ZANCO Journal of Pure and Applied Sciences
The official scientific journal of Salahaddin University-Erbil
https://zancojournals.su.edu.krd/index.php/JPAS

**ISSN (print ):**2218-0230**, ISSN (online):** 2412-3986**, DOI: http://dx.doi.org/10.21271/zjpas**

RESEARCH PAPER

# Molecular Characterization of Some Virulence Genes and Antibiotic Susceptibility Pattern among Uropathogenic *Escherichia coli* Isolated from Patient in Zakho City/Iraq

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## A B S T R A C T:

Uropathogenic *Escherichia coli* (UPEC) is one of the most causative agents which causing urinary tract infections (UTIs) in humans. This study involved the prevalence of the virulence genes among UPEC isolated from patients in various hospitals in Zakho city from July 2018 until January 2019 and their susceptibility to different commonly used antimicrobial agent against UPEC. The different culture media were used for the identification of *Escherichia coli* (*E. coli*). Out of 400 samples, 141 (35.25%) strains of UPEC were isolated from enrolled patients. The antibiotic susceptibility toward different antibiotics was varied among the isolates. Imipenem was the most potent antibiotic with a resistant rate of only 2.84%. While the isolates were resistant to most screened antibiotics, with the highest rate 96.45% to Amoxicillin/ clavulanic. The resistant rates decreased toward other antibiotics at rates varied from 93.62% for Amoxicillin to 43.97% for Norfloxacin. Fifty-seven isolates were selected for PCR analysis, according to the resistance of *E.coli* to various antibiotics. The selected samples were successfully amplified for *E. coli* identification by producing a single band of a target *uidA* gene. In this study, the virulence related genes were detected in only 35 (61.40%) isolates out of 57 isolates. The distribution of the virulence related genes that included; *afa*, *sfa*, *hly*, *cnf* and *pai* were 28.07%, 17.54%, 26.32%, 22.81% and 22.81%, respectively. The study highlight that multidrug resistance UPEC harbors multiple virulence genes circulating in this setting.

KEY WORDS: UPEC, Virulence factors, PCR, Antibiotics resistant.
DOI: http://dx.doi.org/10.21271/ZJPAS.32.2.18
ZJPAS (2020) , 32(2);167- 177 .

## INTRODUCTION

The gram negative *E. coli* bacteria is an opportunistic pathogen which is naturally found in the intestinal tract of humans and other mammals, it may cause serious diseases in the intestinal tract and extraintestinal tract (Russo and Johnson, 2000; Kaper *et al.*, 2004). Among extraintestinal pathogenic *E. coli* (ExPEC), the most common strains of *E. coli* that causes UTIs in human is uropathogenic *E. coli* (UPEC) (Russo and Johnson, 2000). In ascending of the UPEC infections, colonization of the *E. coli* in the urethra leads to the spread upward that causes cystitis and pyelonephritis (Bien *et al.*, 2012). Approximately 70-95% of the community-acquired UTIs and 50% of the nosocomial UTIs are due to the UPEC infections (Wiles *et al.*, 2008; Šišková *et al.*, 2015). So, *E. coli* is considered as an important public health problem (Farell *et al.*, 2003; Zhanel *et al.*, 2006).

Several virulence factors of the UPEC play an important role in the colonization, invasion, and survival within the urinary system of the host and causing UTI (Litza and Brill, 2010; Bien *et al.*, 2012; Behzadi *et al.*, 2016). These different

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**Article History:**
Received:15/09/2019
Accepted: 17/11/2019
Published: 22/04/2020

virulence factors that develop the infectious process includes: adhesins (P and S fimbriae), toxins (cytotoxic necrotizing factor type 1 and α-hemolysin) and siderophores (aerobactin and yersiniabactin). Also, a fimbrial adhesin I and type 1 fimbriae are beneficial in this type of infection (Miyazaki *et al.,* 2002; Dobrindt *et al.,* 2002; Dobrindt *et al.,* 2003). The virulence-associated genes are located on transmissible genetic elements, such as plasmids, bacteriophages and pathogenicity islands (PAIs) (Dobrindt *et al.,* 2003). The PAI is located in a specific region on the chromosome (Farshad *et al.,* 2012), and has led to the coordination of the horizontal transfer of virulence genes between strains of one species or related bacterial species (Johnson and Stell, 2000; Dobrindt *et al.,* 2003). Multidrug-resistant (MDR) among *E. coli* have been increased over the years and this is a major clinical problem in treating the UTI infections caused by *E. coli* (Manikandan and Amsath, 2014). The antimicrobials resistance differs from one region to another and the rates of resistance have increased over the years (Tiwary *et al.,* 2017). Multi-drug resistance and extended spectrum beta-lactamases (ESBLs) producing gram negative bacteria are the major cause of infection of the urinary tract (Poirel *et al.,* 2005).

The limited availability of treatment for *E. coli* has particular clinical significance. Moreover, preventing and controlling the spread of UPEC infection is hampered by a misunderstanding of the population biology of these pathogens (Siu *et al.,* 2008). The spread of resistance for antimicrobial agents used for the first line treatment for uncomplicated UTI such as Ampicillin and Trimethoprim/Sulfamethoxazole lead to the use of the alternative choice of antimicrobial agents such as quinolones and cephalosporins (Hryniewicza *et al.,* 2001). The MDR rates of *E.coli* increase for aminoglycosides, fluoroquinolones and third generation cephalosporins, at rates of 63% during 2012 and further increased to 65 % in 2014 over the consecutive years (Sharma *et al.,* 2016).

The aims of this study were firstly to identify the UPEC and the rate of their distribution among patients in the various hospital in Zakho City, Kurdistan Region-Iraq. Secondly to investigate the antibiogram rates for the isolated UPEC and thirdly to perform PCR analysis of some resistant strains in order to identify the five virulence related gene; *afa*, *sfa*, *hly*, *cnf* and *pai*.

## 1. MATERIALS AND METHODS

### 1.1. Sample Collection

In this study, 400 clinical midstream urine samples were collected in clean fully labelled screw-capped containers from patients with UTI. The samples were collected from various hospitals in Zakho city namely; Zakho General hospital, Maternity hospital and Emergency hospital, from July 2018 until January 2019 (for about 6 days/week). Following collection, each sample was cultured on different culture media and UPEC was identified by cultural characters and biochemical tests: indole production, methyl red reaction, citrate utilization and sugar fermentation with gas production.

### 1.2. Culturing of the urine sample

After delivery of urine samples to the laboratory, a loopful of urine samples of each patient was cultured on Blood and MacConkey agars by streaking method and incubated at 37$^{o}$C for overnight. After overnight incubation, a single colony from the suspected UPEC was selected and sub-cultured on selected media (MacConkey agar) to obtain a pure colony. After incubation of the subcultured bacteria, the pure colonies were cultured on different culture media such as Peptone water, Methyl red broth, Simmon citrate agar and TSI agar to ensure that the isolated bacteria was *E. coli* (Alexander and Strete, 2001; Leboffe and Pierce, 2011).

### 1.3. Antibiotic susceptibility test

Sixteen antibiotics discs supplied by Bioanalyses (Turkey) were used for testing the susceptibility of the isolated UPEC, as most of these antibiotics were commonly prescribed by physicians to patients having UTI infection, as listed in Table 1. The disk diffusion method on the Mueller-Hinton agar was performed to detect the sensitivity and resistant of these antibiotics to the *E. coli*. After 24 hours of incubation, the diameters of inhibition zone around each disc was compared with the standard chart and interpreted as sensitive or resistant depending on the size of the inhibition zone (Coyle, 2005; Cheesbrough, 2006).

**Table 1:** Antibiotic discs

| Antibiotics | Code | Potency (mcg) |
|---|---|---|
| Amikacin | AK | 10 mcg |
| Amoxicillin | AX | 25 mcg |
| Amoxicillin/clavulanic acid | AMC | 20/10 mcg |
| Ampicillin | AM | 20 mcg |
| Cefixime | CFM | 5 mcg |
| Cefotaxime | CTX | 30 mcg |
| Ceftriaxone | CRO | 10 mcg |
| Chloramphenicol | C | 10 mcg |
| Ciprofloxacin | CIP | 10 mcg |
| Gentamicin | CN | 10 mcg |
| Imipenem | IPM | 10 mcg |
| Nalidixic acid | NA | 30 mcg |
| Norfloxacin | NOR | 10 mcg |
| Tetracycline | TE | 10 mcg |
| Trimethoprim | TMP | 10 mcg |
| Trimethoprim/ Sulfamethoxazole | SXT | 1.25/23.75 mcg |

### 1.4. Genomic DNA extraction and determination of the concentration

Fifty-seven of the isolated UPEC were selected for molecular identification according to the resistance rates of *E. coli* to 16 used antibiotics. Genomic DNA was extracted by using a commercial extraction kit (PrimePrep$^{TM}$ Genomic DNA Extraction Kit) supplied by GeNet Bio (Korea). After DNA extraction, the concentration of the genomic DNA were detected by using Nanodrop spectrophotometer (Thermo scientific) and then used for PCR amplification.

### 1.5. Primers

Six primers were used in this study, as listed in Table 2, *uidA* primer was used for amplifying the target *uidA* gene as a species-specific primer for *E. coli*. The five other primers were used to detect the spread of five virulence-related genes including; a fimbrial adhesion, S-fimbrial adhesion, hemolysin, cytotoxic necrotizing factor-1 and pathogenicity island, and the five primers which were represented for the virulence gene were *afa*, *sfa*, *hly*, *cnf* and *pai*, respectivly.

### 1.6. PCR amplification and gel electrophoresis

The PCR amplification reaction was prepared in 20µl as a final volume which contained 5µl of the master mix, 2µl of each primer including forward and reverse (10 pmol/µl except for *hly* 30 pmol/µl and *afa* 20 pmol/µl), 2µl of DNA genome (25-50 ng/µl) and 9 µl of PCR grade water. The amplification condition was shown in Table 3. After amplification, the PCR products were run on gel electrophoresis using 1.2% (w/v) of agarose prepared in 1x Tris-Boric-EDTA (TBE) buffer. The running of the electrophoresis was inserted in 45V for 5 min and then changed to 80V for 1 hour. After running, the DNA bands on agarose gel was visualized using U.V. (Cleaver scientific) light source (Ausubel *et al.,* 2003).

**Table 2:** Primers used for detection of Species-specific gene in UPEC and their virulence genes

| Method | Primer | DNA sequence 5'- 3' (forward and reverse) | Amplified product (bp) | Reference or source |
|---|---|---|---|---|
| **Species-specific PCR** | *uidA* | F-CATTACGGCAAAGTGTGGGTCAAT<br>R-CCATCAGCACGTTATCGAATCCTT | 658 bp | (Adamus-bialek *et al.,* 2009) |
| **Virulence genes** | *afa* | F-GCTGGGCAGCAAACTGATAACTCTC<br>R-CATCAAGCTGTTTGTTCGTCCGCCG | 750 bp | (Le-Bouguenec *et al.,* 1992) |
| | *sfa* | F-GTGGATACGACGATTACTGTG<br>R-CCGCCAGCATTCCCTGTATTC | 240 bp | (Chapman *et al.,* 2006) |
| | *hyl* | F-AGATTCTTGGGCATGTATCCT<br>R-TTGCTTTGCAGACTGTAGTGT | 565 bp | (Mladin *et al.,* 2009) |
| | *cnf* | F-AAGATGGAGTTTCCTATGCAGGAG<br>R-CATTCAGAGTCCTGCCCTCATTATT | 498 bp | (Adamus-bialek *et al.,* 2009; Chapman *et al.,* 2006) |
| | *pai* | F-GGACATCCTGTTACAGCGCGCA<br>R-TCGCCACCAATCACAGCCGAAC | 930 bp | (Chapman *et al.,* 2006; Oliveira *et al.,* 2011) |

**Table 3:** The amplification condition of specific species gene and different virulence gene among *E.coli*

| ***uidA* primer** | | | | | **Reference** |
|---|---|---|---|---|---|
| Initial denaturation | Denaturation | Annealing | Extension | Final extension | |
| 94 $^{o}$C | 92 $^{o}$C | 58 $^{o}$C | 72 $^{o}$C | 72 $^{o}$C | (Adamus-bialek *et al.*, 2009) |
| 10 min | 1 min | 1min | 30 sec. | 5 min | |
| 1 cycle | | 35 cycles | | 1 cycle | |
| ***afa* primer** | | | | | |
| Initial denaturation | Denaturation | Annealing | Extension | Final extension | (Le-Bouguenec *et al.*, 1992) |
| 94 $^{o}$C | 94 $^{o}$C | 63 $^{o}$C | 68 $^{o}$C | 72 $^{o}$C | |
| 5 min | 1 min | 1 min | 3 min | 7 min. | |
| 1 cycle | | 30 cycles | | 1 cycle | |
| ***sfa* primer** | | | | | |
| Initial denaturation | Denaturation | Annealing | Extension | Final extension | (Chapman *et al.*, 2006) |
| 95 $^{o}$C | 94 $^{o}$C | 63 $^{o}$C | 68 $^{o}$C | 72 $^{o}$C | |
| 3 min | 30 sec. | 30 sec. | 4 min | 10 min | |
| 1 cycle | | 30 cycles | | 1 cycle | |
| *hly* primer | | | | | |
| Initial denaturation | Denaturation | Annealing | Extension | Final extension | (Mladin *et al.*, 2009) |
| 94 $^{o}$C | 94 $^{o}$C | 55 $^{o}$C | 72 $^{o}$C | 72 $^{o}$C | |
| 4 min | 30 sec. | 30 sec. | 1 min | 5 min | |
| 1 cycle | | 30 cycles | | 1 cycle | |
| ***cnf* primer** | | | | | |
| Initial denaturation | Denaturation | Annealing | Extension | Final extension | (Chapman *et al.*, 2006; Adamus-bialek *et al.*, 2009) |
| 95 $^{o}$C | 94 $^{o}$C | 68 $^{o}$C | 68 $^{o}$C | 72 $^{o}$C | |
| 3 min | 30 sec. | 30 sec. | 4 min | 10 min | |
| 1 cycle | | 25 cycles | | 1 cycle | |
| ***pai* primer** | | | | | |
| Initial denaturation | Denaturation | Annealing | Extension | Final extension | (Chapman *et al.*, 2006; Oliveira *et al.*, 2011) |
| 94 $^{o}$C | 94 $^{o}$C | 63 $^{o}$C | 72 $^{o}$C | 72 $^{o}$C | |
| 1 min | 1 min | 30 sec. | 1.30 min | 5 min | |
| 1 cycle | | 30 cycles | | 1 cycle | |

## 2. RESULT AND DISCUSSION

### 2.1. Identification of UPEC

A total of 400 clinical urine samples were collected. Out of 400 urine samples, 141 samples (35.25%) were found positive for UPEC, that were recovered from various hospitals in Zakho city. Nearly similar findings have been reported by Muhammad and Ghareb, (2019) in Erbil City in which *E.coli* was responsible for 33.07% of uropathogenic infections. The *E. coli* was identified by the morphological and biochemical characterization on the culture media which have been able to ferment lactose on the MacConkey agar (Alexander and Strete, 2001; Engelkirk and Duben-Engelkirk, 2008). In addition, the isolated bacteria were confirmed to be *E. coli* strains by biochemical tests and the results showed: indole positive, methyl red positive, citrate utilization negative and in TSI test the sugar fermentation with acid accumulation in slant and butt, with gas production and without $H_2S$ production (Leboffe and Pierce, 2011; M *et al.*, 2016).

### 2.2. Antibiotics sensitivity test

All 141 isolated UPEC were tested for their susceptibility toward 16 antibiotics discs. It was obvious from the results that not all the isolates were sensitive to all antibiotics while a wide range of them showed resistance to most of the testes antibiotics, as shown in Table 4.

The most potent antimicrobial agent against *E. coli* was Imipenem with a resistance rate of only 2.84% and this antibiotic is considers as one of the β-lactam antibiotics of carbapenem agent. This finidng was close to study performed by Polse *et al.*, (2016) who found that the sensitivity

rate toward Imipenem was 100%. Likewise, Abdulrahman *et al.,* (2016) revealed that all isolates *E. coli* was sensitive for Imipenem. It is known that carbapenems is stable against the enzymes ESBLs produced by Enterobacteriaceae and is preferred for treating serious bacterial infections caused by bacteria producing β-lactam (Rupp and Fey, 2003; Paterson, 2006).

The isolated *E. coli* were considered as resistant to the most antibiotics, and the resistant rates varied according to the antibiotics used. The resistant rate was high toward penicillin group including; Amoxicillin, Amoxicillin/ clavulanic acid and Ampicillin with rates of 93.62%, 96.45% and 91.49%, respectively. As the resistant rates of these antibiotics are high, therefore, they are limited in use, but some of these agents remain useful for the treatment of certain patients, such as Amoxicillin or Ampicillin. These antibiotics remain the preferred therapy for group B streptococcal and enterococci infection (Nicolle, 2005). The resistant rates of the third generation cephalosporins groups of β-lactam were high. This group includes; Cefixime, Ceftriaxone and Cefotaxime with resistant rates of 83.69%, 87.23% and 87.23%, respectively. The resistance of the *Enterobacteriaceae* to the cephalosporins third-generation is often due to the β-lactamase production and the ESBLs that are able to hydrolyze and disrupt cephalosporins both broad-spectrum and extended-spectrum (Rupp and Fey, 2003; Shah *et al.,* 2004). The resistant rates for Aminoglycoside agent including; Amikacin and Gentamicin with rates of 87.94% and 63.83%, respectively. Furthermore, the quinolones group including; Nalidixic acid, Ciprofloxacin and Norfloxacin with resistant rates of 79.43%, 49.65% and 43.97%, respectively. The resistance rates of Trimethoprim and Trimethoprim/ sulfamethoxazole were 61.70% and 63.12%, respectively. Aminoglycosides, quinolones and trimethoprim-sulfamethoxazole are generally not an eligible therapeutic choice for serious infections caused by *Enterobacteriaceae* producing ESBL enzymes because of ESBL producers are often resistant to those drugs (Lautenbach *et al.,* 2002; Hyle *et al.,* 2005). Moreover, multidrug resistance (MDR) is increasing among *E. coli* producing ESBL, in which *E. coli* is resistant to more than two class of antibiotic (Hyle *et al.,* 2005). The resistance rates of the two other antibiotics used were found to be 63.83% for Tetracycline and 66.67% for Chloramphenicol.

**Table 4:** Antibiotic sensitivity pattern of UPEC isolates in UTI patients

| Antibiotics Disc | Resistance | | Sensitivity | |
|---|---|---|---|---|
| | No. | % | No. | % |
| Amoxicillin | 132 | 93.62 | 9 | 6.38 |
| Amoxicillin/ clavulanic acid | 136 | 96.45 | 5 | 3.55 |
| Ampicillin | 129 | 91.49 | 12 | 8.51 |
| Cefixime | 118 | 83.69 | 23 | 16.31 |
| Ceftriaxone | 123 | 87.23 | 18 | 12.77 |
| Cefotaxime | 123 | 87.23 | 18 | 12.77 |
| Amikacin | 124 | 87.94 | 17 | 12.06 |
| Gentamicin | 90 | 63.83 | 51 | 36.17 |
| Nalidixic acid | 112 | 79.43 | 29 | 20.57 |
| Norfloxacin | 62 | 43.97 | 79 | 56.03 |
| Ciprofloxacin | 70 | 49.65 | 71 | 50.35 |
| Trimethoprim | 87 | 61.70 | 54 | 38.30 |
| Trimethoprim/ sulfamethoxazole | 89 | 63.12 | 52 | 36.88 |
| Imipenem | 4 | 2.84 | 137 | 97.16 |
| Tetracycline | 90 | 63.83 | 51 | 36.17 |
| Chloramphenicol | 94 | 66.67 | 47 | 33.33 |
| Total No. Tested | 141 *E.coli* bacteria | | | |

The resistance of UPEC toward the antibiotics in the present study was similar to other studies performed in Kurdistan Region / Iraq and in other developing countries. For example, Merza and Jubrael, (2015) in Duhok, showed that the isolated UPEC displayed high resistance to Ampicillin 92.6%, Amoxicillin 90.6%, Amoxicillin/Clavulanic acid 90%, Tetracycline 83.3%, Trimethoprim 77.3%, Trimethoprim/ sulfamethoxazole 73.3%, Nalidixic acid 78%, Cefixime 78%, Cefotaxime 78%, Ceftriaxone 71.3%, Gentamicin 70.7%, Ciprofloxacin 52.6%, Norfloxacin 48.6%, Amikacin 46%, Chloramphenicol 30% and Imipenem was 4.6%. Likewise, another study in Kurdistan region reported that UPEC showed variable resistant rates to Ampicillin 85%, Ceftriaxone 65%, Ciprofloxacin 48%, Gentamicin 38%, Amoxicillin 33% and all isolated *E. coli* were sensitive to both Imipenem and Amikacin antibiotics (Assafi *et al.,* 2015). Furthermore, Muhammad and Ghareb, (2019) in Erbil city found that the resistant rates of both Ceftriaxone and Ciprofloxacin were increased against isolates *E. coli* which were 100%. On the other hand, a recent study in Egypt, Hegazy *et al.,* (2018) showed high resistance rates

of UPEC to Ampicillin 100%, followed by Nalidixic acid 91.84%, Trimethoprim/ sulfamethoxazole 87.9%, Norfloxacin 82.65%, Amoxicillin/ clavulanic acid 81.3%, Ceftriaxone 79.60%, Cefotaxime 74.49%, Gentamicin 26.53%, Amikacin 22.45% and Imipenem 20.41%.

For many decades, Amoxicillin/clavulanate, Cephalexin, Fluoroquinolones (for example, Ciprofloxacin) or Trimethoprim/sulfamethoxazole have been used as first-line for the treatment for uncomplicated UTI (Totsika *et al.,* 2012). However, *E. coli* are resistant to typical first-line agents has become substantial and these drugs can no longer be used in many parts of the world as empiric therapy (Gupta *et al.,* 2011). Earlier, Ampicillin was reported to have no greater effect on pathogens in the urinary tract (Sahm *et al.,* 2001), and this is because of the continuous use of Ampicillin among UTIs patients for many years which led to the production of the resistant by UTI pathogens to Ampicillin (Hassan *et al.,* 2011).

It has been reported that the improper empiric antimicrobial treatment for nosocomial-acquired or community-acquired infections, contributed to significantly higher mortality rates in the intensive care unit (ICU). Also, the insufficient antimicrobial therapy of the infection was the most significant independent determinant of hospitals mortality (Kollef *et al.,* 1999). Another important mechanism that facilitates the increase in the resistance of antimicrobial agents to UTIs infections is the introduction and clonal expansion of competitive resistant strains of *E. coli* in the community (Nordstrom *et al.,* 2013).

In all cases of UTIs, the patients were starting antimicrobial therapy before the laboratory results were available for urine culture (Dash *et al.,* 2013). The misuse and self-medication of the antimicrobial agent (where antibiotics could be purchased without any medical prescription) may be a major problem in many countries, including Iraq, and these led to a general rise of resistant of the bacteria to antibiotics (Sahm *et al.,* 2001; Merza and Jubrael, 2015). Furthermore, about 95% of UTI infections were treated without bacteriological investigations (Taghizadeh *et al.,* 2013; Bari *et al.,* 2017). There is a need to introduce strict strategies for dispensing antibiotics in the community in order to prevent the emergence of more resistant isolates of pathogenic bacteria. In addition, physician should depend more on laboratory guidance, and laboratories should provide resistance pattern data for optimum patient management more rapidly (Merza and Jubrael, 2015). Many reports suggested that the resistance of *E. coli* strains to commonly used antimicrobial agents has made the clinical management of UTI complicated by increasing incidence of their infections (Van De *et al.,* 2008). Most of these studies agreed that there is a need to improve on infection control methods (Mukherjee *et al.,* 2013). Genes encoding ESBLs are often found on the same plasmids as genes that encode resistance to aminoglycosides and sulfonamides, and many *Enterobacteriaceae* species have changes that give high-level resistance to quinolones (Paterson, 2006).

### 2.3. PCR Analysis

Out of 141 UPEC isolates, 57 isolated were selected for PCR amplification, and typical all *E. coli* were successfully amplified for specific-species locus by producing a single band of a target *uidA* gene with molecular weight of 670 bps as shown in Figure 1.

Therefore, the amplification of the *uidA* in the selected samples confirmed that all these strains were, in fact, *E.coli.* The result in this study was similar to the study performed by Merza *et al.,* (2016) in Kurdistan region/Iraq for identification *E.coli*, they used the same *uidA* primer and produced 670 bp as molecular weight. However, this molecular weight is different from that obtained by Adamus-bialek *et al.,* (2009) by using the same primer. This might be due to the fact that genes usually contain several repeats of the coding microsatellite (1-10 bp) and minisatellite (>10 bp), which are very dynamic components of genomes and are subcategories of tandem repeats (TRs) that make up genomic repetitive regions (Merza, 2013; Vieira *et al.,* 2016). Therefore, the recombination events within these TRs led to alter in the repetition numbers, which in turn change the sequences (Merza, 2013).

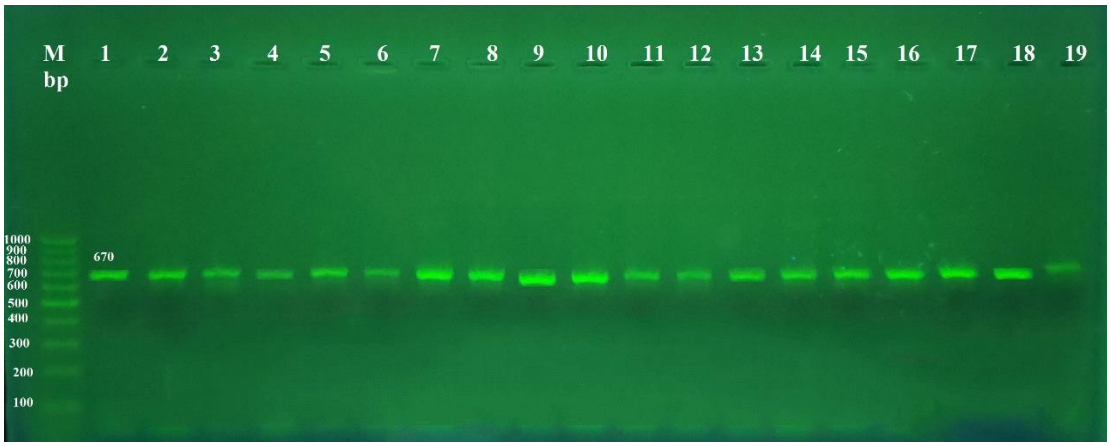


**Figure 1:** The amplification of PCR for *E.coli* species identification:
Using specific-species *uidA* primer with molecular weight 670bp for 57 UPEC isolated strains and the amplicons separation performed on 1.2% agarose gel electrophoresis for 1 hour. Lane M contained DNA molecular weight marker (1000-100bp).

The selected *E. coli* that had *uidA* gene were subjected for the determination of the genes related to virulence and their distribution among UPEC patients in Zakho city, which includes; *afa*, *sfa*, *hly*, *cnf* and *pai* as shown in Figure 2. The most common virulence gene among UPEC isolates was *afa* which was detected in 28.07% (16/57) of the isolates. Following by *hly* that account for 26.32% (15/57). Whereas the prevalence of both *cnf* and *pai* genes displayed among 22.81% (13/57) of the isolates. The *sfa* gene was the lowest prevalence around 17.54% (10/57). According to this study, the prevalence of the virulence related genes among 57 UPEC isolates, it was found that only 35 (61.40%) of these isolated had one of these five virulence related genes. This rate was somewhat in agreement with the related published studies, for example, a total of 59.4% of UPEC isolates contained at least one of the virulence genes (Pourzare *et al.*, 2017). Furthamore, the rates in this study are in agreement with other studies for most of the virulence genes, for example, a study in Duhok province, showed prevalence rates of virulence related genes that includes *afa*, *cnf*, *hly* and *sfa* among UPEC isolates at 32%, 28%, 24% and 16%, respectively, with the exception of the high prevalence rate (70%) of *pai* gene (Merza *et al.*, 2016). In other studies such as, Oliveira and coworkers, (2011) they also determined the virulence factors of the isolated UPEC in the Curitiba/ Brazil and their different prevalence rates as following; *pai* 32%, *sfa* 26%, *cnf*1 18%, *afa* 6% and *hly* 5%. Furthermore, Karimian *et al.*, (2012) found that the presence of difference in the prevalence rates of the virulance genes in the isolated *E. coli* from urine as following; *cnf*1 50.4%, *hly*A 50.4% and *afa* 8.13%.

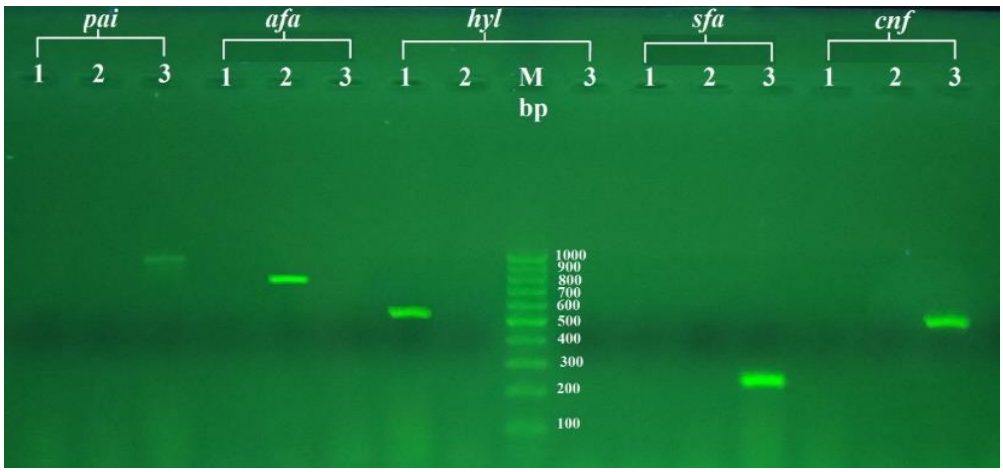


**Figure (2):** The PCR amplification of the five virulence gene using the five primer includes (*afa*, *sfa*, *hly*, *cnf* and *pai*) with different molecular weight.
Lane M contained DNA molecular weight marker (1000-100bp).

The reported differences in the prevalence of UPEC virulence genes might be due to geographical region or to the climatic conditions of each region (Karimian *et al.*, 2012; Firoozeh *et al.*, 2014). Most likely, habits, public health, food, hospital's health and even sampling methods have significant rules for the spread of virulence genes of UPEC strains (Karimian *et al.*, 2012). This study somewhat is giving alarming data regarding multidrug resistant with multiple virulence factors of UPEC spreading in our community. It is highly recommended to survey all UTIs patients for multidrug resistant isolates simultaneously specifying virulence factors, in addition, to limit the random prescription of antibiotics by general practitioners physician in our community.

## 3. CONCLUSION

The *E.coli* is the most common pathogen which causes UTI in human. Furthermore, this pathogen have developed resistance to the most used antibiotics except Imipenem which was highly sensitive against the isolates of pathogenic *E. coli.* The use of *uidA* primer gave successful identification of *uidA* gene in the tested *E. coli* isolates. It is worthwhile to mention that various virulence factors facilitate the spreading of bacteria in human urinary tract causing UTI.

**Conflict of Interest:** there is no conflict of interest.

## References

Abdulrahman, Z.F.A., Salah, H.F. and Mohammad, M.I. 2016. Molecular study and assessment of the medicinal potential of the lepidium sativum plant extracts against antibiotic resistance *E.coli*, *ZANCO Journal of Pure and Applied Sciences*, 28 (1), 1–10.

Adamus-bialek, W., Wojtasik, A., Majchrzak, M., Sosnowski, M. and Parniewski, P. 2009. ( CGG ) 4-Based PCR as a Novel Tool for Discrimination of Uropathogenic *Escherichia coli* Strains : Comparison with Enterobacterial Repetitive Intergenic Consensus-PCR, *Journal of Clinical Microbiology*, 47 (12), 3937–3944.

Alexander, S.K. and Strete, D. 2001. *Microbiology: a photographic atlas for the laboratory*, USA, Daryl Fox.

Assafi, M.S.A., Ibrahim, N.M.R., Hussein, N.R., Taha, A.A. and Balatay, A.A. 2015. Urinary Bacterial Profile and Antibiotic Susceptibility Pattern among Patients with Urinary Tract Infection in Duhok City, Kurdistan Region, Iraq, *International Journal of Pure & Applied Sciences & Technology*, 30(2), 54–63.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. 2003. *Current Protocols in Molecular Biology*, USA, John Wiley & Sons.

Bari, M.A., Arefin, M., Nessa, M., Mostofa, G. and Islam, T. 2017. Recent Antibiotic Sensitivity Pattern of *Escherichia coli* in Urinary Tract Infection, *Journal of Teachers Association*, 30(1), 61–65.

Behzadi, P., Najafi, A., Behzadi, E. and Ranjbar, R. 2016. Microarray long oligo probe designing for *Escherichia coli* : An in-silico DNA marker extraction, *Central European Journal of Urology*, 69(1), 105–111.

Bien, J., Sokolova, O. and Bozko, P. 2012. Role of Uropathogenic *Escherichia coli* Virulence Factors in Development of Urinary Tract Infection and Kidney Damage, *International Journal of Nephrology*, 2012, 1–15.

Le-Bouguenec, C.L.E., Archambaud, M. and Labigne, A. 1992. Rapid and Specific Detection of the pap , afa , and sfa Adhesin- Encoding Operons in Uropathogenic *Escherichia coli* Strains by Polymerase Chain Reaction, *Journal of Clinical Microbiology*, 30 (5), 1189–1193.

Chapman, T.A., Wu, X., Barchia, I., Bettelheim, K.A., Driesen, S., Trott, D., Wilson, M. and Chin, J.J. 2006. Comparison of Virulence Gene Profiles of *Escherichia coli* Strains Isolated from Healthy and Diarrheic Swine, *Enviromental Microbiology*, 72(7), 4782–4795.

Cheesbrough, M. 2006. *District Laboratory Practice in Tropical Countries*, United States of America New York, Cambridge University Press.

Coyle, M., B. 2005. *Manual of Antimicrobial Susceptibility Testing*, USA, Washington, DC : American Society for Microbiology.

Dash, M., Padhi, S., Mohanty, I., Panda, P. and Parida, B. 2013. Antimicrobial resistance in pathogens causing urinary tract infections in a rural community of Odisha, India, *Journal of Family and Community Medicine*, 20(1), 20–26.

Van De, S.-B.N., Grundmann, H., Verloo, D., Tiemersma, E., Monen, J., Goossens, H., Ferech, M., System,

Surveillance, E.A.R. and Groups, E.S. of A.C.P. 2008. Antimicrobial drug use and resistance in Europe, *Antimicrobial Drug Use and Resistance in Europe drug*, 14(11), 1722–1730.

Dobrindt, U., Agerer, F., Michaelis, K., Janka, A., Buchrieser, C., Samuelson, M., Svanborg, C., Gottschalk, G., Karch, H. and Hacker, J. 2003. Analysis of Genome Plasticity in Pathogenic and Commensal *Escherichia coli* Isolates by Use of DNA Arrays, *Journal of Bacteriology*, 185(6), 1831–1840.

Dobrindt, U., Blum-oehler, G., Nagy, G., Schneider, G., Johnn, A. and Gottschalk, G. 2002. Genetic Structure and Distribution of Four Pathogenicity Islands (PAI I 536 to PAI IV 536 ) of Uropathogenic, *American Society for Microbiology*, 70(11), 6365–6372.

Engelkirk, P.G. and Duben-Engelkirk, J. 2008. *Laboratory diagnosis of Infectious Diseases*, USA, Wolters Kluwer Health/Lippincott Williams & Wilkins.

Farell, D.J., Morrissay, I., De Rubeids, D., Robbins, M. and Felmingham, D. 2003. A UK multicenter study and the antimicrobial susceptibility of bacterial pathogens causing urinry tract infection, *Journal of Infection*, 46 (2), 94–100.

Farshad, S., Ranjbar, R., Japoni, A., Hosseini, M., Anvarinejad, M. and Mohammadzadegan, R. 2012. Microbial Susceptibility, virulence Factors, and Plasmid Profiles of Uropathogenic *Escherichia coli* Strains Isolated from children in Jahrom, Iran. *Arch Iran Med*, 15(5), 312–316.

Firoozeh, F., Saffari, M., Neamati, F. and Zibaei, M. 2014. Detection of virulence genes in *Escherichia coli* isolated from patients with cystitis and pyelonephritis, *International Journal of Infectious Diseases*, 29, 219–222.

Gupta, K., Hooton, T.M., Naber, K.G., Wullt, B., Colgan, R., Miller, L.G., Moran, G.J., Nicolle, L.E., Raz, R., Schaeffer, A.J. and Soper, D.E. 2011. Highlights from international clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the infectious diseases society of America and the european society for microbiology and infectious, *Clin Infect Dis.*, 52(5), e103–e120.

Hassan, S.A., Jamal, S.A. and Kamal, M. 2011. Occurrence of multidrug resistant and ESBL producing *E. coli* causing urinary tract infections, *Journal of Basic & Applied Sciences*, 7(1), 39–43.

Hegazy, E.E., Alam El Din, R.A.E., Amin, A.M., Mahgoub, F.M. and El Gamal, S.A.E. 2018. Microbiological Profile of Urinary Tract Infections with special Reference to Antibiotic Susceptibility Pattern of *Escherichia coli* Isolates, *International Journal of Current Microbiology and Applied Sciences*, 7(2), 911–920.

Hryniewicza, K., Szczypab, K., Sulikowskab, A., Jankowskia, K., Betlejewskab, K. and Hryniewiczb, W. 2001. Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Poland, *Journal of Antimicrobial Chemotherapy*, 47(6), 773–780.

Hyle, E.P., Lipworth, A.D., Zaoutis, T.E., Nachamkin, I., Fishman, N.O., Bilker, W.B., Mao, X. and Lautenbach, E. 2005. Risk Factors for Increasing Multidrug Resistance among Extended-Spectrum-Lactamase-Producing *Escherichia coli* and *Klebsiella* Species, *Clinical Infectious Diseases*, 40(9), 1317–1324.

Johnson, J.R. and Stell, A.L. 2000. Extended Virulence Genotypes of *Escherichia coli* Strains from Patients with Urosepsis in Relation to Phylogeny and Host Compromise, *The Journal of Infectious Diseases*, 181(1), 261–272.

Kaper, J.B., Nataro, J.P. and Mobley, H.L.T. 2004. Pathogenic *Escherichia coli*, *Nature Reviews Microbiology*, 2, 123–140.

Karimian, A., Momtaz, H. and Madani, M. 2012. Detection of uropathogenic *Escherichia coli* virulence factors in patients with urinary tract infections in Iran, *African Journal of Microbiology Research*, 6(39), 6811–6816.

Kollef, M.H., Sherman, G., Ward, S. and Fraser, V.J. 1999. Inadequate Antimicrobial Treatment of Infections: A Risk Factor for Hospital Mortality Among Critically Ill Patients, *Chest*, 115(2), 462–474.

Lautenbach, E., Strom, B.L., Bilker, W.B., Patel, J.B., Edelstein, P.H. and Fishman, N.O. 2002. Epidemiological Investigation of Fluoroquinolone Resistance in Infections Due to Extended-Spectrum β-Lactamase–Producing *Escherichia coli* and Klebsiella pneumoniae, *Clinical Infectious Diseases*, 33(8), 1288–1294.

Leboffe, M.J. and Pierce, B.E. (2011). *Photographic Atlas for the microbiology laboratory*, USA, Douglas N. Morton.

Litza, J. and Brill, J. 2010. Urinary tract infections, *Prim Care*, 37(3), 491–507.

M, N.S., Jayakumar, K. and Aravazhi, A.N. 2016. The

effectiveness of antibiotics against a major uropathogen- *Escherichia coli* and its biofilm assay by phenotypic methods, *International Journal of Research in Medical Sciences*, 4(11), 4820–4828.

Manikandan, C. and Amsath, A. 2014. Antibiotic susceptibility pattern of *Escherichia coli* isolated from urine samples in Pattukkottai, Tamilnadu, *International Journal of Current Microbiology and Applied Sciences*, 3(10), 449–457.

Merza, N.S. 2013. Clonal Grouping of Uropathogenic *Escherichia coli* Using Different molecular Typing Methods in Kurdistan Region-Iraq, Ph.D Dissertation, University of Zakho. .

Merza, N.S., Fadhel Al Deen, I.S., Khalid, H.M. and Jubrael, J.M.S. 2016. Molecular Characterization of Extended Spectrum B-lactamase Producing *Escherichia coli* Isolated from Urine in Kurdistan Region-Iraq, *International Journal of Chemical and Biomolecular Science*, 2(2), 51–55.

Merza, N.S. and Jubrael, J.M.S. 2015. The Prevalence of Virulence Factors Among Uropathogenic *Escherichia coli* Strains Isolated From Different Hospitals in Kurdistan Region-Iraq, *International Journal of Bioinformatics and Biomedical Engineering*, 1(3), 338–343.

Miyazaki, J., Ba-Thein, W., Kumao, T., Obata Yasuoka, M., Akaza, H. and Hayshi, H. 2002. Type 1, P and S fimbriae, and afimbrial adhesin I are not essential for uropathogenic *Escherichia coli* to adhere to and invade bladder epithelial cells, *FEMS Immunology and Medical Microbiology*, 33(1), 23–26.

Mladin, C. Usein, C.A. Chifiriuc, M.C. Palade, A. Slavu, C. L. Negut, M. and Damian, M. 2009. Genetic analysis of virulence and pathogenicity features of uropathogenic *Escherichia coli* isolated from patietns with neurogenic bladder, *Romanian Biotechnological Letters*, 14(6), 4900–4905.

Muhammad, I.A. and Ghareb, D.J. 2019. Biofilm Forming Capability , Multidrug Resistance and Detection of Associated Genes in Uropathogenic *Escherichia coli* isolated from Catheterized Patients, *ZANCO Journal of Pure and Applied Sciences*, 31(4), 9–22.

Mukherjee, M., Basu, S., MuKherjee, S.K.M. and Majumder, M. 2013. Multidrug-resistance and extended spectrum beta-lactamase production in uropathogenic *E. coli* which were isolated from hospitalized patients in Kolkata, India, *Journal of Clinical and Diagnostic Research*, 7(3), 449–453.

Nicolle, L E. 2005. Complicated Urinary Tract Infection in Adults, *Can J Infect Dis Med Microbiol*, 16 (6), 349-60.

Nordstrom, L., Liu, C.M. and Price, L.B. 2013. Foodborne urinary tract infections: A new paradigm for antimicrobial-resistant foodborne illness, *Frontiers in Microbiology*, 4(29), 1–6.

Oliveira, F.A., Paludo, K.S., Arend, L.N.V.S., Farah, S.M.S.S., Pedrosa, F.O., Souza, E.M., Surek, M., Picheth, G. and Fadel-Picheth, C.M.T. 2011. Virulence characteristics and antimicrobial susceptibility of uropathogenic *Escherichia coli* strains, *Genetics and Molecular Research*, 10(4), 4114–4125.

Paterson, D.L. 2006. Resistance in gram-negative bacteria: Enterobacteriaceae, *American Journal of Infection Control*, 34 (5 SUPPL.), 20–28.

Poirel, L., Loo, M. Van De, Mammeri, H. and Nordmann, P. 2005. Association of Plasmid-Mediated Quinolone Resistance with Extended-Spectrum β-Lactamase VEB-1, *Antimicrobial Agents and Chemotherapy*, 49(7), 3091–3094.

Polse, R.F., Yousif, S.Y. and Assafi, M.S. 2016. Prevalence and Antimicrobial Susceptibility Patterns of Uropathogenic *E. coli* among People in Zakho, Iraq, *International Journal of Research in Medical Sciences*, 4 (4), 1219–23.

Pourzare, M., Derakhshan, S. and Roshani, D. 2017. Distribution of Uropathogenic Virulence Genes in *Escherichia coli* Isolated from Children with Urinary Tract Infection in Sanandaj, Iran, *Archives of Pediatric Infectious Diseases*, 5(3), 1–6.

Rupp, M.E. and Fey, P.D. 2003. Extended Spectrum β-Lactamase (ESBL) Producing Enterobacteriaceae, *Drugs*, 63(4), 353–365.

Russo, T.A. and Johnson, J.R. 2000. Proposal for a New Inclusive Designation for Extraintestinal Pathogenic Isolates of *Escherichia coli* : ExPEC, *The Journal of Infectious Diseases*, 181(5), 1753–1754.

Sahm, D.F., Critchley, I.A., Kelly, L.J., Karlowsky, J.A., Mayfield, D.C., Thornsberry, C., Mauriz, Y.R. and Kahn, J. 2001. Evaluation of current activities of fluoroquinolones against gram-negative bacilli using centralized in vitro testing and electronic surveillance, *Antimicrobial Agents and Chemotherapy*, 45(1), 267–274.

Shah, A.A., Hasan, F., Ahmed, S. and Hameed, A. 2004. Extended-spectrum β-lactamases (ESBLs): Characterization, epidemiology and detection, *Critical Reviews in Microbiology*, 30(1), 25–32.

Sharma, N., Gupta, A., Walia, G. and Bakhshi, R. 2016. Pattern of antimicrobial resistance of *Escherichia coli* isolates from urinary tract infection patients: A three year retrospective study, *Journal of Applied Pharmaceutical Science*, 6(1), 062–065.

Šišková, P., Černohorská, L., Mahelová, M., Turková, K. and Woznicová, V. 2015. Phenotypes of *Escherichia coli* isolated from urine: Differences between extended-spectrum β-lactamase producers and sensitive strains, *Journal of Microbiology, Immunology and Infection*, 48(3), 329–334.

Siu, H.L., Reddy, S., Cheesbrough, J., Bolton, F.J., Willshaw, G., Cheasty, T., Fox, A.J. and Upton, M. 2008. Major uropathogenic *Escherichia coli* strain isolated in the Northwest of England identified by multilocus sequence typing, *Journal of Clinical Microbiology*, 46(3), 1076–1081.

Taghizadeh, G., Kulkarni, D.K., Delbari, A.S. and Taghizadeh, R. 2013. Antimicrobial susceptibility pattern of *E. coli* among patients attending at apadana hospital in ahwaz, Iran, *International Journal of Research in Ayurveda and Pharmacy*, 4(1), 120–122.

Tiwary, S.K., Sharan, S., Kumar, R. and Kumari, K. 2017. Antimicrobial susceptibility patterns of uropathogenic *Escherichia coli* and their prevalence among people in and around Dhanbad, Jharkhand, *International Journal of Contemporary Medical Research*, 4(8), 1637–1639.

Totsika, M., Moriel, D.G., Idris, A., Rogers, B.A., Wurpel, D.J., Phan, M.-D., Paterson, D.L. and Schembri, M.A. 2012. Uropathogenic *Escherichia coli* Mediated Urinary Tract Infection, *Current Drug Targets*, 13(11), 1386–1399.

Vieira, M.L.C., Santini, L., Diniz, A.L. and Munhoz, C. de F. 2016. Microsatellite markers: What they mean and why they are so useful, *Genetics and Molecular Biology*, 39(3), 312–328.

Wiles, T.J., Kulesus, R.R. and Mulvey, M.A. 2008. Origins and virulence mechanisms of uropathogenic *Escherichia coli*, *Experimental and Molecular Pathology*, 85(1), 11–19.

Zhanel, G.G., Hisanaga, T.L., Laing, N.M., DeCorby, M.R., Nichol, K.A., Weshnoweski, B., Johnson, J. ack, Noreddin, A., Low, D.E. and Karlowsky, J.A. 2006. Antibiotic resistance in *Escherichia coli* outpatient urinary isolates: final results from the North American Urinary Tract Infection Collaborative Alliance (NAUTICA), *Int J Antimicrob Agents*, 27(6), 468–76.