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ORIGINAL ARTICLE

Evaluation of antibiotic resistance changes in *Acinetobacter baumannii* in the era of COVID-19 in Northern Iran

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ABSTRACT

Background and Objectives: During the coronavirus pandemic, the overuse of antibiotics to reduce coinfections and mortality may be contributing to the rise of antimicrobial resistance. In this study, we aim to investigate the antibiotic resistance changes of *Acinetobacter baumannii* post-COVID-19 pandemic in Northern Iran.
Materials and Methods: The current study is a cross-sectional study. Between 2022 and 2023, 2190 clinical samples were collected from patients with healthcare-associated infections (HAIs) at four hospitals in Sari, which served as corona centers after the COVID-19 pandemic. Antimicrobial sensitivity was determined using standard broth macro-dilution, and resistance genes were detected using multiplex PCR.
Results: Based on the results co-amoxiclav had a resistance rate of 100%, while piperacillin/tazobactam showed the least resistance rate of 29.82%. In terms of GM MIC values, colistin was the most potent against multi-drug resistant isolates. The frequency of $bla_{\text{OXA-51}}$, *ampC*, *aphA6*, and bla_{NDM} genes were 100%, 99.12%, 90.35%, and 69.30% respectively.
Conclusion: Our study revealed high multi-drug resistance rates. Piperacillin/tazobactam recommended for treating multi-drug resistant *Acinetobacter baumannii* infections in Northern Iran.

Keywords: COVID-19; Drug resistance; *Acinetobacter baumannii*; Healthcare associated infections; Multiplex polymerase chain reaction

INTRODUCTION

Acinetobacter baumannii (A. baumannii) is a type of bacteria commonly found in healthcare settings and can cause various infections. These infections are known as healthcare-associated infections (HAIs) and can be severe. *A. baumannii* can cause ventilator-associated pneumonia (VAP), meningitis, bacteremia, skin infections, urinary tract infections (UTIs), and endocarditis. It is important to take

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necessary precautions to prevent the spread of this bacteria in healthcare facilities. Unfortunately, the number of these infections has been increasing, and even more concerning is the fact that many people die from them. In fact, the mortality rate can be from 30% to 75%, depending on the infection (1-4). One of the reasons that *A. baumannii* can be so dangerous is that some strains of the bacteria have become resistant to many different antibiotics. These strains are called multi-drug resistant *A. baumannii* (MDRAB), and they are a major cause of HAIs. MDRAB can resist antibiotics through different mechanisms, such as by having genes that prevent antibiotics from activity or by reducing the number of protective proteins on their outer membranes. One of the common antibiotics used to treat *A. baumannii* infections is called β-lactams. However, many strains of the bacteria have developed a resistance to this type of antibiotic. This is because they have enzymes called β-lactamases that can break down the antibiotic and render it ineffective. There are many different types of β-lactamases, including class A (such as bla_{TEM}, bla_{SHV}, and bla_{CTX}), class B (such as bla_{IMPs}, bla_{VIMs}, bla_{GIMs}, bla_{NDMs}, and bla_{SIMs}), class C (cephalosporinases or *ampC*), and class D (Oxacillinases). Many of these enzymes are responsible for the resistance to β-lactams seen in *A. baumannii* infections. *GR6* plasmids or class 1 integrons enable transfer of bla_{OXA-23} and *aphA6* genes between clinical and environmental isolates, causing resistance to multiple drugs (5-8).

Amidst the already complicated situation, the COVID-19 pandemic has made things even more challenging. Reports show that COVID-19 patients can suffer from coinfections and secondary infections, with a wide range of occurrence from 0.6% to 45% globally. Recent studies indicate that up to 3.5% of COVID-19 patients have bacterial coinfections at admission, and up to 15% of patients experience secondary bacterial infections after being hospitalized. The coronavirus pandemic has led to an increase in antimicrobial resistance due to the overuse of antibiotics in treating secondary infections. Studies have suggested a link between COVID-19 and antimicrobial resistance (9). According to a study, *A. baumannii* was found to be the most common bacterial secondary infection in COVID-19 patients. 91.2% of the isolated cases of *A. baumannii* were found to be carbapenem-resistant. Patients infected with *A. baumannii* can transmit the infection in healthcare facilities. MDRAB is a common bacterial co-infection in COVID-19 patients, which leads to severe illness and a negative prognosis (10, 11). The coronavirus pandemic has caused a shift in healthcare priorities, resulting in a decrease in planned healthcare activities and a reversal of previously implemented preventive measures. This has put a significant strain on healthcare systems, leading to a diversion of resources, personnel, and attention away from the diagnosis and management of antimicrobial resistance. As a result, studies on antimicrobial resistance have been impeded and surveillance programs have been either de-emphasized or completely halted (10, 11).

The high prevalence of antimicrobial resistance in hospital settings is a serious concern for both developed and developing countries. It has significant health implications, as well as economic consequences. Clinical findings suggest that antimicrobial resistance affects hospital infection rates and reduces the effectiveness of antibiotics. This, in turn, increases mortality rates, treatment costs, and hospitalization duration. It is crucial to take necessary precautions to prevent the spread of antimicrobial resistance in hospitals and to develop effective treatment protocols for patients affected by this infection (12-14). To address this issue, the study was conducted to investigate the antibiotic resistance changes of *A. baumannii* post-COVID-19 pandemic in North Iran.

MATERIALS AND METHODS

Collecting data and specimens. This cross-sectional study took place between 2022 and 2023 in Mazandaran province, Sari, at four hospitals designated as corona centers during the pandemic. A total of 12834 samples from patients with HAIs were sent to the central laboratory. During the study period, MDR *A. baumannii* strains were isolated from MDR Gram-negative bacteria. Based on the information presented in Table 1, MDR *A. baumannii* strains were collected from various samples, then the isolates were sent to the Pediatric Infectious Diseases Research Center lab.

Ethical approval and consent to participate. The study protocol with code number IR.MAZUMS.REC.1401.13943 was approved by the Ethics Committee of Mazandaran University of Medical Sciences.

Isolation and characterization of *A. baumannii*. The laboratory technician collected the samples and then transferred them to the Pediatric Infectious

Table 1. Patient demographic information

Variable	N (%)
Age	
< 1 Years old	3 (2.63)
1 – 18 Years old	16 (14.04)
> 18 Years old	95 (83.33)
Gender	
Male	85 (74.56)
Female	29 (25.44)
Wards	
Intensive Care Unit (ICU)	68 (59.65)
Internal	26 (22.81)
Pediatric Intensive Care (PICU)	12 (10.53)
Emergency	5 (4.39)
Surgery	2 (1.75)
Pediatric	1 (0.88)
Samples	
Wound	48 (42.11)
Sputum	36 (31.58)
Blood	19 (16.67)
Urine	8 (7.02)
Catheter	2 (1.75)
CSF	1 (0.88)

Diseases Research Center lab. All samples were sub-cultured on MacConkey and blood agar plates (QUELAB, USA), and incubated for 24 h at 37°C. Additionally, the blood samples were injected into aerobic/f culture bottles (Becton Dickinson and Company Spark, Ireland) and were incubated in a BACTEC culture system (BD BACTEC FX140, USA). To identify the isolates, the conventional biochemical tests were used, including urease test, oxidase, citrate test, sugar fermentation, and indole production. By utilizing the multiplex PCR testing and detecting the *OXA-51-like* gene, the confirmation of the strains' identity was achieved. The pure isolates were stored in Trypticase Soy Broth (TSB) (QUELAB, USA) with a glycerol concentration of 20% and maintained at a temperature of -70°C to preserve the isolates for future use.

Antibiotic susceptibility testing. The antimicrobial susceptibility of MDR *A. baumannii* isolates was determined using the standard broth macro-dilution technique. According to the Clinical Laboratory Standards Institute (CLSI, 2020) (15), the minimum inhibitory concentration (MIC) of colistin, meropenem, cefepime, gentamicin, ciprofloxacin, ceftazidime, ampicillin/sulbactam, piperacillin/tazobactam, and amoxicillin/clavulanic acid was determined. A positive control, *A. baumannii* (ATCC No. BAA.1799), was also included.

Detection of ESBLs - producing strains. The combined disk test (CDT), was used to confirm strains producing ESBLs. A suspension of bacterial colonies cultured on Muller-Hinton agar (QUELAB, USA) was prepared and involved placing cefotaxime (CTX; 30µg), ceftazidime (CAZ; 30µg), and cefepime (CPM; 30µg) discs with their combination with clavulanic acid (30 µg/10 µg). The discs were incubated at 37°C for 24 hours. If the inhibition zone on combination with clavulanic acid was enlarged by more than 5 mm compared to the disk without clavulanic acid, it confirmed the presence of ESBL-producing strains. *A. baumannii* (ATCC NO. 19606) was used as the positive control for this study (15, 16).

Extraction of DNA and molecular assay. DNA was extracted using the Genomic DNA Extraction Kit (Yekta Tajhiz, Iran) following the manufacturer's protocol. Specific primers (Bioneer, Korea) listed in Table 2, were used to verify the identity of *A. baumannii* strains and MDR encoding genes using $bla_{\text{OXA-51}}$ and *ampC, aphA6,* and bla_{NDM}, respectively. The multiplex PCR reaction was prepared in a final volume of 20 µl, including 10 µl Taq DNA Polymerase 2× Master Mix RED, 1.5mM $MgCl_2$ from AMPLIQON, Denmark, 0.5 µl of each primer (10 pM), 2 µl DNA template (100 ng), and 4 µl DNase-free distilled water. Multiplex PCR mixtures without DNA templates and with DNA templates of *A. baumannii* (ATCC NO. 17978) were used as negative and positive controls, respectively. The amplification reaction was programmed using the SimpliAmp Cycler (Thermo Fisher Scientific, USA) (Table 2). Finally, the multiplex PCR products were separated on a 1.5% agarose gel and visualized using the gel documentation system (UVIDoc HD6 Touch, USA).

Statistical analysis. Data were analyzed using SPSS version 22. Statistical analysis involved Chi-square and Fisher's exact tests.

RESULTS

In present study, the median age of patients was 40 years (IQR: 32-52 years), with 85 (74.56%) being

Table 2. Sequences of the oligonucleotide primers and cycles of Multiplex PCR amplification (1).

Primers	Primer sequence (5´to 3´)	Product size (bp)	Stages	Temperature (°C)	Time
$bla_{\text{OXA -51-F}}$	CTTGAGCACCATAAGGCAACCA	410	Denaturation	94° C	30 sec
$bla_{\text{OXA- 51-R}}$	ACATCCCATCCCCAACCACTTT				
aphA6-F	AAAATTGGTCAGTCGCCATCGG	631	Annealing	60° C	30 sec
aphA6-R	AGGCATCCTCTCTTAGGCAACG				
$bla_{\text{NDM-F}}$	AATGTCTGGCAGCACACTTCCT	135	Extension	72°C	30 min
$bla_{\text{NDM-R}}$	GATCTGGGCGGTCTGGTCATC				
ampc-F	GCGGGCAATACACCAAAAGACC	824	Final extension	72°C	10 min
ampc-R	TCTTCCCAACCGAGTGCCTGAT				

male and 29 (25.44%) being female. The most common HAIs caused by *A. baumannii* were wound infections (48; 42.11%), sputum infections (36; 31.58%), and bloodstream infections (BSI) (19; 16.67%). The highest and lowest incidence of different types of HAIs caused by MDR *A. baumannii* in various hospital wards were in the Intensive Care Unit (ICU) (68; 59.65%) and the pediatric ward (1; 0.8%), respectively (Table 1).

Antimicrobial susceptibility test. A total of 12834 samples from patients with HAIs were sent to the central laboratory. 114 MDR *A. baumannii* strains were isolated from MDR Gram-negative bacteria. The standard broth macro-dilution technique results showed that the bacteria were 100% resistant to co-amoxiclav and exhibited high resistance to ciprofloxacin, gentamicin, and ceftazidime, with resistance rates of 96.49%, 91.23%, and 90.35% respectively. The most effective antibiotic was found to be piperacillin/tazobactam (Fig. 1).

Table 3 summarizes the MIC_{50}, MIC_{90}, geometric means (GM) MIC, and mode of MICs of ampicillin-sulbactam, ceftazidime, cefepime, ciprofloxacin, colistin, co-amoxiclav, gentamicin, meropenem, and piperacillin-tazobactam against MDR *A. baumannii* strains. In terms of MIC50 values, co-amoxiclav and gentamicin exhibited the lowest activity against MDR *A. baumannii* strains. Regarding GM MIC values, colistin exhibited the most potent activity, while co-amoxiclav showed the lowest activity against MDR *A. baumannii* strains.

Antibiotic-resistant genes in MDR *A. baumannii* isolates. The frequency of resistance genes was $bla_{\text{OXA-51}}$ (100%), *ampC* (99.12%), *apA6* (90.35%), and bla_{NDM} (69.30 %), respectively (Fig. 2). The most common genotypes were co-presence of ($bla_{\text{OXA-51}}$, *apA6*, *ampC*) in 75 (65.79%) isolates, ($bla_{\text{OXA-51}}$, *AmpC*, *apA6*) in 27 (23.68%) isolates, ($bla_{\text{OXA-51}}$, *AmpC*) in eight (7.02%) isolates, ($bla_{\text{OXA-51}}$, *AmpC*, bla_{NDM}) in three (2.63%) isolates, and ($bla_{\text{OXA-51}}$, *apA6*, bla_{NDM}) in 1 (0.88%) isolate, respectively (Table 4). The *apA6* gene was significantly associated with resistance to ceftazidime (0.041), and bla_{NDM} was significantly linked to resistance to meropenem (0.002) (Table 5).

DISCUSSION

Based on present study of 114 strains of MDR *A. baumannii* isolates, it was observed that 113 (99.1%) of them were ESBLs, and 75 (65.79%) were MDR strains. The resistance genes that were most commonly observed were $bla_{\text{OXA-51}}$, *ampC*, *apA6*, and bla_{NDM}. Piperacillin/tazobactam was found to be the most effective treatment for MDR *A. baumannii* infections. However, the isolates displayed high resistance to co-amoxiclav, ciprofloxacin, gentamicin, and ceftazidime. According to the study, a majority of *A. baumannii* isolates (59.65%) were discovered in the ICU. The most common body sites of infection were wounds and sputum. *A. baumannii* is frequently linked to wound infections because of its capability to create biofilms and develop resistance to antimicrobial agents. COVID-19 patients have a hospitalization rate of 5-15%, with some needing ICU care (17). Recent reports suggest that up to 80% of COVID-19 patients who are admitted to the ICU require invasive mechanical ventilation (18). There are several risk factors that increase the hospital stay of

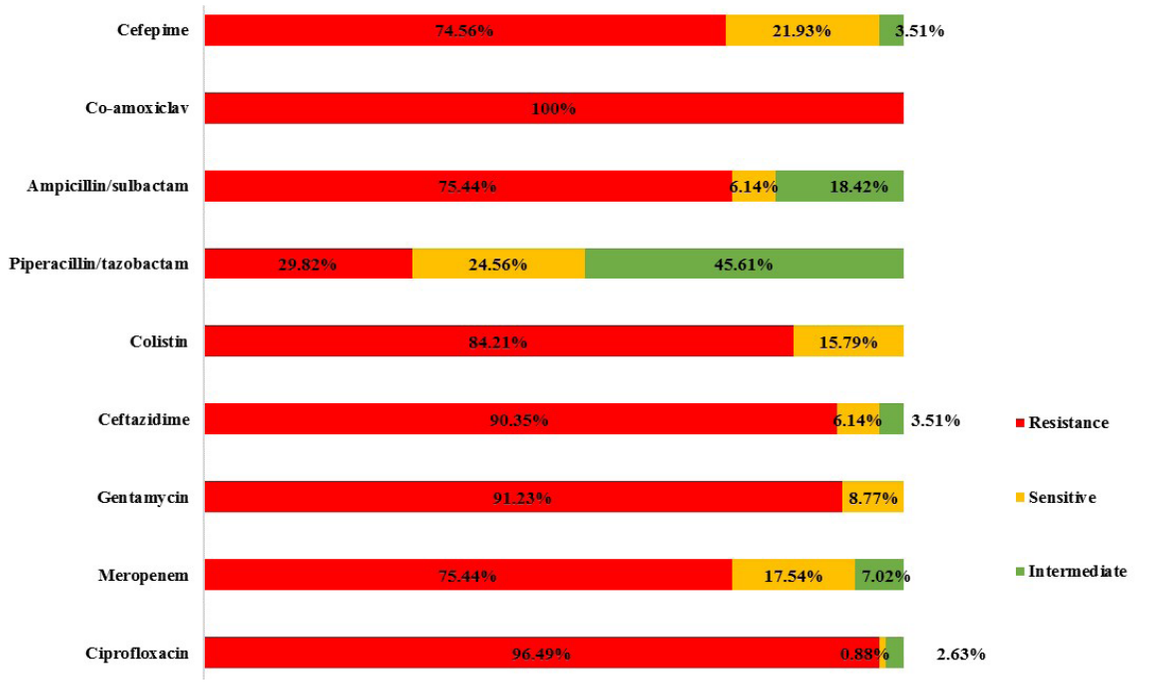

Fig. 1. Antimicrobial susceptibility of MDR *A. baumannii* strains based on broth macro dilution technique.

Table 3. antimicrobial susceptibility of MDR *A. baumannii* strains based on broth macro dilution technique

Antibiotics	Resistant	Intermediate	Sensitive	MIC_{50}	MIC_{90}	GM MIC	Mode
Aminoglycosides							
Gentamicin	91.23%	0	8.77%	1000	1000	441.42	1000
Beta-lactam/Beta-lactamase inhibitor							
Ampicillin / Sulbactam	75.44%	18.42%	6.14%	62.50	1000	86.91	62.50
Co-amoxiclav	114%	0	0	1000	1000	734.82	1000
Carbapenems							
Meropenem	75.44%	7.02%	17.54%	15.60	125	15.81	15.60
Cephalosporins							
Ceftazidime	90.35%	3.51%	6.14%	500	1000	242.07	1000
Cefepime	74.56%	3.51%	21.93%	62.50	250	53.98	125
Fluoroquinolones							
Ciprofloxacin	96.49%	2.63%	0.88%	250	500	148.87	250
Penicillins /ß-lactamase inhibitors							
Piperacillin-Tazobactam	29.82%	45.61%	24.56%	62.50	250	52.27	62.50
Polymyxins							
Colistin	84.21%	0	15.79%	3.90	500	9.71	3.90

MIC_{50} and MIC_{90} refer to the concentration at which 50% and 90% of the isolates were inhibited respectively, GM; geometric mean.

HAIs in the ICU after the COVID-19 pandemic, such as invasive and non-invasive procedures, prolonged hospital stay in the ICU, and high usage of antibiotics (19). According to the recent study, co-amoxiclav and gentamicin are not very effective against MDR *A. baumannii* strains, as their MIC_{50} values are low. For hospitals situated in North Iran, the best treatment option to fight MDR *A. baumannii* infections is piperacillin/tazobactam. Resistance to piperacillin/tazobactam varies across different regions of the

world. For example, while Poland has reported a resistance rate of 94%, India reported only a sensitivity rate of 33.3%. These variations in the results may be attributed to several factors such as geographic location, absence of a standardized definition for MDR strains, different distributions of resistance genes, the number of patients involved and diversity in the sources of the isolates (20).

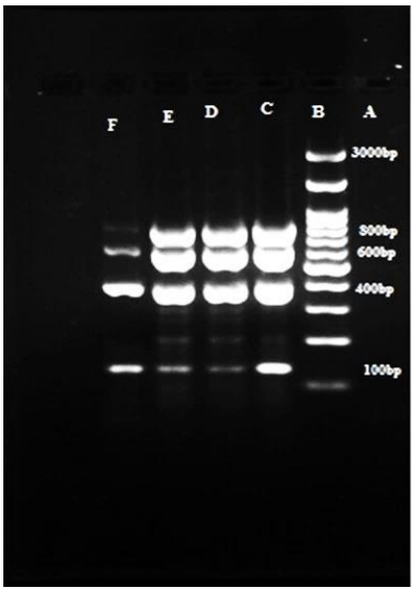

Fig. 2. The results of multiplex PCR in MDR *A. baumannii* isolates. (A): Negative control, (B): Ladder (100-3000 bp), (C): Positive control containing; *AmpC* (824 bp), bla_{NDM} (135 bp), and $bla_{OXA\text{-}51\text{-}like}$ (410 bp), and *aphA6* (631 bp) genes, (D, E, F): The samples from MDR *A. baumannii*.

In our previous surveillance studies conducted in teaching hospitals in Northern Iran before the COVID-19 outbreak, we found that 53.6% of *A. baumannii* isolates were resistant to aminoglycosides, 85.7% to ciprofloxacin, 35.7% to colistin, and 57.1% to imipenem (21). According to a study by Rezai et al., in 2017 (2), ESBL-producing *A. baumannii* isolates were obtained from patients who had VAP. These isolates showed susceptibility to various antibiotics like aminoglycosides (79%), fluoroquinolones (82.8%), colistin (34.5%), carbapenems (55.2%), and cephalosporins (89.7%). However, in our recent surveillance study conducted after the coronavirus pandemic, we noticed a significant increase in the resistance of *A. baumannii* isolates to different antibiotics. Specifically, these isolates have become highly resistant to aminoglycosides (91.23%), fluoroquinolones (96.49%), colistin (84.21%), ceftazidime (90.35%), and carbapenems (75.44%). These findings are concerning and indicate the urgent need for greater attention to antibiotic stewardship and infection control measures to combat the spread of antibiotic-resistant bacteria after COVID-19.

Before the coronavirus pandemic, our surveillance studies in Northern Iran showed that ESBL-producing *A. baumannii* was responsible for 14.15% of cases of VAP in our region (2). However, since the coronavirus pandemic, cases of MDR and ESBL-producing *A. baumannii* have sharply increased in ICUs, which has become a major concern. Recent reports suggest that nearly 19% of patients who have been diagnosed with COVID-19 display co-infections, while 24%

Table 4. Coincidence of antibiotic resistant gene of MDR *A. baumannii* isolates.

Co-incidence of antibiotic-resistant encoding genes	N (%)	Antibiotics' Resistance (%)
$bla_{OXA\text{-}51}$, *AmpC*, *apA6*, bla_{NDM}	75 (65.79%)	AMC (100), CIP (96), CAZ (92), GM (90.67), CL (85.33), MEN (84), CPM (73.33), SAM (77.33), PTZ (30.67)
$bla_{OXA\text{-}51}$, *AmpC*, *apA6*	27 (23.68%)	AMC (100), CIP (96.30), CAZ (92.59), GM (92.59), CL (81.48), CPM (77.78), SAM (74.07), MEN (44.44), PTZ (30.67)
$bla_{OXA\text{-}51}$, *AmpC*	8 (7.02%)	AMC (100), CIP (100), GM (100), MEN (87.50), CPM (75), CL (75), CAZ (62.50), SAM (62.50), PTZ (12.50)
$bla_{OXA\text{-}51}$, *AmpC* , bla_{NDM}	3 (2.63%)	AMC (100), CIP (100), MEN (100), CAZ (100), GM (100), CPM (100), CL (100), SAM (66.67), PTZ (33.33)
$bla_{OXA\text{-}51}$, *apA6*, bla_{NDM}	1 (0.88%)	AMC (100), CIP (100), MEN (100), CAZ (100), SAM (100), CL (100), GM (0), CPM (0), PTZ (0)

AMC: Co-Amoxiclav, SAM: Ampicillin / Sulbactam, CIP: Ciprofloxacin, GM: Gentamicin, CL: Colistin, CAZ: Ceftazidime, MEN: Meropenem, CPM: Cefepime, PTZ: Piperacillin / Tazobactam.

Table 5. *In vitro* susceptibilities of MDR *A. baumannii* isolates containing antibiotic resistance genes.

Positive Genes	Antibiotic	MIC Result			P- value
		Resistant	Intermediate	Sensitive	
bla_{OXA-51}	Gentamicin	91.23%	0	8.77%	NA
	Colistin	84.21%	0	15.79%	NA
	Ciprofloxacin	96.49%	2.63%	0.88%	NA
	Ceftazidime	90.35%	3.51%	6.14%	NA
	Meropenem	75.44%	7.02%	17.54%	NA
	Ampicillin Sulbactam	75.44%	18.42%	6.14%	NA
	Piperacillin-Tazobactam	29.82%	45.61%	24.56%	NA
	Cefepime	74.56%	3.51%	21.93%	NA
	Co-amoxiclav	100%	0	0	NA
ampC	Gentamicin	92.04%	0	7.96%	0.088
	Colistin	84.07%	0	15.93%	1.000
	Ciprofloxacin	96.46%	2.65%	0.88%	1.000
	Ceftazidime	90.27%	3.54%	6.19%	1.000
	Meropenem	75.22%	7.08%	17.70%	1.000
	Ampicillin Sulbactam	75.22%	18.58%	6.19%	1.000
	Piperacillin-Tazobactam	30.09%	46.02%	23.89%	0.246
	Cefepime	75.22%	3.54%	21.24%	0.254
	Co-amoxiclav	100%	0	0	NA
apA6	Gentamicin	90.29%	0	9.71%	0.279
	Colistin	84.47%	0	15.53%	0.819
	Ciprofloxacin	96.12%	2.91%	0.97%	1.000
	Ceftazidime	92.23%	1.94%	5.83%	0.041
	Meropenem	73.79%	7.77%	18.45%	0.625
	Ampicillin Sulbactam	76.70%	17.48%	5.83%	0.398
	Piperacillin-Tazobactam	31.07%	44.66%	24.27%	0.715
	Cefepime	73.79%	3.88%	22.33%	1.000
	Co-amoxiclav	100%	0	0	NA
bla_{NDM}	Gentamicin	89.87%	0	10.13%	0.442
	Colistin	86.08%	0	13.92%	0.412
	Ciprofloxacin	96.20%	3.80%	0	0.189
	Ceftazidime	92.41%	2.53%	5.06%	0.438
	Meropenem	84.81%	5.06%	10.13%	0.002
	Ampicillin Sulbactam	77.22%	16.46%	6.33%	0.767
	Piperacillin-Tazobactam	30.38%	48.10%	21.52%	0.510
	Cefepime	73.42%	5.06%	21.52%	0.616
	Co-amoxiclav	100%	0	0	NA

exhibit superinfections (22). Also, shown that medications that target cytokines, such as IL-1 and IL-6, may increase the risk of superinfections (23). To manage COVID-19 infections, patients are often given broad-spectrum antibiotics, which can increase the risk of developing MDR infections (24).

It is worrying that 65.79% of the isolates in the current study possess all antibiotic-resistant genes (*ampC*, bla_{OXA-51}, bla_{NDM}, *apA6*). Several studies have reported the coexistence of different β-lactamase genes within the same strains. Our research shows that the prevalence of the *ampC* gene was 99.12%. Overexpression of *ampC* is the main cause of resistance to ceftazidime and ampicillin-sulbactam in *A. baumannii* (25). Therefore, our most recent genetic findings confirm the highest percentage of ampicillin-sulbactam resistance in *A. baumannii*.

Our research findings show that the prevalence

of bla_{NDM} gene was observed to be 69.30%. It was also noticed that the incidence of bla_{NDM} gene and resistance to meropenem had a statistically significant correlation (P = 0.002). These results indicate that the spread of CRAB is a serious concern and further investigation is necessary to understand and combat this issue. Boorgula et al. (26), investigated the causes of secondary infections in COVID-19 patients. Their research findings showed that *Klebsiella pneumoniae* was the most commonly identified bacteria, followed by *A. baumannii*. It is noteworthy that the bacterial isolates exhibited a 6% increase in resistance to carbapenems. Additionally, during the pandemic, the frequency of carbapenem-resistant *Enterobacteriaceae* in Italy rose from 5% in 2019 to 50% (27). Also, the co-existence of MBLs genes with oxacillinase genes may have played a role in the development of carbapenem resistance in *A. baumannii*. It is concerning to note that in Iran, the frequency of carbapenem-resistant strains has been high, with a reported rate of 85.1% between 1995 and 2017 (28). Most β-lactams encoding genes are plasmid-mediated enzymes, which can be easily transmitted among bacteria and cause inappropriate or failed antimicrobial therapy (28). The current study has limitations, such as the absence of gene sequencing for bla_{OXA-51}, *AmpC*, bla_{NDM}, and *aphA6* which have a connection with resistance phenotypes.

CONCLUSION

The coronavirus pandemic has led to a troubling rise in antimicrobial resistance in healthcare facilities in Northern Iran. Our study has found a high incidence of strains with the co-presence of genes (bla_{OXA-51}, *ampC*, bla_{NDM}, and *aphA6*) that encode multi-drug resistance. This is significant for broadening empirical antibiotic therapy for critically ill patients. Piperacillin / tazobactam is recommended for treating multi-drug resistant *Acinetobacter baumannii* in patients with healthcare-associated infections in North Iran.

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