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Detection of Multidrug Resistance Uropathogenic *Escherichia coli* in Pregnant Women in Mosul City

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Abstract

One of the main bacterial infections for pregnant women is urinary tract infection (UTI). UTIs during pregnancy are made easier by the physiological and anatomical changes. Bacteria that produce extended spectrum beta-lactamases (ESBLs) have proliferated globally in recent decades. As the primary ESBL-producing bacteria, *Escherichia coli* are highly valued as one of the most significant causal agents of nosocomial infections worldwide.The purpose of the study was genotypic and phenotyping detection of ESBL Producing *Escherichia coli* and its prevalence in pregnant women.All urine samples cultured and diagnosed by biochemical test. The positive culture for *E.coli* streaked on Muller Hinton media for antibiotic susceptibility. Then MDR species were used to assess the presence of ESBL by phentyping and genotyping methods.The antibiotics sensitivity results are 27 (77.1 %) sensitive to CIP and C 25 (71.4 %). resistance to CRO 8 (22.8 %), CTX 10 (28.5 %), AMC 23 (65.7 %). The genotyping showed that from 14 isolates of ESBL, 8 (57.14 %) were positive for CTX-M gene. The most frequent cause of UTIs is E. coli, and the creation of ESBL makes treatment plans more difficult and increases resistance to standard antibiotics.

1. Introduction

Urinary tract infection UTIs are among the most common bacterial outpatient infections, affecting 150 million individuals annually worldwide and accounting for over 50–60% of infections in adult women. UTIs are more common in pregnant women, particularly in the first and second trimesters. [1]Pregnant women are more likely than men to get recurrent UTI. [2] Due to the physiological changes caused by elevated of progesterone, smooth muscle relaxation leads to increase bladder capacity and decreases ureteric peristalsis, encouraging physiological

"hydronephrosis" and urine stasis. UTI may be harmful to the fetus as well as the mother. Among the potential complications are early membrane rupture, preterm delivery, and a higher prevalence of intrauterine growth restriction. [3] Symptomatic UTIs are classified as both upper and lower. Lower UTIs impact the urethra and bladder. Dysuria, or painful urination, frequent urination, and suprapubic pain are indications of bladder infection (cystitis). Painful urination and discharge are common symptoms of urethral infection (urethritis), which is frequently associated with sexually transmitted infection (STIs). Upper UTIs involve the kidneys and ureters. A more serious illness known as kidney infection (pyelonephritis) sometimes manifests as flank pain and discomfort along with Pyuria (pus in the urine). [4] Numerous microbes can cause UTIs, but the most common one is Uropathogenic *Escherichia coli* (UPEC). [5] A number of factors can cause bacteria to develop antibiotic resistance, including through drug target mutations, cellular efflux pumps, and enzymes like as beta-lactamases that deactivate antibiotics. [6]The ability of bacteria to enzymatically break down antibiotic substances is one of the main mechanisms that lead to MDR. The ability of bacterial Beta lactamases to hydrolyze antibiotic substances and render lactam antibiotics inactive is a prime example of these occurrences. [7]Extend Spectrum B-Lactamase (ESBLs) are enzymes that provide resistance to monobactams like Aztreonam (Al) and a wide range of third generation of cephalosporins (TGCs) such as Ceftazidime, Cefotaxime, and Ceftriaxone. [8]The most prevalent enzymes at the moment are the CTX-M beta lactamase type, which have been growing in numerous countries. [9] Although this enzyme is capable of hydrolyzing ceftazidime and cefotaxime, it exhibits considerable resistance to cefotaxime and little activity against Ceftazidime. [10] Since 1995, a variety of clinical bacteria, both within and between species worldwide, have been found to carry the CTX-M enzymes, a class A ESBL. Today, the CTX-M allelic variants are divided into five main phylogenetic groups based on around 130 amino acid sequences: CTX-M1, CTX-M2, CTX-M8, CTX-M9, and CTX-M25. [11] The purpose of this study was for genotypic and phenotyping detection of ESBL Producing *Escherichia coli* in pregnant women suffering from urinary tract infection (UTI) in Mosul city.

2. Materials and Methods

2.1 Study Area and time

The cross sectional study was conducted at Al Salam Teaching Hospital in Mosul city, from October 2024 to February 2025

2.2 Sample Collection and processing

A total of 65 mid-stream urine (MSU) samples were obtained from the pregnant women in sterile containers. Samples were transported to the microbiological laboratory, which immediately processed them. Microscopic and bacteriological using standard method. The samples were culture on nutrient agar, blood agar, MacConkey agar and eosin methylene blue by a sterilized loop and incubated at 37°C for 24 hours. According to IDSA criteria, cultures with a colony count of greater than 10^5 cfu/ml were deemed positive. [12] Following colony expansion, they were recognized by gram staining and using diagnostic tests (API 20, VITEK 2) and other biochemical test. [13] Samples that contain *Escherichia coli* were subcultured on nutrient agar and kept at 4°C for short-term storage. However, four to five E. coli colonies were inoculated in nutritional broth supplemented with glycerol and kept at -80°C for long-term storage. [14]

2.3 Antibiotic Sensitivity Test (AST)

The Kirby-Bauer disk diffusion method was used to test for antimicrobial susceptibility. In short, a pure culture of E. coli was produced in a nutrient broth to create a 0.5 McFarland suspension, which was then cultured onto Muller-Hinton agar and applied the antibiotics present in Table 1. A total of 10 µg of Ceftriaxone (CRO), 5 µg of Cefixime

(CFM), 100 µg of Nitrofurantoin (F), 10 µg of Ciprofloxacin (CIP), 10 µg of Cefotaxime (CTX), 10 µg of Gentamicin (CN), 10 µg of Amikacin (AK), 30 µg of Amoxillin\Clavulanic acid, 10 µg of Trimethoprim (TMP), 10 µg of Imipenem (IPM), 10 µg of Meropenem (MEM), 10 µg of Doxycycline (DO), 10 µg of Chloramphenicol (C), and 30 µg of cephalexin (CL) were tested. All the antimicrobials used for the study were purchased from Bioanalyse Company, Turkey. The media incubates for 24 hours aerobically at 37 ^{o}C. The findings were classified as susceptible (S), intermediate (I), or resistant (R) based on the inhibitory zone's diameter. [15]

Table 1. The antibiotics and their concentration were used in the study

Antibiotics	symbol	Concentration µg
Imipenem	IPM	10
Meropenem	MEM	10
Amikacin	AK	10
Gentamicin	CN	10
Cephalexin	CL	30
Ceftriaxone	CRO	10
Cefotaxime	CTX	10
Cefixime	CFM	5
Amoxillin\Clavulanic acid	AMC	30
Nitrofurantoin	F	100
Trimethoprim	TM	10
Ciprofloxacin	CIP	10
Doxycycline	DOX	10
Chloramphenicol	C	10

2.4 Phenotypic Determination of ESBL Producer E. coli

Double Disc Synergy Test (DDST)

Muller Hinton agar (MHA) was streaked with the bacterial test inoculum (0.5 McFarland turbidity). An amoxicillin/clavulanic acid (AMC-30 µg) disc was positioned 30 mm from the Cefotaxime (CTX-30 µg), Ceftriaxone (CRO-30 µg), Cefixime (CFM-5 µg) and Ceftazidime (CAZ-30 µg) discs, center to center. After incubation for 24 hr. at 37 ^{o}C. A "phantom zone" when formed, is a sign of a positive of bacterial isolates to ESBL, as shown in Fig. 1.

2.5 Genotypic identification of ESBL by molecular technique (PCR)

DNA Extraction

For DNA extraction, E. coli isolates were cultured on nutrient agar for 24 hours at 37°C. A DNA extraction and purification Kit, was provided by the Geneaid Company (Lot No. FK02612). And include a number of steps: sample preparation (by GT buffer and proteinase K), cell lysis (by GB buffer), DNA binding (by absolute ethanol), washing step (by W1 buffer and wash buffer) and elution (by elution buffer). Isolated DNA was kept at -20°C until used for PCR. [16]

Polymerase chain reaction (PCR) for detection of CTX-M gene

The polymerase chain reaction was done after adding the extracted DNA, forward (CGCTTTGCGATGTGCAG) and reverse (ACCGCGATATCGTTGGT) primers and master mix to a PCR vial and putting it in a thermal cycler to start. [17, 18] Initial denaturation of the amplification was performed at 95°C for 300 seconds, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds, extension at 72°C for 30 seconds, final extension at 72°C for 300 seconds and holding temperature for 180 seconds and the program ended. [19]

Gel Electrophoresis

PCR products were resolved using 1.5% agarose gel when the amplification was finished. This was done by dissolving 1.5 g of the agarose powder in 100 ml of a one-fold Tris-borate-EDTA (TBE) buffered solution within a sterile conical flask and after a number of steps in the preparation of the gel the power source was set and run. After that, a short-wave ultraviolet light transilluminator was used to view the DNA bands, and gene gel bioimaging equipment was used to photograph them. After that, the PCR result was examined. [20]

2.6 Statistical Analysis

The analyses were done using SPSS program version 20. The Chi-square test was employed to evaluate differences in the proportions of bacterial isolates causing UTI and their susceptibility to the antibiotics. Gel page was documented using the CS analyzer system.

3. Result

This study involves 65 urine samples collected from the participants in sterile containers. 53 were culture positive with a colony count of more than 10^5 cfu/ml. 35 (66%) bacterial species of UTI were isolated in which *E. coli* was the predominant bacteria, followed by *Klebsiella spp*. 9(16.9 %), Proteus spp. 4(7.5 %), *Staphylococcus saprophyticus* 3 (5.6 %), *Staphylococcus* aures in 2 (3.7 %) as shown in Table 2.

Table 2. Distribution of bacterial spp. causing UTI among the patients in our study

Bacterial species	Frequency (N)	Percentage (%)
E. coli	35	66
Klebsiella spp.	9	16.9
Proteus spp.	4	7.5
S.saprophyticus	3	5.6
S. aureus	2	3.7

MDR by Antibiotic Sensitivity Test (AST) result

The antibiotics sensitivity result shown in Table 3 is reported as following: 35 (100 %) sensitive to Imipenem (IPM) and Meropenem (MEM), 27 (77.1 %) sensitive to CIP and C, 25 (71.4 %) sensitive to F, 17 (48.5 %) sensitive to CFM, 16 (45.7 %) sensitive to CTX, 13 (37.1 %) sensitive to CRO. 25 (71 %) resistance to DOX, 23 (65.7 %) resistance to AMC, 23 (65.7 %) resistance to TM, 16 (45.7 %) resistance to CL, 15 (42.8 %) resistance to AK and CN.

Table 3. Antibiotic Sensitivity pattern for Uropathogenic *Escherichia coli* isolates

Antibiotics	I		R		S	
	N	%	N	%	N	%
IPM	0	0	0	0	35	100
MEM	0	0	0	0	35	100
AK	8	22.8	15	42.8	12	34.2
CN	6	17.	1	42.	1	4

		1	5	8	4	0
CL	8	22.8	16	45.7	11	31.4
CRO	14	40	8	22.8	13	37.1
CTX	9	25.7	10	28.5	16	45.7
CFM	6	17.1	12	34.2	17	48.5
AMC	6	17.1	23	65.7	6	17.1
F	1	2.8	9	25.7	25	71.4
TM	2	5.7	22	62.8	11	31.4
CIP	2	5.7	6	17.1	27	77.1
DOX	6	17.1	25	71.4	4	11.4
C	5	14.2	3	8.5	27	77.1

Multidrug resistance which includes the resistance to two or more antimicrobials. According to antibiotic sensitivity test results, the number of Uropathogenic *Escherichia coli* isolates that were MDR in our study was 24 (68.5%)

among all 35 isolated E.coli. the number of MDR isolates that were being positive to ESBL by the phenotyping method were 14 (58.3 %) and 10 (41.6 %) were negative.

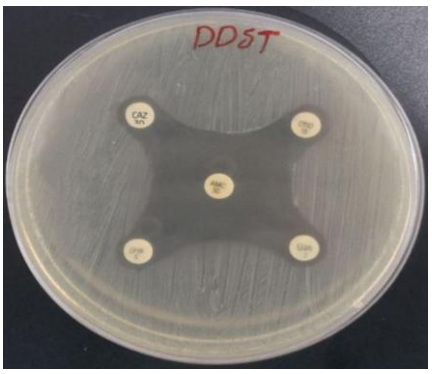

Fig 1. Phenotyping detection of ESBL Uropathogenic E.coli

Genotyping determination of ESBL by PCR

The genotyping method done by PCR which performed under standard conditions using the previously mentioned primers. The CTX-M resistance genes were identified as shown in Fig. 2. From 14 isolates of ESBL they were 8 (57.14 %) positive for CTX-M gene and 6 (42.85 %) were negative for the CTX-M gene.

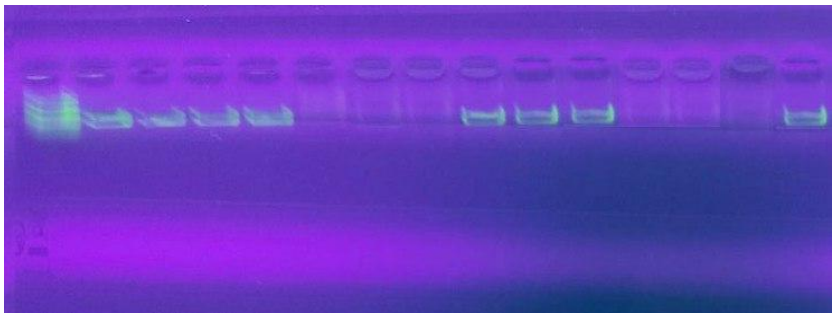

Fig. 2. Agarose gel electrophoresis of CTX-M gene which bands at 175 bp

4. Discussion

Women frequently get UTIs, and the frequency increases significantly during pregnancy. This study involved 65 pregnant women; the age range was from 18 to 43. From 65 urine samples, 53 were culture positive with a colony count of more than 10^5 cfu/ml. 35 (66%) bacterial species of UTI were isolated, in which *E. coli* was the predominant bacteria followed, by *Klebsiella spp*. 9 (16.9%), Proteus spp. 4 (7.5%), *Staphylococcus saprophyticus* 3 (5.6%), *Staphylococcus* aures in 2 (3.7%). The study conducted in Iran by Adekunle C. et al. revealed 29 (14.5%) of the 200 urine samples tested positive for culture. The most common bacteria found in the five UTI bacterial species that were isolated were E. coli (n = 10), followed by Klebsiella spp. in 9 cases (31%), S. saprophyticus in 5 instances (17.2%), S. aureus in 3 patients (10.3%), and *Enterobacter aerogenes* in 2 patients (6.9%). [21] The research conducted in Iraq by Miaad K. Alkhudhairy et al. reported Of the 574 pregnant women who were screened, 386 (67.2%) showed no growth, and 188 (32.8%) had bacteriuria. Of the culture-positive cases, E. coli was the most often isolated organism in 76 patients (40.4%), followed by Klebsiella spp. in 48 (25.5%), Staphylococcus spp. in 39 (20.8%), Proteus spp. in 22 (11.7%), and Pseudomonas spp. in 3 (1.6%).[22] According to Rania Al-Groom et al., 412 urine samples were

analyzed. Urinary tract infections were discovered in 297 females. E. coli was the most common infecting bacteria, with frequencies of 198 (48.1%), 132 (32.0%), 51 (12.4%), 15 (3.6%), 10 (2.4%), and 6 (1.5%), respectively, followed by Staphylococcus saprophyticus, Klebsiella sp., Serratia sp., Enterococci sp., and Proteus spp. [23] Ragab Riham N. et al. reported 79% of the 100 participant samples were culture positive. There were five distinct bacteria found. E. coli is thought to be the most common (n=41, 51.89%). Klebsiella, Proteus, Staphylococcus, and Pseudomonas are among the other identified bacterial strains [24]. Our study reported the antibiotics sensitivity result of 35 (100%) sensitive to Imipenem (IPM) and Meropenem (MEM), 27 (77.1%) sensitive to CIP and C, 25 (71.4%) sensitive to F, 17 (48.5%) sensitive to CFM, 16 (45.7%) sensitive to CTX, 13 (37.1%) sensitive to CRO. 25 (71%) resistance to DOX, 23 (65.7%) resistance to AMC, 22 (62.8%) resistance to TM, 16 (45.7%) resistance to CL, 15 (42.8%) resistance to AK and CN. Adekunle C et al. reported that E. coli; the most common cause of UTIs, had a high percentage of ampicillin resistance and low percentages of ciprofloxacin and penicillin resistance. Every E. coli isolate has levofloxacin sensitivity and meropenem resistance. [21] Kolsoum RK et al. demonstrated that Escherichia coli were resistant to AN (42.85%), GM (28.57%), AM (35.71%), AMC (35.71%), CZ (35.71%), and AZM (50%) antibiotics. Gentamicin had the least amount of antibiotic resistance, whereas ampicillin had the highest sensitivity. [25] The AST by 19 antimicrobial agents was reported by Miaad K. Alkhudhairy et al. With the exception of 16 (88.9%), 11 (61.1%), and 7 (38.9%) isolates that showed resistance to Amoxicillin/Clavulanic acid and Ceftazidime/Clavulanic acid, respectively, all E. coli isolates were 100% resistant to the Cephems, Monobactams, and Penicillins classes under investigation. However, isolates showed varied levels of resistance to trimethoprim 2 (11.1%), gentamicin 4 (22.2%), and tetracycline 5 (27.8%). Resistance to Levofloxacin and Fosfomycin was not reported. Nitrofurantoin, penicillin 18 (31.0%), ampicillin 15 (25.9%), and amoxicillin 9 (15.5%) all cause E. coli to exhibit sensitivity 1 (5.6%). [22] Rania Al-Groom et al. reported the resistance of E. coli to Nalidixic-acid antibiotic was (78.8%), AK (45.5%), GM (28.3%), C (35.5%). CIP (38.9%), TMP (54.5%) and the sensitive of E. coli to Gentamicin was (64.1%), C (62.1%) AK (54.5%), CIP (50.5%). [23]Ragab Riham N. et al. observed that resistance of E. coli to Ampicillin antibiotic was (46.3%), F (4.8%), GM (39%), CRO (78.1%). CTX (69.9%) and the sensitivity of E. coli to Ampicillin antibiotic was (53.6%), F (95.1%), GM (30.9%), CRO (21.9%). CTX (39%) [24]. Multidrug resistance, which includes the resistance to two or more antimicrobials. According to phenotyping results by AST, the number of Uropathogenic *Escherichia coli* isolates that are MDR in our study was 24 (68.5%) among all isolated E. coli. According to Adekunle C et al. they observed that multiple drug resistances (MDR) were present in all the isolated (100%) E. coli [21]. In Our study, the ESBL production was evaluated in all MDR E. coli isolates (24) by the phenotyping method using the double disc synergy test. The number of isolates that were positive for ESBL was 14 (58.3%) and 10 (41.6%) were negative. Miaad K. Alkhudhairy et al. found that out of 76 test isolates, 18 (23.7%) were ESBL producers and approximately 58 (76.3%) isolates gave negative results for ESBL production. The Frequency distribution of non ESBL producing E. coli isolates in this study was found to be highest. [22] Ragab Riham N. et al. observed that 22 (53.65%) of the isolates were gave a positive result for ESBL by the double disk diffusion method from a total (41) isolated E.coli. [24] Rania Al-Groom et al. reported that about 25 (12.6%) were ESBL positive among the 198 isolated E. coli [23]. In our study the genotyping method was done by PCR, which was performed under standard conditions using the previously mentioned primers. The CTX-M resistance genes were identified as shown in figure 2. From 14 isolates of ESBL they were 8 (57.14%) positive for the CTX-M gene and 6 (42.85%) were negative for the CTX-M gene. Adekunle C et al. reported (3) isolates of E. coli, were positive for the CTX-M genes. [21] Kolsoum RK et al. reported (11) of isolates were positive for CTX-M among 14 ESBL isolates E coli. [25] Ragab Riham N. et al. observed the CTX-M gene was found in 12 of 41 E. coli that produce ESBLs. [24] Rania Al-Groom et al. reported that among all ESBL producers E coli 25 (12.6%) only 20 (10.1%) of the E. coli isolates were having the CTX-M gene [23].

5. Conclusion

According to our research, E. coli is the most frequent cause of UTIs and the production of ESBL increases resistance to common antibiotics and makes treatment plans more difficult. Due to their low cost and ease of use, phenotypic approaches for ESBL production testing would be helpful in reducing the rise of antibiotic resistance. Combining genotypic and phenotypic testing provides important information for guiding treatment plans and minimizing possible repercussions in pregnant patients with UTIs.

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