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ORIGINAL ARTICLE

Comparative Study between Community Acquired and Hospital Acquired UTI Caused by *E. Coli*

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ABSTRACT

***Background**: Uropathogenic Escherichia coli (UPEC) are the primary causative agent of urinary tract infection (UTIs). The pathogenic potential of E.coli strains depends on the presence of virulence markers, which in turn are associated with the severity of the infection. **Objectives:** To compare the presence of five virulence genes (fimH, PAI, papG, hlyA, traT), antibiotic susceptibility between community acquired (CA) and hospital acquired (HA) UPEC strains. **Methodology**: A total of 62 UPEC strains divided into 2 groups, 28 CA and 34 HA were tested for antimicrobial resistance against 15 drugs using the disc diffusion method. Screening and confirmatory tests for extended spectrum β lactamases were done as described by clinical laboratory standard institute (CLSI). Strains were tested for the five virulence genes by multiplex polymerase chain reaction. **Results:** FimH gene was the most detected one in the 62 UPEC strains followed by PAI, papG, hlyA, traT (33.87%, 27.42%, 20.97%, 11.29%, 6.45% respectively). No statistical difference was found between CA and HA as regard gene detection. A positive correlation was found between detection of one gene (PAI) and CA (p-value<0.001), detection of multiple genes with urinary catheter in HA (p-values was <0.001). Antibiotic resistance to ceftazidime, cefepime, amikacin, ciprofloxacin, levofloxacin, cotrimoxazole and nitrofurantoin were significantly higher in HA than CA (p-values were 0.009, 0.011, 0.035, 0.001, 0.017, 0.025 and 0.002 respectively). ESBL producing strains, MDR and Quinolone resistance were significantly higher in HA than CA (p-values were 0.028, <0.001 and 0.050 respectively). **Conclusion**: There was no statistical difference between CA and HA as regard the presence of virulence genes. Antibiotic resistance did not correlate with the studied virulence genes.*

INTRODUCTION

Urinary tract infection (UTI) refers to an infection with microbial pathogens at any site in the urinary tract, which includes urethra, bladder, ureters and kidneys. *Escherichia coli* is the most frequent pathogen responsible for up to 80% of UTI [1].

These bacteria are responsible for 85% and 50% of community and hospital acquired UTI, respectively [2]. The severity of the infection depends both on the virulence of the infecting bacteria and on the susceptibility of the host. Urinary infections most often occur in patients with anatomically and functionally normal urinary tracts, and involve spontaneous ascent of bacteria from the urethra to the bladder and in a few patients to the kidney [3].

Uropathogenic *E. coli* (UPEC) strains have special virulence factors, including pili or fimbriae, which mediate attachment to uroepithelial cells. Resistance to human serum bactericidal activity, haemolysin production, and increased amount of k capsular antigen[4].

The most virulence factors dependent upon the UPEC include adhesions (type 1 fimbriae, p fimbriae, curli fimbriae, a fimbrial adhesions and flagellum), aerobactins, hemolysins, and cytotoxic necrotizing factor 1. All these virulence factors are important in colonization of UPEC, extra-intestinal survival, and leading to cytopathic effect. In addition, the expression of special virulence factors of UPEC can contribute to uropathogenicity as well as worsening of UTIs [5].

Over the last decade, the emergence of multidrug resistance of UPEC strains have made UTI treatment more problematic, this phenomenon increase rapidly due to the wide dissemination of UPEC strains harboring determinants for extended spectrum β-lactamase (ESBLs) and resistance to trimethoprim-sulfamethoxazole and fluoroquinolones. The antibiotic resistance is acquired through genetic changes [6].

The aim of this study is to determine the presence of five virulence genes, expressing fimbriae *(fimH*), production of hemolysin (*hlyA*), adherence *papG* allele II, urovirulance *PAI* and *traT* gene coding for serum resistance among *Escherichia coli* isolates obtained from urinary tract infection. Comparing the presence of these genes, antibiotic susceptibility, and ESBL production between community and hospital acquired UPEC strains.

METHODOLOGY

Samples:

This study was conducted on 62 uropathogenic *E. coli* strains isolated from patients suffering from urinary tract infection which were divided into two groups. Group I included 28 community acquired UT infected patients, (19 cystitis and 9 pyelonephritis). Cystitis characterized by supra-pubic pain, dysuria and frequency, while pyelonephritis characterized by fever, rigors and loin pain. Their age ranged from 14 to 73, twenty were females and eight were males. While group II included 34 hospital acquired UT infected patients (21cystitis, and 13pyelonephritis). Their age ranged from 5 to 84, twenty- five were females and nine were males. All patients were subjected to the following: full medical history, demographic data were collected including age, gender, history of recurrent UTI, DM or other chronic diseases. The exclusion criteria included: urinary tract dysfunction, tuberculosis, urinary tract anatomical abnormalities and ureter obstructive hydrocephalus.

All participants provided an informed consent for the collection of samples and subsequent analysis, also this study was approved by the ethics committee, Faculty of Girls Al Azhar University.

Identification of isolates:

Mid-stream urine samples were collected in sterile containers from non-catheterized patients or in sterile syringes under complete aseptic conditions in catheterized patients. Urine samples were subjected to complete physical, chemical, and microscopic examination. Colony count was done on CLED agar plates using a standard calibrated bacteriological loop (0.01 mL). The bacterial colonies grown in significant number ($\geq 10^5$ CFU/ml) were considered pathogenic. Un-centrifuged urine samples were cultured on both blood agar and MacConkey's agar media and incubated at 37°C for 24 hours. Haemolysin production was detected by determining the presence of zone of lysis around each colony on 5% sheep blood agar plate. The bacterial isolates were characterized and identified according to Gram Staining and biochemical tests such as Catalase, indole production, Citrate utilization, triple iron sugar, urease test, and Lisyne as described in standard bacteriological methods[7]. All the above chemicals and media were purchased from Oxaid UK.

Anti-microbial Susceptibility test:

Antimicrobial susceptibility of all isolates, to ampicillin, amoxicillin/clavulinate, cefotaxime, ceftazidime, cefazolin, cefepime, ceftriaxone, imipenem, gentamycin, amikacin, tobramycin, ciprofloxacin, levofloxacin, cotrimoxazole (trimethoprim/ sulphamethoxazol), and nitrofurantoin was performed on Mueller Hinton medium by Kirby Baure disc diffusion method. Interpretation was done according to Clinical Laboratory Standards Institute guidelines (CLSI) [8].

ESBL production testing: was performed according to CLSI 2014 [8]. Screening was done by using three extended spectrum cephalosporins (cefotaxime 30 µg, ceftriaxone 30 µg, and ceftazidime 30 µg), then confirmed by double disc synergy method using the three previously mentioned cephalosporins around amoxicilline-clavulanate disc (20/10 µg). A clearly visible extension of any disc towards the amoxicilline-clavulanate disc was interpreted as positive result.

Molecular identification of UPEC virulence factors: was done by multiplex polymerase chain reaction (PCR) as follows:

DNA extraction:

Genomic DNA templates for PCR amplification were obtained from overnight bacterial isolates growth on nutrient broth. A loop full of bacterial culture was suspended in 400µL sterile deionized water purchased from Promega Company, Madison, WI, USA, and briefly vortexed, then boiled for ten minutes. After cooling on ice for 5 minutes, the samples were centrifuged at 14000 rpm for 3 minutes. The supernatant was applied as the DNA template for PCR.

Polymerase chain reaction amplification:

The PCR was carried out in 25µL Master mix which contains 2.5µL as 10 X PCR reaction buffer with $MgCl_2$ (1.6mm), 0.5µL (200µM) of deoxynucleoside tri-phosphates mixture (dNTps, 10mm), 0.5µL of each primer (10 pm/µL), 2µL of the DNA template (50ng) with 0.5µL Taq DNA polymerase.

The primer sequence used for the studied virulence factors are listed in the following table.

Table 1: Primers sequences used for the multiplex-PCR assay

Gene(s)	*Primer sequence*	*Primer name*	*Size of product (bp)*
papG,	Gggatgagcgggcctttgat	AlleleII f	190
allele II	Cgggcccccaagtaactcg	AlleleII r	
fimH	Tgcagaacggataagccgtgg	*fimH* f	508
	Geagtcacctgccctccggta	*fimH* r	
hlyA	Aacaaggataagcactgttctggct	*hlyA* f	1177
	Accatataagcggtcattcccgtca	*hlyA* r	
traT	Ggtgtggtgcgatgagcacag	*traT* f	290
	Cacggttcagccatccctgag	*traT* r	
PAI	Ggacatcctgttacagcgcgca	*PAI* f	930
	Tcgccaccaatcacagccgaac	*PAI* r	

f, Forward primer; r, Reverse primer

The amplification condition included an initial denaturation at 95° C for 3 minutes, 37 cycles (96 ^{o} C for 30 seconds, 64 ^{o} C for five minutes, 72 ^{o} C for 60 seconds) and a final extension (72 ^{o} C for five minutes. The PCR amplifications were performed on a thermocycler 9700 (Applied Biosystems, USA).

Detection:

PCR products were electrophoresed on agarose gels stained with ethidium bromide and photographed using UV trans-illumination imaging system.

Statistical Analysis:

All statistical analysis was performed using SPSS version 18.0 (SPSS, Chicago, IL, USA). The prevalence of virulence genes and antibiotic resistance patterns were compared between the two studied groups using Pearson chi-square test and Fisher exact test. Continuous variables were compared with the student t-test and Mann-Whitney U test. A p-value ≤0.05 was considered statistically significant, and p-value of ≤0.001 was considered highly significant, while p-value >0.05 was considered insignificant.

Spearman correlation test was used to determine the correlation between different variables. The strength of the relationship was given by the correlation coefficient (r).

RESULTS

By comparing demographic data, history of DM, catheterization, and site of infection between community acquired (group I) and hospital acquired (group II); it was found that diabetic and catheterized patients were significantly higher in HA (group II) than CA (group I) (p-values were 0.02 and 0.043 respectively).

While no statistically significant differences were observed between both groups regarding age, sex, and site of infection, (p-values were 0.531, 0.854, and 0.618 respectively) (Table 2).

From the 62 UPEC strains, 7 showed hemolysis on sheep blood agar but no statistical significant difference was found between hemolysin producing CA strains (group I) and HA (group II). p-value was 0.953. On the other hand, there was statistical significant difference between UPEC causing pyelonephritis and those causing cystitis in both as regard hemolysin production 15% versus 4.55% respectively. The p-value was 0.025 (Table 3).

Comparing the anti-microbial susceptibility between community-acquired (group I) and hospital-acquired (group II) isolates of uropathogenic *E-coli* revealed that community-acquired strains had the highest resistance to ceftriaxone and cotrimoxazol, 64.29% while the lowest resistance was to imipenem 7.14%.

Hospital-acquired strains (group II) had the highest resistance to cotrimoxazol 88.24%, followed by 73.53% to ciprofloxacin, while the lowest resistance was to imipenem 20.59%.

We found that resistances of HA strains to cotrimoxazol, ciprofloxacin, ceftazidime, levofloxacin, nitrofurantoin, cefepime, and amikacin were significantly higher than in CA strains (88.24% vs. 64.29%, 73.53% vs. 32.14%, 61.76% vs. 28.57%, 58.82% vs. 28.57%, 55.88% vs. 17.86%, 44.12% vs. 14.29%, and 38.24% vs. 14.29% respectively (Table 4).

The MDR, ESBL producer and quinolone resistant UPEC strains were significantly higher among HA (group II) than CA (group I) cases 85.29% versus 35.71%, 44.12% versus 17.86%, 44.12% versus 21.43% respectively (p-values were <0.001, 0.028, and 0.050 respectively) (table 5).

From the 62 UPEC strains, 21 (33.87%) carried *fimH* gene, 17 (27.42%) carried PAI gene, 13 (20.97%) carried *papG*, 7(11.29%) carried *hlyA* gene and 4 (6.45%) carried *traT* gene. While 24 (38.71%) UPEC strains gave negative results for the studied 5 genes (figure 1).

In this study, we found that distribution of all genes was higher in HA (group II) than CA (group I) UPEC strains however, the difference between both groups was statistically not significant (table 6).

We found that 19 (30.65%) of UPEC strains harbored single gene, 14 (22.58%) harbored two genes and 5 (8.06%) harbored three or more genes (figure 2).

The presence of single gene was higher in CA (group I) than HA (group II) UPEC strains 53.57% versus 11.76% and it was statistically highly significant p<0.001. On the other hand, the presence of 2 or 3 genes was higher in HA (group II) than CA (group I) UPEC strains 35.29% versus 7.14% and 14.71% versus 0.00% respectively and these differences were statistically significant (p values were =0.008 and 0.034 respectively) (table 7).

No correlation was found between the presence of virulence genes and ESBL production or quinolone resistance. However, there was a significant positive correlation between presence of single gene and CA UPEC strains; between presence of multiple genes and HA UPEC; between multiple genes and catheter in group II; between hemolysin producing strains and pyelonephritis in both groups; and between quinolone resistance and pyelonephritis in group I (table 8).

Table 2: Demographics and clinical characteristics of patients in both groups.

Variables	*Community-acquired Group I (n=28)*	*Hospital-acquired Group II (n=34)*	*p-value*
Age (years):			
Range	14 – 73	5 – 84	0.531
Mean ± SD	50.53 ± 13.88	53.14 ± 17.93	
Gender:			
Female	20 (71.43%)	25 (73.53%)	0.854
Male	8 (28.57%	9 (26.47%)	
Diabetes mellitus:			
Diabetic	4 (14.29%)	14 (41.18%)	0.020*
Non-Diabetic	24 (85.71%)	20 (58.82%)	
Urethral catheter:			
Catheterized	2 (7.15%)	12 (35.29%)	0.043*
Non-catheterized	26 (92.85%)	22 (64.71%)	
Site of infection:			
Cystitis	19 (67.86%)	21 (61.76%)	0.618
Pyelonephritis	9 (32.14%)	13 (38.24%)	

*P- value ≤0.05 is statistically significant.

Table 3: Distribution of hemolysin producers among studied UPEC strains.

	Hemolysin producer UPEC strains (n=7)		*p-value*
	n.	%	
Group I CA UPEC strains (n=28)	2	7.14	0.953
Group II HA UPEC strains (n=34)	5	14.71	
UPEC causing cystitis (n=40)	1	4.55	
UPEC causing pyelonephritis (n=22)	6	15.0	0.025*

*P- value ≤0.05 is statistically significant

Table 4: Resistance of community and hospital-acquired UPEC strains to the tested antimicrobial agents.

Antibiotic	*Group I (n=28)*		*Group II (n=34)*		*p-value*
	Resistant		**Resistant**		
	n	%	n	%	
ampicillin (AMP)	15	53.57	24	70.59	0.167
amoxicillin/Clavulinate (AMC)	10	35.71	15	44.12	0.502
cefotaxime (CTX)	10	35.71	19	55.88	0.113
ceftazidime (CAZ)	8	28.57	21	61.76	0.009*
cefazolin (CZ)	7	25.00	14	41.18	0.180
cefepime (FEP)	4	14.29	15	44.12	0.011*
ceftriaxone (CRO)	18	64.29	19	55.88	0.502
imipenem (IPM)	2	7.14	7	20.59	0.135
gentamycin (CN)	6	21.43	13	38.24	0.153
amikacin (AK)	4	14.29	13	38.24	0.035*
tobramycin (TOB)	10	35.71	18	52.94	0.175
ciprofloxacin (CIP)	9	32.14	25	73.53	0.001*
levoflxacin (LEV)	8	28.57	20	58.82	0.017*
cotrimoxazol (COT)	18	64.29	30	88.24	0.025*
nitrofurantoin (NF)	5	17.86	19	55.88	0.002*

*P- value ≤0.05 is statistically significant. Cotrimoxazol is trimethoprim/sulphamethoxazol.

Table 5: Comparison between CA (group I) and HA (group II) UPEC strains according to ESBL production, MDR, and quinolone resistance.

Variables		*Group I (n=28)*		*Group II (n=34)*		*p-value*
		N	%	N	%	
ESBL production	Positive (n=20)	5	17.86	15	44.12	
	Negative (n=42)	23	82.14	19	55.88	0.028*
MDR strains	Positive (n=39)	10	35.71	29	85.29	
	Negative (n=23)	18	84.29	5	14.71	<0.001*
Quinolone resistance	Positive (n=21)	6	21.43	15	44.12	
	Negative (n=41)	22	78.57	19	55.88	0.050*

*, significant difference.

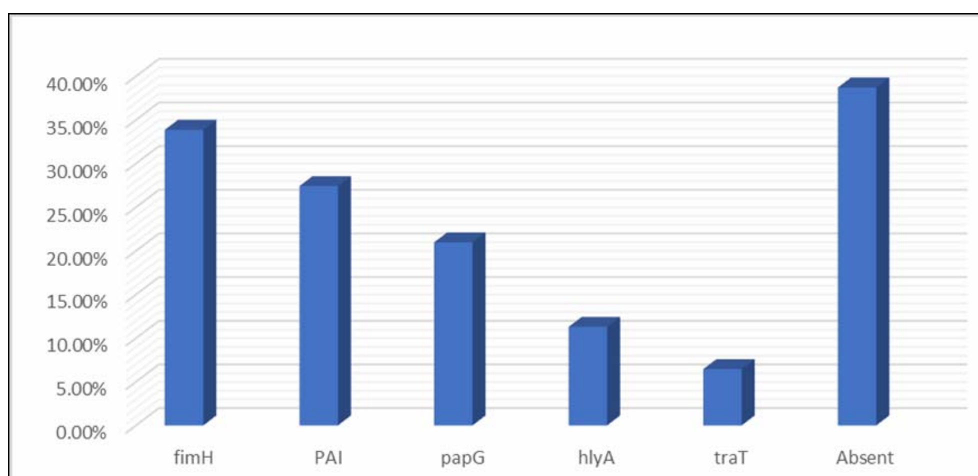

Fig 1: Distribution of the studied genes in the 62 UPEC strains.

Table 6: Comparison between the distribution of genes among CA (group I) and HA (group II) UPEC strains.

Genes	*CA UPEC strains (n=28)*		*HA UPEC strains (n=34)*		*p-value*
	N	%	N	%	
papG	4	14.29	9	26.47	0.241
traT	1	3.57	3	8.82	0.402
FimH	6	21.43	15	44.21	0.060
PAI	6	21.43	11	32.35	0.337
hlyA	2	7.14	5	14.71	0.349

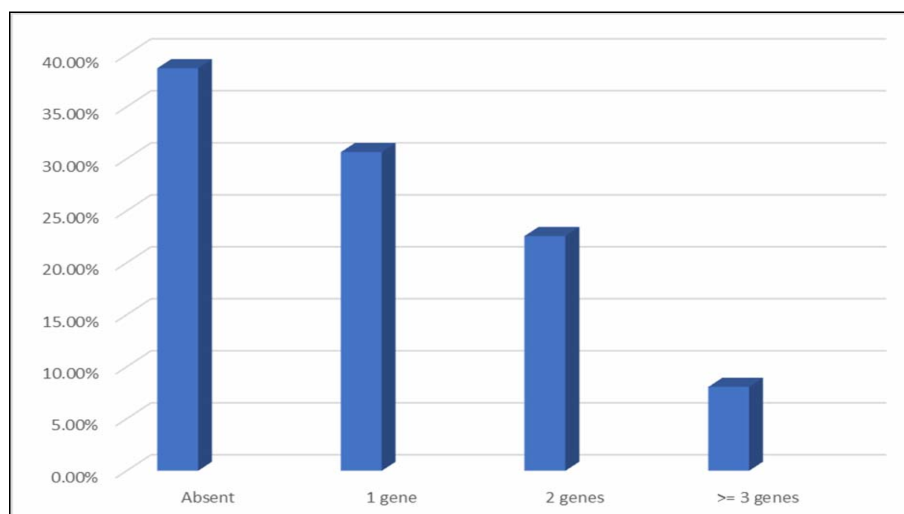

Fig 2: Number of genes in the 62 UPEC strains.

Table 7: Comparison between CA (group I) and HA (group II) UPEC strains according to the number of the presenting genes.

Number of presenting genes	*CA UPEC strains (n=28)*		*HA UPEC strains (n=34)*		*p-value*
	N	%	N	%	
Absent	11	39.29	13	38.24	0.933
Single gene	15	53.57	4	11.76	<0.001*
Two genes	2	7.14	12	35.29	0.008*
Three genes	0	0.00	5	14.71	0.034*

*, Significant difference

Table 8: The significant correlation between different variables among both groups I CA and II HA UPEC strains.

	CA UPEC strains		*HA UPEC strains*	
	r	**p-value**	**r**	**p-value**
fimH/diabetes mellitus	.893	<0.01	.761	<0.001
ESBL production/gene absence	.580	0.001	.886	<0.001
Quinolone resistance/gene absence	.649	<0.001	.642	<0.001
Urinary catheter/multiple genes	1.000	---	.739	<0.001
CA strains/single gene	.451	<0.001		
HA strains/multiple genes			.463	<0.001
Hemolysis/pyelonephritis	.403	0.033	.357	0.038
Quinolone resistance/pyelonephrittis	.572	0.001	.032	0.856

r, correlation coefficient (the closer the r value to 1, the higher the correlation).
P-value<0.01 was considered statistically highly significant.

DISCUSSION

Strains of uropathogenic *Escherichia coli* (UPEC) are the primary cause of community-acquired UTIs (70%–95%) and a large portion of nosocomial UTIs (50%), accounting for substantial medical costs and morbidity worldwide [9].

In this study, we aimed to compare the CA and HA UPEC strains, isolated from 62 patients with UTI as regard: demographic, predisposing factors and clinical characteristics, their resistance patterns to antibiotics and distribution of the *hlyA, PAI, fimH, traT, and papG* virulence genes.

In our study the female patients represented 71.43% in CA (group I) and 73.53% in HA (group II). Similarly, Dormanesh et al. [10] and Khawcharoenporn et al. [11] found in their studies that females were more susceptible to get UTI than males (73.57 and 81% respectively).

Ramesh and Aggrawal, [12] noted that, females' incidence of UPEC is higher than males because of their short urethra and its proximity to the anus as well as sexual activity can increase the chance of urethral bacterial contamination. Also, uterine prolapse causing incomplete bladder emptying can be a contributing factor. Estrogen deficiency with attendant changes in vaginal flora (notably loss of lactobacilli) allows perineal or periurethral colonization with gram negative aerobes, such as *E. coli* that enter the urethra and ascend into the bladder.

In this study patients with Diabetes mellitus (DM) with UTI in HA are significantly higher than those in CA (41.18% versus 14.29% the p-value was 0.020). Chita et al, [13] noted that UTIs are frequent condition associated with DM and it is necessary to improve the care and screening of UTIs in patients to prevent the occurrence of possible associated sever renal complications.

Funfstuch et al., [14] noted that several factors contribute to an increased infection risk in patients with DM such as defects in the host immune mechanisms, incomplete bladder emptying due to autonomic neuropathy, and poor metabolic control. A higher glucose concentration in the urine allows urinary colonization by pathogenic microorganisms.

In the current study the percentage of catheterized patients was significantly higher in HA infection (group II) 35.29% than CA infection (group I) 7.15%. Bacteria can gain access to the bladder through catheter either during insertion or through migration along the track between the catheter and the urethral mucosa.

In a Turkish survey among 483 UTI cases, 63.97% had a urinary catheter. CA UTI was associated with less catheterization than HA UTI [15].

By comparing the antimicrobial susceptibility of CA (group I) and HA (group II) UPEC strains we found that, the resistance of HA strains to cortimoxazol, ciprofloxacin, ceftazidime, levofloxacin, nitrofurantoin, cefepime and amikacin were significantly higher than CA (group II) strains (88.24% versus 64.29%, 73.53%

versus 32.14%, 61.76% versus 28.57%, 58.82 versus 28.57%, 55.88% versus 17.86%, 44.12% versus 14.29% and 38.24% versus 14.29%, respectively).

Shariff et al., [16] reported that the resistance of HA strains of UPEC to cortimoxazol, ciprofloxacin, ciftazidime and amikacin were significantly higher in HA than CA strains (70.3% versus 39%, 98% versus 32.5%, 40% versus 39%, 9% versus 2%).

Massoud et al., [17] compared 50 CA UPEC strains with 50 HA UPEC strains in Alexandia, Egypt. They observed significant higher antibiotic resistance to ceftazidim, ceftriaxone, cefipime, ciprofloxacin, levofloxacin and nitrofurantoin in HA than CA strains (60% versus 24%, 60% versus 20%, 60% versus 16%, 72% versus 32%, 72% versus 32%, 72% versus 32%, respectively).

In this study, imipenem was the least resistant antibiotic for both CA and HA UPEC strains with 7.14% and 20.59% respectively.

In accordance with our results, Prakash and Saxena [18] reported 7.74% imipenem resistance in CA UPEC and 15.48% in HA UPEC strains.

Also, Zaki et al. [19] reported 7% resistance to imipenem in hospital strains of UPEC at Mansura University Hospital, Egypt.

Messai et al. [20] and Massoud et al. [17] reported that, carbapenems appeared to be the drug of choice for serious infections caused by MDR *E. coli* especially ESBL producer strains.

ESBL producing organisms especially enterobacteriaceae have been a major concern involved in infectious diseases since their discovery at 1983 and became a major challenge in HA as well as CA infections [21].

In the current study ESBL, MDR and quinolone resistance strains were significantly higher in HA (group II) than CA (group I) UPEC strains (44.12% versus 17.86, 85.29% versus 35.71% and 44.12% versus 21.43 % respectively).

The increasing rate of ESBL producer E. coli strains among HA is considered a burden for both microbiology laboratories and clinicians. This is most probably due to widespread and misuse of β-lactam antimicrobials in most health care setting [21].

Near to our results Zaki et al. [19] reported 49% of hospital acquired UPEC strains were ESBL producers. Baral et al.[22] reported 52% of HA UPEC were ESBL producers, while 85% were MDR. Tillekeratne et al. [23] reported 63.2% of CA and 34.2% were in patients with diabetes were ESBL producing UPEC strains. Tariq and Reyaz [24] found 85% of HA UPEC were MDR, while Massoud et al. [17] reported 42% MDR. Khawcharoenporn et al. [11] reported 21% quinolone resistance among community UPEC strains.

The observed increased antibiotic resistance to quinolones were striking, because these antibiotics considered one choice of UTI empirical treatment for clinicians. In the United States quiolones are recommended as empiric therapy for complicated UTIs due to the reported low rate of resistance [25 26].

Resistant bacterial strains will continue to appear in an advanced pattern forming a major public health problem as long as antimicrobial agents are misused. The regular monitoring of antibiotic resistance seems necessary to improve the guidelines for empirical antibiotic therapy [27].

UPEC are genetically heterogenous groups that possess several virulence factors necessary for persistence and colonization of the bacteria in the urinary tract [28].

FimH is involved in adhesion, invasion and apoptosis of urothelial cells and initiation of bladder pathology by binding to the uroplakin receptor [29].

In the current study, 33.87% of UPEC strains carried fimH gene. Asadi et al. [28] reported 56.7% of studied strains carried fimH, while Tarchouna et al. [30] reported higher percentage 68%. Munkhdelger et al. [31] found higher prevalence of fimH 89.9% among 148 UPEC.

In our study, 27.42% of strains carried PAI gene. Near to this percent was reported by Asadi et al. [28] where 23.3% of studied strains carried PAI gene. The rate was higher in previous two studies by Oliveira et al. [32], Johnson and Stell [33] where PAI was 32% and 67% respectively.

Pap genes are the principal adherence organelles of UPEC, allow bacteria to adhere to epithelial surfaces and protect them against urine lavage [34].

In our study, 20.97% of strains carried papG II. Near to this result was reported by both Mohajeri et al. [34] and Firoozeh et al. [35] with 20.50% and 16.7% respectively. But the percent was higher in another two studies by Bogyiova et al. [36] and Al-Myahie [37] (74% and 34% respectively).

The hlyA gene is considered as cytolycin coding for hemolysin that form pores in host cells leading to their destruction [38].

In this study, hlyA gene was detected in 11.29% of UPEC strains. Trachouna et al. [30] detected this gene in 19% of studied strains, while it was detected in 43.33% in another study by Dormanesh et al. [10]. A much lower percent 5% was detected by another study by Olivera et al. [32].

The traT protein confers serum resistance by interfering with complement-mediated killing without affecting complement deposition or inactivating soluble complement [39].

In the current study, 6.45% of UPEC strains carried traT gene which was much lower than that reported by Munkhdelger et al. [31], Oliveira et al. [32], Johanson and Stell. [33] (66.2%,76% and 63% respectively).

In spite of the higher percent of the five detected genes (papG, traT, fimH, PAI, and hlyA) in HA (group II) than CA (group I) In this study (26.47% versus 14.29%, 8.82% versus 3.57%, 44.21% versus 21.43%, 32.35% versus 21.43% and 14.71 versus 7.14%) we did

not find any statistical significant difference between them, p-values were 0.241, 0.402, 0.060, 0.337 and 0.349 respectively. The same was noted by Massoud et al. [17] who did not find any significant difference in studied virulence factors between CA and HA UPEC strains.

In this study, the detection of one gene was higher in CA (group I) than HA (group II) 53.57% versus 11.76% and the difference was statistically highly significant p-value was<0.001. While detection of two or three genes were significantly higher in HA (group II) than CA (group I) 35.29% versus 7.14% and 14.71% versus 0% p-values were 0.008 and 0.034 respectively. Regarding gene combination papG II appeared always in association with one or more of the other four genes. This is in agreement with the previous study from Lane and Mobley [40] who noted that all papG allele II isolates were positive for multiple genes association including fimH (100%) and hlyA (72.7%).

In the current study, 38.71% of UPEC isolates harbored none of the studied five genes. Their absence correlated positively with ESBL production and Quinolone resistance (r= 0.727 and 0.621 respectively p<0.001). This was in agreement with a previous study by Massoud et al. [17] who found that ESBL producer strains did not possess any of the studied virulence genes while MDR and Quinolone resistant strains carried virulence genes. Similarly, Johnson and Stell [33] reported 93% prevalence genes in their studied UPEC strains and their existence were associated with decreased antibiotic resistance.

As regard, significant correlations between different variables in this study we found significant positive correlation between fimH gene and Diabetes Mellitus in both CA and HA groups (r=.893 and .761 p-value<0.001). Taganna et al. [41] reported increased prevalence of fimH in diabetic women suffering from UTI.

Also, we detected a significant positive correlation between ESBL producing strains, Quinolone resistance with absence of the five studied virulence genes in both CA and HA groups p-values were <0.001. In agreement with these results Massoud et al. [17] did not detect any of studied virulence genes in ESBL producer strains in spite of different gene examination than ours (papC, cnfI). They also noted that, both quinolone resistance and MDR isolates exhibited significantly lower virulence score than did the susceptible isolates. In contrary to our results Shi-Wei et al. [4٢] reported that, the virulence gene number was positively related with the resistance number and with ESBL and negatively related with the sensitivity.

In this study, we found a positive correlation between hemolysis with pyelonephritis in both groups CA and HA (r=.403, .357 and p-values 0.033 and 0.038 respectively). Kean et al [٤٣] noted that Alpha-hemolysin (AH) is a 110,000-dalton protein secreted extracellularly by certain Escherichia coli. This protein is an acknowledged virulence factor for E. coli and has been implicated as an important determinant in the pathogenesis of E. coli pyelonephritis. Quinolone resistance was correlated positively with pyeloneghritis in CA UPEC strains (r=.572 and p-value=0.001). This could be due to excessive use of quinolones as empirical treatment by physicians which leads to emergence of resistant strains.

CONCLUSION

HA UPEC are more resistant to antibiotics (MDR, ESBL, and Quinolone) than CA strains. Resistance did not correlate with the virulence genes. Catheterization and DM are considered risk factors for acquisition of virulence genes by UPEC.

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REFERENCES

1. Wojnicz D. Virulence factors of uropathogenic *Escherichia coli* strains isolated from children with chronic pyelonephritis. *Adv Clin Exp Med* 2007; 16(5):651
2. Agarwal J, Srivastava S, Singh M. Pathogenomics of uropathogenic *Escherichia coli. Indian J Med Microbiol* 2012; 30(2):141–9
3. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Am J Med* 2002; 113 (1A): 5S–13S
4. Emody L, Kerenyi M, Nagy G. Virulence factors of uropathogenic *Escherichia coli*. *Int J Antimicrob Agents.* 2003;22(2):29–33
5. Mladin C, Usein CR, Chifiriuc M, Palade A, Slavu CL, Negut M, et al. Genetic analysis of virulence and pathogenicity features of uropathogenic *Escherichia coli* isolated from patients with neu-rogenic bladder. *Rom Biotech Lett* 2009; 14(6):4906–11
6. Espinosa R M, Castro R H, Delgado G, Mendez J L, Navarro A, Manjarrez A, Cravioto A. UPEC strain characterization isolated from Mexican patients with recurrent urinary infections. *J Infect Dev Ctries* 2016; 10 (4):317-328
7. Crichton PB. Enterobacteriaceae: *Escherichia*, *Klebsiella, Proteus* and other genera. In: Colle JG, Fraser AG, Marmion BP,and Simmons A (ed), Mackie and McCarteney Practical Medical Microbiology 14th ed; Churchill Livingstone: New York 1996; 361-84
8. Clinical and Laboratory Standard Institute. Performance standards for antimicrobial

susceptibility testing; 5th informational supplement. CLSI document M100-S24 vol. 34 no 1. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA, 2014

9. Kudinha T, Kong F, Johnson JR, Andrew SD, Anderson P, Gilbert GL. Multiplex PCR-based reverse line blot assay for simultaneous detection of 22 virulence genes in uropathogenic *Escherichia coli*. Appl Environ Microbiol.2012;78: 1198-1202
10. Dormanesh B, Dehkordi F, Momtaz H, Mirnejad R, Hoseini M, Yahaghi E, et al. Virulence Factors and O-Serogroups Profiles of Uropathogenic *Escherichia Coli* Isolated from Iranian Pediatric Patients. Iran Red Crescent Med J. 2014; 16(2):14627
11. Khawcharoenporn T, Vasoo S, Singh K. Urinary Tract Infections due to Multidrug-Resistant Enterobacteriaceae: Prevalence and Risk Factors in a Chicago Emergency Department. Emergency Medicine International. 2013;ID258517
12. Ramesh HC, Aggarwal KK. Urinary Tract Infections in Women. Indian Journal of Clinical Practice. 2012;23(4):187-192
13. Chita T, Timar B, Muntean D, Baditoiu L, Horhat F, Hogea E, Moldovan R, Timar R**,** Licker M. Urinary tract infections in Romanian patients with diabetes: prevalence, etiology, and risk factors. Therapeutics and Clinical Risk Management 2017; 13:1-7
14. Fünfstück R, Nicolle LE, Hanefeld M, Naber KG**.** Urinary tract infection in patients with diabetes mellitus. *Clin Nephrol* 2012;77(1):40–48
15. Tasbakan MI, Durusoy R, Pullukcu H, Oguz Resat Sipahi OR. Hospital-acquired urinary tract infection point prevalence in Turkey: Differences in risk factors among patient groups. Annals of Clinical Microbiology and Antimicrobials. 2013,' ID 1476-0711-12-31
16. Shariff VA, Shenoy MS, Yadav T, Radhakrishna M, The Antibiotic Susceptibility Patterns of Uropathogenic *Escherichia coli*, With Special Reference to the Fluoroquinolones. Journal of Clinical and Diagnostic Research. 2013;7(6):1027-1030
17. Massoud BZ, El Sherbini EA, Rizk NG, Arafa SA. Characterization of Uropathogenic *Escherichia coli* Strains Isolated from Community Acquired and Hospital Acquired Infections in Alexandria. Egyptian Journal of Medical Microbiology. 2007;16(3):513-519
18. Prakash D, Saxena RS. Distribution and antimicrobial susceptibility pattern of bacterial pathogens causing urinary tract infection in urban community of meerut city, India. ISRN Microbiol. 2013;ID749629
19. Zaki ME, Refai EM, Mansour FA, Hadek SE. Interleukin 6 Response in Children with *Echerichia Coli* Associated Urinary Tract Infection, One Centre Study. J Advances in Medicine. 2012;1(1):25-36
20. Messai Y, Benhassine T, Naim M, Paul G., Bakour R. Prevalence of beta-lactams resistance among *Escherichia coli* clinical isolates from a hospital in Algiers. Rev Esp Quimioter. 2006;19:144-151
21. Leal AL, Corts JA, Arias G, Ovalle MV, Saavedra SY, Buitrago G, et al. Emergence of resistance to third generation cephalosporins by Enterobacteriaceae causing community- onset urinary tract infections in hospitals in Colombia. Enferm Infec Microbiol Clin. 2013;31(5):298-303
22. Baral P, Neupane S, Marasini BP, Ghimire KR, Lekhak B, Shrestha B. High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal. BMC Res Notes. 2012;5:38
23. Tillekeratne L, Vidanagama D, Tippalagama R, Lewkebandara R, Joyce M, Nicholson B P, et al. Extended-spectrum β-lactamase-producing *Enterobacteriaceae* as a common cause of urinary tract infections in Sri Lanka. Infect Chemother 2016;48(3):160-165
24. Tariq AL, Reyaz AL. Threapeutic Analysis of Terminalia Chebula Against Uropathogenic *Escherichia Coli* (UPEC). Global Journal of Pharmacology. 2012;6(3):160-165
25. Christiansen N, Nielsen L, Jakobsen L, Stegger M, Hansen LH, Frimodt-Moller N. Fluoroquinolone resistance mechanisms in urinary tract pathogenic *Escherichia coli* isolated during rapidly increasing fluoroquinolone consumption in a low-use Country. Microb Drug Resist. 2011 ;17(3):395-406
26. Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis. 2011; 52:103-120
27. Farshad S, Ranjbar R, Japoni A, Hosseini M, Anvarinejad M, Mohammadzadegan R. Microbial susceptibility, virulence factors, and plasmid profiles of uropathogenic *Escherichia coli* strains isolated from children in Jahrom, Iran. Arch Iran Med. 2012;15:312-316
28. Asadi S, Kargar M, Solhjoo K, Najafi A, Sadegh Ghorbani-Dalini S. The Association of Virulence Determinants of Uropathogenic *Escherichia coli* with Antibiotic Resistance. Jundishapur J Microbiol. 2014;7(5),ID 9936
29. Mark A. Schembri MA, Kjaergaard K, Evgeni V, Sokurenko EV, Klemm P. Molecular Characterization of the *Escherichia coli fimH*

Adhesin. Oxford Journal University of Denmark. 2001;183(1):28-31

30. Tarchouna M, Ferjani A, Ben-Selma W, Boukadida J. Distribution of uropathogenic virulence genes in *Escherichia coli* isolated from patients with urinary tract infection. Int J Infect Dis. 2013;17:450-453
31. Munkhdelger Y, Gunregiav N, Dorjpurev A, Juniichiro N, Sarantuya J. Detection of virulence genes, phylogenetic group and antibiotic resistance of uropathogenic *Eschereichia coli* in Mangolia. J Infect Dev Ctries 2017; 11(1):51-57
32. Oliveira FA, Paludo KS, Arend LN, Farah SM, Pedrosa FO, Souza EM, et al. Virulence characteristics and antimicrobial susceptibility of uropathogenic *Escherichia coli* strains. Genet Mol Res. 2011;10:4114-4125
33. Johnson JR, Stell AL. Extended Virulence Genotypes of *Escherichia coli* Strains from Patients with Urosepsis in Relation to Phylogeny and Host Compromise. J Infect Dis. 2000;181(1):261-272
34. Mohajeri P, Khademi H, Ebrahimi R, Farahani A, Rezaei M. Frequency distribution of virulence factors in uropathogenic *Escherichia coli.* isolated from Kermanshah in 2011-2012. International Journal of Applied basic medical Research. 2014;4(2):1 11-116
35. Firoozeh F, Saffari M, Neamati F, Zibaei M. Detection of virulence genes in *Escherichia coli* isolated from patients with cystitis and pyelonephritis. Int J Infect Dis. 2014;29:219-222
36. Bogyiova E, Kmetov M, Biros E, Siegfried L. Detection of pap-, sfa- and afa-specific DNA sequences in *Escherichia coli* strains isolated from extraintestinal material. Folia Microbiol. 2002;47(6):723-726
37. Al-Mayahie SM. Vaginal colonization by papG allele II+ *Escherichia coli* isolates from pregnant and nonpregnant women as predisposing factor to pyelonephritis. Infect Dis Obstet Gynecol. 2013;19:1-6
38. Justice SS, Htmg C, Theriot JA, Fletcher DA, Anderson GG, Footer MJ, et al. Differentiation and developmental pathways of uropathogenic *Escherichia coli* in urinary tract pathogenesis. Proc Natl Dead Sci USA. 2004;l01:1333-1338
39. Wiles TJ, Kulesus RR, Mulvey MA. Origins and virulence mechanisms of uropathogenic *Escherichia coli*. Exp Mol Pathol. 2008;85:11-19
40. Lane MC, Mobley HL. Role of P-flmbrial-mediated adherence in pyelonephritis and persistence of uropathogenic *Escherichia coli* (UPEC) in the mammalian kidney. Kidney Int. 2007;72: 19-25
41. Taganna J, de Boer AR, Wuhrer M, Bouckaert J. Glycosylation changes as important factors for the susceptibility to urinary tract infection. Biochem Soc Trans. 2011; 39(1):349-354
42. Shi-Wei L, Xiao-Yang X, Jie X, Jiu-Yun Y, Wei-Kang W, Ning Z, Ze-Liang C**.** Multi-drug resistant *Eschereichia coli* and its treatment by Chinese medicine. Chin J Integr Med 2016; 27:1-7
43. Keane W F, Welch R, Gekker G, Peterson P K. Mechanism of *Escherichia coli* α-hemolysin induced injury to isolated renal tubular cells. Am J Pathol.1987;16(2):350-357.