Emergence of Extended Spectrum β-lactamases and Metallo-β-Lactamase Genes producing *Escherichia coli* among urinary tract infected women
Running title/ Emergence of β-lactamases Genes in *Escherichia coli* .

Ghofran Khudhair Ismail
Department of Biology College of Sciences, The University of Diyala, Diyala- Iraq
scibioms222304@uodiyala.edu.iq (corresponding author)
Lina Abdulameer S. Alsaadi
Department of Biology College of Sciences, The University of Diyala, Diyala- Iraq
linaabdulameer@uodiyala.edu.iq

Received: 13 December 2024 \ Revised: 2 January 2025 \ Accepted: 10 January 2025

ABSTRACT

Background: In recent times, urinary tract infection (UTI) is one of the most widely recognized bacterial diseases all over the planet.The emergence of multidrug-resistant *Escherichia coli* is a major public health threat worldwide.**Objectives**: This study aims to determine the frequency of some virulence factor genes (ESBLs and MBL) and Antibiogram profile from the uropathogenic *Escherichia coli* isolated from Iraqi Women in Diyala. **Materials and Methods**: A total 250 urine specimens from women suffering from UTI symptoms. Diagnoses were then confirmed using biochemical tests , VITEK-2 system and molecular screening of β-lactamases genes were done by PCR. **Results:** Conducted study showed the most participants have *E. coli* growth 53 (21. Susceptibility testing showed that isolates had varying levels of resistance to different antibiotics, highest resistance to ampicillin (94.3%), amoxicillin-clavulanate (92.5%), cefotaxim(90.6%), Piperacillin -tazobactam (79.2%), Piperacillin (73.6%), Ceftriaxone (75.5%), Resistance patterns for the isolates was also calculated and 22(41.5%) isolates were MDR, 29 (54.7%) were XDR and the remaining 2(3.8%) were MDS . Results of present study showed the positivity of *E. coli* isolates produce enzymes were (22.6%) MBL and (17.%) ESBL. The results achieved by using multiplex PCR revealed that 19(95%) isolates have *TEM* gene, while 7 (35%) isolates carried bla_{OXA} gene. None of the 20 *E .coli* isolates had *SHV* gene. Results by monoplex PCR detect MBLs genes show that 4 (20%) of isolates have *VIM* gene, while 2 (10%) isolates carried *NDM* gene. **Conclusion:** *E. coli* isolates showed high resistance rates to most of the antibiotics tested. ESBL- MBLs producing *E. coli* showed high prevalence.

Keywords- E.coli , UTIs, MBLs genes, ESBLs genes, PCR, Antibiotic Resistance, XDR

Introduction

Urinary tract infections (UTIs) are among one of the most prevalent bacterial infections contracted in both community and hospital settings. In individuals absent of anatomical or functional defects, urinary tract infections are typically self-limiting but have a tendency to recur[1]. Atimely diagnosis of UTIs is essential to alleviate symptoms and prevent complications. The present urinary tract infection screening using urine test strip analysis and microscopic examination exhibits inadequate diagnosis accuracy [2]. *Escherichia* coli is the primary bacterial agent responsible for urinary tract infections, constituting the majority of cases, while other bacterial species contribute to a lesser degree[3]. Multi-drug resistance (MDR) has markedly increased in community-acquired uropathogens

causing urinary tract infections (UTIs), predominantly *Escherichia coli*. Uropathogenic *E. coli* accounts for 80% of uncomplicated community-acquired urinary tract infections, particularly in pre-menopausal women [4]. Polycystic ovarian syndrome (PCOS) is a prevalent endocrine condition impacting 4–20% of women of reproductive age globally, contingent upon the diagnostic criteria employed[5]. The aetiology of PCOS remains unclear; however, factors such as obesity, ovarian follicle maturation, insulin sensitivity, and chronic systemic inflammation have been suggested as potential contributors[6]. Women with PCOS demonstrate heightened susceptibility to infections compared to those without the condition[7]. Metallo-beta-lactamase (MBL) generating Gram-negative bacteria are increasingly reported from many nations and have become a prevalent and clinically relevant mechanism of carbapenem resistance[8]. Pathogens that produce metallo-β-lactamase can hydrolyse all categories of β-lactams, including penicillins, cephalosporins, carbapenems, and cephamycins, with the exception of monobactams such as aztreonam [9].New Delhi Metallo-beta-lactamases (NDM) were found to be widespread in India among patients travelling to various nations[10]. Verona imipenemase (VIM) metallo-beta-lactamases were initially identified in Japan [11]. Gram-negative bacteria, such as ESBL-resistant forms of *E. coli*, are pivotal in the worldwide dissemination of drug-resistant illnesses. ESBL strains render all beta-lactam antibiotics ineffective, with the exception of cephamycins and carbapenems [12,13]. The possibility of resistance to many antibiotic classes poses a considerable obstacle in managing infections induced by ESBL strains [14,15]. Extended-spectrum beta-lactamase (ESβL) is produced by several bacterial genes, with a notable increase in prevalence following the extensive use of the beta-lactam class of antibiotics in both human and veterinary medicine to combat numerous bacterial diseases[16]. [17] shown that the indiscriminate use of antibiotics resulted in the emergence of numerous bacterial strains resistant to beta-lactam agents. The prevalence of β-lactam resistance in Gram-negative bacteria has emerged as a significant global clinical issue, thereby constraining therapeutic options and diminishing the likelihood of identifying suitable antibiotic treatments for newer beta-lactam-resistant Enterobacteriaceae isolates [18]. Within the region of Diyala, Iraq, there is a limited amount of information available concerning the distribution of MBL and ESBL-producing isolates as well as the associated clonal infections. In this particular experiment, the purpose was to determine the extent to which MBL and ESBL genes were present in *E. coli* that had been isolated from clinical specimens obtained from a number of hospitals located in Diyala.

Materials and Methods

Collection, isolation and identification of clinical specimens

This study included 250 Iraqi women samples, were collected from women ages ranging 18 to 65 years old, Patients were selected from the Infertility, Al Batool Educational Hospital, Public Health Laboratoried Baquba City, and Baqubah General Hospital and have been investigated in this study for the collection of urine samples from patients. This study extended from December 2023 to February 2024. Two groups (PCOS with UTI and UTI without PCOS). Urine was collected midstream in a disposable sterile screw urine cap, labeled with the patient's ID, and kept in a cooling icebox until it was transported to the laboratory for diagnosis. 50 µl of each sample were cultured on Blood agar and MacConkey agar (Himedia/India) and placed in an incubator at 37 oC for 24 hours. In addition, morphological (gram staining) and biochemical tests, and it was confirmed that it was *E. coli* bacteria using the Vitek 2 compact system.

Antibiotic Susceptibility Testing

The Kirby-Bauer method was used to evaluate the isolates' sensitivity to a few chosen antibiotics in accordance with [19]. Until the turbidity of MacFarland's standard (1.5 x 108 cells/mL) was reached, three to five colonies grown on nutrient agar were transferred to tubes containing normal saline. The isolate was interpreted as either sensitive, intermediate, or resistant to particular antibiotics including of aminoglycosides (Amikacin (AK), Gentamicin (CN) , penicillins (Ampicillin AMP), Piperacillin(PRL) , carbapenems (Imipenem (IPM) , Meropenem (MEM) , tetracyclines (Tetracycline (TE) , fluoroquinolones (Ciprofloxacin (CIP), Levofloxacin (LEV), cephalosporins (Cefriaxone (CRO) , Cefoxitins (FOX) ,Ceftazidime (CAZ) , Cefotaxime (CTX), (Aztreionam (ATM), Amoxicillin-Calvulanic acid(AMC) , Piperacillin-Tazobactam (TPZ), (Trimethoprim (TMP) by comparing the inhibition zone with the standards set by CLSI[20].

phenotypic Detection of ESBL β-Lactamase Enzymes

The Double Disc Synergy Method for ESBL was implemented as follows [21]; A bacterial suspension was prepared by combining bacterial colonies with 15 mL of normal saline, achieving turbidity that corresponded to the

MacFarland standard solution. A sterile cotton swab was immersed in the bacterial suspension and subsequently spread across 8.5 cm diameter petri plates containing Muller-Hinton agar to achieve uniform growth. The dishes were allowed to dry and absorb the grown bacteria for 5 minutes. An amoxicillin/clavulanic acid antibiotic disc (30mg) was positioned in the center of the petri dish, while the antibiotic discs (Cefotaxime, Piperacillin, Cefixime) were arranged around it, each separated by a distance of 2cm. The zone of inhibition is observed with the fusion of both discs, Cefotaxime, Piperacillin, and Cefixime, towards the central disc of amoxicillin/clavulanic acid, indicating a positive outcome for the production of ESBL.

Phenotypic detection of MBL

Imipenem-EDTA combined disc test (CDST) was utilised for the purpose of identifying MBL-producing isolates in accordance with [22].

Genotyping Detection

Extraction of DNA

Among fifty three *E. coli* isolates, only 20 XDR isolates have been used for genotyping study. The genomic DNA was isolated from bacterial growth following the ZR fungal/yeast/bacterial DNA MiniPrep protocol according to manufacturers' instruction (ZYMO) from the USA.

PCR Amplification

The PCR amplification protocol for the genetic detection of local isolates of *Escherichia coli* All 20 XDR isolates were subjected to normal conventional PCR screening using particular primers as indicated in Table(1).

Table (1): Primers Utilised for the Detection of ESBL and MBL Genes

Primer		Oligo sequence (5'-3')	Product size bp	Annealing temp°C	Re.
SHV	***ESBLs genes***	F: CTT TAT CGC CCC TCA CTC AA R : AGG TGC TCA TCA TGG GAA AG	273	62 C° 90 sec	23
TEM		F: CGC CGC ATA CAC TAT TCT CAG AAT GA R: ACG CTC ACC GGC TCC AGA TTT AT	445	62 C° 90 sec	23
blaOXA		F:ACACAATACATATCAACTTCGC R: AGTGTGTTTAGAATGGTGATC	814	62 C° 90 sec	23
NDM	***MBLs genes***	F: TGGCAGCACACTTCCTATC R: AGATTGCCGAGCGACTTG	488	58 C° 60 sec	24
VIM		F: AGTGGTGAGTATCCGACAG R: TCAATCTCCGCGAGAAG	212	52 C° 60 sec	24

Final volume for PCR mixture was 25 µl (12.5 of Master Mix 2x, 5 µl template DNA, 1 µl primers for each forward and reverse primer, finally, 5.5 µl nuclease free water) in uniplex PCR Eppendorf tubes but amount changed in multiplex PCR, mixed briefly via vortex then been placed in thermocycler polymerase chain reaction. The program used for each multiplex PCR mixture was illustrated in the tables (2).

Table (2): Amplification program of primer.

Amplified gene	Initial denaturation	No. of cycle	Denaturation	Annealing	Elongation	Final extension
SHV	**95°C/ 5min**	**35**	**94°C/ 45sec**	**62°C/90 sec**	**72°C/30 sec**	**72°C/7min**
TEM	**95°C/ 5min**	**35**	**94°C/ 45sec**	**62°C/90sec**	**72°C/45sec**	**72°C/7min**
blaOXA	**95°C/ 5min**	**35**	**94°C/ 45sec**	**62°C/90sec**	**72°C/60sec**	**72°C/7min**
NDM	**95°C/ 5min**	**35**	**94°C/ 45sec**	**58°C/60sec**	**72°C/45sec**	**72°C/7min**
VIM	**95°C/ 5min**	**35**	**94°C/ 45sec**	**52°C/60sec**	**72°C/45sec**	**72°C/7min**

Statistical analysis

Study groups	Total positive	*E.coli*	%	P value
UTI infected non- pregnant woman without PCOS	86	17	19.76%	P<0.001***
UTI infected pregnant woman without PCOS	91	21	23.07%	P<0.001***
UTI infected woman with PCOS	46	15	32.60%	P<0.001***
Total	223	53	23.76%	P<0.001***
P value	P>0.05			

Nominal and ordinal data were described as frequency and percentages. The differences among percentages were calculated by Chi-square test at significant level P≤0.05. SPSS version 22.0 was depended for analysis current data.

Ethical approval

The study was conducted with the patients' verbal and analytical consent prior to subject recruitment. The research approval number 4689, dated September 23, 2023, indicates that the Diyala University, College of Sciences, Department of Biology evaluated and approved the study protocol, subject information, and consent form.

Results

Results of present research showed the positivity of *E. coli* was highest in UTI infected woman with PCOS (32.60%), following UTI infected pregnant woman without PCOS (23.07%), and then infected UTI non- pregnant woman without PCOS (19.76%) with significant different (p<0.05). In contrast, the differences among positivity of *E. coli* within study groups was no significant (p>0.05) (table 3).

Table (3): distribution of study groups according to bacterial growth

Results of conducted study showed the most participants have *E. coli* (21%), another bacteria (68%) and no bacterial growth (11%) with significant different (p<0.05) (table4).

Table (4): frequency and percentages of bacterial growth

Bacteria	N	%	P value
E. coli	53	21%	P<0.01**
Another bacteria	170	68%	
No growth	27	11%	
Total	250	100%	

Antibiogram pattern of *Escherichia coli*

Fifty-three E. coli isolates were evaluated for 16 antibiotic discs from various classes of antibiotics using the disc diffusion method, revealing that the isolates exhibited differing degrees of resistance to the antibiotics. Results of current study showed the *E. coli* isolates scored highest resistance toAM(94.3%), AMC (92.5%),CTX (90.6%),TPZ (79.2%), PRL(73.6%), CRO (75.5%), and CAZ (92.5%). Based on sensitive, current study showed the IMP and MEM scored highest efficiency (69.81 %) and (84.9%) against *E. coli* isolates. The differences among antibiotic resistance against *E. coli* isolates were significant (p<0.05) Figure (1).

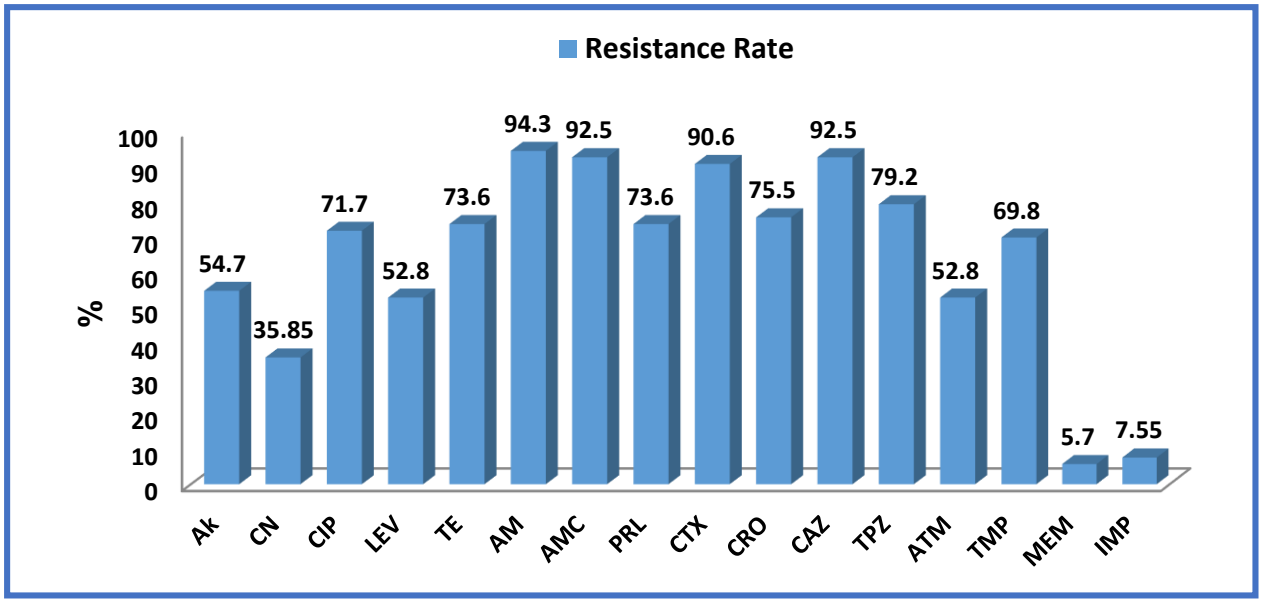


Figure (1): Antibiotic Susceptibility profile of *E. coli* . AK: amikacin; CN: Gentamicin; CIP: Ciprofoloxacin; LVE: Levofloxacin; TE : Tetracyclin ; AMP Ampicillin: AMC Amoxicillin-Calvulanic acid: PRL: Piperacillin; CTX: Cefotaxime; CRO: Ceftriaxone; CAZ: ceftazidime; TZP: Piperacillin -tazobactam; ATM Aztreionam; TMP Trimethoprim: MEM: meropenem; IPM: Imipenem.

In this investigation, antibiotic susceptibility testing of *E. coli* isolates showed that 22 (41.5%), 29(54.7%) and 2 (3.8%) of the isolates multi drug resistant MDR, extensively drug resistant (XDR) and multi drug sensitive (MDS), respectively (figure 2).

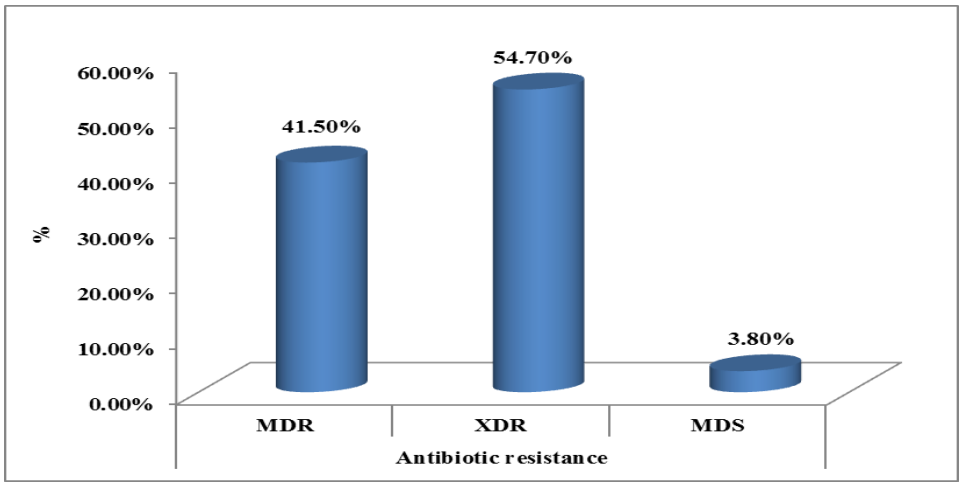


Figure (2): Multidrug resistance pattern of of *Escherichia coli*

Phenotypic Detection of β-Lactamase production.

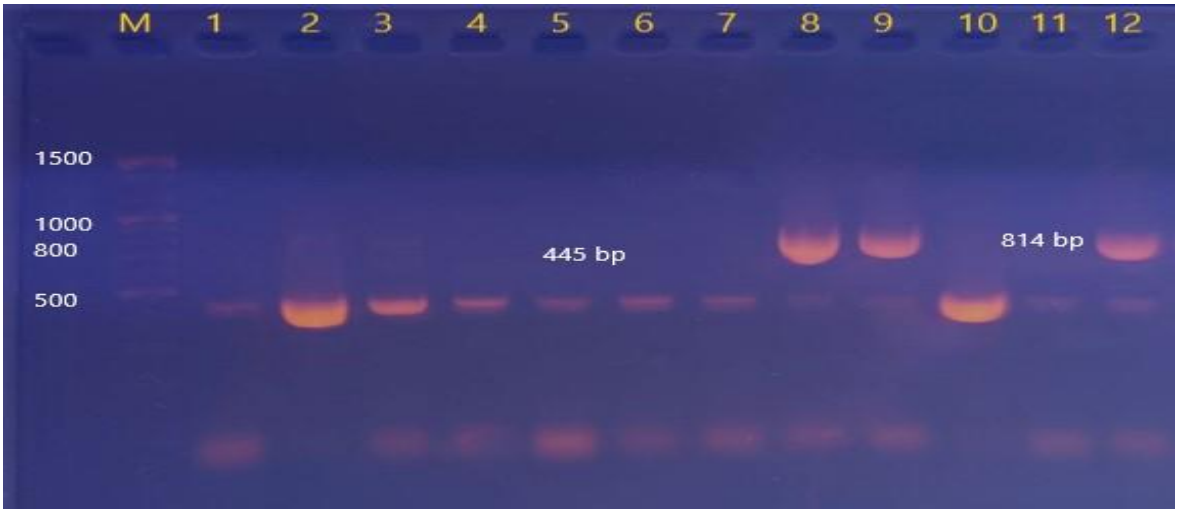
Results of present study showed the positivity of *E. coli* isolates produce enzymes were as following; MBL (22.6%), ESBL(17.0%), and AMPC (26.4%) compared to negativity (77.4%,83.0 %,73.6 %) respectively, with significant differences ($p<0.05$) (table 5).

Table (5): frequency and percentages of β-Lactamase enzymes of *E. coli*

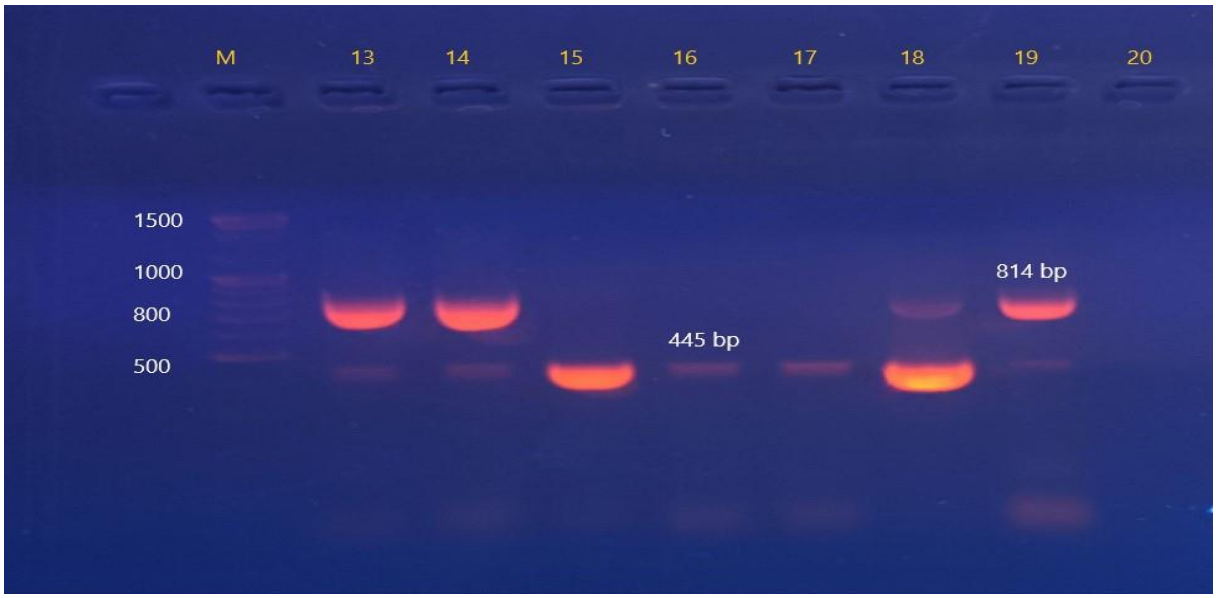
	Value	Count	Percent	P value
MBL	Negative	41	77.4%	P<0.001***
	Positive	12	22.6%	
ESBL	Negative	44	83.0%	P<0.001***
	Positive	9	17.0%	
AMPC	Negative	39	73.6%	P<0.001***
	Positive	14	26.4%	

Molecular Characterization of ESβL genes *SHV, TEM* and *bla*$_{OXA}$

This study was carried out in order to detect ESBL genes in 20 extensively drug resistance *E .coli* isolates. ESBLs genes *SHV*, *TEM* and *bla*$_{OXA}$ was screened by Multiplex PCR technique. The results of gel electrophoresis for PCR product by using specific primers for this genes showed that 19(95%) isolates were positive for *TEM* gene as shown in Figure (3,4). Results revealed that *TEM* gene was the most spread gene.While The results of current study showed that 7(35%) isolates were positive for *bla*$_{OXA}$ gene as shown in Figure (3,4). Noteworthy, none of the 20 extensively drug resistance *E .coli* isolates had *SHV* ESBL gene.



Figure(3): Agarose gel electrophoresis of *E .coli* (1.5% agarose,70v/cm2 for 90 min) for ***bla***$_{OXA}$ gene (814 bp amplicon) ; ***TEM*** gene (445 bp amplicon) and ***SHV*** gene (273 bp amplicon) respectively. Lane M represent M100bp DNA Ladder, lanes 1-7 and 10-11 (- + -) , lanes 8-9 and 12 (+ +-).



Figure(4): Agarose gel electrophoresis of *E .coli* (1.5% agarose,70v/cm2 for 90 min) for ***bla$_{OXA}$*** gene (814 bp amplicon) ; ***TEM*** gene (445 bp amplicon) and ***SHV*** gene (273 bp amplicon) respectively. Lane M represent M100bp DNA Ladder, lanes 15,16 and 17 (- + -) , lanes13-14 and 18-19 (+ + -), lane 20 (- - -)

Molecular Characterization of MBL genes *VIM* and *NDM*

Among extensively drug resistance *E .coli* twenty (20) isolates the results achieved by using PCR revealed that 4 (20%) of isolates have *VIM* gene as in Figure (5), while 2 (10%) isolates carried *NDM* gene Figure (6,7).

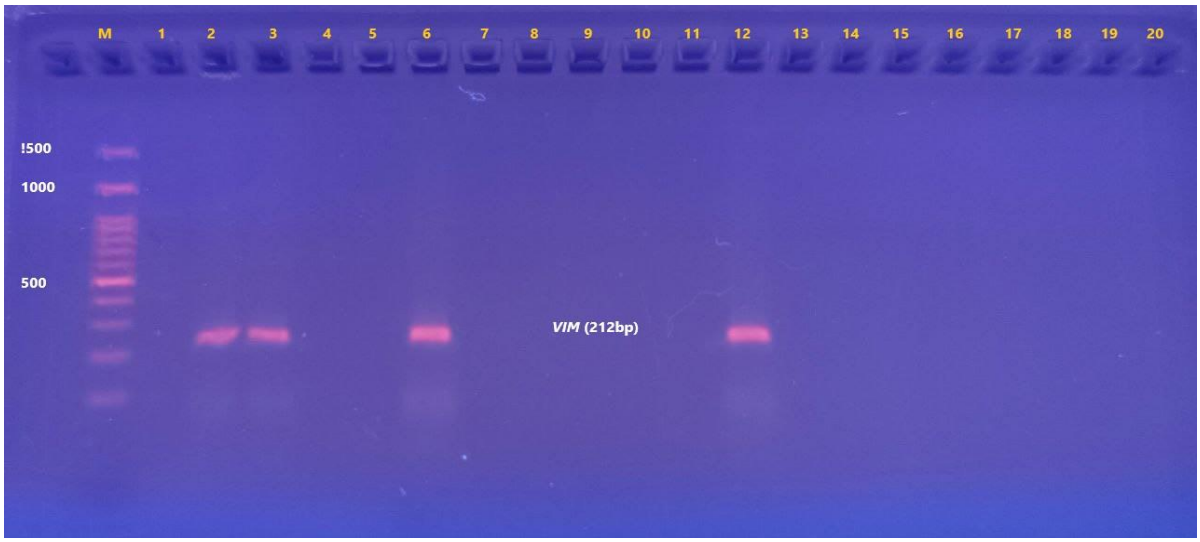


Figure (5): Agarose gel electrophoresis of *E. coli* (1.5% agarose,70v/cm^2 for 90 min) for ***VIM*** gene (212 bp amplicon). lane M represent M100bp DNA Ladder, lanes 2,3,6 and 12 Positive for ***VIM*** gene .

Figure (6): Agarose gel electrophoresis of *E. coli* (1.5% agarose,70v/cm^2 for 90 min) for *NDM* gene (488 bp amplicon). lane M represent M100bp DNA Ladder, lanes 9 (Positive for *NDM* gene) , 1-8 and 10 (Negative for *NDM* gene).

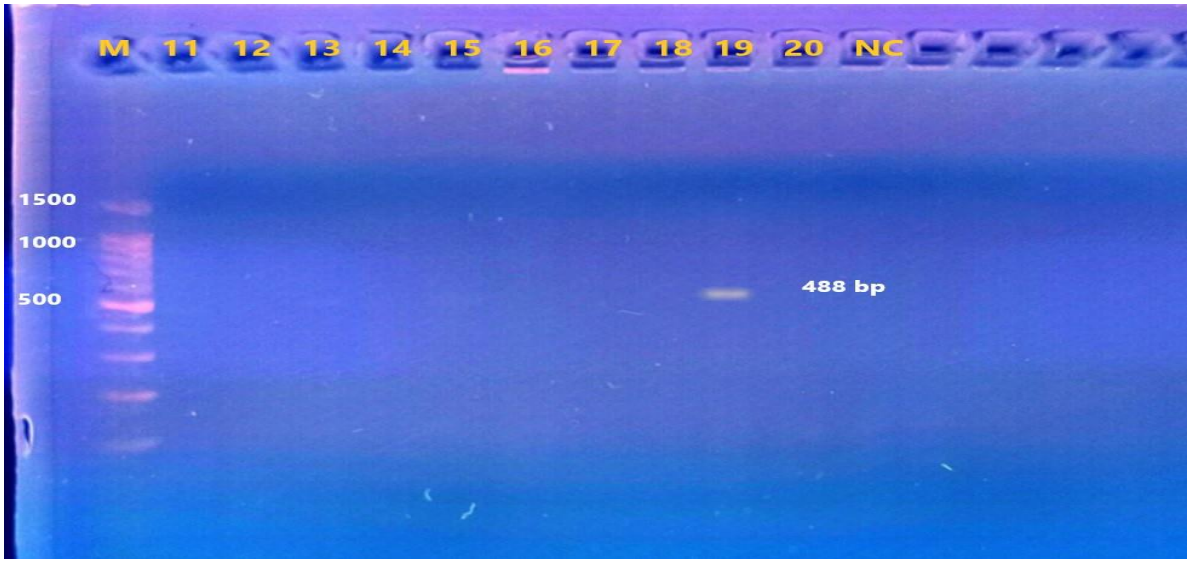


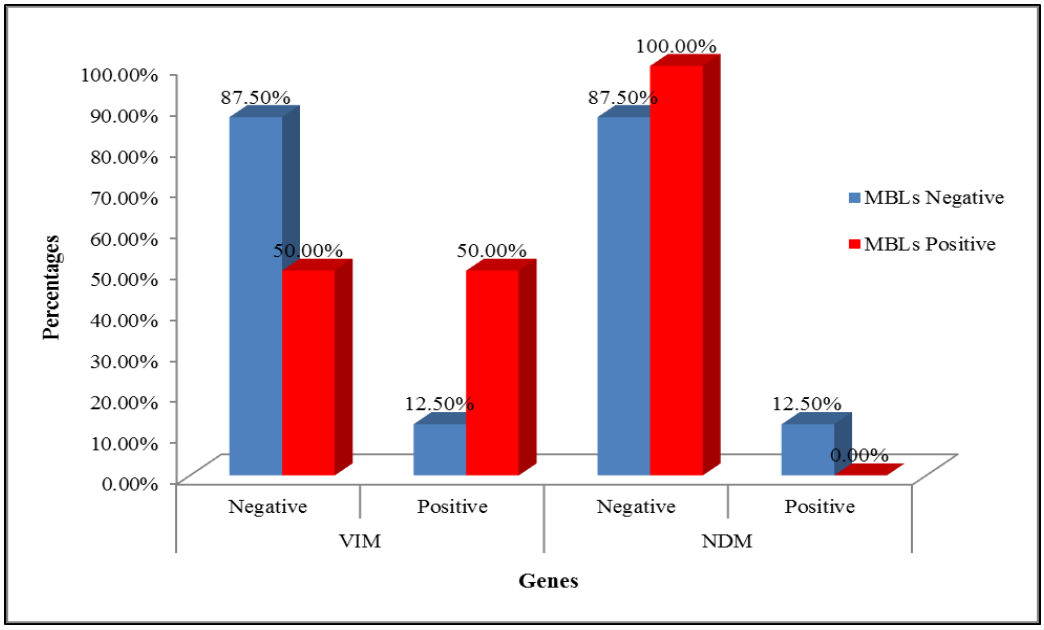
Figure (7): Agarose gel electrophoresis of *E. coli* (1.5% agarose,70v/cm^2 for 90 min) for *NDM* gene (488 bp amplicon). lane M represent M100bp DNA Ladder, lanes 19 (Positive for *NDM* gene) , 11-18 and 20 (Negative for *NDM* gene).

Comparative of VIM, NDM genes with phenotyping detection of MBLs

Findings of present study showed there is no significant differences ($p>0.05$) between VIM, NDM genes with phenotyping detection of MBLs of *E. coli* (table 6).

Table (6): comparative of VIM, NDM genes with phenotyping detection of MBLs

MBLs genes			MBLs		Total	P value
			negative	positive		
VIM	negative	N	14	2	16	P<0.01**
		%	87.5%	50.0%	80.0%	
	positive	N	2	2	4	
		%	12.5%	50.0%	20.0%	
NDM	negative	N	14	4	18	P>0.05
		%	87.5%	100.0%	90%	
	positive	N	2	0	**2**	
		%	12.5%	0.0%	**10%**	



Figure(8): Comparative of SHV, TEM, BlaOXA genes with ESBL

Comparative of VIM, NDM genes with phenotyping detection of ESBLs

Outcomes of current study showed there is no significant differences (p>0.05) between *SHV*, *TEM* and Bla_{OXA} genes with ESBL of *E. coli* . In contrast, present study showed ESBL scored highest positivity (100.0%) in *E. coli* isolate than not has bla_{OXA} gene compared to isolate has gene (0.0%) with significant differences (P<0.05) (table 7).

Table (7): comparative of *SHV*, *TEM*, *BlaOXA* genes with ESBL

ESBL genes			ESBL		Total	P value
			negative	positive		
SHV	negative	N	14	6	20	1.00
		%	100.0%	100.0%	100%	
TEM	negative	N	1	0	1	P>0.05
		%	7.1%	0.0%	5%	
	positive	N	13	6	19	
		%	92.9%	100.0%	95%	
blaOXA	negative	N	7	6	13	P<0.05*
		%	50.0%	100.0%	65%	
	positive	N	7	0	7	
		%	50.0%	0.0%	35%	

Discussion

Urinary tract infections are a primary source of morbidity and significant healthcare costs across all age groups. Young, sexually active women are more susceptible, however additional factors obviously play a role. At-risk populations comprise the elderly and individuals utilising genitourinary devices or catheters. Urinary tract infection (UTI) constitutes a major public health issue impacting millions annually. The study results indicated that 53 subjects (21%) experienced growth attributed to *E. coli*. This aligns with the results of an Iraqi investigation, which indicated that 20% of Gram-negative isolates were *E. coli* [25]. E. coli are the primary bacteria responsible for urinary tract infections, as indicated by the current study, which corroborates several studies showing a high incidence of *E. coli* in Duhok city and Babylon, Iraq [26,27]. The results indicated that E. coli was detected in urinary tract infections (UTIs) among pregnant women without polycystic ovary syndrome (PCOS) at a rate of 23.07%, compared to 19.76% in non-pregnant women without PCOS, with a statistically significant difference ($p<0.05$). The findings of the current study align with those of Simba et al. (2022), which indicated a prevalence of 23.5% in Kenya [28]. Another study in Baghdad, Iraq indicated that E. coli is the predominant uropathogen in individuals with PCOS, accounting for 42.8% of UTIs, compared to 44.4% in those without PCOS [29].

A study by Tahir (2022) found a substantial ($p < 0.05$) incidence of urinary tract infections (UTIs) among pregnant women compared to non-pregnant women, with the highest percentage of patients in the age bracket of 36-45 years. In relation to identified gram-negative bacteria in pregnant women, *E. coli* accounted for 40.8% [30]. The results of the current research indicated that the prevalence of E. coli was highest in women with urinary tract infections and polycystic ovary syndrome (PCOS) at 32.60%. These findings align with those of [31] who reported that *E. coli* isolates constituted 22 (36.67%) of the most prevalent isolates in women with PCOS.

The results of the current study indicated that the *E. coli* isolates exhibited the highest resistance to the penicillin group, specifically ampicillin (94.3%). This percentage aligns with a prior study conducted in Iraq [32]. Additionally, 92.5% of the isolates demonstrated resistance to Amoxicillin+Clavulanic acid, consistent with findings from another study [33]. The

increased resistance may be attributed to the production of beta-lactamase enzymes by the majority of *E. coli* isolates. The resistance percentages differed within the cephalosporin group, with ceftazidime at 92.5% and cefotaxime at 90.6%. The findings of the current study align with those reported by [34]. The results demonstrated differing degrees of resistance across the isolates. Resistance rates were notably high for ampicillin (92.6%), amoxicillin (93.6%), ceftriaxone (88.9%), and ciprofloxacin (82.4%), while lower resistance rates were recorded for meropenem (8.2%) and imipenem (12.4%). The current study concurs with [35] regarding Al-Diwaniya, Iraq, indicating that E. coli had a strong resistance profile to ampicillin (97.9%) and ceftriaxone (81.3%), while demonstrating significant susceptibility to meropenem (97.9%) and amikacin (97.6%).

In this study were showed the IMP and MEM scored highest efficiency(69.81 %) and (84.9%) against *E. coli* isolates , the result agree with study by [36] showed the highest levels of sensitivity were found for Meropenem (97.3%) and Imipenem (95.6%). Results showed 41.5% *E.coli* strains showed multidrug-resistant (MDR), 54.7% extremely-resistant (XDR) and 3.8% multidrug-sensitive (MDS) ,but not found pandrug-resistant(PDR). The current study's findings corresponds with what was found by [37] that found the total multi-drug resistant (MDR) strains that formed (46.4%) and the extensively-drug resistant (XDR) strains (25.4%). While current study disagree with by [38] that show 92.7% of UPEC strains showed multidrug-resistant (MDR) , 6.7% extremely-resistant (XDR) and 0.6% pandrug-resistant PDR. Also disagree with study [39] that found 37 (88.09%) were found to be MDR while 5 isolates (11.90%) were XDR. Results of present study showed the positivity of *E. coli* isolates produce enzymes were as following; MBL (22.6%), ESBL(17.0%), this results agree with study by [40] that Results 15% of isolates tested positive for ESBL enzymes. Another study were found ESBL was produced by 37% of the isolates [41]. In study by [42] The prevalence of ESBLs in the *E. coli* isolates was 41%.

The results achieved by using PCR revealed that 4 (20%) of isolates have *VIM* gene as while 2 (10%) isolates carried *NDM* gene. This study agrees with [43] results for the *VIM* gene showed 19 (23.75%) positive isolates of *E. coli*, this result close with study by [44] that found the most prevalent MBL gene in this study is the ^{bla}*VIM* gene (18.8%) which mediate MBL production in Gram negative bacteria . The results of study by [45] that found of resistance genes have appeared in two isolates that have the resistance gene *blaVIM* (5%) and one isolate has *blaNDM* genes (2.5%).

ESBLs genes *SHV*, *TEM* and bla_{OXA} showed that 19(95%) isolates were positive for *TEM*, 7(35%) isolates were positive for bla_{OXA} gene Noteworthy, none of the 20 extensively drug resistance *E .coli* isolates had *SHV* ESBL gene, it is agree with study by[18] showed that *blaTEM* gene was 93%,while *blaOXA* and *blaSHV* were the least common, at 10% and 6%, respectively. While this study disagree with study by [26] show ^{bla}*SHV* (12.5%), ^{bla}*TEM* (6.3%) for *E. coli* isolates. another study that found *TEM* gene (46.2%) and the *SHV* gene (17.2) [46]. In the study by [24] found all ESβL producing uropathogenic *E. coli* phenotypically by conventional PCR. The result showed that *bla*TEM, *bla*SHV genes were 46 (95.83%), 39 (81.25%) respectively .

Conclusions

The present study concluded high prevalence of uropathogenic *Escherichia coli* (UPEC) with Multidrug-resistant (MDR) isolated from urinary tract infection in Diyala province – Iraq. This study concluded that there is high prevalence of MBL and ESBL producing *E. coli* in our clinical settings. Therefore, the detection of *blaVIM* and *blaNDM*, *SHV*, *TEM*, *BlaOXA* positive *E. coli* isolates in this study indicates importance of strengthening surveillance to prevent the nosocomial infection and dissemination of *bla* genes in Diyala.

References

1- Foxman, B. The epidemiology of urinary tract infection. *Nat Rev Urol* **7**, 653–660, 2010, https://doi.org/10.1038/nrurol.2010.190.

2- Mattoo, T.K., Spencer, J.D. Biomarkers for urinary tract infection: present and future perspectives. *Pediatr Nephrol* **39**, 2833–2844 ,2024. https://doi.org/10.1007/s00467-024-06321-9.

3- Hasan, Thualfakar Hayder, et al. Antibiotic susceptibility pattern of E. coli causing urinary tract infection in pregnant women in Al-Najaf Province, Iraq. In: *AIP Conference Proceedings*. AIP Publishing, 2023. https://doi.org/10.1063/5.0182049

4- Khan A, Saraf VS, Siddiqui F, Batool T, Noreen Z, Javed S, Ahmad A, Alonazi WB, Ibrahim M, Pucciarelli S, Bokhari H. Multidrug resistance among uropathogenic clonal group A *E. Coli* isolates from Pakistani women with uncomplicated urinary tract infections. BMC microbiology. 2024 Mar 7;24(1):74. https://doi.org/10.1186/s12866-024-03221-8

5- Deswal, Ritu, et al. The prevalence of polycystic ovary syndrome: a brief systematic review. *Journal of human reproductive sciences*, 2020, 13.4: 261-271. https://doi.org/10.4103/jhrs.JHRS_95_18

6- Ibáñez L, Oberfield SE, Witchel S, Auchus RJ, Chang RJ, Codner E, Dabadghao P, Darendeliler F, Elbarbary NS, Gambineri A, Garcia Rudaz C. An international consortium update: pathophysiology, diagnosis, and treatment of polycystic ovarian syndrome in adolescence. Hormone research in paediatrics. 2017 Dec 12;88(6):371-95. https://doi.org/10.1159/000479371

7- Juber NF, Abdulle A, Ahmad A, AlAnouti F, Loney T, Idaghdour Y, Valles Y, Ali R. Associations between Polycystic Ovary Syndrome (PCOS) and Antibiotic Use: Results from the UAEHFS. Antibiotics. 2024 Apr 26;13(5):397.https://doi.org/10.3390/antibiotics13050397

8- Rasheed JK, Kitchel B, Zhu W, Anderson KF, Clark NC, Ferraro MJ, Savard P, Humphries RM, Kallen AJ, Limbago BM. New Delhi metallo-β-lactamase–producing enterobacteriaceae, United States. Emerging infectious diseases. 2013 Jun;19(6):870. . http://dx.doi.org/10.3201/eid1906.121515

9- Bush K. Bench-to-bedside review: The role of β-lactamases in antibiotic-resistant Gramnegative infections. Crit Care. 2010;14 (3): 224.

10- P. Nordmann, T. Naas, and L. Poirel, "Global Spread of Carbapenemase-producing Enterobacteriaceae," Emerging Infectious Diseases, vol. 17, no. 10, pp. 1791–1798, 2011. DOI: 10.3201/eid1710.110655

11- R. I. Manenzhe, H. J. Zar, M. P. Nicol, and M. Kaba, "The spread of carbapenemase-producing bacteria in Africa: a systematic review," Journal of Antimicrobial Chemotherapy, vol. 70, no. 1, pp. 23–40, 2014. DOI: 10.1093/jac/dku356 .

12- Cassier P, Lallechere S, Aho S, Astruc K, Neuwirth C, Piroth L, et al. Cephalosporin and fluoroquinolone combinations are highly associated with CTX-M β-lactamase-producing *Escherichia coli*: a case-control study in a French teaching hospital. Clin Microbiol Infect. 2011;17(11):1746-51. doi:10.1111/j.1469-0691.2010.03349.

13- Pitout JD, Laupland KB. Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet infect Dis. 2008;8(3):159-66. doi:10.1016/S1473-3099(08)70041-0 PMid:18291338

14- Thuo TG. Antimicrobial Resistance Profiles and Clonal Relatedness of Pseudomonas aeruginosa Strains Recovered from Wounds Infections of Patients Presenting in Tigoni District Hospital, Kenya: JKUAT-COHES; 2021. http://doi.localhost/xmlui/handle/123456789/5563

15- Joji RM, Al-Mahameed AE, Al Jishi T, Fatani DI, Saeed NK, Jaradat A, et al. Molecular detection of plasmid-derived AmpC β-lactamase among clinical strains of Enterobacteriaceae in Bahrain. Ann Thoracic Med. 2021;16(3):287. doi:10.4103/atm.ATM_523_20

16- Warnke PH, Lott AJ, Sherry E, Wiltfang J, Podschun R. The ongoing battle against multi-resistant strains: in-vitro inhibition of hospital-acquired MRSA, VRE, Pseudomonas, ESBL E. coli and Klebsiella species in the presence of plant-derived antiseptic oils. Journal of Cranio-Maxillofacial Surgery. 2013 Jun 1;41(4):321-6. https://doi.org/10.1016/j.jcms.2012.10.012

17- El-Badawy MF, Tawakol WM, Maghrabi IA, Mansy MS, Shohayeb MM, Ashour MS. Iodometric and molecular detection of ESBL production among

clinical isolates of *E. coli* fingerprinted by ERIC-PCR: the first Egyptian report declares the emergence of *E. coli* O25b-ST131clone harboring bla GES. Microbial drug resistance. 2017 Sep 1;23(6):703-17. https://doi.org/10.1089/mdr.2016.0181

18- Haji, Sayran Hamad, et al. Molecular detection of SHV-Type ESBL in *E. coli* and K. pneumoniae and their antimicrobial resistance profile. *Zanco Journal of Medical Sciences (Zanco J Med Sci)*, 2018, 22.2: 262-272. https://doi.org/10.15218/zjms.2018.035

19- Vandepitte J. Basic laboratory procedures in clinical bacteriology. World Health Organization; 2003 Dec 31.

20- Clinical and laboratory standards institute (CLSI). Performance standards for antimicrobial susceptibility testing 33rd ed. CLSI supplement M100(ISBN 978-1-68440-170-3. Clinical and laboratory standards institute,USA,2023.

21- Pitout JD, Laupland KB. Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public-health concern. The Lancet infectious diseases. 2008 Mar 1;8(3):159-66. DOI: 10.1016/S1473-3099(08)70041-0

22- El-taie, M. I.; El-hasso, M. Z. Detection of some types of beta-lactamase enzymes in Rhizobium leguminosarum biovar viciae. *J. Education and Science*, 2009, 22.35: 149-158.

23- Thangavelu, Sathiamoorthi, et al. Isolation, identification, characterization, and plasmid profile of urinary tract infectious Escherichia coli from clinical samples. *Evidence-Based Complementary and Alternative Medicine*, 2022, 2022.1: 7234586.

24- Kaboré, Boukaré, et al. Emergence of New Delhi Metallo-β-Lactamase (NDM) Genes Detected from Clinical Strains of Escherichia coli Isolated in Ouagadougou, Burkina Faso. *International journal of microbiology*, 2023, 2023.1: 4813225.

25- Mouhammed, K., Gdoura, R. Study of the Genomic Characterization of Antibiotic-Resistant *Escherichia Coli* Isolated From Iraqi Patients with Urinary Tract Infections. Indian J Microbiol **64**, 457–466 :2024. https://doi.org/10.1007/s12088-023-01123-

26- Albazaz RI, Yassin NA. Prevalence and molecular detection of virulence genes among multidrug resistant *Escherichia coli* from human clinical samples and poultry in Duhok city, Iraq. Med J Babylon, 2024;21:S81-7. DOI: 10.4103/._204_23

27- Abbood DH, Obaid Alwan ZH. Molecular detection of genes encoding for adhesion factors in biofilm formation among uropathogenic *Escherichia coli* isolates. Med J Babylon 2023;20:258-63. DOI: 10.4103/MJBL.MJBL_233_22

28- Simba, SM, Omwenga, EO, and Musyoki, SK. Prevalence of *E. coli* as a causative agent of urinary tract infections and its drug susceptibility patterns among pregnant mothers seeking medicare at Kisii teaching and referral hospital, Kenya. Int J Community Med Public Heal . 2022: 9:1161–9. doi: 10.18203/2394-6040.ijcmph20220671

29- Ibraheem RS, Hussain SS, khalawe Tektook N. Biofilm Formation And Antibiotic Resistance For *E. Coli* Isolated From Urinary Tract Infections From Iraqi Women With The Polycystic Ovarian Syndrome. Journal of Pharmaceutical Negative Results. 2023 Mar 17:3194-201. https://doi.org/10.47750/pnr.2023.14.03.400

30- Tahir PH. Prevalence of Urinary Tract Infections Caused by Some Gram-Negative Bacteria Among Pregnant Women in Kirkuk Province – Iraq. Microbiol Infect Dis. 2022; 6(2): 1-4 https://doi.org/10.1038/ki.1994.370

31- El-beaty WA, Altaii HA. DETECTION OF GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA FROM IRAQI WOMEN INFECTED WITH POLYCYSTIC OVARY SYNDROME. InObstetrics and Gynaecology Forum 2024 Jun 19 (Vol. 34, No. 3s, pp. 1976-1989). https://doi.org.obstetricsandgynaecologyforum.com/618

32- Abbood DH, Obaid Alwan ZH. Molecular detection of genes encoding for adhesion factors in biofilm formation among uropathogenic *Escherichia coli* isolates. Med J Babylon 2023;20:258-63. DOI: 10.4103/MJBL.MJBL_233_22

33- Ibraheem RS, Hussain SS, khalawe Tektook N. Biofilm Formation And Antibiotic Resistance For *E. Coli* Isolated From Urinary Tract Infections From Iraqi Women With The Polycystic Ovarian Syndrome. Journal of Pharmaceutical Negative Results. 2023 Mar 17:3194-201. https://doi.org/10.47750/pnr.2023.14.03.400

34- Abdullah HM, Haleem B, Tariq M, Akhlaq S, Manawar S, Afzal MI. Unraveling the resistance puzzle: Antibiotic susceptibility and genetic insights into extra-intestinal *E. coli* Strains in UTIs. The

Professional Medical Journal. 2024 May 31;31(06):899-906.46- https://doi.org/10.29309/TPMJ/2024.31.06.7871

35- Alfetlawi B, Jasim A. Determining the Prevalence of Upper and Lower Urinary Tract Infections' Pathogens and Their Antibiotic Susceptibility Profile for Adult Patients in Al-Diwaniya, Iraq (Conference Paper). Iraqi Journal of Pharmaceutical Sciences 2022;31(Suppl.):86-91.48 https://doi.org/10.31351/vol31issSuppl.pp86-91

36- Abdulrahman IS. Antimicrobial susceptibility pattern of pathogenic bacteria causing urinary tract infections at Azadi Hospital in Duhok City\Kurdistan Region of Iraq. Science Journal of University of Zakho. 2018 Jun 30;6(2):46-50.54- https://doi.org/10.25271/2018.6.2.435

37- JAWAD, Rawaa; ALSHAMI, Zina; JIHAM, Haidar. Prevalence of Colistin Pan Resistance among Multidrug-Resistant and Extensively Drug-Resistant Escherichia Coli O157: H7. *Journal of Medical and Health Studies*, 2024, 5.2: 48-53.

38- Ballesteros-monrreal, Manuel G., et al. Virulence and resistance determinants of uropathogenic Escherichia coli strains isolated from pregnant and non-pregnant women from two states in Mexico. *Infection and Drug Resistance*, 2020, 295-310.

39- AL-HASNAWY, Huda Hadi; JUDI, Mohammad Ridha; HAMZA, Hiba Jasim. The dissemination of multidrug resistance (MDR) and extensively drug resistant (XDR) among uropathogenic E. coli (UPEC) isolates from urinary tract infection patients in Babylon Province, Iraq. *Baghdad Science Journal*, 2019, 16.4 Supplement: 986-922.

40- Tabassi Z, Vahedpoor-fard Z, Sedaghat H, Rouhi V, Rahimi M, Gharehhssanlou S. Examination of broad-spectrum betalactamase-producing *E. coli* vaginal colonization and antibiotic resistance in pregnant women; a pilot study in Iran. Int Arch Health Sci. 2024;11(2):128-134. doi: 10.48307/IAHSJ.2024.417607.1066

41- Eman, Salama Abdelmegeed; RASHA, Barwa; KHALED, H. A. E. G. Comparative study on prevalence and association of some virulence factors with extended spectrum beta-lactamases and AmpC producing *Escherichia coli*. *African Journal of Microbiology Research*, 2015, 9.17: 1165-1174. **https://doi.org/10.5897/AJMR2015.7463**

42- Mohebi, Samane, et al. Pathogenic features of urinary *Escherichia coli* strains causing Asymptomatic Bacteriuria during Pregnancy. 2020. **https://doi.org/10.1016/j.genrep.2022.101559**

43- Kakian, Farshad, et al. Identification of VIM and IMP genes and metallo-betalactamase enzymes in Escherichia coli isolates by molecular and phenotypic methods in shahrekord educational hospitals. *Journal of Shahrekord University of Medical Sciences*, 2020, 22.1: 46-52. **doi: 10.34172/jsums.2020.08**

44- Nwike, Ifeanyi Emmanuel, et al. Phenotypic and molecular characterization of enteropathogenic Escherichia coli and Salmonella spp. causing childhood diarrhoea in Awka, South-Eastern Nigeria. *Bulletin of the National Research Centre*, 2023, 47.1: 97. **https://doi.org/10.1186/s42269-023-01076-z**

45- Mahdi, Zinah Mohammed, et al. Detection of resistance genes (Blandm and Blavim) of Escherichia coli in the aquatic environment. *Iraqi Journal of Science*, 2023, 6193-6203.

46- Ali, G.H., Yakout, M.A. Comparative Study of ESBL Production Among Uropathogenic *Escherichia coli* Clinical Isolates from Pre- and Post-menopausal Women in Egypt. *Curr Microbiol* **78**, 3516–3525 (2021). https://doi.org/10.1007/s00284-021-02599-2.