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Fecal Carriage of ESBL and Carbapenem Resistance among hospitalized patients in Tehran, Iran

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ABSTRACT

Background & Objective: Enterobacteriaceae, a family of Gram-negative bacteria in the normal gut flora, are common human pathogens. The rising fecal carriage of carbapenemase-producing (CPE) and extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-PE) poses significant resistance to beta-lactam antibiotics. Accordingly, this study investigated the prevalence and molecular characterization of beta-lactamase genes (blaKPC, blaIMP, blaVIM, blaNDM, blaSHV, blaTEM, blaOXA, and blaCTX-M) in Enterobacteriaceae isolates from hospitalized patients in Shahriar, Iran.

Materials & Methods: A total of 85 stool samples were collected from hospitalized patients in the ICU and general wards of Imam Sajjad Hospital, Shahriar. Bacterial isolates were identified using standard microbiological methods for identification of Enterobacteriaceae. Antibiotic susceptibility profile of isolates was analysed by using the disc diffusion, and Minimum inhibitory concentrations (MICs) method based on the Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines. Phenotypic detection of ESBL and such as metallo-beta-lactamase (MBL) resistance, was performed using combined disc tests with specific inhibitors. PCR amplification with specific primer was used for detection beta-lactamase genes including *bla$_{KPC}$, bla$_{IMP}$, bla$_{VIM}$, bla$_{NDM}$, bla$_{SHV}$, bla$_{TEM}$, bla$_{OXA}$* and *bla$_{CTX-M}$*.

Results: From 85 samples, 19 isolates of *Klebsiella pneumoniae* (20.43%) and 66 isolates of *Escherichia coli* (79.57%) were identified. Among these, 31 isolates exhibited ESBL production, and 10 were identified as MBL [-producing isolates]. Among the 31 carbapenem-resistant or intermediate isolates, all carried the blaCTX-M gene. Additionally, 2 isolates carried the blaNDM-1 gene, and another 2 harbored the blaIMP gene. However, the blaKPC and blaVIM genes were not detected in any of the isolates.

Conclusion: The *bla$_{CTX-M}$* gene is one of the most common resistant genes in Iran. According to studies, the prevalence of antibiotic resistance in Iran is rising dramatically, which reduces the choice of antibiotics to treat severe infections in the future.

Keywords: Fecal Carriage, ESBL, Carbapenem Resistance, *Escherichia coli*, *Klebsiella pneumoniae*

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1. Introduction

Enterobacteriaceae is known as a large family of Gram-negative bacteria. They are found in water, food, animals, and humans and are distributed worldwide (1, 2). Enterobacteriaceae include many pathogenic genera such as *Salmonella, Shigella, Escherichia,* and *Klebsiella* (3). Although they belong to the normal gut microbiota in humans, they can cause urinary tract infections, intra-abdominal infections, and hospital- and healthcare-associated pneumonia. In this context, *Escherichia coli* and *Klebsiella pneumoniae* specifically are associated with serious nosocomial infections; for example, *E. coli* is a frequent cause of urinary tract infections, and antibiotic resistance in this species is associated with human infections. β-lactam antibiotics are known as the treatment of choice for Enterobacteriaceae infections. Because of their genetic adaptability, these bacteria have been the subject of numerous laboratory studies, and they have developed resistance to β-lactam antibiotics, which is an important problem and requires immediate attention (4, 5).

The first antibiotic was identified by Alexander Fleming in 1928, and antibiotic resistance was soon described in 1945 because of the widespread use of antibiotics. Antibiotic resistance is on the rise in various bacterial species, especially Gram-negative bacteria, and is a major concern for multidrug-resistant strains, which are responsible for more than half of all deaths associated with healthcare infections (6). As mentioned in previous studies, β-lactams have been used as the treatment of choice for Enterobacteriaceae infections, but in Enterobacteriaceae, the production of β-lactamase is one of the most important factors in the development of resistance to these drugs. β-lactamases, as hydrolytic enzymes produced by bacteria, hydrolyze β-lactams and inactivate them. Infection with extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae is a serious concern for humans and the environment (5). Carbapenems are β-lactam antibiotics with a broad spectrum of antibacterial activity against Gram-positive and Gram-negative bacteria. They are highly effective against many bacterial species and are not susceptible to most beta-lactam resistance determinants, making them one of the most reliable treatment options for infections caused by multidrug-resistant Gram-negative bacteria. (7, 8).

Identification of other isolates of ESBLs as newer β-lactamases that have acquired this ability through plasmids has led to resistance to a wider range of antibiotics. Carbapenemases are one such group of enzymes, and the first report in Enterobacteriaceae in 1993 was in 1993, and they have been increasingly reported in the last 10 years (9). So far, carbapenemases belonging to three classes of beta-lactamases have been identified. *K. pneumoniae* carbapenemases (KPC), as the most common enzyme in class A carbapenemases, were first identified in 1996 in the eastern United States and have recently reported from Puerto Rico, Colombia, Greece, and China (10, 11). Class B metallo-β-lactamases (MBLs) include three types of Verona integron-encoded metallo-β-lactamase (VIM), imipenemase (IMP) and New Delhi metallo-β-lactamase (NDM) enzymes, and their endemicity has been reported in Greece, Taiwan, and Japan (12). This class of carbapenemases can hydrolyze all β-lactams except aztreonam. OXA-48 makes up Class D and was first identified from a *K. pneumoniae* strain isolated in Turkey in 2003 (13).

Fecal carriage of carbapenemase-producing Enterobacteriaceae (CPE) has expanded in recent years, which has received less attention compared with the carriage of isolates producing extended-spectrum β-lactamases (ESBLs) (14). The emergence of carbapenem-resistant Enterobacteriaceae is a rapidly advancing global public health problem and calls for immediate action bythe international scientific community.

According to studies, most *E. coli* and *K. pneumoniae* isolates from human clinical extraintestinal infections are resistant to several drugs, and reports of carbapenemase-producing cases are increasing. In both species of these bacteria, multidrug resistance occurs with the acquisition and maintenance of MDR plasmids.

It should be noted that the plasmids associated with ESBLs and carbapenemases are different, and whenever both resistances occur in the same strain, they are usually on separate plasmids, so that the spread of ESBLs such as blaCTX-M and carbapenemases such as blaNDM and blaKPC depends completely on the presence of MDR plasmids. IncF family plasmids seem to be responsible for the global spread of blaCTX-M in *E. coli* (15, 16).

In a study conducted in Hamedan in 2016, out of 307 Enterobacteriaceae isolates collected, only 40 isolates were reported to be resistant to carbapenems. Using the modified Hodge test (MHT), it was found that out of 40 carbapenem-resistant Enterobacteriaceae (CRE) isolates, 29 isolates were positive for carbapenemase enzymes. In that study, blaIMP, blaVIM, blaNDM, and blaOXA-48 genes were identified in only 6 isolates (17).

Due to the improper and inappropriate use of antibiotics, especially β-lactams, the emergence of MDR strains of Enterobacteriaceae and the increasing prevalence of β-lactamases and carbapenemases among these bacteria, this study aimed to isolate and molecularly characterize Enterobacteriaceae strains resistant to β-lactam and carbapenem antibiotics isolated from stool samples of hospitalized patients.

2. Materials and Methods

2.1 Sampling

In this cross-sectional study conducted from July to August 2020, 85 stool samples were collected from hospitalized patients in the general wards and the ICU of Imam Sajjad Hospital in Shahriar, Iran.

The samples were then transported in sterile tubes at 4 °C to the microbiology laboratory. Regardless of the type of disease and the cause of hospitalization, were collected due to the presence of risk factors such as ICU admission and prior antibiotic therapy.

Inclusion criteria included patients hospitalized in the general wards and ICU with risk factors such as antibiotic therapy and ICU stay, while exclusion criteria included patients lacking informed consent or those with

incomplete medical records. Ethical approval was obtained from the Human Research Ethics Committee of the Islamic Azad University, Tehran Medical Branch (IR.IAU.PS.REC.1399.079). Written informed consent was obtained from all patients or their guardians, and the study adhered to relevant guidelines and regulations, ensuring compliance with ethical principles for human research.

2.2 Isolation and phenotypic detection of ESBLs

The samples were examined phenotypically based on the method proposed by the CDC (18). In brief, a sterile swab was inserted into the stool samples; then, a stool-soaked swab was placed in a sterile tube containing 5 ml of Tryptic Soy Broth medium (HiMedia, India), and a 30-µg cefotaxime disk (Mast, UK) was inserted into the tube. Tubes were mixed by vortexing and incubated at 37 °C overnight. All tubes in which growth was observed were first vortexed, and then 100 µL of bacterial suspension was cultured on MacConkey agar medium. Then, a 30-µg cefotaxime disk (Patanteb, Iran) was placed on the plate, and finally, the plates were incubated at 37 °C overnight. Colonies that grew around the 30-µg cefotaxime disk with an inhibition zone diameter of ≤ 27 mm were considered as potential extended-spectrum β-lactamase (ESBL)-producing isolates (19) (Figure 1).

2.3 Bacterial Identification

Isolates were identified at the species level using standard biochemical tests and microbiological methods such as colony morphology, motility, carbohydrate fermentation tests for glucose, lactose, and sucrose, Triple Sugar Iron (TSI), Simmons citrate, SIM (Sulfide, Indole, Motility), methyl red, and urease (20).

2.4 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was carried out for Enterobacteriaceae isolates using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (19).

Inocula with turbidity equal to a 0.5 McFarland standard were lawn-cultured on Mueller-Hinton agar (MHA) plates. Then the antimicrobial disks were placed on the MHA plates. Finally, the plates were incubated at 37 ºC for 24 h.

The antibiotic disks (Patanteb, Iran) included: ceftriaxone (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), amikacin (30 µg), imipenem (10 µg), meropenem (10 µg), ertapenem (10 µg), gentamicin (10 µg), and ciprofloxacin (5 µg). The results of susceptibility testing were validated using the American Type Culture Collection (ATCC) quality control strain *E. coli* ATCC 25922.

Table 1. Primer used for the detection of beta-lactamases

Primer name	target	Primer sequence (5´ →3´)	Amplicon size (bp)	Annealing temperature (ºC)	Reference
KPC	*bla*$_{KPC}$	F-CGTCTAGTTCTGCTGTCT R-CTTGTCATCCTTGTTAGGCG	798	60	(24)
VIM	*bla*$_{VIM}$	F-GTTTGGTCGCATATCGCAAC R-AATGCGCAGCACCAGGATAG	389	58	(24)
IMP	*bla*$_{IMP}$	F-GGAATAGAGTGGCTTAATTCTC R-GGTTTAACAAAACAACCACC	232	50	(24)
NDM	*bla*$_{NDM}$	F-GGTTTGGCGATCTGGTTTTC R-CGGAATGGCTCATCACGATC	621	55	(24)
CTX-M	*bla*$_{CTX-M}$	F- CGCTTTGCGATGTGCAG R- ACCGCGATATCGTTGGT	550	54	(25)
SHV	*bla*$_{SHV}$	F-TCAGCGAAAAACACCTTG R-TCCCGCAGATAAATCACC	472	56	(26)
TEM	*bla*$_{TEM}$	F-CTTCCTGTTTTTGCTCACC R-AGCAATAAACCAGCCAGC	632	58	(26)
OXA	*bla*$_{OXA}$	F-GCGTGGTTAAGGATGAACAC R-CATCAAGTTCAACCCAACCG	438	55	(27)

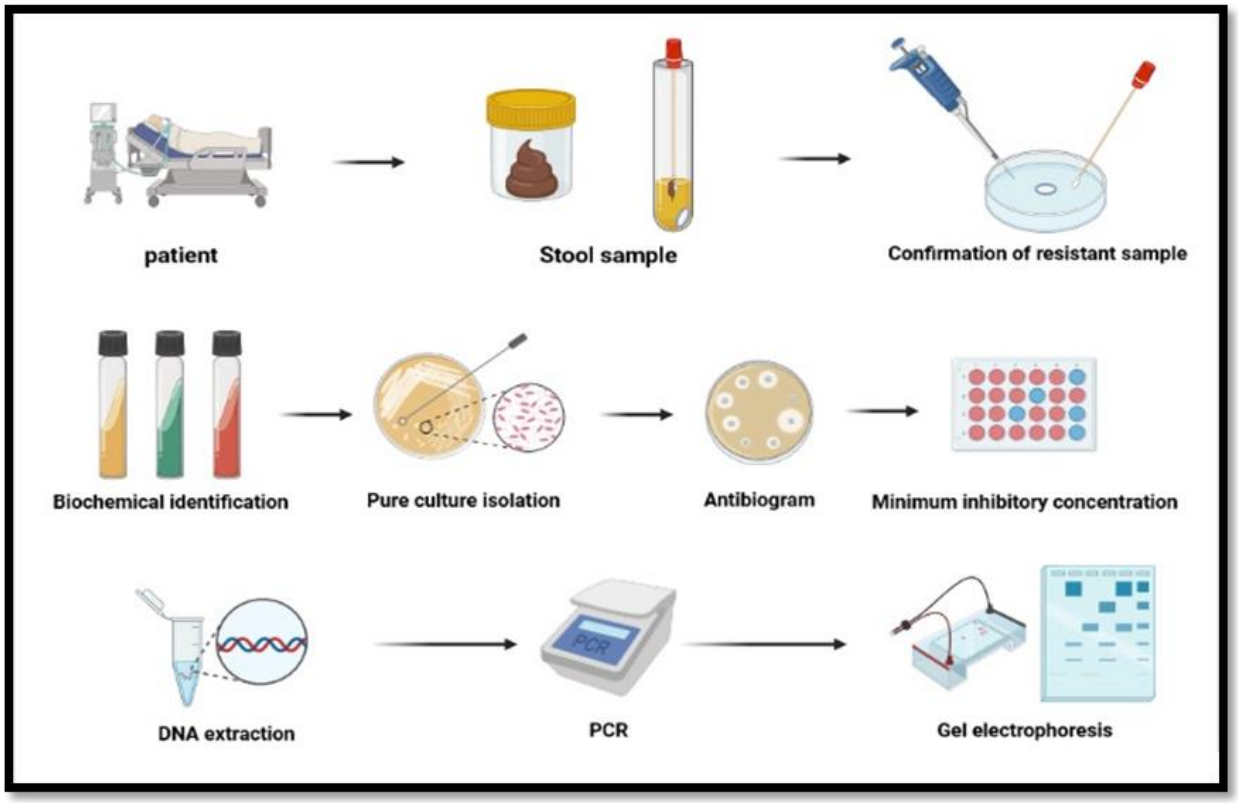

Figure 1. Practical processes used in this study (**Prepared by Authors, 2026**).

2.5 Phenotypic Detection of Metallo-β-Lactamases (MBL)-producing strains

The detection of MBL production in bacterial isolates was performed using the method described by Bahramian et al. Initially, the isolates that were resistant or showed intermediate sensitivity to carbapenems (imipenem, meropenem, and ertapenem) were evaluated using the MIC method. The isolates identified as resistant to carbapenems according to CLSI guidelines susceptibilitybreakpoint ≤ 2 mg/L and resistance breakpoint ≥ 8 mg/L) were subsequently assessed for phenotypic MBL production using the combined disk test (CDT). The CDT was performed according to the CLSI 2020 guidelines. In this method, isolates were first screened for carbapenem resistance using a 10-µg meropenem disk (MAST, UK) on Mueller-Hinton agar (MHA) plates. Bacterial suspensions were adjusted to a 0.5 McFarland turbidity standard and uniformly lawn-cultured on MHA plates. Following incubation at 37 °C for 24 hours, isolates that showed reduced susceptibility to meropenem (inhibition zone diameter of ≤ 21 mm) were further tested for MBL production. Two meropenem disks (10 µg) were used, with one disk containing 10 µL of 0.5 M EDTA (Sigma-Aldrich, USA), an MBL inhibitor. Both disks were placed at least 25 mm apart on the inoculated MHA plates. After overnight incubation at 37 °C, the results were interpreted by comparing the inhibition zones around the meropenem disk with and without EDTA. An increase of ≥ 7 mm in the inhibition zone diameter around the meropenem-EDTA disk compared to the meropenem-only disk indicated MBL production **(21)**.

2.6 Molecular Detection of Carbapenemases and ESBL Genes

Chromosomal DNA of isolates was extracted according to the method described by Azadi et al. In brief, a few colonies of bacteria grown on blood agar medium were added to 200 µL of TE buffer (1 mM EDTA, 10 mM Tris [pH 8]) and boiled for 15 minutes; then the microtube was placed in a −20 °C freezer for 20 minutes; this procedure was repeated twice. Afterwards the suspension was centrifuged at 8,000 ×g for 10 min and the supernatant was transferred to a new microtube and centrifuged at 13,000 ×g for 20 min. The pellet was resuspended in 50 µL of TE buffer and stored at −20 °C **(22)**.

The detection of β-lactamase genes (blaKPC, blaVIM, blaIMP, blaNDM and blaCTX-M) in all strains was confirmed using PCR with specific primers (Table 1). The PCR was carried out in a total reaction volume of 25 µL, containing 50 ng of DNA template, a master mix (Cinnagen, Iran), and 25 pmol of both forward and reverse primers. Thermal cycling was performed using a Mastercycler (Bio-Rad, USA) with 30 amplification cycles.

The conditions included an initial denaturation at 95 °C for 5 minutes, annealing at 55 °C for 30 seconds, and extension at 72 °C for 45 seconds, followed by a final extension at 72 °C for 6 minutes.

The amplified PCR products were analyzed via electrophoresis on a 1% agarose gel stained with Safe Stain (Sinaclon, Iran) in Tris-Borate-EDTA buffer (Merck, Germany).
Negative controls included water, while positive controls consisted of *K. pneumoniae* ATCC 700603, *P. aeruginosa* ATCC 27853, and *E. coli* KX342011 (23).

2.7 Statistical analysis

Data were entered and statistically analyzed using Microsoft Excel 2019 (Microsoft Corporation, USA) and the Statistical Package for the Social Sciences (SPSS) software version 22 (IBM SPSS Statistics, USA). The results were presented as descriptive statistics in the form of relative frequencies.

3. Result

3.1 Prevalence

In this study, a total of 85 bacterial isolates were identified from stool samples. These samples were obtained from 42 male and 43 female patients. The patient cohort consisted of two groups: 24 samples were collected from patients hospitalized in the ICU, and 69 samples were obtained from patients hospitalized in the general wards. Of the 85 bacterial isolates, 19 (20.43%) were identified as *Klebsiella pneumoniae* and 66 (79.56% [77.65%]) were identified as *Escherichia coli*. The distribution of bacterial isolates between ICU patients and those in the general wards showed a notable pattern. ICU patients were found to have a higher proportion of *K. pneumoniae* isolates compared to those in the general wards, where *E. coli* was the predominant species. (Table 2).

Table 1. details of samples and bacterial isolates

Sample Category	Total Samples	*K. pneumoniae* (n, %)	*E. coli* (n, %)
Male Patients	42	9 (21.42%)	33 (78.57%)
Female Patients	43	10 (23.25%)	33 (76.74%)
ICU Patients	24	8 (33.33%)	16 (66.66%)
General Ward Patients	69	11 (15.94%)	58 (84.05%)
Total	85	19 (20.43%)	66 (79.56%)

3.2 Antimicrobial Susceptibility

The results of AST showed that 59 out of the 74 *E. coli* isolates (79.72%) and 11 out of the 19 *K. pneumoniae* isolates (57.89%) were identified as ESBL producers. These ESBL-producing isolates exhibited high levels of resistance to ceftriaxone (100%, 70/70) and ceftazidime (90%, 63/70). In contrast, resistance to gentamicin (15.71%, 11/70), ciprofloxacin (14.28%, 10/70), and amikacin (11.42%, 8/70) was relatively lower. Of the 70 ESBL-producing isolates, 31 isolates (44.28%) were found to be resistant or intermediate to carbapenem antibiotics, including imipenem, meropenem, and ertapenem. Among these carbapenem-resistant or intermediate isolates, 26 (83.87%) were *E. coli* and 5 (16.13%) were *K. pneumoniae*. (Table 3) (28). Moreover, based on the MIC and CDT testing results, 10 isolates were confirmed as MBL-producing strains.

Table 2. Antimicrobial susceptibility results of isolates

	E. coli 74			*K. pneumonia* 19		
Antibiotic	Sensitive N (%)	Intermediate N (%)	Resistance N (%)	Sensitive N (%)	Intermediate N (%)	Resistance N (%)
Cefotaxime	14 (18.91)	1 (1.35)	59 (79.72)	0 (0)	0 (0)	11 (57.89)
Ceftriaxone	14 (18.91)	1 (1.35)	59 (79.72)	0 (0)	0 (0)	11 (57.89)
Ceftazidime	15 (20.27)	4 (5.40)	55 (74.32)	0 (0)	3 (15.78)	8 (42.10)
Gentamicin	49 (66.21)	17 (22.97)	8 (10.81)	5 (26.31)	3 (15.78)	3 (15.78)
Amikacin	53 (71.62)	14 (18.91)	7 (9.45)	8 (42.10)	2 (10.52)	1 (5.26)
Imipenem	49 (66.21)	20 (27.02)	5 (6.75)	6 (31.57)	2 (10.52)	3 (15.78)
Meropenem	49 (66.21)	20 (27.02)	5 (6.75)	6 (31.57)	2 (10.52)	3 (15.78)
Ertapenem	49 (66.21)	20 (27.02)	5 (6.75)	6 (31.57)	3 (15.78)	2 (10.52)
Ciprofloxacin	33 (44.59)	31 (41.89)	10 (13.51)	4 (21.05)	7 (36.84)	0 (0)

3.3 Carbapenemase and ESBL Genes

To check the presence of the above genes with the specific primers mentioned, the PCR test was performed on all isolated carbapenem-resistant strains. For this purpose, 31 isolates were selected that were resistant or intermediate to one of the imipenem, meropenem, and ertapenem antibiotics from the carbapenem family. Among all 31 isolates that were resistant or intermediate to carbapenem, all of them had the bla_{CTX-M} gene. Also, just 2 isolates had the bla_{NDM-1} gene and 2 isolates had the bla_{IMP} gene both of them belong to *E. coli* isolates and simultaneous presence of bla_{CTX-M} gene was observed. Also, the simultaneous presence of carbapenemase-producing genes was not observed among the isolates. Finally, the prevalence of bla_{SHV}, bla_{TEM}, bla_{OXA}, bla_{KPC} and bla_{VIM} genes was not observed among the isolates.

Also, among the 10 isolates that are phenotypically resistant based on MIC results, only 4 contained the genes bla_{IMP} and bla_{NDM-1}, and one of the isolates also had negative MIC results (Table 4).

Table 3. Carbapenemase-encoding genes identified in CRE isolates studied

Species	carbapenemase gene detected							
	bla_{NDM}	bla_{KPC}	bla_{IMP}	bla_{VIM}	bla_{CTX}	bla_{SHV}	bla_{TEM}	bla_{OXA}
E. coli	2	0	2	0	26	0	0	0
K. pneumoniae	0	0	0	0	5	0	0	0

4. Discussion

The emergence and dissemination of β-lactamase genes among Enterobacteriaceae represent a significant global public health threat (29). β-lactamases, enzymes that confer resistance to β-lactam antibiotics, undermine the efficacy of one of the most widely used classes of antimicrobial agents. β-lactam antibiotics, including penicillins, cephalosporins, and carbapenems, are cornerstone treatments for a variety of bacterial infections. The rise of ESBLs and carbapenemases limits therapeutic options, leading to higher morbidity, mortality, and healthcare costs. Effective surveillance and control measures are imperative to curb the spread of these resistant genes and preserve the utility of β-lactam antibiotics (30).

In this study, we assessed the prevalence and molecular characterization of β-lactamase genes in Enterobacteriaceae isolates from hospitalized patients in Shahriar, Iran. Out of the 85 isolates, *Escherichia coli* was the most prevalent species (79.56%), followed by *K. pneumoniae* (20.43%). A substantial portion of these isolates were identified as ESBL producers, with 79.72% of *E. coli* and 57.89% of *K. pneumoniae* showing resistance. The isolates demonstrated high resistance to ceftriaxone and ceftazidime. The prevalence of carbapenem-resistant Enterobacteriaceae (CRE) in our study (31 out of 85 isolates) compared with previous research, is relatively high, such as the study conducted in Hamedan, which reported a lower prevalence of carbapenem resistance (40 out of 307 isolates) (31). Such variations may be attributed to differences in hospital settings, patient populations, and antibiotic stewardship practices.

Our results showed that all carbapenem-resistant or carbapenem-resistant or intermediate isolates carried the blaCTX-M gene, while a smaller subset was positive for both blaNDM-1 and blaIMP genes. Interestingly, blaKPC and blaVIM genes were not detected, indicating a potential regional variation in carbapenemase gene distribution. Additionally, the study identified that MBL production was confirmed in 12.35% of the isolates, primarily linked to the presence of blaNDM-1 and blaIMP genes. These findings highlight the growing concern of multidrug resistance in the region.

The high prevalence of ESBL-producing *E. coli* and *K. pneumoniae* in our study aligns with global trends indicating a widespread dissemination of ESBL genes, particularly blaCTX-M. Similar findings have been reported in various regions, including studies from Finland and Germany, where blaCTX-M was also predominant among ESBL producers (32, 33).

The universal presence of blaCTX-M in carbapenem-resistant isolates underscores its pivotal role in conferring resistance and highlights the urgent need for targeted interventions to control its spread (34).

The findings of our study highlight the significant concern posed by the presence of resistance genes such as blaNDM-1 and blaIMP, even in a limited number of *E. coli* isolates. These MBLs can hydrolyze a wide spectrum of β-lactams, including carbapenems, which are often considered one of the last lines of defense in treating resistant infections. When compared to other studies conducted in our region, the prevalence of these genes in our isolates appears to be lower (35).

However, the detection of these MBL genes, even at a limited scale, raises concerns about their potential for wider dissemination. For instance, studies conducted in Tehran and Isfahan have reported a

higher prevalence of blaNDM-1 and blaIMP genes, indicating regional differences in the spread of carbapenem-resistant Enterobacteriaceae (36). Our results showed that the isolates did not harbor blaKPC or blaVIM genes. The absence of blaKPC and blaVIM in our isolate's contrasts with other studies from regions like Greece and China, where these genes are more prevalent (37, 38). This discrepancy may reflect local antimicrobial usage patterns, infection control practices, or the introduction of specific clones into the hospital setting. The antimicrobial susceptibility profiles revealed that ESBL-producing isolates exhibited high resistance to third-generation cephalosporins, consistent with their enzymatic activity. However, lower resistance rates to aminoglycosides (gentamicin and amikacin) and ciprofloxacin suggest that these antibiotics may still retain efficacy against certain resistant strains. This pattern is supported by other studies which have also observed varying resistance rates depending on the antibiotic class and local resistance mechanisms (39, 40). Nonetheless, the presence of MDR plasmids complicates treatment strategies, as the concurrent carriage of multiple resistance genes can lead to limited therapeutic options (41). The identification of only blaCTX-M, blaNDM-1, and blaIMP genes suggests that other resistance mechanisms, such as efflux pumps or porin modifications, may also be contributing to the observed resistance patterns, warranting further investigation (42). Moreover, the lack of detection of blaSHV, blaTEM, blaOXA, blaKPC, and blaVIM genes in our isolates is noteworthy. While blaCTX-M is evidently the dominant ESBL gene, the absence of other β-lactamase genes could indicate either their low prevalence in the region or potential limitations in the detection methods employed (42, 43).

5. Conclusion

Our study underscores the alarming prevalence of β\betaβ-lactamase-producing Enterobacteriaceae isolates, particularly ESBL-producing *E. coli* and *K. pneumoniae*, among hospitalized patients in Shahriar, Iran. The high resistance rates to third-generation cephalosporins, coupled with the detection of β\betaβ-lactamase genes such as *blaCTX-M*, *blaNDM-1*, and *blaIMP*, emphasize the growing challenge of antimicrobial resistance in clinical settings. The absence of *blaKPC* and *blaVIM* genes in our isolates suggests regional variations in the distribution of carbapenem resistance genes. Despite the relatively low occurrence of metallo-β\betaβ-lactamase genes, their presence, even in a limited number of isolates, highlights the need for vigilant monitoring and stringent infection control measures to prevent the further spread of these multidrug-resistant organisms. Our findings call for the implementation of effective antibiotic stewardship programs and continuous surveillance to mitigate the impact of resistance and preserve the efficacy of current therapeutic options.

6. Declarations

6.1 Acknowledgments

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6.2 Ethical Considerations

This study was approved by the Islamic Azad University, Tehran Medical Branch (IR.IAU.PS.REC.1399.079).

6.3 Authors' Contributions

The study was conceived by FS and RH. patients were enrolled and clinical history data were provided by AO. All culture work, molecular work, and data analysis was done by AO and RS. The manuscript was written by AO, MRM and ML supported by VN and MRM, FA. FS had done the final editing and approved the final manuscript. All authors have read and approved the final manuscript.

6.4 Conflict of Interest

The authors declare that they have no conflict of interest.

6.5 Fund or Financial Support

This work was supported by a personal budget.

6.6 Using Artificial Intelligence Tools (AI Tools)

The authors were not utilized AI Tools.

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