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RESEARCH ARTICLE

Detection of Colony Adhesion Factors and Genetic Background of Adhesion Genes Among Multidrug-Resistant Uropathogenic *Escherichia coli* Isolated in Iraq

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Abstract

***Escherichia coli* remain significant problem caused urinary tract infection; Results indicated a higher urinary tract infection in women compared with men across all age groups. Phylogenetic analysis showed that majority of uropathogenic isolated *E. coli* belong to phylogroup B2 followed by D. The isolates showed the existence of the first type of fimbriae, maximum P fimbriae positive isolates 74/94 (80.43%) were widely correlating with common UTI. colony adhesion factor (CAFÐ) represented in 40 (43.47%) and 5 (5.43%) colony adhesion factor (CAFØ). Six major clusters (A-F) were identified depending on antibiograms typing. B2 is the most phylogenetic type showed wide range of resistant from 1 to 12 resistant to antibiotic of remaining strains. 14 isolates (15.21%) were detected as ESâLs producers and 30 isolates (32.60%) of them were AmpC β-lactamases producers. prevalence of virulent genes occurred in 51 *papC* (55.43%), 66 *fim H* (71.73%) and *SfaDE* detected in low occurrence 21(22.82). These results emphasize the low or moderate resistant to antibiotics focused in isolates with high genetic content .These results indicated that the greater the resistance to antibiotics the less the genetic expression of the virulence factors, this confirms the reverse relationship. The genes required for uropathogenicity in a single isolate may not reflect virulence in another isolate. Pathogenicity is a multi-factor characteristic, the result of adhesion-related genes which interact in separate set in various genetic backgrounds.**

Keywords: UTI ,*E. coli* , Phylogenetic analysis, adhesion factor, virulent genes.

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INTRODUCTION

Urinary tract infections (UTIs) are common bacterial infections associated with much morbidity and health care cost[1]. *Escherichia coli* strains capable of causing disease outside the gastrointestinal tract belong to a diverse group of isolates referred to as uropathogenic *E. coli* (UPEC)[2]. UPEC mediated several virulence factors, which help bacteria to colonize the urinary tract and begin infections[3]. Fimbriae are categorized serologically by their hemagglutination pattern and receptor specificities as mannose sensitive (MSHA) or mannose resistance hemagglutination (MRHA) [4]. Despite the vast subclass of adhesins that have been reported in UPEC, Type I (MSHA) and P (MRHA) are the widespread fimbriae in UPEC strains. They play a remarkable role in binding and invasion of the bladder and kidney[5]. In *E. coli* pathogenicity associated island (PAI) many virulence gene were expressed . The important ones are the 'adhesions, which help to break the inertia of urinary bladder mucus and help to attach to them [6]. Type I pili, are mediated and expressed by a *fim* gene cluster in the most of UPEC strains [7]. *pap* genes coded P pili (pyelonephritis-associated pili, PAP), which have essential role in upper UTIs [8] and S fimbrial adhesion (*sfa*) [9]. UTI multi-drug resistant *E. coli* has gradually increased. essentialy as a result of the incidence of fluoroquinolone-resistant and ESBL-producing isolates [10]. The decreased expression in virulence mediated genes and invasive capacity associated with MDR strains [11]. Many predispose disease are associated with many factors lead to genetic background [12]. There is real proof that the connection among virulence ability of *E. coli*, phylogenetic background, and resistance to antibiotics are incident, resulting from their diverse mutual [13].

The distribution pattern of adhesion virulence factor encoding *fim H, SfaDE* and *pap C* genes in the multidrug resistance UPEC isolates are as yet indistinct in different parts of Iraq. So, this study is aimed to determine the coordination between the genetic backgrounds, coexist, correlation of phenotypic and genotypic adhesive virulence factors and antibiotic resistance profile among the UPEC.

MATERIAL AND METHODS

Study Population

Urine samples from 450 cases (both male and female of age 15-70 years) of UTI were collected during Jan 2012 to June 2012, for isolation of *E. coli*. Patients on antibiotic therapy were excluded from the study.

Collection of Urine Samples

Early morning midstream urine samples were collected using a sterile plastic container with screw cap tops with name, age, sex, time of collection [14].

Isolation and identification of *E. coli*

All urine samples were cultured and incubated overnight at 37°C on blood agar and MacConckey agar; further incubation for 24 hours were done if the remained negative. A positive specimen yields colonies ($\geq 10^5$ cfu/ml) and the microscopic detection of bacteriuria when PMNs ($\geq$8 leukocytes/mm^3). No mixed infections were encountered [15]. further identification of isolates were done by specific biochemical tests, and API 20E test system (Bio- Merieux) considered confirmatory test [16].

Antibacterial Agents Susceptibility

Antibacterial susceptibility of *E. coli* was tested on Mueller-Hinton agar by the disk diffusion method according to [17]. The antibiotic discs used in this study were Amoxicillin/clavulanic acid (30mg; 20:10), Amoxicillin (30μg), Cefoxitin (30μg), Cefotaxime (30μg), Cefotazidime, (30μg), ciprofloxacin (5μg), Cefepime (30μg), Imipenem (10μg), Levofloxacin (5μg), Kanamycin (30μg).

ESBL and AmpC screening

Production of ESBL was detected by double disc synergy test according to CLSI guidelines [17]. For *AmpC* screening done according to [18].

Detection of colony adhesion factors (CAF)

Three types of colony adhesion factors I,II and III were detected according to [19].

Bacterial adherence to epithelial cells, screening

The adherence ability of the *E. coli* isolates to uroepithelial cells was assayed as described by [20].

Preparation of bacterial DNA

Template DNA was prepared by boiling method that described by [21].

Phylogenetic groups procedure

Phylogenetic groups were defined as described by [22].

PCR amplification of virulence factor genes procedure:

Detection of *papC* (type P pili) gene was performed by PCR. The primer sequences were reported by [23] and obtained from an Alpha DNA company (USA). The program ,for *pap*C gene the reactions condition included an initial denaturation at 94°C for 1 min consisted of 30 cycles of 94°C for one min, specific annealing temperature 63°C for 30 seconds, and a final extension at 72°C for 90 seconds. For *fim* H and *SfaDE,* the primers sequences were previously reported by(24).The initial denaturation of reactions mixtures at 94°C for 5 min followed with 30 cycles of 94°C for one min, specific annealing temperature 58°C (*fim* H) and 63°C (*SfaDE*) for one min and 68°C for three min, and a final extension at 72°C for 7 min.The detection of PCR products was performed on 1.5 % agarose gels by electrophoresis and visualized under UV light.

RESULTS

A total number of 450 Urine samples, from which 148 (32.88%) were found to contain heavy and appreciable bacterial growth (significant bacteriuria) while 302 (67.11%) had no appreciable bacterial growth. Their ages ranging from 15 to 70 years , 392 females and 58 males. The majority of *Escherichia coli* in UTI cases, 67 cases, were predisposed by adult women married. 25 cases of girls unmarried and 11 cases of children 15 year for both sex. The laboratory criterion for acute *E. coli* UTI was the presence of a positive culture response with at least 10^5 CFU of *E. coli* per ml of clean-voided urine. Table 1.

Analysis of Phylogenetic group's demonstrated that *E. coli* isolates presented into four phylogenetic groups: groups A, B1, B2, and D. The distribution of phylogroup B2 (57 isolates) was at high percentage as compared with phylogroup D (16 isolates), while B1and A groups distributed as (10, 9 isolates) respectively.

In the present study, 80.43% of the UTI isolates revelation the existence of type 1 fimbriae (MSHA). While only 43.47% of UTI *E.coli* isolates have P fimbriae (MRHA). Simple UTI and cystitis showed highly expression of type 1 fimbriae. detection of colony adhesion factor (CAFĐ) represented in 40 (43.47%) uropathogenic *E. coli*, while only 5 (5.43%)isolates were positive for colony adhesion factor (CAFIII) .

Adherence of *E. coli* isolates to uroepithelial cells were showed in all isolates 100%. Although, all isolates considered uropathogenic according to number of bacteria/cells, Table 2.

The dendrogram of the antibiogram profiles created by UPGMA showed the presence of high similarity in banding patterns of isolates among every group which was about 23% - 95%. All 92 local isolates under study design and distributed to Six major clusters (A-F) were identified with a

Table 1. Data of *Escherichia coli* UTI patients

Data	Variable
15-70 years	Age of patients
Gender of patients	392:58 Female : male
208 cases	Adult pregnant women UTI
92 cases	Adult women UTI
81cases	Girls UTI
58cases	Men UTI
11cases	Children UTI

Table 2. The adhesion factor and Percentage of colony adhesion factors pattern of *E. coli* clinical isolates:

Adhesion factor				Isolates
CAFIII	CAFII	CAFI	Adherence to uroepithelial cells	
5	40	74	92	Number of adhesion
5.43	43.47	80.43	100	Percentage % (N 92)

small dissimilarity between clusters depending on the type of multidrug resistance, in order to facilitate the distribution process. Table 3.

Phylogenetic groups A, B1, B2, and D, which showed different patteren in susceptibility to antibiotics. Multidrug resistance showed in all isolates. B2 is the most phylogenetic type showed wide range of resistant from 1 to 12 resistant to antibiotic of remaining strains.

In the present study, Out of 92 of *E. coli*, 14 isolates (15.21%) were detected as ESβLs producers and 30 isolates (32.60%) of them were AmpC β-lactamases producers by phenotypic method ESBL positive isolates were more resistant to all tested antibiotics except for imipenem compared to the non-ESBL isolates.

The local uropathogenic *E. coli* isolates distributed to three clusters depending on the type of Quinolones and Fluroquinolones resistance. The highest rate of multidrug resistance was observed with GroupC 26 (31.52%) isolates, in which this isolate was able to resist all Quinolones and Fluroquinolones antimicrobial agents.as well as Group B distinguished in 11(11.96%) isolates. While the lowest Quinolones and Fluroquinolones resistance was notice with Group A 55(59.78%) isolates in which sensitive for all these antimicrobial agents.

Table 3. Distribution of *E. coli* phylogenetic group according to antibiogram profiles dendrogram analysis

Phylogenetic group	Number of antibiotics	Name of isolates which resisted by isolates	Groups
B2	1	E_{70}	Group A
2-B2; 1-D	1-2	E_{63} E_{66} E_{72}	Group B B_1
B1	4	E_{79}	B_2
3-A ; 6-D;2-B1;16-B2	10-12	E_9 E_{48} E_{52} E_{17} E_{24} E_{30} E_{34} E_{32} E_{36} E_{42} E_{43} E_{44} E_{49} E_2 E_{75} E_4 E_6 E_{15} E_1 E_3 E_{21} E_{64} E_{67} E_{14} E_{41} E_{69} E_{89}	C_1
			Group C
2-A; 3-B2	8-9	E_{71} E_{73} E_{18} E_{84} E_{19}	C_2
2-A ; 2-D;1-B1; 6-B2	5-6	E_{37} E_{38} E_{39} E_{54} E_{16} E_{60}	D_1
		E_{80} E_{26} E_{37} E_{91} E_{92}	Group D
1-D; 2-B1; 6-B2	4-6	E_{31} E_{81} E_{82} E_{87} E_{90} E_{83} E_{45} E_{85} E_{86}	D_2
1-A ; 3-D; 4-B2	5-6	E_{11} E_{27} E_{65} E_{10} E_{56} E_{68} E_{74} E_{88}	D_3
1-D;1-B1;7-B2	6-7	E_{13} E_{33} E_{50} E_{58} E_{47} E_{22} E_{35} E_{76} E_{40}	D_4
B2	2	E_7	Group E E_1
1-A ; 2-D;3-B1;3-B2	3	E_8 E_{12} E_{28} E_{29} E_{51} E_{53} E_{55} E_{57} E_{61}	E_2
2-B2	5-Apr	E_{23} E_{62}	E_3
3-B2	3-Feb	E_{25} E_{46} E_{59}	E_4
3-B2	6	E_{20} E_{77} E_{78}	Group K

Isolates with multidrug resistance antibiotics was found it to be related with colony adhesion factor, 16 (17.39%) isolates have first colony adhesion factor (CAFI) resisted 5-6 antibiotics represented high rate of isolates harboring CAFI, while resistant to 12 antibiotics appear only in 3 isolates .Percentage %1.08 of isolates which resisted 12 antibiotics and have only one isolate have second colony adhesion factor (CAFII), while there was a clear rule in the production of moderate resistance 5-6 antibiotics pattern in 13 isolates with high percentage value 14.13% between CAFII isolates. In the same manner isolates have third colony adhesion factor (CAFIII), resist 5-6 antibiotics were predominant in percentage 22.8%. Table 4.

Collection of 92 uropathogenic *E. coli* isolates were screen for selectable adhesion virulence factor encoding *fim H , SfaDE* and *pap C* genes . Results showed that the prevalence of

Table 4. The percentage of correlation between colony adhesion factors and multidrug resistance pattern of all *E. coli* isolates

	N R	12	11	10	9	9	7	5-6	4-3	2-1
CAF I	NMDR	3	15	8	5	12	6	16	7	2
	%	3.26	16.3	8.69	5.43	13.04	6.52	17.39	7.6	2.17
CAFΠ	N R	12	11	10	9	9	7	5-6	4-3	2-1
	NMDR	1	5	1	3	9	1	13	6	1
	%	1.08	5.43	1.08	3.26	9.78	1.08	14.13	6.52	1.08
CAFIΠ	N R	12	11	10	9	9	7	5-6	4-3	2-1
	NMDR	2	8	5	2	10	6	21	7	4
	%	2.17	8.69	5.43	2.17	10.86	6.52	22.82	7.6	4.34

N R Number of antibiotics which resisted by isolates
NMDR Numbers of the multidrug resistance isolates and continue CAF %
Percentage of % N92 CAF

virulent genes occurred in 51 *pap*C (55.43%), 66 *fim H* (71.73%) and *SfaDE* it can detected in low occurrence 21(22.82) isolate of *E.coli* , *FimH* gene was the common occurrence virulence factor detected. Fiften isolate gave negative results for all adhesive virulence genes. Distributed based on the appearance of genes in in each isolate, all studied uropathogenic *E. coli* possess 8 patterns of virulence gene , indicate to as EG (Table 5).The pattern EG6 characterized by *fim H* was one of the most common patterns that had only one gene spread and was observed in 23 isolates. Among the isolates that owned *fim H* and *papC* gene, from where 39.39% % of isolates that was *fim* positive, represented by 26 isolates and which was predominate pattern (EG2).

Table 5. Pattern of Adhesive virulance gene in *E. coli*

No. of isolates	Adhesive virulence gene			
	sfaBC III	Fim H	pap C	Pattern
14	+	+	+	EG1
26	-	+	+	EG2
4	+	-	+	EG3
7	-	-	+	EG4
3	+	+	-	EG5
23	-	+	-	EG6
0	+	-	-	EG7
15	-	-	-	EG8
	21	66	51	Total

DISCUSSION

The study indicates that a higher ratio of UTI in females 392 than in males 58. In this regard [25] noted the incidence of UTI is higher in women compared with men across all age groups. It came in agreement with [26] which showed the majority of *Escherichia coli* in UTI cases in 92 females than in males 47 cases only.

The result of current study on the line with study by [27] that showed high parentage of UTI of pregnant woman, is due to physiological changes . While didn't agree with study in Iraq by [28] that reported was only 3.44% of pregnant woman

infected UTI for age between 21-25 years. More than 85% of UTIs caused by *E. coli*, according to [29].

Prevalence of phylogenetic group B2 followed by D among others, represented that the virulence isolates most of time indicated UTI. Many studies state that *E. coli* strains causing UTI mostly belong to phylogroups B2 and D rather than B1 and D [30].

From the study result, the absence of fimbriae or other surface adhesion systems effect on bacterial adhesion. Irreversible attachment happened by Fimbriae to the uroepithelial cell membrane. While recently reported, 74 isolates (80.43%) were able to express type 1fimbriae and another strains expressed type P but not type 1 fimbriae. Current study relatively agree with [26] that show (89%) of *E. coli* expression of P fimbriae. While it decreased to 17(63%) UTI isolates of *E. coli* expression of P fimbriae *in* Iraqi study by [32].

In the study observation, most P fimbriae positive isolates were so associated with simple UTI. in the same manner [33] showed there is no relation between the incident or symptoms severity and the site of infection and expression of fimbria in *E. coli* isolates from urine.

However (34) studies reported type 3 fimbriae expression mediated by conjugative plasmid in *E. coli* in .The results identified colony adhesion factor (CAFIII) rare in uropathogenic *E. coli* agree with the showed low rate of CAFIII in uropathogenic *E. coli* 3.2% .

The present study indicated the relation between site of infection (lower urinary tract infections) and expression of P fimbriae. It should be noted that many of the isolates under the study have become intrusive in the possession of adhesion factors and this may indicate that adhesion is a necessary and essential step to start the infection, especially in urinary tract infections[35]. Reported the number of bacteria adherent to uroepithelial cells used to differentiate between uropathogenic and fecal strains.

Dendrogram indicates division isolates depending on the number of antibiotics which resisted. Only 1 isolate were placed in clusters A. Cluster D contained the majority of the isolates. In this cluster some isolates were a located in groups of higher or lower similarity and most strains were discriminated. The isolate no. 70 showed low percentage of similarity in the dendrogram which was only 23%. This low percentage of similarity , probably as a result of the variation of the isolates source (patient's gender and age group). The cluster A contains only one isolate distinguished by being sensitive to all antibiotic and only resist to Gentamycin , in the other hand showed possession positive band in virulence gene detection only for *fim* H ,while showed positive result in phenotype detection for adherence to uropathogenic cells and for CAFI and CAFII. The cluster B at a linkage distance of 54 units can be grouped into 2 sub clusters B_1 and B_2. All 4 isolates in these groups are isolated from pregnant women and distinguish by sensitivity to all Fluroquinolones drugs, whereas variable in the virulence recipes. The cluster C consisted of only 26 isolates which differed from other cluster by having high resistance to 10-12 antibiotics. The cluster D which is the bigger one from other cluster can be grouped into 5 sub clusters. All the isolates in this cluster have moderate multi drug resistance. The cluster E consisted of only 16 isolates which having low resistance to 2-5 antibiotics. The cluster F consisted of three isolates

The results of the study showed that there is a correlation between the nature of resistance to antibiotics and the ability of bacteria to produce the virulence factors in uropathogenic *E. coli*. Actually, when study assessment the antibiograms of *E. coli* strains having virulence factor.

The antibiotic resistance rates generally increased over time and the last set of isolates collected were more resistant to antibiotics, as noted in a group of β-lactam antibiotics such as Amoxicillin and a group of Quinolones antibiotics such as Ciprofloxacin the isolates collected were more resistant reach to 100% and 38% respectively. A high concern to the limited treatment options of multidrug-resistant clinical isolates between ESBL producing *E. coli* [36].

Non-detection of certain phylogenetic group that it is resistance to antibiotics in this study , but the important noticed that phylogenetic group B2 were normally distribution in all antibiotic resistance pattern , it may indicated Phytogenic and multidrug resistance types remained largely unchanged within patients in this study.

ESBL harboring *E. coli* isolates have been found to be resistant to other antibiotics,

especially, Fluroquinolones. Resistance to ciprofloxacin has also been observed in ESBL producing *E. coli* compared to non-ESBL isolates [37]. Many reason for this coexistence of resistance to β-lactam and Quinolones, one possible explanation suggested by [38] is that the bacteria are able to acquire the ability to produce ESBLs can be selected by intensive Quinolones use. Moreover, the presence of Quinolones resistance on plasmids is concerning. Carrying both ESBLs enzymes and Quinolones resistance genes no such plasmid could speed the development and speared of multidrug resistance phenotype [39].

Ciprofloxacin and Nalidixic acid resistance was observed among quinolones group associated with ESBL producer isolates. ESBL and quinolone resistance genes are usually carried on mobile genetic elements [40].

The results indicated that when the resistance to antimicrobial agent rate is relatively low, there is an increase in productivity of adhesion factors gradually. And clearly the theory of genetic background, that there is an inverse relationship between resistance to antibiotics and the efficacy of isolates for virulence factors, which was evident in this study. Some research replied that ability of resistance may be coordinate with the virulence factors loss in *E. coli* isolates [41]. While [42] who observing association between highly virulent and multiresistant *E. coli* isolates . Finally [26] find a relationship between UPEC reduced virulence and multidrug resistance.

To indicate theory of genetic background and relationship between resistance to antibiotics and the efficacy of isolates for virulence factors, some selectable adhesion genes was studded to determine the correctness of the theory obtained from the results of phenotypic characteristics out of the adhesion virulence genes, *fim H* was the most spread gene and was positive in 66 (71.73%) isolates, followed by *papC* gene was present in 5 isolates among total 92 isolates. this results agree with [43] whom found that *fim* gene was the most widespread detected in 95% of the *E. coli* uropathogenic isolates. Next, pap gene was identified in 57% isolates .This result is not in line with the result of Firoozeh *et al*. (2014) in which they reported *pap* was the most spread gene and was identified in 25 (16.7%) isolates. In another study conducted by [26] they found that *papC* gene which was predominant followed by *Fim H*. The other isolates exhibited distinct diversity of gene patterns. From total of 92 local isolates 14 isolates (15.21%) own three different adhesive studied genes. *afa* gene were negative in isolates, indicating *afa* alone is infrequent between uropathogenic isolates. This result was in agreement with the study of [44] which recorded that *afa* gene not found in there strain. The results indicated that *afa* sequence is may be related with horizontal gene transfer, this agreement to [45] the referred to the *afa* gene clusters encode a fimbrial adhesions (Afas) that are expressed by uropathogenic and diarrhea-associated *E. coli* strains .But the low appearance of *afa* sequence gene is depending on acquired the gene by horizontal transmission [46].

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