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Original article

Characteristics of uropathogenic *Escherichia coli* isolated from pregnant women: a cross section study

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ABSTRACT

Background and aim: Urinary tract infection (UTI) is the most common bacterial infection in pregnancy and is associated with premature delivery and low birth-weight infants. We aimed to determine the virulence factors of uropathogenic *Escherichia coli* (UPEC) strains isolated from pregnant women with or without clinical signs and symptoms of UTI and their association with antimicrobial resistance pattern. **Methods:** Isolation and detection of UPEC isolates, antimicrobial susceptibility followed by phenotypic detection of ESBL and biofilm production. Virulence and resistant genes were amplified by PCR. **Results:** Urine samples collected from 432 pregnant women of which significant bacteriuria represented 155/432 (35.9%). Patients were divided into 4 groups: cystitis, pyelonephritis, asymptomatic bacteriuria (ASB) and insignificant bacteriuria. UPEC was the most frequent organism 58/155 (37.4 %). The highest resistance rates were against ceftazidime and lowest resistance was to fosfomycin. ESBL producing UPEC represented 24/58 (41.4%) of samples while biofilm formation was 32/58 (55.2%). Moreover, 54/58 (93.1%) of UPEC isolates were found to be MDR. However, 53.1% of positive biofilm isolates were ESBL positive, all biofilm-producing isolates were MDR. Generally, fimH, fyuA and iutA genes were the most frequently detected virulence genes. Pap G and afa genes were significantly higher among patients with pyelonephritis. Pap G, and IutA genes were more frequent in positive biofilm group. **Conclusion**: Most of the isolates were MDR. Fosfomycin can be used precautionary in resistant cases. Biofilm producing isolates were more resistant to antibiotics and higher virulent than non-biofilm producers.

Introduction

Urinary tract infection (UTI) is the most common bacterial infection during pregnancy [1]. Acute pyelonephritis, acute cystitis, and asymptomatic bacteriuria (ASB) are the three clinical forms of UTI associated with pregnancy. [2]. Uropathogenic *Escherichia coli* (UPEC) is the most common bacterial pathogen causing 75–95% of all cases of uncomplicated cystitis and pyelonephritis [3].

The likelihood of UTI for pregnant women goes up from week 6 and reaches the highest point during week 22-24 of pregnancy [3]. UPEC have virulence factors that increase their ability to colonize and invade the urinary tract as well as fitness factors that enable uropathogens to survive by invading the bladder epithelium, producing

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toxins to obtain nutrients from the host cells, and synthesizing siderophores to take iron [4].

While multi-drug resistance (MDR) is acquired non susceptibility to at least one agent in three or more antimicrobial categories, extensive drug resistance (XDR) defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories both are facilitated by repeated antibiotic exposure. Extended spectrum ß-lactamase (ESBL) producing UPEC strains are posing a critical danger to patients in healthcare settings [5]. The main types of ESBL variants include TEM, SHV, CTX-M, and OXA. BlaCTX−M has rapidly increased and is widely found in clinically isolated UPEC across the world [6].

Biofilm associated infections have a major impact on pregnant women often with relapsed infections [7]. Biofilms protect the bacteria from the host immune response and impede the effects of antibiotics. High antimicrobial concentrations are imperative to inactivate organisms growing in a biofilm, and this may increase antibiotic resistance [8]. Treatment for UTIs may soon be difficult due to the lack of novel antibiotics, which is making the problem worse [9, 10]. Therefore, we aimed to determine the virulence factors of UPEC strains isolated from pregnant women with or without clinical signs and symptoms of UTIs and their association with antimicrobial resistance pattern.

Materials and methods

Study type, settings, and duration:

This was a cross-sectional study in which urine specimens were collected from 432pregnant women in Women's Heath Hospital of Assiut University during the period from May 2021 to December 2022. The study included 4 groups:

Group 1: pregnant women with cystitis (n=52) (complaining of urinary frequency (abnormally frequent urination e.g., once every hour or two), internal dysuria (any discomfort associated with urination) and suprapubic or pelvic pain) [2].

Group 2: pyelonephritis(n=50) (complaining of fever, dysuria, urgent voiding, flank pain, nausea and vomiting) [3].

Group 3: pregnant women with asymptomatic bacteriuria (n=53) (Prescence of >105 CFU /ml of urine and pyuria >10.000 leucocyte/ml of urine in the absence of signs and symptoms of UTI) [2].

Group 4: pregnant women with insignificant bacteriuria (viable bacterial count ≤105 CFU/mL) who served as controls, n=277.

Demographic data of study patients were collected and analyzed. All pregnant women were invited to participate in the study. Informed consent was obtained from each patient before participation in the study. We excluded pregnant women on antibiotics and patients with any anatomical or functional abnormalities in urinary tract function. The Faculty of Medicine Medical Ethics Committee Assiut University reviewed and approved the research proposal. IRB no: 17100940.

Study procedure

Clean-catch midstream urine sample was collected from each participant. All specimens were transported within 2 hours of collection in an icebox to bacteriology laboratory. The examination process included gross examination of urine specimens, direct microscopic examination for detection of pyuria, chemical examination of all collected urine samples by reagent strips for detection of leucocyte esterase and nitrite.

Viable count by calibrated loop technique was done (calibrated loop 0.01-mL was vertically held and immersed just below the surface of a well-mixed uncentrifuged urine specimen and delivered onto nutrient agar. A straight line was done down the center of the plate and the urine was streaked by making the series of passes at 90 degree, nutrient agar was incubated overnight in 35 to 37°C. For positive culture, colonies were counted on each plate and the number of CFUs was multiplied by100 to determine the number of microorganisms per milliliter in the original specimen). In freshly voided urine (number of bacteria is ≥ 105 CFU/mL) has usually been regarded as a cutoff for UTI [1]. UPEC isolates were identified by colony morphology on different culture media and biochemical confirmatory tests as CLED (Himedia, India M792), EMB (Himedia, India M317), chromogenic (Himedia, India M1353A), MacConkey media (Himedia, India M081), citrate utilization test (Himedia, India M099) and TSI test (OXOID CM0277).

Antimicrobial susceptibility testing

Antimicrobial susceptibility of the isolates was determined by Kirby-Bauer disc diffusion method according to CLSI guidelines [11], thirteen antimicrobial agents (HiMedia Laboratory Diagnostics Pvt Ltd, India) were selected for testing:

ampicillin (AMP)10mcg, amoxicillin clavulanate (AMC) 30mcg, pipercillin tazobactam (PTZ)100/10mcg, ceftazidime (CAZ)30mcg, cefotaxime (CTX)30mcg, ceftizoxime (CZX)30mcg, ceftriaxone (CTR)30mcg, azitronam (AT)30mcg , imipenem (IPM)10mcg, azithromycin(AZM)15mcg ,trimethoprim – sulphamethoxazole (COT)23.75/1.25 mcg, fosfomycin (FO)200mcg, nitrofurantoin(NIT)300mcg.

Phenotypic detection of ESBL and biofilm

For detection of ESBL production, resistance to ceftazidime, cefotaxime and ceftriaxone was screened by disc diffusion method. If resistance to one or more of these discs, phenotypic confirmatory tests should be used to ascertain the diagnosis [11]. Confirmatory tests included Double Disk Synergy Test (DDST) (five antibiotics were used for DDST namely aztreonam (30mcg), amoxicillin-clavulanic acid (20/10mcg), ceftriaxone (30mcg), ceftazidime (30mcg) and cefotaxime (30mcg). At the center amoxicillin-clavulanic acid disc was placed and these discs were placed at a distance of 1.5cm apart (center to center) on an inoculated agar plate. A clear extension of the edge of the inhibition zone toward the disc containing clavulanate (keyhole like) after 24hrs incubation was interpretated as synergy indicating the presence of ESBL). Phenotypic combination disc diffusion test (PCDDT) cefotaxime (30 mcg) disc and a combination of clavulanic acid (10 mcg) and cefotaxime (30 mcg) disc were used. Both discs were placed on Muller Hinton agar plates which were earlier swabbed by culture and incubated for 24hrs. at 37°C. A difference of ≥ 5mm between the zone diameters of the cefotaxime disc and cefotaxime /clavulanate disc is taken to be phenotypic confirmation of ESBL production [11]. Biofilm production was determined by Congo-red Agar method (CRA) (MedEx, Egypt RD067). The positive isolate was indicated by black and dry crystalline colonies and negative isolate was indicated by red colored colonies [7].

DNA extraction and gene detection

DNA extraction by boiling method was done (three to four colonies were picked from an overnight culture plate and suspended into 200µl of distilled water in eppendorf tube, tubes were placed in a heat block at 95°C for 15 min., then they were centrifuged at 14000 rpm for 5 min. to pellet the cellular debris and the supernatant was transformed into a properly labeled eppendorf tube, and stored at -20°C [12]. Single PCR reactions for amplification of virulence and resistant genes, specific pairs of primers (Invitrogen, USA) **(Table 1).** PCR was performed using the thermal cycler (Thermofisher, SimpliAmp, Singapole). The amplification reactions were performed at a defined volume of 25 µL (Dream taq TM Green PCR Master Mix (2x) 12.5 µL, primers (forward and reverse) 0.5 µL, distilled water 8.5 µLand Purified DNA 3 µL). Annealing temperature was calculated by Tm calculator program by thermoscientific company. PCR products were resolved on 1.5% agarose gel with ethidium bromide dye. The gel was visualized under a UV transilluminator. Translated nucleotide sequences of virulence and resistant genes were compared with corresponding reference protein sequences using BLAST software of NCBI; National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast).

Statistical analysis

All statistical calculations were done using SPSS (statistical package for the social science; SPSS Inc., Chicago, IL, USA) version 22. *P*-value is always significant at 0.05 level.

Table 1. Sequences of the primers used for amplification of virulence and resistance genes of UPEC and their expected sizes of amplicons.

<	Primer Sequence (5′-3')	Product Size	Reference	Primary annealing temperature
Fim H (adhesin virulence gene)	F: AACAGCGATGATTTCCAGTTTGTGTG R: ATTGCGTACCAGCATTAGCAATGTCC	465 bp	[13]	68°C for 30 s
*PapG class*Ⅲ (adhesin Virulence gene)	F: CATGGCTGGTTGTTCCTAAACAT R: TCCAGAGACTGTGCAGAAGGAC	421 bp	[14]	59.9°C for 30 s
Sfa (adhesin virulence gene)	F: CTCCGGAGAACTGGGTGCATCTTAC R: CGGAGGAGTAATTACAACCTGGCA	410 bp	[15]	63.2°C for 30 s
Afa (adhesin virulence gene)	F: GCTGGGCAGCAAACTGATAACTCTC R: CATCAAGCTGTTTGTTCGTCCGCCCG	750 bp	[15]	63.9°C for 30 s
Hly A (cytotoxic virulence gene)	F: AACAAGGATAAGCACTGTTCTGGCT R: ACCATATAAGCGGTCATTCCCGTCA	1177 bp	[15]	62.1°C for 30 s
Aer (aerobactin virulence gene)	F: TACCGGATTGTCATATGCAGACCGT R: AATATCTTCCTCCAGTCCGGAGAAG	602 bp	[15]	62.6°C for 30 s
Cnf1 (Cytotoxic necrotizing factor 1)	F: AAGATGGAGTTTCCTATGCAGGAG R: CATTCAGAGTCCTGCCCTCATTATT	498 bp	[15]	60.7°C for 30 s
Tra T (protectin virulence gene)	F: GGTGTGGTGCGATGAGCACAG R: CACGGTTCAGCCATCCCTGAG	290 bp	[16]	65.3°C for 30 s
Fyu A (iron acquisition virulence gene)	F: GTAAACAATCTTCCCGCTCGGCAT R:TGACGATTAACGAACCGGAAGGGA	850 bp	[17]	64°C for 30 s
IutA (iron acquisition virulence gene)	F: AAAGAGCTGAAAGACGCACTGG R: TGTCGGAACGTGAAGAGTTGAG	150 bp	[18]	62°C for 30 s
Bla-CTX−M1 (resistant gene)	F: AGT TCA CGC TGA TGG CGA CG R: GAC GAT TTT AGC CGC CGA CG	839 bp	[19]	61°C for 30 s
BlaTEM (resistant gene)	F: ATG AGT ATT CAA CAT TTC CGT R: TTA CCA ATG CTT AAT CAG TGA	861 bp	[20]	57.1°C for 30 s

F: forward, R; reverse. FimH = type-1 fimbriae, sfa = S-fimbriae, pap = pyelonephritis-associated pilus, afa = afimbrial adhesion, hlyA = α-haemolysin, UPEC= uropathogenic *Escherichia coli*.

Results

Viable count detection of the examined urine samples

The study included 432 pregnant women, 155 women had significant bacteriuria, 53 asymptomatic cases, 52 cystitis cases, 50 pyelonephritis cases and 277 cases were insignificant bacteriuria **(Figure 1).**

Demographic data of the participants

The demographic data of the studied participants was summarized in **table (2)** with no significant difference between them (p>0.05)

Bacteriological analysis of the urine samples

Distribution of isolated organisms

Our results showed that UPEC was the most frequently detected organism among the studied cases 58/155 (37.4%) isolates **(Figure 2).**

Antibiotic resistance profile of UPEC isolates

The highest resistance rate was observed against ceftazidime documented in 54 cases (93.1%), followed by cefotaxime in 50 cases (86.2%), cotrimoxazole in 45 cases (77.6%) while, the least resistant antibiotic was fosfomycin documented in four cases (6.9%).

Detection of ESBL production in UPEC isolates

ESBL production was detected in 24 (41.4%) isolates. A significant association was observed between the ESBL-production and examined resistant genes as ESBL positive isolates were positive to blaTEM, blaCTX-M1 and both genes (62.5%, 91.7% and 62.5%, respectively). No significant difference among the three studied groups concerning ESBL production was detected.

Detection of biofilm formation in UPEC isolates

Our results showed that biofilm formation was detected in 32 (55.2%) isolates. No significant difference among the three studied groups concerning biofilm formation was detected.

Detection of MDR of UPEC isolates

Multi-drug resistance (MDR) was detected in 54/58 (93.1%) of UPEC isolates. The frequency of MDR to three, four or five of total classes of antibiotics

was 22 (38.0%), 18 (31.0%), 14 (24.0%), respectively. Of the 54 (93.1%) MDR isolates, the most prevalent pattern was resistance to three classes of antibiotics followed by resistance to four classes of antibiotics as shown in **figure (3**). No significant difference among the three studied groups concerning MDR was detected.

Molecular detection of virulence and resistance genes

UPEC isolates were tested for the prescence of virulence and resistance genes by PCR. Generally, fimH, fyuA and iutA genes were the most frequently detected virulence genes (81.0%, 74.1%, 63.3%, respectively) **(Table 3).** No significant difference was observed between the three studied groups in virulence genes (p>0.05), except for pap G & afa genes which were significantly higher among patients with pyelonephritis (p= <0.001, and 0.010) respectively.

Association between biofilm formation and ESBL production and MDR

There was a significant difference between biofilm producing and non-producing isolates in ESBL production (p= 0.044), as 53.1% of positive biofilm production group was detected as ESBL positive. In addition, 100.0% of biofilm-producing isolates were MDR (p= 0.035) **(Table 4).**

Association between biofilm production, virulence and resistance genes

PapG, and iutA genes were significantly higher in positive biofilm production group (p= 0.028 and 0.046, respectively) than non-producing.

Association between biofilm production and antibiotic resistance

In our results, a significant higher resistance to cotrimoxazole (p =0.001), ceftizoxime (p =0.003), imipenem (p =0.020), azitreonam and azithromicin (p <0.001), was observed in biofilm forming isolates while the resistance to other studied antibiotics showed no significant difference with biofilm formation.

Association between virulence, resistance genes and antibiotic resistance

Relationship between the distribution of virulence genes and resistance to multiple drugs was also investigated. Among the twelve evaluated genes, A significantly higher resistance to selected antibiotics in tra T gene, blaTEM , pap G gene, cnf1 gene, blaCTX-M1gene and fyuA gene positive isolates than negative one was detected as shown in **tables (5a,b).**

Table 2. Demographic and clinical data of the studied participants.

Variable name	Cystitis n=52(%)		Pyelonephritis n=50(%)		Asymptomatic bacteriuria n=53(%)		Insignificant bacteriuria n=277(%)		*P* value
Age (years)									0.262
• Mean ± SD	26.96 ± 5.46		26.08 ± 4.78		27.73 ± 6.76		27.79 ± 6.04		
• Range	18 – 40		18 – 38		17 – 43		17 – 50		
Trimester									0.596
• 1st	8	(15.4%)	9	(18.0%)	5	(9.4%)	34	(12.3%)	
• 2nd	14	(26.9%)	10	(20.0%)	9	(17.0%)	54	(19.5%)	
• 3rd	30	(57.7%)	31	(62.0%)	39	(73.6%)	189	(68.2%)	
No. of pregnancy									0.115
• Median (range)	3 (1 – 9)		3 (1 – 6)		4 (1 – 9)		3 (1 – 12)		
Education									0.603
• Educated	36	(69.2%)	37	(74.0%)	38	(71.7%)	182	(65.7%)	
• Illiterate	16	(30.8%)	13	(26.0%)	15	(28.3%)	95	(34.3%)	
Residence									0.068
• Urban	25	(48.1%)	30	(60.0%)	27	(50.9%)	114	(41.2%)	
• Rural	27	(51.9%)	20	(40.0%)	26	(49.1%)	163	(58.8%)	
Occupation									0.347
• Employed	14	(26.9%)	8	(16.0%)	15	(28.3%)	78	(28.2%)	
• House wife	38	(73.1%)	42	(84.0%)	38	(71.7%)	199	(71.8%)	

Data are presented as mean ± SD and range, data are presented as number (percentage), significance defined by $p < 0.05$, n=432.

Table 3. Frequency of virulence and resistance genes in 58 UPEC isolates from urine samples.

Genes	Positive	
	N	(%)
FimH gene	47	(81.0)
FyuA gene	43	(74.1)
IutA gene	35	(60.3)
BlaCTX-M1 gene	33	(56.9)
Cnf1 gene	29	(50.0)
***TraT* gene**	29	(50.0)
Aer gene	26	(44.8)
BlaTEM gene	26	(44.8)
Sfa gene	25	(43.1)
HlyA gene	23	(39.7)
PapGIII gene	12	(20.7)
Afa gene	8	(13.8)

Qualitative data are presented in the form of number (%), UPEC= uropathogenic *Escherichia coli*, n=number. FimH = type-1 fimbriae, sfa = S-fimbriae, pap = pyelonephritis-associated pilus, afa = afimbrial adhesion, hlyA = α-haemolysin.

Table 4. Association between biofilm production, MDR and ESBL production detected by PCDDT in UPEC isolates.

	Biofilm production (n=58)				
	Negative n=26 (%)		**Positive n=32 (%)**		***P* value**
ESBL production					**0.044**
• Negative	19	(73.1%)	15	(46.9%)	
• Positive	7	(26.9%)	17	(53.1%)	
MDR	22	(84.6%)	32	(100.0%)	**0.035**

Qualitative data are presented as number (percentage). Significance defined by $p < 0.05$, ESBL = extended-spectrum β- lactamase, UPEC= uropathogenic *Escherichia coli*, PCDDT= Phenotypic Combination Disc Diffusion Test, n=number.

Table 5a. The association between virulence, resistance genes and antibiotic resistance among patients with UPEC isolates.

Virulence genes	**Ceftazidime**		**Cefitraxone**		**Cotrimoxazole**		**Ampicillin**		**Nitrofurantoin**		**Ceftizoxime**		**Pipercillin tazobactam**	
***FimH* gene**	44	(81.5%)	30	(73.2%)	34	(75.6%)	34	(77.3%)	16	(69.6%)	28	(73.7%)	17	(85.0%)
***TraT* gene**	26	(48.1%)	20	(48.8%)	21	(46.7%)	18	(40.9%)*	10	(43.5%)	20	(52.6%)	9	(45.0%)
BlaTEM	25	(46.3%)	19	(46.3%)	21	(46.7%)	22	(50.0%)	16	(69.6%)*	21	(55.3%)*	9	(45.0%)
PapG	10	(18.5%)	8	(19.5%)	11	(24.4%)	8	(18.2%)	3	(13.0%)	11	(28.9%)	4	(20.0%)
***Sfa* gene**	23	(42.6%)	15	(36.6%)	19	(42.2%)	17	(38.6%)	9	(39.1%)	13	(34.2%)	8	(40.0%)
***Afa* gene**	7	(13.0%)	5	(12.2%)	7	(15.6%)	5	(11.4%)	4	(17.4%)	7	(18.4%)	1	(5.0%)
***HlyA* gene**	20	(37.0%)	15	(36.6%)	17	(37.8%)	17	(38.6%)	5	(21.7%)	17	(44.7%)	9	(45.0%)
***Cnf1* gene**	26	(48.1%)	21	(51.2%)	23	(51.1%)	23	(52.3%)	10	(43.5%)	23	(60.5%)*	12	(60.0%)
***Aer* gene**	26	(48.1%)	19	(46.3%)	21	(46.7%)	20	(45.5%)	11	(47.8%)	18	(47.4%)	9	(45.0%)
***BlaCTX-M1* gene**	31	(57.4%)	28	(68.3%)*	31	(68.9%)*	31	(70.5%)*	19	(82.6%)*	27	(71.1%)*	16	(80.0%)*
***FyuA* gene**	41	(75.9%)	34	(82.9%)*	33	(73.3%)	31	(70.5%)	18	(78.3%)	29	(76.3%)	15	(75.0%)
***IutA* gene**	32	(59.3%)	24	(58.5%)	29	(64.4%)	26	(59.1%)	13	(56.5%)	26	(68.4%)	13	(65.0%)

Data are presented as number (percentage). *=significant ($p < 0.05$). *FimH* = type-1 fimbriae, *sfa* = S-fimbriae, *pap* = pyelonephritis-associated pilus, *afa* = afimbrial adhesion, *hlyA* = α-haemolysin, UPEC= uropathogenic *Escherichia coli*, n=58, n=number.

Table 5b. The association between virulence, resistance genes and antibiotic resistance among patients with UPEC isolates.

Virulence genes	Imipenem		Amoxiclav		Azitreonam		Azithromicin		Fosfomycin		Cefotaxime	
FimH gene	14	(77.8%)	29	(87.9%)	14	(73.7%)	14	(77.8%)	4	(100.0%)	41	(82.0)
TraT gene	8	(44.4%)	17	(51.5%)	11	(57.9%)	10	(55.6%)	2	(50.0%)	23	(46.0%)
BlaTEM	12	(66.7%)	12	(36.4%)	12	(63.2%)	11	(61.1%)	2	(50.0%)	25	(50.0%)
PapG gene	5	(27.8%)	7	(21.2%)	5	(26.3%)	5	(27.8%)	3	(75.0%)*	10	(20.0%)
Sfa gene	6	(33.3%)	18	(54.5%)	7	(36.8%)	7	(38.9%)	1	(25.0%)	24	(48.0%)
Afa gene	2	(11.1%)	4	(12.1%)	5	(26.3%)	5	(27.8%)	1	(25.0%)	7	(14.0%)
HlyA gene	7	(38.9%)	11	(33.3%)	7	(36.8%)	7	(38.9%)	4	(100.0%)	20	(40.0%)
Cnf1 gene	9	(50.0%)	15	(45.5%)	11	(57.9%)	11	(61.1%)	3	(75.0%)	25	(50.0%)
Aer gene	8	(44.4%)	14	(42.4%)	9	(47.4%)	8	(44.4%)	1	(25.0%)	22	(44.0%)
Bla CTX-M1 gene	16	(88.9%)*	17	(51.5%)	15	(78.9%)*	14	(77.8%)*	2	(50.0%)	30	(60.0%)
FyuA gene	14	(77.8%)	25	(75.8%)	13	(68.4%)	13	(72.2%)	2	(50.0%)	36	(72.0%)
IutA gene	13	(72.2%)	19	(57.6%)	13	(68.4%)	13	(72.2%)	2	(50.0%)	29	(58.0%)

Data are presented as number (percentage). *=significant ($p < 0.05$). *FimH* = type-1 fimbriae, *sfa* = S- fimbriae, *pap* = pyelonephritis-associated pilus, *afa* = afimbrial adhesion, *hlyA* = α-haemolysin, UPEC= uropathogenic *Escherichia coli*.

Figure 1. Flowchart demonstrating the results of the viable count of the examined urine samples.

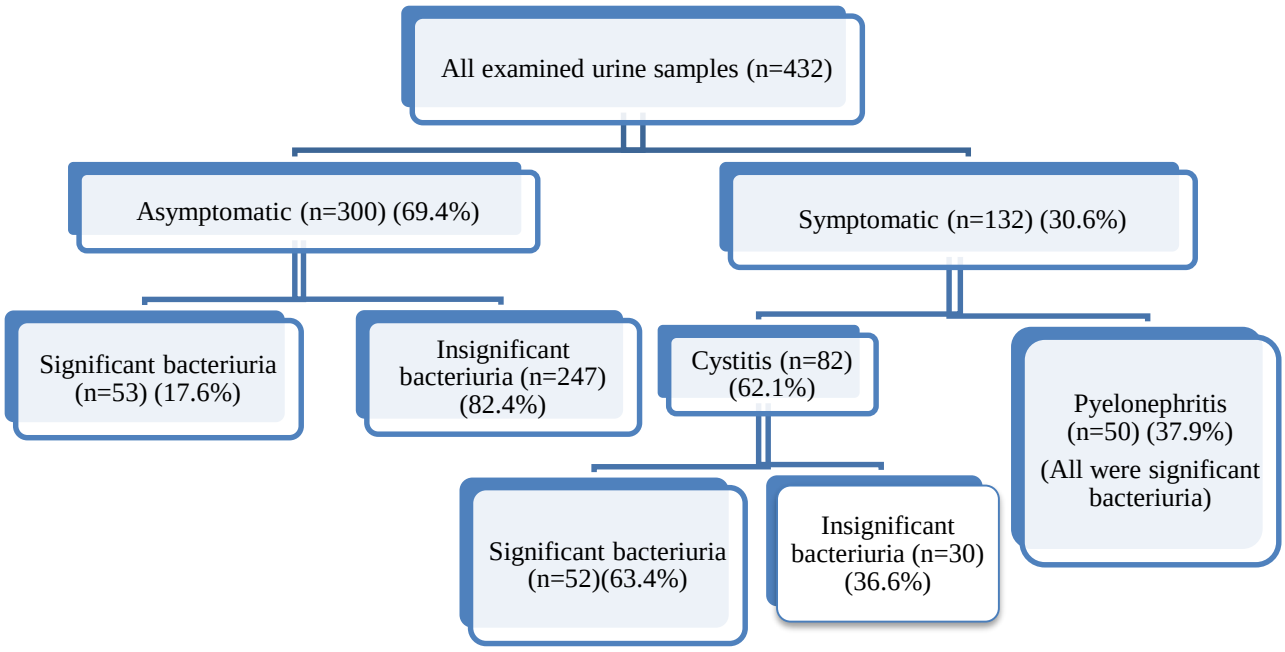


Figure 2. Frequency of isolated organisms in urine samples.

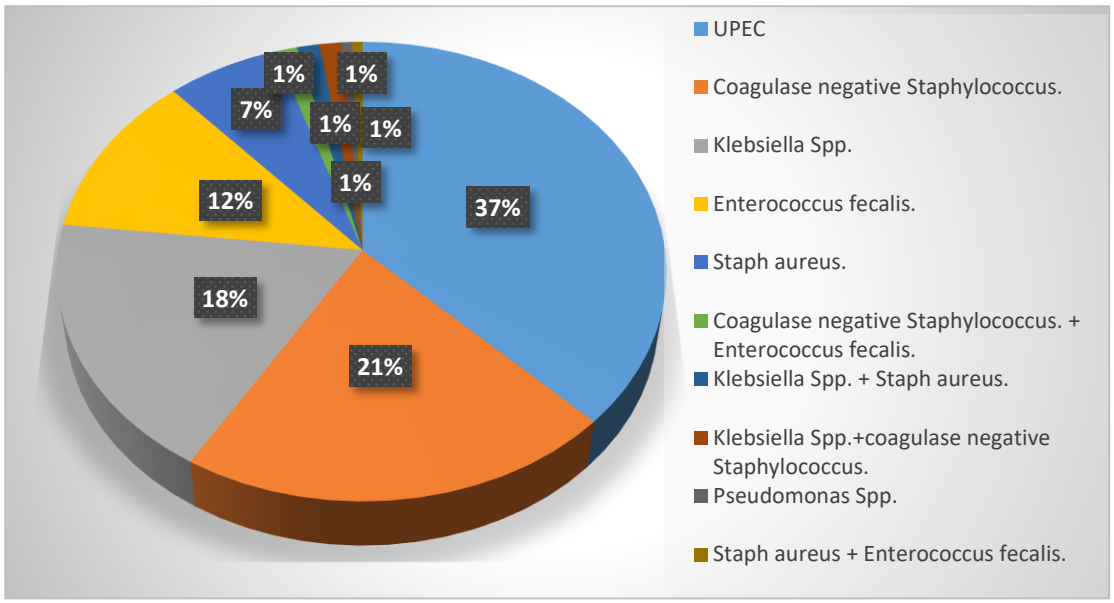
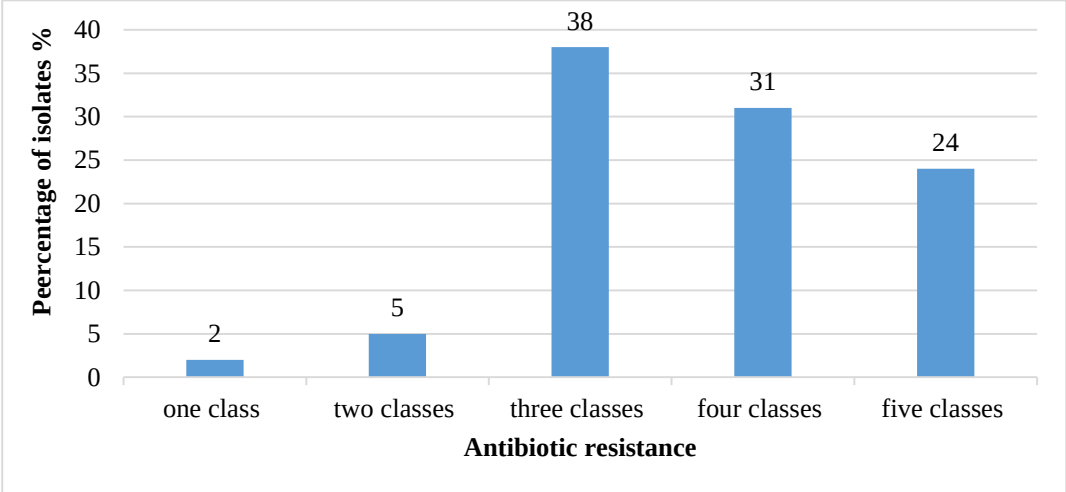


Figure 3. Frequency of MDR UPEC according to its resistance to three or more antibiotic classes.



Discussion

According to the findings of our research, the overall proportion of UTI among pregnant women was 155/432 (35.9%). This was similar to earlier researches done in Egypt; **Shaheen et al.** [21] found that the proportion was 32% in the Menoufia governorate, **Ahmed** [22] found that it was 30.5% in the Cairo governorate. Even though our results were lower than another Egyptian research conducted by **Metwally et al.** [23] who found that the frequency was 45%. On the other hand, our findings exceeded those of a research conducted in Khartoum [24] which discovered the frequency to be 14%. The variation in UTI rates across various studies could be due to the environmental, cultural, social and religious factors that influence sexual practices in different communities [22].

In our study, UPEC was the predominant isolated microorganism, occurring in 58/155 isolates (37.4 %). According to a similar study in Egypt [23] UPEC was the most common isolated bacteria, appearing in 34% of cases. UPEC is the most common microorganism in the vaginal and rectal area [23]. In contrast to our findings, a study by **Ahmed** [22] discovered that *S. saprophyticus* had the highest percentage of isolation (35.0%), followed by UPEC (26.2%), and *S. aureus* (19.4%). And this may be explained by error in obtaining midstream urine samples.

Our results showed the highest level of antibiotic resistance against 3rd generation

cephalosporins and the lowest level of resistance against fosfomycin. According to a similar study in Egypt [22], UPEC had the highest level of resistance against ampicillin, penicillin, ceftriaxone, nitrofurantoin, and ampicillin/sulbactam. However, lowest level of resistance was shown against cotrimoxazole. The highest rate of resistance against third generation cephalosporins in our study may be attributed to overuse of third generation cephalosporins in empirical treatment for outpatients and in hospital settings.

In our study, fosfomycin susceptibility was found to be high. In agreement with our findings, **Alrowais et al.** [25] reported that UPEC was fosfomycin sensitive, with resistance rate of less than 5%. In our area, in vitro susceptibility tests against bacterial clinical isolates are infrequent and fosfomycin's clinical use is restricted so, infections brought on by MDR UPEC may be treated differently with fosfomycin. Similarly, **Dzib-Baak et al.** [26] reported that infections caused by MDR UPEC, and in which the antibiotic treatment is increasingly becoming ineffective, fosfomycin may be an option. As the number of available treatments for MDR UPEC clinical isolates is decreasing, epidemiological surveillance is crucial.

The current study found that 41.4% UPEC isolates produced ESBL-enzyme, which was lower than a percentage of 52.4% reported by a similar study in Nigeria [27]. However, the proportion of ESBL-producing UPEC was higher than a study by **El-Khizzi et al.** [28] who found a percentage of 15.8%. In general, percentage of UPEC isolates producing ESBL in UTI cases varies according to geographical location and antimicrobial usage [28].

Among ESBL production group, blaCTX-M1 was the most common examined ESBL gene followed by blaTEM (91.7%, 62.5%, respectively). In agreement with a study by **Mohebi et al.** [29], who found that the percentage of blaCTX-M1 and blaTEM genes in ESBL producers was 93% and 79%, respectively. In the current investigation, biofilm production made up 55.2% of the UPEC isolates. which was more than a rate of 47.6% [30] but was lower than a rate of 72% [31].

In the current investigation, 93.1% of the isolates were MDR. Our findings were close to that reported in the study by **Halah et al.** [32] who found that most UPEC isolates (98.23%) were MDR. Our results showed that 100% of biofilm producer UPEC isolates were MDR. In agreement with another study [33] which reported that 64% UPEC isolates with biofilm production were MDR. Within bioflm producing UPEC, 53.1% of isolates were ESBL producers. In agreement another study [34] which detected that ESBL production and biofilm formation were positively related. This may be explained by the matrix of biofilm helps to stabilize and increase the movement of genetic elements, which allows ESBL genes to move between UPEC isolates [34].

The current study fimH gene appeared with the highest percentage (81.0%) in urine isolates. This finding was similar to those of studies conducted in Egypt [23], and in Sudan [35]. UPEC strains need fim H gene to stick to, enter and stay in the urinary bladder after settling there [7]. A higher number of afa gene in isolates from patients with pyelonephritis (33.3%) may be attributed to that afa gene may play a role in causing chronic interstitial nephritis this was in line with another study [36]. Also, papG III gene was higher in cases of pyelonephritis than in cases of cystitis (53.3% and 19.0%, respectively). Similarly, a study conducted in France revealed that pyelonephritis was more frequently associated with papG III gene and explained this by strains that have papG III gene can attach to kidney and causing pyelonephritis [37].

In the current investigation, the expressions of the papG III and iutA genes were higher in biofilm-forming UPEC isolates. Another study found a correlation between the expression of the sfa and afa genes and the production of biofilm-forming UPEC isolates [38]. The relationship between some of antimicrobial resistance and virulence genes, may be due to that resistance to antimicrobial agents is often associated with the spread of transmissible plasmids, which may also carry virulence genes [38]. The significant association between biofilm production and antibiotic resistance was similar to other studies which concluded that bacteria that form biofilm gain benefits such as becoming resistant to antibiotics, expressing several virulence factors and increasing resistance against phagocytosis [39,40].

Conclusion

The majority of UPEC strains were MDR with increased resistance to the empirically used antibiotics. Biofilm producing isolates were more resistant to antibiotics and higher virulent than non-biofilm producers. Fosfomycin was effective in biofilm positive UPEC. Isolates linked to antibiotic resistance and ESBL production were associated

with certain genes, such as Bla CTX-M1. The nature of biofilm and virulence genes of UPEC will help in the treatment of UTI.

Limitation of the study

We didn't use any of the aminoglycosides or quinolones which could have helped to better define multidrug-resistant pattern, as quinolones and aminoglycosides classified as class C or D according to food and drug administration (FDA) categories of medications in pregnancy. So not be used in the hospital policy for treatment of UTI in pregnancy.

Competing interests

The authors declare that they have no competing interests.

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