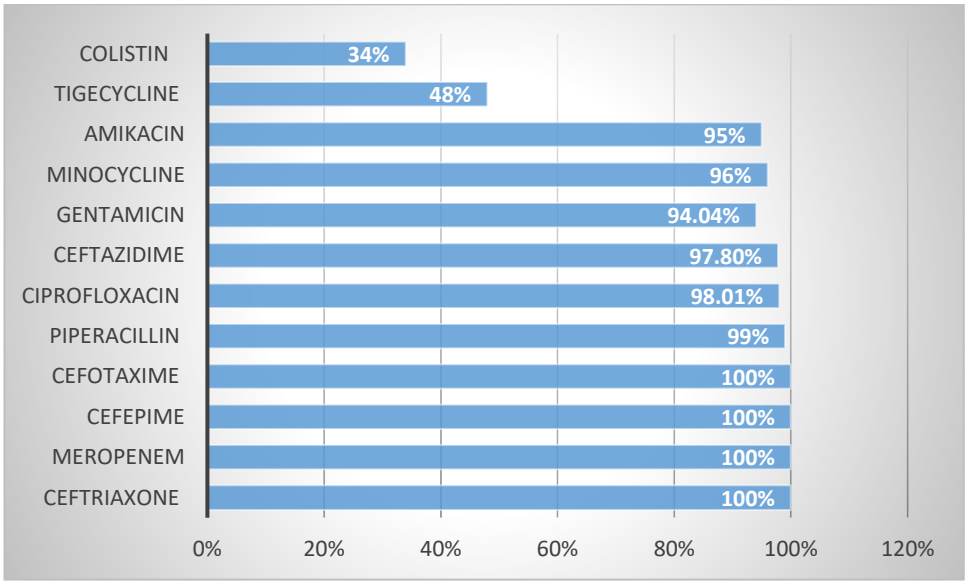

Figure 2: **Antibiotic resistance pattern in three hospitals**

***PCR*:** The results of the *pmrA* and *pmrB* genes in PCR indicated that of the 100 isolates of the studied *A. baumannii*, 90 isolates had *pmrA* gene, 10 isolates lacked the *pmrA* gene, 79 isolates had the *pmrB* gene, and 21 isolates lacked the *pmrB* gene (Figures 3 and 4). This analysis was performed on isolates at three (Table 3, 4 and 5).

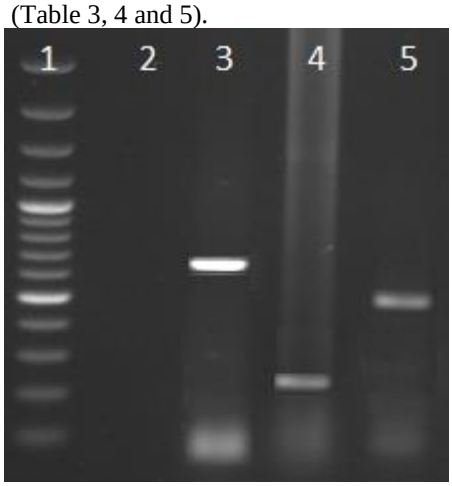

Figure 3:**1: Ladder 100 bp, 2: Negative control sample, 3: Positive sample *pmrB* gene (size 539 bp), 4: Positive sample pmrAB (size 252 bp), 5: Positive sample pmrA (size)720 bp.**

hospitals. There is no significant difference between antibiotic resistance and the presence of pmrA and pmrAB genes, however, Ciprofloxacin antibiotic resistance was significantly higher in the presence of the pmrB gene.

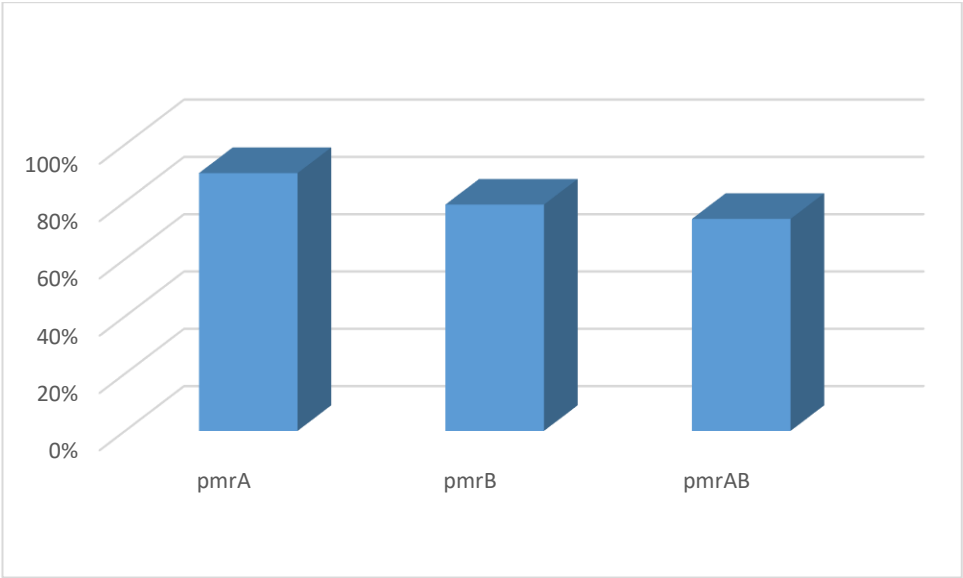

Figure 4: **Percentage of *Acinetobacter baumannii* isolates carrying *pmrA*, *pmrB* and *pmrAB* genes**

Table 3: **The table below indicates resistance to various antibiotics in the presence and absence of the pmrA gene. Based on Fisher's exact test results, no significant difference was observed between the antibiotic resistance between the two conditions (p <0.05).**

Antibiotic	pmrA+ (n=90)		pmrA- (n=10)		p-value
	frequency	percent	frequency	percent	
Colistin	30	33.3%	4	40.0%	.731
Piperacilllin	89	98.9%	10	100.0%	1.00
Ciprofloxacin	88	97.8%	10	100.0%	1.00
Ceftazidime	88	97.8%	9	90.0%	.273
Gentamicin	84	93.3%	10	100.0%	1.00
Minicycline	87	96.7%	9	90.0%	.348
Amikacin	86	95.6%	10	100.0%	1.00
Tigecycline	46	51.1%	2	20.0%	.094

Table 4: **The table below indicates resistance to various antibiotics in the presence and absence of the pmrB gene. Based on Fisher's exact test results, resistance to Ciprofloxacin antibiotic was significantly higher in the presence of the pmrB gene (p = 0.042). No significant difference was observed between the degree of antibiotic resistance between the presence and absence of the pmrB gene for other antibiotics (p <0.05).**

Antibiotic	pmrB+ (n=79)		pmrB- (n=21)		p-value
	frequency	percent	frequency	percent	
Colistin	24	30.4%	10	47.6%	.138
Piperacilllin	78	98.7%	21	100.0%	1.00
Ciprofloxacin	79	100.0%	19	90.5%	.042
Ceftazidime	77	97.5%	20	95.2%	.511
Gentamicin	75	94.9%	19	90.5%	.603
Minicycline	75	94.9%	21	100.0%	.576
Amikacin	76	96.2%	20	95.2%	1.00
Tigecycline	38	48.1%	10	47.6%	1.00

Table 5: **The table below indicates resistance to various antibiotics in the presence and absence of the pmrAB gene. Based on Fisher's exact test results, no significant difference was observed in the antibiotic resistance between the two conditions (p <0.05).**

Antibiotic	pmrAB+ (n=71)		pmrAB- (n=29)		p-value
	frequency	percent	frequency	percent	
Colistin	21	29.6%	13	44.8%	.144
Piperacilllin	70	98.6%	29	100.0%	1.00
Ciprofloxacin	69	97.2%	29	100.0%	1.00
Ceftazidime	69	97.2%	28	96.6%	1.00
Gentamicin	68	95.8%	26	89.7%	.352
Minicycline	70	98.6%	26	89.7%	.072
Amikacin	68	95.8%	28	96.6%	1.00
Tigecycline	37	52.1%	11	37.9%	.270

Discussion

Many life threatening and deadly diseases occur through nosocomial infections. In recent decades, *A. baumannii* has been a significant factor in developing fatal nosocomial infections, especially in ICUs and burn hospitals. *A. baumannii* is the second most common non-fermentative bacterium isolated from humans after *Pseudomonas*. Patients with immunodeficiency due to long-term hospitalization are less likely to be treated for *A. baumannii* infections. *A. baumannii* isolates in patients hospitalized in different hospital wards were isolated from different samples. In a study on *A. baumannii* by Rebic et al., most isolates were found in septicemia, urinary, and wound samples, and the lowest isolates were obtained in the abscess(13). In this study on *A. baumannii*, most isolates were from the wound and urine samples, respectively, and the least isolates were from blood samples that can be stated that this difference in isolations of different samples indicates the ability of *A. baumannii* to cause various nosocomial infections. *A. baumannii* has become increasingly resistant to various antibiotics due to its prolonged survival in the hospital. There are significant problems in treating patients due to the drug resistance of the bacterium. In addition to the adverse clinical effects, it is costly to treat. In a study conducted by AraujÂ jo Castilho et al., the highest antibiotic resistance was observed in Cefepime (96.4%), Ceftazidime (92.8%), Ciprofloxacin (91.0%), Imipenem (76.7%), Meropenem (76.7%), Piperacillin/Tazobactam (69.6%), and Gentamicin (57.1%), and the lowest resistance was obtained to Tigecycline and Colistin (14). In this paper, the highest resistance of clinical samples of *A. baumannii* was observed to Ceftriaxone (100%), Meropenem (100%), Cefepime (100%), Cefotaxime (100%), Piperacillin (99%), Ciprofloxacin (98.01%), Ceftazidime (97.8%), Gentamicin (97.04%), Minocycline (96%), and Amikacin (95%).

Dent et al. reported that *Acinetobacter* strains were 58% resistant to imipenem and 46% to aminoglycosides, cephalosporins, broad-spectrum penicillins, and quinolones (15). The higher antibiotic resistance percentage is due to the widespread use of imipenem and colistin in different hospital wards and the community's indiscriminate and arbitrary drug use.

Similar to Hu et al(16) and Smolyakov et al(17) this study found that most strains were resistant to Amikacin, Ceftazidime, gentamicin, imipenem, meropenem, Cefepime, and Piperacillin/tazobactam.

As in the study of Hujer et al (18) and contrary to Shahcheraghi et al(19)., Karlowsky et al(20)., and Kilic et al(21) susceptibility to meropenem is low in *Acinetobacter baumannii* isolates, and *Acinetobacter baumannii* strains are highly resistant to these antibiotics. This difference is due to the excessive use of this antibiotic, which has led to a highly resistant strain in these hospitals.

The lowest resistance to antibiotics was found in Tigecycline and Colistin, which indicate an increasing resistance of *A. baumannii* to various antibiotics around the world. The treatment line for this bacterium was Carbapenem, Tigecycline, Colistin, and phage therapies, respectively. There are few options for treating infections caused by this bacterium due to its resistance. One of the latest treatment options for *A. baumannii* is colistin. Unfortunately, the colistin resistance of this bacterium is increasing worldwide today, with the highest resistance reported from Asia. Compared to other studies on the resistance of *A. baumannii*, the increased resistance of *A. baumannii* to colistin was 34% vs. 6% in AraujÂ jo Castilho et al. (14). Given that colistin is the last resort for treating *A. baumannii*, it is essential to consider the mechanisms of resistance of this bacterium to colistin. The colistin resistance mechanism of *A. baumannii* includes excluding lipopolysaccharide (LPS) (22) and adding phosphoethanolamine (PEA) to LPS by a mutation in the *PmrA/B* two-component system (23,24). The *pmrA*, *pmrAB*, and *pmrB* gene frequencies were investigated in the isolates, which indicated an increase in the abundance of these genes in *A. baumannii* isolates indicating an increase in resistance of this bacterium to colistin. Therefore, it is essential to know the status of *Acinetobacter* spp resistance to common antibiotics in hospitals, to determine the treatment policy in the initial treatment and to control the resistance of this bacterium to antibiotic therapies.

Conclusions

The antibiotic sensitivity of bacteria isolated from patients indicates that it is difficult to use any antibiotic without resistance. The study indicated that resistance to colistin is increasing due to its excessive use. Antibiotic administration is limited due to the toxicity of this antibiotic on the kidneys, and controlling such infections is essential in the early stages of treatment. Achieving epidemiological information in order to discover the distribution of resistance of this bacterium in hospitals and to help promptly identify the mechanisms of resistance using PCR is also vital to examine the percentage and frequency of resistance genes.

Ethics approval

The study was conducted in accordance with good clinical practice guidelines. Ethics approval was granted by the Islamic Azad University of Falavarjan Clinical Research Ethics Committee(IR, IAU, FALA, REC,2020.027).

References

1. Lin MF, Lin YY, Yen HW, Lan CY. Role of the BaeSR two-component system in the regulation of Acinetobacter baumannii adeAB genes and its correlation with tigecycline susceptibility. BMC Microbiol. 2014; 9:14-119
2. Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: emergence of a successful pathogen. Clin Microbiol Rev. 2008; 21: 538–582.
3. Centers for Disease and Prevention (2004). Acinetobacter baumannii infections among patients at military medical facilities treating injured U.S. service members, 2002–2004.

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[2]Shiraz HIV/ AIDS Research Center, Institute of Health, Shiraz University of Medical Sciences, Shiraz, Iran
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4. Uwingabiye J, Lemnouer A, Baidoo S, Frikh M, Kasouati J, Maleb A, *et al*. Intensive care unit-acquired Acinetobacter baumannii infections in a Moroccan teaching hospital: epidemiology, risk factors and outcome. Germs. 2017; 7(4): 193–205.
5. Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, *et al*. International study of the prevalence and outcomes of infection in intensive care units. JAMA. 2009;302(21):2323-9.
6. Faye C, Dexter C, Kostoulias X, Uddin MI, Peleg AY.The Mechanisms of Disease Caused by *Acinetobacter baumannii.* Front Microbiol. 2019; 10: 1601.
7. McConnell MJ, Actis L, Pachon J. Acinetobacter baumannii: human infections, factors contributing to pathogenesis and animal models. FEMS Microbiol Rev. 2013;37(2):130-55
8. Mansura S, Ekta E. Kamble, Tawre MS, Karishma R, Pardesi KR.Emerging Strategies to Combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review. Front Microbiol.2019; 10: 539.
9. Moubareck CA, Halat DH. Insights into Acinetobacter baumannii: A Review of Microbiological, Virulence, and Resistance Traits in a Threatening Nosocomial Pathogen. Antibiotics. 2020; 9:119.
10. Poirel L, Jayol A, Nordmann P. Polymyxins: Antibacterial Activity, Susceptibility Testing, and Resistance Mechanisms Encoded by Plasmids or Chromosomes. Clin Microbiol Rev. 2017;30(2): 557–596.
11. Ahmed MA, Zhong L, Shen C, Yang Y, Doi Y, Tian GB. Colistin and its role in the Era of antibiotic resistance: an extended review (2000–2019). Emerg Microbes Infect. 2020; 9(1): 868–885.
12. Mu X, Wang N, Li X, Zhou Z , Yu Y , Hua X .The Effect of Colistin Resistance-Associated Mutations on the Fitness of Acinetobacter baumannii. Front Microbiol 2016; 7:1715.
13. Rebic V , Masic N, Teskeredzic S, Aljicevic M, Abduzaimovic A, Rebic D. The Importance of Acinetobacter Species in the Hospital Environment. Med Arch. 2018;72(5): 325–329.
14. Araújo Castilho SR, Miranda Godoy CS, Guilarde AO, Juliana Lamaro Cardoso JL , Porfirio André MC , Junqueira-Kipnis AP , *et al* .*Acinetobacter baumannii* strains isolated from patients in intensive care units in Goiânia, Brazil: Molecular and drug susceptibility profiles. PLoS One. 2017;12(5): 0176790.
15. Dent LL, Marshall DR, Pratap S, Hulette RB. Multidrug resistant Acinetobacter baumannii: a descriptive study in a city hospital. BMC Infect Dis. 2010; 10:196.
16. Hu H, Hu Y, Pan Y, Liang H, Wang H, Wang X, et al. Novel plasmid and its variant harboring both a blaNDM-1 gene and type IV secretion system in clinical isolates of Acinetobacter lwoffii. Antimicrob Agents Chemother. 2012;56(4):1698-702.
17. Smolyakov R, Borer A, Riesenberg K, Schlaeffer F, Alkan M, Porath A, et al. Nosocomial multi-drug resistant Acinetobacter baumannii bloodstream infection: risk factors and outcome with ampicillin-sulbactam treatment. J Hosp Infect. 2003;54(1):32-8.
18. Hujer KM, Hamza NS, Hujer AM, Perez F, Helfand MS, Bethel CR, et al. Identification of a new allelic variant of the Acinetobacter baumannii cephalosporinase, ADC-7 β-lactamase: defining a unique family of class C enzymes. Antimicrob Agents Chemother. 2005;49(7):2941-8.
19. Shahcheraghi F, Abbasalipour M, Feizabadi M, Ebrahimipour G, Akbari N. Isolation and genetic characterization of metallo-β-lactamase and carbapenamase producing strains of Acinetobacter baumannii from patients at Tehran hospitals. Iran J Microbiol. 2011;3(2):68.
20. Karlowsky JA, Draghi DC, Jones ME, Thornsberry C, Friedland IR, Sahm DF. Surveillance for antimicrobial susceptibility among clinical isolates of Pseudomonas aeruginosa and Acinetobacter baumannii from hospitalized patients in the United States, 1998 to 2001. Antimicrob Agents Chemother. 2003 May 1;47(5):1681-8.
21. Kilic A, Li H, Mellmann A, Basustaoglu AC, Kul M, Senses Z, et al. Acinetobacter septicus sp. nov. association with a nosocomial outbreak of bacteremia in a neonatal intensive care unit. J Clin Microbiol. 2008 Mar 1;46(3):902-8.
22. Cafiso V, Stracquadanio S, Verde FL, Gabriele G. Colistin Resistant *A. baumannii*: Genomic and Transcriptomic Traits Acquired Under Colistin Therapy. Front Microbiol. 2018; 9: 3195.
23. Nurtop E, Bilman FB, Menekse S, Gabriele G , Mezzatesta ML , Caio C , *et al* .Promoters of Colistin Resistance in Acinetobacter baumannii Infections . Microb Drug Resist. 2019;25(7):997-1002.
24. Beceiro A, Llobet E, Aranda J, Doumith M, Hornsey M, Dhanji H, *et al*. Phosphoethanolamine Modification of Lipid A in Colistin-Resistant Variants of Acinetobacter baumannii Mediated by the pmrAB Two-Component Regulatory System. Antimicrob Agents Chemother. 2011; 55(7):3370–337.