Antimicrobial Resistance, Virulence Factor-Encoding Genes, and Biofilm-Forming Ability of Community-Associated Uropathogenic Escherichia coli in Western Saudi Arabia

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Abstract

To explore the prevalence of multidrug-resistant community-associated uropathogenic Escherichia coli (UPEC) and their virulence factors in Western Saudi Arabia. A total of 1,000 urine samples were examined for the presence of E. coli by selective plating on MacConkey, CLED, and sheep blood agar. Antimicrobial susceptibility patterns were determined using Vitek® 2 Compact (MIC) and the disc diffusion method with Mueller-Hinton agar. Genes encoding virulence factors (kpsMTII, traT, sat, csgA, vat, and iutA) were detected by PCR. The overall prevalence of UTI-associated E. coli was low, and a higher prevalence was detected in samples of female origin. Many of the isolates exhibited resistance to norfloxacin, and 60% of the isolates showed resistance to ampicillin. No resistance to imipenem, meropenem, or ertapenem was detected. In general, half of the isolates showed multiple resistance patterns. UPEC exhibited a weak ability to form biofilms, where no correlation was observed between multidrug resistance and biofilm-forming ability. All uropathogenic E. coli isolates carried the kpsMTII, iutA, traT, and csgA genes, whereas the low number of the isolates harbored the sat and vat genes. The diversity of virulence factors harbored by community-associated UPEC may render them more virulent and further explain the recurrence/relapse cases among community-associated UTIs. To the best of our knowledge, this study constitutes the first exploration of virulence, biofilm-forming ability, and its association with multidrug resistance among UPEC isolates in Saudi Arabia. Further investigations are needed to elucidate the epidemiology of community-associated UPEC in Saudi Arabia.

Keywords: uropathogenic E. coli, antimicrobial resistance, virulence factors, biofilm formation, urinary tract infection, Saudi Arabia

Introduction

Urinary tract infections (UTIs) remain a common and leading cause of morbidity worldwide, and more than 150 million people are affected annually (Flores-Mireles et al. 2015). The social and health care burden of UTIs in the United States alone has been estimated to equal approximately 3.5 billion US dollars per year (Flores-Mireles et al. 2015). However, it has been suggested that the estimated burdens of UTIs may exceed...
the reported cost because UTIs are not included among the diseases that require mandatory notification (Öztürk and Murt 2020). UTIs are either community-associated or hospital-acquired UTIs (CA-UTIs or HA-UTIs, respectively). CA-UTIs are the most common disease observed in outpatient cases, and HA-UTIs are among the most frequently encountered nosocomial infections (Tandogdu and Wagenhler 2016; Paul 2018).

The etiology of UTIs varies because different bacterial, fungal, and parasitic agents are the cause of the infections. Among causative bacterial agents, Escherichia coli is the most frequently reported (Medina and Castillo-Pino 2019; Sokhn et al. 2020). Other common bacterial agents that cause UTI are Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, staphylococci (e.g., Staphylococcus aureus and Staphylococcus saprophyticus), and Enterococcus faecalis (Medina and Castillo-Pino 2019). Although UTIs have been reported in individuals of all ages and both genders, epidemiological data suggest that females are more prone to UTIs than males, and the frequency of UTIs in females increases with increasing age (Medina and Castillo-Pino 2019).

E. coli is a common inhabitant of the gastrointestinal tract of humans and warm-blooded animals and is also the most common etiology of UTIs (Kaper et al. 2004). It is widely accepted that the intestinal tract is the source of UTI-associated E. coli; however, strains implicated in UTIs may differ from other commensal strains. Thus, more than 90% of CA-UTI cases are attributed to uropathogenic E. coli (UPEC), and nearly 50% of HA-UTI cases are caused by UPEC (Vila et al. 2016). UPEC may differ from other commensal intestinal E. coli strains because they are more adaptable to different environments, such as the urethra, bladder, and kidneys (Kaper et al. 2004; Vila et al. 2016). Moreover, UPEC strains possess an arsenal of virulence factors that facilitate their transition from the intestinal tract to the urinary tract and their abilities to attach, invade host tissues, and evade the immune system (Vila et al. 2016).

The pathogenicity of UPEC is a complicated multifactorial process mediated by various virulence factors. UPEC strains encode various virulence attributes that allow this pathogen to adapt, colonize, and survive in the environment of the urinary tract and successfully evade the host immune system. Some of the UPEC virulence factors include adhesions (e.g., type 1 fimbriae, P fimbriae, and curli fimbriae); toxins such as α-hemolysin, endotoxin, and cytotoxic-necrotizing factor 1; iron acquisition system (i.e., haem receptors and siderophores); and genes involved in mechanisms related to evading the immune system, such as immune system suppression, serum resistance, and protection against phagocytosis. In addition, the ability of UPEC to form biofilms can add further success to their virulence and aid their pathogenicity (Lüthje and Brauner 2014; Flores-Mireles et al. 2015; Jahandeh et al. 2015; Terlizzi et al. 2017). Curli fimbriae (csgA) serve as an adhesion factor that facilitates UPEC invasion into host cells and aids the entrance of the bacteria into the bloodstream resulting in progressive acute infection and urosepticemia. Ferric aerobactin receptor (iutA) or siderophores are crucial factors in aiding the persistence of UPEC strains in the urinary tract, and such genes are more prevalent in persisting UPEC strains than in those causing sporadic infections. Capsular polysaccharide synthesis K1 (kpsMTII) protects UPEC from unfavorable conditions and helps the bacteria evade the immune system response, particularly by escaping phagocytosis and antiserum activities. Serum survival (traT) protects against the complement system in serum, and secreted autotransporter toxin (sat) has cytotoxic activity and thus, causes damage to host cells and increases the reproductive ability of UPEC strains. Vacuolating autotransporter (vat) can induce cytotoxic effects on the bladder and kidney (Lüthje and Brauner 2014; Kudinha 2017; Parvez and Rahman 2018).

Antimicrobial drug resistance is a global problem with severe public health implications. Because urinary tract infections are, in most cases, treated empirically, UTIs have the highest rank among all diseases in terms of the number of antibiotic prescriptions used to treat infected individuals (Paul 2018). High rates of antimicrobial resistance among bacterial uropathogens, particularly UPEC strains, have been reported (Terlizzi et al. 2017; Sokhn et al. 2020; Pasillas Fabian et al. 2021). Due to the increased resistance of UPEC to cephalosporins and fluoroquinolones, care should be considered when using these drugs as the first choice for treating UTI-associated E. coli, especially in complicated UTI cases caused by UPEC (Shariff et al. 2013; Kot 2019). Similarly, increasing trends in the numbers of UPEC strains resistant to amoxicillin, ampicillin, tetracycline, amikacin, gentamicin, and ciprofloxacin have been reported (Moroh et al. 2014; Stephenson and Brown 2016; Vila et al. 2016; Terlizzi et al. 2017). Due to the increasing emergence of multidrug-resistant and extended-spectrum β-lactamase (ESBL)-producing E. coli associated with UTI cases, the treatment of choice for complicated and uncomplicated infections is suggested to involve carbapenems (imipenem and ertapenem), trimethoprim/sulfamethoxazole, nitrofurantoin, and fosfomycin (Vila et al. 2016; Terlizzi et al. 2017; Pasillas Fabian et al. 2021).

Considerable attention has been given to UTIs in Saudi Arabia in recent years. Nonetheless, few related studies have been reported. In fact, between 1988 and 2021, only 24 published reports focused mainly on the etiology and antimicrobial susceptibility patterns among various groups of patients (searched in PubMed, Scopus,
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ISI Web of Science, and Google Scholar). For instance, Akbar (2001) and Al-Rubeaan et al. (2013) investigated the causative agent among HA- and CA-UTIs in diabetic and nondiabetic individuals, and UTIs in Saudi children were investigated by Al-Ibrahim et al. (2012), Albalawi et al. (2018) and Hameed et al. (2019). Although several published studies have investigated HA-UTI cases in various regions in Saudi Arabia (El-Tahawt and Khalaf 1988; Abdal-Fattah 2005; Al-Tawfiq and Anani 2009; Alzohairy and Khaderi 2011; Alkatheri 2013; Balkhi et al. 2018; Amin et al. 2021), the studies have emphasized the prevalence of CA-UTIs in Saudi Arabia were also reported (Eltahawt and Khalaf 1988; Al-Tawfiq and Anani 2009; Al Sibiani 2010; Alzohairy and Khaderi 2011; Faidah et al. 2013; El-Kersh et al. 2015; Alaznai 2018; Ahmad 2019; Ahmed et al. 2019; Alshabi et al. 2019). In all these studies, E. coli was identified as the predominant causative agent, and the observed susceptibility patterns to antimicrobial agents exhibit various trends. Only three focused on antimicrobial resistance among UTI-associated E. coli strains in Saudi Arabia (Al-Otaibi and Bukhari 2013; Al-Yousef et al. 2019; Al-Sibiani 2010; Alzohairy and Khaderi 2011; Faidah et al. 2013; El-Kersh et al. 2015; Alaznai 2018; Ahmad 2019; Ahmed et al. 2019; Alshabi et al. 2019), although these studies did not further investigate the pathogenicity of these isolates, i.e., types of virulence determinants, ability to form biofilms, the association between biofilm formation and virulence factors and/or antimicrobial resistance.

The aim of this study was to investigate the prevalence of UPEC in CA-UTIs, their antimicrobial susceptibility patterns, the genes encoding virulence factors, and the isolate’s ability to form biofilms. The association between biofilm formation and antimicrobial resistance among UPEC strains was also explored. To the best of our knowledge, this study is the first to shed some light on the virulence traits of community-associated UPEC in Saudi Arabia.

**Experimental**

**Materials and Methods**

**Samples.** A total of 1,000 midstream urine samples were collected from various clinical establishments in Makkah city, Western Saudi Arabia. The samples comprised 774 samples of male origin and 226 samples from females visiting the outpatient department. The samples were collected in sterile urine collecting bottles between September and December 2020. All the samples were transported to the laboratory on ice in darkness, and microbiological assays were initiated on the day of sampling.

**Isolation of E. coli from urine samples.** Each urine sample was streaked on MacConkey and cysteine-lactose-electrolyte-deficient (CLED) agar plates (Oxoid, UK) using a calibrated loop (0.01 ml), and the plates were incubated at 37°C for 24 h (Karigoudar et al. 2019). Presumptive E. coli colonies on MacConkey and CLED agar plates were streaked onto eosin methylene blue (EMB) agar (HiMedia, India) plates for purification of the isolates and primary differentiation between E. coli and other Gram-negative UTI causative agents based on the distinctive colonial characteristics of E. coli on EMB agar, and the EMB agar plates were incubated at 37°C for 24–48 h (Leininger et al. 2001).

**Identification of E. coli isolates.** Presumptive E. coli isolates on EMB agar were subjected to the following confirmatory tests: catalase, oxidase, motility, and indole production in SIM medium (HiMedia, India); fermentation of lactose with acid, and gas production in MacConkey broth (Oxoid, UK) at both 37°C and 44°C; reaction on triple sugar iron (TSI) agar slants, and utilization of sodium citrate on Simmons citrate (HiMedia, India) slants at 37°C (Alshabi et al. 2019). Further identification was performed using API 20E strips (bioMerieux, France) according to the manufacturer’s instructions (Munkhdelger et al. 2017), and additional identification was also achieved with a Vitek® 2 Compact system (bioMerieux, France) (Al-Tawfiq and Anani 2009; Ahmed et al. 2019). All isolates were maintained at –80°C in nutrient broth (Oxoid, UK) containing 30% (v/v) glycerol for further testing.

**Antimicrobial susceptibility testing.** Antimicrobial susceptibility testing was performed using a Vitek® 2 Compact system (bioMerieux, France) (Al-Tawfiq and Anani 2009; Ahmed et al. 2019; González et al. 2020). The minimum inhibitory concentrations (MICs) of antimicrobial agents belonging to nine antimicrobial classes were measured according to the Clinical and Laboratory Standard Institute (CLSI 2021). The following antimicrobial agents were tested: ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam (penicillins); ceftazidime, cefepime (cephalosporins); ertapenem, imipenem, meropenem (carbapenems); amikacin, gentamicin (aminoglycosides); ciprofloxacin, norfloxacin (fluoroquinolones); tigecycline (tetracyclines); and fosfomycin, nitrofurantoin, and trimethoprim/sulfamethoxazole (miscellaneous agents). Random confirmation of the Vitek® 2 Compact results was achieved using Etest strips and the Kirby-Bauer disc diffusion method. This step was performed using random isolates with random antimicrobial agents to confirm the Vitek® 2 Compact results. A 0.5 McFarland standard suspension was prepared for each of the tested isolates; the suspension was spread on Mueller-Hinton agar (HiMedia, India), antibiotic discs and Etest strips were placed on each plate, and the plates were incubated at 37°C for 18 h (Munkhdelger et al. 2017; van den Bijlalaardt et al. 2018; Al-Saad et al. 2020). Interpretation of the zone diameter and MIC values obtained with
the Etest was performed according to the guidelines provided by CLSI (2021). The susceptibility to meropenem and imipenem was examined using Etest strips (bioMerieux, France), and the disc diffusion method was used to examine the following antimicrobial agents, ampicillin (10 µg), cefepime (30 µg), imipenem (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), and nitrofurantoin (300 µg) (Oxoid, UK).

**Phenotypic detection of antimicrobial resistance mechanisms and virulence factors.** The phenotypic detection of ESBL and *K. pneumoniae* carbapenemase (KPC) was achieved by subculturing confirmed *E. coli* isolates on CHROMagar ESBL, and CHROMagar KPC (CHROMagar, France) and the plates were incubated at 37°C for 24–48 h (Hornsy et al. 2013; Khater and Sherif 2014; Farra et al. 2016). Sheep blood agar plates (Oxoid, UK) were used to detect hemolysin-producing phenotypes as one of the virulence factors harbored by UPEC (Aghemwenhio et al. 2017).

**Assay of biofilm formation.** The ability of UPEC isolates to form biofilms was measured using the 96-well microtiter plate method as described by Naves et al. (2008), using nutrient broth as growth media (HiMedia, India). The biofilm formation abilities were classified as strong (S), moderate (M), and weak (W) by comparing the absorbance of crystal violet solubilized in 95% ethanol at 610 nm against that of the negative control. The classification of UPEC biofilm-forming abilities is listed in Table I.

**Molecular detection of the genes encoding virulence factors.** Bacterial genomic DNA was extracted by boiling, and a volume of 2 µl was used as the template DNA (Abulreesh 2014). The following genes encoding virulence factors were detected in all confirmed *E. coli* isolates; *kpsMTII* (capsular polysaccharide synthesis K1), *traT* (serum survival), *sat* (secreted autotransporter toxin), *csgA* (curli fimbriae), *vat* (vacuolating autotransporter), and *iutA* (ferric aerobactin receptor). The specific primers for each gene, the product size, and the annealing temperature are listed in Table II.

**Molecular detection of KPC and ESBL genes.** The presence of the *bla*KPC and *blaCTX-M* genes, which represent class A carbapenemase and ESBL, respectively, in all confirmed *E. coli* isolates were detected by PCR. The primers used in this assay, their annealing temperature, and their product size are shown in Table II.

### Table I
Calculations of Biofilm classification values.

| Biofilm formation ability | Range     | Strong (S) | Moderate (M) | Weak (W) | Negative control |
|--------------------------|-----------|------------|--------------|----------|-----------------|
| BF = AB – CW             | ≥0.200 – 0.299 | 0.100 – 0.199 | <0.100      | <0.100   |
| BF = AB + CW             | 4.00 – 5.99   | 2.00 – 3.99   | <2.00 – 1.00 | <1.00   |

BF – biofilm formation, AB – stained attached bacteria, CW – stained control wells

### Table II
The sequence, annealing temperatures and the product size of the primers used to amplify genes encoding virulence factors and antimicrobial resistance.

| Gene/ primer | Sequence | Product size (bp) | Annealing temperature |
|--------------|----------|------------------|----------------------|
| *kpsMTII*    | FP: 5'-GCCGATTGTGCTGATCCTGTG-3' | 270 | 62 |
|              | RP: 5’-CATCCAGACGATAAGCATGAGCA-3' |              |            |
| *traT*       | FP: 5'-GGCGATGATGCGATGACCAGAC-3' | 288 | 62 |
|              | RP: 5’-CAGGCTTACGACCTCCCTAGG-3' |              |            |
| *sat*        | FP: 5'-CTCAAGAAGCTCAGGGAATATTGTTGGCGATGG-3' | 931 | 59 |
|              | RP: 5’-CCATTATCACCAATGCAAAGCCACC-3' |              |            |
| *csgA*       | FP: 5'-GGCGGAAATGTTGCGATGGCGATGTTG-3' | 301 | 60 |
|              | RP: 5’-CGTATGACCATAAGCTTCTTCCCCGA-3' |              |            |
| *vat*        | FP: 5'-AAGCGATGCGGATCAATCCGAAACATCC-3' | 418 | 58 |
|              | RP: 5’-AGGCCTTCTAGAATGGGCGAGTA-3' |              |            |
| *iutA*       | FP: 5'-GGCGATGACATGCGGGAATGCGTG-3' | 302 | 85 |
|              | RP: 5’-CGTCGAGGAACGGGTGAGTACC-3' |              |            |
| *blaKPC*     | FP: 5'-GATAACAGTGGCTTTCGTTGGCGATGAC-3' | 246 | 50–60 |
|              | RP: 5’-GCATATGCCTTGGGCGATGAC-3' |              |            |
| *blaCTX-M*   | FP: 5'-TTTGGGATGTCGATGACCTGAAACATCC-3' | 544 | 62 |

**BF** – biofilm formation, **AB** – stained attached bacteria, **CW** – stained control wells
The PCR conditions were previously described by Al-Sa’ady et al. (2020).

**Control strains.** *E. coli* ATCC® 25922™, was used as a positive control for culture media, confirmatory tests, detection of hemolysis, and antimicrobial susceptibility testing. *K. pneumoniae* ATCC® 700603™ was used as a positive control for CHROMagar ESBL and negative control for CHROMagar KPC. *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC® 14028™ was a negative control for culture media. *P. aeruginosa* NCTC10662 was used as a positive control for oxidase test, and *S. aureus* ATCC® 25923™was applied as a negative control throughout the study.

**Statistical analysis.** The statistical analyses were performed using SPSS Statistics for Windows version (SPSS version 21.0). Chi-squared ($\chi^2$) test was used to test the hypothesis that the prevalence of UPEC is not different among male and female patients; a $p$-value less than 0.05 was considered to indicate significance and to measure the linear association between biofilm-forming ability and multidrug-resistance in UPEC, Pearson correlation coefficient was used.

### Results

**Prevalence of community-associated UPEC.** Of the 1,000 urine samples examined for UTI-associated *E. coli*, only 50 samples (5%) were positive for UTIs, and all 50 samples yielded positive UPEC cultures. The prevalence of *E. coli* was significantly higher ($\chi^2 \leq 0.001$) in samples of female origin (12.4%, 28 positives, $n=226$), whereas 2.84% of the samples of male origin yielded UPEC isolates ($n=774$) (Table III).

**Antimicrobial susceptibility patterns of UPEC.**

Antimicrobial susceptibility testing revealed that 82% ($n=50$) of all UPEC isolates were resistant to norfloxacin (fluoroquinolones), and 60% ($n=50$) of the isolates were resistant to ampicillin (penicillins) (Table III). Moreover, 44% ($n=50$) of all UPEC isolates showed resistance to trimethoprim/sulfamethoxazole, and 38% ($n=50$) of the isolates exhibited resistance to cephalosporins (ceftazidime and cefepime). In contrast, very low resistance to gentamicin (aminoglycosides) (12%), amoxicillin/clavulanic acid (8%), and piperacillin/tazobactam (penicillins) (4%) was observed. None of the 50 UPEC isolates examined in this study exhibited resistance to carbapenems (imipenem, meropenem, and ertapenem) or amikacin (aminoglycosides) (Table III).

Almost half of the UPEC isolates investigated in this study exhibited multidrug resistance (MDR) patterns (resistance to at least three classes of antimicrobial agents), as shown in Table IV. Approximately 23 (46%) of the 50 UPEC isolates showed MDR, mainly to four antimicrobial classes (13 isolates, 56.5%). Nine of the 23 UPEC of male origin exhibited resistance to at least three antimicrobial classes, and 14 isolates of female origin showed this pattern (Table IV). The most noticeable MDR pattern involved resistance to ampicillin (penicillins), ceftazidime and cefepime (cephalosporins), ciprofloxacin and norfloxacin (fluoroquinolones), and trimethoprim/sulfamethoxazole. This pattern was more frequently observed in the isolates of female origin than in the isolates of male origin (Table IV).

**Detection of ESBL, KPC, and α-hemolysin phenotypes of UPEC isolates.** Phenotypic detection of ESBL-positive strains of UPEC showed that 36% (18 isolates, $n=50$) of the isolates were ESBL positive, as detected by the Vitek® 2 Compact system and CHROMagar ESBL, while 54% (27, $n=50$) of all UPEC exhibited β-hemolytic activity on sheep blood agar (Table V). Overall, the number of ESBL-positive and hemolysin-producing UPEC of female origin was not higher than that of their male counterparts (Table V). Most of the ESBL- and KPC-positive phenotypes exhibited MDR patterns, except for the isolates UPEC 666 and UPEC 1000, which were not multidrug-resistant strains (Table V). Despite detecting 17 KPC-positive phenotypes, none of the 50 UPEC isolates were resistant to carbapenems, as demonstrated using the Vitek® 2 compact system and Etest strips (Table III).

### Table III

Antimicrobial resistance (Intermediate resistance*) profiles of uropathogenic *E. coli* in western Saudi Arabia.

| Sample origin | N  | P (%) | AM | AC | PT | CEFT | CEFE | ERT | IMI | MER | AMI | G | CIP | NOR | NIT | TS |
|---------------|----|-------|----|----|----|------|------|-----|-----|-----|-----|---|----|-----|-----|-----|
| Male          | 774| 22 (2.84) | 12 | 1, (1*) | 1 | 7 | 7 | 0 | 0 | 0 | 0 | 2 | 6 | 19 | 2 | 9 |
| Female        | 226| 28 (12.4) | 18 | 1, (1*) | 1 | 12 | 12 | 0 | 0 | 0 | 0 | 4 | 8 | 22 | (2*) | 13 |
| Total         | 1,000| 50 (5) | 30 | 4 | 2 | 19 | 19 | 0 | 0 | 0 | 0 | 6 | 14 | 41 | 4 | 22 |
| $p$           |    | <0.001 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |

$N$ – total number of urine samples examined for *E. coli*, $P$ – total number of samples positive for *E. coli*, % – percentage of positive samples, $p$ – $p$-values from chi-square ($\chi^2$), * – strains with intermediate resistance

AM – ampicillin, AC – amoxicillin/clavulanic acid, PT – piperacillin/tazobactam, CEFT – ceftazidime, CEFE – cefepime, ERT – ertapenem, IMI – imipenem, MER – meropenem, AMI – amikacin, G – gentamicin, CIP – ciprofloxacin, NOR – norfloxacin, NIT – nitrofurantoin, TS – trimethoprim/sulfamethoxazole
**Table IV**

Multidrug-resistance patterns among uropathogenic E. coli.

| Isolate code | Origin | Antibiotic resistance patterns | No. of antimicrobial classes | ESBL† | KPC‡ |
|--------------|--------|--------------------------------|-----------------------------|-------|------|
| **UPEC 2**   | F      | AM, CEFT, CEF, NOR, TS        | 3                           | –     | –    |
| **UPEC 6**   | F      | AM, CEFT, CEF, NOR, TS        | 4                           | +     | +    |
| **UPEC 12**  | F      | AM, CEFT, CEF, NOR, TS        | 4                           | +     | +    |
| **UPEC 13**  | F      | AM, CEFT, CEF, NOR, TS        | 4                           | +     | +    |
| **UPEC 17**  | M      | AM, CEFT, CEF, NOR, TS        | 4                           | –     | –    |
| UPEC 20      | F      | NOR                           | 1                           | –     | –    |
| **UPEC 25**  | F      | AM, AC, PT*, CIP, NOR, NIT*   | 3                           | –     | –    |
| **UPEC 32**  | F      | AM, CEFT, CEF, NOR, TS        | 4                           | +     | +    |
| **UPEC 39**  | M      | AM, CEF, CEF, NIT             | 3                           | +     | +    |
| **UPEC 45**  | M      | AM, CEF, CEF, NOR, TS         | 4                           | +     | +    |
| UPEC 88      | F      | AM, NOR                        | 2                           | –     | –    |
| **UPEC 95**  | F      | AM, CEF, CEF, NOR, TS         | 4                           | +     | +    |
| **UPEC 119** | F      | AM, CEF, CEF, NOR             | 3                           | +     | +    |
| UPEC 127     | M      | NOR                           | 1                           | –     | –    |
| UPEC 138     | F      | NOR                           | 1                           | –     | –    |
| UPEC 145     | F      | NOR                           | 1                           | –     | –    |
| UPEC 151     | F      | NOR                           | 1                           | –     | –    |
| UPEC 156     | M      | NOR                           | 1                           | –     | –    |
| UPEC 168     | F      | NOR                           | 1                           | –     | –    |
| **UPEC 192** | M      | AM, CEF, CEF, NOR, TS         | 4                           | +     | +    |
| **UPEC 213** | M      | AM, CEF, CEF, CIP, NOR, TS    | 4                           | +     | +    |
| **UPEC 225** | M      | AM, G, CIP, NOR, TS           | 4                           | –     | –    |
| UPEC 226     | M      | NOR                           | 1                           | –     | –    |
| UPEC 230     | M      | NOR                           | 1                           | –     | –    |
| UPEC 242     | M      | NOR                           | 1                           | –     | –    |
| **UPEC 243** | F      | AM, G, CIP, NOR, TS, NIT*     | 4                           | –     | –    |
| **UPEC 316** | F      | AM, CEF, CEF, G, CIP, NOR, TS | 5                           | +     | +    |
| **UPEC 326** | F      | AM, CEF, CEF, G, CIP, NOR, TS | 5                           | +     | +    |
| UPEC 331     | M      | NOR                           | 1                           | –     | –    |
| **UPEC 353** | F      | AM, CEF, CEF, TS              | 3                           | +     | –    |
| **UPEC 425** | M      | AM, AC, PT, NOR, TS           | 3                           | –     | –    |
| UPEC 432     | M      | NOR                           | 1                           | –     | –    |
| **UPEC 450** | M      | AM, CEF, CEF, NOR, TS         | 5                           | +     | +    |
| **UPEC 544** | F      | AM, AC*, CEF, CEF, G, CIP, NOR, TS | 4 | – | – |
| **UPEC 549** | M      | AM, CEF, CEF, CIP, TS         | 4                           | +     | +    |
| **UPEC 661** | F      | AM, CEF, CEF, TS              | 3                           | +     | +    |
| UPEC 662     | F      | AM                            | 1                           | –     | –    |
| UPEC 666     | F      | AM, CEF, CEF                  | 2                           | +     | +    |
| UPEC 738     | M      | AM                            | 1                           | –     | –    |
| UPEC 829     | F      | AM, TS                         | 2                           | –     | –    |
| UPEC 950     | M      | AM, TS                         | 2                           | –     | –    |
| UPEC 1000    | M      | AM, NIT                       | 2                           | +     | +    |

* – intermediate resistance
** – multidrug resistant – a single isolate is resistant against more than 3 antimicrobial classes
† – ESBL positive phenotypes detected by CHROMagar ESBL
‡ – KPC positive phenotypes detected by CHROMagar KPC

AM – ampicillin, AC – amoxicillin/clavulanic acid, PT – piperacillin/tazobactam, CEF – cefazidime, CEF – cefepime,
G – gentamicin, CIP – ciprofloxacin, NOR – norfloxacin, NIT – nitrofurantoin, TS – trimethoprim/sulfamethoxazole
Biofilm formation of UPEC isolates. The ability of all 50 UPEC isolates to form biofilms was examined using microtiter plates with crystal violet staining. As shown in Table VI, a substantial number of UPEC isolates were either unable (56%, n = 50) or exhibited a weak (84%, n = 50) ability to form biofilms, as demonstrated using two different formulae (Table VI). Pearson correlation coefficients were used to study the relationship between MDR and biofilm formation, and no significant association (r = –0.0948) and (r = –0.1475) (Table VI) was found. Notably, no association was observed between biofilm formation and resistance to any particular drug.

Molecular detection of genes encoding virulence factors and antimicrobial resistance in UPEC isolates. The PCR detection of genes encoding virulence factors revealed that all 50 UPEC isolates examined in this study harbored genes encoding capsular polysaccharide synthesis K1 (kpsMTII), ferric aerobactin receptor (iutA), serum survival (traT) and curli fimbriae (csgA) (Table VII and Fig. 1). The genes encoding secretion autoinducer toxin (sat) and vacuolating

| Isolates origin | Total number of UPEC | ESBL+* (%) | ESBL+† (%) | KPC+ (%) | β-hemolysis (%) |
|-----------------|----------------------|------------|------------|----------|-----------------|
| Male            | 22                   | 7 (32)     | 7 (32)     | 7 (32)   | 11 (50)         |
| Female          | 28                   | 11 (39.3)  | 11 (39.3)  | 10 (36)  | 16 (57.2)       |
| Total           | 50                   | 18 (36)    | 18 (36)    | 17 (34)  | 27 (54)         |

*+ phenotypes were detected via Vitek 2 Compact screening
†+ phenotypes were detected on CHROMagar ESBL
+ phenotypes were detected on CHROMagar KPC
β-hemolysis – phenotypes were detected on sheep blood agar

Fig. 1A. Agarose gel electrophoresis shows positive results of kpsMTII virulence gene at (270 bp) in UPEC.
M – DNA marker, C – negative control

Fig. 1B. Agarose gel electrophoresis shows positive results of csgA virulence gene at (301 bp) in UPEC.
M – DNA marker, C – negative control

Fig. 1C. Agarose gel electrophoresis shows some positive results of vat virulence gene at (418 bp) in UPEC.
M – DNA marker, C – negative control
Semiquantitative classification of biofilm formation using two different formulas and the association of biofilm-forming ability and multidrug-resistance in uropathogenic E. coli.

| Isolate code | BF\textsuperscript{†} | BF\textsuperscript{‡} | AMR |
|-------------|---------------------|---------------------|-----|
| UPEC (2)    | N (0.058)           | W (1.167)           | 3   |
| UPEC (6)    | M (0.185)           | N (0.420)           | 4   |
| UPEC (12)   | S (0.343)           | W (1.988)           | 4   |
| UPEC (13)   | S (0.365)           | M (2.051)           | 4   |
| UPEC (17)   | N (0.066)           | W (1.190)           | 4   |
| UPEC (20)   | S (0.313)           | W (1.902)           | 2   |
| UPEC (25)   | M (0.121)           | W (1.348)           | 2   |
| UPEC (32)   | N (0.067)           | W (1.193)           | 4   |
| UPEC (39)   | W (0.39)            | M (2.123)           | 2   |
| UPEC (45)   | N (0.086)           | W (1.247)           | 4   |
| UPEC (88)   | N (0.066)           | W (1.190)           | 2   |
| UPEC (95)   | N (0.086)           | W (1.247)           | 4   |
| UPEC (119)  | N (0.046)           | W (1.132)           | 4   |
| UPEC (127)  | S (0.339)           | W (1.976)           | 1   |
| UPEC (138)  | S (0.239)           | W (1.688)           | 1   |
| UPEC (145)  | M (0.151)           | W (1.435)           | 1   |
| UPEC (151)  | M (0.135)           | W (1.389)           | 1   |
| UPEC (156)  | W (0.057)           | W (1.164)           | 1   |
| UPEC (168)  | W (0.031)           | W (1.089)           | 1   |
| UPEC (192)  | W (0.034)           | W (1.097)           | 4   |
| UPEC (213)  | W (0.074)           | W (1.213)           | 4   |
| UPEC (225)  | M (0.187)           | W (1.538)           | 4   |
| UPEC (226)  | M (0.121)           | W (1.348)           | 1   |
| UPEC (230)  | N (0.068)           | W (1.178)           | 1   |
| UPEC (242)  | N (–0.015)          | N (0.960)           | 1   |
| UPEC (243)  | N (0.062)           | W (1.162)           | 4   |
| UPEC (316)  | N (0.016)           | W (1.041)           | 5   |
| UPEC (326)  | N (–0.027)          | N (0.929)           | 5   |
| UPEC (331)  | N (0.004)           | W (1.010)           | 1   |
| UPEC (353)  | N (0.031)           | W (1.081)           | 3   |
| UPEC (425)  | N (–0.017)          | N (0.955)           | 3   |
| UPEC (432)  | N (0.027)           | W (1.070)           | 1   |
| UPEC (450)  | N (0.054)           | W (1.141)           | 5   |
| UPEC (544)  | N (0.061)           | W (1.159)           | 4   |
| UPEC (549)  | S (0.216)           | W (1.565)           | 4   |
| UPEC (601)  | N (0.024)           | W (1.062)           | 0   |
| UPEC (654)  | N (0.013)           | W (1.034)           | 0   |
| UPEC (661)  | N (0.088)           | W (1.230)           | 3   |
| UPEC (662)  | M (0.125)           | W (1.327)           | 1   |
| UPEC (666)  | N (0.068)           | W (1.178)           | 2   |
| UPEC (704)  | S (0.328)           | W (1.858)           | 0   |
| UPEC (738)  | N (–0.017)          | N (0.955)           | 1   |
| UPEC (829)  | N (–0.027)          | N (0.929)           | 2   |
| UPEC (842)  | N (0.074)           | W (1.193)           | 0   |
| UPEC (848)  | S (0.379)           | W (1.992)           | 0   |
| UPEC (882)  | N (0.09)            | W (1.235)           | 0   |

All values were measured at OD_{620nm}.

- BF\textsuperscript{†} = biofilm formation was determined by applying formula \( BF = AB – CW \)
- BF\textsuperscript{‡} = biofilm formation was determined by applying formula \( BF = AB/CW \)

AMR = antimicrobial resistance (showing resistance to the number of antimicrobial classes)

- AB = stained attached bacteria
- CW = stained control wells
- S = strong
- M = moderate
- W = weak
- N = negative

\( r \) = the value from Pearson correlation coefficient, no significant relationship between biofilm-forming ability and multidrug-resistant in all 50 UPEC isolates

NS = not significant, no significant association between biofilm-forming ability and multidrug-resistance to antimicrobial agents

autoinducer (vct) were found in 49 (98%) and 19 (38%) of the isolates, respectively (Table VII and Fig. 1). The prevalence of genes encoding antimicrobial resistance, namely, \( bla_{CTX-M} \) (ESBL) and \( bla_{KPC} \) (class A carbapenemase) was low because these were detected in 36% (18, \( n = 50 \)) and 24% (12, \( n = 50 \)) of the UPEC isolates, respectively (Table VII). The isolates UPEC 168; 192; 213; 316; 326, and 1000 harbored the \( bla_{KPC} \) gene; these isolates grew on CHROMagar KPC plates despite their susceptibility to imipenem, meropenem, and ertapenem, as determined using the Vitek® 2 Compact system and Etest strips. Other UPEC isolates harboring the \( bla_{KPC} \) gene included UPEC 829, 842, 848, 882, and 900. None of these isolates grew on CHROMagar KPC, and all of the isolates were susceptible to all antimicrobial agents (Table IV).

**Discussion**

Uropathogenic E. coli remains the leading cause of CA-UTIs worldwide (Flores-Mireles et al. 2015). Epidemiological records have shown that UPEC accounts for more than 70% of CA-UTIs in the USA and UK (Foxman 2010; 2014; Öztürk and Murt 2020), European countries (Cullen et al. 2012; François et al. 2016), South and far Eastern Asia (Banerjee 2009; Tan and Chlebicki 2016; Setu et al. 2016), Africa (Moroh et al. 2014) the Middle East (Al-Gasha’a et al. 2020), Australia (Cunningham et al. 2021), and Central and Latin America (Medina and Castillo-Pino 2019; Pasillas...
Fabian et al. 2021). Our results also showed that all positive UTI cultures contained UPEC isolates, and this high prevalence of UPEC is following the world trend and previous studies conducted in Saudi Arabia (Faidah et al. 2013; El-Kersh et al. 2015; Ahmad 2019). This higher prevalence of UPEC compared with other uropathogens in CA-UTIs may be explained by the fact that *E. coli* is more abundant in the faces than other Gram-negative causative agents and faces-derived Gram-positive enterococci which is a less common cause of CA-UTIs (Flores-Mireles et al. 2015). Even among HA-UTIs, UPEC accounts for approximately 50–60% of the reported cases, 40% of the cases are attributed to *Klebsiella*, *Proteus*, *Pseudomonas*, and *Serratia* (Gram-negative), and 10% of the cases are caused by Gram-positive cocci (enterococci and staphylococci) (Flores-Mireles et al. 2015).

Females may be at a higher risk of developing UTIs, particularly community-associated UPEC infections, than males. The published records show that more than 50% of adult women may develop UTIs (Tan and Chlebicki 2016; Medina and Castillo-Pino 2019) at some point in their life because the anatomical distance between the urethra and anus, where *E. coli* usually exists, is closer in females than in males. Additionally, the female urethra is shorter than the male urethra, which gives the bacteria more straightforward access to the bladder (Flores-Mireles et al. 2015). Thus, our results showed a higher prevalence of UPEC in females than in male patients, which agrees with previously published accounts worldwide (Foxman 2010; 2014; Flores-Mireles et al. 2015; Medina and Castillo-Pino 2019; Öztürk and Murt 2020).

UTIs remain a leading cause of morbidity, with more than 10 million visits to clinics and three million emergency admissions annually around the globe (Paul 2018; Kot 2019). UTI is not a self-limiting infection, and failure to treat UTI may result in various life-threatening complications; thus, a UTI requires a treatment regimen with antibiotics (Godbole et al. 2020). There are recommendations for UTI treatment based on the type of infection, e.g., nitrofurantoin, trimethoprim, and cefalexin are recommended as first-choice empirical treatments for CA-UTIs in women, whereas amoxicillin, trimethoprim/sulfamethoxazole, and amoxicillin/clavulanic acid are recommended as second-line choices if the first choice yields no improvement (Godbole et al. 2020). Ciprofloxacin is effective in treating complicated UTIs (Kot 2019). Although the currently recommended choice of antibiotics for treating UTI caused by UPEC includes trimethoprim/sulfamethoxazole, ciprofloxacin, and ampicillin (Flores-Mireles et al. 2015). It was suggested that the treatment choice should be based on the actual susceptibility testing of the patients’ bacterial culture (McLellan and Hunstad, 2016).

In the present study, we observed high rates of resistance to norfloxacin (82%), followed by ampicillin (60%), and trimethoprim/sulfamethoxazole (44%), which are among the recommended treatment choices. Other studies in Saudi Arabia have revealed similar observations (Eltahowt and Khalaf 1988; Al-Yousef et al. 2016; Balkhi et al. 2018; Ahmad 2019). Ciprofloxacin (fluoroquinolones) has been highly effective in treating complicated UPEC-associated UTIs and an alarming increase in the number of UPEC strains resistant to ciprofloxacin worldwide has been detected, suggesting that avoid considering ciprofloxacin as first-line treatment of UPEC-associated UTIs. It should be considered in severe infections or as an alternative when the recommended agents cannot be used (Kot 2019). We also found that 28% of our isolates were resistant to ciprofloxacin, as has also recorded in previous studies in Saudi Arabia (Al-Yousef et al. 2016; Balkhi et al. 2018). With the emergence of ESBL-producing UPEC strains, the use of carbapenems (e.g., imipenem, ertapenem, and meropenem) and aminoglycosides (e.g.,

### Table VII

| Isolates origin | Total number of UPEC | Genes encoding for virulence factors | Genes encoding for antimicrobial resistance |
|-----------------|----------------------|-------------------------------------|------------------------------------------|
|                 |                      | kpsMTII (%) | iutA (%) | traT (%) | csgA (%) | Sat (%) | Vat (%) | blaCTX-M (%) | blaKPC (%) |
| Male            | 22                   | 22 (100)    | 22 (100) | 22 (100) | 22 (100) | 22 (100) | 9 (41)   | 8 (37)      | 7 (32)     |
| Female          | 28                   | 28 (100)    | 28 (100) | 28 (100) | 28 (100) | 27 (97)  | 10 (36)  | 10 (36)     | 5 (18)     |
| Total           | 50                   | 50 (100)    | 50 (100) | 50 (100) | 50 (100) | 49 (98)  | 19 (38)  | 18 (36)     | 12 (24)    |
amikacin) has been recommended for the treatment of UPEC because the majority of the strains (more than 98%) show susceptibility to these agents (Terlizzi et al. 2017). We also observed complete susceptibility (100%) to these agents among our isolates, which is in accordance with the observations from other studies in Saudi Arabia (Alsultan et al. 2013; Ahmad 2019). We also observed very low resistance to amoxicillin/clavulanic acid and piperacillin/tazobactam, which may be effective against ESBL-producing UPEC strains (Terlizzi et al. 2017). The results obtained in this study and other previously published accounts generally indicate that UPEC strains in Saudi Arabia are susceptible to the commonly recommended antibiotics for the treatment of UTIs; however, the choice of treatment in each UTI case must be decided after susceptibility testing to avoid the overuse of particular drugs that might lead to the promotion of drug resistance.

MDR has become a worrying issue from a public health standpoint (Samreen et al. 2021). The emergence of multidrug-resistant Gram-negative bacteria, particularly members of the Enterobacteriaceae implicated in UTI cases, has increased the burden on managing UTIs worldwide, particularly in regions where antimicrobials are readily available over the counter (Paul 2018). The treatment of infections related to antimicrobial-resistant strains is estimated to cost approximately $2.2 billion annually in the USA alone (Nguyen et al. 2019). In the current study, MDR was observed in 46% of the UTI-associated UPEC isolates, and approximately 61% of the MDR isolates were of female origin. The most notable resistance patterns included resistance to ampicillin, cefepime, ceftazidime, ciprofloxacin, norfloxacin, and trimethoprim/sulfamethoxazole, which are frequently encountered in UPEC strains of female origin. Resistance patterns to β-lactams, cephalosporins, and trimethoprim/sulfamethoxazole as observed in this study, are increasingly emerging among UPEC isolates in Saudi Arabia and other parts of the world (Alsultan et al. 2013; Alanazi et al. 2018; Critchley et al. 2019; Sokhn et al. 2020). This resistance pattern may be due to overuse or misuse of fluoroquinolones, trimethoprim/sulfamethoxazole, and cephalosporins. Such widespread use of these agents as first-choice treatments for UTIs may have promoted resistance, particularly in regions where patients tend to take antibiotics without prescription (Sokhn et al. 2020). In this study, we observed a worrying increasing prevalence of ESBL phenotypes in UPEC isolates (36%, n = 50), particularly among isolates exhibiting resistance to fluoroquinolones, cephalosporins, and trimethoprim/sulfamethoxazole. This observation agrees with other published results (Critchley et al. 2019; Sokhn et al. 2020), and may be explained by the overuse of cephalosporins, and the global distribution of UPEC clones carrying the bla \text{CTX-M} gene. This gene was detected in 36% of the isolates tested in this study. UPEC clones carrying bla \text{CTX-M} are widely disseminated and exhibit MDR to fluoroquinolones, trimethoprim/sulfamethoxazole, and aminoglycosides (Critchley et al. 2019). Thus, the continuous surveillance of MDR strains and the enforcement of the judicious use of antimicrobial agents are vital for control and combat strategies against emerging UTI-associated MDR-UPEC strains and the overall management of UTI treatment.

Carbapenems have proven high efficacy in treating various severe bacterial infections, particularly those caused by ESBL-producing agents (Codjoe and Donkor 2018). Although the alarming increase in the emergence of carbapenem-resistant UPEC is of great public health concern worldwide (Terlizzi et al. 2017; Codjoe and Donkor 2018), none of the 50 UPEC isolates tested in this study exhibited resistance to carbapenems, as detected phenotypically by using the Vitek® 2 Compact system and Etest strips. Several studies conducted in Saudi Arabia and other parts of the world reported complete susceptibility to carbapenems among UPEC strains responsible for HA- and CA-UTIs (Critchley et al. 2019; Sokhn et al. 2020). However, using CHROMagar KPC, we observed that 34% of the isolates, particularly those exhibiting MDR patterns yielded positive cultures. Furthermore, the molecular detection of bla \text{KPC} produced positive bands (246 bp) from 24% of the isolates, even isolates with no resistance to any antimicrobial agent. Similar results have been observed with a collection of UPEC isolates from Saudi Arabia, and this finding was thought to be due to a lack of sensitivity or even a failure of automated systems to detect carbapenem resistance (AITamimi et al. 2017). It has been suggested that CHROMagar KPC exhibits more sensitivity (100%) and specificity (98.4%) than the PCR detection of KPC genes and the disk diffusion, Etest, and automated system (Vitek® 2 Compact; MicroScan (Siemens Healthcare, Germany)) (Codjoe and Donkor 2018). Another possible explanation for our results is the probability that the bla \text{KPC} gene detected in our isolates may have been under repression and that it was not expressed; thus, no resistance against meropenem, imipenem, and ertapenem was detected with the Vitek® 2 Compact system and Etest strips. In light of these results, we suggest that interpretations of carbapenem susceptibility in clinical settings should be met with care because these solely rely on automated systems and/or disc diffusion techniques, and additional confirmatory methods should therefore be used.

Biofilm-forming ability is believed to play an important role in the pathogenesis of bacterial infections. The clinical relevance of the biofilm-forming capability of pathogenic bacteria includes the ability of these bacteria to resist antimicrobial agents and to become
persistent sources of infections and their ability to exchange genetic elements for virulence and drug resistance. In pathogenic bacteria, biofilm formation appears to be a survival mechanism that helps these bacteria adapt to environmental conditions, and this ability thus has profound public health implications (Muhammad et al. 2020). The biofilm-forming ability of UPEC enhances the ability of this pathogen to persist and thrive in the urinary tract environment, particularly in the bladder, by evading the immune system, and it has been suggested to play a role in recurrent infection (Kaper et al. 2004; Vila et al. 2016; Kudinha 2017). Thus, many studies have reported that UPEC strains, particularly hospital-acquired strains, exhibit a strong ability to form biofilms (Zamani and Salehzadeh 2018; Tewawong et al. 2020).

In contrast, the substantial number of the 50 community-associated UPEC isolates examined in this study exhibited either no (84%) or a weak ability (56%) to form biofilms. Similar results were reported by De Souza et al. (2019), who found that community-associated UPEC strains are less able to form biofilms than hospital-acquired UPEC strains, which reportedly show stronger biofilm-forming ability. De Souza et al. (2019) hypothesized that the poor ability of community-associated UPEC to form biofilms is due to low cell hydrophobicity observed in community-associated UPEC.

On the other hand, a strong correlation has been found between cell hydrophobicity and biofilm formation in hospital-acquired UPEC because medical devices such as catheters are produced from hydrophobic materials (De Souza et al. 2019). The observations on our isolates are similar to those reported by Naziri et al. (2021), who found that the frequency no or weak biofilm-forming ability is significantly higher ($p < 0.05$) among community-associated UPEC than among hospital-acquired isolates. Karigoudar et al. (2019) observed that hospital-acquired UPEC tended to exhibit stronger biofilm-forming ability than community-associated UPEC strains. More recently, Zhao et al. (2020) reported that even though the majority of UPEC (84%) isolates can form biofilms, more than 40% of these isolates exhibit weak biofilm-forming abilities in accordance with the results reported in this study.

It is well established that the biofilm-forming ability of UPEC may play a role in the increasing emergence of MDR in this pathogen. Several studies have explored the relationship between biofilm-forming ability and MDR in UPEC and have suggested that the acquisition of multiple resistance in UPEC strains is strongly associated with their biofilm formation capacities (Karigoudar et al. 2019; Zhao et al. 2020). Other researchers did not observe an association between biofilm formation and MDR in general; however, a strong connection between biofilm formation and the development of resistance to particular drugs, such as ampicillin, ciprofloxacin, and norfloxacin, has been suggested (Tewawong et al. 2020). In contrast, the results of the current study suggest the lack of a relevant association ($r = -0.0948$) between MDR and biofilm formation in our community-associated UPEC isolates. Similar observations have been reported by Behzadi et al. (2020), who examined 250 UPEC isolates with various resistance profiles to determine possible relationships between their biofilm-forming ability and resistance profiles. Moreover, no relationships between biofilm-forming ability and the development of MDR have been observed in other uropathogens, e.g., Acinetobacter baumannii, as determined by phenotypic and genotypic methods (Avila-Novoa et al. 2019). Thus, it is difficult to draw definitive conclusions on this topic due to conflicting reports regarding the association between biofilm formation and the development of MDR in UPEC.

UPEC differ from other commensal strains because they possess various virulence factors that facilitate their ability to invade, penetrate, attach to, and persist in the urinary tract and evade the response of the human immune system. It has been hypothesized that food from animal sources (e.g., poultry meat) may constitute a potential reservoir for community-associated UPEC strains (Vincent et al. 2010; Manges 2016). After humans acquire these strains, they become common inhabitants of the human intestinal tract and may transfer their virulence to common commensal E. coli via a horizontal gene transfer (Sarowska et al. 2019). We observed a high prevalence of virulence factors in the community-associated UPEC strains examined in this study; a prevalence of 100% ($n = 50$) was found for the csgA, iutA, traT and kpsMTII genes, and the prevalence rates of the sat and vat genes were 94% and 38%, respectively. This finding agrees with recent studies that revealed a high prevalence of these genes in UPEC isolates in Iraq (Al-Sa’ady et al. 2020) and Egypt (Abd El-Baky et al. 2020). The detection of α-hemolysin in our isolates revealed that an overall prevalence of this toxin is equal to 54%, and a higher prevalence was found among isolates of female origin. It has been suggested that persisting and/or recurrent UTIs are caused by UPEC strains with more virulence factors and that produce α-hemolysin, as has been observed in CA-UTIs among females (Ejrnaes et al. 2011); thus, the virulent isolates examined in this study may cause persistent UTIs. It appears that community-associated UPEC isolates produce more extensive virulence factors and exhibit increased antimicrobial resistance than hospital-acquired isolates, which appears to be a worldwide trend (De Souza et al. 2019). A comparative study between community-associated and hospital-acquired UPEC strains revealed that community-associated UPEC possesses more ($p < 0.05$) virulence factors than...
nosocomial strains (Shevade and Agrawal 2015). This finding supports the results obtained in this study, which reveal a higher prevalence of virulence factors among community-associated UPEC strains in Saudi Arabia. No previous studies have investigated the prevalence and diversity of genes encoding virulence factors among UPEC strains in Saudi Arabia; thus, to the best of our knowledge, this study constitutes the first investigation of the virulence of UPEC in general and community-associated UPEC strains in particular.

**Conclusion**

UPEC remains the leading cause of UTI in females in Saudi Arabia. The increasing emergence of multidrug-resistant UPEC strains, particularly those showing resistance to first-line drugs, is very alarming, and continued surveillance is mandatory to identify resistance patterns and implement appropriate treatment management. Community-associated UPEC strains appear to have weak biofilm-forming abilities, and conflicting reports regarding the association between biofilm formation and the development of MDR in UPEC strains make it difficult to draw definitive conclusions. The diversity of virulence factors possessed by community-associated UPEC strains may render these strains more virulent and may further explain the frequency of recurrence/relapse among cases of community-associated UTIs. To our knowledge, this study constitutes the first exploration of the virulence, biofilm-forming ability, and its association with MDR of UPEC strains in Saudi Arabia. Further similar investigations are needed to elucidate the epidemiology of community-associated UPEC strains in Saudi Arabia.

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**Abbreviations**

CA-UTI – community associated urinary tract infection
CLED – cystine-lactose-electrolyte-deficient
EMB – eosin methylene blue
ESBL – extended spectrum β-lactamase
HA-UTI – hospital acquired urinary tract infection
KPC – Klebsiella pneumoniae carbapenemase
MDR – multidrug resistance
MIC – minimum inhibitory concentration
TSI – triple sugar iron
UPEC – uropathogenic *Escherichia coli*
UTIs – urinary tract infections

**Ethical statement**

This study has been reviewed and approved by the Department of Biology postgraduate and research ethics committee and also approved by the Faculty of Applied Science postgraduate and research ethics committee, approval number (3421209144114) on 5th of May 2020. All urine samples analyzed in this study were anonymous, and only the gender of the sample provider was disclosed. Personal, clinical and epidemiological data related to these samples were not provided or disclosed during the study.

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**Author contributions**

Conceptualization: H.H.A, and N.A.O; Methodology: H.H.A, S.H.A, K.E., and N.A.O; Investigation: S.H.A, H.H.A, M.S.A, W.A.A. and F.H.A; Data curation: S.H.A, H.H.A, N.A.O; Formal analysis: H.H.A. and S.H.A; Resources: S.R.O. and K.E.; Writing – original draft preparation: H.H.A and S.H.A; Writing – review and editing: H.H.A and I.A. Supervision: H.H.A. All authors have read and agreed to the published version of the manuscript.

**Conflict of interest**

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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