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Research Article

Frequency of Metallo-β-Lactamase-Producing *Escherichia coli* Bacteria in Urinary Tract Isolates

Abbas Atiya Hammoudi

College of Health and Medical Technologies, Al-Bayan University, Baghdad, Iraq
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I N F O

E-mail Id:
abbas.at @albayan.edu.iq
Orcid Id:
https://orcid.org/0009-0008-6619-4780
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A B S T R A C T

Introduction: The most popular facultative Gram-negative anaerobic bacillus that causes community-acquired urinary tract infection (UTI) illness is *Escherichia coli*. The present study sought to detect *E. coli* isolates that produced MBLs in patients who suffered from UTIs and to determine the antibiotic susceptibility pattern in these isolates.

Methods: A total of 210 urine specimens were collected from patients with clinical signs and symptoms of a UTI. only 52 (42.6%) specimens demonstrated *Escherichia coli* growth. Metallo-lactamase (MBL) enzymes were examined using three separate phenotypic techniques: the Double Disk Synergy Test (DDST), the Combined EDTA Disk Test (CMDT), and the Modified Hodge Test (MHT), all of which included the selection of a 10 µg meropenem antibiotic disk as a positive test. The commercially available antibiotic disks were used in the susceptibility test.

Results: Results demonstrated that 30 out of the 52 isolates (57.7%) tested positive for the production of the MBL enzymes. DDST test demonstrated a significant zone of enhancement between the meropenem disk and the EDTA disk in eight isolates. CMDT test revealed 17 (56.7%) for the 30 *E. coli* isolates showed zone inhibition widths of at least 7 mm surrounding the meropenem-linked EDTA disk, and 15 out of 30 isolates of carbapenem-resistant *E. coli* tested positive for MHT. The most resistance of the selected antibiotics were ceftriaxone and co-trimoxazole (76.9%), ceftazidime (73.1%), tobramycin (71.2%), and the most effective against every isolate of *E. coli* was imipenem (86.5%), followed by meropenem (84.6%) and nitrofurantoin (75.0%).

Conclusion: The findings emphasise the urgent need for routine surveillance of antibiotic resistance, judicious use of broad-spectrum antibiotics, and improved infection control and diagnostic practices. Implementing these strategies can help reduce the burden of MDR infections and guide appropriate treatment to curb the spread of resistant strains in clinical settings.

Keywords: UTIs, *E coli*, Metallo-β-Lactamase Enzymes, Antimicrobial Sensitivity Tests

Introduction

Urinary tract infections (UTIs) constitute a leading cause of mortality and morbidity. Diagnostic and clinical reports estimate that 150 million people around the world suffer from UTIs each year.[1,2] Gender, age, urological devices and equipment, weakened immunity, lodged urinary catheters, and comorbidities like diabetes mellitus are some of the key factors that influence the progression of UTIs.[3] *Escherichia coli (E. coli), Klebsiella pneumoniae, Staphylococcus aureus, Enterococcus faecalis,* and *Proteus mirabilis* are the popular bacteria that cause UTIs. Community-acquired UTI is most commonly caused by *E. coli* bacteria, which are numerous in the gastrointestinal microflora of humans.[4,5] Despite being a beta-lactam antibiotics used for treatment of UTI, *E. coli* bacteria become multidrug-resistant (MDR) strains against all beta-lactam antibiotics.[6,7]

The worldwide distribution of *E. coli* harbouring metallo-β-lactamases (MBLs) is a serious threat, and due to MBL production, carbapenem resistance is progressively spreading among clinical isolates of *E. coli*.[8] Antimicrobial resistance to these life-saving medications is thus a significant global public health issue.[9] The clinical signs and symptoms of UTI are used to make the diagnosis, which is supported by laboratory evidence of pyuria and bacteriuria.[10] This study used three phenotypic methods—the Double disk synergy test (DDST), the Combined disc test (CMDT) with EDTA, and the Modified Hodge test (MHT)—to detect *E. coli* isolates that produced MBLs in patients who suffered from UTIs in Baghdad City. It also sought to determine the antibiotic susceptibility pattern in these isolates.

Material and Methods

Isolation and Identification

The present prospective cross sectional study included 210 patients with clinical signs and symptoms of UTI who visited the consulting clinics of the Al-Karama Teaching Hospital and a private laboratory in the Baghdad governorate of Iraq between January 3 and August 30, 2021. A total of 210 urine specimens were collected with the advice to obtain midstream specimens and were transported to the laboratory as soon as possible in sterile containers. *E. coli* was isolated and identified from urine samples using standard bacteriological techniques. This study used three phenotypic methods—the Double disk synergy test (DDST), the Combined disc test (CMDT) with EDTA, and the Modified Hodge test (MHT)—to identify MBL-producing *E. coli* in individuals suffering from UTIs. Furthermore, the pattern of antibiotic susceptibility was analysed in these isolates. DDST is simple test used for Extended-Spectrum Beta-Lactamase (ESBL) production in gram-negative bacteria, such as *E. coli* and K. pneumonia. Inclusion criteria patients with UTIs whether male or female. Exclusion criteria include patients with any chronic systemic diseases and elderly patients of more than 60 years.

Biochemical Tests

After the colonies were visible to be an *E. coli* bacterium (HiMedia Company, India), followed that the urine samples were grown on blood agar, MacConkey medium, and eosin-methylene blue agar. The specimens were examined after a 24-hour incubation period at 37°C. The isolates were determined to be *E. coli* using Gram staining (pink colour) and manual biochemical tests (Voges–Proskauer, methyl red, indole, H_2S production, gas production, oxidase, citrate utilisation, catalase, and urease), performed in accordance with the manufacturer's instructions.[11] Three different phenotypic techniques were applied to screen bacterial isolates for the presence of MBL enzymes, with each technique using a 10 µg meropenem antibiotic disk.

Double Disk Synergy Test (DDST)

The Mueller–Hinton agar (MHA) plates were inoculated with the test organism for DDST. A 10 µg meropenem disk and a blank Whatmann No. 1 filter paper disk (both 6 mm in diameter) were placed 10 mm apart (edge to edge). After an 18-hour incubation period at 37°C, 10 µL of 0.5 M EDTA solution was applied to the blank disk. The test was considered positive EDTA synergy test when the zone that extends toward the saturated EDTA disk.[12] An EDTA- and meropenem-saturated disk was seen in the present test results.

Combined EDTA Disk Test (CMDT)

An overnight broth culture of the studied organism strain was used for this test, and it was inoculated onto a plate of MHA with an opacity calibrated to 0.5 McFarland standard. The antibiotic disks were quickly dried in an incubator after being mixed with 4 mL of the sterilised EDTA solution, and were then stored at -20°C in airtight vials without desiccants until further use.[13] On a dry MHA plate,[14] two 10 µg meropenem disks and one meropenem disk treated with EDTA were spaced 20 mm apart and incubated for 24 hours at 37°C. After the combined meropenem and EDTA-saturated disk was incubated, an increase of a minimum of 7 mm was seen in the inhibition zone diameter.[15]

Modified Hodge Test (MHT)

According to Lee et al., MHT testing was performed on each isolate under investigation. To create an overnight culture suspension, 5 mL ordinary saline was mixed with two to three isolated colonies of the *E. coli* strain.[16] Before being streaked across the MHA plate, 1 mL of suspension was diluted with 4 mL of 0.85% NaCl. Then, the mixture was adjusted to 0.5 McFarland standard. After the 10 µg meropenem disks have been dried, up to four different test

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isolates were streaked in a straight line from the edge of the plate towards its centre. Every strain under examination had a zone of inhibition that resembled a clover leaf, which is regarded as a positive indicator that the strain produced carbapenemases. A negative MHT results showed that *E. coli* ATCC 25922 growth has not developed alongside the test organism.[17]

Antimicrobial Sensitivity Tests

Antimicrobial susceptibility testing was carried out using the Kirby–Bauer disc diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) recommendations. MHA plates (HiMedia, India) were used for this test. The excess fluid was then drained by rotating sterile cotton swabs against the upper interior wall of the tube after they had been dipped in the culture broth. The entire agar surface of the plate was streaked three times with the swab, with each streak involving a 60° rotation of the plate.

Using an antibiotic disc dispenser, amikacin (30 µg), imipenem (10 µg), tobramycin (10 µg), ciprofloxacin (5 µg), nitrofurantoin (50 µg), amoxicillin/clavulanic acid (20 µg/ 10µg), ceftazidime (30 µg), ceftriaxone (30 µg), co-trimoxazole (25 µg), meropenem (10 µg) and levofloxacin (10 µg) were all applied to the agar surface of the plate. The medium was left for five minutes to dry. The discs were properly placed on the media using sterile forceps. The plates were examined after 24 hours of incubation at 37°C. The diameters of the zones of inhibition were interpreted in accordance with the CLSI guidelines.[17] *E. coli* ATCC 25922 was used as the control strain for the susceptibility tests.

Ethical Approval

This study was approved by the Institutional Review Board (IRB) of Al-Bayan University. Informed consent was signed from all participants prior to sample collection. All procedures were conducted in accordance with the Declaration of Helsinki.

Statistical Analysis

Descriptive statistics were used to summarise bacterial distribution and antibiotic susceptibility. The chi-square test was applied to assess associations between MBL production and resistance rates. A p value < 0.05 was considered statistically significant. All analyses were performed using Statistical Package for Social Science (SPSS) software (IBM SPSS Statistics for Windows 24.0, IBM Corp., Armonk, NY, USA).

Results and Discussion

The study's sample included 125 men (59.5%) and 85 women (40.5), aged 18–55 years. The following bacteria were identified in 122 samples (out of 210 sample swab isolates): *E. coli* (52, 42.6%), coliform (5, 4.1%), *Enterococcus* (6, 4.9%), Proteus spp. (9, 7.4%), Staphylococcus aureus (10, 8.2%), *Pseudomonas spp.* (10, 8.2%), and *Klebsiella pneumoniae* (30, 24.6%) (Table 1). Additionally, 88 mixed growth isolates showed the following strains: *E. coli* and *Pseudomonas spp.* (8, 9.1%), *E. coli* and *Proteus spp.* (6, 6.8%). Coliform and *Proteus spp.* (8, 9.1%), *Enterococcus spp.* and *Staphylococcus aureus* (12, 13.6%), *Klebsiella pneumoniae* and *Proteus spp.* (9, 10.2%), *Klebsiella pneumoniae* and *Proteus spp.* (9, 10.2%), and *Pseudomonas spp.* and *Klebsiella pneumoniae* (20, 22.7%) (Table 2).

Table 1.Types, Frequencies and Percentages of Isolated Bacteria

S. No.	Type of Bacteria	Frequency	Percentage
1	*E. coli*	52	42.6
2	*Klebsiella pneumoniae*	30	24.6
3	*Pseudomonas spp.*	10	8.2
4	*Staphylococcus aureus*	10	8.2
5	*Proteus spp.*	9	7.4
6	*Enterococcus*	6	4.9
7	Coliform	5	4.1
Total		122	100.0

In many countries, including Iraq and other Middle Eastern nations, UTIs were the most prevalent ailment. Numerous pathogens, including bacteria and fungi, were found to infect persons of all ages in different parts of the world.[18] The human colon is home to the Gram-negative, rod-shaped bacterium *E. coli*,[19] which was found to be the most frequently (52, 42.6%) isolated pathogen in the present study. This outcome is consistent with research that found that uropathogenic *E. coli* (UPEC) is responsible for around 90% of UTIs.[20]

Additionally, a recent investigation revealed that 65 (73.9%) *E. coli* isolates were identified from the clinical samples, which is consistent with the present study.[21] While other mixed growth rates varied, our analysis found that isolates of *E. coli* and *Klebsiella pneumoniae* had the highest rates of mixed growth (28.4%) (Table 2). This might have happened as a result of poor urine collection methods, which allowed bacteria from the skin or vaginal area to contaminate urine samples. These results are supported by the findings of Folaranmi et al., which revealed that mixed growth urine cultures (MGUCs) were reported for urine specimens processed to detect bacterial pathogens and that *Enterococcus spp*. (30.1%) and *E. coli* (27.5%) were the most frequently isolated organisms, with this

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pathogen combination being the most common, accounting for 24% of all isolates.[22] At least one *Enterobacterales* was present in 82.5% of all MGUCs, and two or more organisms were present in 65.5% of all cultures.[22] Our investigation discovered that isolates of *E. coli* and *Klebsiella pneumoniae* had the highest rates of mixed growth (28.4%), whereas other mixed growth rates varied (Table 2).

Table 2.Types, Frequencies, and Percentages of Mixed Isolated Microorganisms

S. No.	Types of Mixed Bacteria	Frequency	Percentage
1	*Enterococcus spp. and Staphylococcus aureus*	12	13.6
2	*Coliform bacteria and Proteus spp.*	8	9.1
3	*E. coli and Klebsiella pneumoniae*	25	28.4
4	*Pseudomonas spp. and Klebsiella pneumoniae*	20	22.7
5	*Klebsiella pneumoniae and Proteus spp.*	9	10.2
6	*E. coli and Proteus spp.*	6	6.8
7	*E. coli and Pseudomonas spp.*	8	9.1
Total		88	100.0

Metallo-β-Lactamases Detection

Clinical strains that produce MBLs have been implicated in an increasing number of nosocomial infections that escalate to more serious illnesses.[23-25] To check for the presence of MBL enzymes in frequently occurring *E. coli* isolates, the following phenotypic approaches have been used: 30 out of the 52 isolates tested positive for the production of the MBL enzymes, yielding a proportion of 57.7%, according to the results of the tests. A study by Giri et al. found that *E. coli* (32%) and *Klebsiella pneumoniae* (58%) were the two most prevalent carbapenem resistant *Enterobacteriaceae*,[26] while Bahramian et al. reported that 16 (66.7%) of the 24 isolates of carbapenem-resistant *E. coli* generated this enzyme[25]. Furthermore, a study by Wang et al. revealed that *K. pneumoniae* isolates expressing carbapenemase were less prevalent than *E. coli* isolates.[27]

Double Disk Synergy Test (DDST) Results

Out of 30 *E. coli* isolates, the results of DDST in this study demonstrated a significant zone of enhancement between the meropenem disk and the EDTA disk in eight isolates, suggesting a successful outcome. In contrast to Panchal et al.'s investigation, which showed higher rates of positivity in DDST (17, 56.67%).[28] In other studies carried out in South India, 15 out of 51 MBL-producing *E. coli* isolates were identified using combined disc diffusion and DDST.[29]

Combined Edta Disk Test (Cmdt) Results

After applying CMDT, 17 (56.7%) for the 30 *E. coli* isolates, they demonstrated successful results, with zone inhibition widths of at least 7 mm surrounding the meropenem-linked EDTA disk that is saturated. These results are quite similar to those of a study by Bahramian et al., which showed that 16 (66.7%) of 24 samples of *E. coli* were carbapenem-resistant.[25] These results disagree with Husain, who stated that only 2 isolates (66%) were positive in the Combined EDTA Disk test out of 34 carbapenem-resistant *E. coli* isolates, and 1 isolate (34%) was negative.[30] Furthermore, according to Nagaraj et al., 15 out of 51 carbapenem-resistant *E. coli* isolates were found to be positive using mixed disc diffusion.[29]

Modified Hodge Test (MHT) Results

The results showed that each tested strain had an inhibitory zone around the meropenem disk; this was regarded as a strain generating carbapenemases or as a positive group. In the current investigation, 15 out of 30 isolates of carbapenem-resistant *E. coli* tested positive for MHT. The results of this study were consistent with those of a study by Amjad et al., which discovered that 69% of the isolates that were carbapenem intermediate or sensitive could be detected as having carbapenemase using only MHT.[31] A later investigation carried out in 2007 at the Centers for Disease Control and Prevention in Atlanta, Georgia, showed MHT to be a very sensitive and reliable test for the identification of carbapenemases.[32]

Antimicrobial Sensitivity Patterns

After testing for antibiotic sensitivity against the chosen drugs, all 52 *E. coli* isolates were found to have the following resistance patterns to the selected antibiotics: ceftriaxone and co-trimoxazole (76.9%), ceftazidime (73.1%), tobramycin (71.2%), amoxicillin/ clavulanic acid (69.2%), ciprofloxacin (63.5%), levofloxacin (34.6%), amikacin (26.9%), nitrofurantoin (25.0%), meropenem (15.4%), and imipenem (13.5%) (Table 3). The antibiotic that was most effective against every isolate of *E. coli* was imipenem

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(86.5%), followed by meropenem (84.6%), nitrofurantoin (75.0%), levofloxacin (57.7%), and amikacin (55.7%), while the least effective antibiotics were ciprofloxacin (36.5%), tobramycin (28.8%), co-trimoxazole (23.1%), amoxicillin/clavulanic acid (17.3%), ceftriaxone (13.5%), and ceftazidime (11.5%).

A study by Bahramian et al. discovered that more than 70% of the isolates were resistant to the third-generation cephalosporins (TGCs), which is consistent with the findings of the current inquiry.[25] In a different investigation, Jamil et al. found that 25 (33.3%) out of 75 isolates were resistant to ceftriaxone, ceftazidime, cefotaxime, and imipenem.[33] A study by Niranjan and Malini found that there was a substantial resistance to ceftazidime, amoxicillin, and clavulanic acid.[34] The isolates in the current study showed high sensitivity to imipenem and meropenem, two antibiotics widely regarded as the best treatments for many conditions caused by Gram-positive and Gram-negative bacteria.[35] Additionally, carbapenems, one of the most potent broad-spectrum antibiotics, are frequently used to treat infections caused by various bacteria, notably those that cause nosocomial infections. Carbapenems offer a wider antibacterial spectrum than the penicillin and beta-lactamase inhibitor combos that are currently available.[36,37] The results of this study may be explained by the low rate at which these antibiotics are used to treat bacterial diseases in our country. Adam and Elhag concurred that imipenem was the most successful (94%) antimicrobial among all isolates.[37] In contrast to Nagaraj et al.'s investigation, this study indicated the prevalence of carbapenem-resistant *K. pneumoniae* and *E. coli* to be 75.0% and 66.6%, respectively.[29] Levofloxacin, amikacin, and ciprofloxacin each had a susceptibility percentage of 57.7%, 55.7%, and 36.5%, respectively, in the current study. Although amikacin (78, 92.3%) was found to be more effective against *E. coli* isolates, ciprofloxacin and levofloxacin were found to be more effective against *E. coli* isolates that were not carbapenem-resistant, in a study by Huang et al.[24] According to a different study by Dwomoh et al., 69.4% of *E. coli* isolates were resistant to sulfamethoxazole-trimethoprim, amoxicillin-clavulanate, ciprofloxacin, and levofloxacin, with antimicrobial resistance rates of 86.8%, 75.0%, 70.8%, and 65.0%, respectively.[38]

The findings of the isolates in this investigation are consistent with those of the study conducted by Kwami et al., which showed that *E. coli* isolates had the highest percentage of tobramycin resistance (53.8%).[39] The current study showed that 37 (71.2%) isolates were resistant to tobramycin. The results of this investigation did not agree with those of the study by Dehkordi et al., which showed that the *E. coli* isolates (15.90%) had the highest prevalence of tobramycin resistance.[41] The MDR *E. coli* strain has been found to be complicating the therapeutic management of infectious diseases and raising mortality rates, morbidity lengths and severity, and healthcare costs.[41-43]

Table 3.Antibiotic Susceptibility Patterns of Certain Strains of *E. coli*

Antibiotics	Sensitivity n (%)	Intermediate n (%)	Resis -tance n (%)
Amikacin	29 (55.7)	9 (17.3)	14 (26.9)
Ciprofloxacin	19 (36.5)	-	33 (63.5)
Meropenem	44 (84.6)	-	8 (15.4)
Imipenem	45 (86.5)	-	7 (13.5)
Ceftazidime	6 (11.5)	8 (15.4)	38 (73.1)
Ceftriaxone	7 (13.5)	5 (9.7)	40 (76.9)
Amoxicillin/ clavulanic acid	9 (17.3)	7 (13.5)	36 (69.2)
Tobramycin	15 (28.8)	-	37 (71.2)
Nitrofurantoin	39 (75.0)	-	13 (25.0)
Co-trimoxazole	12 (23.1)	-	40 (76.9)
Levofloxacin	30 (57.7)	4 (7.7)	18 (34.6)

Conclusion

The emergence and spread of MBL-producing *E. coli* represent a significant public health concern due to their role in MDR, which limits therapeutic options for UTIs. In this study, 57.7% of *E. coli* isolates demonstrated MBL production, indicating a substantial prevalence of carbapenemase activity among UTI pathogens in Baghdad. Among the three phenotypic methods used for MBL detection, CMDT and MHT proved to be more reliable than DDST, highlighting their diagnostic utility in clinical laboratories. Antimicrobial susceptibility testing revealed high resistance rates to commonly used antibiotics, particularly third-generation cephalosporins, co-trimoxazole, and fluoroquinolones. However, carbapenems (imipenem and meropenem) and nitrofurantoin retained good efficacy against most isolates, making them suitable candidates for empirical therapy. The findings emphasise the urgent need for routine surveillance of antibiotic resistance, judicious use of broad-spectrum antibiotics,

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and improved infection control and diagnostic practices. Implementing these strategies can help reduce the burden of MDR infections and guide appropriate treatment to curb the spread of resistant strains in clinical settings.

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