Original Article

High Prevalence of Antimicrobial Resistance Genes in Multidrug-Resistant Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* Clinical Isolates after COVID-19 Pandemic, North Iran

Golnar Rahimzadeh[1], Shaghayegh Rezai[2], Reza Valadan[3], Raha Rezai[1], Saman Soleimanpour[2], Laleh Vahedi[4], Somayeh Sheidaei[4], Masoud Moradi[5], Mohammad Sadegh Rezai[1], Ebrahim Nemati[1]

[1]Pediatric Infectious Diseases Research Center, Communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran, [2]Department of Medical Microbiology and Virology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, [3]Molecular and Cell Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran, [4]Department of Pathology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran, [5]Student Research Committee, Faculty of Health, Mazandaran University of Medical Sciences, Sari, Iran

Abstract

Background: Amid the COVID-19 pandemic, the surge in hospital admissions and widespread use of broad-spectrum antibiotics have heightened the risk of hospital-acquired infections from multidrug-resistant (MDR) organisms, particularly *Escherichia coli*. It is imperative to implement stringent measures to curb the spread of antimicrobial resistance in hospitals and devise robust treatment strategies for patients grappling with such infections. To confront this challenge, a comprehensive study was undertaken to examine MDR extended-spectrum beta-lactamase (MDR-ESBL)-producing *Escherichia coli* isolates from patients with nosocomial infections following the COVID-19 pandemic in Northern Iran.

Materials and Methods: The current study was conducted as a cross-sectional study. A total of 12,834 samples were collected from patients with healthcare-associated infections at four designated corona centers in Northern Iran, following the COVID-19 pandemic. Antimicrobial resistance was determined using standard broth micro-dilution, while resistance genes were accurately detected using the multiplex PCR method.

Results: The results indicated that meropenem and ciprofloxacin had a resistance rate of 100% and 98.2%, respectively, while piperacillin-tazobactam showed the highest sensitivity rate at 54.4%. The frequency of specific genes, including bla_{IMP}, bla_{TEM}, *AcrA, AcrB,* bla_{CTX}, bla_{OXA-58}, *aaclb,* bla_{SHV}, and *aacla*, were found to be 100%, 100%, 99.1%, 99.1%, 91.2%, 80.7%, 64.9%, 44.7%, and 37.7%, respectively.

Conclusions: In the current study, over 50% of MDR-ESBL-producing *Escherichia coli* isolates exhibited resistance to antibiotics. A combination of antibiotics, including piperacillin-tazobactam and colistin, is recommended for treating extensively drug-resistant *Escherichia coli* infections.

Keywords: COVID-19, *Escherichia coli*, extended-spectrum beta-lactamase (ESBL), multidrug-resistant, multiplex polymerase chain reaction

Address for correspondence: Dr. Mohammad Sadegh Rezai, Pediatric Infectious Diseases Research Center, Communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran.
E-mail: drmsrezaii@yahoo.com
Submitted: 27-Jul-2024; **Revised:** 11-Aug-2024; **Accepted:** 13-Aug-2024; **Published:** 28-Oct-2024

Introduction

During the COVID-19 pandemic, there has been a troubling disregard for international guidelines on prescribing antibiotics. Consequently, we have seen a surge in microbial resistance in both community-acquired infections (CAIs)

Access this article online

Quick Response Code:

Website: www.advbiores.net

DOI: 10.4103/abr.abr_341_24

How to cite this article: Rahimzadeh G, Rezai S, Valadan R, Rezai R, Soleimanpour S, Vahedi L, *et al*. High prevalence of antimicrobial resistance genes in multidrug-resistant extended-spectrum beta-lactamase-producing *Escherichia coli* clinical isolates after COVID-19 pandemic, North Iran. Adv Biomed Res 2024;13:105.

and hospital-acquired infections (HAIs). The prolonged hospitalization of COVID-19 patients, particularly in intensive care units (ICUs) and the extensive use of invasive procedures and immunosuppressive agents have contributed to the rise of secondary bacterial infections and the overuse of antibiotics. Regrettably, these factors have fueled the escalation of antimicrobial resistance (AMR).[1] According to estimates, nearly 4.95 million deaths occur each year due to AMR. Without the implementation of effective control measures, this number could double to 10 million by 2050.[2]

Escherichia coli (E. coli) is a significant cause of both HAIs and CAIs, including respiratory tract infections (RTIs), urinary tract infections (UTIs), enteric infections, sepsis, neonatal meningitis, wound infections, and pneumonia.[3]

In the last two decades, there has been a noticeable increase in infections caused by antibiotic-resistant *E. coli*, which has had an impact on patient outcomes. Multidrug-resistant (MDR) *E. coli*, particularly those producing extended-spectrum β-lactamase (ESBL), are a major concern due to their growing prevalence and resistance to a wide range of antibiotics. Many strains of *E. coli* have developed resistance to multiple, extensive, or all available drugs (MDR, XDR, and PDR), posing a significant challenge for infection treatment. This bacterium has acquired a combination of antibiotic genes from both chromosomal and plasmid sources. The acquisition of antibiotic-resistant genes through plasmids and other mobile agents, along with mutations under antibiotic pressure, has led to the emergence of highly resistant strains of MDR and XDR *E. Coli* in clinical settings. Multidrug resistance is a serious issue, where microorganisms exhibit resistance to at least one agent in three or more antimicrobial categories.[4]

The main mechanism of antimicrobial resistance to β-lactams is through the production of β-lactamase enzymes. There exist different types of β-lactamases: class A (e.g., bla_{TEM} bla_{SHV}, and bla_{CTX}), class B (e.g., bla_{IMPs}, bla_{VIMs}, bla_{GIMs}, bla_{NDMs}, and bla_{SIM}), class C (cephalosporinases or *AmpC*), and class D (Oxacillinases). Many of these enzymes play a pivotal role in imparting resistance to β-lactam antibiotics. The most well-known ESBL genotypes worldwide include bla_{CTXM}, bla_{SHV}, and bla_{TEM}, which are encoded by plasmid. The emergence of *E. coli* strains with ESBLs and AmpC β-lactamase carriers also render them resistant to other antibiotics such as aminoglycosides, fluoroquinolones, tetracycline, chloramphenicol, sulfonamides, and carbapenems.[4,5] Quinolone resistance in *E. coli* strains involves complex mechanisms, including the presence of quinolone resistance determining regions (QRDR) and the expression of *gyrA* and *parC* genes, which target the quinolones. In addition, resistance can occur through the acquisition of plasmid-mediated quinolone resistance (PMQR), which may include genes such as *qnrA, qnrB, qnrC, qnrD,* and *qnrS*.[4] In addition, efflux pump systems contribute to MDR by expelling antibiotics outside the bacterial cell. These systems fall into six groups and include the *AcrAB-TolC* efflux pump, which is the main efflux pump in virulent *E. coli* strains. Aminoglycoside resistance can occur through mutation of the 16S rRNA, reduced permeability, efflux pumps, and enzymatic inactivation, which is the most prevalent in the clinical setting. Resistance to newer semisynthetic aminoglycosides is diverse and involves the production of aminoglycoside-modifying enzymes such as aminoglycoside acetyltransferases (AAC) and aminoglycoside nucleotidyl transferases (ANT, or AAD).[6]

The high prevalence of AMR in hospitals is a serious global concern, varying by region. Research shows that AMR contributes to HAIs and reduces antibiotic effectiveness, leading to increased mortality rates, treatment costs, and hospital stays.[1] Investigating the spread of AMR in hospitals for patients with HAIs in different regions is crucial in the post-COVID-19 era. Therefore, the current study was conducted to investigate the emergence of MDR extended-spectrum beta-lactamase (ESBL)-producing *E. coli* isolates from patients with HAIs after the COVID-19 pandemic in teaching hospitals in northern Iran.

Materials and Methods

Study design, and sample collection

This cross-sectional study was conducted between 2022 and 2023 at four hospitals in Mazandaran province, 12,834 patient samples with HAIs were included. The study focused on isolating MDR *E. coli* strains from MDR gram-negative bacteria (MDR-GNB). Table 1 presents the data illustrating the collection of MDR *E. coli* strains from various samples. Samples were cultured on McConkey and blood agar (QUELAB, USA) and incubated for 24 hours at 37°C. Conventional biochemical tests were used to identify the isolates.[1,7] The study protocol was approved under the code IR.MAZUMS.REC.1401.13949 and received ethical approval from the esteemed Ethics Committee of Mazandaran University of Medical Sciences.

Antibiotic sensitivity determination

The antimicrobial susceptibility of MDR *E. coli* isolates was determined using the standard microdilution method recommended by the Institute of Clinical Laboratory Standards guideline (CLSI 2020). The minimum inhibitory concentration (MIC) of various antibiotics was measured.[1,7]

Detection of Extended-Spectrum Beta-Lactamase (ESBL)-Producing E. coli isolates

To detect ESBL-producing strains phenotypically, a combined disk test (CDT) was performed. The bacterial colony suspension equivalent to 0.5 McFarland (1.5×10^8 CFU/mL) was prepared and cultured on Muller-Hinton agar (QUELAB, USA).[1,7]

DNA extraction and molecular assays

DNA of MDR *E. coli* isolates was extracted using an extraction kit (Yekta Tehiz, Iran) according to the manufacturer's protocol. The specific primers included bla_{IMP}, bla_{TEM}, *AcrA, AcrB,* bla_{CTX},

bla_{OXA-58}, *aaclb*, bla_{SHV}, and *aacla*. Multiplex PCR reaction was prepared, including Taq DNA polymerase (AMPLIQON, Denmark), primer (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 (*CTXM*, TEM, and SHV) and *E. coli* ATCC NO. 35218 (*AcrA, AcrB, aaclb, aacla*))[7,8] were used as negative and positive controls, respectively. In summary, the amplification process involved a denaturation step at 94°C for 30 seconds, followed by 35 cycles at 61°C for 30 seconds, 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).[7]

Table 1: Patient demographic information

Variable	*n* (%)
Age	
<1 year old	18 (15.8)
1–18 years old	10 (8.8)
>18 years old	86 (75.4)
Gender	
Male	46 (40.4)
Female	68 (59.6)
Unit	
Emergency	41 (36)
Surgery	8 (7)
Internal	18 (15.9)
Intensive care units (ICUs)	13 (11.4)
Ear, Nose, and Throat (ENT)	1 (0.9)
Orthopedic	19 (16.7)
Neurology	3 (2.6)
Oncology	2 (1.8)
Burn ward	6 (5.3)
Pediatric ward	3 (2.6)
Sample	
Urine culture (UC)	97 (85.1)
Wound	6 (5.3)
Sputum	3 (2.5)
Bronchoalveolar lavage (BAL)	1 (0.9)
EYE	2 (1.8)
Ascites	1 (0.9)

Statistical analysis

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

Results

The median age of patients was 58 years (IQR: 53 (18–71) years), with 46 (40.4%) being male and 68 (59.6%) being female. UTIs (97; 58.1%) the highest incidence of type of HAIs caused by MDR *E. coli* in various hospital wards were in the emergency (41; 36%), orthopedic ward (19; 16.7%), internal (18; 15.9%), and ICUs (13; 11.4%) [Table 1].

Antibiotic sensitivity testing

A total of 12,834 samples from HAI patients were tested, and 2190 gram-negative isolates were identified, among which 114 were MDR *E. coli* strains. The micro-dilution technique results showed that the bacteria were 100% resistant to meropenem and exhibited high resistance to ciprofloxacin, ampicillin-sulbactam, ceftazidime, and gentamicin, with resistance rates of 98.2%, 72.8%, 69.3%, and 64.9%, respectively. The most effective antibiotic was found to be piperacillin-tazobactam, with a sensitivity rate of 54.4%.

Table 2 shows a summary of the MIC_{50}, MIC_{90}, and geometric means (GM) MIC. In terms of MIC50 values, meropenem and ciprofloxacin showed the lowest activity against MDR *E. coli* isolates. When it comes to GM MIC values, colistin demonstrated the most potent activity against MDR *E. coli* isolates.

Molecular epidemiology of MDR E. coli isolates

The frequency of resistance genes is as follows: bla_{IMP} 100%, bla_{TEM} 100%, *AcrA* 99.1%, *AcrB* 99.1%, bla_{CTX} 91.2%, bla_{OXA-58} 80.7%, *aaclb* 64.9%, bla_{SHV} 44.7%, *aacla* 37.7%. Strains with the bla_{SHV} gene had antibiotic resistance rates of 51%–100% and sensitivity rates of 0%–49%. Strains with the bla_{TEM} gene had antibiotic resistance rates of 51.8%–100% and sensitivity rates of 0%–54.4%. Strains with the bla_{CTX} gene had antibiotic resistance rates of 48.1%–100% and sensitivity rates of 0%–51.9%. Strains with the *AcrA* gene had antibiotic resistance rates of 46%–100% and sensitivity rates of 0%–54%. Strains with the *AcrB* gene had antibiotic resistance rates of 46%–100% and sensitivity rates of 0%–54%. Strains with the *aacIa* gene had antibiotic

Table 2: Antimicrobial susceptibility of MDR *E. Coli* isolates based on the microdilution technique

Antibiotics	Resistant	Sensitive	MIC_{50}	MIC_{90}	GM MIC	Mode
Piperacillin-Tazobactam	45.6%	54.4%	11.7	500	22.24	500
Colistin	51.8%	48.2%	31.2	500	12.44	0.9
Cefepime	54.4%	45.6%	31.2	500	15.21	0.9
Gentamicin	64.9%	35.1%	31.2	1000	39.77	500
Ceftazidime	69.3%	30.7%	62.5	500	40.56	500
Ampicillin-Sulbactam	72.8%	27.2%	62.5	500	45.62	500
Ciprofloxacin	98.2%	1.8%	62.5	1000	128.67	31.2
Meropenem	100.0%	0.0%	500	1000	197.13	500

resistance rates of 20.9%–100% and sensitivity rates of 0%–79.1%. Strains with the *aaclb* gene had antibiotic resistance rates of 40.5%–100% and sensitivity rates of 0%–59.5%. Strains with the bla_{IMP} gene had antibiotic resistance rates of 45.6%–100% and sensitivity rates of 0%–54.4%. Strains with the *OXA-58* gene had antibiotic resistance rates of 48.9%–100% and sensitivity rates of 0%–51.1%. In all the above strains, meropenem showed the highest resistance, while piperacillin-tazobactam had the highest sensitivity.

The bla_{SHV} gene was significantly associated with resistance to ceftazidime (<0.001), and ampicillin-sulbactam (0.039). The bla_{CTX} gene was significantly associated with resistance to gentamicin (0.014), colistin (0.017), and cefepime (<0.001). The *AcrA, AcrB* genes were significantly associated with resistance to ciprofloxacin (0.018). The *aacla, aaclb* genes were significantly associated with resistance to gentamicin (<0.001), and colistin (<0.001). The $bla_{OXA\text{-}58}$ gene was significantly associated with resistance to piperacillin-tazobactam (<0.001), and ampicillin-sulbactam (<0.001) [Figure 1]. Table 3 presents a concise overview of antibiotic sensitivity in both adults and children. The results obtained from the microdilution method reveal a significant difference in sensitivity to ampicillin-sulbactam between the two groups (0.024).

Discussion

In the current study, 114 strains of MDR-ESBL-producing *E. coli* isolates were assessed. The results revealed that

Table 3: Antimicrobial susceptibility of isolated MDR *E. Coli* from adults and children based on the microdilution technique

Antibiotics' resistance (R)*	Child *n*=28	Adult *n*=86	*P*
Gentamicin	19 (67.9%)	55 (64%)	0.707
Colistin	17 (60.7%)	42 (48.8%)	0.275
Ciprofloxacin	28 (100%)	84 (97.7%)	>0.99
Ceftazidime	17 (60.7%)	62 (72.1%)	0.257
Meropenem	28 (100%)	86 (100%)	-
Ampicillin-Sulbactam	25 (89.3%)	58 (67.4%)	0.024
Piperacillin-azobactam	15 (53.6%)	37 (43%)	0.330
Cefepime	18 (64.3%)	44 (51.2%)	0.226

*Resistance

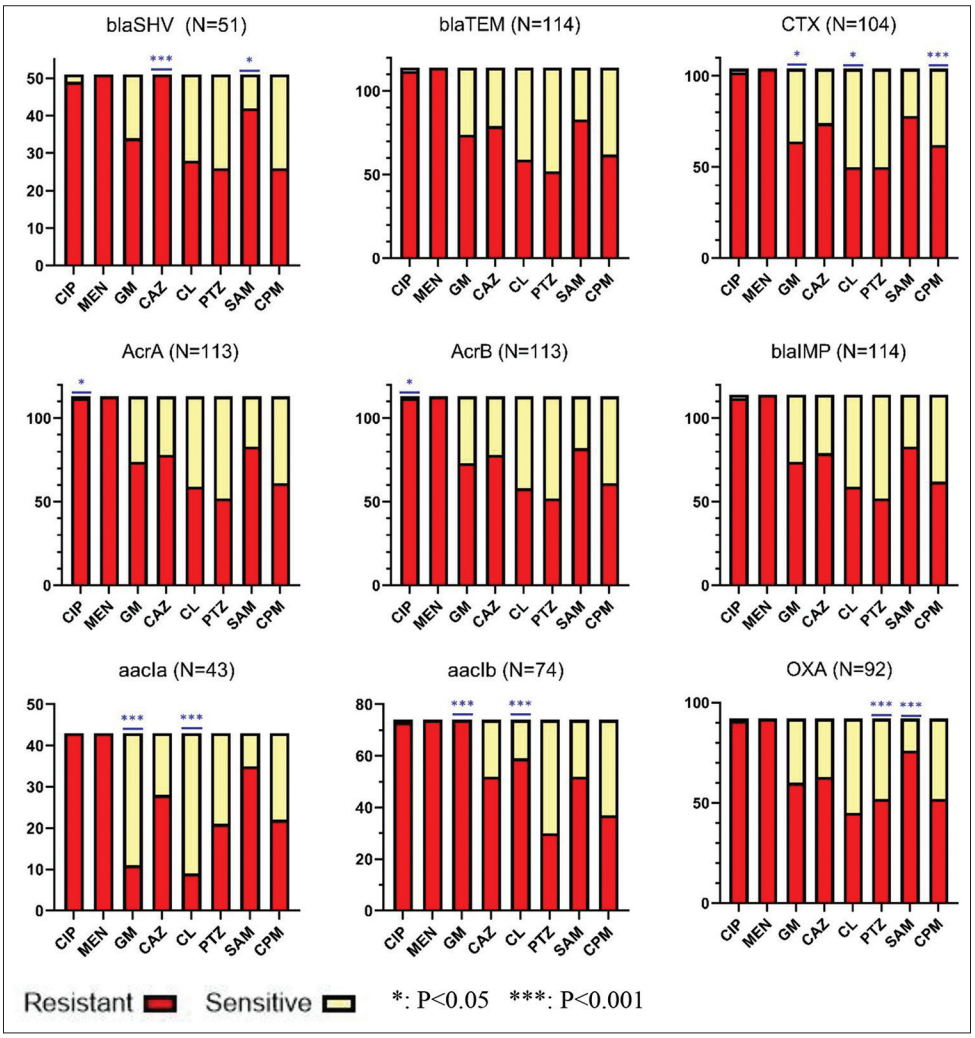

Figure 1: Correlation between antibiotic resistance genes and susceptibility to antibiotics among MDR *E. coli* isolates. SAM: Ampicillin-Sulbactam, CIP: Ciprofloxacin, GM: Gentamicin, CL: Colistin, CAZ: Ceftazidime, MEN: Meropenem, CPM: Cefepime, PTZ: Piperacillin-Tazobactam

110 (96.49%) of the strains were ESBL-producing *E. coli*, and 76 (66.66%) were MDR strains. The most prevalent resistance genes detected were bla_{IMP}, bla_{TEM}, *AcrA, AcrB,* bla_{CTX}, bla_{OXA-58}, *aaclb,* bla_{SHV}, and *aacla*. The isolates showed significant resistance to meropenem, ciprofloxacin, ampicillin-sulbactam, ceftazidime, gentamicin, and cefepime. The study also highlighted UTIs as the most common infection. UTIs affect about 150 million people worldwide annually. In developed countries, uropathogenic *E. coli* (UPEC) is responsible for 80% of UTIs due to two key virulence factors: adhesin receptors (fimbriae type I, P, and S) and toxins (hemolysin and tumor necrosis factor). Based on our results and supporting European reports, it is irrefutable that UTIs caused by *E. coli* rank as the most prevalent HAIs.[9,10] Bagheri-Nesami *et al*.[11] indicated that *E. coli* was responsible for 77.18% of ventilator-associated pneumonia (VAP) cases and 29% of catheter-associated urinary tract infections (CAUTI). These findings underscore the urgency of implementing robust prevention and management strategies to combat E. coli-related infections in healthcare facilities.

Our results demonstrate a substantial 66.66% increase in ESBL-producing *E. coli*, marking a significant surge compared to our 2015 surveillance (30.5%)[7] and the study of Bagheri-Nesami *et al*.[11] (8.69%). This highlights the urgent need for further research and action to address. However, the prevalence of ESBL-producing *E. coli* was notably lower in countries such as India (27%), Lebanon (13.3%), Korea (9.2%), and Turkey (17%).[11] The varying factors contributing to this distinction include differences in sample sizes, types of HAIs, geographical locations, patients hospitalized in different wards, and patterns of antibiotic prescription.

In our study, was detected the most common ESBL genes, namely bla_{TEM}, bla_{CTX}, and bla_{SHV}. According to a systematic review in Iran, bla_{TEM} is reported as the most prevalent ESBL-producing gene at 51%.[12] Our previous study also supports this finding, showing that the bla_{TEM} gene was identified most frequently at 49%, followed by bla_{SHV} at 44%, and bla_{CTX} at 28%.[7] Furthermore, Bagheri-Nesami *et al*.[11] 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}. A meta-analysis (2016) reported that ESBL strains had a 14% prevalence with a 5.38% annual increase. Asia and Africa had a higher prevalence (15%–46%) than Europe and the Americas (2%–6%). The ESBL strains pose a serious challenge for effective treatment as ESBL-producing bacteria are resistant to a wide range of antibiotics and carry genes that confer resistance to multiple other antibiotics. In addition, the transferability of ESBL genes, often found on large plasmids, enables easy transmission between bacterial strains and even different species, contributing to high patient mortality and a substantial financial burden on the health system.[13,14]

In our study, was utilized the microdilution technique to assess antibiotic sensitivity. The results revealed that over half of the MDR and XDR *E. coli* isolates were resistant to ciprofloxacin, ampicillin-sulbactam, ceftazidime, gentamicin, colistin, meropenem, and piperacillin-tazobactam. Pre-COVID-19 pandemic, our study showed that MDR *E. coli* isolates were most susceptible to carbapenems (66%) and aminoglycosides (58%).[7] Bagheri-Nesami *et al*.[11] reported that *Enterobacteriaceae* were generally sensitive to carbapenems. Pervious study. demonstrated that only 3.62% of isolates exhibited resistance to amikacin. The impact of the COVID-19 pandemic on the healthcare system has been substantial, leading to a significant increase in AMR. Studies revealed a concerning rise in AMR in bacteria such as *E. coli, K. pneumoniae, S. aureus*, and *A. baumannii*, particularly in the MDR strains during the pandemic, with rates ranging from 24% to 37.5% (19-25). An international study reported the occurrence of AMR in COVID-19 patients to be as high as 54%.[15]

Our results showed that the bla_{SHV} gene was significantly linked to resistance to ceftazidime (<0.001) and ampicillin-sulbactam (0.039). In addition, the bla_{CTX} gene was significantly associated with resistance to gentamicin (0.014), colistin (0.017), and cefepime (<0.001). These results suggest the overuse of broad-spectrum antibiotics in healthcare settings and the transmission of ESBL-encoding genes on plasmids. Some of these genes are carried within transposons or integrons, which facilitate their transfer between different organisms.[16-18] The frequency of bla_{CTX-M}, bla_{TEM}, and bla_{SHV} genes, and resistance to cefotaxime, ceftazidime, cefepime, and ceftriaxone indicate a strong link between the presence of resistant genes and high expression of resistance to ESBLs. These strains present a significant therapeutic challenge as they often display resistance to various antimicrobial drugs, including aminoglycosides, quinolones, and cotrimoxazole.[19,20]

Our findings revealed a significant correlation between the presence of *AcrA* and *AcrB* genes and resistance to ciprofloxacin (0.018). The *AcrA-B* secretory pumps are a major factor in innate resistance to fluoroquinolones and also confer resistance to chloramphenicol, tetracycline, trimethoprim, beta-lactams, and macrolides.[21] Previous studies in various regions of Iran have reported *E. coli* resistance to ciprofloxacin ranging from 10.2% to 85%. Abdi *et al*.[22] reported a slightly lower rate of 51%, but in the United States, the resistance rate was 16.8%, and in China, it ranged from 43% in 2013 to 48% in 2015.[23,24]

In our study, the genes *aacla* and *aaclb* were found to be significantly associated with resistance to gentamicin (<0.001) and colistin (<0.001). Our results revealed that *E. coli* isolates displayed significant resistance to aminoglycosides. Interestingly, a study in Iran[25] reported lower resistance rates of 21% for gentamicin, 24.6% for tobramycin, 23.18% for kanamycin, and 3.62% for amikacin. These findings emphasize the urgent need to address the notable levels of resistance to aminoglycoside antibiotics in MDR *E. coli* isolates in Northern Iran.

Our study revealed a significant difference in resistance to ampicillin-sulbactam between adults and children ($P = 0.024$).

According to our results, prescribing ampicillin-sulbactam in combination with other antibiotics is recommended for treating pediatric infectious diseases caused by MDR-ESBL-producing *E. coli* at hospitals in Northern Iran. The current study has limitations, such as the absence of sequencing for MDR and ESBL-producing genes.

Conclusion

The current study revealed a high frequency of ESBL genes (bla_{SHV}, bla_{TEM}, bla_{CTX}), carbapenemase-producing genes (bla_{IMP}), aminoglycoside resistance genes (*aacIb* and *aacIa*), and efflux pump-expressing genes (*AcrA, AcrB*) in MDR-ESBL-producing *E. coli* isolates, indicating an increase in antimicrobial resistance in healthcare facilities in Northern Iran following the COVID-19 pandemic. Our results showed that over 50% of MDR-ESBL-producing *E. coli* isolates are resistant to antibiotics. We recommend a combination of antibiotics, including piperacillin-tazobactam and colistin, for treating extensively drug-resistant *E. coli* infections.

Acknowledgments

We gratefully acknowledge Mrs.Fatemeh Bagherzadeh, and Shahram Divsalar for their supportive help during performing laboratory tests.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Rahimzadeh G, Valadan R, Rezai SH, Khosravi M, Vahedi Larijani L, Sheidaei S, *et al*. Evaluation of antibiotic resistance changes in *Acinetobacter baumannii* in the era of COVID-19 in Northern Iran. Iran J Microbiol 2024;16:314-22.
2. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A, *et al*. Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. Lancet 2022;399:629-55.
3. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol 2000;66:4555-8.
4. Galarce N, Arriagada G, Sánchez F, Venegas V, Cornejo J, Lapierre L. Antimicrobial use in companion animals: Assessing veterinarians' prescription patterns through the first national survey in Chile. Animals (Basel) 2021;11:348-54.
5. World Health Organization. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Available from: https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed. [Last accessed on 2023 Oct 30].
6. Chetri S, Bhowmik D, Paul D, Pandey P, Chanda DD, Chakravarty A, *et al*. AcrAB-TolC efflux pump system plays a role in carbapenem nonsusceptibility in *Escherichia coli*. BMC Microbiol 2019;19:210-8.
7. Rezai MS, Salehifar E, Rafiei A, Langaee T, Rafati M, Shafahi K, *et al*. Characterization of multidrug resistan extended-spectrum beta-lactamase-producing *Escherichia coli* among uropathogens of pediatrics in North of Iran. Biomed Res Int 2015;2015:309478.
8. Rihacek M, Kuthanova M, Splichal Z, Adam V, Hrazdilova K, Vesely R, *et al*. *Escherichia coli* from human wounds: Analysis of resistance to β-lactams and expression of RND efflux pumps. Infect Drug Resist 2023;16:7365-75.
9. Kumar G, Kumar Y, Kumar G, Tahlan AK. Characterization of uropathogenic *E. coli* from various geographical locations in India. J Taibah Univ Med Sci 2023;18:1527-35.
10. Maniam L, Vellasamy KM, Ong TA, Teh CSJ, Jabar KA, Mariappan V, *et al*. Genotypic characteristics of Uropathogenic *Escherichia coli* isolated from complicated urinary tract infection (cUTI) and asymptomatic bacteriuria—A relational analysis. Peer J 2023;11:e15305.
11. Bagheri-Nesami M, Rafiei A, Eslami G, Ahangarkani F, Rezai MS, Nikkhah A, *et al*. Assessment of extended-spectrum β-lactamases and integrons among Enterobacteriaceae in device-associated infections: Multicenter study in north of Iran. Antimicrob Resist Infect Control 2016;5:52-60.
12. Sheykhsaran E, Bannazadeh Baghi H, Soroush Barhaghi MH, Alizadeh N, Memar MY, Etemadi S, *et al*. The rate of resistance to tetracyclines and distribution of tetA, tetB, tetC, tetD, tetE, tetG, tetJ and tetY genes in Enterobacteriaceae isolated from Azerbaijan, Iran during 2017. Physiol Pharmacol 2018;22:205-12.
13. Rahimzadeh G, Rezai MS, Farshid F. Genotypic pat-terns of multidrug-resistant Acinetobacter baumannii: A systematic review. Adv Biomed Res 2023;12:56.
14. Rahimzadeh G, Rezai MS. Detection extended spectrum beta lactamase and carbapenemase producing Enterobacteriaceae isolates from clinicalsamples; Narrative review. J Isfahan Med Sch 2022;40:743-58.
15. Conway Morris A, Kohler K, De Corte T, Ercole A, De Grooth HJ, Elbers PWG, *et al*. Co-infection and ICU-acquired infection in COIVD-19 ICU patients: A secondary analysis of the UNITE-COVID data set. Crit Care 2022;26:236.
16. Mariana C, Simner PJ, Bradford PA. Extended-spectrum β-lactamases: An update on their characteristics, epidemiology and detection. JAC Antimicrob Resist 2021;3:dlab092.
17. Pruss A, Kwiatkowski P, Masiuk H, Bilska I, Giedrys-Kalemba S, Dołęgowska B. Epidemiological analysis of extended-spectrum β-lactamase-producing Klebsiella pneumoniae outbreak in a neonatal clinic in Poland. Antibiotics (Basel) 2023;12:50.
18. Husna A, Rahman MM, Badruzzaman ATM, Sikder MH, Islam MR, Rahman MT, *et al*. Extended-spectrum β-lactamases (ESBL): Challenges and opportunities. Rev Biomedicines 2023;11:2937.
19. Livermore DM, Day M, Cleary P, Hopkins KL, Toleman MA, Wareham DW, *et al*. OXA-1 β-lactamase and nonsusceptibility to penicillin/β-lactamase inhibitor combinations among ESBL-producing *Escherichia coli*. J Antimicrob Chemother 2019;74:326-33.
20. Arakawa Y, Shibata N, Shibayama K, Kurokawa H, Yagi T, Fujiwara H, *et al*. Convenient test for screening metallo-beta-lactamase-producing gram-negative bacteria by using thiol compounds. J Clin Microbiol 2000;38:40-3.
21. Anne D-R, Jean-Marie P, Aurélie F. Clinical Status of Efflux Resistance Mechanisms in Gram-Negative Bacteria. Review. Antibiotics 2021;10:1117.
22. Abdi S, Ranjbar R, Vala MH, Jonaidi N, Bejestany OB, Bejestany FB. Frequency of blaTEM, blaSHV blaCTX-M, and qnrA among *Escherichia coli* isolated from urinary tract infection. Arch Clin Infect Dis 2014;9:e18690.
23. Johnson JR, Johnston B, Kuskowski MA, Sokurenko EV, Tchesnokova V. Intensity and mechanisms of fluoroquinolone resistance within the H30 and H30Rx subclones of *Escherichia coli* sequence type 131 compared with other fluoroquinolone-resistant *E. coli*. Antimicrob Agents Chemother 2015;59:4471-80.
24. Korona-Glowniak I, Skrzypek K, Siwiec R, Wrobel A, Malm A. Fluoroquinolone-resistance mechanisms and phylogenetic background of clinical *Escherichia coli* strains isolated in southeast Poland. New Microbiol 2016;39:210-5.
25. Soleimani N, Aganj M, Ali L, Shokoohizadeh L, Sakinc T. Frequency distribution of genes encoding aminoglycoside modifying enzymes in Uropathogenic *E. coli* isolated from Iranian hospital. BMC Res Notes 2014;7:842.