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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ABSTRACT:

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.

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
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 adhesion 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 (Poirol 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 (Hryniewicz 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°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 measured and interpreted as sensitive or resistant depending on the size of the inhibition zone (Coyle, 2005; Cheesbrough, 2006).
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 fimbral 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*, respectively.

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 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        |
| Ciprofloxicin                    | 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 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.

**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-CATTACGGCAAGTGTGGGTCAAT R-CCATCAAGCTGTGGTATCGAATCCTT | 658 bp | (Adamus-bialek et al., 2009) |
|                  | *afa*  | F-GCTGGGCAGCAACTGATAACTCTC R-CATCAAGCTGTGGTATCGAATCCT | 750 bp | (Le-Bouguenec et al., 1992) |
| Virulence genes                                         | 240 bp | (Chapman et al., 2006)                  |
| *hyl*           |        | F-AGATTCGGCCAGCATTCCCTGTTATCCTT | 565 bp | (Mladin et al., 2009)          |
| *cnf*           |        | F-AAAGTGAGGTTTATCTGAGGAG R-CATTCAAGCTGTGGTATCGAATCCTT | 498 bp | (Chapman et al., 2009; Chapman et al., 2006) |
| *pai*           |        | F-GGACATCCGTTACAGCGCCGA R-TCGCAACCAATCAGCGCCGAAC | 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*

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

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 H2S 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 finding 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 (Nicoll, 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 Norfloxacn 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 No. | Sensitivity % |
|------------------|---------------|--------------|
| Amoxicillin      | 132           | 93.62        |
| Amoxicillin/ clavulanic acid | 136 | 96.45 |
| Ampicillin       | 129           | 91.49        |
| Cefixime         | 118           | 83.69        |
| Ceftriaxone      | 123           | 87.23        |
| Cefotaxime       | 123           | 87.23        |
| Amikacin         | 124           | 87.94        |
| Gentamicin       | 90            | 63.83        |
| Nalidixic acid   | 112           | 79.43        |
| Norfloxacn       | 62            | 43.97        |
| Ciprofloxacin    | 70            | 49.65        |
| Trimethoprim     | 87            | 61.70        |
| Trimethoprim/ sulfamethoxazole | 89 | 63.12 |
| Imipenem         | 4             | 2.84         |
| Tetracycline     | 90            | 63.83        |
| Chloramphenicol  | 94            | 66.67        |

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%, Norfloxacn 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).
The selected \textit{E. coli} that had \textit{uidA} gene were subjected for the determination of the genes related to virulence and their distribution among UPEC patients in Zakho city, which includes; \textit{afa}, \textit{sfa}, \textit{hly}, \textit{cnf} and \textit{pai} as shown in Figure 2. The most common virulence gene among UPEC isolates was \textit{afa} which was detected in 28.07\% (16/57) of the isolates. Following by \textit{hly} that account for 26.32\% (15/57). Whereas the prevalence of both \textit{cnf} and \textit{pai} genes displayed among 22.81\% (13/57) of the isolates. The \textit{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 \textit{et al.}, 2017). Furthermore, 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 \textit{afa}, \textit{cnf}, \textit{hly} and \textit{sfa} among UPEC isolates at 32\%, 28\%, 24\% and 16\%, respectively, with the exception of the high prevalence rate (70\%) of \textit{pai} gene (Merza \textit{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; \textit{pai} 32\%, \textit{sfa} 26\%, \textit{cnf} 18\%, \textit{afa} 6\% and \textit{hly} 5\%. Furthermore, Karimian \textit{et al.}, (2012) found that the presence of difference in the prevalence rates of the virulence genes in the isolated \textit{E. coli} from urine as following; \textit{cnf} 50.4\%, \textit{hly}A 50.4\% and \textit{afa} 8.13\%.

\textbf{Figure 1:} The amplification of PCR for \textit{E.coli} species identification: Using specific-species \textit{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).

\textbf{Figure (2):} The PCR amplification of the five virulence gene using the five primer includes (\textit{afa}, \textit{sfa}, \textit{hly}, \textit{cnf} and \textit{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.

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