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Frequency of Extensively Drug Resistance and Metallo-Beta-Lactamase Genes among Uropathogenic *Escherichia coli* Isolates from Nasiriya, Iraq

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Authors
Ihab Rasmi Hassan, *MSc*[1]
Seyedeh Elham Rezatofighi, *PhD*[1*]
Hossein Motamedi, *PhD*[1]
Tanvir Rahman, *PhD*[2]

[1] Department of Biology; Faculty of Science; Shahid Chamran University of Ahvaz; Ahvaz; Iran
[2] Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Banglades

** Correspondence*
Department of Biology; Faculty of Science; Shahid Chamran University of Ahvaz; Ahvaz; Iran
E-mail: e.tofighi@scu.ac.ir

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ABSTRACT

Background: This study aimed to evaluate the frequency of extensively drug-resistant (XDR) uropathogenic *Escherichia coli* (UPEC) isolates and to detect their metallo-beta-lactamase (MBL) genes.
Materials & Methods: Three hundred urine samples collected from patients with suspected urinary tract infection (UTI) were evaluated for the presence of UPEC isolates. These isolates were subjected to antibiotic susceptibility testing to determine multidrug-resistance (MDR) and XDR profiles. Imipenem or meropenem-resistant isolates were evaluated for MBL production using modified carbapenem inactivation (mCIM) and EDTA-CIM (eCIM) methods. PCR was carried out to identify the presence of MBL genes, including bla_{GIM}, bla_{SIM}, bla_{VIM-1}, bla_{VIM-2}, bla_{SPM-1}, bla_{IMP-1}, bla_{IMP-2}, bla_{NDM}, and bla_{KPC}.
Findings: Out of 300 urine samples, 200 (66.66%) were positive for UTI. Among these, 150 were caused by UPEC. The highest antimicrobial resistance was against cefepime (88%) and ampicillin (85.3%), while the highest susceptibility was against imipenem (91.7%) and fosfomycin (84%). MDR and XDR profiles were detected in 145 (96.66%) and 5 (3.33%) isolates, respectively. Overall, five UPEC isolates were XDR and resistant to imipenem and meropenem. All these isolates were positive for mCIM, while four were positive for eCIM. The bla_{NDM} gene was found in all five isolates, while the other MBL genes were not found.
Conclusion: The existence of MDR and XDR bacteria poses a significant risk to public health. bla_{NDM} is circulating in UPEC strains at least in Nasiriya province, Iraq. This could lead to increased resistance to carbapenems among *Enterobacteriaceae*, a serious threat to public health.

Keywords: Uropathogenic *Escherichia coli,* Urinary tract infection, Antibiogram, Drug resistance, Beta-lactamases

CITATION LINKS

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Introduction

Urinary tract infection (UTI) is diagnosed with a combination of urinary symptoms and a positive urine culture [1]. Uropathogenic *Escherichia coli* (UPEC) is regarded as a highly virulent pathogen causing 80 to 90% of acute community-acquired UTIs owing to its efficient virulence factors, contributing to the development and progression of the disease [2, 3].

Antimicrobial resistance (AMR) is one of the most important threats to public health, food safety, and livestock production. According to the World Health Organization (WHO), antimicrobial resistance is defined as the ineffectiveness of drugs in the treatment of diseases caused by microorganisms. AMR creates problems in the therapy of many infections like UTIs, burn infections, respiratory tract infections, and many other diseases that are difficult to treat, thereby facilitating the sustainability and spread of infections [3].

Antimicrobial resistance could be grouped into multiple grades, including multidrug resistance (MDR) (resistance against at least one antibiotic in three or more different antibiotic groups), extensively drug resistance (XDR) (resistance against at least one agent in all but two or fewer antimicrobial groups), and pan-drug-resistance (PDR) (resistance to all antibiotics in all different antibiotic groups) [4].

It is estimated that more than 10 million people will die by 2050 due to MDR E. coli strains, especially carbapenem-resistant strains that are spreading globally. The only effective drug against these strains (i.e. colistin) has already lost its effectiveness [5, 6].

Extended-spectrum β-lactamases (ESBLs) are enzymes that are capable of hydrolyzing most beta-lactams including cephalosporins, penicillins, and monobactams. In this situation, carbapenems such as meropenem, imipenem, and ertapenem are used as the last-line drugs; however, resistance to these antibiotics has also increased recently partly due to the emergence of isolates producing metallo-beta-lactamases (MBLs). Clinical isolates of *E. coli* gradually develop carbapenem resistance (CR) as a result of MBL synthesis [7].

A variety of MBL genes have been identified worldwide, the most common of which are bla_{NDM}, bla_{VIM}, and bla_{IMP} genes [7]. Patients infected with carbapenem-resistant Enterobacteriaceae (CRE) frequently carry transposon genes, which are responsible for resistance to several drugs. Eradication of these infections is more challenging due to the high level of antimicrobial resistance and the limited number of therapeutic choices available [8].

Several studies have reported the presence of MDR *E. coli* isolates resistant to carbapenems in Iraq and its neighboring country, Iran [9-12]. Carbapenem-resistant isolates detected in a study conducted in Baghdad, Iraq, carried bla_{IMP} and $bla_{OXA\text{-}48}$ but not bla_{KPC}, $bla_{NDM,}$ or bla_{VIM} [9]. Al-Moslem et al. (2023) reported that all UPEC strains isolated from patients in Basrah city in Iraq were MDR [10]. In another study in Karbala province in Iraq, among *E. coli* strains isolated from different clinical sources, 2 (8.4%) isolates were positive for bla_{NDM} [12].

Objectives: Given the importance of the issue and the rarity of studies on XDR isolates and their MBL genes in Iraq, this study was designed to evaluate the frequency of extensively drug-resistant UPEC isolates and to detect their MBL genes.

Material and Methods

Sample collection and identification: A total of 300 urine samples were collected from patients with UTI symptoms in Al-Nasiriyah city in southern Iraq from March to October 2023. These patients were referred to the laboratories and hospitals by medics

in order to detect the pathogen and perform the antibiogram test.

The ethical permission of this study was taken from the Ethics Committee of Shahid Chamran University of Ahvaz according to the Declaration of Helsinki (IR.SCU.REC.1403.074).

The midstream urine specimens were collected and stored in sterile screw-capped containers for further analysis. A general urine examination (GUE) was conducted to check for the existence of pus, epithelial cells, erythrocytes, bacteria, and other substances. The specimens were then immediately cultured on blood agar (Biolife Italiana; Italy) and MacConkey agar (Biolife Italiana; Italy) and incubated to find any bacterial growth. UTI was considered as the existence of at least 10^5 CFU/mL of pathogenic agent in urine. People who had recently taken antibiotics were excluded from the study.

To select UPEC isolates, in a nutshell, lactose-positive bacteria grown on MacConkey agar were cultivated onto eosin methylene blue medium (EMB, Merck; Germany). Pink colonies (lactose-positive) on MacConkey agar or colonies with metallic shine on EMB medium were subjected to further conventional biochemical tests such as oxidase, sulfur indole motility (SIM), lysine decarboxylase production, methyl red/ Voges-Proskauer (MR/VP), and Simmon's citrate tests. Typical E. coli colonies were identified using the automated VITEK system (bioMérieux, France).

Antimicrobial susceptibility patterns: The Kirby-Bauer disc diffusion method was performed according to Clinical and Laboratory Standards Institute 2022 (CLSI-2022) guidelines to investigate the antibiotic susceptibility pattern of UPEC isolates [13]. The antibiotic susceptibility test was performed on Mueller-Hinton agar (MHA) using the following antibiotic discs: nalidixic acid (30 µg), ciprofloxacin (5 µg), ampicillin (10 µg), streptomycin (10 µg), imipenem (10 µg), sulfamethoxazole-trimethoprim (75 µg), tetracycline (30 µg), gentamycin (300 µg), fosfomycin (200 µg), cefotaxime (10 µg), cefixime (5 µg), and nitrofurantoin (10 µg). Then resistance of these isolates to cefoxitin (30 µg), ampicillin-sulbactam (1 µg), aztreonam (30 µg), cefepime (30 µg), ceftriaxone (10 µg), and piperacillin/ tazobactam (100 µg) was investigated. All antimicrobial discs were from Padtan Teb Co. (Iran). *E. coli* ATCC 25922 was used as a control isolate. UPEC isolates resistant to three or more drug families were considered MDR, while XDR isolates were those resistant to at least one agent in all but two or fewer antimicrobial groups.

Modified carbapenem inactivation method (mCIM) and EDTA-CIM (eCIM): mCIM test was used to detect carbapenemase producers among the tested isolates. However, eCIM was performed only for mCIM-positive isolates to differentiate serine carbapenemase and MBL producers. For the mCIM test, 1 µL of each isolate was completely mixed in 2 mL of tryptone soya broth (TSB). After that, meropenem or imipenem discs were placed in the inoculated TSB and incubated at 37 °C for 4 hrs. E. coli ATCC-25922 (0.5-McFarland standard) was cultured on MHA. The discs were removed and placed in the center of the MHA culture plate.

Then the plates were incubated at 37 °C for 24 hrs. Carbapenemase-producing isolates showed a clear zone with a 6–15 mm diameter or formed pinpoint colonies within a 16–18 mm zone around carbapenem discs [13]. The eCIM test was performed similarly to the mCIM, except that 20 µL of 0.5 M EDTA was added to the bacterial suspensions. MBL-producing isolates showed an increase of ≥5 mm in inhibition zone in the eCIM test compared to mCIM, while carbapenemase-producing isolates had no change in clear zone diameter or an increase of ≤4 mm [13].

Detection of metallo-beta-lactamase genes by PCR: DNA was extracted from XDR UPEC isolates by boiling and chilling method [14]. PCR (polymerase chain reaction) was used to check the presence of MBL genes, including bla_{VIM-1}, bla_{VIM-2}, bla_{SPM-1}, bla_{SIM}, bla_{NDM}, bla_{IMP-1}, bla_{IMP-2}, $bla_{GIM,}$ and bla_{KPC}. Primers, PCR reactions, and conditions were prepared as previously described (Table 1) [15-19]. All reactions were done as follows: initial denaturation at 95 °C for 5 min; 30 cycles of denaturation at 94 °C for 1 min, annealing (temperature according to Table 1) for 1 min, and extension at 72 °C for 1 min; and a final extension at 72 °C for 7 min. MBL-producing isolates confirmed in another study [20] were used as positive controls.

Statistical analysis: Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) (Ver. 21). The frequency of variables was presented as percent. If necessary, Chi-square and Fisher's exact tests were used to calculate data correlation. The level of significance was $p< .05$.

Findings

Bacterial isolates: In this study, out of 300 urine samples, 200 (66.66%) samples were positive for UTI. The colony counts of these samples were more than 10^5 CFU/mL. Among UTI-positive samples, UPEC isolates were identified in 150 samples and subjected to antimicrobial susceptibility testing.

Antimicrobial resistance patterns: The resistance and susceptibility of the isolates against antimicrobial agents was investigated by Kirby-Bauer disc diffusion method. As shown in Table 2, the highest resistances was against cefepime (88%) and ampicillin (85.3%), while the highest susceptibility was against imipenem (91.7%), fosfomycin (84%), and cefoxitin (72.7%). MDR and XDR profiles were detected in 145 (96.66%) and 5 (3.33%) isolates, respectively.

Phenotypic detection of MBL-producing UPEC isolates: In total, five UPEC isolates showed XDR profile and resistance to imipenem. These isolates were also resistant to meropenem and investigated for MBL

Table 1) Sequences of PCR primers used for the detection of metallo-beta-lactamase genes

Gene Name	Primer Sequence (5'→3')	Product Size (bp)	Annealing Temperature	References
bla_{IMP-1}	F-ACCGCAGCAGAGTCTTTGCC	587	55	15
	R-ACAACCAGTTTTGCCTTACC			
bla_{IMP-2}	F-GTT TTA TGT GTA TGC TTC C	678	52	15
	R-AGC CTG TTC CCA TGT AC			
bla_{VIM-1}	F-AGTGGTGAGTATCCCGACAG	261	55	15, 18
	R-ATGAAAGTGCGTGGAGAC			
bla_{VIM-2}	F-ATGTTCAAACTTTTGAGTAAG	801	56	17, 18
	R-CTACTCAACGACTGAGCG			
bla_{SPM-1}	F-GCGTTTTGTTTGTTGCTC	786	55	15
	R-TTGGGGATGTGAGACTAC			
bla_{SIM}	F-TACAAGGGATTCGGCATCC	570	53	16
	R-TAATGGCCTGTTCCCATG			
bla_{NDM}	F-GGCGGAATGGCTCATCACGA	286	58	19
	R-CGCAACACAGCCTGACTTTC			
bla_{GIM}	F-TCGACACACCTTGGTCTG	477	55	16
	R-AACTTCCAACTTTGCCAT			

Table 2) Antimicrobial susceptibility profile of uropathogenic Escherichia coli isolates

Antibiotics	AMP N (%)	FEP N (%)	CTX N (%)	CRO N (%)	FOX N (%)	CFM N (%)	IMP N (%)	AZT N (%)	FOF N (%)	GEN N (%)	STR N (%)	TET N (%)	CIP N (%)	NAL N (%)	SXT N (%)	NIT N (%)	TZP N (%)	SAM N (%)
Resistant	128 (85.3)	132 (88)	80 (53.3)	118 (78.7)	40 (26.7)	115 (76.7)	5 (3.3)	121 (80.7)	18 (12)	48 (32)	102 (68)	110 (73.3)	108 (72)	114 (76)	109 (72.7)	44 (29.3)	99 (66)	73 (48.7)
Intermediate	1 (0.7)	0 (0)	4 (2.7)	0 (0)	1 (0.7)	2 (1.3)	3 (2)	4 (2.7)	6 (4)	6 (4)	4 (2.7)	3 (2)	4 (2.7)	6 (4)	3 (2)	6 (4)	2 (1.3)	5 (3.3)
Susceptible	21 (14)	18 (12)	66 (44)	32 (21.3)	109 (72.7)	33 (22)	142 (91.7)	25 (16.7)	126 (84)	96 (64)	44 (29.3)	37 (24.7)	38 (25.3)	30 (20)	38 (25.3)	100 (66.7)	49 (32.7)	72 (48)

UPEC: Uropathogenic Escherichia coli, XDR: extensive drug resistance, AMP: ampicillin, FEP: cefepime, CTX: cefotaxime, CRO: ceftriaxone, FOX: cefoxitin, CFM: cefixime, IMP: imipenem, AZT: aztreonam, FOF: fosfomycin, GEN: gentamicin, STR: streptomycin, TET: tetracycline, CIP: ciprofloxacin, NAL: nalidixic acid, SXT: sulfamethoxazole trimethoprim, NIT: nitrofurantoin, TZP: piperacillin-tazobactam, SAM: ampicillin-sulbactam

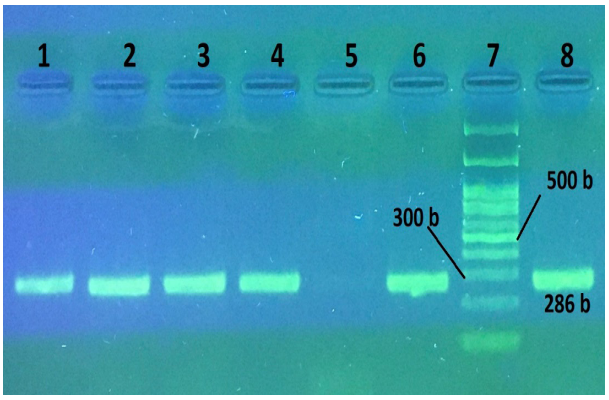

Figure 1) PCR result of bla_{NDM} gene of UPEC isolates. Lanes 1-4 and 6: bla_{NDM} gene (286 bp) of UPEC isolates; lane 7: DNA ladder (100 bp), lane 5: negative control; lane 8: positive control

production. All five XDR UPEC isolates were positive for mCIM, while one isolate was negative for eCIM. Therefore, according to phenotypic tests, four isolates were considered MBL producers, and one isolate was serine carbapenemase producer.

Detection of MBL genes: PCR method was applied to detect MBL genes, including bla_{IMP1}, $bla_{IMP2,}$ $bla_{VIM1,}$ $bla_{VIM2,}$ $bla_{SPM1,}$ $bla_{SIM,}$ $bla_{GIM,}$ and bla_{NDM}. All five imipenem-resistant isolates that also showed the XDR profile harbored bla_{NDM}, while the other MBL genes were not found among the isolates (Fig. 1).

Discussion

Today, MDR and XDR bacteria pose a significant risk to public health, especially inflicting high morbidity and mortality rates due to postoperative infections. The eradication of these infections is more difficult because of their resistance to multiple antibiotics. Researchers have tried to treat such infections and overcome antimicrobial resistance by understanding the underlying mechanisms of this phenomenon [21].

In the present study, five (3.33%) UPEC isolates were identified with XDR profiles, while the rest (96.66%; n=145) displayed MDR profiles. Previous studies have showed that the prevalence of MDR isolates in southern Iraq is higher than in other regions of the country [22, 23]. Al-Hasnawy and colleagues (2019) investigated the prevalence of MDR and XDR UPEC isolates in Babylon province, Iraq, and reported that 88.09 and 11.90% of the isolates detected were MDR and XDR, respectively [24]. In another study in Baghdad city, MDR, XDR, and PDR profiles were found in 98.23, 21.24%, and 1.77% of *E. coli* clinical isolates, respectively [25]. Al-Moslem et al. (2023) reported that all UPEC isolates found in Basrah city were multidrug-resistant [10]. While Asia and Africa have been reported to have higher rates of MDR UPEC than other regions, North America and Europe seem to have the lowest rates. This could be due to the improper and indiscriminate usage of antibiotics in Asian and African countries in the past [22]. The preferred antibiotics for the treatment of severe infections caused by Gram-negative bacteria are carbapenems; nonetheless, several studies have shown that MBL-producing clinical strains are widely distributed worldwide [19].

This present study aimed to identify MBL genes, including bla_{IMP-1}, bla_{IMP-2}, bla_{VIM-1}, bla_{VIM-2} bla_{SIM}, $bla_{GIM,}$ bla_{SPM-1} and bla_{SPM-1}, in carbapenem-resistant UPEC isolates. The findings indicated that 5 (3.33%) isolates carried the bla_{NDM} gene, while none of them possessed bla_{IMP-1}, bla_{IMP-2}, bla_{VIM-1}, bla_{VIM-2}, bla_{SIM}, $bla_{GIM,}$ and bla_{SPM-1} genes. Therefore, the bla_{NDM} gene seems to be the main cause of carbapenem resistance in these isolates. Taqi and Hadi (2023) in Mosul, Iraq, found that all *E. coli* isolates were imipenem and meropenem resistant and harbored bla_{NDM} but not other beta-lactamases [12]. In another study by Al-Hasso (2023) on Gram-negative bacilli isolated from different sources, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{OXA48}, and bla_{KPC} were detected in 1 (7.7%), 1 (7.7%), 1 (7.7%), 2 (15.4%), and 5 (38.5%) *E. coli* clinical isolates, respectively [26]. Ahmed et al. (2023) investigated the presence of bla_{NDM} and $bla_{OXA\text{-}48}$ genes among *E. coli* clinical isolates and found that 22.9 and 12.2% of these isolates were positive

for bla_{OXA48} and bla_{NDM}, respectively [27]. A study on UPEC strains isolated from cancer patients showed that bla_{VIM}, bla_{KPC}, bla_{IMP}, and bla_{SPM} were detectable in 59.1, 22.73, 22.73, and 18.18% of the isolates, respectively [28]. Compared to the frequencies reported for bla_{NDM} in countries around the world, the frequency observed in this study is significantly higher. In a study in Iran, bla_{NDM} was predominant in carbapenem-resistant UPEC isolates [20]. In fact, NDM is a recently identified MBL gene that confers resistance to all β-lactam antibiotics. Bacteria like *Klebsiella pneumoniae*, *E. coli,* and *Acinetobacter baumannii* have been determined to be the main hosts of NDM-MBL. NDM was first identified in a Swedish patient of Indian origin with *K. pneumoniae* and *E. coli* [19, 29]. Currently, NDM is widely dispersed in almost 40 countries [29].

The high prevalence of NDM gene in our UPEC isolates might be attributed to the fact that a large number of Iraqi patients travel to India for medical care and undergo various surgical operations in this country, leading to the transfer of these resistance genes to Iraqi hospitals. Rapid dissemination of carbapenem resistance among *Enterobacteriaceae* occurs through clonal and plasmid-mediated propagation of clinical carbapenem-resistant bacterial strains [27, 30].

Plasmids are the primary means of transferring antibiotic resistance and virulence genes, particularly CR genes, to bacteria. However, since plasmids impose costs on bacteria and limit their energy resources, the ability of these microorganisms to adapt to different plasmids is restricted, and it is unclear whether recipient bacteria could increase their pathogenicity, antimicrobial resistance, or both. According to research findings, pathogenicity is not significantly modified by NDM carriage or expression, but there is a fitness penalty [29]. Hasoon and Hamed (2019) demonstrated that the increase in global travel and the influx of foreign labor, particularly from endemic regions of the Indian subcontinent, Bangladesh, and other countries, might have contributed to the notable rise in the prevalence of carbapenem-resistant bacteria in Iraq over the past 20 years, particularly after the 2003 war. Factors such as geographical location, type of infection, source of specimen, and antibiotic selection could affect the prevalence of carbapenemase-producing isolates. Discrepancies between the results of different studies may also be linked to variations in study populations and antibiotic administration rates in hospitals [30].

The emergence and spread of distinct variants of carbapenemase-producing pathogenic bacteria in Iraq may be significantly influenced by armed conflicts across the country, travel abroad to receive medical care, and cross-border transmission, particularly by immigrants and labor [30]. In order to lower the risk of antibiotic resistance and closely monitor the emergence of MDR bacteria, especially CRE, which is connected to high mortality rates (according to the CDC), it is essential to restrict public access to antibiotics and provide them only with a physician's prescription. Thus, it is clear that more regular reporting of *Enterobacteriaceae* antibiotic resistance is required to track propagation routes and identify underlying mechanisms of resistance [31]. Although different mechanisms could lead to resistance to carbapenems, only the production of metallo-beta-lactamases was investigated in the present study. Another limitation of this study was the lack of evaluation of the isolates' susceptibility to the polymyxin family due to the unavailability of colistin.

Conclusion

The high prevalence of MDR isolates and the identification of several XDR strains indicate

the widespread propagation of antimicrobial resistance among pathogenic bacteria in this region, posing a significant risk to public health. Also, the NDM gene was found to be circulating among UPEC strains at least in Nasiriya province of Iraq. This could lead to increased resistance to carbapenems among Enterobacteriaceae, which is a serious threat to public health. Therefore, appropriate infection control measures, including preventive and diagnostic measures, as well as appropriate antibiotic stewardship measures should be implemented to hurdle the spread of these pathogens.

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Conflicts of interests: The authors declare that they have no competing interests.

Authors' contributions: SER, IHR, HM, and MTR participated in searching for subjects and data as well as performing the research. SER conducted the research. SER and IHR analyzed and interpreted the data. All authors read, revised, and approved the final manuscript.

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