Original Article

Updating the Antimicrobial Resistance Pattern among Critical Priority Pathogens in the Intensive Care Unit in Northern Iran Post COVID-19 Pandemic

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Abstract

Background: During the COVID-19 pandemic, the widespread and indiscriminate prescription of antibiotics led to a significant increase in antibiotic resistance and the emergence of multi-drug-resistant (MDR) strains. This study aims to evaluate the prevalence of antibiotic resistance in MDR Gram-negative isolates in the intensive care unit (ICU) of northern hospitals in Iran following the COVID-19 pandemic.

Materials and Methods: This is a cross-sectional study. The samples were collected from patients with healthcare-associated infections at ICU of hospitals in northern Iran. Antimicrobial resistance was assessed using standard broth macrodilution, and resistance genes were accurately identified using the multiplex polymerase chain reaction method.

Results: The present study revealed that the ICU had the highest frequency of MDR *Acinetobacter baumannii* infections (32.1%) and the lowest frequency of *E. coli* infections (12.6%). The frequency of resistance genes of *A. baumannii* is as follows: bla_{OXA-51} (100%), *ampC* (99.12%), *apA6 (*90.35%), and bla_{NDM} (69.30%). Co-amoxiclav showed a 100% resistance rate, while Piperacillin-tazobactam had the lowest resistance rate at 38.2%.

Conclusions: This study identified a high prevalence of MDR *A. baumannii* in ICU patients with healthcare-associated infections at northern hospitals in Iran, following COVID-19. Recommended treatments include Piperacillin-tazobactam or Meropenem.

Keywords: COVID-19 pandemic, Gram-negative bacterial infection, healthcare-associated infections, intensive care unit, multidrug resistance, multiplex polymerase chain reaction

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Introduction

In the past 20 years, infectious diseases have undergone significant changes due to the rapid increase in infections and the emergence and re-emergence of pathogens. A major event in this transformation was the emergence of SARS-CoV-2 in 2020. The COVID-19 pandemic has worsened the issue of antimicrobial resistance (AMR) because recent reports suggest that there are up to 80% of COVID-19 patients who are admitted to the intensive care unit (ICU); the use of invasive procedures like ventilators, catheters, and immunosuppressive medications; and that

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there is a rise in secondary bacterial infections caused by multi-drug-resistant (MDR) bacteria.[1,2]

Globally, healthcare-associated infections (HAIs) present a significant challenge for healthcare systems. A World Health Organization (WHO) survey reveals that developed countries experience a 25% rate of HAIs in ICUs, while developing countries face a higher rate of 50%. Notably, the incidence of HAIs in ICU patients far exceeds that of other hospital wards. Tragically, mortality rates from HAIs in the ICU can reach as high as 10%–80%.[1-3] In the ICU, despite significant treatment advancements, prolonged hospitalization and the use of invasive procedures like vascular catheters, ventilators, intubation, immunosuppression, and blood transfusions significantly increase the risk of HAIs. These infections not only raise treatment costs and prolong hospital stays but also can be transmitted to other patients, leading to increased mortality and morbidity.[4]

The fermenter and no-fermenter Gram-negative bacteria are the leading causes of HAIs and outnumbering Gram-positive bacteria. This is attributed to their outer membrane structure, the presence of efflux pumps, genetic flexibility, and insufficient infection control measures.[4] In 2017, the WHO highlighted 12 groups of disease-causing agents in three priority categories (critical, high, and moderate). Notably, critical pathogens among Gram-negative pathogens included *Acinetobacter baumannii* (*A. baumannii*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) resistant to carbapenems, as well as the *Enterobacteriaceae* producing extended-spectrum beta-lactamase (ESBLs).[5-7]

The HAIs caused by Gram-negative bacteria include ventilator-associated pneumonia (VAP), healthcare-associated pneumonia (HAP), urinary tract infections (UTIs), especially catheter-Associated UTIs (CAUTI), and bloodstream infections (BSIs).[4,5] Not only Gram-negative microorganisms have caused widespread outbreaks of HAIs but they also pose a significant challenge due to their high level of antibiotic resistance. These bacteria can exhibit both intrinsic and acquired resistance to antibiotics. Resistance factors in Gram-negative bacteria include the production of beta-lactam enzymes (beta-lactamases), aminoglycoside-modifying enzymes (AMEs), increased efflux pump expression, and reduced OprD protein production.[6,7]

Beta-lactamases causing antibiotic resistance are classified into four groups: A, B, C, and D according to Ambler's classification. Classes A, C, and D use the serine mechanism, while class B requires zinc (Zn) for activity. Metallobetalactamases (MBLs), also known as Class B beta-lactamases, are highly effective against a wide range of beta-lactam antibiotics, including carbapenems. In carbapenem-resistant strains, the reduced expression of OprD, along with the production of class A beta-lactamases like *KPC* and *GES*; metallo-beta-lactamases such as *IMP, NDM, GIM, FIM, SPM,* and *VIM*; and class D enzymes like high-level *OXA-48*, results in resistance to carbapenems. These enzymes play a crucial role in the emergence of ESBL strains. ESBL strains are highly resistant to most beta-lactam antibiotics, including penicillins, cephalosporins, and aztreonam.[4-8] In strains that are resistant to multiple drugs (MDR) and extensively drug-resistant (XDR) strains, not only do they contain genes that are resistant to carbapenems and cephalosporins (such as beta-lactamase class C (*AmpC*), bla_{SHV}, bla_{TEM}, bla_{CTXM}) but they also show resistance to quinolones like ciprofloxacin and levofloxacin. This resistance is associated with the inhibition of DNA gyrase and topoisomerase IV, changes in topoisomerase 2 and 4, and the expression of plasmid-dependent efflux pump *OqxA* and *OqxB*.[8]

During the COVID-19 pandemic, there was an increase in irrational antibiotic use worldwide, leading to significant antibiotic resistance. This resulted in the spread of strains resistant to multiple antibiotics. Varying resistance patterns have been reported in different regions, and experimental antibiotic prescriptions without knowledge of resistance patterns have contributed to this challenge.[4] Given this context, the present study was conducted to determine the prevalence of antibiotic resistance in multidrug resistance Gram-negative isolates in the ICU at the Northern Hospital, Iran, after the COVID-19 pandemic.

Materials and Methods

Study design and sample collection

The cross-sectional study was conducted at designated coronavirus healthcare centers in Mazandaran province from 2022 to 2023 during the pandemic. A total of 12,834 patient samples with HAIs were included in the study. The focus was on MDR nonfermenting Gram-negative bacilli (NF-GNB) and MDR fermentative Gram-negative bacteria (GNB) collected from the ICUs. The MDR strains resisted to at least one agent across three or more antimicrobial classes. Gram-negative isolates that were non-MDR, Gram-negative that were isolated from other wards, and Gram-positive isolates were excluded from the study.

Table 1 contains the data illustrating the collection of MDR-NF-GNB and MDR-F-GNB isolates from various samples. The samples were cultured on McConkey and blood agar (QUELAB, USA) and then incubated for 24 hours at 37°C. Standard biochemical tests, including the urease, oxidase, catalase, citrate, sugar fermentation, and indole production tests, were used to identify the isolates. The study protocol was approved under the code IR.MAZUMS.REC.1403.214 and received ethical approval from the esteemed Ethics Committee of Mazandaran University of Medical Sciences.[9,10]

Susceptibility testing

The MDR-GNB was assessed for antibiotic susceptibility using the standard broth macrodilution technique, in accordance with the Clinical and Laboratory Standards Institute (CLSI; 2020). We obtained antibiotics including Ampicillin-Sulbactam, Ceftazidime, Cefepime, Ciprofloxacin, Colistin, Co-amoxiclav,

Gentamicin, Meropenem, and Piperacillin-tazobactam from Bio Basic, Canada.[9,10]

Detection of ESBL-producing isolates

To identify ESBL-producing isolates, a combined disk test (CDT) was performed using the cephalosporins Cefotaxime, Cefepime, Ceftazidime, and Ceftriaxone (Padtan Teb, Iran). The presence of ESBLs was confirmed with antibiotic disks of Ceftazidime/clavulanic acid, Cefotaxime, and Cefepime (30/10 µg). A standard strain of *K. pneumoniae*, ATCC No. 700603, was used as a positive control.[9,10]

DNA extraction and molecular assays

DNA of MDR-GNB isolates was extracted using an extraction kit (Yekta Taghiz, Iran) according to the manufacturer's protocol. The specific primers of *E. coli* included bla_{IMP}, bla_{TEM}, *AcrA, AcrB*, bla_{CTX}, $bla_{OXA\ -58}$, *aaclb*, bla_{SHV}, and *aacla*. The specific primers of *A. baumannii* included bla_{OXA-51}, *ampC, apA6*, and $_{blaNDM}$. The specific primers of *K. pneumoniae* included $bla_{SHV,}$ bla_{CTX}, bla_{TEM}, acrAB, *OqxAB*, and bla_{IMP}. The specific primers of *P. aeruginosa* included bla_{SHV}, $bla_{\text{CTX-M}}$, bla_{AmpC}, bla_{IMP}, bla_{SPM}, and bla_{SIM}. Multiplex polymerase chain reaction (PCR) was prepared, including Taq DNA polymerase (AMPLIQON, Denmark), primers (10 pM), template DNA (100 ng), and DNase-free distilled water. Multiplex PCR mixtures without template DNA and with DNA control (*K. pneumoniae* ATCC NO.7881 (bla_{CTXM}, bla_{TEM}, and bla_{SHV}) and *E. coli* ATCC NO. 35218 (*AcrA, AcrB, aaclb, aacla*) were used as negative and positive controls, respectively. In summary, the amplification process involved a denaturation step at 94°C for 30 s, followed by 35 cycles at 61°C for 30 s, 72°C for 30 minutes, and a final extension step at 72°C for 10 minutes. The multiplex PCR products were separated on a 1.5% agarose gel and were visualized using the gel documentation system (UVIDoc HD6 Touch, USA).[9,10]

Table 1: Patient demographic information

Variable	*n* (%)
Age	
<1 Years old	16 (6.5%)
1 – 18 Years old	37 (15%)
>18 Years old	193 (78.5%)
Gender	
Male	164 (66.7%)
Female	78 (31.7%)
Unit	
ICU	128 (52%)
BICU	44 (17.9%)
PICU	31 (12.6%)
CCU	27 (11%)
NICU	16 (6.5%)
Samples	
Sputum	76 (30.9%)
urinary catheter infections	66 (26.8%)
Wound	44 (17.9%)
Blood	30 (12.2%)
ETT	13 (5.3%)
BAL	8 (3.3%)
CSF	3 (1.2%)
Catheter	3 (1.2%)
EYE	2 (0.8%)
Pleural	1 (0.4%)

Statistical analysis

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

Results

In Table 1, the study involves 246 patients, aged between 2 days and 94 years, with an average age of 48.93 years (SD = 27.82). The median age of the patients was 54 years (IQR: 43 (29–72)), and 66.7% were male. The data reveal that the most prevalent HAIs were sputum infections (76; 30.9%) and urinary catheter infections (66; 26.8%). The ICU had the highest frequency of MDR *A. baumannii* infections at 79 (32.1%) and the lowest frequency at 31 (12.6%) with *E. coli* infections [Figure 1].

Antimicrobial susceptibility test

Tables 2 and 3 present a comprehensive overview of the antimicrobial susceptibility of MDR-GNB isolates utilizing the macro dilution technique and MIC. The summary includes the geometric means (GM) MIC and mode of MICs for Ampicillin-sulbactam, Ceftazidime, Cefepime, Ciprofloxacin, Colistin, Co-amoxiclav, Gentamicin, Meropenem, and Piperacillin-Tazobactam against MDR-GNB.

The antibiotic sensitivity test revealed that the bacteria were 100% resistant to co-amoxiclav. The MDR-GNB isolates showed high resistance to Ciprofloxacin, Ceftazidime, Gentamicin, Ampicillin-Sulbactam, and Colistin with resistance rates of 86.2%, 82.5%, 80.9%, 80.5%, and 80.1%, respectively, and the lowest resistance to piperacillin-tazobactam at a resistance rate of 38.2%.

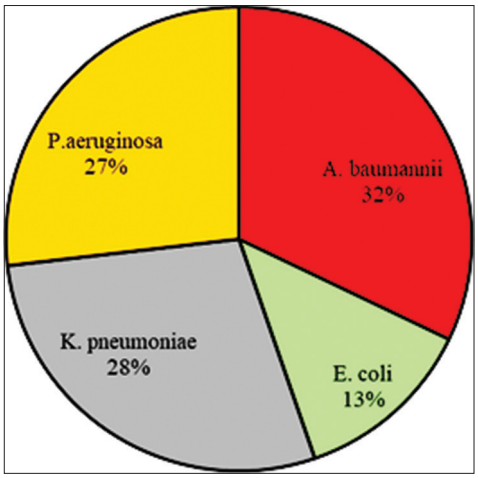

Figure 1: Various species of nonfermenters and fermenters MDR-GNB isolates from ICU

Table 2: MIC_{50}, MIC_{90}, GM MIC base on Macro dilution Method

Antibiotic	R	S	I	MIC_{50}	MIC_{90}	GM MIC	Mode
Ciprofloxacin	86.2%	8.5%	5.3%	250	500	79.33	250
Meropenem	67.9%	26%	6.1%	15.6	250	16.88	0.9
Gentamicin	80.9%	17.5%	1.6%	500	1000	206.21	<1000
Ceftazidime	82.5%	15%	2.4%	250	1000	151.68	<1000
Colistin	80.1%	15%	4.9%	7.8	1000	19.17	3.9
Piperacillin-Tazobactam	38.2%	36.6%	25.2%	62.5	500	39.87	62.5
Ampicillin-Sulbactam	80.5%	13.4%	6.1%	125	1000	114.94	<1000
Co- Amoxiclav	100%	0%	0%	1000	1000	521.95	>1000
Cefepime	71.5%	24.8%	3.7%	125	1000	54.14	250

Resistant, Sensitive, Intermediate

Table 3: Antimicrobial susceptibility of MDR-GNB isolates based on the macro dilution technique

Antibiotics	*A. baumannii* (*n*=79)					*E. coli* (*n*=31)					*K. pneumoniae* (*n*=70)					*P. aeruginosa* (*n*=66)				
	R	S	I	MIC		R	S	I	MIC		R	S	I	MIC		R	S	I	MIC	
				50	90				50	90				50	90				50	90
Ciprofloxacin	97.5	0	2.5	250	650.3	96.8	3.2	0	250	250	81.4	8.6	10	187.5	500	72.7	21.2	6.1	125	500
Meropenem	78.5	15.2	6.3	15.6	125	100	0	0	62.5	250	68.6	22.9	8.6	31.2	250	39.4	54.5	6.1	1.9	650
Gentamicin	94.9	5.1	0	1000	1000	74.2	25.8	0	250	500	75.7	18.6	5.7	749.5	1000	72.7	27.3	0	500	1000
Ceftazidime	94.9	2.5	2.5	500	1000	74.2	25.8	0	125	1000	90	8.6	1.4	500	1000	63.3	31.8	4.5	187.5	1000
Colistin	83.5	16.5	0	3.9	650.3	58.1	41.9	0	7.8	849.2	88.6	11.4	0	7.8	1000	77.3	4.5	18.2	15.6	1000
Piperacillin-Tazobactam	31.6	19	49.4	62.5	250	35.5	64.5	0	0.9	250	45.7	37.1	17.1	62.5	1000	39.4	43.9	16.7	32	1000
Ampicillin-Sulbactam	75.9	6.3	17.7	62.5	1000	71	29	0	250	900	85.7	14.3	0	750	1000	84.8	13.6	1.5	125	1000
Co-Amoxiclav	100	0	0	1000	1000	100	0	0	250	1000	100	0	0	500	1000	100	0	0	500	1000
Cefepime	81	16.5	2.5	62.5	250	54.8	45.2	0	250	250	88.6	7.1	4.3	250	1000	50	43.9	6.1	16	1000

Resistant, Sensitive, Intermediate

Molecular epidemiology of MDR-NGB isolates

The frequency of resistance genes of *E. coli* is as follows: bla_{IMP} 100%, bla_{TEM} 100%, *AcrA* 99.1%, *AcrB* 99.1%, bla_{CTX} 91.2%, $bla_{OXA\text{-}58}$ 80.7%, *aaclb* 64.9%, bla_{SHV} 44.7%, and *aacla* 37.7%.

The frequency of resistance genes of *A. baumannii* is as follows: $bla_{OXA\text{-}51}$ (100%), *ampC* (99.12%), *apA6 (*90.35%), and $_{blaNDM}$ (69.30%).

The frequency of resistance genes of *K. pneumoniae* is as follows: bla_{SHV} (74.56), bla_{CTX} (88.60), bla_{TEM} (99.12), *acrAB* (92.98), *OqxAB* (67.54), and bla_{IMP} (64.04).

The frequency of resistance genes of *P. aeruginosa* is as follows: bla_{SHV} (91. 3%), $bla_{CTX\text{-}M}$ (76%), bla_{AmpC} (91.3%), and bla_{IMP} (95.2%). It is noted that the bla_{SPM} and bla_{SIM} were not seen among isolates.

In *E. coli* isolates, the bla_{SHV} gene was significantly associated with resistance to Ceftazidime ($P < 0.001$) and Ampicillin-Sulbactam ($P = 0.039$). The bla_{CTX} gene was significantly associated with resistance to Gentamicin ($P = 0.014$), Colistin ($P = 0.017$), and Cefepime ($P < 0.001$). The *AcrA, AcrB* genes were significantly associated with resistance to Ciprofloxacin ($P = 0.018$). The *aacla, aaclb* genes were significantly associated with resistance to Gentamicin ($P < 0.001$) and Colistin ($P < 0.001$). The $bla_{OXA\text{-}58}$ gene was significantly associated with resistance to Piperacillin-Tazobactam ($P < 0.001$) and Ampicillin-Sulbactam ($P < 0.001$).

In *A. baumannii* isolates, the *apA6* gene was significantly associated with resistance to Ceftazidime ($P = 0.041$), and bla_{NDM} was significantly linked to resistance to Meropenem ($P = 0.002$).

In *P. aeruginosa* isolates, the $bla_{CTX\text{-}M}$ gene was significantly associated with resistance to Ciprofloxacin ($P = 0.006$), and bla_{IMP} gene was significantly associated with resistance to Meropenem ($P = 0.037$).

In *K. pneumoniae* isolates, the bla_{SHV} gene was significantly associated with resistance to Gentamicin ($P = 0.004$), bla_{CTX} *gene* was significantly associated with resistance to Gentamicin ($P = 0.024$), and bla_{IMP} gene was significantly associated with resistance to Ciprofloxacin, Ceftazidime, Meropenem, Piperacillin-Tazobactam, and Cefepime ($P = 0.001$, $P < 0.001$, $P < 0.001$, $P = 0.002$, and $P < 0.001$, respectively). The *OqXB* gene was significantly associated with resistance to Gentamicin, Ciprofloxacin, and Piperacillin-Tazobactam ($P = 0.028$, $P = 0.002$, and $P = 0.024$, respectively).

Discussion

In the present study, we assessed 246 MDR ESBL-producing GNB isolates from patients with HAIs in ICU. Both nonfermentative and fermentative bacteria were identified. The most frequently collected sample was sputum, and the predominant microorganism was identified as *A. baumannii*. Based on MIC50 values, a high resistance rate of 100% to Co-amoxiclav was exhibited, while Piperacillin-Tazobactam had a lower resistance rate of 38.2%.

Our research found that Gram-negative nonfermenting organisms are alarmingly common in the ICU. Acinetobacter species have become a significant cause of opportunistic infections in the ICU, showing resistance to a wide range of antibacterial agents through both intrinsic and extrinsic mechanisms. These resistant organisms thrive in moist environments commonly found in ICU equipment such as nebulizers, dialysis fluids, saline, catheters, and other devices. Additionally, the widespread use of broad-spectrum antibiotics, invasive devices, prolonged hospital stays, and inadequate infection control practices contribute to the higher prevalence of MDR-ESBL-producing GNB isolates in the ICU. Notably, a post-COVID-19 pandemic surveillance study conducted by Rahimzadeh *et al*. revealed a significant 59.65% prevalence of MDR *A. baumannii* isolates in the ICU, with wound and sputum being the dominant sites of infection.[9,11]The results of the study of Chaudhary *et al*.[12] correlate with our results; out of 500 samples, 76% showed growth of Gram-negative bacteria, and the most resistant isolates were found to be Acinetobacter species (100%) with 83.15% exhibiting multidrug resistance. Additionally, Saad *et al*.[13] and Chawla *et al*.[14] reported a significant increase in antimicrobial resistance among nonfermenting respiratory pathogens due to prophylactic antibacterial therapy, prolonged stays in the ICU, and mechanical ventilation following COVID-19.

Before the COVID-19 pandemic, Ghasemian *et al*.[15] and Babamahmoodi *et al*.[16] reported that pneumonia caused by Enterobacter, a Gram-negative fermenter, was the most common HAI in the ICU at teaching hospitals in northern Iran.

Our study and another in the same regions found a significant shift in pathogens responsible for HAI after the COVID-19 pandemic. This change may be due to our sampling from various intensive care units, including the pediatric (PICU), neonatal (NICU), and burn (BICU) units. *A. baumannii* is the most common bacterial secondary infection in COVID-19 patients, with 91.2% of cases being carbapenem-resistant, allowing for transmission within healthcare centers.[9]

In the current study, based on macro dilution results, Co-amoxiclav was found to be ineffective against MDR-GNB. However, Piperacillin-Tazobactam exhibited the lowest resistance. In terms of GM MIC values, Meropenem was identified as the most effective antibiotic against MDR-GNB isolates. Our findings on carbapenem sensitivity are consistent with previous studies. Soni *et al*.[17] reported a susceptibility rate of 79.3%, Yadav *et al*.,[18] 79.7%, Siwakoti *et al*.,[19] 81%, and Parajuli *et al*.,[20] 86.4%. This suggests promise for using carbapenems to treat MDR Acinetobacter spp, although some studies recommend Colistin as the preferred drug for this purpose.

Before the COVID-19 pandemic, our surveillance studies revealed that 53.6% of MDR Gram-negative isolates were resistant to several antibiotics: aminoglycosides (85.7%), ciprofloxacin (35.7%), colistin (57.1%), and imipenem (21%).[21] A 2017 study by Rezai *et al*.[22] indicated that ESBL-producing *A. baumannii* isolates were found in patients with VAP. These isolates demonstrated susceptibility to aminoglycosides (79%), fluoroquinolones (82.8%), colistin (34.5%), carbapenems (55.2%), and cephalosporins (89.7%).

However, in our recent surveillance study conducted after the COVID-19 pandemic, we noted a significant increase in the resistance of MDR Gram-negative isolates to various antibiotics. Specifically, these isolates have shown a marked increase in resistance to aminoglycosides (94.9%), fluoroquinolones (97.5%), colistin (83.5%), ceftazidime (94.9%), and carbapenems (78.5%).

Before COVID-19 pandemic, our studies conducted in northern Iran indicated that ESBL-producing *A. baumannii* was responsible for 14.15% of VAP cases in the region.[21-23] However, since the coronavirus pandemic, cases of ESBLs—respiratory pathogens—have significantly increased in ICU, which is a major concern. In a retrospective study, colonization by carbapenem-resistant fermenter isolates increased from 6.7% to 50% from 2019 to 2020.[5-7] The rise in antimicrobial resistance among GNB in the COVID-19 era can be attributed to several factors. These include prior antimicrobial exposure, the use of broad-spectrum antibiotics for COVID-19 patients, difficulties in distinguishing between viral and bacterial infections, prolonged mechanical ventilation, changes in hospital operations during the pandemic, and healthcare worker-mediated microbe transmission.[4,7]

The current study identified that both nonfermenting and fermenting respiratory pathogens significantly had the resistant-encoding genes such as bla_{IMP}, bla_{TEM}, *AcrA-B*, bla_{CTX}, $bla_{OXA\ -58}$, *aaclb*, bla_{SHV}, and *aacla*. Before COVID-19, our previous study found that the bla_{TEM} gene was identified most frequently at 49%, followed by bla_{SHV} at 44% and bla_{CTX} at 28%.[21,22] Furthermore, Bagheri Nesami *et al*.[23] reported a high frequency of ESBL-related genes, with 94.3% for bla_{SHV}, 48.6% for bla_{CTX}, 22.9% for bla_{VEB}, and 17.14% for bla_{GES}.

Our recent research showed that the bla_{SHV} gene was significantly associated with resistance to Ceftazidime ($P < 0.001$) and Ampicillin-sulbactam ($P = 0.039$). Additionally, the bla_{CTX} gene was significantly associated with resistance to Cefepime ($P < 0.001$). These results suggest that the overuse of broad-spectrum antibiotics in healthcare settings leads to the transmission of ESBL-encoding genes on plasmids,

making it easier for them to be transferred between different organisms. This creates a strong link between the presence of resistant genes and high expression of resistance to ESBLs, posing a significant therapeutic challenge as these strains often display resistance to various antimicrobial drugs.[5-7] Furthermore, the *AcrA-B* genes were significantly associated with resistance to Ciprofloxacin ($P = 0.018$). It is important to note that the *AcrA-B* secretory pumps are crucial for resisting fluoroquinolones, chloramphenicol, tetracycline, trimethoprim, beta-lactams, and macrolides.

Our study revealed that fermenter and nonfermenter respiratory pathogens carrying the genes bla_{NDM} and bla_{IMP} were significantly linked to resistance to Meropenem ($P = 0.002$) ($P = 0.001$). The spread of carbapenem-resistant *A. baumannii* is a major concern, and further investigation is urgently needed to understand and address this issue. Similar to our findings, Boorgula *et al*.[24] reported that *K. pneumoniae* and *A. baumannii* were the most commonly identified bacteria causing secondary infections in COVID-19 patients. There has been a dramatic rise in carbapenem-resistant Enterobacteriaceae in Italy, from 5% in 2019 to a staggering 50% in 2020. Additionally, the coexistence of metallo-β-lactamases (MBLs) genes with Oxacillinases genes has likely contributed to the development of carbapenem resistance in *A. baumannii*. The potential for plasmid-mediated transmission of β-lactam encoding genes among bacteria is a cause for serious concern as it can lead to ineffective antimicrobial therapy. The current study has limitations, particularly the absence of sequencing for resistant genes, which could enhance the overall findings.

Conclusion

This study identified a significant prevalence of antibiotic-resistant genes as well as an increase in MDR Gram-negative bacterial isolates among patients with healthcare-associated infections in the ICU at North Hospital in Iran following COVID-19. It is recommended to prescribe Piperacillin-tazobactam or Meropenem for the empirical treatment of these infections caused by MDR Gram-negative bacteria at North Hospital.

Ethics statement

The study was approved by the Ethics Committee of the Mazandaran University of Medical Science (IR.MAZUMS.REC.1403.214), Sari, Iran.

Author contributions

Sh R, G R, RV, RR, M SH, N GH, and MS R designed the project, collected data, and wrote and performed a critical review of the manuscript. Sh R, GR, RV, and RR did the microbiological and molecular tests. M M carried out statistical interpretation. All authors contributed to the article and approved the submitted version.

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Conflicts of interest

There are no conflicts of interest.

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