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Molecular Detection of *pap*E Virulence Genes in *Escherichia coli* Isolated from Women with Urinary Tract Infection

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Abstract **Background:** Urinary Tract Infections (UTIs) are among the most prevalent bacterial infections in women, often caused by uropathogenic *Escherichia coli* (UPEC). The *pap*E gene, a component of the P fimbriae operon, is recognised as a key virulence factor facilitating bacterial adhesion and colonization of uroepithelial cells. **Objective:** This study aimed to detect the presence of the *pap*E virulence gene in clinical *E. coli* isolates obtained from women with UTIs using molecular methods. **Methods:** A total of 116 urine samples from women with clinically diagnosed UTIs were collected. *E. coli* isolates were identified using standard microbiological methods and confirmed by the VITEK® 2 Compact system. Genomic DNA was extracted from the isolates and PCR amplification of the *pap*E gene was performed using gene-specific primers. Amplification products were analysed by electrophoresis on 1.5% agarose gels. **Results:** According to Sex Distribution: Out of the 116 clinical bacterial isolates, 41 (36.6%) were from male patients, while 71 (63.4%) were from female patients. The isolates' resistance to quinolone antibiotics, which includes nalidixic acid, ciprofloxacin and levofloxacin, was at 82.5, 56.3 and 50%, respectively. Cephalosporins, which include cefoxitin, cefotaxime, ceftazidime, cefepime and ceftriaxone, had a resistance of 53.3, 60, 76.6, 66.6 and 53.3%, respectively, for each aforementioned antibiotic. The isolates' resistance to carbapenems, which includes meropenem and imipenem, was at 22 and 35%, respectively. As for β-lactam combinations, which include Augmentin and piperacillin-tazobactam, the resistance was found to be at 60 and 40%. And as for the penicillin group of antibiotics that includes piperacillin, the resistance was found to be 46.6%. Tetracycline from the antibiotic group of the same name and chloramphenicol from the antibiotic group phenicol both had a resistance rate of 46.6%. Aztreonam, from the antibiotic group minocycline, had resistance at 73.3%. The isolates were capable of producing biofilms; 20 isolates (66.7%) were biofilm producers out of 30 isolates,7 isolates accounting for 35% were strong biofilm producers, while 8 isolates (40%) were moderate biofilm producers. The remaining 5 isolates, accounting for 25 % of the total isolates, were weak biofilm producers. **Conclusions:** The detection of the *pap*E gene in clinical *E. coli* isolates highlights its potential role in UTI pathogenesis and underscores the importance of molecular surveillance of virulence factors in guiding therapeutic strategies. Future studies should explore the association of *pap*E with antimicrobial resistance patterns and biofilm formation to develop targeted interventions.

Key Words Biofilm, *Escherichia coli*, Molecular Detection, PCR, *pap*E gene, Urinary Tract Infection, Virulence Factors

INTRODUCTION

Urinary Tract Infections (UTIs) are one of the most common bacterial infections in women with the majority caused by uropathogenic *Escherichia coli* (UPEC). The pathogenicity of UPEC is largely due to a suite of virulence factors which mediate adhesion, invasion and evasion of the host immune response. P fimbriae in particular are important for bacterial binding to uroepithelial cells allowing subsequent colonization and persistence of the urinary tract [1]. Antibiotic resistance One of the key global health crises which takes the lives of thousands of people is antibiotic resistance and it's estimated to get even worse in the near future. It is estimated that by 2050, it will kill 10 million people each year) [2]. *E. coli* is a multi-drug-resistant pathogen with a potential to resistance to a wide range of antibiotics through various mechanism including production

of enzymes like β-lactamase, which enable resistance to β-lactam antibiotics and other enzymes that lead to resistance to Quinolones, Aminoglycosides and many more [3].

The pap operon includes the P-fimbrial structural and regulatory genes. Notably the *pap*E gene is involved in the biosynthesis of the fimbrial tip structure which is critical for interactions with host tissues. *pap*E detection could be considered as a bio-marker for screening possible virulent *E. coli* in UTI [4]. With the increasing prevalence of recurrent and multidrug-resistant forms of UTI, knowledge of the distribution of both urovirulence genes, such as *pap*E, can provide information for clinical risk management and therapeutic strategy planning [5]. The aim of the present study is to evaluate the *pap*E gene among the *E. coli* isolated from clinical UTIs of women by Polymerase Chain Reaction (PCR) to get better understanding of the molecular properties of UPEC in this study population [6]. The present work was conducted in response to the absence of such studies on UTI-causing *E. coli* and its pathotypes in the eastern Diyala Province of Iraq and aims to: (i) Screen the provided samples for UPEC and non-UPEC isolates; (ii) Determine the VFs and ISR of the isolated *E. coli* UPEC and Non-UPEC strains; (iii) Test for antibiotic sensitivity of the isolated *E. coli* UPEC and non-UPEC strains and (iv) identifying of structural genes for VF and ISR of the isolates.

This study was undertaken to address knowledge gaps regarding the prevalence and clinical significance of the *pap*E gene in uropathogenic *Escherichia coli* (UPEC). In order to ensure clarity and alignment between aims and outcomes, the objectives were organized into primary and secondary levels as follows:

- To determine the prevalence of the *pap*E gene among clinical isolates of UPEC obtained from patients with urinary tract infections
- To evaluate the antimicrobial resistance profiles of the collected isolates using standard susceptibility testing
- To investigate the potential association between the presence of the *pap*E gene and multidrug resistance (MDR) patterns in UPEC strains
- To compare the prevalence of the *pap*E gene in our study with findings reported from other geographic regions, thereby providing contextual relevance
- To assess the potential clinical role of the *pap*E gene as a virulence marker that may contribute to the persistence and severity of UPEC infections

METHODS

Sample Collection

About 116 urine samples were collected from women with symptoms of Urinary Tract Infection (UTI) between February 2025 and May 2025 at Al-Batoul Teaching Hospital. The women's ages ranged from 15 to 45 years. Include 41(36.6%) male and 71(63.4 %) females. All samples were collected aseptically and transported to the microbiology laboratory for immediate processing. A total of 72 (64.3 %) samples tested positive for UTI, while the remaining 40 (35.7%) tested negative growth. After morphological, microscopic and biochemical tests and the application of the Vitek 2 system, 35(48.6%) *E. coli* isolates were obtained from the 72 positive samples of the total.

Bacterial Isolation and Identification

Urine samples were cultured on MacConkey and blood agar plates and incubated at 37°C for 24 hours. Colonies with morphology suggestive of *E. coli* were subcultured and confirmed using standard biochemical tests (e.g., indole, citrate, urease, TSI) and/or automated identification systems.

Characteristic of Cultural Isolation

Samples were cultured and diagnosed using the appropriate culture mediathen the right characteristics of *E. coli* grown colonies were noticed, on MacConkey agar, shiny pink colonies were noted [7]. Pure colonies were taken and cultured on EMB (Eosin Methylene Blue) which is a differentiating mediatory *E. coli*. The grown of the bacteria gives colonies with a green metallic sheen [8].

Identification of *E. coli* with Vitek2 Compact System

Bacteria were subjected for identification by VITEK 2 compact system according to the instruction provided by the company. Three to Five well-isolated colonies from each isolate were transferred to a glass tube containing 3 ml distal water to measure and adjust turbidity that represents bacterial cells number per 1 ml which must be equal to 0.5 Macfarlane standard. Sample entered to VITEK 2 compact system machine to transferred to bacterial suspension to cassette by negative pressure, then incubate cassette to complete biochemical reaction within 12 hours. Interpretations of results were performed according to VITEK 2 compact system special software to identify bacterial species and strains.

Antibiotic Susceptibility Testing

Following the standards set forth by the Clinical and Laboratory Standards Institute (CLSI), the Kirby-Bauer disk diffusion method was used to ascertain the antibiotic susceptibility profiles of the Escherichia coli isolates. Every *E. coli* isolate was used to create a standardized bacterial suspension that met the 0.5 McFarland turbidity criterion. The suspension was evenly applied to the Mueller-Hinton agar plate surface using a sterile swab. Sterile forceps were used to apply commercial antibiotic discs (Oxoid, UK or comparable) on the inoculation plates. For 18 to 24 hours, plates were incubated at 37°C. Based on the CLSI breakpoint, the millimeter-diameter zones of inhibition were classified as susceptible, moderate or resistant [9]. All antibiotic discs used in this study are listed in Table 1.

Determination of Biofilm Formation

A total of 20 MDR isolates were tested for their biofilm production ability using a plastic microtiter plate, nine isolates recorded as strong biofilm producers as detailed in Table 2. To estimate the on-biofilm production of these

Table 1: The antibiotic discs

Antibiotic discs	Company (origin)	Concentration (µg/disc)
Ampicillin	Mastdiscs (U.K)	10
Nalidixic acid	Mastdiscs (U.K)	30
levofloxacin	Mastdiscs (U.K)	10
Ciprofloxacin	Mastdiscs (U.K)	10
Amoxicillin-Clavulante (Augmentin)	Mastdiscs (U.K)	25
piperacillin-tazobactam	Mastdiscs (U.K)	300
piperacillin	Mastdiscs (U.K)	10
ceftriaxone	Mastdiscs (U.K)	10
Aztreonam	Mastdiscs (U.K)	30
Cefepime	Mastdiscs (U.K)	30
Cefotaxime,	Mastdiscs (U.K)	30
Ceftazidime	Mastdiscs (U.K)	30
Cefoxitin	Mastdiscs (U.K)	30
Tetracycline	Mastdiscs (U.K)	25
Imipenem	Mastdiscs (U.K)	10
Meropenem.	Mastdiscs (U.K)	10

Table 2: Formula for interpreting the absorption results for MTP

No.	Absorption value	Interpretation
1	ODC≥ ODI	Incapable of forming biofilm
2	2ODC<ODI	Strong biofilm producer
3	ODC< ODI≤ 2ODC	Intermediate biofilm producer

ODI: Optical density of isolates, ODC: Optical density of control

isolates, five strong producers' strains were selected. OD of each well was read at 630 nm using a microtiter plate reader.

PCR Detection of *pap*E Gene

DNA Extraction

Genomic DNA was extracted from confirmed **E. coli** isolates utilizing either the boiling method or a commercial DNA extraction kit, such as Qiagen or Promega's Wizard genomic DNA purification kit, adhering to the respective manufacturer's protocols. Following extraction, the DNA quality and concentration were evaluated through methods including spectrophotometry (Nanodrop) or agarose gel electrophoresis. The extracted DNA was stored at -20°C pending further analysis. The Wizard genomic DNA purification kit specifically comprises Cell Lysis Solution, Nuclei Lysis Solution, Protein Precipitation Solution, DNA Rehydration Solution and RNase A Solution.

Estimation of DNA purity and concentration

The purity and concentration of DNA samples were measured by nanodrop spectrophotometer, the 260/280 ratio and concentration were measured by using 2 µl of each sample, 1.8-1.9 ratio was considered pure and a concentration of 10-100 ng/µl was accepted.

Polymerase Chain Reaction (PCR)

The *pap*E gene is one of the genes associated with P fimbriae (pyelonephritis-associated pili) in *Escherichia coli*. Detecting *pap*E is important for:

- Identifying uropathogenic *E. coli* (UPEC) strains
- Studying virulence factors associated with urinary tract infections (UTIs)
- PCR (Polymerase Chain Reaction) is commonly used because it allows rapid, sensitive and specific detection of target genes

Materials and Reagents

- DNA template: Genomic DNA extracted from bacterial isolates
- Primers: Specific for *pap*E gene (forward and reverse).
- PCR reagents:
- Taq DNA polymerase
- dNTPs
- $MgCl_2$
- PCR buffer
- Nuclease-free water
- Thermocycler
- Agarose gel electrophoresis equipment
- DNA ladder (to determine amplicon size)

Primer Design

A typical primer set for *pap*E:

Primer	Sequence (5' → 3')	Amplicon size
Forward (*pap*E-F)	ATGACCGTGTTGCTGCTG	~300-500 bp
Reverse (*pap*E-R)	TACGACGACGACGATGAC	~300-500 bp

Exact sequences should be based on published literature or NCBI database for *pap*E.

PCR Protocol

The PCR amplification mixture which used for detection of each gene includes FIREPol® Master Mix 5X (4 µl), 5 µl of DNA template, 1 µl (1 mM) of each forwarded and

Table 3: PCR mixture components for *pap*E gene

Component	Volume (µl)	Final Concentration
FIREPol® Master Mix, 5X	5	1 X
Forward primer	1	1 Mm
Reverse primer	1	1 µM
DNA template	2	25 ng
Nuclease free dH2O	11	
Final volume	20	

Table 4: The optimum condition of gene detection

No.	Phase	Temperature (°C)	Time	No. of cycle
1	Initial denaturation	95°C	3 min.	1 cycle
2	Denaturation	95°C	45 sec	
3	Annealing	60°C	45 sec	30 cycles
		55°C		
4	Extension	72°C	50 sec	1 cycle
5	Final extension	72°C	10 min.	

reversed primers and 9 µl of nuclease-free water to complete the amplification mixture to 20 µl (Table 3).

After preparing the reaction volume in the PCR tube the mixture was spin down and then the PCR tube was placed in the PCR thermocycler and the amplification reactions were started according to the program described in the Table 4. Agarose gel was mixed with 5 µl red safe stain. DNA band was visualized by electrophoresis and captured by gel documentation system to the observed band.

Agarose Gel Electrophoresis

- Prepare 1.5% agarose gel with ethidium bromide or safer alternatives (e.g., GelRed)
- Load 5-10 µL PCR product with loading dye
- Run gel at 80-100 V for 30-45 min
- Visualize bands under UV light
- Compare bands to DNA ladder to confirm expected size (~300-500 bp for *pap*E)

Data Analysis

The frequency of *papE*-positive isolates was calculated and correlations with clinical or demographic data (if available) were assessed using descriptive statistics.

RESULTS AND DISCUSSION

Sample Collection

According to Sex Distribution: Out of the 116 clinical bacterial isolates, 41 (36.6%) were from male patients, while 71 (63.4%) were from female patients. As shown in Table 5.

A study in Iraq by Jassim *et al.* [10] on bacterial infections in patients with UTIs showed a similar trend, with a higher percentage of UTIs caused by *E. coli* and other pathogens in female patients. In their cohort, 65% of the isolates were from females, which is very close to your study's finding of 63.4%. They also observed a significant difference ($p<0.01$) in the frequency of infections between males and females, similar to your results.Another Iraqi study by Al-Kubaisy *et al.* [11] found that females were more frequently affected by UTIs and had a higher isolation rate of *E. coli* and other bacterial pathogens (about 60-70%), suggesting that sex-specific factors, such as anatomy, hormonal differences and higher rates of self-inoculation, might contribute to the higher susceptibility in women.

A study by Hirsch *et al.* [12] from the United States on bacterial infections in UTIs showed that females accounted for about 70% of the UTI cases. This is consistent with your study in terms of the higher prevalence of UTIs in females, although the percentages vary slightly. They also reported a significant association ($p<0.01$) between sex and the likelihood of having a UTI, with females showing a higher incidence.

In European studies, particularly in Germany, males accounted for around 40% of UTI isolates in a study by Berg *et al.* [13], with a similar Chi-Square result indicating a significant association between sex and bacterial infections. These studies emphasize that although women are more frequently diagnosed with UTIs, males with underlying conditions like diabetes or urinary tract abnormalities may also be at higher risk for infection.

The results of this study are consistent with both local and international trends, where female patients are disproportionately affected by urinary infections. These findings could be attributed to factors such as the shorter urethra in females, which facilitates easier access for bacteria to the urinary tract, as well as hormonal differences that may influence the immune response. In addition, anatomical differences and sexual activity are also significant contributors to increased UTI risk in women. This test result of $p = 0.0046$ confirms that the difference in the number of isolates between males and females is not random, further supporting the hypothesis that females are more prone to urinary tract infections.

Bacterial Isolation and Identification

Presumptive *Escherichia coli* 116 isolates were initially identified based on colony morphology on MacConkey and blood agar, followed by standard biochemical tests. *E. coli* isolates were identified depending on their chemical characteristics. The isolate appeared as bright pink colonies when cultured on MacConkey agar and the colonies appeared green metallic sheen on EMB media. This results from MacConkey agar containing crystal violet and bile salts which allows Gram-negative bacteria to grow while

Table 5: Distribution of samples according to Sex

Sex	No. of isolates (%)
Male	41 (36.6%)
Female	71 (63.4%)
Total	116 (100%)
Chi-Square (χ^2)	8.035**
p-value	0.0046

**p≤0.01

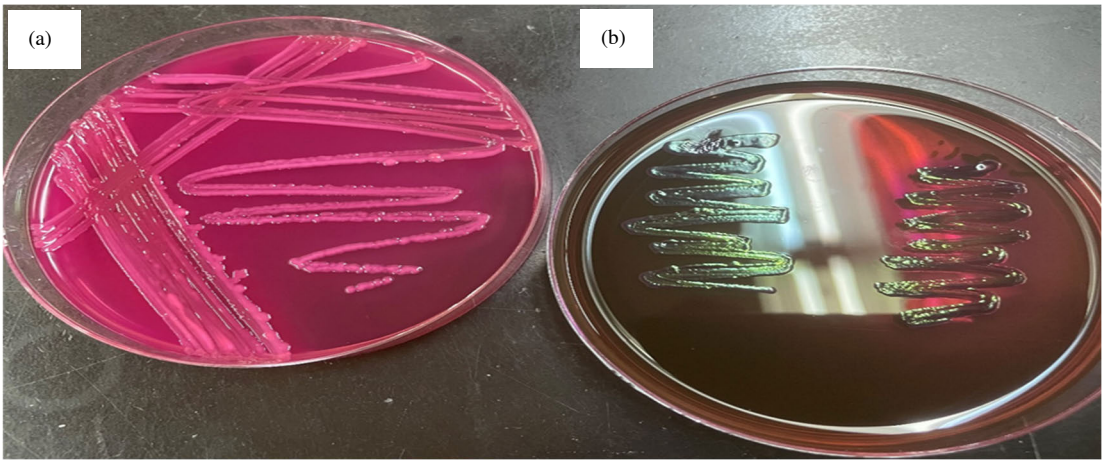

Figure 1(a-b): Bacterial growth on 3 different mediums, (a) Bacterial colonies on MacConkey agar, notice the bright pink colonies and (b) Sheen green metallic colonies on EMB media, (c) Hemolysis on blood agar plates, circular, convex and smooth colonies

inhibiting Gram-positive bacteria growth. It was found that the bacteria were sugar fermenters as shown in Figure 1a, on Eosin Methylene Blue (EMB), which is a differential medium for *E. coli* used to differentiate it from other Enterobacteriaceae members. Sheen green metallic colonies were observed, which means that the colonies produced organic acids due to glucose and lactose fermentation, which, in the presence of eosin and methylene, gives a sheen green metallic color, as shown in Figure 1b. The isolates were also cultured on sheep blood agar to test their ability to lyse red blood cells and as shown in Figure 1, the isolates did lyse red blood cells and cause hemolysis.

Microscopic Examination

A smear was made from a colony grown on MacConkey agar and it was stained with Gram's stain. The bacteria appeared short rods, negative to Gram's stain and not spore forming and this is what was mentioned in Jochum *et al.* [14].

Biochemical Tests

Biochemical Tests All the isolates All the isolates were subjected to biochemical tests and represented as in Table 6 and for all the isolates gave (+) result for catalase test because it dissolved the reagent to water and oxygen [12]. Oxidase test was equally carried out on the isolates and there was no colour changing to burgundy; hence all the isolates were reported negative as the isolates do no possess cythrome oxidase as a hydrogen receptor. Urease test was carried out and all were negative, because the isolates lack the urease enzyme and therefore unable to hydrolyze urea. The bacterial isolates had a positive result to IMVIC test including the indole and methyl red tests that distinguish *E. coli* from the rest of Enterobacteriaceae. The medium pH was significantly reduced by glucose and peptose consumption as indicated by a color change observed in the Methyl red test [8]. The VP test indicated a yellowish-brown color media since the bacteria were unable to utilize glucose in order to produce acetoin. The citrate test was negative, no color change occurred, thus the bacteria were not able to use citrate as their carbon source. The VITEK 2 System was used to validate our identification of the bacterial isolates. The GNID card was also applied to Gram-negative bacteria. The System allows 64 tests within a period of 5-8 hours, which presents a real time to diagnose the isolates without having any mutation occur and with very high sensitivity (approximately 99%) and very low potential of having any error. The vit 2 results are summarized in Table 6 and the vit 2 report in Appendix 2.

Bacterial Resistance to Antibiotics

Thirty *E. coli* isolates were tested for 16 antibiotic discs from different kinds of antibiotics using disc diffusion method and it was found that isolates had varying levels of resistance to different antibiotics (Figure 2).

The study showed that the isolates' resistance to quinolone antibiotics, which includes nalidixic acid, ciprofloxacin and levofloxacin, was at 82.5, 56.3 and 50%, respectively. Cephalosporins, which include cefoxitin, cefotaxime, ceftazidime, cefepime and ceftriaxone, had a resistance of 53.3, 60.0, 76.6, 66.6 and 53.3%, respectively,

Table 6: Identification of *E. coli* by biochemical tests

Isolate	Biochemical test								
	Lactose fermentation	Catalase	Oxidase	Urease	KIA	Indole	MR	VP	*Simmon's citrate*
E. coli	+	+	-	-	A/A, H2S	+	+	-	-

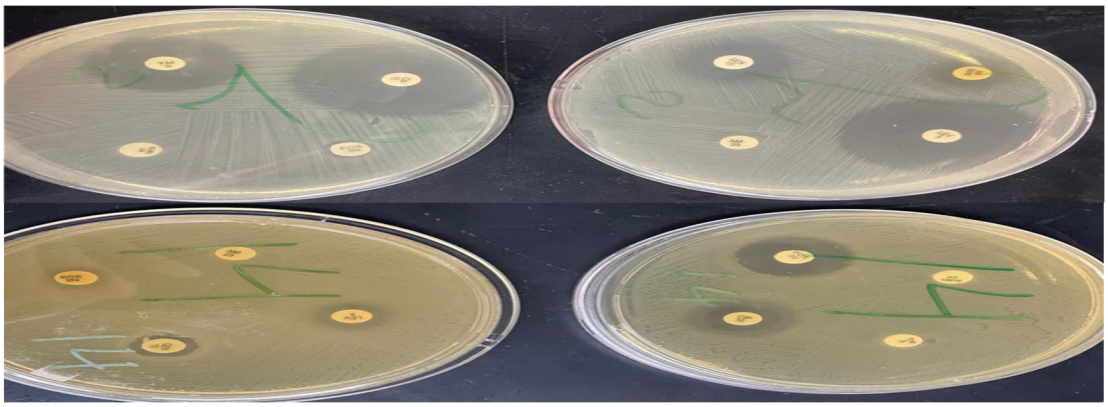

Figure 2: Inhibition zone of Bacterial Resistance to Antibiotics

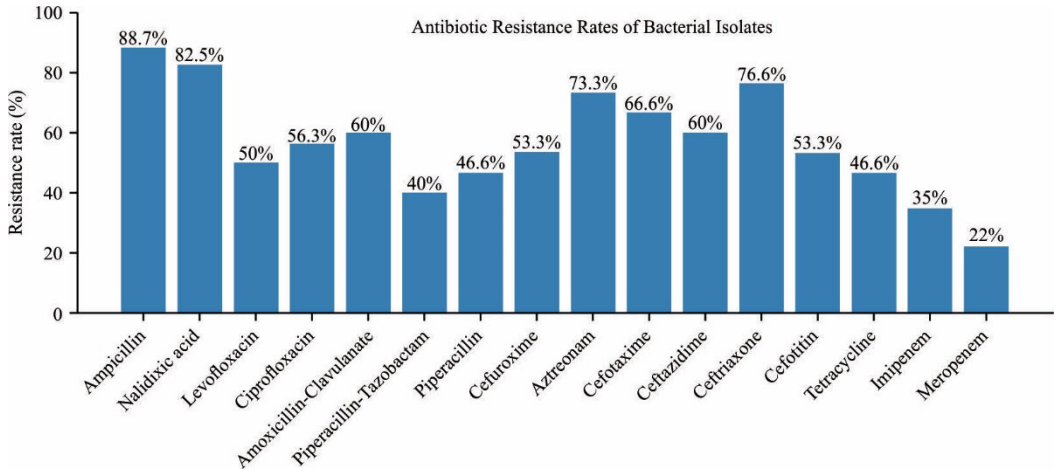

Figure 3: Resistance Rates of the Isolates

for each aforementioned antibiotic. The isolates' resistance to carbapenems, which includes meropenem and imipenem, was at 22% and 35%, respectively. As for β-lactam combinations, which include Augmentin and piperacillin-tazobactam, the resistance was found to be at 60 and 40%. And as for the penicillin group of antibiotics that includes piperacillin, the resistance was found to be 46.6%. Tetracycline from the antibiotic group of the same name and chloramphenicol from the antibiotic group phenicol both had a resistance rate of 46.6%. Aztreonam, from the antibiotic group minocycline, had resistance at 73.3%. Table 7 detail the resistance rates and the number of isolates that were resistant (Figure 3).

The findings of this study highlight significant resistance rates among bacterial isolates to multiple antibiotic classes. These results are consistent with growing global concerns regarding antimicrobial resistance (AMR), particularly in regions with high antibiotic usage and limited regulation. Quinolones (Nalidixic acid, Ciprofloxacin, Levofloxacin). Current study resistance rates were 82.5% (nalidixic acid), 56.3% (ciprofloxacin) and 50% (levofloxacin). A study conducted in Wasit province reported resistance to nalidixic acid at 44.7% and ciprofloxacin at 26.3% among *E. coli* isolates from outpatients with UTIs. In Kirkuk, resistance rates to nalidixic acid and ciprofloxacin were 58.3% and 28.57%, respectively. Comparison: This study shows markedly higher resistance rates, possibly reflecting differences in antibiotic usage patterns or population sampling. Globally, quinolone and cephalosporin resistance remain high but is often lower than observed in Iraq [15]. Carbapenems still show relatively low resistance worldwide, particularly in high-resource settings with antibiotic stewardship programs. Studies from Europe and North America report ciprofloxacin resistance rates ranging from 10% to 35% and carbapenem resistance below 10% in *E. coli*. The high resistance rates observed in Iraq, including in your study, likely reflect overuse, misuse and lack of consistent infection control policies [16].

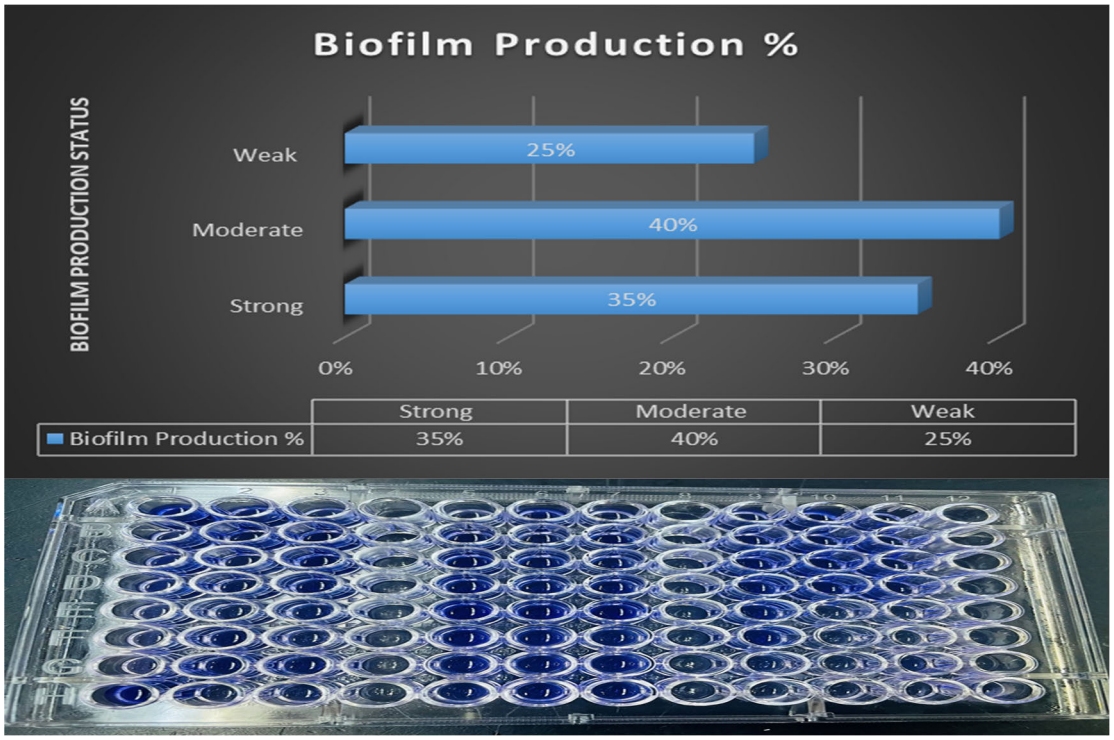

Figure 4(a-b): Biofilm production testing: MTP method results

Biofilm Detection

A microtiter plate (MTP) was also used to detect biofilm production in the isolates and it was found that most of the isolates were capable of producing biofilms; 20 isolates (66.7%) were biofilm producers out of 30 isolates,7 isolates accounting for 35% were strong biofilm producers, while 8 isolates (40%) were moderate biofilm producers. The remaining 5 isolates, accounting for 25% of the total isolates, were weak biofilm producers, as shown in Figure 4(a-b) shows a chart of MTP results. MTP is a quantitative method using 96 wells to determine the strength of the biofilm produced by the isolates using spectrophotometer at a wavelength of 630 nm to give a final digital value representing the quantity of biofilms produced by the bacterial suspension in the wells.

Hassuna *et al.* [17] evaluated biofilm formation in Escherichia coli isolates from urinary tract infections in Iraq using the MTP method. They reported that 62.5% of isolates were biofilm producers, with 30% strong, 42.5% moderate and 27.5% weak biofilm producers [17]. This aligns closely with your findings and underscores the consistency of biofilm formation among *E. coli* isolates in Iraq. Al-Kubaisy *et al.* [11] also found that approximately 70% of UTI-causing *E. coli* isolates in Baghdad were biofilm producers, emphasizing the high prevalence of biofilm formation in clinical strains within the country [18].

According to a study in India by Soto *et al.* [19] using the MTP method reported 65% of uropathogenic *E. coli* isolates as biofilm producers, of which 25% were strong producers, 40% moderate and 35% weak. This is highly comparable to your results and supports the generalizability of your findings. While Soto *et al.* [20] in Spain reported that 56% of *E. coli* isolates from UTIs were biofilm producers, which is slightly lower than your study's finding but still indicates a significant proportion of clinical isolates exhibiting this trait.

The high prevalence of biofilm production observed in your study is consistent with both regional and international research. Biofilms provide bacteria with protection against antibiotics and host immune responses, leading to persistent and recurrent infections. Strong and moderate biofilm producers pose particular challenges in clinical settings due to their increased tolerance to antimicrobial therapy and potential to cause chronic infections [10]. The MTP method employed in your study is a reliable and cost-effective quantitative tool widely used in microbiological research. Its use allows for direct comparison with previous studies and contributes to a growing body of evidence emphasizing the importance of targeting biofilms in infection control strategies.

Identification of *E. coli* by Using *pap*E Gene

Whole genomic DNA was extracted from *E. coli* isolates and electrophoresis in 1.5% agarose. The polymerase chain reaction procedure was used for amplifying the *pap*E gene by using appropriate primers. Distinct yields for band sizes that are approximately equal to 545 bp were obtained compared with the ladder that was used (Figure 5).

PCR Amplification of *pap*E Gene

This was employed to detect the presence of the *pap*E gene-a key virulence determinant associated with the P fimbriae

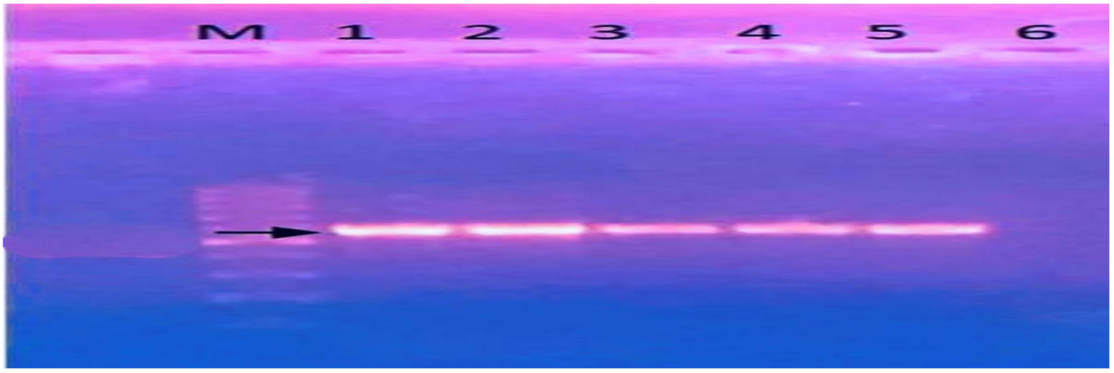

Figure 5: A single-gene PCR amplification of, pap, genes of *Escherichia coli* samples were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-15 on each image resemble, 545bp (Pap), PCR products

in uropathogenic *E. coli*. Specific primers targeting the *pap*E gene sequence were used. The PCR conditions, including annealing temperature and cycle number, were optimized to achieve reliable amplification.

Gel Electrophoresis Results

Amplified PCR products were visualized on a 1.5% agarose gel stained with ethidium bromide. A 100 bp DNA ladder was used as a molecular weight marker to determine the size of the amplified fragments. The results showed distinct bands at approximately 545 bp, indicating the successful amplification of the *pap*E gene from the tested isolates.

Jassim *et al.* [10] used PCR to detect the pap operon genes (*pap*C and *pap*E) in uropathogenic *E. coli* isolates. They reported successful amplification of *pap*E at a similar expected size (~545 bp), confirming the gene's presence in a significant proportion of the isolates [10]. Al-Kubaisy *et al.* [11] also reported amplification of pap genes from UTI-causing *E. coli* strains using similar PCR methods. The amplicon size was comparable (approximately 550 bp), aligning with your findings [11].

Soto *et al.* [20] conducted PCR detection of pap genes and found consistent amplicon sizes (~550 bp) using gene-specific primers in clinical *E. coli* isolates from Spain [20]. Totsika *et al.* [21] in Australia also reported amplification of *pap*E in UPEC strains using PCR, with expected band sizes ranging from 540-550 bp depending on primer design [21].

The successful detection of a 545 bp band corresponding to the *pap*E gene confirms its presence in the tested isolates and aligns with previous studies in Iraq and globally. The consistent amplicon size validates the specificity and reliability of the primers used and highlights the importance of the *pap*E gene in the pathogenicity of uropathogenic *E. coli.* This result supports the molecular characterisation of virulence factors in clinical isolates, which is essential for understanding bacterial pathogenicity and developing targeted infection control strategies [12].

The *pap*E gene, as part of the P fimbrial operon in uropathogenic *E. coli*, may have diagnostic value. Its detection can help distinguish pathogenic UPEC from commensal *E. coli* and is often associated with upper urinary tract infections such as pyelonephritis. PCR-based identification of *pap*E offers a rapid supplementary tool for diagnosis, although its reliability increases when combined with other virulence markers (e.g., *pap*C, fimH). Further validation is needed before routine clinical application.

Primary Recommendation

- Complete and report analysis of *pap*E gene results
- Suggest expanding sample size and including multi-center data
- Recommend formal analysis of gene-antibiotic/biofilm associations
- Reinforce strict ethical and methodological adherence in future work

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