ORIGINAL ARTICLE

Uropathogenic *Escherichia coli* in the high vaginal swab samples of fertile and infertile women: virulence factors, O-serogroups, and phenotyping and genotyping characterization of antibiotic resistance

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Abstract

Transmission of urinary tract infections into the reproductive system is unavoidable. The present research was performed to assess the distribution of virulence genes, O-serogroups and antibiotic resistance properties of uropathogenic *Escherichia coli* (UPEC) strains isolated from the high vaginal swab samples of fertile and infertile women. A total of 460 high vaginal swab samples were taken from fertile and infertile women. Distribution of virulence factors and serogroups and antibiotic resistance properties of the *E. coli* isolates were assessed. Sixty-five out of 460 (14.13%) swab samples were positive for *E. coli*. Prevalences of *E. coli* in samples taken from fertile and infertile women were 13.63% and 14.58%, respectively. O1 (7.69%), O2 (6.15%) and O6 (6.15%) were the most frequently detected serogroups. The most frequently detected virulence genes were *sfa* (72.72%), *afa* (72.72%), *cnf1* (72.72%) and *fim* (72.72%). The most commonly detected antibiotic-resistance genes were *tetA* (95.45%), *CITM* (88.63%), *aac(3)-IV* (86.36%) and *sul1* (72.72%). UPEC strains harboured the highest prevalence of resistance against tetracycline (88.63%), ampicillin (79.54%), gentamicin (77.27%) and enrofloxacin (52.27%). Seventeen out of 26 (65.38%) UPEC strains isolated from infertile women were resistant toward more than ten antibiotic agents. Infertile women with a history of urinary tract infections had the higher prevalence of UPEC strains and also the other characters. High prevalence of the virulent and resistant UPEC strains in the high vaginal part of the infertile women with a history of urinary tract infections may show an important role of these pathogens as causes of female infertility. However, further research is required to confirm this hypothesis.

Keywords: Antibiotic resistance, high vaginal swabs, uropathogenic *Escherichia coli*, virulence factors women's infertility
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Introduction

Infertility is an important issue and is defined as the failure to attain a pregnancy after 12 months or more of *consecutive* unprotected sex [1–4]. Infertility affects 5.00%–25.70% of couples globally, and about 73 million couples are considered to be infertile [4]. Documented data show that the main reason for infertility in about 30% of cases is still unknown [1–4]. In addition, infertility can lead to divorce, suicide, guilt, blame, stress and depression, especially in women [5]. From a clinical perspective, it is important to understand novel aspects of infertility in females.

Most infertile women are faced with severe changes in the local immunity of the vagina. Studies report that infections of the oviduct and vagina are the main factors causing such

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inflammatory changes [6]. Infectious agents usually contribute to chronic inflammation of the high vagina, cervix and endometrium. Infections can also cause significant changes in the secretions of the reproductive tract, interference in the normal physiology of the embryo and gamete, and structural damage to the vagina [7,8].

High prevalence of *Escherichia coli* in the vaginal of women with explained and unexplained infertility has been reported in recent investigations [9–17], but types of bacteria isolated were unclear. *Escherichia coli* strains have been classified into six types: enterohaemorrhagic *E coli*, enterotoxigenic *E. coli*, attaching and effacing *E. coli*, enteropathogenic *E. coli*, shiga toxin-producing *E. coli* and uropathogenic *E. coli* (UPEC) [18–20]. The UPEC strains are estimated to be a major cause of urinary tract infections (UTIs) all around the world [18,19]. Overall, more than half of women have a UTI during their lives [21].

Urinary and reproductive systems are closely associated with each other and infections of one system can easily transmit to another. It is documented that women who have recurrent UTIs have a higher frequency and magnitude of vaginal colonization with *E. coli* [22,23]. Bacterial examination of the vaginal epithelial cells of women with recurrent UTIs confirmed the high presence of *E. coli* strains [23]. Presence of putative virulence factors in the UPEC strains causes diverse inflammatory reactions and changes in the hormonal secretions of the vagina [24]. Adhesions, P fimbriae (*pap*), haemolysin (*hly*), cytotoxic necrotizing factor I (*cnf-1*), aerobactin (*aer*), type I fimbriae, S fimbriae (*sfa*), a fimbrial adhesin I (*afaI*), *iroN*, *usp*, *set-1*, *kpsMT*, *fimH*, *ompT*, group II capsule synthesis, *astA*, *iha*, S and F1C fimbriae, *traT*, *sfa/foc* and *iutA* are the most important virulence factors of the UPEC strains in different types of human clinical infections. These genes are involved in the pathogenicity of the UPEC bacteria [16,18]. Adhesion factors, systems of the iron uptake and also cytotoxins, haemolysin and specified O:K:H serotypes are responsible for the pathogenicity of UPEC infections. UTIs caused by the UPEC strains mainly belong to O1, O2, O4, O6, O7, O8, O15, O16, O18, O21, O22, O25, O75 and O83 serogroups [18–20].

Antibiotic therapy is one of the most important protocols for the treatment of infections caused by UPEC strains. However, *E. coli* strains isolated from different types of clinical infections show a high prevalence of resistance against several classes of antibiotics [25–34].

Antibiotic-resistant UPEC strains cause more severe diseases for longer periods of time with higher therapeutic expenses [18,35,36]. According to the recent epidemiological studies, UPEC strains displayed considerable levels of resistance (50%–100%) against routine antibiotic agents [18,35,36]. Several important antibiotic-resistance genes are responsible for the occurrence of resistance against commonly used antibiotic agents such as kanamycin, tetracycline, ampicillin, gentamycin, imipenem, amikacin, cefotaxime, ciprofloxacin, cotrimoxazole, norfloxacin and cephalothin [18,35,36].

According to the high importance of UPEC bacteria in human clinical infections and their unknown roles in the vagina of women with a history of recurrent UTIs, the current research was carried out to assess the distribution of virulence factors, O-serogroups and antibiotic resistance properties of UPEC strains isolated from the high vaginal swab samples of fertile and infertile women.

Materials and methods

Ethical approval

This study was confirmed by the Ethical Council of the Infertility and Sterility Centre, Iran (Fatemeh-Zahra infertility and Sterility Centre, Babol, Iran). Corroboration of the research project and the licenses related to sampling procedures were also confirmed by Prof. Hassan Momtaz and Prof. Sedigheh Esmaeilzadeh. Informed consent was obtained from all patients. All samples were taken from volunteer women who were referred to the Infertility and Sterility Hospital, Babol, Iran.

Samples, inclusion and exclusion criteria, and E. coli identification

From October 2014 to October 2015, 240 high vaginal swab specimens were collected from infertile women with unknown causes of infertility. A woman after a year of unprotected sex without any successful pregnancy was considered infertile. The selected women were screened by transvaginal sonography in follicular phase and underwent pelvic ultrasound scans to exclude individuals with polycystic ovarian syndrome, uterine fibroids (>5 cm in size or impinging on the uterine cavity), endometriosis and other structural anomalies of the genital tract. Screening also included a basal hormone evaluation between days 2 and 5 of the ovarian cycle to exclude women with abnormal levels of serum luteinizing hormone, follicular-stimulating hormone, prolactin and thyroid-stimulating hormone. Women with a diagnosis of male factor infertility, as determined by an abnormal semen analysis of the male partner, were also screened and excluded. Women without certain causes of female infertility were included in the present study. Specimens were obtained from the ventral fornix without any interaction with urine and external parts of the reproductive system using a speculum and commercial sterile cotton-tipped swabs. Specimens were taken by a skilled midwife. Two-hundred and twenty vaginal swab specimens were also taken directly from fertile women. History of UTIs was recorded for each sample.

TABLE 1. The oligonucleotide primers and PCR condition used for detection of O-serogroups, virulence factors and antibiotic-resistance genes of *Escherichia coli* strains isolated from infertile and fertile women [16,18,25,39]

Target gene	Primer sequence (5′–3′)	PCR product (bp)	PCR volume (50 µL)	PCR programs
E. coli 16S rRNA	F: AGAGTTTGATCMTGGCTCAG R: CCGTCAATTCATTTGAGTTT	919	5 µL PCR buffer 10 × 1.5 mM $MgCl_2$ 200 µM dNTP (Thermo Fisher Scientific, St Leon-Rot, Germany) 0.5 µM of each primers F & R 1.25 U *Taq* DNA polymerase (Thermo Fisher Scientific, St Leon-Rot, Germany) 2.5 µL DNA template	1 cycle: 95 C for 6 min 30 cycles: 94 °C for 45 s 59 °C for 60 s 72°C for 60 s 1 cycle: 72°C for 5 min
O1	F: GTGAGCAAAAGTGAAATAAGGAACG R: CGCTGATACGAATACCATCCTAC	1098	5 µL PCR buffer 10 × 2 mM $MgCl_2$	1 cycle: 94°C for 5 min
O6	F: GGATGACGATGTGATTTTGGCTAAC R: TCTGGGTTTGCTGTGTATGAGGC	783	200 µM dNTP 0.5 µM of each primers F & R	30 cycles: 95°C for 30 s
O7	F: CTATCAAAATACCTCTGCTGGAATC R: TGGCTTCGAGATTAAACCTATTCCT	610	1.5 U *Taq* DNA polymerase 5 µL DNA template	55°C for 60 s 72°C for 60 s
O8	F: CCAGAGGCATAATCAGAAATAACAG R: GCAGAGTTAGTCAACAAAAGGTCAG	448		1 cycle: 72°C for 8 min
O16	F: GGTTTCAATCTCACAGCAACTCAG R: GTTAGAGGGATAATAGCCAAGCGG	302		
O21	F: CTGCTGATGTCGCTATTATTGCTG R: TGAAAAAAAGGGAAACAGAAGAGCC	209		
O75	F: GAGATATACATGGGGAGGTAGGCT R: ACCCGATAATCATATTCTTCCCAAC	511		
O2	F: AGTGAGTTACTTTTTAGCGATGGAC R: AGTTTAGTATGCCCCTGACTTTGAA	770	5 µL PCR buffer 10 × 2 mM $MgCl_2$	1 cycle: 94°C for 5 min
O4	F: TTGTTGCGATAATGTGCATGTTCC R: AATAATTTGCTATACCCACACCCTC	664	200 µM dNTP 0.5 µM of each primers F & R	25 cycles: 94°C for 60 s
O15	F: TCTTGTTAGAGTCATTGGTGTATCG R: ATAAAACGAGCAAGCACCACACC	183	1.5 U *Taq* DNA polymerase 5 µL DNA template	56°C for 60 s 72°C for 60 s
O18	F: GTTCGGTGGTTGGATTACAGTTAG R: CTACTATCATCCTCACTGACCACG	551		1 cycle: 72°C for 8 min
O22	F: TTCATTGTCGCCACTACTTTCCG R: GAAACAGCCCATGACATTACTACG	468		
O25	F: AGAGATCCGTCTTTTATTTGTTCGC R: GTTCTGGATACCTAACGCAATACCC	230		
O83	F: GTACACCAGGCAAACCTCGAAAG R: TTCTGTAAGCTAATGAATAGGCACC	362		
iss	F: ATCACATAGGATTCTGCCG R: CAGCGGAGTATAGATGCCA	309	5 µL PCR buffer 10 × 2 mM $MgCl_2$	1 cycle: 94°C for 3 min
irp2	F: AAGGATTCGCTGTTACCGGAC R: AACTCCTGATACAGGTGGC	413	200 µM dNTP 0.5 µM of each primers F & R	25 cycles: 94 °C for 30 s
tsh	F: ACTATTCTCTGCAGGAAGTC R: CTTCCGATGTTCTGAACGT	824	1.5 U *Taq* DNA polymerase 5 µL DNA template	58°C for 30 s 68°C for 3 min
vat	F: TCCTGGGACATAATGGTCAG R: GTGTCAGAACGGAATTGT	981		1 cycle: 72°C for 10 min
cva	F: TGGTAGAATGTGCCAGAGCAAG R: GAGCTGTTTGTAGCGAAGCC	1181		
usp	F: ACATTCACGGCAAGCCTCAG R: AGCGAGTTCCTGGTGAAAGC	440	5 µL PCR buffer 10 × 2 mM $MgCl_2$ 200 µM dNTP 0.5 µM of each primers F & R 1.5 U *Taq* DNA polymerase 5 µL DNA template	1 cycle: 94°C for 2 min 30 cycles: 94 °C for 30 s 58°C for 30 s 73°C for 30 s 1 cycle: 72°C for 10 min
iha	F: CTGGCGGAGGCTCTGAGATCA R: TCCTTAAGCTCCCGCGGCTGA	827	5 µL PCR buffer 10 × 2 mM $MgCl_2$	1 cycle: 94°C for 2 min
iron	F: AAGTCAAAGCAGGGGTTGCCCG R: GACGCCGACATTAAGACGCAG	665	200 µM dNTP 0.5 µM of each primers F & R	30 cycles: 94°C for 30 s
ompT	F: ATCTAGCCGAAGAAGGAGGC R: CCCGGGTCATAGTGTTCATC	559	1.5 U *Taq* DNA polymerase 5 µL DNA template	58°C for 30 s 73°C for 30 s 1 cycle: 72°C for 10 min
kpsMT	F: CCATCGATACGATCATTGCACG R: ATTGCAAGGTAGTTCAGACTCA	400	5 µL PCR buffer 10 × 2 mM $MgCl_2$ 200 µM dNTP 0.5 µM of each primers F & R 1.5 U *Taq* DNA polymerase 5 µL DNA template	1 cycle: 94 °C for 10 min 30 cycles: 94 °C for 60 s 60 °C for 60 s 72 °C for 60 s 1 cycle: 72 °C for 5 min
papGI	F: TCGTGCTGAGGTCCGGAATTT R: TGGCATCCCCCAACATTATCG	461	5 µL PCR buffer 10 × 2 mM $MgCl_2$	1 cycle: 95°C for 2 min
papGII	F: GGGATGAGCGGGCCTTTGAT R: CGGGCCCCCAAGTAACTCG	190	200 µM dNTP 0.5 µM of each primers F & R	30 cycles: 94°C for 60 s
papGIII	F: GGCCTGCAATGGATTTACCTGG R: CCACCAAATGACCATGCCAGAC	258	1.5 U *Taq* DNA polymerase 5 µL DNA template	69°C for 30 s 72°C for 2 min 1 cycle: 72°C for 10 min
luc	F: ATGAGAATCATTATTGACATAATTG R: CTCACGGGTGAAAATATTTT	1482	5 µL PCR buffer 10 × 2 mM $MgCl_2$	1 cycle: 94 °C for 60 s
fim	F: GAGAAGAGGTTTGATTTAACTTATTG R: AGAGCCGCTGTAGAACTGAGG	559	200 µM dNTP 0.5 µM of each primers F & R	40 cycles: 94 °C for 60 s 58°C for 70 s

Continued

TABLE 1. Continued

Target gene	Primer sequence (5′–3′)	PCR product (bp)	PCR volume (50 µL)	PCR programs
			1.5 U *Taq* DNA polymerase	72°C for 70 s
			5 µL DNA template	1 cycle:
				72°C for 3 min
set-1	F: GTGAACCTGCTGCCGATATC	147	5 µL PCR buffer 10 ×	1 cycle:
	R: ATTTGTGGATAAAAATGACG		2 mM $MgCl_2$	94°C for3 min
sen	F: ATGTGCCTGCTATTATTTAT	799	200 µM dNTP	30 cycles:
	R: CATAATAATAAGCGGTCAGC		0.5 µM of each primers F & R	94°C for 30 s
astA	F: ATGCCATCAACACAGTATAT	110	1.5 U *Taq* DNA polymerase	55°C for 60 s
	R: GCGAGTGACGGCTTTGTAGT		5 µL DNA template	72°C for 60 s
sigA	F: TCCTCGGTATTATTTTATCC	408		1 cycle:
	R: CGTAACCCCTGTTGTTTCCAC			72°C for 5 min
sap	F: TACCCTCCACAACAGAGAATG	832		
	R: TACCCTCCACAACAGAGAATG			
pap	F: GCAACAGCAACGCTGGTTGCATCAT	336	5 µL PCR buffer 10 ×	1 cycle:
	R: AGAGAGAGCCACTCTTATACGGACA		2 mM $MgCl_2$	94°C for 5 min
cnf1	F: AAGATGGAGTTTCCTATGCAGGAG	498	200 µM dNTP	30 cycles:
	R: TGGAGTTTCCTATGCAGGAG		0.5 µM of each primers F & R	94°C for 60 s
hlyA	F: AACAAGGATAAGCACTGTTCTGGCT	1177	1.5 U *Taq* DNA polymerase	63°C for 30 s
	R: ACCATATAAGCGGTCATTCCCGTCA		5 µL DNA template	72°C for 90 s
sfa	F: CTCCGGAGAACTGGGTGCATCTTAC	410		1 cycle:
	R: CGGAGGAGTAATTACAAACCTGGCA			72°C for 10 min
afa	F: GCTGGGCAGCAAACTGATAACTCTC	750		
	R: CATCAAGCTGTTTGTTCGTCCGCCG			
aadA1	F: TATCCAGCTAAGCGCGAACT	447	5 µL PCR buffer 10 ×	1 cycle:
	R: ATTTGCCGACTACCTTGGTC		2 mM $MgCl_2$	95°C for 15 min
aac(3)-IV	F: CTTCAGGATGGCAAGTTGGT	286	200 µM dNTP	30 cycles:
	R: TCATCTCGTTCTCCGCTCAT		0.5 µM of each primers F & R	94°C for 30 s
sul1	F: TTCGGCATTCTGAATCTCAC	822	1.5 U *Taq* DNA polymerase	58°C for 30 s
	R: ATGATCTAACCCTCGGTCTC		5 µL DNA template	72°C for 60 s
blaSHV	F: TCGCCTGTGTATTATCTCCC	768		1 cycle:
	R: CGCAGATAAATCACCACAATG			72°C for 10 min
CITM	F: TGGCCAGAACTGACAGGCAAA	462		
	R: TTTCTCCTGAACGTGGCTGGC			
cat1	F: AGTTGCTCAATGTACCTATAACC	547		
	R: TTGTAATTCATTAAGCATTCTGCC			
cmlA	F: CCGCCACGGTGTTGTTGTTATC	698		
	R: CACCTTGCCTGCCCATCATTAG			
tet(A)	F: GGTTCACTCGAACGACGTCA	577		
	R: CTGTCCGACAAGTTGCATGA			
tet(B)	F: CCTCAGCTTCTCAACGCGTG	634	5 µL PCR buffer 10 ×	1 cycle:
	R: GCACCTTGCTGATGACTCTT		2 mM $MgCl_2$	94°C for 8 min
dfrA1	F: GGAGTGCCAAAGGTGAACAGC	367	200 µM dNTP	32 cycles:
	R: GAGGCGAAGTCTTGGGTAAAAAC		0.5 µM of each primers F & R	95°C for 60 s
qnr	F: GGGTATGGATATTATTGATAAAG	670	1.5 U *Taq* DNA polymerase	55°C for 70 s
	R: CTAATCCGGCAGCACTATTTA		5 µL DNA template	72°C for 2 min
				1 cycle:
				72°C for 8 min

Specimens were directly transported to laboratory at 4°C using ice packs. The swab samples were inoculated onto MacConkey agar (Oxoid, Basingstoke, UK) and 5% sheep blood agar (Oxoid), and then incubated at 37°C for 24 hours. Positive samples were determined by growth of typical colonies of *E. coli*. The *E. coli* isolates were then identified based on morphological properties and biochemical tests including Gram-staining, indole, methyl red, Voges–Proskauer and citrate (IMViC) fermentation, triple sugar iron, urease, and nitrate reduction tests (Merck, Darmstadt, Germany). *Escherichia coli* isolates were also identified by the API 20E system (Analytab Products, Plainview, NY, USA).

Antimicrobial susceptibility testing

Patterns of antibiotic resistance of the *E. coli* isolates were assessed using the simple disc diffusion method. The isolates were cultured onto the Mueller–Hinton agar (HiMedia Laboratories, Mumbai, India; MV1084). Antibiotic discs including kanamycin (1000 µg/disc), tetracycline (30 µg/disc), ampicillin (10 µg/disc), gentamycin (10 µg/disc), imipenem (30 µg/disc), amikacin (30 µg/disc), mezlocillin (30 µg/disc), cefotaxime (30 µg/disc), piperacillin (30 µg/disc), ciprofloxacin (5 µg/disc), cotrimoxazole (30 µg/disc), norfloxacin (30 µg/disc), ceftazidime (30 µg/disc), nitrofurantoin (300 µg/disc), ofloxacin (5 µg/disc), ceftriaxone (30 µg/disc), nalidixic acid (30 µg/disc), tobramycin (30 µg/disc), clindamycin (2 µg/disc) and cephalothin (30 µg/disc) (Oxoid) were placed on the cultured Mueller–Hinton agar and all media were incubated aerobically at 37°C for 24 hours. All examinations and also interpretation of the findings were performed according to the instructions and guidelines of the CLSI [37]. *Escherichia coli* ATCC 8739 was used as a control organism.

DNA extraction and E. coli confirmation using PCR

A single colony of the *E. coli* isolates was inoculated on 5 mL of Luria–Bertani broth media (Merck) and incubated at 37°C for

24 hours. Genomic DNA was extracted from the bacterial colonies using a commercial DNA extraction kit (Thermo Fisher Scientific, Bremen, Germany). DNA extraction was performed according to the manufacturer's instruction's. Purity (A_{260}/A_{280}) and concentration of extracted DNA were then checked (NanoDrop, Thermo Scientific, Waltham, MA, USA). The quality of extracted DNA samples was assessed on a 2% agarose gel stained with ethidium bromide (0.5 μg/mL) (Thermo Fisher Scientific, Germany) [38]. PCR amplification of the *16SrRNA* gene was used to confirmed of the *E. coli* colonies [39,40] (Table 1).

Detection of O-serogroups, virulence genes and antibiotic resistance genes

Table 1 shows the sequence of primers, size of products and PCR conditions used for detection of O-serogroups, virulence and antibiotic-resistance genes [16,18,25,39]. PCR amplification was performed using a programmable DNA thermo-cycler device (Eppendorf Mastercycler; Eppendorf, Hamburg, Germany). Ten microlitres of PCR product was exposed to electrophoresis in a 2% agarose gel in 1 × TBE buffer at 80 V for 30 min, stained with SYBR Green (Thermo Fisher Scientific, Germany). The UVI doc gel documentation system (Grade GB004, Jencons PLC, London, UK) was used for analysis of images. Positive DNA samples and PCR-grade water were used as positive and negative controls, respectively.

Statistical analysis

Microsoft Excel software (Microsoft Corp., Redmond, WA, USA) was used for data classification. Statistical analysis was performed using the SPSS 21.0 statistical software (SPSS Inc., Chicago, IL, USA). The χ^2 test and Fisher's exact two-tailed test were used to assess any significant relationship between the prevalence of UPEC strains and their virulence and antibiotic resistance properties. A p value < 0.05 was considered statistically significant.

Results

Demographic characteristics

Table 2 shows the demographic characteristics of the studied individuals. Findings revealed that the mean age in fertile and infertile women was 36.2 and 37.6 years, respectively. The frequencies of women with a history of UTIs in fertile and infertile groups was 33.33% (80/240) and 37.560% (90/240). There were no statistically significant differences between the demographic properties of fertile and infertile women included in the present study.

Distribution of E. coli strains in different specimens

Table 3 shows the prevalence of *E. coli* bacteria in high vaginal swabs of the fertile and infertile women. Of 460 high vaginal swab samples studied, 65 (14.13%) specimens were positive for *E. coli*. Thirty-five out of 240 (14.58%) high vaginal swab samples of infertile women and 30 out of 220 (13.63%) high vaginal swab samples of fertile women were positive for *E. coli* (p 0.77). Total distribution of *E. coli* in the women with a history of UTIs was higher than in those without a history of UTIs (p 0.053).

Distribution of O-serogroups

Table 3 shows the distribution of O-serogroups in the *E. coli* strains isolated from fertile and infertile women. The most commonly detected O-serogroups in all studied samples were O1 (7.69%), followed by O2 (6.15%) and O6 (6.15%). O25 (29.41%) and O1 (17.64%) were the most commonly detected serogroups in the infertile women with a history of UTIs, but there were no positive results for O1 serogroup and the incidence of O25 serogroup was 11.11% in women without a history of UTIs. A statistically significant difference was seen for the distribution of O25 serogroup between infertile women with a history of UTIs and those without a history of UTIs ($p < 0.05$). Statistically significant differences were also obtained for the prevalence of O1 and O5 serogroups between UPEC bacteria isolated from fertile women with a history of UTIs and those without a history of UTIs ($p < 0.05$). Similarly, a statistically significant difference was found for the distribution of O1 and O5 serogroups between UPEC bacteria isolated from infertile women with a history of UTIs and fertile women without a history of UTIs ($p < 0.05$).

Monthly prevalence of E. coli strains

Fig. 1 shows the numbers of *E. coli* strains isolated from fertile and infertile women in different months of the year. Results showed that high vaginal swab samples collected in July had the highest numbers of isolated *E. coli* in all four groups of women. We found statistically significant difference for the numbers of isolated *E. coli* between cold and warm months ($p < 0.05$).

Frequency of virulence factors

Table 4 shows the distribution of different virulence factors in the *E. coli* strains isolated from fertile and infertile women. *sfa* (72.72%), *afa* (72.72%), *cnf1* (72.72%), *fim* (72.72%), *papGI* (65.90%) and *pic* (63.63%) were the most frequently detected virulence factors in the *E. coli* strains. Distributions of *cva*, *irp2*, *vat*, *iss*, *sap* and *ompT* genes were 11.36%, 15.90%, 20.45%, 22.72%, 27.27% and 29.54%, respectively. A statistically significant difference was found for the distribution of the *sfa* gene between the infertile women with a history of UTIs and those

TABLE 2. Demographic characteristics of the studied individuals

Demographic characteristics	Fertile women (n = 240)	Infertile women (n = 240)	p value
Age (years), mean (SD)	36.2 (8.4)	37.6 (9.5)	NS
Weight (kg), mean (SD)	65.2 (10.1)	67.3 (10.4)	NS
BMI (kg/m^2), mean (SD)	25.4 (2.8)	26.3 (3.1)	NS
History of UTIs (n)	80	90	NS

BMI, body mass index; NS, not significant; SD, standard deviation.

without a history of UTIs ($p < 0.05$). Statistically significant differences were also obtained for the prevalence of *sfa* and *afa* genes between fertile women with a history of UTIs and those without a history of UTIs ($p < 0.05$). Similarly, statistically significant differences were found for the distribution of *sfa* and *afa* genes between UPEC bacteria isolated from infertile women with a history of UTIs and fertile women without a history of UTIs ($p < 0.05$).

Antibiotic resistance pattern of UPEC isolates

Table 5 shows the antibiotic resistance pattern of *E. coli* strains isolated from the fertile and infertile women. Strains of *E. coli* harboured the highest prevalence of resistance against tetracycline (88.63%), ampicillin (79.54%), gentamicin (77.27%), enrofloxacin (52.27%) and cephalothin (52.27%), and the lowest prevalence of resistance against chloramphenicol (6.81%), imipenem (9.09%) and tobramycin (15.90%). Statistically significant differences were found for the prevalence of antibiotic resistance against tetracycline, ampicillin and gentamicin between UPEC bacteria isolated from infertile women with a history of UTIs and those without a history of UTIs ($p < 0.05$), fertile women with a history of UTIs and those without a history of UTIs ($p < 0.05$) and between infertile women with a history of UTIs and fertile women without a history of UTIs ($p < 0.05$).

Distribution of antibiotic-resistance genes

Table 6 shows the distribution of antibiotic-resistance genes of the *E. coli* strains isolated from fertile and infertile women. *TetA* (95.45%), *CITM* (88.63%), *aac(3)-IV* (86.36%) and *sul1* (72.72%) were the most frequently detected antibiotic-resistance genes in the UPEC bacteria isolated from fertile and infertile women. Statistically significant differences were found for the distributions of *tetA*, *aadA1*, *dfrA* and *CITM* antibiotic resistance genes between UPEC bacteria isolated from infertile women with a history of UTIs and those without a history of UTIs ($p < 0.05$), fertile women with a history of UTIs and those without a history of UTIs ($p < 0.05$) and also between infertile women with a history of UTIs and fertile women without a history of UTIs ($p < 0.05$).

Prevalence of multidrug-resistant strains

Fig. 2 shows the numbers of multidrug-resistant *E. coli* strains isolated from fertile and infertile women. All *E. coli* strains of both fertile and infertile groups were resistant to at least one examined antibiotic agent. UPEC bacteria isolated from infertile women had the higher prevalence of multidrug resistance than those of fertile women ($p < 0.05$). One out of 18 (5.55%) *E. coli* strains isolated from fertile women and 17 out of 26 (65.38%) *E. coli* strains isolated from infertile women were resistant to more than ten antibiotic agents.

Discussion

Infections of the reproductive tract might be aetiological factors of female infertility. Hormonal disturbances in infertile women can cause a decrease in the level of local immunity in the vagina, which facilitates UPEC colonization and survival [41]. Reported data showed that follicular fluids of women are not sterile. Indeed, infertility causes a decrease in the local

TABLE 3. Distribution of O-serogroups in the *Escherichia coli* strains isolated from fertile and infertile women

Groups of women (No. of samples)		No. of *E. coli* (%)	Distribution of O-serogroups (%)														
			O1	O2	O4	O6	O7	O8	O15	O16	O18	O21	O22	O25	O75	O83	Other
Infertile women	History of UTIs (90)	17	3	2	1	2	—	1	—	—	1	—	—	5	1	—	1
	No history of UTIs (150)	18	—	—	—	1	1	1	1	1	—	1	1	2	—	1	8
	Total (240)	35 (14.58)	3 (8.57)	2 (5.71)	1 (2.85)	3 (8.57)	1 (2.85)	2 (5.71)	1 (2.85)	1 (2.85)	1 (2.85)	1 (2.85)	1 (2.85)	7 (20)	1 (2.85)	1 (2.85)	9 (25.71)
Fertile women	History of UTIs (80)	14	2	2	1	1	—	1	—	1	1	—	—	3	1	—	1
	No history of UTIs (140)	16	—	—	—	—	—	—	1	1	—	1	1	1	—	—	11
	Total (220)	30 (13.63)	2 (6.66)	2 (6.66)	1 (3.33)	1 (3.33)	—	1 (3.33)	1 (3.33)	2 (6.66)	1 (3.33)	1 (3.33)	1 (3.33)	4 (13.33)	1 (3.33)	—	12 (40)
Total (460)		65 (14.13)	5 (7.69)	4 (6.15)	2 (3.07)	4 (6.15)	1 (1.53)	3 (4.61)	2 (3.07)	3 (4.61)	2 (3.07)	2 (3.07)	2 (3.07)	11 (16.92)	2 (3.07)	1 (1.53)	21 (32.30)

Abbreviations: UPEC, uropathogenic *Escherichia coli*; UTI, urinary tract infection.

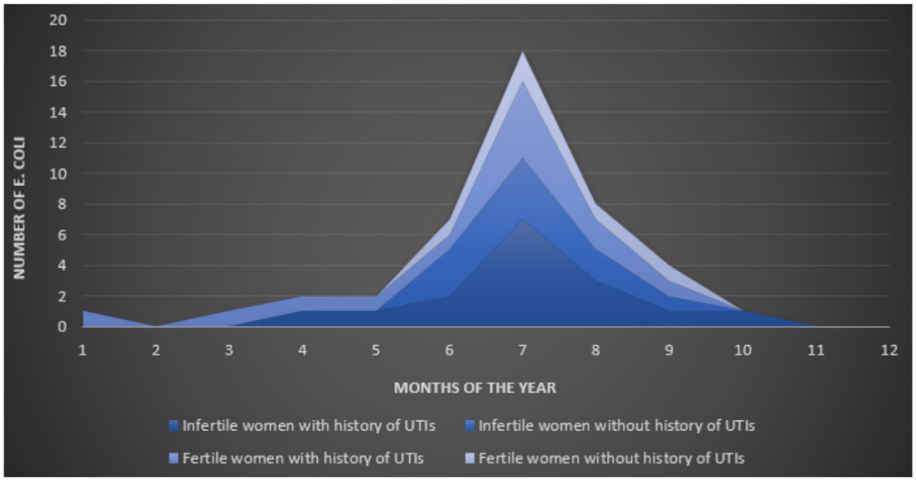

FIG. 1. Monthly distribution of *Escherichia coli* strains isolated from fertile and infertile women.

immunity of the vagina, particularly chemokines, cytokines and even related growth factors [42], secretion of the ovarian steroid hormones [42], and growth and reversion of corpus luteum [43]. Therefore, infective agents can easily grow in the vagina and even be transferred from anus and/or urinary tract into the reproductive organs. Previous studies showed that women who have recurrent UTIs have a higher frequency and magnitude of vaginal colonization with UPEC strains [44,45]. Our research reported a high prevalence of resistant and virulent UPEC strains in the high vaginal swab samples of fertile and infertile women, particularly those with a history of UTIs. Lack of timely treatment caused probable transmission of UPEC strains from the urinary tract into the reproductive system. Damage caused by the virulence genes and the high prevalence of resistance in bacteria against commonly used antimicrobial agents are possibly predisposing factors involved in the persistence of infection in the high vaginal region of infertile women.

Total prevalence of *E. coli* in the high vaginal swab samples examined in the present study was 14.13%. Different prevalence rates of *E. coli* in the vagina have been reported [9–13]. Pdia *et al.* [46] reported that the prevalence of *E. coli* colonization in the high vaginal swab samples of women was 25%, which was higher than our findings. Kazi *et al.* [47] reported that the prevalence of *E. coli* in the high vaginal regions of women examined in Pakistan was 28%. Obata-Yasuoka *et al.* [48] reported that the prevalence of *E. coli* in the high vaginal regions of Japanese women was 3.41%. Kaur and Prabha [49] reported that the presence of *E. coli* in the vagina/vaginal tract might play a significant role in female infertility. A marked monthly distribution was found for incidence of the *E. coli* strains in the high vaginal swab samples of the present study. Samples collected from patients in July had the highest prevalence of *E. coli*. Changes in weather conditions and also differences in atmospheric pressure may influence the distribution of bacteria in July. These factors cause decreases in the level of human immunity. Based on the conclusion of Freeman *et al.* [50], summer peaks in the prevalence of *E. coli* may be a result of multifaceted seasonal variations in human behaviour. Changes in behaviour could increase the risk of contact with *E. coli* and also the risk of contact with *E. coli* carried by other humans. Changes in levels of personal hygiene, sexual actions and even dietary behavior are clear examples of behavioral changes linked to the seasons. Levels of hygiene decrease during warmer seasons of the year. Therefore, the possibility of bacterial growth is higher in warmer months. Similar results have been reported by Al-Hasan *et al.* [51], Perencevich *et al.* [52], Schwartz *et al.* [53], Dehkordi *et al.* [54], Nejat *et al.* [55] and Hasanpour Dehkordi *et al.* [56].

A high prevalence of putative virulence factors was reported in the UPEC strains of the present study. Obata-Yasuoka *et al.* [48] reported that *E. coli* recovered from the vagina harboured certain virulence factors and serotypes similar to those of extra-intestinal *E. coli*. They revealed that the most frequently detected virulence factors were *PAI* (78%), *pap* (45%), *K1* (44%), *ibeA* (32%), *hlyA* (22%) and *cnf1* (19%), which was relatively similar to our findings. Our results revealed that *sfa*, *afa*, *cnf1*, *hlyA* and *fim* were the most frequently detected virulence genes among the UPEC strains of the studied women. Comparable findings have been reported by Momtaz *et al.* [33] and Dormanesh *et al.* [19]. Tiba *et al.* (2008) [58] revealed that the prevalence of *sfa*, *afa*, *hlyA* and *fim* virulence factors in UTIs in Brazil were 27.80%, 6.20%, 25.30% and 97.50%, respectively. High prevalence of *afa*, *sfa*, *fim* and *hlyA* virulence genes in cases of recurrent UTIs have been determined by Arabi *et al.* [59], Asadi *et al.* [36] and Karimian *et al.* [60].

TABLE 4. **Distribution of virulence factors in the *Escherichia coli* strains isolated from fertile and infertile women**

Groups of women (No. of UPEC strains)		Distribution of virulence factors (%)																							
		set1	astA	sigA	sap	pic	sfa	afa	Cnf1	hlyA	iuc	fim	kspMT	omPT	usp	iss	Irp2	vat	cva	pap	papGI	papGII	papGIII	iha	Iron
Infertile women	History of UTIs (16)	10	9	8	6	11	16	15	14	16	13	16	5	4	14	5	4	4	2	16	15	14	13	9	13
	No history of UTIs (10)	7	8	5	3	8	5	4	6	4	6	3	5	5	3	2	1	3	1	5	4	4	4	3	5
	Total (26)	17 (65.38)	17 (65.38)	13 (50)	9 (34.61)	19 (73.07)	21 (80.76)	19 (73.07)	20 (76.92)	21 (80.76)	19 (73.07)	19 (73.07)	10 (38.46)	9 (34.61)	17 (65.38)	7 (26.92)	5 (19.23)	7 (26.92)	3 (11.53)	21 (80.76)	19 (73.07)	18 (69.23)	17 (65.38)	12 (46.15)	18 (69.23)
Fertile women	History of UTIs (13)	9	8	7	3	9	11	12	10	11	9	12	5	4	8	3	2	2	2	11	9	8	8	4	7
	No history of UTIs (5)	2	1	1	—	—	—	1	2	1	1	1	—	—	—	—	—	—	—	1	1	1	—	—	1
	Total (18)	11 (61.11)	9 (50)	8 (44.44)	3 (16.66)	9 (50)	11 (61.11)	13 (72.22)	12 (66.66)	12 (66.66)	10 (55.55)	13 (72.22)	5 (27.77)	4 (22.22	8 (44.44)	3 (16.66)	2 (11.11)	2 (11.11)	2 (11.11)	12 (66.66)	10 (55.55)	9 (50)	8 (44.44)	4 (22.22	8 (44.44)
Total (44)		28 (63.63)	26 (59.09)	21 (47.72)	12 (27.27)	28 (63.63)	32 (72.72)	32 (72.72)	32 (72.72)	33 (75)	29 (65.90)	32 (72.72)	15 (34.09)	13 (29.54)	25 (56.81)	10 (22.72)	7 (15.90)	9 (20.45)	5 (11.36)	33 (75)	29 (65.90)	27 (61.36)	25 (56.81)	16 (36.36)	26 (59.09)

Abbreviations: UPEC, uropathogenic *Escherichia coli*; UTI, urinary tract infection.

TABLE 5. **Antibiotic-resistance pattern of the *Escherichia coli* strains isolated from fertile and infertile women**

Groups of women (No. of UPEC strains)		Antibiotic-resistance pattern (%)																					
		Kan[a]	TE30	S_{10}	C_{30}	SXT	GM_{10}	NFXS	CF_{30}	CIP5	TMP_5	F/M_{300}	AM_{10}	NLX	imp	AM_{30}	MZL	Cef_{30}	pip	CTMX	CLN	CFTI	tob
Infertile women	History of UTIs (16)	9	16	8	2	11	16	10	9	11	10	9	16	6	3	10	5	6	6	8	9	7	5
	No history of UTIs (10)	2	9	5	—	2	5	4	5	2	1	—	5	1	—	1	1	1	—	4	5	3	—
	Total (26)	11 (42.30)	25 (96.15)	13 (50)	2 (7.69)	13 (50)	21 (80.76)	14 (53.84)	14 (53.84)	13 (50)	11 (42.30)	9 (34.61)	21 (80.76)	7 (26.92)	3 (11.53)	11 (42.30)	6 (23.07)	7 (26.92)	6 (23.07)	12 (46.15)	14 (53.84)	10 (38.46)	5 (19.23)
Fertile women	History of UTIs (13)	7	12	6	1	9	12	8	7	9	9	8	12	2	1	7	4	3	4	3	4	4	2
	No history of UTIs (5)	1	2	1	—	1	1	1	2	—	—	—	2	—	—	—	—	—	—	1	2	1	—
	Total (18)	8 (44.44)	14 (77.77)	7 (38.88)	1 (5.55)	10 (55.55)	13 (72.22)	9 (50)	9 (50)	9 (50)	9 (50)	8 (44.44)	14 (77.77)	2 (11.11)	1 (5.55)	7 (38.88)	4 (22.22)	3 (16.66)	4 (22.22)	4 (22.22)	6 (33.33)	5 (27.77)	2 (11.11)
Total (44)		19 (43.18)	39 (88.63)	20 (45.45)	3 (6.81)	23 (52.27)	34 (77.27)	23 (52.27)	23 (52.27)	22 (50)	20 (45.45)	17 (38.63)	35 (79.54)	9 (20.45)	4 (9.09)	18 (40.90)	10 (22.72)	10 (22.72)	10 (22.72)	16 (36.36)	20 (45.45)	15 (34.09)	7 (15.90)

Abbreviations: UPEC, uropathogenic *Escherichia coli*; UTI, urinary tract infection.
[a]Kan, kanamycin (1000 μg/disc), TE_{30}, tetracycline (30 μg/disc); S_{10}, streptomycin (10 μg/disc); C_{30}, chloramphenicol (30 μg/disc); SXT, sulfamethoxazole (25 μg/disc); GM_{10}, gentamycin (10 μg/disc); NFX_5, enrofloxacin (5 μg/disc); CF_{30}, cephalothin (30 μg/disc); CIP_5, ciprofloxacin (5 μg/disc); TMP_5, trimethoprim (5 μg/disc); F/M_{300}, nitrofurantoin (300 μg/disc); AM_{10}, ampicillin (10 μg/disc); NLX, nalidixic acid (30 μg/disc); imp, imipenem (30 μg/disc); AM_{30}, amikacin (30 μg/disc); MZL, mezlocillin (30 μg/disc); Cef_{30}, cefotaxime (30 μg/disk); pip, piperacillin (30 μg/disk); CTMX, cotrimoxazole (30 μg/disk); CLN, clindamycin (2 μg/disc); CFTI, ceftriaxone (30 μg/disc); tob, tobramycin (30 μg/disc).

TABLE 6. Distribution of antibiotic-resistance genes in the *Escherichia coli* strains isolated from fertile and infertile women

Groups of women (No. of UPEC strains)		Antibiotic-resistance genes (%)										
		aadA1	tetA	tetB	dfrA1	qnr	aac (3)-IV	Sul1	blaSHV	CITM	cat1	cmlA
Infertile women	History of UTIs (16)	11	15	7	12	14	16	13	7	16	4	1
	No history of UTIs (10)	4	10	4	3	5	8	7	2	9	1	—
	Total (26)	15 (57.69)	25 (96.15)	11 (42.30)	15 (37.69)	19 (73.07)	24 (92.30)	20 (76.92)	9 (34.61)	25 (96.15)	5 (19.23)	1 (3.84)
Fertile women	History of UTIs (13)	8	12	5	9	10	12	11	5	12	1	—
	No history of UTIs (5)	1	3	1	1	1	2	1	—	2	—	—
	Total (18)	9 (50)	17 (94.44)	6 (33.33)	10 (55.55)	11 (61.11)	14 (77.77)	12 (66.66)	5 (27.77)	14 (77.77)	1 (5.55)	—
Total (44)		24 (54.54)	42 (95.45)	17 (38.63)	25 (56.81)	30 (68.18)	38 (86.36)	32 (72.72)	14 (31.81)	39 (88.63)	6 (13.63)	1 (2.27)

Abbreviations: UPEC, uropathogenic *Escherichia coli*; UTI, urinary tract infection.

High prevalence of these genes in the UPEC strains isolated from high vaginal areas of women is associated with the occurrence of serious damage in these areas. The *hlyA* gene is able to lyse nucleated host cells and immune cells, and improves admission to host nutrients and iron stores [16,18,19]. The *sfa* gene is responsible for binding to epithelial and endothelial cells and facilitates bacterial dissemination within host tissues [16,18,19]. The *cnf1* gene is produced by a quarter of all pyelonephritis strains, and may also be involved in kidney damage, polymorphonuclear cell phagocytosis and epithelial cell apoptosis [16,18,19]. Clinical findings recommended that the UPEC strains that harbour *afa* adhesins have a higher ability for occurrence of pyelonephritis, recurrent and chronic UTIs [16,18,19].

Some O-serogroups show considerable prevalence in the UPEC strains isolated from high vaginal swab samples. O1, O2, O6 and O25 serogroups had greater distribution than others. Similarly, these serogroups had a high distribution in the UPEC strains isolated from UTIs [18,19]. These serogroups are mainly associated with special adhesion and invasion into the urinary and reproductive tissues [35]. Similar results have been reported by Momtaz *et al.* [18], Dormanesh *et al.* [19] and Arabi *et al.* [59].

UPEC strains in the present study harboured the highest prevalence of resistance against tetracycline, ampicillin, gentamicin, enrofloxacin and cephalothin. Additionally, isolated bacteria harboured high distribution of *tetA*, *CITM*, *aac(3)-IV* and *sul1* antibiotic-resistance genes. Similar prevalence of antibiotic resistance of the UPEC strains has been reported previously [18,19,35,36]. Indiscriminate and irregular prescription of antibiotic agents without attention to the results of disc diffusion tests is the main reason for the high occurrence of antibiotic resistance. Some of the UPEC strains examined harboured resistance toward more than ten antibiotic agents. Ali *et al.* [61] revealed that 59% of the UPEC strains isolated from different hospital infections in Pakistan harboured complete resistance to at least three antibiotic agents. Prevalence of multidrug-resistant UPEC strains in Indian hospitals was 82.6% [62]. Prevalences of multidrug-resistant UPEC strains in Iranian [63] and American [64] hospitals were 74% and 7.10%,

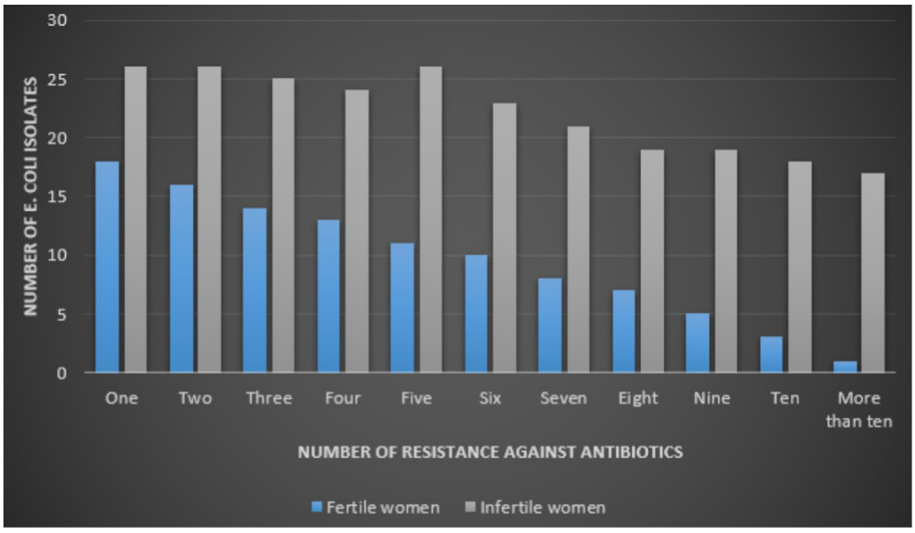

FIG. 2. Distribution of multidrug-resistant uropathogenic *Escherichia coli* strains isolated from high vaginal swab samples of fertile and infertile women.

respectively. Our findings showed that infertile women with a history of UTIs harboured a higher prevalence of multidrug-resistant UPEC strains.

Put together, invasion of UPEC into the vaginal epithelial cells has been demonstrated in various studies [39]. Moreover, the negative impact of vaginal infections on fertilization has been demonstrated [40,41]. O'Brien *et al.* [65] reported on the ability of *E. coli* to colonize the murine vagina and ascend to the uterine horns, consistent with our observations of *E. coli* colonizing the cervix and uterine horns. In keeping with these findings, resistant and virulent UPEC bacteria can easily colonize the high vaginal regions and induce severe invasion and subsequent injuries, which can lead to infertility in women. However, our future research on high vaginal *E. coli* colonization and invasion may help in the proper diagnosis of UTIs and distinguish women with infertility from other cases.

The present survey is a preliminary report of prevalence, antibiotic resistance and molecular properties of UPEC strains in fertile and infertile women with and without a history of UTIs. It is limited by the absence of a control group of infertile women with known cause of infertility and also a lack of pathological examination of the high vaginal regions of the women examined. In addition, lack of consideration of the presence of *E. coli* in sexual partners or husbands of the women examined is another important limitation. However, using four different groups of women, assessment of both phenotypic and genotypic determination of antibiotic resistance, and the molecular detection of virulence factors are all strengths of the present survey.

Conclusions

In conclusion, we identified a large number of UPEC strains, *afa*, *sfa*, *fim*, *cnf1* and *hlyA* virulence genes, O1, O2, O6 and O25 serogroups, high prevalence of resistance against tetracycline, ampicillin, gentamicin, enrofloxacin and cephalothin and high distribution of *tetA*, *CITM*, *aac(3)-IV* and *sul1* antibiotic-resistance genes in high vaginal swab samples of fertile and infertile women. Infertile women with a history of UTIs had significantly higher distribution of *E. coli* and their virulence factors, antibiotic resistance genes and O-serogroups. Infertile women with a history of UTIs also harboured higher distribution of multidrug-resistant UPEC strains. The UPEC strains may have a significant role in the occurrence of infertility in women with a history of UTIs. However, additional investigations are required to determine the exact role of UPEC strains as a probable cause of female infertility.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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