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ORIGINAL ARTICLE

The Prevalence of Biofilm Formation, Antimicrobial Resistance and Adhesive Pap Gene (Pyelonephritis Associated Pili) among *Escherichia Coli* Strains Isolated from Outpatients and Inpatients with Urinary Tract Infection

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ABSTRACT

Background*: E.coli is the most common cause of urinary tract infection (UTI) both in the community and hospital settings. Uropathogenic E.coli (UPEC) possesses a number of virulence factors that allow it to colonize and persist in the urinary tract. Of particular interest are the P pili and biofilm formation. The emergence of drug resistant E.coli is a global threat to the public health.* ***Objectives:*** *To determine the biofilm producing ability, antimicrobial susceptibility pattern, ESBL production and the presence of the adhesive pap gene (pyelonephritis associated pili) in E-coli strains isolated from Outpatients and Inpatients diagnosed with UTI.* ***Methodology:*** *The study was conducted on 40 Inpatients and 40 Outpatients diagnosed with UTI attending Ain Shams University Hospitals. E. coli Isolates were tested for antimicrobial susceptibility using disk diffusion method, ESBL production by ESBL detection discs, in vitro formation of biofilm on Congo red media, and detection of Pap gene using conventional PCR technique.* ***Results:*** *The prevalence of antibiotic resistance was significantly higher in E.coli isolates from inpatients group than those from the outpatients group. The prevalence of ESBL production, biofilm formation and pap gene among E. coli isolates was 55%, 82.5% & 40% respectively. ESBL production and biofilm formation were significantly higher in the inpatients with upper UTI than in patients with lower UTI (90-38.5%) & (100-61.5%) respectively. There was no statistically significant difference between the 2 groups as regards the prevalence of pap gene.* ***Conclusion:*** *The prevalence of biofilm producing, ESBL producing and antibiotic resistant E.coli strains is more in the inpatients population particularly those with upper UTI. Special concern should be addressed to the spread of ESBL producing E.coli in the community. Pap gene is expressed equally in the outpatients and inpatients groups which highlights its importance in the establishment of UTI.*

INTRODUCTION

Urinary tract infections (UTIs) are the second most common type of infections in humans[1]. Uropathogenic *Escherichia coli* (UPEC) is the most common cause of UTIs both in community and hospital settings with significant morbidity and mortality worldwide [2].

UPEC possesses many virulence factors that allow its colonization, persistence, and pathogenesis in the urinary tract. Among these factors; fimbriae, biofilm formation, and toxins the most important[3].

P fimbriae are encoded by the pap genes and play an important role in binding and invasion to bladder and kidney epithelial cells. They are required for colonization and invasion of the human upper urinary tract and thus play an important role in the pathogenesis of pyelonephritis[4].

Biofilm of UPEC provides a nutrient-rich environment which promotes growth and persistence of microorganisms at the site of infection, and protects bacteria from antimicrobial substances. They are crucial for UPEC persistence causing relapses and recurrence[5,6].

Emergence of drug resistance to broad-spectrum beta lactams among UPEC strains increase the serious threat to global public health [7]. This study aimed to determine the biofilm producing ability, antimicrobial susceptibility pattern, ESBL production and the presence of the adhesive pap gene (pyelonephritis associated pili) in *E-coli* strains isolated from out patients and in patients diagnosed with UTI in Ain Shams University Hospital.

METHODOLOGY

Patients:

The present study was conducted on 80 patients suffering from UTI attending Ain Shams University Hospitals in the period from June 2014 to June 2015. Patients were divided into 2 groups: Group 1: 40 inpatients and Group2: 40 outpatients from the outpatient clinic. Informed consents were obtained from all patients. This work had been conducted after approval of Ain Shams University Ethical Committee and in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans.

Bacterial Isolates and Testing:

A forty (40) midstream urine (MSU) samples were collected from outpatients and (30) MSU samples and (10) urine samples from urinary catheters were collected from inpatients under aseptic conditions in sterile universal containers and processed for microbiological examination.

Microbiologic analysis:

Bacterial strains, media and growth conditions:

Urine samples that showed significant pyuria by microscopic examination and significant bacteriuria by bacterial counting were subjected to isolation and identification of microorganisms by conventional methods. Identification of isolated *E.coli* strains was done according to *Cheesebrough*[8]; based on colonial morphology, microscopic examination of Gram stained films, biochemical tests using Analytical Profile Index (API) 20 E test (BioMerieux, Marcy L'Etoile, France). For viable bacterial counting: uncentrifuged urine was cultured on Cystine Lactose Electrolyte Deficient (CLED) agar. The results were expressed as CFU/ml**.** All media and reagents were supplied from (Oxoid, UK).

Antimicrobial susceptibility testing:

Antibiotic susceptibility of isolated strains was done by disc diffusion method, using Muller-Hinton agar plates (discs & agar supplied by Oxoid, UK). Interpretation of results was done according to CLSI guidelines. Nalidixic acid, ciprofloxacin, nitroforantion, cephalexin, cefotaxime, amoxicillin/clavulonic acid, amikacin, levofloxacin, ampicillin, imipenem, and aztreonam were used for the antibiotic susceptibility testing [9].

Detection of ESBL production:

E.coli isolates were tested for ESBL production by ESBL detection discs (Mast discs, USA). Test procedure, contents of ESBL discs and interpretation of results were done according to Clinical Laboratory Standard Institute[9]. The ESBL detection discs cefotaxim (30 ug) versus cefotaxime/clavulanic acid (30/10ug) were placed onto a Muller-Hinton agar plate lawned with the test organism using a sterile forceps. After incubation at 37°C for 24 hours, the diameter of any observed zone of inhibition was measured. Regardless of the zone diameters, a > 5mm increase in a zone diameter for cefotaxime tested in combination with clavulonic acid versus its zone size when tested alone, indicates probable ESBL production (Fig. 1).

Fig. 1: Detection of ESBL producing *E.coli* isolate by ESBL detection discs cefotaxime (30ug) versus cefotaxime/clavulanic acid (30/10ug).

Detection of biofilm formation using congo red binding assay:

Plates of congo red agar medium were inoculated with *E.coli* strains and incubated aerobically for 24 hours at 37°C. A positive result was indicated by black colonies with a dry crystalline consistency. Non-slime producers usually remained pink, though occasional darkening at the centre of the colonies was observed. An indeterminate result was indicated by a darkening of the colonies but with the absence of a dry crystalline colonial morphology[10]. (Fig. 2).

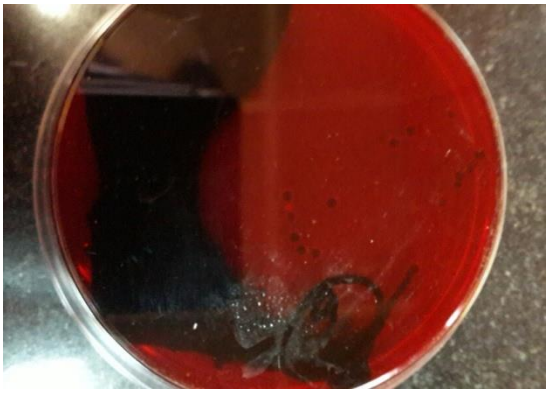

Fig. 2: Congo red agar plate showing biofilm forming black *E.coli* colonies.

***PCR for detection of the adhesive pap gene (pyelonephritis associated pili)* [11]:**

DNA was extracted from the bacterial isolates using the spin column method using Qiagen DNeasy (Qiagen, USA), according to manufacture instructions[12]. PCR amplification was carried out using thermal cycler (BioRad, USA) with specific primers for PAP-gene (table 1). Agarose gel electrophoresis was used for examining the amplified products; PCR products were run on 2% agarose gel, stained with ethidium bromide visualized under UV light and photographed.

Table 1: Primers for the PCR assay:

Virulence factor	Target gene	Name	Primer sequence (5`-3`)	Size of amplicon (bp)
P fimbriae	Pap	Pap1	Gacggctgtactgcagggtgtggcg	328
		Pap2	Atatcctttctgcagggatgcaata	

Statistical analysis:

Data was collected, tabled and statistically analyzed using SPSS vs. 15. Non parametric data was expressed as number and percentage. Parametric data was expressed as minimum, maximum, mean and standard deviation. Comparison between two groups as regards non parametric data was done using Chi-square (x2). Comparison between two groups as regards parametric data was done using student t test. Two tailed p value was considered significant if less than or equal to 0.05 and insignificant if more than 0.05.

RESULTS

E.coli isolates were detected in 40 (50%) of total urine samples collected from 80 patients; 17/40 (42.5%) & 23/40 (57.5%) from outpatients and inpatients respectively. The demographic and clinical data of both inpatients and outpatients groups are shown in table 2.

Table 2: Demographics & clinical data of *E.coli* positive inpatients and outpatients groups

	Inpatients group (n=23) N (%)	**Outpatients group (n=17) N (%)**		**p**
Age			t	0.6
Minimum-maximum	32-86	30-72	3.2	
Mean±SD	59.3±11.4	47.23±12.2		
Gender N (%)			X2	0.6
Male	7 (30.4)	4 (23.5)	0.23	
Female	16 (69.6)	13 (76.5)		
Type of infection N (%)			X2	0.6
Lower UTI	13(56.5)	11 (64.7)	0.27	
Upper UTI	10 (43.5)	6 (35.3)		

p>0.05 insignificant

Antibiotic susceptibility of *E. coli* isolates:

The prevalence of antibiotic resistance was higher in *E.coli* isolates from inpatients group than those from the outpatients group. This result was statistically significant for ampicillin, cephalexin & amoxicillin-clavulanate. However, *E.coli* isolates from outpatients were significantly more resistant to nitrofurantoin than isolates from inpatients (table 3).

Table 3: Antibiotic susceptibility results of *E.coli* strains isolated from inpatients and outpatients groups:

Antibiotic	Inpatients group (n=23) N (%)	Outpatients group (n=17) N (%)	X2	P
Ciprofloxacin			4.6	0.09
R	15 (65.2)	8 (47.1)		
S	8 (34.8)	6 (35.3)		
I	0	3 (17.6)		
Gentamycin			5.58	0.06
R	14 (60.9)	4 (23.5)		
S	8 (34.8)	12 (70.6)		
I	1 (4.3)	1 (5.9)		
Amikacin			2.03	0.3
R	5 (22)	4 (23.5)		
S	11 (47.8)	11 (64.7)		
7	7 (30.4)	2 (11.8)		
Cefoxitin			3.78	0.1
R	16 (69.6)	7 (41.2)		
S	6 (26.1)	7 (41.2)		
I	1 (4.3)	3 (17.6)		
Levofloxacin			3.92	0.1
R	15 (65.2)	8 (47.1)		
S	6 (26.1)	9 (52.9)		
I	2 (8.7)	0		
Nitrofurantoin			28.8	0.0001*
R	2 (8.7)	16 (94.1)		
S	19 (82.6)	1 (5.9)		
I	2 (8.7)	0		
Ampicillin			7.14	0.02*
R	21 (91.3)	13 (76.5)		
S	2 (8.7)	0		
I	0	4 (23.5)		
Cephalexin			6.95	0.03*
R	21 (91.3)	11 (64.6)		
S	1 (4.3)	0		
I	1 (4.3)	6 (35.3)		
Amoxicillin-Clavulanate			7.16	0.02*
R	15 (65.2)	4 (23.5)		
S	1 (4.3)	3 (17.6)		
I	7 (30.4)	10 (58.8)		
Nalidixic acid			2.1	0.3
R	16 (69.6)	8 (47.1)		
S	5 (21.7)	6 (35.3)		
I	2 (8.7)	3 (17.6)		
Imipenem			0	1
R	0	0		
S	23 (100)	17 (100)		
I	0	0		
Aztreonam			0.36	0.8
R	10 (43.4)	7 (41.2)		
S	9 (39.1)	8 (47)		
I	4 (17.4)	2 (11.8)		

p≤0.05 significant* R: Rresistant S: Sensitive I: Intermediate

The prevalence of resistance to ciprofloxacin, cefoxitin, levofloxacin, nalidixic acid & aztreonam was significantly higher among inpatients with upper UTI than in patients with lower UTI for (table 4).

Table 4: Antibiotic Susceptibility results of *E.coli* strains isolated from patients with lower and upper UTI

	Inpatients group				Outpatients group			
	Lower UTI (n=13) N (%)	**Upper UTI (n=10) N (%)**	**X2**	**P**	**Lower UTI (n=11) N (%)**	**Upper UTI (n=6) N (%)**	**X2**	**P**
Ciprofloxacin								
R	6 (46.2)	10 (100)	7.7	0.02*	4 (36.4)	4 (66.7)	2.4	0.3
S	6 (46.2)	0			4 (36.4)	2 (33.3)		
I	1 (7.6)	0			3 (27.2)	0		
Gentamycin							0.9	0.6
R	6 (46.2)	8 (80)	2.94	0.2	2 (18.2)	2 (33.3)		
S	6 (46.2)	2 (20)			8 (72.7)	4 (66.7)		
I	1 (7.6)	0			1 (9.1)	0		
Amikacin							0.38	0.8
R	1 (7.6)	4 (40)	5.16	0.07	3 (27.3)	1 (16.6)		
S	6 (46.2)	5 (50)			7 (63.6)	4 (66.7)		
I	6 (46.2)	1 (10)			1 (9.1)	1 (16.6)		
Cefoxitin							2.68	0.2
R	7 (53.8)	10 (100)	6.24	0.04*	3 (27.3)	4 (66.7)		
S	5 (38.5)	0			5 45.5)	2 (33.3)		
I	1 (7.7)	0			3 (27.2)	0		
Levofloxacin							1.4	0.2
R	6 (46.2)	9 (90)	6.32	0.04*	4 (36.4)	4 (66.7)		
S	6 (46.2)	0			7 (63.6)	2 (33.3)		
I	1 (7.6)	1 (10)			0	0		
Nitrofurantoin							0.5	0.4
R	1 (7.6)	1 (10)	0.8	0.9	1 (9.1)	0		
S	11 (64.6)	8 (80)			10(90.9)	6 (100)		
I	1 (7.6)	1 (10)			0	0		
Ampicillin							4.8	0.08
R	11 (64.6)	10 (100)	1.6	0.1	9 (81.8)	4 (66.7)		
S	0(0)	0(0)			0	2 (33.3)		
I	2 (15.4)	0 (0)			2 (18.2)	0		
Cephalexin							5.85	0.054
R	11 (64.6)	10 (100)	1.68	0.4	7 (63.6)	4 (66.6)		
S	1 (7.6)	0			4 (36.4)	0		
I	1 (7.6)	0			0	2 (33.3)		
Amoxicillin-Clavulanate							0.28	0.8
R	7 (53.8)	8 (80)	5.1	0.07	3 (27.2)	1 (16.7)		
S	5 (38.5)	0			2 (18.2)	1 (16.7)		
I	1 (7.7)	2 (20)			6 (54.5)	4 (66.6)		
Nalidixic acid								
R	6 (46.1)	10 (100)	7.74	0.02*	4 (36.3)	4 (66.6)	2.4	0.3
S	5 (38.5)	0			4 (36.3)	2 (33.3)		
I	2 (15.4)	0			3 (27.2)	0		
Imipenem			0	1			0	1
R	0	0			0	0		
S	13 (100)	10 (100)			11 (100)	6 (100)		
I	0	0			0	0		
Aztreonam			9.82	0.007*			1.2	0.5
R	2 (15.4)	8 (80)			4 (36.3)	3 (50)		
S	8 (61.5)	1 (10)			5 (45.5)	3 (50)		
I	3 (23.1)	1 (10)			2 (18.2)	0		

p≤0.05 significant* R: Resistant S: Sensitive I: Intermediate

ESBL production by *E.coli* isolates:

ESBL production among *E. coli* isolates was 55% (22 out of 40 *E.coli* isolates). The prevalence of ESBL production by *E.coli* isolates was higher in inpatients group (60.9%) than in outpatients group (47.1%). However, this difference was not statistically significant. ESBL production was significantly higher in the inpatients with upper UTI (90%) than in patients with lower UTI (38.5%) (table 5).

Table 5: ESBL production among *E.coli* isolates:

	Inpatients group				Out patients group			
	Lower UTI (n=13) N (%)	Upper UTI (n=10)N (%)	X2	P	Lower UTI (n=11) N (%)	Upper UTI (n=6) N (%)	X2	P
ESBL PRODUCTION	5(38.5)	9(90)	6.3	0.01*	5 (45.5)	3 (50)	0.03	0.8
Total	14 (60.9)				8(47.1)			
X2	0.75							
p	0.3							

p≤0.05 significant*

ESBL: Extended spectrum B-lactamase.

Biofilm formation:

The percentage of biofilm producing *E.coli* was (82.5%); 33 out of 40 *E.coli isolates.* Biofilm formation was detected in 18 (78.3%) and in 15 (88.2%) isolated *E. coli* strains from inpatients and outpatients respectively. Biofilm formation was significantly higher among inpatients with upper UTI (100%) than inpatients with lower UTI (61.5%) (table 6). There was statistically significant positive correlation between biofilm formation and resistance to gentamycin, cefoxitin, levofloxcacin, amoxicillin clavulanate & nalidixic acid (table 7).

Table 6: Biofilm formation among *E.coli* isolates:

	Inpatients group				Outpatients group			
	Lower UTI (n=13) N (%)	Upper UTI (n=10) N (%)	X2	P	Lower UTI (n=11) N (%)	Upper UTI (n=6) N (%)	X2	P
Biofilm formation	8 (61.5)	10 (100)	4.9	0.02*	10 (90.9)	5 (83.3)	0.2	0.6
Total	18 (78.3)				15 (88.2)			
X2	0.67							
P	0.4							

Table (7): The relation between biofilm formation and antibiotic resistance among *E.coli* isolates:

	Inpatients Group (n=23) N (%)		**Outpatients group (n=17) N (%)**		**Biofilm positive P**
	Biofilm formation		**Biofilm formation**		
	N	**P**	**N**	**P**	
Ciprofloxacin					
I	0	1 (4.3)	0	3 (17.6)	0.08
R	2/23 (8.6)	14 (60.8)	2 (11.8)	6 (35.3)	
S	3 (13.1)	3 (13.1)	0	6 (35.3)	
P	0.1		0.2		
Gentamycin					0.02*
I	0	1 (4.3)	0	1 (5.9)	
R	3 (13.1)	12 (52.2)	1 (5.9)	3 (17.6)	
S	2 (8.6)	5 (21.7)	1 (5.9)	11 (64.7)	
P	0.7		0.6		
Amikacin					0.6
I	3 (13.1)	4 (17.4)	0	2 (11.8)	
R	0	5 (21.7)	1 (5.9)	3 (17.6)	
S	2 (8.6)	9 (39.1)	1 (5.9)	10 (58.8)	
P	0.1		0.6		
Cefoxitin					0.02*
I	1 (4.3)	0	0	3 (17.6)	
R	2 (8.6)	14 (60.9)	2 (11.8)	5 (29.4)	
S	2 (8.6)	4 (17.4)	0	7 (41.2)	
P	0.08		0.1		
Levofloxacin					0.02*
I	0	2 (8.6)	0	0	
R	2 (8.6)	13 (56.6)	2 (11.8)	6 (35.3)	
S	3 (13.1)	3 (13.1)	0	9 (52.9)	
P	0.1		0.1		
Nitrofurantoin					0.5
I	1 (4.3)	1 (4.3)	0	0	
R	0	2 (8.6)	0	1 (5.9)	
S	4 (17.4)	15 (65.2)	2 (11.8)	14 (64.7)	
P	0.4		0.7		
Ampicillin					0.09
I	1 (4.3)	1 (4.3)	0	4 (23.5)	
R	4 (17.4)	17 (73.9)	2 (11.8)	11 (64.7)	
S	0	0	0	0	
P	0.3		0.4		
Cephalexin					0.2
I	0	1 (4.3)	0	6 (35.3)	
R	4 (17.4)	17 (73.9)	2 (11.8)	9 (52.9)	
S	2 (8.6)	0	0	0	
P	0.03*		0.2		
Amoxicillin-Clavulanate					0.004*
I	1 (4.3)	6 (26.1)	0	10 (58.8)	
R	3 (13.1)	12 (52.2)	2 (11.8)	2 (11.8)	
S	1 (4.3)	0	0	3	
P	0.1		0.02*		
Nalidixic acid					0.03*
I	1 (4.3)	1 (4.3)	0	3 (17.6)	
R	1 (4.3)	15 (65.2)	2 (11.8)	6 (35.3)	
S	3 (13.1)	2 (8.6)	0	6 (35.3)	
P					
Imipenem					-
I	0	0	0	0	
R	0	0	0	0	
S	5 (21.7)	18 (78.3)	2 (11.8)	15 (88.2)	
P	-		-		
Aztreonam					0.3
I	0	4 (17.4)	0	2 (11.8)	
R	1 (4.3)	9 (39.1)	2 (11.8)	5 (29.4)	
S	4 (17.4)	5 (2.7)	0	8 (47.1)	
P	0.09		0.1		

P<0.05 is statistically significant.

PAP gene detection by PCR:

PAP gene was detected in 16/40 (40%) of *E. coli* isolates; 9 (39.1%) and 7(41.7%) from inpatients and outpatients respectively. There was no statistically significant difference between the 2 groups (table 8) & (figure 3).

Table 8: The presence of Pap gene among E.coli isolates:

	Inpatients group				Outpatients group			
	Lower UTI (n=13) N (%)	**Upper UTI (n=10) N (%)**	**X2**	**P**	**Lower (n=11) N (%)**	**Upper (n=6) N (%)**	**X2**	**P**
PAP gene	6 (46.2)	3 (30)	0.6	0.4	3 (27.3)	4 (66.7)	2.4	0.1
Total	9 (39.1)				7 (41.2)			
X2	0.01							
P	0.8							

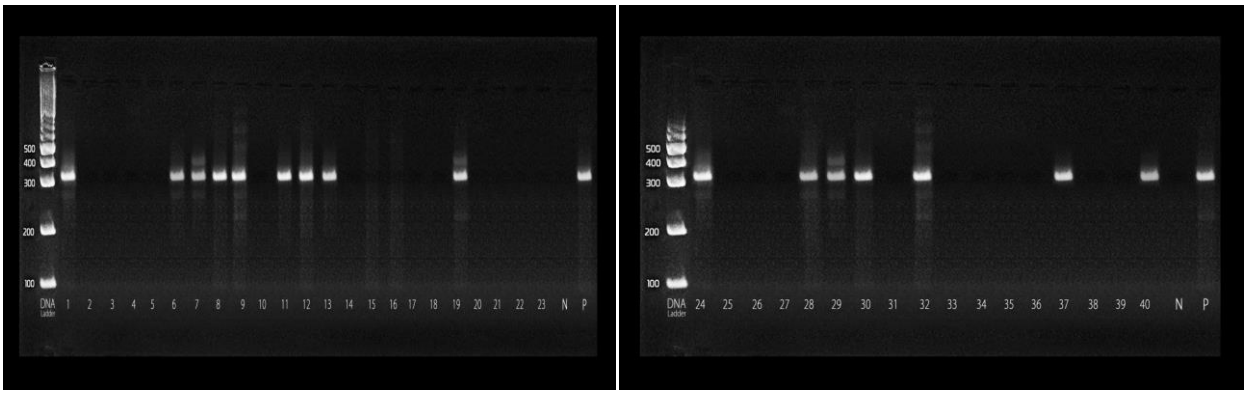

Fig. 3: Agrose gel electrophoresis for PAP gene in *E.coli* isolates

P: positive control, N: negative control

100 bp DNA ladder; different strains of *E.coli* with PAP gene products detected at 328 bp, strains 1,6,7,8,9,11,12,13,19 are positive for the PAP gene among *E.coli* isolates from inpatients, strains 24,28,29,30,32,37,40 are positive for the PAP gene among *E.coli* isolates from outpatients.

DISCUSSION

E.coli is the main cause of both community-acquired and hospital UTIs, accounting for significant medical costs and morbidity and mortality worldwide**.** The ability of UPEC to cause symptomatic UTIs is associated with expression of a broad spectrum of virulence factors, with adhesive molecules and biofilm formation being the most important determinants of pathogenicity. P fimbriae is particularly associated with pyelonephritis and is encoded by pap genes[4,13].

The present study showed that the prevalence of antibiotic resistance was higher in *E.coli* isolates from inpatients group than the out patients group. This difference was statistically significant for ampicillin, cephalexin & amoxicillin-Clavulanate. However, *E.coli* isolates from outpatients were significantly more resistant to nitrofurantoin than isolates from inpatients. These results agree with those done by Saperston et al., 2014 in Taiwan in which the antibiotic resistance among *E.coli* isolates was significantly higher among inpatients more than outpatients for trimethoprim-sulphamethazole and cephalothin [14]. Another study in Iran also reported the antibiotic resistance among *E.coli* isolates is higher in inpatients than out patients for cefotaxime, ciprofloxacin, ofloxacin, norfloxacin, gentamicin, nalidixic acid and nitrofurantoin[15].

In the present study the resistance to ciprofloxacin, cefoxitin, levofloxacin, nalidixic acid, and aztreonam *was* significantly higher in *E.coli* isolates among inpatients with upper UTI than in patients with lower UTI. These results agree with those done by Kudinha et al.[16] who found significant higher levels of resistance to multiple antibiotics such as ampicillin, amoxicillin-clavulanate, cephalothin, trimethoprim-sulphamethazole among pyelonephritis patients more than cystitis patients. In contrast to these results Tabasi et al.[17] found that there were significant higher levels of resistance to

multiple antibiotics such as nalidixic acid, ceftriaxone, and cotrimoxazole among acute cystitis patients more than pyelonephritis patients.

Our results showed that resistance rates were higher to antimicrobials that have been used for a long time as empirical therapy in the hospital such as ampicillin and amoxicillin-Clavulanate. Low levels of resistance to nitrofurantoin among inpatients and higher levels of resistance in outpatients may be linked to the uncommon use of this antimicrobial for in patients in our hospital whereas; it is routinely prescribed for outpatients. Similar findings were reported by Asadi et al.[1] in Iran and Dash et al.[18] in India in which the rate of resistance to nitrofurantoin was the least among inpatients.

ESBL production in the present study was found to be 55% (22 out of 40 *E.coli* isolates). These results agree with those done by Ali et al.[19] in Pakistan and Hassan et al.[20] in India in which ESBL was 43.25% &54% respectively. Our studies showed that ESBL production was higher in inpatients group (60.9%) than in outpatients group (47.1%); however, this difference was not statistically significant. These results agree with those done by Senbayrak et al. in Turkey[21], Al Mously et al. in Saudia Arabia[22] in which ESBL production was higher among inpatients than outpatients (44.7% - 22.8% & 65-36% respectively). In the present study ESBL production was higher in inpatients with upper UTI (90%) than in patients with lower UTI (38.5%) and the difference was statistically significant. In concordance with our results, Kudinha et al.[16] found significant higher levels of ESBL production in patients with upper UTI (9%) than in patients with lower UTI (5%).

The results of our study show that community-associated ESBL rates must not be ignored. ESBL producing *E.coli* is increasingly recognized as an important cause of UTI among patients who did not have a history of recent contact with a health care facility. Colonization by ESBL producing *E.coli* can occur after previous infection, previous hospitalization or both. [23].

Regarding biofilm results in this study, the percentage of biofilm producing *E.coli* was (82.5%). These findings come in accordance with Murugan et al.[24] and Tabasi et al.[17] who showed that among UPEC isolates 84.37% and 85.3% were biofilm producers respectively.

Our study shows that biofilm formation was significantly higher among *E.coli* strains isolated from inpatients with upper UTI (100%) than inpatients with lower UTI (61.5%). Tapiainen et al.[25] also showed that biofilm formation was significantly higher among inpatients with upper UTI (89%) than inpatients with lower UTI (71%). Bacteria that adhere to the uroepithelium and form biofilm have higher tendency to invade the renal tissue causing pyelonephritis [26].

Our results showed a statistically significant positive correlation between biofilm formation and resistance to multiple antibiotics. These results agree with those done by Tabasi et al.[17] & Tajbakhsh et al.[27].

Microorganisms growing in a biofilm are intrinsically resistant to many antibiotics increasing the antibiotic resistance up to 1000 folds and high antimicrobial concentrations are required to inactivate organisms growing in a biofilm [28].

P fimbriae are required for colonization and invasion of the human upper urinary tract. PAP gene has been detected in the majority of patients with acute pyelonephritis and cystitis who have a normal immune system[29].

In the present work the prevalence of PAP gene among *E.coli isolates* from inpatients and out patients groups was 39.1% and 41.2% respectively with no statistically significant difference between the two groups. These results agree with those done by Usein et al. in Romania[30] and Santo et al. in Brasil[31]. In contrast, Tarchouna et al. in Tunisia[32] showed higher PAP gene among *E.coli isolates* from inpatients than in outpatients groups.

In the hospital environment, many patients have multiple risk factors including immunocompromise, indewelling urinary catheters and exposure to a wide range of antimicrobial drugs. From this standpoint, hospital acquired UTI might result not only from infections by typical UPEC but can also be produced by various *E.coli* strains with unusual virulence genes [33].

CONCLUSION

Our results show that resistance to many antimicrobials are more prevalent in the inpatients population than in the community. However, the ESBL producing isolates in the outpatients population is comparable to those from inpatients. This draws the attention to the necessity of complying with strict antimicrobial strategies to prevent the spread of resistant strains both in hospital settings and the community. Our results also indicate the vulnerability of inpatients particularly those with upper UTI to ESBL and biofilm producing strains. Pap gene is also an important virulence factor which is expressed equally in the out patients and inpatients groups. Further studies compromising a larger repertoire of virulence genes are necessary to evaluate their role in pathogenesis of UTI.

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