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Research Article

The association between multidrug resistance and phylogenetic group with virulence factors in Uropathogenic Escherichia coli (UPEC) isolated in pregnant women of Basra City

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Abstract:
Background: UTIs are the second most prevalent pregnancy problem and may harm the mother and fetus if left untreated. Uropathogenic Escherichia coli (UPEC) is also categorized by antibiotic resistance, serogroup, and pathogenicity. This research examined the incidence of UPEC isolate phylogenetic groups, multi-drug resistance isolates, and virulence characteristics in pregnant women with UTI in Basrah city. This research included 250 urine samples from pregnant and non-pregnant women with UTIs (125 pregnant and 125 control) aged 15–45. Basrah Teaching Hospital, Sadr Teaching Hospital, and Al-Mwana Teaching Hospital provided these pregnant and non-pregnant patient samples. This trial ran from November 2023 until March 2024.
Methods: The isolates were diagnosed using culture medium (MacConkey Agar, Blood Agar, Eosin Methylene Blue Agar (EMB), CLED, and Hi Crome). After microscopic examination, Vitek-2 recognized all isolates. Hi, Crome Agar distinguished E. coli from other isolates.
Results: In 125 urine samples from pregnant women, several bacterial species were found. In pregnant women, E. coli 72 (34.7%), Klebsiella pneumoniae 22 (10.6%), Staph aureus 12 (5.7%), Staph epidermidis 5 (2.4%), and Pseudomonas 4 (1.9%) were the most common bacteria isolates. UTI bacteria were isolated, and E. coli isolates were chosen to evaluate the predominance of virulence genes (Chu A, yja A, Arp A, TspE4.C2). The investigation yielded five groups: A, B1, B2, C, and D. The most prevalent was group C, with 20 (27%), followed by B2 (18%), B1 (17.7%), D (15.2%), and A (8.3%), based on the virulence genes indicated above.
Conclusions: This study creates a database for UPEC dispersion, virulence factors, and genetic makeup in Basrah Province, highlighting the necessity for an epidemiological program to track its spread and genes locally. Women in eastern Basrah Province are more likely to get Escherichia coli, especially pregnant women.

Keywords: Escherichia coli, phylogenetic, UPEC, UTI, MDR, ESBL

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1. Introduction

The presence of bacteria in any sterile organ within the urinary system is a characteristic of urinary tract infections UTI (1). Gram-negative bacteria belonging to the Enterobacteriaceae family are the primary cause of UTI, including uropathogenic Escherichia coli (UPEC), which accounts for 80% of UTI infections globally (2). Although the majority of E. coli strains are commensal, pathogenic strains may induce various intra-intestinal disorders, including diarrhea, as well as extra-intestinal diseases, such as urinary tract infections (3). Currently, enteric E. coli pathotypes, referred to as Diarrhoeagenic E. coli (DEC), are classified into six categories according to their serological virulence features and other phenotypic attributes, The six types include enterotoxigenic E. coli (ETEC), enteropathogenic E. coli (EPEC), enterohaemorrhagic E. coli (EHEC), verodoxin or Shiga toxin-producing E. coli (VTEC/STEC), enter invasive E. coli (EIEC), diffusely adherent E. coli (DAEC), and enteroaggregative E. coli EAEC (4,5). Known virulence factors (VFs) include several forms of encapsulation, adhesins, toxins, a siderophore, and a protease (6). Pregnant women are at increased risk for UTI starting in week 6 through week 24 because uterus sits directly on top of the bladder and displaces it, shift in the position of the urinary tract and hormonal changes during pregnancy make it easier for bacteria to travel up the urethras to the kidneys additionally (7). Antibiotic resistance is one of the main worldwide health issues responsible for thousands of deaths and that is expected to get worse over time (8). Drug resistance in E. coli and other bacteria can result from natural evolutionary processes, selective pressures, and antibiotic misuse or overuse, Here are some key factors (9). Extended Spectrum Beta-Lactamase (ESBL) is a set of enzymes generated by some bacteria that hydrolyze and inactivate several beta-lactam medicines, including penicillin's and cephalosporins (10). By 2050, it is projected that over 3 million people would die due to MDR E. coli strains, particularly carbapenem-resistant ones, these bacteria have already spread worldwide, and the sole current therapy, colistin, is becoming less effective (11). Nevertheless, the improper and inappropriate use of these antibiotics has resulted in a significant and widespread rise in antimicrobial resistance in recent times, the emergence of resistance and cause serious clinical complication (12). He used a triplex PCR technique using the ChuA and YjaA genes, as well as the TspE4.C2 fragment, to categorize E. coli into four separate strain groups: A, B1, B2, and D (13). This modification led to the development of a quadruple PCR method, which allowed for the classification of E. coli strains into eight distinct phylogenetic groups: A, B1, B2, D (14).

2- Materials and methods

2.1 Sample Collection Steps

Mid-stream urine samples from 250 pregnant and non-pregnant women suspected of urinary tract infections based on the examining physician's clinical symptoms. From November 2023 to March 2024, 125 pregnant and 125 non-pregnant samples were distributed. This sample includes 140 outpatients and 110 15-63-year-olds. All patients sent to Al-Teaching Basrah, Sadr, and Mawani hospitals. The samples were stored in the preservation box at the
and MacConkey Agar at 37 °C for 24 hours.

2.2 Isolation and Purification of the Bacterial Isolates

The samples were cultured and diagnosed using the right medium. Growing E. coli colonies showed predicted characteristics. Bright pink colonies were seen on MacConkey agar (15). When isolated colonies were cultured on EMB (Eosin Methylene Blue), a culture medium used to distinguish E. coli, the bacteria proliferated and formed shiny green metallic colonies (16).

2.3 Detection of E. coli isolates

HiCrome Agar is recommended for E. coli detection. HiCrome Agar detects E. coli bacteria by measuring glucuronidase enzyme activity, which forms blue-green colonies (17). The Microtiter Plate Method (MTP) included transferring two to four isolates produced on nutrient agar to nutrient broth, where their turbidity was compared to the MacFarland standard turbidity. 200 µL of bacterial suspension was administered onto a 96-well polystyrene plate. The method was performed thrice for each isolate, using a bacterial-free nutritional broth as a negative control. The plate was sealed and incubated for 24 hours at 37 degrees Celsius.

2.4 Diagnosis by Vitek- 2 System Compact

The Vitek 2 System was used to accurately identify the specific kind of bacteria present. It consists of 64 biochemical tests. Following the completion of biochemical testing and Gram staining, the bacterial isolates were confirmed to be identical.

Detection of uropathogenic groups

The genes of uropathogenic E. coli (A,B1,B2, D) groups were amplified using polymerase chain reaction (PCR) utilizing specific primers (18). In the table (2-1).

Table (2-1) The specific primer Sequence for uropathogenic E coli groups

Genes	Name of the primer	Primer sequence (5-3)	Size of the product(bp)	Length(bp)	Reference
ChuA	Reverse	TGCCGCCAGTACCAAAGACA	288	20	(19)
ChuA	forward	ATGGTACCGGACGAACCAAC	288	20	(19)

YjaA	Reverse	AATGCGTTCCTCAACCTGTG	211	20	(19)
YjaA	forward	CAAACGTGAAGTGTCAGGAG	211	20	(19)
TspE4.c2	Reverse	CACTATTCGTAAGGTCATCC	152	20	(19)
TspE4.c2	forward	AGTTTATCGCTGCGGGTCGC	152	20	(19)
Arp A	Reverse	TCTCCCCATACCGTACGCTA	400	20	(19)
Arp A	forward	AACGCTATTCGCCAGCTTGC	400	20	(19)

2.5 Diagnosis of phylogenetic groups of Uropathogenic E. coli (A, B1, B2, D) groups

Genomic DNA was extracted from bacterial isolates using Presto™ Mining DNA Bacteria. Description of Gene Aid Korea kit. Gel electrophoresis analyzed 5 microliters of 1000 base pair DNA ladder and 5 microliters of uropathogenic E. coli. A 1.5% agarose gel in 1×TBE buffer with 0.2 microliters of ethidium bromide was electrophoresed for 45 minutes at 70V in a casting tray. Products were inspected using UV light. This study employed this method for all gene PCR testing.

2.6 Virulence factors detection for uropathogenic E. coli groups

Detection of virulence factors for uropathogenic E. coli (A, B1, B2, C, D) groups by using. **Congo red agar biofilm** production assay the bacteria's biofilm-forming ability was tested using Congo Red Agar (CRA). Brain Heart Infusion Agar was dissolved with 50 g/l sucrose and 0.8 g/l Congo red dye to make the test medium. The bacteria were injected onto Congo red agar and incubated at 37°C for 24 hours. Biofilm producers generate black colonies on CRA, whereas pink or white bacteria are intermediate biofilm producers. Non-biofilm-producing strains form red colonies.

2-7 Antimicrobial Susceptibility

The antibiotic susceptibility test was conducted using the Vitek® 2 AST Reference number 413083 antibiotic susceptibility Kit card, manufactured by bioMérieux in France. The Kit card offers a variety of antimicrobial testing. and Laboratory Standards Institute (CLSI). The antibiotics discs and their concentrations Which were used for determining the sensitivity of isolated bacteria is provided in table (2.2).

Antibiotic disc (Symbol)	Conc. (mg /disc)	Inhibition zone diameter (mm)			Use
		S	I	R	
B-lactam/carbapenem					
Meropenem (MEM)	10	≥23	20 – 22	19≤	Susceptibility testing
Impenem (IMP)	10	≥23	20 – 22	19≤	Susceptibility testing
Pencillin					
Piperacillin (PI)	100	≥21	18 – 20	17≤	Susceptibility testing
B-lactam combinations					
Amoxicillin-clavulante (AMC)	30	≥18	14 – 17	13≤	Susceptibility and beta-lactamase enzyme testing
Piperacillin-tazobactam (PIT)	30	≥21	18 – 20	17≤	Susceptibility testing
Monocyclin					
Aztreonam (ATM)	30	≥21	18 – 20	17≤	Susceptibility and beta-lactamase enzyme testing
Cephems (including cephalosporins)					
Cefotaxime (CTX)	30	≥26	23 – 25	22≤	Susceptibility and beta-lactamase enzyme testing
Ceftriaxone (CRO)	30	≥23	20 – 22	19≤	Susceptibility and beta-lactamase enzyme testing
Ceftazidime (CAZ)	30	≥21	18 – 20	17≤	Susceptibility and beta-lactamase enzyme testing
Cefoxitins (FOX)	30	≥18	17 – 15	14≤	Susceptibility and beta-lactamase enzyme testing
Cefepime (CPM)	30	≥25	19 – 24	18≤	Susceptibility testing, beta-lactamase enzyme testing and MIC determination.
Fluroquinolone					
Ciprofloxacin (CIP)	5	≥26	22 – 25	21≤	Susceptibility testing
Nalidixic acid (NA)	5	≥21	17 – 20	16≤	Susceptibility testing
Levofloxacin (LVX)	30	≥19	14 – 18	13≤	Susceptibility testing
Tetracycline					
Tetracycline (TE)	30	≥15	12 – 14	11≤	Susceptibility testing
Phenicol					
Chloramphenicol (C)	30	≥18	13 – 17	12≤	Susceptibility testing

2-8 Detection of Extended Spectrum β-lactamase (ESBL) Double disk synergy test (DDST)

The Double Disk Synergy Disk method was implemented as follows (20).

3- Results and discussion

This study collected 250 samples, 125 pregnant and 125 non-pregnant. The 125 urine samples from pregnant women included various bacterial species. The most common bacteria in pregnant women were Escherichia

coli 72 (34.7%), Klebsiella pneumoniae 22 (10.6%), Staphylococcus aureus 12 (5.7%), S. epidermidis 5 (2.4%), and P. aeruginosa 4 (1.9%). Escherichia coli was the most prolific isolated in all samples. Show in figure (3.1).

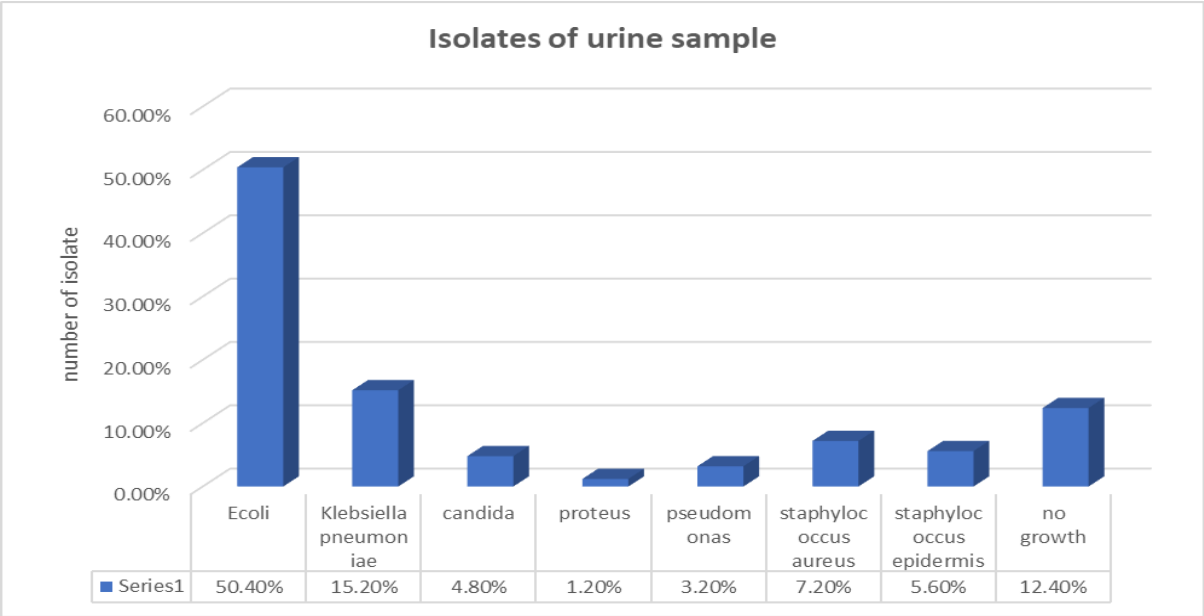


Figure 3.1: Distribution of isolated bacteria

The high prevalence of E. coli in UTI cases underscores its efficiency as a pathogen due to its natural habitat, anatomical proximity, virulence factors, and adaptability (21). These characteristics, combined with host factors and behaviors, make E. coli the most common causative agent in UTIs. According to this research, the age group with the highest infection rate was 25-34 (54.3%), followed by 35-45 (27.7%) and 15-24 (18%). This may be because women are more fertile at this period. Because of this, individuals are more likely to engage in sexual activity, which may introduce bacteria to the urinary system and cause infection (22).4.8Bacterial Resistance to Antibiotics

3-1 Bacterial Resistance to Antibiotics

Bacterial resistance to antibiotics denotes the capacity of bacteria to endure and proliferate in the presence of antimicrobial agents intended to eradicate or suppress them (23). This is a significant global health concern that complicates the management of bacterial infections and heightens the risk of severe consequences. A total of 72 E. coli isolates were subjected to testing with 27 antibiotic discs representing various types of antibiotics. The results revealed that the isolates exhibited diverse degrees of resistance to different antibiotic figure (3.2).

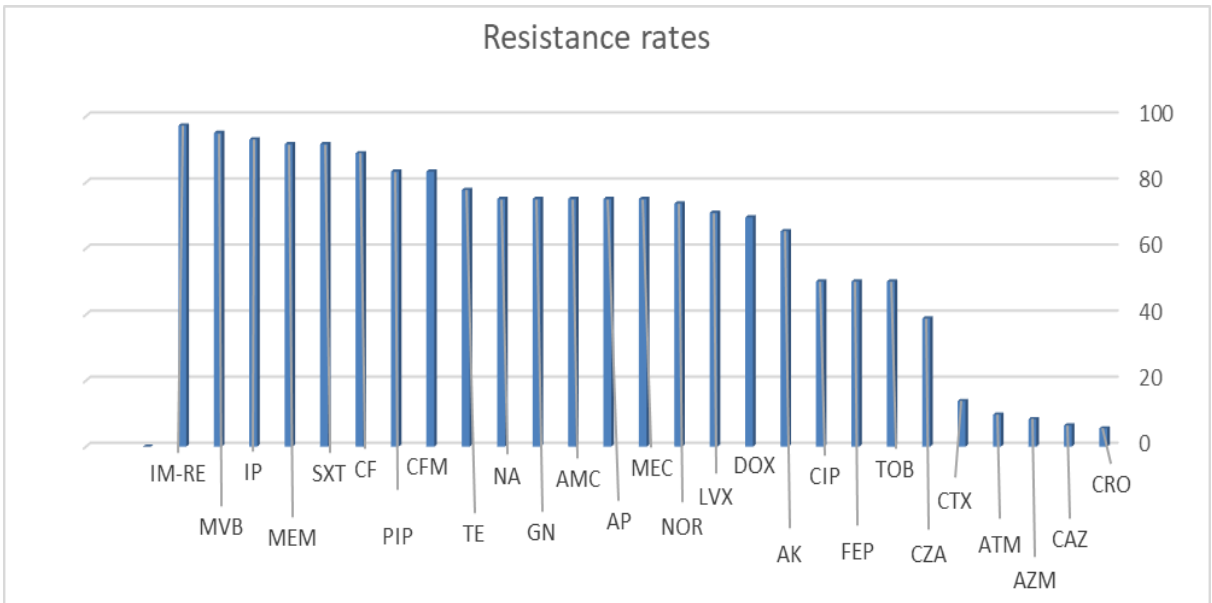


Figure 3.2: Resistance rates of the UPEC 72 isolates. Amikacin (AK), Ampicillin (AP), Tetracycline (TE), Doxycycline(DOX), Amoxicillin /clavulanic acid (AUG), Ciprofloxacin (CIP), Cefotaxime (CTX), Imipenem (IP), Gentamicin(GN), Meropenem(MEM), Nalidixic acid(NA), Ceftazidime(CAZ),Piperacillin(PIP),Mecillinam(MEC) , Ceftazidime-avibactam(CZA), Imipenem-relebactam(IM-RE), Meropenem-vaborbactam(MVB), Cefazolin(CF), Aztreonam(ATM), Cefixime(CFM), Tobramycin(TOB), Azithromycin(AZM), Levofloxacin(LVX), Norfloxacin(NOR), Trimethoprim-sulfamethoxazole(SXT), Cefepime(FEP), Ceftriaxone(CRO).

The study found that 77.7%, 75%, 75%, and 65.2% of the isolates were resistant to fluroquinolone medicines. The β-lactam combinations ceftazidime-avibactam, Ampicillin-sulbactam, Amoxicillin-clavulanate, Meropenem-vaborbactam, and Imipenem-relebactam had resistance rates of 88.8%, 73.6%, 70.8%, 6.9%, and 5.5% Meropenem and Imipenem had 9.7% and 8.3% resistance, respectively. Cephems like Ceftriaxone, Ceftazidime, Cefotaxime, Cefepime, Cefixime, and Cefazolin have resistance rates of 97.2%, 95%, 91.6%, 83.3%, 50%, and 38.8%. The resistance rates for Aminoglycosides, Tobramycin, Amikacin, and Gentamicin were 83.3%, 75%, and 69.4%Doxycycline Tetracycline resistance was 75%, 50%. Azithromycin, Aztreonam, Mecillinam, Ampicillin, and Piperacillin were 93%, 91.6%, 75%, 73.6%, and 50% resistant.

Escherichia coli antibiotic resistance analysis your Escherichia coli isolate antibiotic resistance data shows a broad range of resistance levels across classes. Different fluoroquinolone antibiotics had 77.7%, 75%, 75%, and 65.2% resistance rates. high fluoroquinolone resistance suggests extended usage in treating urinary and other infections has reduced efficacy. Chloramphenicol resistance may develop when an enzyme that normally adds acetyl groups to chloramphenicol antibiotics becomes inactive, altering the antibiotic in a way that makes it more resistant to the original drug (24).

Resistance Rates for β-Lactam Combinations 88.8% ceftazidime-avibactam. High resistance to carbapenem-lactam, ceftazidime-avibactam combinations (88.8%) emerging resistance mechanisms, such as mutations in the blaKPC or blaOXA genes, may affect the effectiveness of Avibactam (25). Ampicillin-sulbactam: 73.6% Amoxicillin-clavulanate 70.8% Meropenem-vaborbactam 6.9%, Imipenem-relebactam 5.5% Resistance to carbapenem combinations (e.g., meropenem-vaborbactam, imipenem-relebactam) is uncommon, but strong resistance to other β-lactam combinations suggests substantial β-lactamase activity in these isolates (26).

Carbapenems resistance rates, Meropenem 9.7%, Imipenem 8.3%. Low resistance rates to carbapenems are encouraging, but the emergence of carbapenem resistance is concerning, as these drugs are often the last line of defense against multi-drug-resistant E. coli (27).

Cephalosporins resistance rates, Ceftriaxone 97.2%, Ceftazidime 95%, Cefotaxime 91.6%, Cefepime 83.3%, Cefixime: 50%, Cefazolin 38.8%. High resistance to third-generation cephalosporins (e.g., ceftriaxone, cefotaxime) and cefepime reflects the widespread production of extended-spectrum beta-lactamases (ESBL) by these isolates (28).

Aminoglycosides resistance rates, Tobramycin: 83.3%, Amikacin 75%, Gentamicin 69.4%. High aminoglycoside resistance suggests a reduction in the effectiveness of these antibiotics, possibly due to enzymatic modification mechanisms (29).

Tetracyclines resistance rates, Doxycycline 75% , Tetracycline 50%, Moderate resistance rates to tetracyclines indicate some retained efficacy, but resistance may limit their use (30).

The high resistance rates to these antibiotics can be attributed to several interconnected factors, including bacterial adaptability, misuse of antibiotics, Causes of High Resistance to Azithromycin (93%) Efflux pumps Many E. coli strains possess efflux pump systems (e.g., AcrAB-TolC) that actively pump macrolides like azithromycin out of bacterial cells (31).

The high resistance to Aztreonam (91.6%) is mostly attributed to Extended-spectrum beta-lactamases (ESBLs), since several E. coli strains generate ESBLs that hydrolyze aztreonam, rendering it useless. Resistance genes, including blaTEM, blaCTX-M, and blaSHV, are often located on plasmids, facilitating fast dissemination among bacteria (32).

Resistance to Mecillinam (75%) and Ampicillin (73.6%) arises from analogous processes, namely the formation of beta-lactamase, since both are beta-lactam antibiotics. Numerous E. coli strains synthesize beta-lactamases (e.g., TEM-1, TEM-2) that degrade these drugs (33). Resistance to Piperacillin (50%) arises from several factors, including the presence of beta-lactamases, including as extended-spectrum beta-lactamases (ESBLs) and carbapenemase, these enzymes can hydrolyze piperacillin and are becoming more prevalent in E. coli (34).

3-2 Patterns of Multi-drug Isolates

All isolates in the investigation exhibited a 100% rate of multi-drug resistance (MDR, XDR) to the tested antibiotics. The results indicated that isolates E20, E33, E34, E50, E52, E53, and E64 shown resistance to 3 antibiotic groups, which was the lowest number of antibiotic groups resisted by all isolates. On the other hand, isolates E7, E18, E27, E43, E44, E62, E63, and E67 exhibited resistance to 4 antibiotic groups. E2, E9, E15, E19, E23, E28, E29, E36, E49 and E68 exhibited resistance to five antibiotic classes. Isolate E8, E38, E39,E41,E47,E48,E54,E56,E57,E60,E65,and E55 was discovered to be resistant to the largest number of antibiotic classes, namely 7 groups. Isolates E5, E13, E17, E22, E31, E59, and E71 exhibited resistance to eight different antibiotic classes. A separate cluster of isolates consisting of E4, E10, E58, E61, E69, and E70 exhibited resistance to nine different classes of antibiotics, as shown in table (3.1).

Table 3.1 Resistance type for each isolate

No,of Isolates	Isolates	No. of antibiotic Groups resisted	Resistance Type
9	E1,E14,E20,E33,E34,E50,E52,E53,E64	3	MDR
7	E7,E18,E27,E46,E62,E63,E67	4	MDR
12	E2,E9,E15,E19,E23,E28,E29,E36,E45,E49, E66,E68	5	MDR
12	E8,E38,E39,E41,E47,E48,E54,E56,E57,E60 ,E65,E72	6	MDR
18	E3,E6,E11,E12,E16,E21,E24,E25,E26,E32, E30,E35,E37,E42,E43,E44,E51,E55	7	MDR
8	E5,E13,E17,E22,E31,E40,E59,E71	8	MDR
6	E4,E10,E58,E61,E69,E70	9	XDR

The study's findings indicated that all of the isolates were multidrug-resistant (MDR), which may be attributed to either a mutation or the acquisition of a plasmid that confers a high degree of resistance to the cell. The extensive and indiscriminate use of broad-spectrum antibiotics has resulted in the emergence of several multi-drug resistance E. coli strains (35). The significant level of antibiotic resistance is seen as a crucial health concern, particularly in the case of E. coli, which is responsible for causing urinary tract infections (UTIs). UTIs are recognized as one of the most significant health challenges worldwide (36).

3-2 Distribution of UPEC phylogroups among in pregnant women

In this study, phylogenetic groups of UPEC among 72 E. coli isolated in pregnant women were identified based on quadraplex PCR assay. The maximum frequency of UPEC isolates has been in group C 20(27.8%) then in groupB2 18(25 %) followed by the phylogroups B1 17(23.7%), D (15.2%) and A 6(8.3%). These phylogroups shown in table (3.2).

Table 3.2: Distribution of UPEC phylogenetic groups among 72 E. coli isolates in pregnant women.

Type of phylogenetic groups	Pregnant women	
	Positive	percent
C	20	27.8 %
B2	18	25.0 %
B1	17	23.7 %
D	11	15.2 %
A	6	8.3 %
Chi squared test P value S / NS	23.74 0.000028 S	

S: significant association between groups

NS: Non-significant association between groups.

Result of PCR technique revealed Arp A gene 65(90.2%), Chu A 62(86.1), TspE4C2 54 (75%), YjaA 45(62,5%). This result shown in figure (3.3).

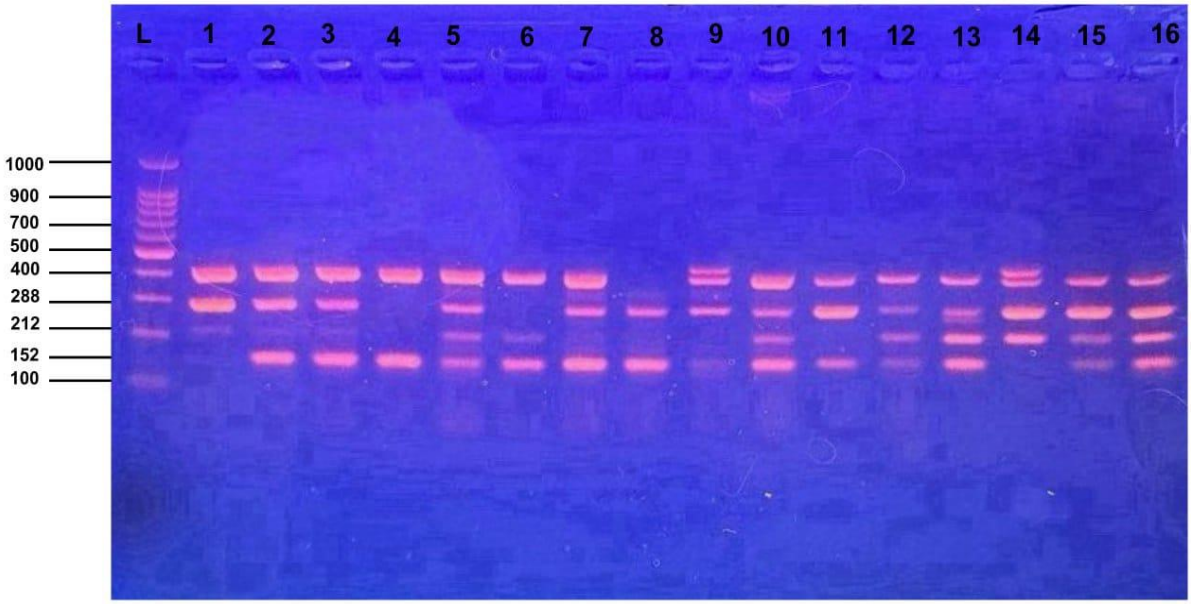
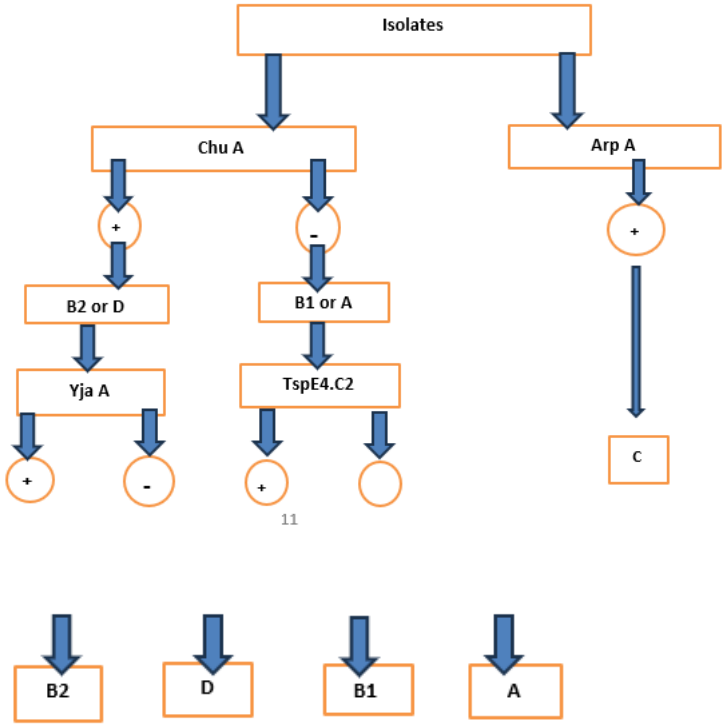


Figure (3.3): **Agarose gel electrophoresis of the polymerase chain reaction product for the detection of TspE4C1gene (152bp) using 1.5 % agarose for 60 minutes at 70 V, stained with ethidium bromide, L: DNA ladder 100 basis points, passages (2,3,4,5,6,7,8,9.10,11,12,13,14,15, , and 16) TspE4C2gene (152bp) and pathways (1 and 14): negative. And Yja A gene (212bp) passages (1 ,2 ,3 ,5, 6, 10, 12, 13, 1, 15 and 16) .Yja A gene pathways (4 ,7, 8 and 11) : negative. And Chu A gene (288bp) passages (1 ,2 ,3 ,5, 7 ,8 ,9 ,10 ,11 ,12 ,13 and 14)) Chu A gene pathways (4 and 6)): negative. And Arp A gene (400bp) passages (1 ,2 ,3 ,4 ,5 ,6 ,7 ,9 ,10 ,11 ,12 ,13 ,14 ,15 and 16) and pathways (8): negative for the gene.**

The present result of these four-amplification gene made depending on the dichotomous decision tree figure 3.4 (37).



Figur3.4 Dichotomous decision tree to determine the phylogenetic groups of *E.coli* strain by using the result of PCR amplification of the Chu A, Yja A, Arp A, and TspE4.C2.

This shows UPEC strains from pregnant women have a similar colonization capability.

Gene function ArpA controls cell adhesion and entry, which are essential for infection (42). This gene helps bacteria penetrate cells and connect to host tissues, enabling chronic infections. The ArpA gene was detected in all positive isolates from pregnant women (65 positive, 7 negative), showing its importance in UPEC pathogenicity independent of patient type.

3.3 Phenotypic Detection of Virulence Factors of Escherichia coli

The isolates' phenotypic evaluation was reviewed in the following sections, which examined different E. coli virulence factors.

3.3.1 Biofilm Detection

3.3.1.1Micro titer plate (MTP) method

MTP was employed to identify biofilm development in isolates. The majority of isolates may form biofilms. Of the 72 isolates studied, 60 (83.3%) produced biofilm. Twelve isolates (16.7%) were non-biofilm producers. Figure 3.4 show that MTP is a quantitative method that uses 96 wells to measure isolate biofilm intensity. This is done by measuring light absorption at 630nm using a spectrophotometer. The digital value represents the bacterial suspension's biofilm production in the wells. Results on biofilm development have been consistent across investigations (43). discovered that 76.5% of their isolates could generate biofilms, whereas (44). found 68%.

The distribution of UPEC phylogenetic groupings among 38 pregnant women was studied in West Iraq. Group B2 9 (23.6%) had the most UPEC isolates, followed by B1 5 (13.1%), A 4 (10.5%), D 3 (7.8%), C 1 (2.6%), E 0(0%), F 6 (15.7%), 4 (10.5%), and Nontippable 6 (15.7%) ,(37). Another research is done at Hamedan, west of Iran. Group B2 50 (44.2%) had the most UPEC isolates, followed by D 35 (31%), A 23 (20.4%), and B1 5 (4.4%), (38**).**

E. coli commensal strains are A and B1, whereas external gut pathogens include B2, C, and D. (McGarry,2024). By searching the gene library of several E. coli strain phylogenetic groups for gene fragments, certain genes may be used as markers (38). See markers below. Chu A is required for E. coli O157 heme transport. YjaA encodes an unknown protein, whereas TSPE4C2 encodes a DNA fragment (39). Study Results, the ChuA gene was detected in 62 positive, 10 negative UPEC isolates from pregnant women, suggesting a substantial involvement in the increased virulence observed during pregnancy.

Its purpose While its processes are unknown, gene YjaA is considered an indication for pathogenic E. coli strains (40). suggesting illness potential. A strain with gene YjaA is more likely to be virulent. Results showed a significant difference in gene YjaA presence between pregnant women (45 positive, 27 negative**).**

TspE4C2 helps the bacteria colonies the urinary system in the early stages of infection (41). Study Results 54 positive and 18 negative patients have TspE4C1 gene.

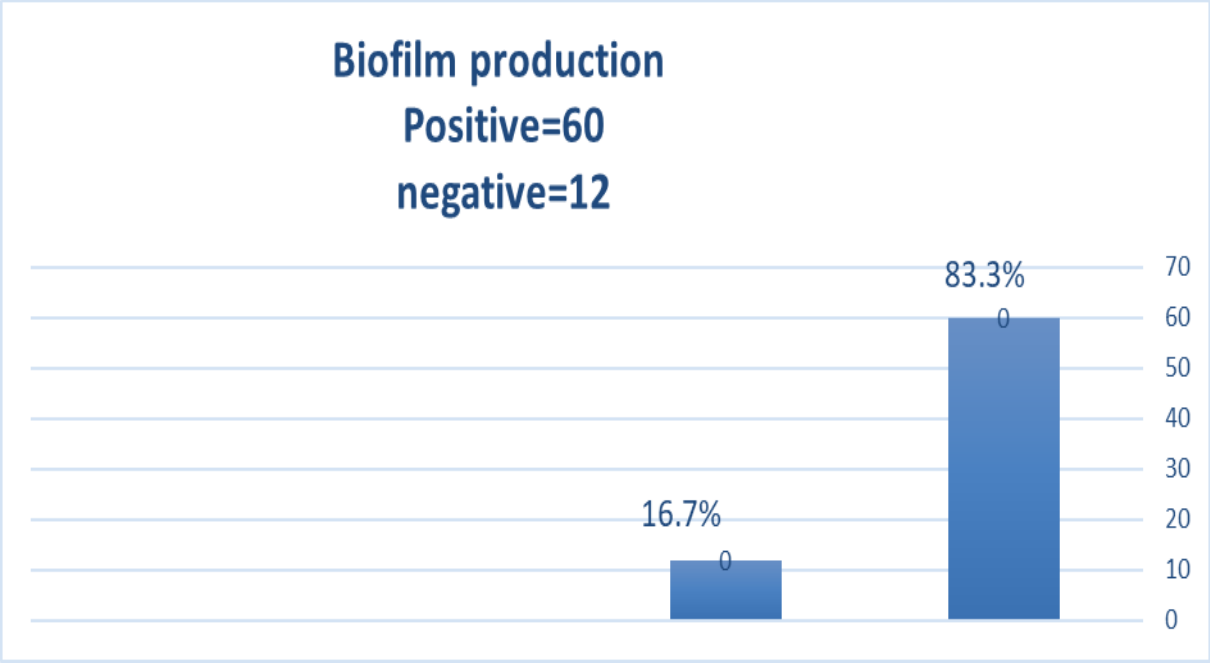


Figure 3.4: Biofilm production testing for E.coli of pregnant women.

3.3.1.2 Congo Red Agar Results for Biofilm Formation

This Congo red agar culture tests E. coli biofilm composition. Positive biofilm production results in dark (black) colonies. Figure (3.4) shows that non-biofilm-forming Escherichia coli strains create soft red colonies, whereas the tested strain can build a biofilm.

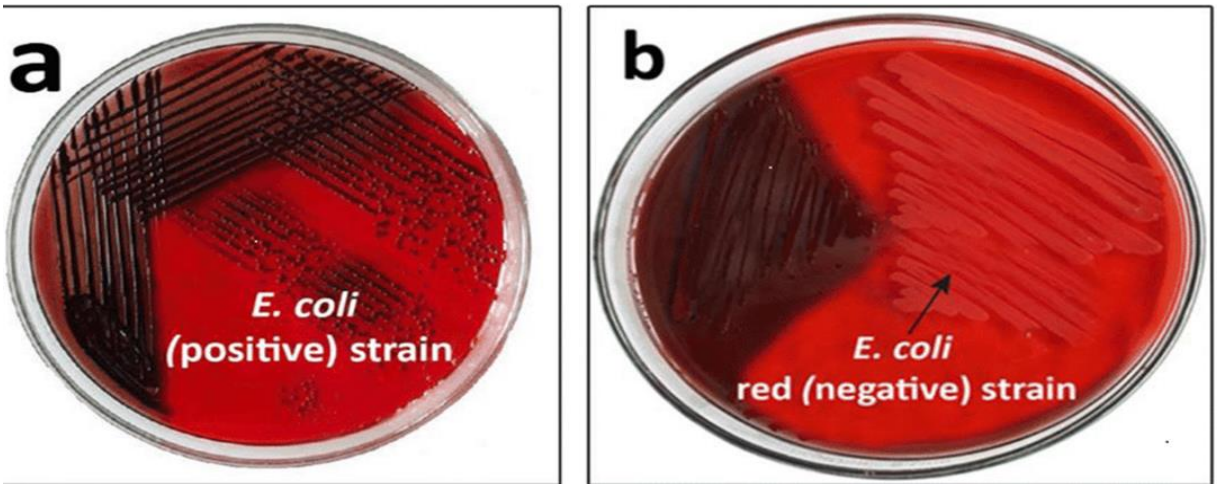


Figure 3.4: Biofilm production results on Congo Red Agar medium

Biofilms are extracellular polymeric matrix-coated enzymes. In Nepal (Pandit et al., 2020), 40.3% of isolates generated ESBL Hassuna et al. (47) found 59.7% ESBL production in Egyptian isolates. Ethiopian isolates produced 66.7% ESBLs (48). The diversity of isolates used in this research, the differences in local antibiotic consumption within each country, the excessive use of broad-spectrum antibiotics, particularly third-generation cephalosporins, and the pathogens' drug resistance may all contribute to f production variability (49). The presence of β-lactamase is a key determinant in the pathogenicity and antibiotic resistance of E. coli. This occurs via cleaving the β-lactam ring in certain drugs.

bacteria that cling to surfaces (44). Recent study has showed that curli and cellulose genes help E. coli form biofilm. Biofilm production envelops complete bacterial populations with a water-repellent matrix, allowing UPEC to stay in the urethra longer (45). Biofilms reduce the efficiency of antimicrobials and activate human immune systems, resulting in UPEC in the urinary tract and severe UTI and antibiotic resistance (46).

3.4 Extended Spectrum Beta-lactamase (ESBL) Production

The double disc synergy test (DDST) determined isolate ESBL enzyme production (Figure 4.12). There were 65 (90.3%) ESBL-producing isolates and 7 (9.7%) without

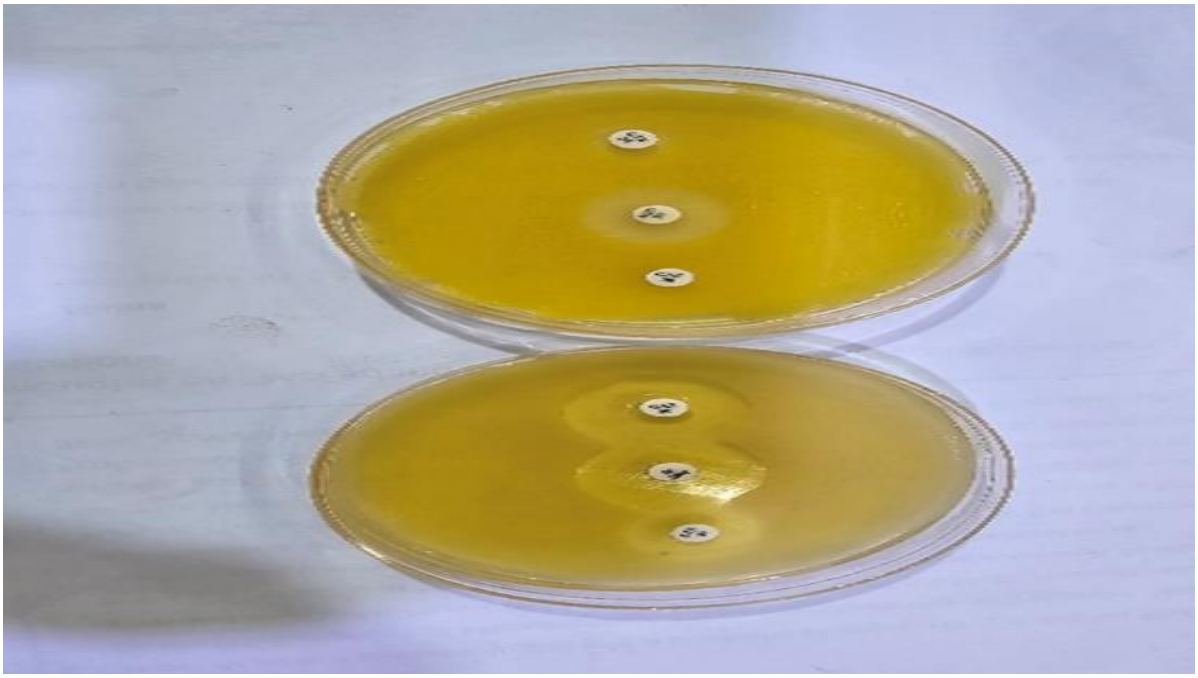


Figure 3.5: ESBL from E. coli on Mueller Hinton Agar

3.6 Motility

The motility of the isolates was assessed using a semi-solid medium, and it was determined that all of the isolates exhibited motility.

3.7 Association between Antibiotic Resistance and Virulence Factors

The relationship between antibiotic resistance and virulence factor has developed over time due to several factors, including bacterial species, resistance mechanism, virulence factors, biological niche, environmental variables, and host immune system. Age and host vulnerability also affect infection risk (50). The study results revealed that each isolate exhibited multidrug resistance (MDR), indicating resistance to a minimum of two distinct antibiotic classes. The result shown in (Figure 3.5). Isolates that formed biofilm had 1000 times greater antibiotic resistance than those that did not. This suggests that biofilm development and isolate resistance are linked (51). Bacteria may evade urinary tract defenses and tolerate potent antibiotics by forming biofilms. This, in turn, facilitates the development of multidrug resistant (MDR) strains that cause more severe urinary tract infections (52). The ESBL enzyme, produced by mutations in the genes bla-TEM1, bla-TEM2, and bla-SHV-1, is the major cause of E. Coli resistance to 3rd and 4th generation cephalosporins (53). The plasmid with Extended-Spectrum Beta-Lactamase (ESBL) encodes ampicillin and other antibiotic resistance. The findings also showed that UPEC virulence variables affect UTI symptoms. In particular, UPEC's virulence components that adhere to and invade the urinary epithelium increased UTI-related mucosal inflammation and discomfort (54). Antimicrobial resistance and pathogenicity genes were discovered in 92.6% of UPEC isolates, which were resistant to all antibiotics. 100% of isolates were multidrug-resistant, resisting three or more antibiotics. This supports Indian (55) and Iranian (56) investigations. Additionally, virulence gene-containing stains were more antibiotic-resistant. As previously shown (57). UPEC virulence genes have a major role in medication resistance. Antimicrobial drug resistance is linked to transmissible plasmids, which may also be virulent. Microorganisms may gain from resistance and virulence. This might cause ecological shifts and ferocious antibiotic-resistant microorganisms to dominate (58).

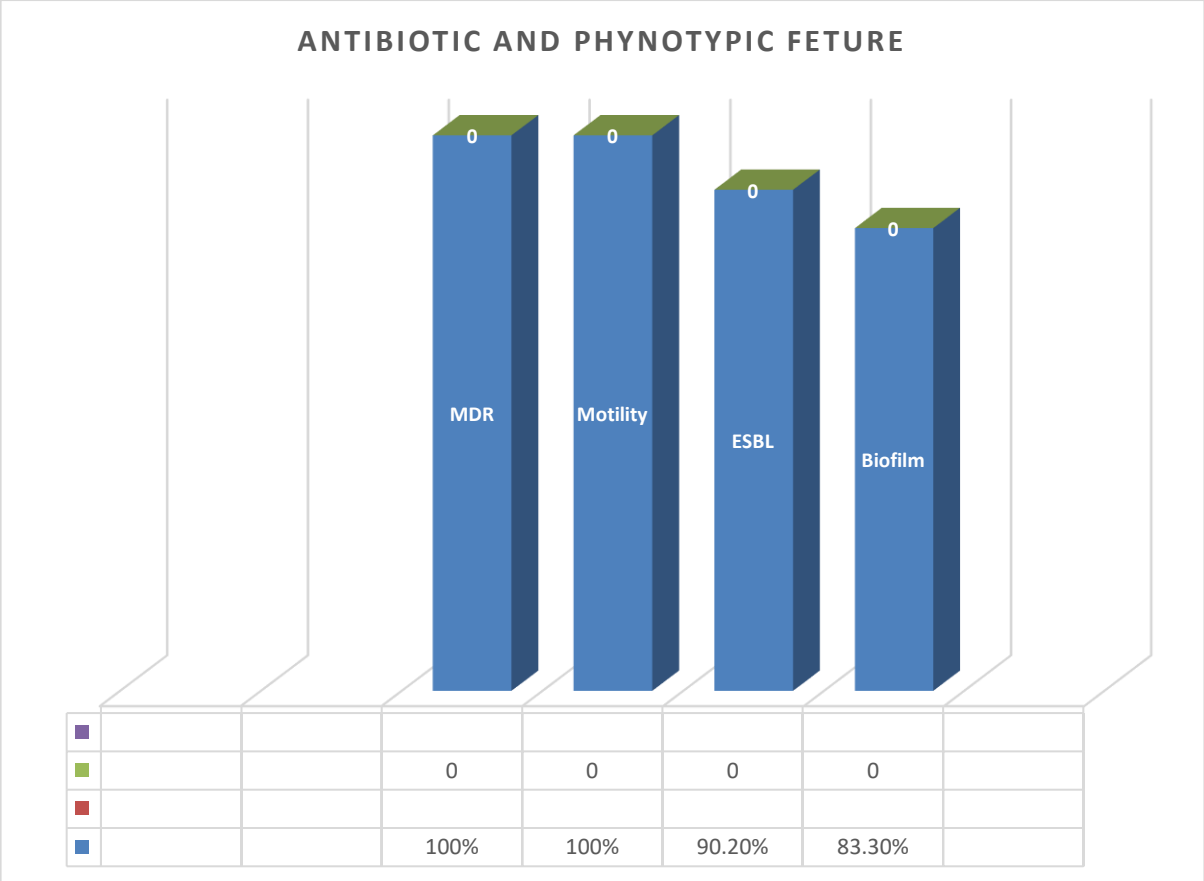


Figure 3.6: Association between phenotypic features and antibiotic resistance of the isolate

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