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Detection of Uropathogenic Specific Protein Gene (*usp)* and Multidrug Resistant Bacteria (MDR) of Pathogenic *Escherichia coli* Isolated from Baghdad City

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Abstract:

Escherichia coli is gram negative bacteria and represents a typical resident of the digestive systems of both humans and animals. The stability and equilibrium of the luminal microbial flora are significantly influenced by E. coli. The Escherichia coli uropathogenic-specific protein (Usp) represents type of genotoxins produced by uropathogenic E. coli rather than fecal E. coli isolates. In the current study E. coli was isolated from urine and stool and usp gene was detected in it. Sensitivity test was evaluated by using different types of antibiotics and the usp gene was detected by PCR in all bacterial isolates. Antibiotics sensitivity test showed variable degrees of sensitivity and resistance. High percentage of sensitivity was achieved against amikacin (86%) and that for ciprofloxacin, ceftazidime, gentamicin and tobramycin it was 80%. Whereas trimethoprim and aztreonam showed 64% and 60% sensitivity respectively. In this study, most isolates were resistant to amoxicillin 92%; while they showed different degrees of resistance against other types of antibiotics (from tetracycline 62% to amikacin 4%). The frequency of multi drug resistant (MDR) bacteria was about 64 % (32 isolates), 30 from urine and 2 from the stool. The results showed that usp gene was found in 26 bacterial isolates (52%), whereas other 24 (48%) isolates didn't have this gene. It was concluded that source of bacterial isolates carry usp gene was urine, with the exception of one isolate from f stool, and E. coli recorded as multidrug resistant bacteria (MDR).

Keywords: *E.coli*, *usp* gene, UTI, PCR, MDR.

الكشف عن جين البروتين النوعي الممرض البولي (*usp*) والبكتيريا المقاومة للأدوية المتعددة (MDR) من الإشريكية القولونية الممرضة المعزولة من مدينة بغداد

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الخلاصة:

الإشريكية القولونية هي بكتريا سالبة لصبغة غرام تتواجد بشكل طبيعي في الجهاز الهضمي للانسان والحيوانات. يتاثر الإستقرار والتوازن المايكروبي المعوي بشكل كبير بوجود هذه البكتريا. يمثل البروتين النوعي البولي (Usp) نوعا من السموم الجينية التي تنتج بوساطة بكتريا الإشريكية القولونية البولية مقارنة بافرازه من قبل بكتريا الإشريكية القولونية البرازية. في الدراسة الحالية تم عزل بكتريا الإشريكية القولونية من البول والبراز

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وكذلك الكشف عن جين الـ *usp* فيها. في هذه الدراسة تم تقييم اختبار الحساسية بإستخدام أنواع مختلفة من المضادات الحيوية وتم الكشف عن جين *usp* بواسطة تفاعل البلمرة المتسلسل في جميع العزلات البكتيرية. اظهر اختبار حساسية المضادات الحيوية درجات متفاوتة من الحساسية والمقاومة. تم تحقيق نسبة عالية من الحساسية تجاه الأميكاسين بنسبة 86٪ والسيبروفلوكساسين، السيفتازيديم ،الجنتاميسين والتوبراميسين بنسبة 80٪، بينما تريميثوبريم وأزترونام بنسبة 64٪ و 60٪ على التوالي. في هذه الدراسة كانت معظم العزلات مقاومة للأموكسيسيلين 92٪. بينما أظهرت العزلات درجات مختلفة من المقاومة ضد الأنواع الأخرى من المضادات الحيوية تراوحتبين62٪ ضد التتراسيكلين إلى 4٪ ضدالأميكاسين. بلغ تردد البكتريا متعددة المقاومة للمضادات الحيوية (MDR) حوالي 64٪ (32 عزلة) و 30 عزلة من البول و 2 من البراز ، وأظهرت النتائج أن جين *usp* وجد في 26 عزلة بكتيرية (52٪) ، في حين أن 24 عزلة أخرى (48٪) غير موجودة.. نستنتج من هذه الدراسة ان مصدر العزلات البكتيرية التي تحمل الجين *usp* هو البول باستثناء عزلة واحدة كان فيها مصدر الجين المذكور هو البراز وان بكتريا الإشريكية القولونية تعتبر متعددة المقاومة للمضادات الحيوية.

1.Introduction

Escherichia coli normally lives in the gut as a commensal bacteria. However, certain pathogenic strains that release virulence factors can result in a range of extraintestinal and intestinal illnesses. The severity of urinary tract infection (UTI) conncected to *E. coli* is due to the expression of a wide spectrum of virulence factors [1]. The uropathogenic specific protein (*usp*) gene was discovered when looking for homologues of the *Vibrio cholerae* zot gene in the uropathogenic *E. coli* (UPEC) strain Z42 recovered from a prostatitis patient [2]. Despite the fact that the *usp* gene might also be identified in non-UPEC isolates, usp-positive bacteria predominate in UTI isolates [3]. Additionally, due to its ability to endow non-pathogenic *E. coli* with infectious capability, Usp is thought to be a crucial factor for UPEC infection [4].

The *usp gene* and its associated genes, imu1-3, are found in strains of *E. coli* that cause prostatitis, pyelonephritis and bacteremia of urinary tract origin. The activities of the three related proteins and the Usp C-terminal domain are unknown, despite the fact that they share similarities with DNase-like colicins and pyocins [5, 6].

Based on sequence homology research, Parret and Mot hypothesized that Usp might be a bacteriocin [7, 8]. Most nuclease-type bacteriocins have three functional domains: the translocation, receptor recognition and nuclease domain, each of which is accountable for recognizing a specific receptor protein on the goal cell membrane, allowing the protein to enter the target cell, and degrading the chromosomal DNA of the goal cell [9, 10]. Usp has not been given a specific function, but it has been reported that it is more frequently associated with UPEC strains than fecal *E. coli* isolates and that it increases the infectious potential of *E. coli* strains in mouse models of pyelonephritis, which may indicate that Usp is involved in the pathogenesis of UPEC [4].

As the number of *E. coli* strains resistant to many drugs has risen, due to overuse and misuse of antibiotics, recent years have seen a rapidly increasing prevalence of antibiotic-resistant *Escherichia coli* globally, so treatment options have become more limited [11, 12, 13]. Numerous research has revealed that UPEC antibiotic resistance is rising annually [14]. *E. coli* infections in hospitals and the community are frequently treated with cephalosporins, fluoroquinolones and trimethoprim-sulfamethoxazole. Resistance to these medications causes delays in the administration of the proper therapy which increases morbidity and death [15, 16]. This study was aimed to detect *usp* gene in pathogenic *E. coli* and investigate the antimicrobial sensitivity for different types of antibiotics against these bacteria.

2.Materials and Methods:

2.1 Isolation and identification

Urine and stool samples, totaling 120, were gathered in sterile containers from several hospitals of Baghdad. The obtained samples were streaked immediately onto MacConkey, Eosin methylene blue (EMB), and HiCrome UTI agars (Himedia/India) in the laboratory under aseptic circumstances where they were then incubated for 24 hours at 37°C. On a different MacConkey and EMB agar. pink colonies were selected and re-cultured. Depending on Forbes *et al.* research [17], biochemical tests were done for further identification of bacterial isolates Vitek 2 compact system test was performed (BIOMÉRIEUX) for the confirmation.

2.2 Antibiotic sensitivity test:

Antimicrobial susceptibility testing was performed by Kirby-Bauer test [18]. In brief: A McFarland 0.5 adjustment was made to the suspension of overnight bacterial growth. A sterile cotton swab was used to apply the bacterial suspension to the Muller-Hinton agar plate's surface. After being inoculated, the plates were later left at room temperature for 30 minutes so that any extra moisture could be absorbed. Then using sterile forceps, the antibiotic disks were adhered to the agar surface. Each isolate was tested for antibiotics susceptibility: amoxicillin (A) 10 µg, tobramycin (TOB) 30 µg, gentamicin (GEN) 10 µg, tetracyclin (TE) 30 µg, ciprofloxacin (CIP) 5 µg, aztreonam (ATM) 30 µg, ceftazidime (CAZ) 30 µg, amikacin (AK) 30 µg, piperacillin (PRL) 30 µg, and trimethoprim (TMP) 5 µg (Himedia\India). The plates were then incubated for 24 hours at 37°C. Each antibiotic disk's inhibition zone was measured using a metric ruler and the results were recorded as resistant, sensitive, or intermediately resistant in accordance with the Clinical and Laboratory Standards Institute (CLSI) breakpoints, 2020 (Table 1).

Table 1: Antimicrobial agents used in the current study (CLSI, 2020)

Id	Antimicrobial Agent	Disc Potency (µg /Disc)	Diameter of Zone Inhibition (mm)		
			Sensitive	Intermediate	Resistant
1	Ceftazidime (CAZ)	30	≥ 21	18-20	≤ 17
2	Ciprofloxacin (CIP)	5	≥ 26	22-25	≤ 21
3	Gentamicin (GEN)	10	≥ 15	13-14	≤ 12
4	Aztreonam (ATM)	30	≥ 21	18-20	≤ 17
5	Trimethoprim (TMP)	5	≥ 16	11-15	≤ 10
6	Amikacin (AK)	30	≥ 17	15-16	≤ 14
7	Amoxicillin (A)	10	≥ 18	14-17	≤ 13
8	Tetracyclin (TE)	30	≥ 15	12-14	≤ 11
9	Tobramycin (TOB)	30	≥ 15	13-14	≤ 12
10	Piperacillin (PRL)	30	≥ 21	18-20	≤ 17

2.3 Screening the genotoxins producing isolates.

Local *E. coli* isolates were examined to find the most effective isolates for producing DNase. Ability to produce DNase was tested after incubating these isolates at 37°C on DNase test agar with toluidine blue. The DNA hydrolyzing effect was identified by changing the color from blue-purple to pink [19].

2.4 Detection of *usp* gene by PCR:

2.4.1 DNA extraction

In this study, 50 isolates of *E. coli* were selected for detecting *usp* gene, 35 isolates from urine and 15 from diarrheagenic stool. Genomic DNA was extracted from these isolates by using commercial Wizard genomic DNA purification kit, (Promega, USA). And then DNA concentration and purity were determined by using Quantus Fluorometer**.**

2.4.2 PCR amplification

According to a prior study, the specific pair of primers' sequence was employed [20]. PCR reaction was used to detect bacteria that possess *cdtB and clbA* genes. The PCR reactions were done in 25 µl volume and comprised 12.5 µl of green master mix (Promega, USA), 1 µl of each primer (10 Pmol) and 2 µl DNA template. Deionized distilled water was used to bring the final reaction volume to 25 µl. The thermocycler cycling conditions were 1 cycle of denaturation at 94°C for 2 min, annealing for 1 min, elongation at 72°C for 1 min then 30 cycles of denaturation at 94°C for 1 min. The PCR reaction products were stored at -20°C or immediately separated on 2% agarose gels.

3.Results and Discussion:

3.1 Isolation and Identification

Fifty bacterial isolates from *E. coli* were achieved after culturing it on different culture media such as MacConkey, EMB and HiCrome UTI agars. The uropathogenic. *E. coli* represented 35 isolates (70%), while the other 15 isolates (30%) were isolated from stool and represented enteropathogenic *E. coli*. The current study findings showed that the majority of *E. coli* isolates were from urine samples (70%) compared to stool (30%) since it is the primary causal pathogen in women's recurrent UTI which accounted for 80% of all infection episodes [21]. The current study findings showed that urine samples contained a higher percentage of *E. coli* isolates than stool samples (70 percent versus 30 percent) likely because this pathogen is the primary culprit in recurrent urinary tract infections (UTI) in women which accounts for about 80% of all episodes of infection [22]. Around 90% of urinary tract infections are caused by uropathogenic *E. coli*; the bacteria invade from feces or the perineum and ascend into the urinary tract and then to the bladder [17, 23]. Specific virulence traits that are strongly associated to bacterial colonization and persistence in the urinary system, are used to identify UPEC strains. These elements consist of poisons, siderophore systems, and adhesins or fimbriae [24, 25, 26].

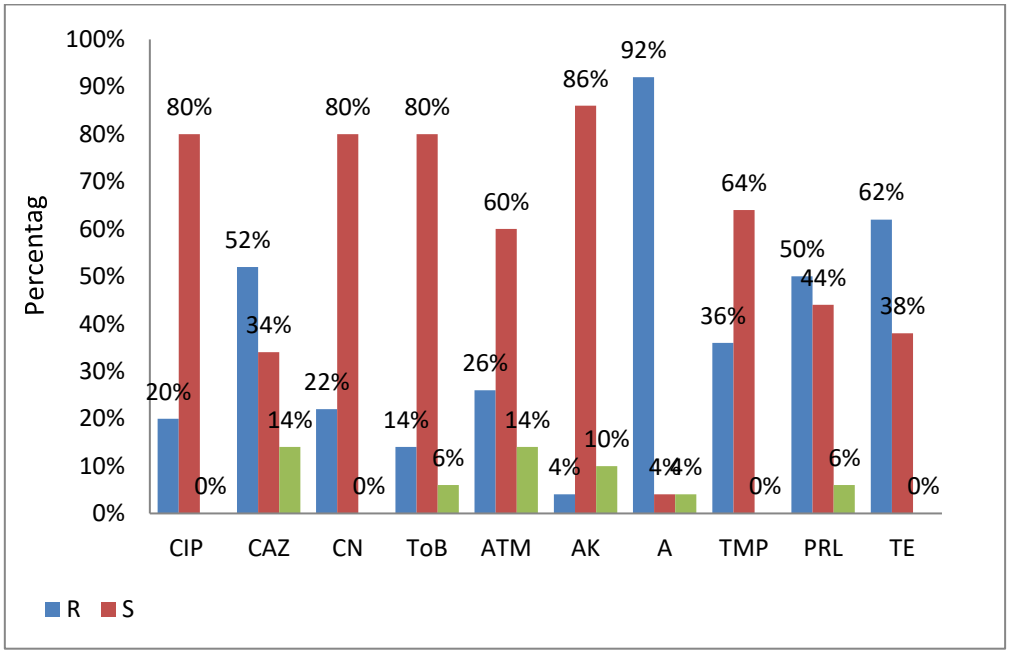
A Local study by Shukur [27] reported that *E. coli* isolates formed 42 (85%) of urine specimens. Another local study by Alfuraiji *et al.,* [28] reported that UPEC prevalence amongst the urine specimens collected from male and female pediatric patients was 34.44% and 40.00% respectively.

3.2 Antibiotic sensitivity test:

Antibiotic susceptibility test was done according to Kirby-Bauer method by using ten types of different antibiotic discs with different concentrations. The results showed variations in the susceptibility of isolates to different types of antibiotics depending on the antibiotic type and the source of bacterial isolate.

High sensitivity ratio was achieved by amikacin, ciprofloxacin, tobramycin and gentamicin, 86% for amikacin and 80% for each one of other antibiotics respectively. Aztreonam and trimethoprim showed 60% and 64% sensitivity respectively. In this study, most isolates were resistant to amoxicillin (92%). Furthermore, the isolates that showed resistance to other antibiotics involved tetracycline (62%), ceftazidim (52%) and piperacillin (50%). Whereas only

10 (20%) isolates were resistant to ciprofloxacin, 11 isolates (22%) to gentamicin, 7 isolates (14%) to tobramycin, 13 isolates (26%) to aztreonam, 18 isolates (36%) to trimethoprim and 2 isolates only (4%) to amikacin (Figure 1). A Local study by Sweedan *et al*. [29] reported that uropathogenic *E. coli* resisted to all antibiotics under study such as ceftazidime, cefotaxime, amikacin, amoxicillin, ciprofloxacin, trimthoprim, kanamycin, norfloxacillin, and cefalexine, tetracycline, doxycillin. Another study by Mohammed and Ibrahim [30] reported that *E. coli* was highly resistant to trimethoprim (82%) and cefotaxime (82%) which disagrees with the results of present study, hence concluding that *E. coli* was 64% sensitive to trimethoprim.



R= Resistant, S= Sensitive, I= Intermediate

Figure 1: The percentage of susceptibility pattern for *Escherichia coli* isolates against antibiotics.

All isolates that showed resistance to at least three antibiotics belonged to three different classes, were regarded as multidrug-resistant (MDR) [31]. In the current study, the percentage of MDR bacteria represented 64%), whereas, 30 isolates were recorded as MDR among the 35 isolates taken from the urine. It was observed in this study that only two isolates out of 15 taken from stool were MDR. A Local study by Abdul-Ghaffar and Abu-Risha [32] also reported *E. coli* as multidrug resistant bacteria which agrees with the present study.

Over the past few decades, multidrug-resistant *E. coli* has emerged in a number of nations. Concerns over how to treat *E. coli* sickness are growing due to its growth in cephalosporin resistance, particularly given the parallel rise in the prevalence of multidrug-resistant *E. coli* [33].

Multidrug-resistant *E. coli* has been seen more frequently over the past few decades. The developing cephalosporin resistance, notably the accompanying rise in the prevalence of multidrug-resistant *E. coli*, is raising concerns about the treatment of *E. coli* disease [33, 34]. Multidrug-resistant *E. coli* strains have become more common which has limited treatment options [11, 12]. According to several researches, antibiotics resistance in UPEC is rising annually [13]. Antibiotic therapy is crucial for the treatment of bacterial infections. Gram-negative and -positive bacterial infections are commonly treated with the second-generation fluoroquinolone antibiotic CIP. In a different study on UTIs in Iran, the MDR *E. coli* isolates

percentage was 82.1% [35, 36]. A study by Al-Hasnawy *et. al.* [37] concluded a high prevalence of UPEC with MDRisolated from urinary tract infection in Babylon province, Iraq. Major repercussions of MDR include the empirical treatment of *E. coli* infections and a potential co-selection of antibiotic resistance which is mediated by MDR plasmids [38, 28].

3.3 Screening the genotoxins producing isolates.

The results of current study revealed that all *E. coli* isolates have the ability to produce DNase enzyme and caused DNA damage, so all bacterial isolates may have genotoxic activity **(Figure 2).**

As a result of the indicator metachromatic qualities, the area around the organisms that have the DNase enzyme appears to have a vivid rose-pink color. It is advised for the detection of DNase in gram-negative microbes because toluidine blue may be inhibitory to some gram-positive species [39].

A.Positive result on DNase agar. B. DNase agar (Control)

Figure 2: *E. coli* on DNase agar

A quick agar plate method for demonstrating the DNase activity of microorganisms was published by Jeffries *et. al*. in 1957 [40]. This approach made use of a semi-synthetic medium that also contained a nucleic acid solution. Placing a flood of 1 N hydrochloric acid on the plate allows for the detection of enzyme activity (HCl). A clear zone around growth denotes a favorable response. A metachromatic dye is present in DNase test agar with toluidine blue to do away with the need to add reagents to the agar after incubation. Toluidine blue should only be used with Enterobacteriaceae since it may be harmful to some gram-positive cocci [14].

3.4 Detection of usp gene by PCR:

This study was carried out to detect *usp* gene in all 50 *E. coli isolates* by PCR technique. Specific primers for this gene were used for detecting its presence which is responsible for the production of urinary specific protein. The results showed that 26 isolates were positive for the *usp* gene, whereas 24 isolates didn't have this gene (Table 1). All these isolates were taken from urine, except one that was from stool. The product of PCR was detected by using gel electrophoresis (Figures 3, 4 and 5).

Table 1: Frequency of usp gene in *E. coli* isolated from urine and stool.

Isolates	Source	*usp* Gene	Isolates	Source	*usp* Gene
E1	Stool	-	E26	Urine	+
E2	Stool	-	E27	Urine	-
E3	Stool	-	E28	Urine	+
E4	Stool	-	E29	Urine	+
E5	Stool	-	E30	Urine	-
E6	Stool	-	E31	Urine	+
E7	Stool	-	E32	Urine	+
E8	Stool	-	E33	Urine	-
E9	Stool	-	E34	Urine	+
E10	Stool	-	E35	Urine	+
E11	Stool	-	E36	Urine	+
E12	Stool	-	E37	Urine	-
E13	Stool	-	E38	Urine	-
E14	Stool	+	E39	Urine	+
E15	Stool	-	E40	Urine	+
E16	Urine	+	E41	Urine	+
E17	Urine	+	E42	Urine	+
E18	Urine	-	E43	Urine	+
E19	Urine	-	E44	Urine	-
E20	Urine	-	E45	Urine	+
E21	Urine	+	E46	Urine	+
E22	Urine	+	E47	Urine	+
E23	Urine	+	E48	Urine	+
E24	Urine	+	E49	Urine	+
E25	Urine	+	E50	Urine	-

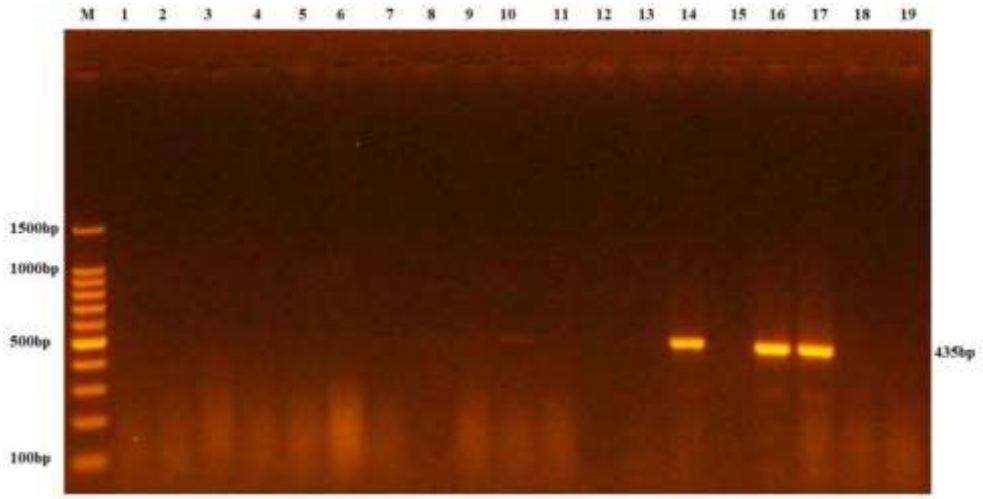


Figure 3: Results of the amplification of *usp gene* of *Escherichia coli* samples were fractionated on 1.5% agarose gel electrophoresis stained with ethidium bromide. Lane M: 100bp DNA ladder marker, lanes 1-19: PCR products (435bp).

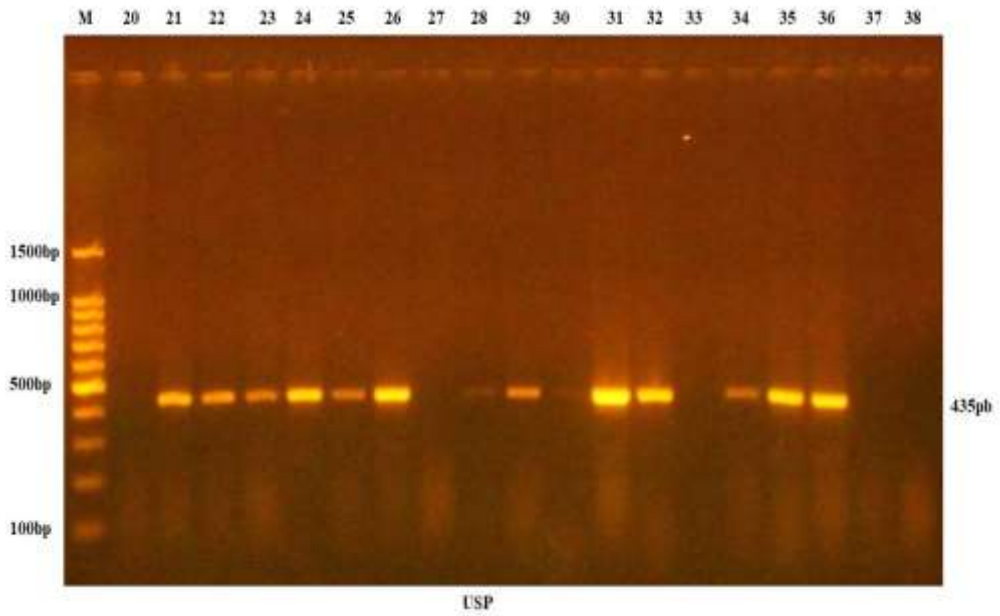


Figure 4: Results of the amplification of *usp gene* of *Escherichia coli* samples were fractionated on 1.5% agarose gel electrophoresis stained with ethidium bromide. Lane M: 100bp DNA ladder marker, lanes 20-38: PCR products (435bp).

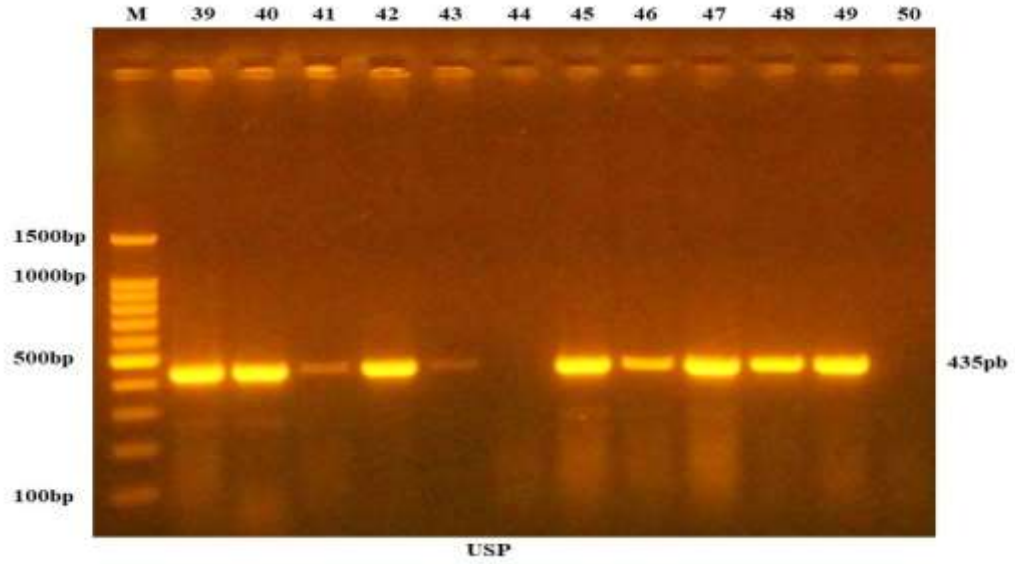


Figure 5: Results of the amplification of *usp gene* of *Escherichia coli* samples were fractionated on 1.5% agarose gel electrophoresis stained with ethidium bromide. Lane M: 100bp DNA ladder marker, lanes 39-50: PCR product (435bp).

Majority of uropathogenic *E. coli* strains seen in instances of pyelonephritis, prostatitis, and urinary tract bacteremia carry the uropathogenic-specific gene, *usp*, which is encoded inside a tiny pathogenicity island [41]. Although the *usp* gene might also be identified in non-UPEC isolates, *usp*-positive bacteria dominate in UTI isolates [42]. Additionally, *usp* is believed to be a significant component contributing to UPEC infection due to its capacity to confer infectious potential to non-pathogenic *E. coli* [4]. In contrast to previous studies of collections of uropathogenic *E. coli* strains, majority of the uropathogenic *E. coli* strains isolated from patients with prostatitis and pyelonephritis (93.4% and 88%, respectively) encoded the uropathogenic-specific gene, *usp*, while only 24% of the strains isolated from fecal specimens from healthy

individuals did so [4]. Additionally, the *usp* gene is highly prevalent in *E. coli* strains recovered from people with urosepsis [43].

In pediatric patients' urine samples, another study discovered that the prevalence of UPEC bacteria was 37.50 percent which was lower than the prevalence recorded in Nepal (68.40%), Saudi Arabia (75.70 %) and Qatar (32.4 %) [44], and higher than its prevalence in Uganda (10.00%) [45] and Ethiopia (25.34%) [46].

4.Conclusions

The current study revealed high frequency of *usp* gene in uropathogenic *E. coli*. MDR spreads highly among uropathogenic and non-hospitalized patients resulting in life threatening infections. Our finding showed that most. *E. coli* isolates were resistant to amoxicillin, while being highly sensitive to amikacin, ciprofloxacin, tobramycin and gentamicin.

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