ORIGINAL ARTICLE

Identification of metallo-β-lactamases and AmpC production among *Escherichia coli* strains isolated from hemodialysis patients with urinary tract infection

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Abstract

Background This study aimed to identify metallo-β-lactamases (MBLs) and AmpC β-lactamases-producing *Escherichia coli* isolates obtained from hemodialysis (HD) patients with urinary tract infections (UTI).

Methods and results A total of 257 HD patients with UTI were included in this study, from which 47 *E. coli* isolates were collected. Antibiotic susceptibility was tested by disc diffusion method. MBLs and AmpC production were phenotypically detected by imipenem-ethylenediaminetetracetate and cefoxitin/boronic acid assays, respectively. The presence of MBLs and AmpC genes was examined by polymerase chain reaction (PCR). Fosfomycin and ampicillin were the most and the least effective antibiotics against *E. coli* isolates, respectively. Moreover, 61.7% (29/47) of *E. coli* isolates were multidrug-resistant with seven different antibiotypes. Antibiotype V (AMP–CIP–IMP–MEM–CPD–CRO–CTX–GEN–LEV–SXT–TOB) was the most prevalent profile. Besides, 24 (51.1%) isolates were simultaneously resistant to imipenem and meropenem. Phenotypic assay showed MBL production in 16 (66.7%) of the 24 carbapenem-resistant *E. coli* isolates. The distribution of MBL genes in carbapenem-resistant *E. coli* was as follows: bla_{IMP} 18 (72%), bla_{VIM} 7 (28%), and bla_{NDM} 1 (4%). AmpC was detected in 61.7% (29/47) of the isolates using the phenotypic method. The presence of AmpC genes was confirmed by PCR in only 26 of 29 (86.7%) AmpC producers. The frequencies of bla_{DHA-1}, bla_{ACC}, and bla_{CMY-2} were 6 (20.7%), 11 (37.9%), and 21 (72.4%), respectively.

Conclusions The emergence of MBL and AmpC coproducing *E. coli* isolates calls for an urgent surveillance program for timely diagnosis and screening of these genes in our healthcare systems.

Keywords AmpC · *Escherichia coli* · Hemodialysis patients · Metallo-β-lactamases · Urinary tract infections

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Introduction

Dialysis is a procedure to remove excess fluids and waste products from the human body. There are two different methods of dialysis: peritoneal dialysis and hemodialysis (HD). The second method uses a machine located outside the body to remove blood and pump it into a dialyzer [1]. HD patients are at high risk for various infections, not only due to the invasiveness of this method, but also because of the immunosuppression caused by the inflammation and uremia [2]. In HD patients with chronic renal disease, infection is the leading cause of death. These patients are at risk of urinary tract infections (UTIs) which can lead to serious complications. Because of the problems in collecting urine samples from anuric or oliguric HD patients, UTIs are initially difficult to diagnose. Previous evidence suggests that multidrug-resistant bacterial

pathogens of UTIs are more common in HD patients compared with those with normal renal function [3]. Despite extensive studies on UTIs in many countries, little is known about hemodialysis patients.

Infections such as UTIs, cause high economic costs and morbidity rates, making them one of the most significant diseases in hospitals and communities. In Asia, UTIs are the main causes of mortality and morbidity. According to diagnostic and clinical reports, approximately 150 million people become infected with UTI each year worldwide [4, 5]. The prevalence of UTIs is mainly influenced by a variety of factors including gender, age, urological instruments, impaired immunity, indwelling urinary catheters, and underlying diseases such as diabetes mellitus [6]. *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Proteus mirabilis* are the commonest bacteria causing UTIs. Community-acquired UTI is most commonly caused by *E. coli* bacteria, which are abundant in the gastrointestinal microflora of humans [7, 8].

Treatment of infections has been complicated by the emergence of multidrug-resistant (MDR) strains of *E. coli*. Beta-lactam antibiotics are prescribed drugs for UTI treatment. These agents are a class of extended-spectrum antibiotics and include all antibiotics that have a β-lactam ring in their structure [9, 10]. The worldwide distribution of *E. coli* harboring metallo-β-lactamases (MBLs) and AmpC β-lactamases (AmpC) is a serious threat, and due to MBL production, carbapenem resistance is progressively spreading among clinical isolates of *E. coli* [11]. Varied types of MBL genes have been identified around the world. The bla_{NDM}, bla_{VIM}, and bla_{IMP} are among the most common MBLs [12].

The increasing emergence of AmpC (*pAmpC*) β-lactamases mediated by plasmids is also of growing concern. Six families of *pAmpC* β-lactamases have been defined by Pérez-Pérez and Hanson as EBC, MOX, FOX, CIT, DHA, and ACC. Among *E. coli* strains, the CMY-2 type is the most commonly known *pAmpC*. In recent decades, the frequency of AmpC-producing *E. coli* strains, which are mostly MDR, has increased significantly. Therefore, the detection of AmpC-positive strains is of utmost importance for appropriate treatment [13].

Considering the importance of these issues and knowing that studies on the prevalence of β-lactamase genes in *E. coli* strains isolated from HD patients with UTIs from all over the world, and especially from Iran are very rare, this study aimed to identify the MBLs and AmpC β-lactamases-producing *E. coli* isolates in HD patients with UTIs.

Methods

Study design and specimen collection

This descriptive cross-sectional study was conducted from October 2019 to July 2020. Written informed consent was obtained from all patients who participated in this study before the start of the work. A total of 257 HD patients with clinical suspicion of UTI (fever, chills, burning sensation during urination, cloudy urine, frequent and low urine output, unpleasant odor of urine) admitted to Army hospitals in Tehran city, were included in this study. The Army hospitals are located in Tehran, the capital of the country of Iran. These hospitals are for general referral and have all specialized and advanced medical departments. All patients with suspected UTI symptoms had their urine samples collected by clean-catch midstream protocol. A urine specimen was deemed positive for UTI if it contains a single microorganism with a count of ≥ 10^5 CFU/ml. *E. coli* isolates were identified by phenotypic and biochemical tests including Gram staining, lactose fermentation on MacConkey agar, triple sugar iron agar reaction, indole production, motility test, and Simmons' citrate test [14]. All culture media used in this study, were prepared and purchased from Merck Co, Germany.

Antibiotic susceptibility testing (AST)

AST was conducted using disc diffusion method, according to the recommendations of Clinical and Laboratory Standards Institute (CLSI) [15]. The AST was performed with the following antibiotic discs: imipenem (IMP, 10 µg), meropenem (MEM, 10 µg), amikacin (AMK, 30 µg), gentamicin (GEN, 10 µg), tobramycin (TOB, 10 µg), ampicillin (AMP, 10 µg), cotrimoxazole (SXT, 25 µg), fosfomycin (FOS, 10 µg), piperacillin–tazobactam (PTZ, 100/10 µg), ceftriaxone (CRO, 10 µg), ceftazidime (CAZ, 30 µg), cefpodoxime (CPD, 10 µg), cefotaxime (CXT, 30 µg), nitrofurantoin (NF, 300 µg), ciprofloxacin (CIP, 10 µg), and levofloxacin (LEV, 10 µg) (Mast, UK). *E. coli* ATCC 25922 was used as a control strain for the susceptibility tests. Isolates resistant to at least three classes of antibiotics were considered as MDR isolates.

Phenotypic detection of MBLs

The presence of MBLs was screened by the combined imipenem-ethylenediaminetetracetate (IMP-EDTA) disc test. Briefly, isolates to be tested were inoculated on a Mueller Hinton Agar (Merck Co, Germany) plate using lawn culture method. One disc of IMP (10 µg) alone and another

disc in combination with EDTA (750 µg/ml) were applied 20 mm apart and incubated for 18–24 h at 37ºC. Strains were confirmed as MBL producers once an increase of ≥7 mm in the inhibition zone of IMP-EDTA combination disc was observed compared to the IMP disc alone [16].

Phenotypic detection of AmpC

The cefoxitin (FOX) disc (30 µg)/boronic acid was used to screen AmpC-producing isolates as previously described [17]. Isolates with the inhibition zones of <18 mm in diameter against FOX discs were considered potentially positive for AmpC screening and were subjected to further testing, using FOX disc alone and in combination with boronic acid (Sigma, USA). The presence of a 5-mm or larger increase in the inhibition zone diameter of the FOX disc in combination with boronic acid compared to the FOX alone, was considered as positive AmpC production.

Molecular detection of MBLs and AmpC genes

The presence of AmpC genes (bla_{CMY-2}, bla_{DHA-1}, and bla_{ACC}) [18] and MBLs genes (bla_{IMP}, bla_{VIM}, and bla_{NDM}) [19] was detected by polymerase chain reaction (PCR). Genomic DNA extraction was performed using the boiling method [20]. The PCR was performed in a final volume of 25 µl consisting of DNA template (50 ng), dNTPs (100 µM), Taq buffer (5×), Taq DNA polymerase (1 U; Cinnagen, Iran), and forward and reverse primers (25 pM each). The PCR mixtures were subjected to thermal cycling. PCR reactions included 30 amplification cycles in a Mastercycler (Eppendorf, Germany) under the following conditions: denaturation at 95 °C/5 min, annealing at 55 °C/30 s, and extension at 72 °C/45 s, with a final extension at 72 °C/6 min. Amplified products were visualized using electrophoresis on a 1% agarose gel stained with safe stain (Sinaclon, Iran), in a Tris-Borate-EDTA buffer (Promega, USA). Water was used as a negative control in the study, and the positive controls were *K.pneumoniae* ATCC 700603, *P.aeruginosa* ATCC 27853, *P.aeruginosa* ST 147, *K.pneumoniae* KP696465, *E.coli* KX 342010 and *E.coli* KX342011.

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) [21]. The results were presented as descriptive statistics in the form of relative frequency.

Results

Bacterial isolates and antibiotic resistance

In this study, a total of 47 *E. coli* isolates were obtained from urine samples of HD patients, with an overall prevalence of 18.3% (47/257). The isolates were obtained from 21 (44.7%) males and 26 (55.3%) females (female/male ratio =1.23), respectively. The mean age of the patients was (31 ± 1) years, and ranged from 2 to 65 years. The *E. coli* isolates showed the highest susceptibility (87.2%) and esistance (100%) to FOS and AMP antibiotics, respectively. PTZ and NF were the second and third most effective antibiotics, respectively. More than 70% of the isolates were resistant against third-generation cephalosporin (TGC) antibiotics. As well, 24 (51.1%) isolates were simultaneously resistant to IMP and MEM. The susceptibility rate results for all antibiotics tested are shown in Table 1. The results of AST showed that 61.7% (29/47) of *E. coli* isolates were MDR with seven different antibiotypes (Table 2). Antibiotype V (AMP-CIP-IMP-MEM-CPD-CRO-CTX-GEN-LEV-SXT-TOB) was the most prevalent profile (31.0%).

Detection of MBLs and AmpC genes

The results of the IMP-EDTA combined disc test confirmed the MBL production in 16 (66.7%) out of 24 carbapenem-resistant *E. coli* isolates. PCR results showed that all 24 carbapenem-resistant *E. coli* isolates had at least one MBL gene. The distribution of MBL genes among

Table 1 The results of antibiotic susceptibility testing

Antibiotic	Total *Escherichia coli*, N (%)		
	Sensitive	Intermediate	Resistant
Gentamicin	12 (25.5)	8 (17.0)	27 (57.4)
Amikacin	28 (59.6)	10 (21.3)	9 (19.1)
Levofloxacin	27 (57.4)	2 (4.3)	18 (38.3)
Ciprofloxacin	18 (38.3)	–	29 (61.7)
Tobramycin	14 (29.8)	5 (10.6)	28 (59.6)
Fosfomycin	41 (87.2)	–	6 (12.8)
Cefpodoxime	7 (14.9)	4 (8.5)	36 (76.6)
Cefotaxime	9 (19.1)	4 (8.5)	34 (72.3)
Ceftriaxone	7(14.9)	–	40 (85.1)
Ceftazidime	4 (8.5)	7 (14.9)	36 (76.6)
Ampicillin	–	–	47 (100)
Cotrimoxazole	9 (19.1)	–	38 (80.9)
Meropenem	23 (48.9)	–	24 (51.1)
Imipenem	19 (40.4)	4 (8.5)	24 (51.1)
Piperacillin–tazobactam	38 (80.9)	–	9 (19.1)
Nitrofurantoin	36 (76.6)	–	11 (23.4)

Table 2 Different antibiotypes of 29 multidrug-resistant (MDR) *E. coli* isolates

MDR profile	Antibiotypes	Number of isolates (%)
I	CIP–CPD–CRO–CTX–SXT–TOB–NF	1 (3.4)
II	AMP–CAZ–CIP–PTZ– IMI–MEM–CPD–CRO–CTX–FOS–GEN–SXT–TOB–LEV	4 (13.8)
III	AMP–CAZ–CIP–PTZ–IMI–MEM–CPD–CRO–CTX–GEN–LEV–SXT–TOB	5 (17.2)
IV	AMP–AN–CIP–CPD–CRO–CTX–GEN–SXT–TOB–NF	3 (10.3)
V	AMP–CIP–IMI–MEM–CPD–CRO–CTX–GEN–LEV–SXT–TOB	9 (31.0)
VI	AMP–AN–CIP–IMI–MEM–CPD–CRO–CTX–GEN–SXT–TOB–NF	6 (20.7)
VII	CIP–CPD–CRO–CTX–SXT–NF	1 (3.4)

AMP Ampicillin, *AN* Amikacin, *CAZ* Ceftazidime, *CIP* Ciprofloxacin, *CPD* Cefpodoxime, *CRO* Ceftriaxone, *CTX* Cefotaxime, *FOS* Fosfomycin, *GEN* Gentamicin, *IMI* Imipenem, *LEV* Levofloxacin, *MEM* Meropenem, *NF* Nitrofurantoin, *PTZ* Piperacillin–tazobactam, *SXT* Cotrimoxazole, *TOB* Tobramycin

carbapenem-resistant *E. coli* isolates was as follows: bla_{IMP} 18 (72%), bla_{VIM} 7 (28%), and bla_{NDM} 1 (4%). Two isolates showed coexistence of MBL genes. Results from FOX disc/boronic acid detected 61.7% (29/47) potential AmpC producers. However, the presence of AmpC genes was confirmed by PCR in only 26 of 29 (86.7%) AmpC producers. The frequency rates of the resistance genes bla_{DHA-1}, bla_{ACC}, and bla_{CMY-2} among AmpC β-lactamase-producing strains were 6 (20.7%), 11 (37.9%), and 21 (72.4%), respectively. Ten isolates showed coexistence of AmpC genes. Overall, the frequencies of MBL and AmpC genes were 51.1% (24/47) and 55.3% (26/47), respectively. Fifteen (31.91%) *E. coli* isolates had no MBL and AmpC genes, and 18 (38.3%) isolates had both of these genes, simultaneously (Table 3). The electrophoresis image of some MBL and AmpC positive isolates is shown in Fig. 1.

Discussion

At present, a paucity of epidemiological information is available on *E. coli* strains causing UTIs, as well as on their antibiotic resistance profiles and resistance mechanisms in HD patients from many countries including Iran. The novelty of this study was to address these issues in a region of Iran. The present study investigated the prevalence and antibiotype patterns of UTI-causing *E. coli* isolates in HD patients against 16 antibiotics.

The results showed an overall prevalence of 18.3% for UTI-causing *E. coli* isolates in HD patients. This rate was much lower than the rate (43%) reported by Sadeghi et al. [22] from Isfahan, Iran, but in kidney transplant patients. In another research in USA, a frequency rate of 29.3% was reported for *E. coli* isolates in HD patients [23]. The rationale behind these discrepancies is likely the differences in studied population, patient screening programs, and predominant pattern of UTI-inducing pathogens in each region.

According to the results of AST, MEM and IMP were among the relatively least effective antibiotics, with resistance rates of about 50%. These results were in contradiction with previous reports by Critchley et al. [24] from USA, Raeispour et al. [25] from Iran, and Duicu et al. [26] from Romania who showed the efficacy of more than 90% for these two antibiotics. The emergence of carbapenem-resistant Gram-negative bacteria in recent years has posed a new challenge to medical centers, both in Iran and other parts of the world [27, 28]. This phenomenon may arise from the high prevalence of different carbapenemase genes that can develop resistance to carbapenem antibiotics [28]. Although FOS was the most effective antibiotic, its resistance rate (12.8%) was higher than the rate reported in South Korea (6.7%) [29].

Of the three aminoglycosides studied here, AMK showed the highest efficacy compared to GEN and TOB, supporting previous studies from USA [24] and Gabon [30]. However, a survey by Kushwaha et al. [31] from Nepal showed an opposite result: GEN had higher efficacy against UTI-inducing *E. coli* isolates than AMK and TOB. In this study, more than half of the *E. coli* isolates were resistant to GEN and TOB, which was higher than the report of Naziri et al. [32] from Iran.

This higher resistance rate could be explained by the excessive parenteral administration of these antibiotics in our region. Our study explored a high resistance rate, ranging from 72.3 to 85.1%, against TGC antibiotics, which was in agreement with previous researches conducted by Raeispour et al. [25] and Lee et al. [33], but in contrast to findings achieved from USA [24] and Gabon [30]. The high resistance rates against TGCs is possibly rooted in increasing the spread of extended-spectrum β-lactamase genes in UTI pathogens in Iran and other countries during recent decades, which were not investigated in the current study [34, 35].

Table 3 MBLs and AmpC profiles of all 47 *Escherichia coli* isolates

Strain ID	Gender	AmpC producer	MBL producer	AmpC genes	MBL genes
Ec 1	M	+	+	bla_{DHA-1}, bla_{ACC}	bla_{IMP}
Ec 2	F	+	−	bla_{CMY-2}	bla_{IMP}, bla_{VIM}
Ec 3	M	−	+	−	bla_{IMP}
Ec 4	M	+	+	bla_{CMY-2}	bla_{VIM}
Ec 5	F	−	−	−	−
Ec 6	F	+	−	bla_{DHA-1}, bla_{ACC}, bla_{CMY-2}	bla_{VIM}
Ec 7	M	+	+	bla_{CMY-2}	bla_{IMP}
Ec 8	F	+	−	bla_{CMY-2}	bla_{VIM}
Ec 9	F	−	−	−	−
Ec 10	M	−	+	−	bla_{IMP}
Ec 11	F	+	+	−	bla_{VIM}
Ec 12	F	+	−	bla_{ACC}, bla_{CMY-2}	−
Ec 13	F	+	−	bla_{CMY-2}	bla_{IMP}
Ec 14	M	−	+	−	bla_{IMP}
Ec 15	M	−	−	−	−
Ec 16	F	+	+	bla_{ACC}, bla_{CMY-2}	bla_{VIM}
Ec 17	F	−	−	−	−
Ec18	M	+	+	bla_{CMY-2}	bla_{IMP}
Ec 19	F	−	−	−	−
Ec 20	M	+	+	bla_{DHA-1}, bla_{ACC}	bla_{IMP}
Ec 21	F	−	−	−	−
Ec 22	M	+	+	bla_{ACC}	bla_{VIM}, bla_{NDM}
Ec 23	M	+	−	bla_{CMY-2}	−
Ec 24	F	+	−	bla_{CMY-2}	bla_{IMP}
Ec 25	M	−	−	−	−
Ec 26	M	+	−	bla_{ACC}	−
Ec 27	F	+	−	bla_{CMY-2}	bla_{IMP}
Ec 28	M	−	−	−	−
Ec 29	F	+	−	bla_{CMY-2}	−
Ec 30	M	−	−	−	−
Ec 31	F	+	−	bla_{DHA-1}, bla_{CMY-2}	bla_{IMP}
Ec 32	F	+	−	−	−
Ec 33	M	−	−	−	−
Ec 34	M	+	−	bla_{CMY-2}	bla_{IMP}
Ec 35	F	+	−	bla_{CMY-2}	−
Ec 36	F	+	+	bla_{DHA-1}, bla_{CMY-2}	bla_{IMP}
Ec 37	M	−	+	−	bla_{IMP}
Ec 38	F	+	−	bla_{ACC}	−
Ec 39	F	+	−	bla_{ACC}, bla_{CMY-2}	−
Ec 40	M	−	−	−	−
Ec 41	F	+	−	−	−
Ec 42	M	−	+	−	bla_{IMP}
Ec 43	F	+	+	bla_{ACC}, bla_{CMY-2}	bla_{IMP}
Ec 44	F	−	−	−	−
Ec 45	F	+	−	bla_{CMY-2}	−
Ec 46	M	−	−	−	−
Ec 47	F	+	+	bla_{DHA-1}, bla_{ACC}, bla_{CMY-2}	bla_{IMP}

Ec Escherichia coli, *F* female, *M* male, *MBL* Metallo-β-lactamase, *AmpC* AmpC beta-lactamase
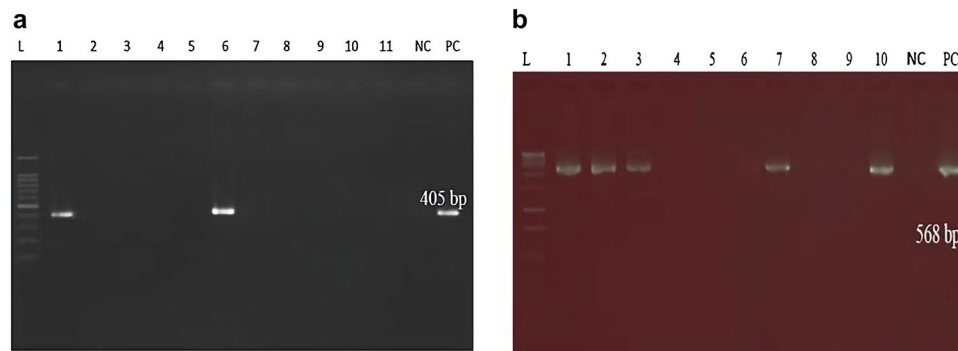
a

b

Fig. 1 Agarose gel electrophoresis (1.0%) of bla_{DHA} PCR products. From left to right: L indicates DNA Ladder (100 bp), lanes 1 and 6 demonstrate bla_{DHA}-positive (405 bp) *Escherichia coli* isolates, lanes 2,3,4,5,7,8,9,10, and 11 indicate bla_{DHA}-negative *E. coli* isolates, NC indicates negative control, PC indicates positive control of bla_{DHA} gene (405 bp) (**a**). Agarose gel electrophoresis (1.0%) of bla_{IMP} PCR

products. From left to right: L indicates DNA Ladder (100 bp), lanes 1, 2, 3, 7, and 10 demonstrate bla_{IMP}-positive (568 bp) *E. coli* isolates, lane 4, 5, 6, 8, and 9 show bla_{IMP}-negative *E. coli* isolates, *NC* indicates negative control, *PC* indicates positive control of bla_{IMP} gene (568 bp) (**b**)

The resistance rates against CIP and LEV were 61.7% and 38.3%, respectively. Reports from Iran by Halaji et al. [34] and Raeispour et al. [25] stated the resistance rates of 54.3% and 34% for CIP, respectively. These rates were lower than those of the current study. The administration of CIP, as the first-line therapy, against UTI-inducing bacteria might be the reason for this higher resistance rate. Another finding obtained in the present work was the exceptional efficacy of NF with a susceptibility rate of 76.6%.

In line with our result, Lee et al. [33] reflected the high susceptibility rates (74.3%-100%) of NF against different UTI-inducing bacteria and stated that 99.2% of *E. coli* isolates were NF-susceptible. Similarly, Raeispour et al. [23] implied that 90% of UTI-inducing *E. coli* isolates were susceptible to this antibiotic, as well. In another study in Gabon, which was comparable with ours, Mouanga Ndzime et al. [30] identified NF as the most effective drug against UTI-inducing *E. coli* isolates.

Likewise, a high proportion (80.9%) of *E. coli* isolates were susceptible to PTZ, confirming the results observed by Critchley et al. [24] in the USA and Halaji et al. [34] in Iran. In this study, AMP and SXT (with resistance rates of 100% and 80.9%, respectively) were among the less effective antibiotics, a result consistent with those observed in the Gabon [30], USA [24], and Iran [34].

This result for AMP was not far-fetched because in recent years, many Gram-negative bacilli carrying β-lactamase enzymes have readily become resistant to a wide range of β-lactam antibiotics. As SXT has always been one of the first-line drugs for the treatment of UTIs, according to the observed evidence, it is recommended to prescribe this drug with regard to the results of antibiotic susceptibility tests.

One notable finding of this study was the relatively high prevalence of MDR (61.7%) *E. coli* isolates. Previous studies have reported various MDR frequencies, ranging from

55.1 to 100% for UTI-causing *E. coli* isolates [25, 30, 34]. In this study, 7 different antibiotypes were seen in MDR isolates. Previous study from Saudi Arabia showed 70 different antibiotypes in *E. coli* isolates collected from clinical samples [36]. In another study by Alanazi et al. [37], 7 different antibiotypes were seen in UTI-causing *E. coli* isolates against ciprofloxacin, ampicillin and co-trimoxazole antibiotics. In this study, according to the results of the antibiogram, the most effective antibiotic against these MDR isolates was the FOS that can be considered for treatment of the MDR UTI-causing *E. coli*. These results necessitate the development of a surveillance program for antibiotic consumption to control the spread of MDR *E. coli* isolates in healthcare systems of our country. In recent years, the outbreak of *E. coli* sequence type 131 (ST131) isolated from urine culture has become one of the global health problems due to the high prevalence of multidrug resistance. This clone has a wide range of virulence factors including siderophores, adhesins, and toxins which disrupt host defense mechanisms. The majority of these factors are found on mobile genetic elements (MGEs) or pathogenicity islands (PAI), which are capable of being horizontally transmitted among different species [38, 39]. MGEs have a huge impact on bacterial genomes, including causing marked differences in genome size and pathogenicity. Although bacteria have several mechanisms for resisting lateral gene transfer, MGEs play a major role in bacterial evolution and contribute greatly to adaptation to new and changing ecological niches. Most of the resistance genes are acquired by horizontal transfer of plasmids and other MGEs, and this process has been associated with the successful dissemination of particular lineages. Also, MGEs have an important role in virulence gene acquisition and forming new subpopulations among pandemic clones such as *E. coli* ST131 [38, 39].

This study investigated the production of MBL and AmpC β-lactamases in UTI-inducing *E. coli* isolates in HD patients. To the best of our knowledge, this was the first study in this regard in Iran. Today, there are multiple phenotypic methods for detecting β-lactamases, but owing to the multiplicity of different types of these enzymes, none of the phenotypic methods can identify all types. Therefore, their detection is mostly based on molecular methods such as PCR or real-time PCR [40, 41].

In our survey, AmpC and MBL phenotypes were detected in 29 (61.7%) and 16 (34%) UTI-causing *E. coli* isolates, respectively. Similar to these results, Helmy et al. [42] reported a prevalence of 66.6% for AmpC-producing *E. coli* isolates in Egyptian UTI patients using phenylboronic acid method. A higher prevalence rate (87.5%, n = 175/200) was also reported for the detection of phenotypic AmpC in UTI-inducing *E. coli* isolates collected from New Zealand by Drinkovic et al. [43]. In opposition to our findings, Jamil et al. [44] stated a lower frequency (16%, n = 12/75) of UTI-causing *E. coli* isolates harboring MBLs in Pakistan using phenotypic method. Variations in the results seem to arise from differences in the phenotypic test methods, quality of materials used, and dissimilarity in geographical area.

The results of PCR assay revealed a frequency of 20.7%, 37.9%, and 72.4% for bla_{DHA-1}, bla_{ACC}, and bla_{CMY-2} AmpC β-lactamases-producing isolates, respectively. In conformity with our results, findings by Helmy et al. [42] from Egypt and Drinkovic et al. [43] from New Zealand represented the bla_{CMY-2} (89% [n = 90/101]) as the predominant type of AmpC in UTI-inducing *E. coli* isolates. The extensive dissemination of bla_{CMY-2} among *E. coli* isolates could be linked to unique transposon-like element IS*Ecp1*, which is thought to play a role in the transmission of bla_{CMY-2} from the *Citrobacter freundii* chromosome to other *Enterobacteriaceae* [42]. In another study conducted in Egypt, the prevalence rate of 50% was reported for bla_{DHA} AmpC in UTI-causing *E. coli*, while other AmpC genes were not detected [45]. In a study from Nepal, two AmpC genes bla_{CIT} and bla_{DHA} were detected in 30.6% and 31.3% of clinical *E. coli* isolates, respectively [46]. Another experiment by Shayan and Bokaeian [47], showed a prevalence of 5.0% and 0.0% for bla_{CMY-2} and bla_{FOX} genes, respectively. They did not investigate the remaining AmpC genes. In a recent study from Iran, no bla_{MOX} and bla_{ECC} genes were found in uropathogenic *E. coli* isolates, while, bla_{CIT}, bla_{FOX}, bla_{DHA}, and bla_{EBC} were detected in 73.6%, 10.5%, 10.5%, and 15.8% of *E. coli* isolates, respectively [48]. Currently, the clinical microbiology laboratories do not have a reliable method of detecting and confirming AmpC β-lactamases. For this reason, the molecular characterization of β-lactamases, in particular the AmpC family, is of crucial importance in in terms of molecular epidemiology and genetic contributing factors [48].

This study revealed the high prevalence rate of MBL genes in 24 carbapenem-resistant isolates, as all of them harbored at least one MBL gene. In the current study, the bla_{IMP} (72%) was the most prevalent MBL gene followed by bla_{VIM} (28%) and bla_{NDM} (4%). The emergence of MBL-producing UTI-inducing *E. coli* isolates could be considered a serious threat to health communities because this pathogen is resistant to numerous antibiotics. In a study by Naeem et al. [49], the NDM-positive rate was 38.5% (n = 10/26) among *E. coli* strains isolated from urine samples that was higher than the current result. In another study from Sudan [50], the bla_{VIM} (16.7%) was the most prevalent MBL gene in clinical *E. coli* isolates followed by bla_{IMP} (8.3%) and bla_{NDM} (2.8%). There is a paucity of data regarding the bla_{VIM} and bla_{IMP} harboring UTI-causing *E. coli* in Iran. In a recent study from southwest Iran, each of bla_{IMP-1} and bla_{IMP-2} genes were detected in 8.3% of uropathogeneic *E. coli* isolates, while the bla_{NDM} was detected in 75.0% of isolates. Also, the bla_{VIM-1} and bla_{VIM-2} were not detected [51]. In another study by Deldar Abad Paskeh et al. [52] from Iran, a lower occurrence rates of bla_{IMP} (8.7%) and bla_{VIM} (9.8%) genes were reported in UTI-causing *E. coli* compared to the current study. In a recent study from Taiwan, an increasing rate of NDM-positive *E. coli* isolates has been reported in clinical samples. They reported a prevalence rates of 39.1%, 30.4%, 21.7%, and 8.7% for NDM, IMP-8, KPC-2, and VIM-1, respectively [53]. NDM is a member of the amber class B β-lactamases, which can hydrolyze virtually all β-lactams except monobactam [53]. In China and its neighboring countries, IncX3 plasmids are the most common types of plasmids carrying bla_{NDM} in *Enterobacteriaceae* [54], while in a recent study from Iran, bla_{NDM-1} gene was located on both conjugative plasmids: IncFII ~86-kb to ~140-kb and IncA/C [55]. There are various classes of chromosomal and plasmidic MBLs including Sao Paulo metallo-β-lactamase (SPM), German imipenemase (GIM), and Adelaide imipenemase (AIM) that were not screened in this study and could pave the way for further epidemiological research in the future [56].

The present research showed that 38.3% of the isolates harbored both MBL and AmpC genes simultaneously. In this regard, co-occurrence of β-lactamase genes has been reported in several investigations [45, 57], which was in harmony with the results of this study. In recent years, the occurrence of different β-lactamases in various Gram-negative bacteria has been reported in various studies [58–61]. Due to the financial and traffic constraints caused by the COVID-19 virus pandemic, the current study has the following limitations: the lack of MBLs and AmpC genes sequencing, the lack of antibiogram results for some newer and reserved antibiotics including ceftazidime/avibactam, imipenem/relebactam, ceftolozane/tazobactam, colistin, and tigecycline, and lack of evaluation of other bacteria contributed to UTI in HD patients. Also, due to the non-shareable

privacy of those patients referred to Army hospitals, we did not have any clinical data of individuals to evaluate the correlation of infections by MDR *E. coli* in HD patients with other important predisposing conditions to detect if any significant trends exist in the prevalence of such MDR strains among different susceptible groups.

Conclusion

This survey portrayed the high resistance rates of UTI-causing *E. coli* isolates harboring AmpC and MBLs in HD patients against AMP, SXT, and TGCs. In light of this information, it is recommended to choose treatments based on the antibiogram results, in order to hinder the further spread of MDR isolates. Moreover, the higher efficacy of FOS, PTZ and NF against UTI-inducing *E. coli* isolates, compared to other antibiotics, made them suitable options for empirical therapy. The emergence of MBL and AmpC co-producing *E. coli* isolates calls for an urgent surveillance program for timely diagnosis and screening of these genes in our healthcare systems. In future, such program can be of great help in preventing the spread of antibiotic resistance in our country, Iran. Another suggestion is to evaluate the other infections and their etiological pathogens such as septicemia in HD patients to prepare a suitable epidemiological data bank in this regard in Iran.

Data availability All analyzed data within this study can be obtained from the corresponding author on request.

Code availability Not applicable.

Declarations

Conflict of interest The authors have no conflict of interest.

Consent to participate Before the initiation of the study, written informed consents were provided by all the HD patients who enrolled in this survey.

Consent for publication Not applicable.

Ethical approval This descriptive cross-sectional study was approved by the Ethics Committee of Army University of Medical Sciences, Tehran, Iran (IR.AJAUMS.REC.1400.105) in accordance with the Declaration of Helsinki.

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