

## **Microbiological and Molecular Investigation of Urinary and Uterine Infections in Physiologically Normal Reproductive-Age Women**

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### **Abstract :**

**Background:** Urinary tract infections (UTIs) are among the most common bacterial infections affecting reproductive-age women, including those who are anatomically and physiologically normal. Uterine infections, particularly endometritis, are significant inflammatory conditions that may lead to serious gynaecological complications if untreated. Although these infections involve different anatomical sites, potential microbiological and molecular associations between urinary and uterine pathogens remain insufficiently explored. This study aimed to investigate the microbiological and molecular characteristics of bacterial isolates from urinary and uterine infections in physiologically normal reproductive-age women and to assess the presence of shared virulence determinants.

### **Keywords:**

(16s *rRNA*, *fimH* , *UTIs* , *papC*, High vaginal swab , Endocervical swab  
Introduction)

**Methods:** A total of 80 female patients aged 15–45 years diagnosed with UTIs were recruited from Al-Hilla Teaching Hospital and the Children and Maternity Hospital in Al-Hilla city between January and March 2025. Midstream urine samples, high vaginal swabs, and endocervical swabs were collected under aseptic conditions. Samples were cultured on blood agar and MacConkey agar for bacterial isolation and identification using standard microbiological and biochemical methods. *Escherichia coli* isolates were further

confirmed biochemically. Molecular identification and detection of virulence genes (16S rRNA, fimH, papC, and usp) were performed using polymerase chain reaction (PCR).

**Results:** Out of 80 samples, 25 isolates (31.25%) were confirmed as *Escherichia coli*. All confirmed isolates (100%) carried the 16S rRNA gene. The virulence genes fimH and usp were detected in 96% of isolates, while papC was identified in 92%. The high prevalence of these virulence determinants indicates strong pathogenic potential among the isolates obtained from physiologically normal women.

**Conclusion:** This study demonstrates a significant presence of virulence-associated genes among *Escherichia coli* isolates recovered from urinary and uterine samples in reproductive-age women. The integration of conventional microbiological techniques with molecular diagnostics provides enhanced accuracy in pathogen identification and characterization. Understanding shared virulence profiles may help clarify the potential association between urinary and uterine infections and support improved diagnostic, therapeutic, and preventive strategies.

Urinary tract infections (UTIs) are among the most common bacterial infections in women of reproductive age, with an estimated 150–250 million cases annually worldwide. Although the urinary system in young women is usually anatomically and physiologically normal, several biological and hormonal factors increase susceptibility to these infections(1). These factors include the shortness of the female urethra and its proximity to the vagina and anus, as well as changes in the microenvironment during menstruation or sexual intercourse. On the other hand, uterine infections—particularly endometritis—represent a significant source of gynaecological morbidity worldwide. These infections often arise from the spread of bacteria from the lower reproductive organs to the uterus, and may extend to the fallopian tubes and ovaries, causing serious complications such as infertility, chronic pelvic pain, recurrent miscarriages, and systemic infections in advanced cases(2). The relationship between urinary tract infections and uterine infections raises important questions in clinical and microbiological science(3). Multiple studies suggest the possibility of microbiological and genetic overlap between the pathogens of infection in both the urinary and reproductive systems(4). Some viral and genetic factors of the bacteria themselves—such as genes associated with cell adhesion, biofilm formation, and antibiotic resistance—may be shared in both locations, enhancing their ability to colonize different tissues and cause multisite infections(5). This research aims to highlight the microbiological and molecular relationship between urinary tract infections

and uterine infections in women., utilizing the integration of traditional culture and microbiological diagnostic methods with molecular analysis, which supports the development of targeted therapeutic and preventive strategies

## **2. Methods and Materials**

### **The Study Design and Ethical Issues**

Between January and March 2025, a cross-sectional study was carried out at Al-Hilla Teaching Hospital involving 10 female patients aged 15–40 years, who were infected with *Escherichia coli*. Patients who were immunocompromised, undergoing antibiotic treatment, or who did not provide consent were excluded. Verbal consent was obtained from each participant before sample collection. The study was approved by the Committee of Publication Ethics at the College of Medicine, Babylon University, Iraq (Approval No. 300-29/1/2025). All samples were collected in accordance with international ethical standards, and written informed consent was obtained from all participants prior to data collection.

### **Collecting Samples, Isolating Them, and Identifying Them**

An 10 samples (urine, High vaginal swab It is taken from the upper part of the vagina, just away from the cervix.) were gathered from female patients, aged between 15 and 45 years. with suspected urinary tract infections. These samples were promptly transferred to the Microbiology and Antimicrobial Substances Research Unit in the Department of Microbiology at Al-Hillah Teaching Hospital for further analysis. It is taken from the upper part of the vagina, just away from the cervix. The specimens of the study were initially cultured on blood agar and MacConkey agar for primary isolation, where *Escherichia coli* colonies were presumptively identified. Subsequently, the isolates were subcultured onto mannitol agar and incubated at 37° Celsius for a day (24 hrs) to encourage bacterial growth and isolation. Detection of *E. Coli* was carried out using standard microbiological and biochemical techniques, involving inoculation on mannitol agar. The isolates were further characterised depending on colony morphology, pigmentation on blood agar, and their biochemical properties such as oxidase, catalase, citrate utilisation, urease, and indole production.

### **High vaginal swab**

Insert a speculum to widen the vagina and allow visualisation and access to the upper vaginal area. Using a sterile swab, collect the sample from the upper vaginal area, near the cervix, avoiding direct contact with the labia majora or cervix to minimise contamination. Gently move the swab along the upper vaginal wall to collect secretions and microorganisms. Place the swab in a sterile transport tube. Deliver the sample to the laboratory immediately or store it at the appropriate temperature, depending on the type of analysis (usually 4°C if not cultured directly). Cultivate the sample on conventional growth media to detect bacteria. Analysis by PCR (polymerase chain reaction) can identify pathogenic genes or antibiotic resistance.

### **Antibiotic Susceptibility Assessment**

The antibiotics used in susceptibility testing were levofloxacin, amoxicillin-clavulanic acid, cefepime, amikacin, and ampicillin. Every *E. Coli* sample grew on nutrient agar at 37° Celsius overnight. Two to three colonies were then cultured into Muller-Hinton broth (MHB) and left overnight at 37° Celsius. The bacterial suspension was injected into Muller-Hinton agar plates with cotton swabs the next day. Following Clinical and Laboratory Standards Institute standards, the antibiotic resistance of every bacterial sample was evaluated using the disk diffusion technique. If strains showed resistance to at least one agent in three or more antimicrobial categories, they were labelled as multidrug resistant (MDR).

#### **DNA extraction:**

Around 300 µL of Lysis Solution was added. Along with 20 µL of Universal Sorbent. Each added to pre-labelled Eppendorf tubes. Labels matched each sample number. After that, 100 µL of the sample was pipetted in. A filter tip was used. Aerosol-safe. Tubes were closed firmly. Mixed briefly on a vortex mixer. Then incubated. 65 °C for 5 minutes. After incubation, tubes were vortexed again. Quick mix. Then left at room temp for 2 minutes. Just to settle. Next, centrifugation. 10,000 rpm. 30 seconds. Pellet at bottom. Supernatant? Removed gently. Without touching the pellet. Then, Washing Buffer. About 1 mL. Added to each tube. Vortexed hard. Needed full resuspension of the sorbent. Tubes were uncapped. Placed at 65 °C. Let dry. To  
ok around 5 to 10 minutes. For elution, 65 µL of TE Buffer went in. Tubes vortexed again. Sorbent fully resuspended. Then another incubation at 65 °C. Five more minutes. Final spin—12,000 rpm for 1 min. DNA in the supernatant. Collected carefully. Stored at –20 °C. Ready for later steps.

### **The detection of PCR for genes associated with virulence**

We used specific oligonucleotide primers. Picked from previously published sequences. The goal? To detect virulence genes. The list of primers used it's in Table 1. Each one selected for gene-specific amplification. Traditional PCR was also done. To confirm the isolates were *Escherichia coli*. That part targeted the 16S rRNA gene. Species-specific primers were used for that too. Refer again to Table 1. PCR amplification needed a 25  $\mu$ L reaction mix. Pretty standard. It had 5  $\mu$ L of PCR Master Mix. Plus 1  $\mu$ L of each primer (forward and reverse). Then 2  $\mu$ L of the DNA template. The rest was nuclease-free water. To make it up to volume. Reactions were run on a SimpliAmp™ Thermal Cycler (Applied Biosystems). Conditions? Initial denaturation at 95°C for 5 min. Then 30 cycles. Each cycle: 94°C for 1 min (denaturation), annealing for 1 min (check Table 2 for temps), and extension at 72°C for 30 sec. Final step extension again at 72°C for 5 min. Done. Results were checked by electrophoresis. We used 1.5% agarose gel. Stained with SYBR Safe (Invitrogen). Bands were visualized under UV. A transilluminator was used for that. Clear bands meant successful amplification.

**Table 1:** Primer Sequences, Amplicon Sizes, and Annealing Temperatures for Detection of UPEC Virulence Genes

Gene name	Gene sequence	Amplicon size	Tm (°C)
<b>fimH</b>	F:AACAAGGATAAGCACTGTTCTGGCT R:ACCATATAAGCGGTCATTCCCGTCA	500	61
<b>usp</b>	F:AAGATGGAGTTTCCTATGCAGGAG R:TGGAGTTTCCTATGCAGGAG	498	58
<b>papC</b>	F:ACATTCACGGCAAGCCTCAG R:GCGAGTTCCTGGTGAAAGC	408	62
<b>16s rRNA</b>	F:CATGCCGCGTGTATGAAGAA R:CGGGTAACGTCAATGAGCAAA	100	59

**Gel electrophoresis:**

Gel electrophoresis was performed after PCR. 2% Agarose gel electrophoresis was used to evaluate the PCR results. Additionally, ethidium bromide was used to detect the products. For forty minutes, the power supply condition was set at 100V. The Electrophoresis Power Supply was the power source utilised.

### **Statistical analysis:**

All frequency data was analysed. Pearson's chi-squared test was used. Also, Fisher's exact test is used when needed. The analysis wasn't too complex. SPSS version 22 was the main tool. That's the Statistical Package for Social Sciences. Data went in, stats came out. Everything is processed inside SPSS. Results, mostly shown as percentages. Simple and clear.

### **Ethical approval :**

Ethical approval for this study was obtained from the ethical committee at Hilla Surgical Teaching Hospital. Furthermore, all individuals participating in the study were informed about the research, and their consent for both conducting the experiments and publishing the results was obtained prior to sample collection. This study was also approved by a local ethics committee at the College of Medicine, University of Babylon. and hospital ethics committee under document number [ IRB: ٦-٦6, 7/8/202٥].

## **3. Results**

### **Microbiological Characterisation of Urinary and Vaginal Bacterial Isolates in**

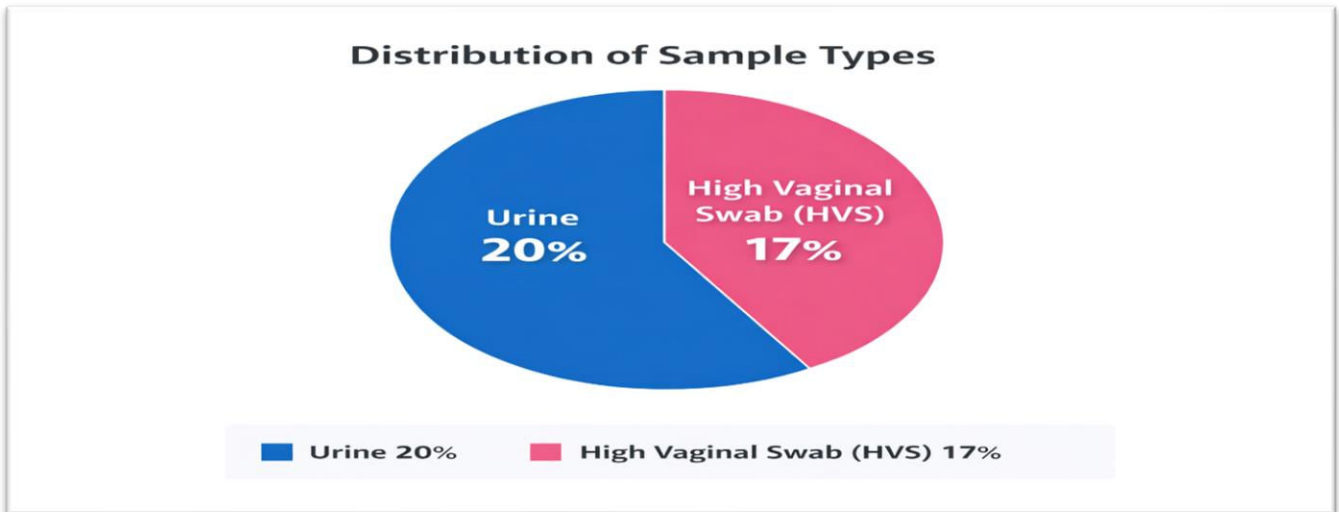
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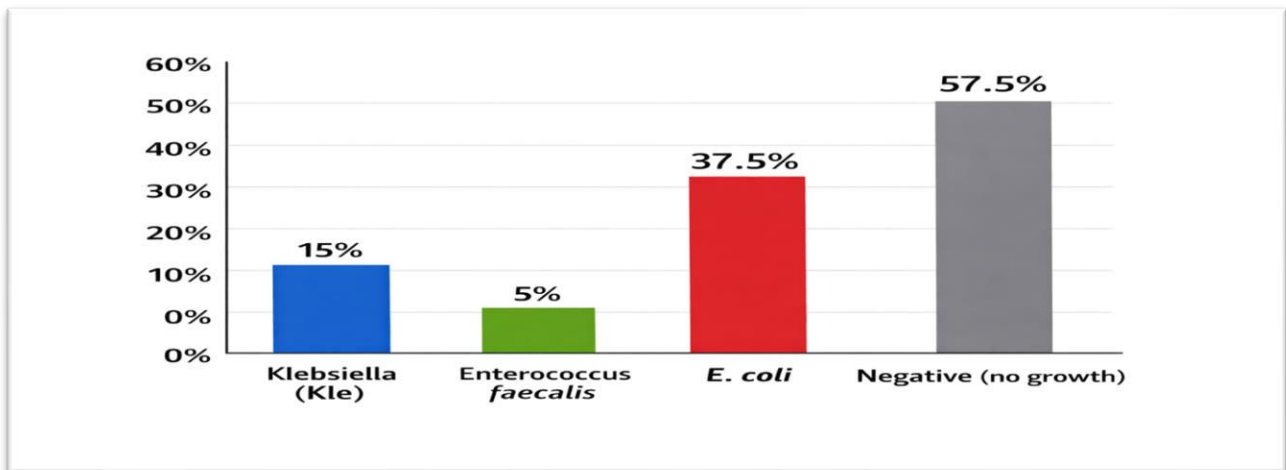
## **Women with Suspected Urinary Tract Infections**

Eighty samples were collected from women aged 15 to 45 years who were clinically diagnosed with a suspected urinary tract infection. The samples included 20% urine and 17% high vaginal swabs (HVS). HVS swabs were taken from the upper part of the vagina, away from the cervix, to avoid direct contamination of the cervix or labia. All samples were immediately transported to the Microbiology and Antimicrobial Research Unit of the Microbiology Department at Hilla Teaching Hospital for laboratory analysis. Samples were cultured on blood agar and MacConkey agar to isolate the primary bacteria, with initial identification of suspicious colonies (15% Kle, 5% Enterococcus faecalis, and 37.5% Escherichia coli) as in Figure(1), 37.5% Patients clinically diagnosed with urinary tract infections (UTIs) provided urine samples. Following normal aseptic procedures to reduce contamination, E. Coli positive cases were distributed across age groups as shown in Table (2). Midstream clean-catch urine samples were collected in sterile containers and sent straight to the lab for examination. Arriving samples were grown on MacConkey agar and blood agar plates and incubated aerobically at 37°C for 18–24 hrs. Selected for additional research were colonies displaying the usual *Escherichia coli* shape on MacConkey agar—lactose-fermenting pink colonies. Gram staining for identification turned up Gram-negative, rod-shaped bacteria. Standard biochemical tests covering indole generation, methyl red, Voges-Proskauer, citrate use (IMViC), and urease testing. recognised as E. Coli are isolates with a positive reaction for indole and methyl red and negative for Voges-Proskauer, citrate, and urease.

The isolates were then re-cultured on mannitol agar and incubated at 37°C for 24 hours to promote bacterial growth and isolation. E. coli was diagnosed using standard microbiological and biochemical tests. Colony Morphology, Biochemical Characteristics: Oxidase, Catalase, Citrate, Urease, and Indole Production



**Figure(1):**Distribution of Sample Types (Urine and High Vaginal Swab)



**Figure(2):**Distribution of bacterial isolates and negative culture results

Table(2): Distribution of *E. Coli* Positive Cases Across Age Groups and Statistical Significance

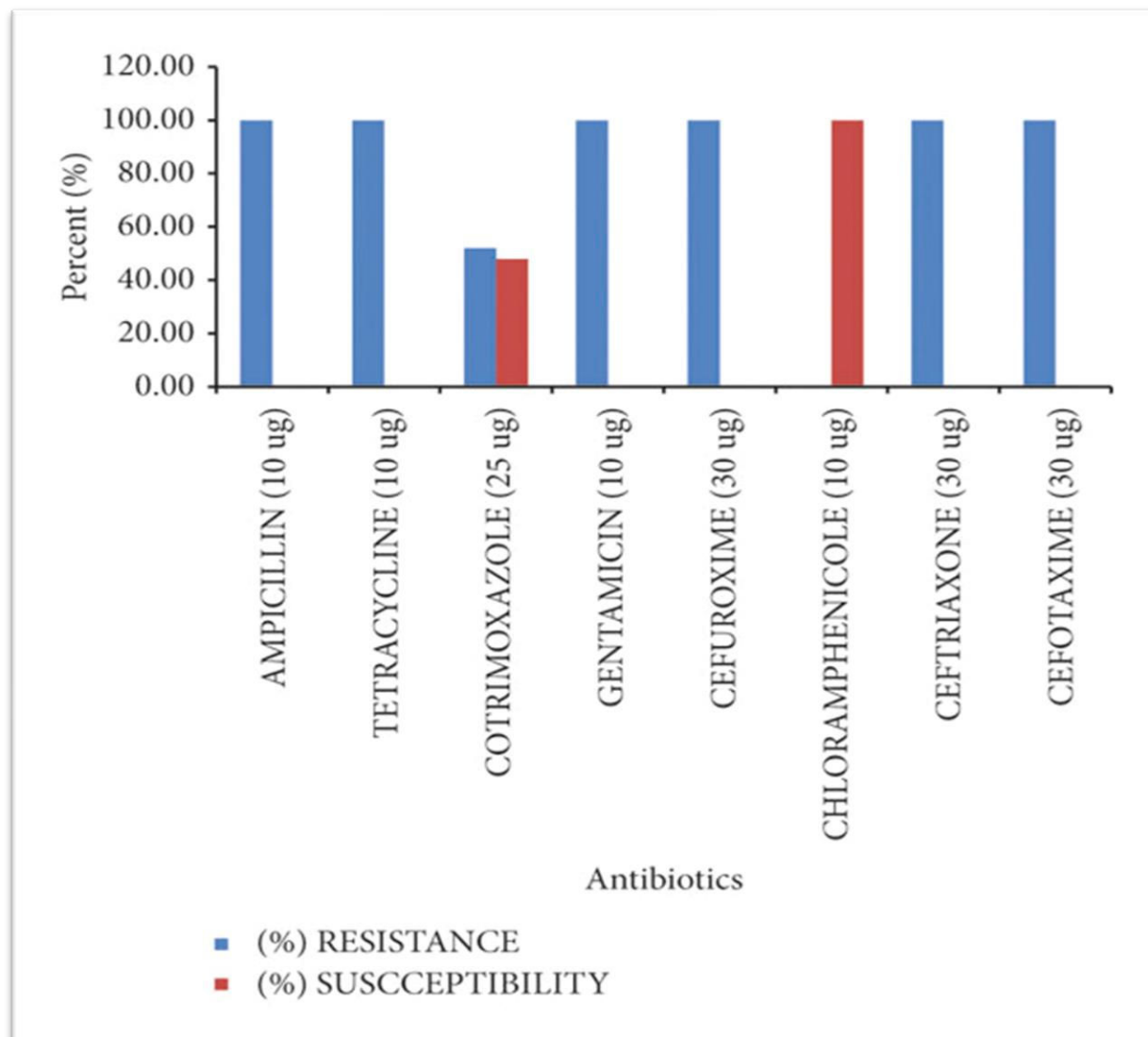
Age	<i>E. Coli</i> positive cases	p-value
15-20	15	<b>0.005</b>
21-30	45	
31-45	35	

Being presented with *E. Coli* positive cases distributed throughout several age categories, the p-value is 0.005. This shows ( $p < 0.05$ ) a statistically significant variation in the distribution of positive cases between age groups.

#### **Antimicrobial Susceptibility Test :**

Levofloxacin, amoxicillin-clavulanic acid, cefepime, amikacin, and ampicillin were among the several classes of antibiotics used in assessments of the UPEC isolates' antibiotic susceptibility profile (Table 3). Table 3 shows that 44% (n = 11) showed resistance to amoxicillin-clavulanic acid, 80% (n = 25) of the isolates were resistant to ampicillin. Observed in 20% (n = 5) and 16% (n = 4) of the isolates respectively were resistance to levofloxacin and amikacin. Especially, 4 isolates (16%) were found to be multidrug-resistant (MDR), showing resistance to three or more drugs. By comparison, three isolates—12%—were displayed to be sensitive to each tested antibiotic. The Zone of Inhibition (mm) for *E. Coli* samples treated with varying antibiotics is illustrated in

Fig. 1.



Figure(3):Antibiotic resistance and susceptibility pattern of bacterial isolates.

### Virulence Gene Prevalence in *E. Coli* Isolates (n = 25)

Using PCR amplification of the 16S rRNA gene, twenty-five isolates from urinary tract infections (UTIs) were confirmed to be *Escherichia coli*. Figures 1 show all isolates tested positive for the *E. coli*-specific 16S rRNA gene. Table 4 shows the frequency of virulence genes among these isolates: *sat* was detected in 24 isolates (96%), *usp* in 24 isolates (96%), *hlyA* in 23 isolates (92%), whereas *cnf* was not detected in any isolate (0%).

**Table 3. Prevalence of Virulence Genes in *E. Coli* Isolates (n = 25)**

Gene	Number of Positive Isolates	Percentage (%)
16S rRNA	25	100%
<b>fimH</b>	24	96%
usp	24	96%
<b>papC</b>	23	92%

### Gel Electrophoresis

After PCR, gel electrophoresis was done. To check the bands. And to see if amplification worked. A 2% agarose gel was made. Agarose powder was melted into 1X TBE buffer. Heated, then cooled. Let it set right. Ethidium bromide was added—0.5 µg/mL. That helped make DNA bands visible under UV. PCR products were mixed with loading dye. Then added into the wells. Each sample had its own well. Alongside a DNA ladder—either 100 bp or 1 kb. Used as a reference. Electrophoresis was run at 100 volts. Constant. For 40 minutes. The Wix Electrophoresis Power Supply was used. After running, the gel was put on a UV transilluminator. Bands showed up. Bright and sharp. Photos were taken for documentation. Band sizes were compared with the expected ones. That helped confirm if target virulence genes were amplified correctly. This gel check was done after every PCR. Band migration through agarose is shown in Figures (2, 3, 4, 5, 6). Everything looked as it should. Mostly

### Detection of virulence genes by PCR :

By amplifying the 16S rRNA gene using PCR, 25 UPEC bacteria were discovered and confirmed as *E. Coli*. These strains were obtained from individuals with UTIs. Each of the 25 samples tested positive for the presence of the *E. Coli* 16S rRNA gene (Figure 3). Table 4 demonstrates that among all the *E. Coli* isolates, the following virulence factors were the most prevalent:**fimH** (96%) and usp (96%) as well as **papC** (23%) .



Fig. 3: PCR products were analysed. 16S rRNA gene primer was used. A sharp band appeared—100 bp in size. Primers had a  $T_m$  of about 63°C.

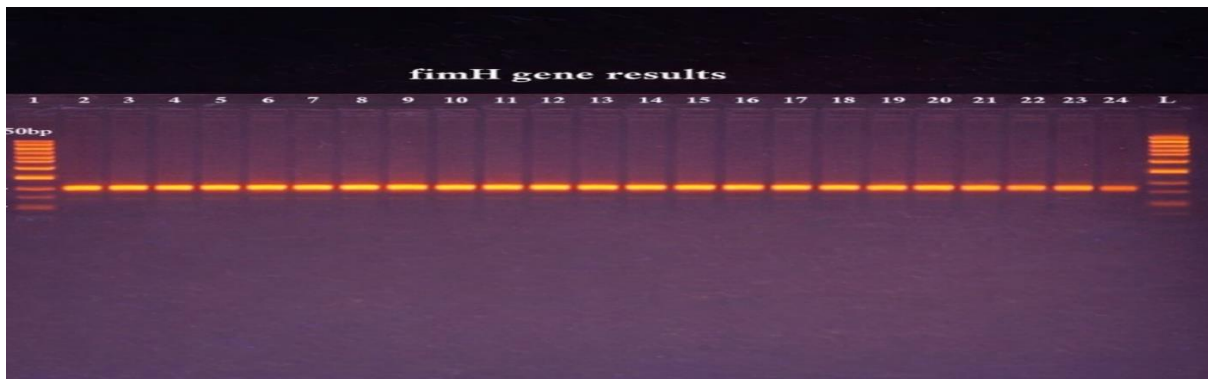


Fig. 4: PCR product was run on a gel. The *fimH* primer was used. A clear 100 bp band showed up. Pretty expected. Primer  $T_m$  was around 59°C.

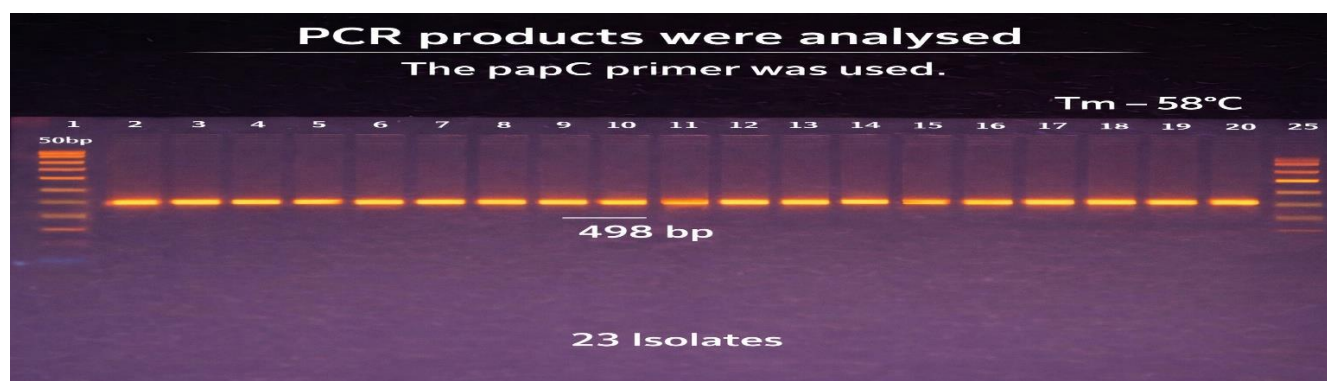


Fig. 5: Gel electrophoresis was done on the PCR product. The **papC** primer was used. A single band at 498 bp was observed. Primers had a  $T_m$  around  $58^\circ\text{C}$ .

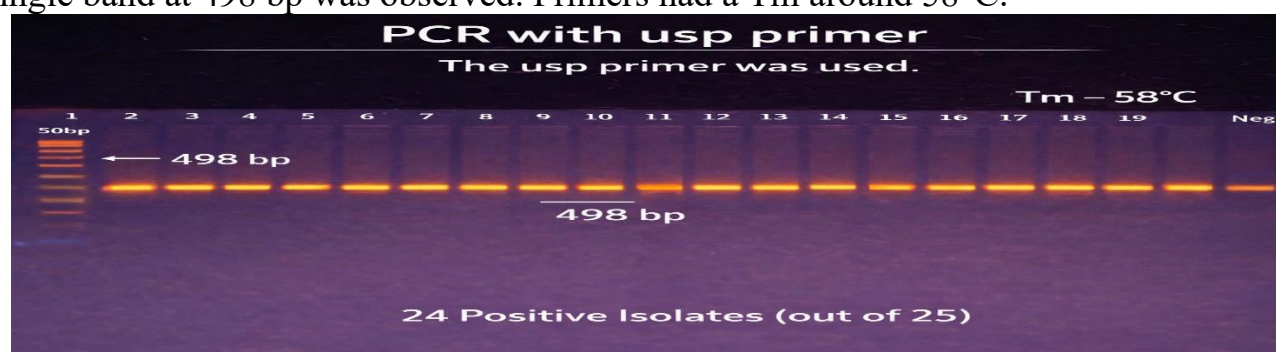


Fig. 6: Gel electrophoresis was done on the PCR product. The **usp** primer was used. A single band at 498 bp was observed. Primers had a  $T_m$  around  $58^\circ\text{C}$

## Discussion

In this study, a total of 80 samples were collected from women aged 15–45 years who were clinically suspected of having urinary tract infections (UTIs). Samples included 20% urine and 17% high vaginal swabs (HVS). The careful collection of HVS from the upper vagina, avoiding contact with the cervix and labia, minimized contamination and ensured reliability of microbial isolation. Samples were immediately transported to the microbiology laboratory, cultured on blood agar and MacConkey agar, and preliminary identification revealed that *E. coli* accounted for 37.5% of isolates, followed by *Klebsiella* spp. (15%) and *Enterococcus faecalis* (5%). The predominance of *E. coli* is consistent with multiple contemporary studies. For example, Flores-Mireles et al. (2015) highlighted that *E. coli* is responsible for approximately 70–95% of uncomplicated UTIs in women, with uropathogenic strains (UPEC) harboring virulence factors that facilitate adherence, invasion, and persistence in the urinary tract [1]. Similarly, a recent Iraqi study reported that *E. coli* represented the majority of UTI isolates in women, confirming the local epidemiological relevance [2]. Analysis of *E. coli* positive cases across age groups revealed a statistically significant variation ( $p = 0.005$ ), with the highest prevalence in

women aged 21–30 years. This age-dependent susceptibility aligns with previous findings indicating that sexually active women and those of reproductive age are at higher risk for UTI due to hormonal influences and anatomical predisposition [3]. Antibiotic susceptibility testing revealed a high resistance rate among UPEC isolates: 80% resistant to ampicillin and 44% resistant to amoxicillin-clavulanic acid, while resistance to levofloxacin and amikacin was lower (20% and 16%, respectively). Notably, 16% of isolates were multidrug-resistant (MDR), showing resistance to three or more antibiotic classes. These findings are in line with global reports of rising antibiotic resistance among UPEC strains, highlighting the urgent need for rational antibiotic prescription and local susceptibility monitoring [4,5]. Comparatively, studies from neighboring regions have reported similar resistance trends. For instance, Al-Badr and Al-Shaikh (2013) reported high ampicillin resistance (75–85%) and variable fluoroquinolone resistance in UPEC isolates, emphasizing that local surveillance is crucial to guide empiric therapy [6]. Molecular analysis confirmed all 25 *E. coli* isolates by 16S rRNA PCR, and revealed high prevalence of fimH (96%), usp (96%), and papC (92%). The fimH adhesin is critical for attachment to uroepithelial cells, while papC mediates P fimbriae formation, facilitating colonization of the urinary tract. The usp gene contributes to survival under host immune pressure. No isolates carried cnf, consistent with prior studies suggesting that certain virulence genes are strain-specific and geographically variable [7]. These results support previous evidence that the presence of multiple virulence genes in UPEC strains correlates with enhanced pathogenicity and increased risk of recurrent or complicated infections [8]. The combination of phenotypic identification and molecular characterization provides a comprehensive assessment of the pathogenic potential of isolates, which is critical for both clinical management and epidemiological surveillance.

### **Conclusion :**

This study confirms that *E. coli* is the predominant pathogen in UTIs among women and carries multiple virulence factors that enhance its infectivity. The high rates of antibiotic resistance and the presence of virulence genes emphasise the need for **regular surveillance, targeted antimicrobial therapy, and integration of molecular diagnostics** to improve clinical management and prevent recurrent infections.

## Conflicts of Interest

No conflicts of interest.

## Funding

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