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Virulence Profile, Fluoroquinolone and Quinolone Resistance of Uropathogenic *Escherichia coli* Isolates Recovered from Thabet Hospital-Tulkarm, Palestine

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GA, KA and BI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript and managed literature searches. Authors GA and BI managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Backcground: *Escherichia coli* (*E. coli*) is the most common cause of urinary tract infection (UTI). Virulence factors are mainly responsible for the severity of these emerging infections.
Aims: To analyze virulence factors and resistance phenotype to quinolones and fluoroquinolones in a collection of *E. coli* strains isolated from UTIs with previously known phylogenetic groups.
Place and Duration of Study: Department of Biology and Biotechnology, An-Najah N. University, Palestine, during May-December 2012.
Methodology: Fifty clinical *E. coli* isolates were previously recovered from urine specimens obtained from patients suffered from urinary tract infections at Thabet Hospital, Tulkarm-Palestine. Multiplex PCR technique was used to detect the presence of 18 virulence genes and ERIC-PCR

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was used to detect the heterogeneity of these strains. All *E. coli* isolates were examined for resistance to different antibiotics using disk diffusion method.
Results: It is found that the prevalence of virulence genes ranged from 0% for P-fimbria adhesion variant 1 (*pap G I*) allele and α- hemolysin (*hly A*) to 86% for Type1 fimbriae adhesion (f*im H)* and Serum resistance-associated gene (*tra T*) in strains tested. The results showed that the mean virulence score for group D was 8.2 and ranged from 2 to15, while for group A was 6.2 and ranged from 1 to14 (P=6.2 x 10^{-4}). The mean virulence score for strains resistant to fluoroquinolones and /or quinolones was 7.3, while for strains sensitive to both fluoroquinolones and quinolones was 8.1. Quinolones and/or fluoquinolones sensitive strains related to group D showed an increased prevalence of catecholate siderophore receptor (*iron*) than resistant strains. It was also found that *traT* gene was the most common prevalence among strains resistant to nalidixic acid, fluoroquinolones and trimethoprim/sulphamethoxazole and it was 90.1% (30/33), 95.8 (23/24) and 90.6% (29/32), respectively. ERIC-PCR revealed that the 50 isolates were genetically diverse and comprised a heterogeneous population with at total 10 ERIC-PCR profiles at a 50% similarity level.
Conclusion: The molecular analysis of strains belonged to groups A and D, results showed that group D had higher mean virulence score than group A. It seems that there is no single virulence factor or virulence profile that is entirely specific to UTI in general.

Keywords: Virulence genes; UPEC; group D; group A; fluoroquinolones; quinolones; UTI.

1. INTRODUCTION

Escherichia coli (*E. coli*) is considered as a major cause of urinary tract infection (UTI) and it accounts for approximately 70 to 95% of community-acquired cases and 50% of all hospital-acquired infections [1]. *E. coli* is responsible for significant social and economic costs for both communities and public health resources and is associated with significant mortality [2].

Uropathogenic *E. coli* (UPEC) strains associated with a variety of virulence factors that allow their successful transition from the gastrointestinal tract to the urinary system, facilitate colonization of host epithelia surfaces, overcome host immune defenses and establish infection. These virulence genes are transmitted vertically within the resulting virulent clones or sometimes can be transmitted horizontally to other lineages as part of pathogenicity-associated islands (*PAIs*) [3]. Determination the prevalence of certain virulence factors in UPEC strains, suggests that they might make useful targets for vaccines and other protective interventions [4].

It was shown that strains of *E. coli* belonged to groups B2 and D often carry virulence genes that are lacking in other groups or showed highest presence of virulence genes [3,5-8]. In regard to the association of antimicrobial resistance genes and virulence factors, there are controversial reports [9-12]. A lower prevalence of B2 group was detected in resistant than in fluoroquinolones-susceptible UPEC strains and the combination of certain virulence determinants with group B2 and with susceptible isolates was confirmed [10,13-17].

Enterobacterial repetitive intergenic consensus (ERIC) PCR is a PCR-fingerprinting technique but it is not arbitrary. The ERIC sequences are present in many copies in the genomes of different *Enterobacteriaceae*. ERIC elements are highly conserved at the nucleotide level, their positions in enterobacterial genomes varies between different species and has been used as a genetic marker to characterize isolates within a bacterial species. In ERIC-PCR a band pattern is obtained by amplification of genomic DNA located between successive repetitive ERIC elements or between ERIC elements and other repetitive DNA sequences for subtyping different Gram-negative enteric bacteria [18].

The relationship among virulence factors of *E. coli*, phylogenetic background and quinolones and fluoroquinolones is a complex phenomenon. To clarify whether the lack virulence factors is directly associated with quinolones and fluoroquinolones resistance or, instead, depends on a phylogenetic distribution, our study aimed to analyze virulence factors and resistance phenotype to quinolones and fluoroquinolones in a collection of 50 *E. coli* strains isolated from UTIs with previously known phylogenetic groups [19], This study has not been investigated previously in Palestine.

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification

Fifty clinical isolates of *E. coli* were previously recovered from urine specimens obtained from patients suffered from urinary tract infections at Thabet Hospital, Tulkarm-Palestine, during May-December 2012. These isolates were identified in laboratory of Thabet Hospital and also were confirmed in Microbiology laboratories at An-Najah National University-Nablus, Palestine. These isolates were cultured on MacConkey and/or EMB agars, Gram stain, motility test and biochemical tests such as IMViC tests, H_2S production were used [19].

2.2 Phylogenetic Groups and Antibiotic Resistance

Antimicrobial susceptibility and phylogenetic groups for these strains were detected previously [19]. Strains were assigned to one of the four *E. coli* phylogenetic groups (A, B1, B2 and D) using a triplex PCR based on the presence or absence of *chuA*, *yjaA* genes and TSPE4.C2 fragment [20]. Antimicrobial susceptibility was determined according to the Clinical and Laboratory Standard Institute (CLSI) using the disk diffusion method [21]. All *E. coli* isolates were examined for resistance to tetracycline (30µg), streptomycin (10µg), gentamicin (10µg), kanamycin (30µg), nalidixic acid (30µg), norfloxacin (10µg), ciprofloxacin (10µg), ofloxacin (5µg), Levofloxacin (5µg), ceftriaxone (30µg), ceftazidime (30µg), cefazolin (30 µg), Trimethoprim/Sulfamethoxazole (1.25/23.75µg) and erythromycin (15 µg). All used antibiotics were purchased from Oxoid. Zones of inhibition were determined in accordance with procedures of the Clinical and Laboratory Standard Institute [21].

2.3 DNA Extraction

E. coli DNA was prepared for PCR according to the method described previously [22]. Briefly, cells were scraped off an overnight nutrient agar plate with a sterile loop, washed with 1 ml of 1X Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]), then the pellet was resuspended in 0.5 ml of sterile distilled H_2O, and boiled for 10-15 min. The cells then were incubated on ice for 10 min. The debris was pelleted by centrifugation at 11,500 X *g* for 5 min. DNA concentration was determined using a spectrophotometer and the samples were stored at -20ºC until use for further DNA analysis.

2.4 ERIC PCR Assay

Enterobacterial repetitive intergenic consensus (ERIC) PCR was performed using Primer ERIC1 and Primer ERIC2 which described previously [23]. Each PCR reaction mix (25 µL) was performed using 12.5 µL of PCR premix (ReadyMix^{TM} Taq PCR Reaction Mix with $MgCl_2$, Sigma), 0.6 µM of each primer, 3 µL DNA template, concentration of dNTPs was modified to 0.35 mM , $MgCl_2$ to 2.5 mM and Taq DNA polymerase to 1.5 U. DNA amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf) according to the following thermal conditions: initial denaturation for 2 min at 94ºC was followed by 30 cycles of denaturation at 94ºC for 50 s, annealing at 50ºC for 40 s and extension at 72ºC for 1 min, with a final extension step at 72ºC for 5 min. The PCR products were analyzed by electrophoresis on 1.5% agarose gel. The gel images were scored using binary scoring system that recorded the presence and absence of bands as 1 and 0, respectively. A binary matrix was analyzed by the unweighted pair group method for arithmetic averages (UPGMA), using SPSS statistics software version 20 (IBM).

2.5 Virulence Factors

The presence of 18 virulence genes was investigated using multiplex PCR divided into seven pools and their amplicon sizes are listed in Table 1 [24]. The genes detected were f*imH*, *traT*, *iron*, *hly*, afimbrial adhesin (*afa*), S/F1 C fimbriae (*sfa/foc*), P fimbriae (*papG* three alleles), cytotoxic necrotizing factor 1 (*cnf 1*), secreted autotranspoter toxin (*sat*), aerobactin (*iutA)*, yersiniabactin receptor for ferric yersiniabactin uptake (*fyuA*), iron-regulated gene A homologue adhesin (*iha*), group 2 capsule (*kpsMTII*), outer membrane protease T (*ompT*) , pathogenic island marker (*malX*) and uropathogenic specific protein (*usp*). Each PCR reaction mix (25 µL) was performed using 12.5 µL of PCR premix with $MgCl_2$ (ReadyMix^{TM} Taq PCR Reaction Mix with $MgCl_2$, Sigma), 0.4 µM of each primer and 3 µL DNA template. DNA amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following thermal conditions: initial denaturation for 4 min at 94ºC followed by 25 cycles of denaturation at 94ºC for 30 s, annealing at 60ºC for 30 s and extension at 72ºC for 1 min, ending with a final

extension step at 72ºC for 5 min. The PCR products (10 µL) were analyzed by electrophoresis on 2% agarose gel. The sizes of the amplicons were determined by comparing them with a 100-bp DNA ladder. The virulence factors were scored using binary scoring system that recorded the presence and absence of the virulence factor as 1 and 0, respectively. A binary matrix for virulence factors was analyzed by the UPGMA, using SPSS statistics software version 20 (IBM).

2.6 Statistical Analysis:

The virulence scores were calculated for each isolate as the sum of all virulence factors in each strain [13]. Comparisons of proportions were tested using the Chi^2 (χ^2) test or Fisher exact test. Comparisons of mean virulence scores were done by using the Mann-Whitney U-test. $P<0.05$ values were considered statistically significant. Statistical analyses were performed with SPSS statistics software version 20 (IBM).

3. RESULTS

Detection of virulence genes coding for different adhesions, toxins, iron uptake system factors, cell protection factors, *malX* (a marker for a pathogenicity-associated island (PAI) from archetypal ExPEC strain CFT073) and *usp* (uropathogenic specific protein) was carried out by multiplex PCR. The prevalence of virulence genes ranged from 0% for *papG allele I* and *hlyA* to 86% for f*imH* and *traT* in strains tested. Virulence genes *iha*, *kpsMTII*, *usp* and *fyuA* had the following prevalence 74%, 64%, 64%, 58% and 52%, respectively (Table 2). Distribution of *fimH* gene was 86.1% and 84.6% in group D and A, respectively. The prevalence of genes tested in each strain was ranged from 5.6% to 83.3%. The mean virulence score for group D was 8.2 and ranged from 2 to15, while for group A was 6.2 and ranged from 1 to14. There was statistically significant difference between the mean virulence scores of these groups at $P=6.2 \times 10^{-4}$. Also it was found that 69.2% (9/13) of strains belonged to group A carried 6 or less virulence factors, while 66.7% (24/36) of strains belonged to group D carried 7 or more virulence factors. Our results showed that mean virulence score for strains resistant to fluoroquinolones and/or quinolones was 7.3, while for strains sensitive to both fluoroquinolones and quinolones was 8.1. Quinolones and/or fluoquinolones sensitive strains belonged to group D showed an increase in prevalence of *iroN* virulence gene than resistant strains belonged to the same group. In group A, average of prevalence of *papGII* was increased and *fimH* decreased in quinolones and fluoquinolones sensitive strains. Sensitive strains to quinolones and fluoquinolones showed an increase in prevalence of *ompT* virulence gene (Table 3). Cluster analysis of virulence factors showed that 3 large clusters depend on the presence/absence of *cnfI* and *afa/dra* genes, but clustering is independent into phylogenetic groups (Fig. 1). In addition, it was found that *traT* gene was the most common prevalence among strains resistant to Nalidixic acid, fluoroquinolones and Trimethoprim/Sulphamethoxazole and it was 90.1% (30/33), 95.8 (23/24) and 90.6% (29/32), respectively. ERIC-PCR analysis revealed that these 50 isolates were genetically diverse and comprised a heterogeneous population with at total 10 ERIC-PCR profiles at a 50% similarity level (Fig. 2) and there was no specific correlation between ERIC-PCR profiles, virulence factor profiles and phylogenetic groups.

4. DISCUSSION

According to human, commensal strains usually derive from phylogenetic groups A and B1, while most of extraintestinal pathogenic *E. coli* (ExPEC) strains usually belong to the B2 and D groups and harbor large number of various virulence factors, which allow them to induce diseases in both healthy and compromised hosts. In human, ExPEC were currently defined as *E. coli* isolates containing two or more of the following genes: *papA*, and/or *papC*, *sfa/foc*, *afa/dra*, *kpsMT II* and *iutA* [25].

Among the 18 virulence genes studied, *fimH* and *traT* genes were the most frequently detected. *fimH* gene was almost equally distributed in isolates of phylogenetic groups A and D. The high prevalence of *fimH* gene in UPEC has been reported [24,26-29]. This was explained by the playing role of *fimH* adhesin encoded by *fimH* gene in the initiation of colonization and infection. In this research, it was found that the 63% of strains carried *usp* gene. This result is consistent with other report [30], which showed that 72.48% of UPEC carried this gene. Results of this study showed that all strains were *hlyA* negative which were in contrast to other reports which identified this gene in 34%-37% of UPEC strains [8,31].

Table 1. Virulence gene primers [20]

Pool	Virulence gene	Primer forward/reverse	Forward sequence 5′→3′	Reverse sequence 5′→3′	Product size (bp)
Pool I	*fimH*	FimHf/FimHr	TGCAGAACGGATAAGCCGTGG	GCAGTCACCTGCCCTCCGGTA	508
	malX	malXf/malXr	GGACATCCTGTTACAGCGCGCA	TCGCCACCAATCACAGCCGAAC	930
Pool II	*sfa/foc*	sfa1/sfa2	CTCCGGAGAACTGGGTGCATCTTAC	CGGAGGAGTAATTACAAACCTGGCA	410
	iutA	AerJf/AerJr	GGCTGGACATCATGGGAACTGG	CGTCGGGAACGGGTAGAATCG	300
	papGIII	allele IIIf/allele IIIr	GGCCTGCAATGGATTTACCTGG	CCACCAAATGACCATGCCAGAC	258
	fyuA	yuaf/fyuar	TGATTAACCCCGCGACGGGAA	CGCAGTAGGCACGATGTTGTA	880
Pool III	*hlyA*	hlyf/hlyr	AACAAGGATAAGCACTGTTCTGGCT’	ACCATATAAGCGGTCATTCCCGTCA	1117
	KpsT II	kpsIIf/kpsIIr	GCGCATTTGCTGATACTGTTG	CATCCAGACGATAAGCATGAGCA	272
	papGI	allele If/allele Ir	TCGTGCTCAGGTCCGGAATTT	TGGCATCCCCCAACATTATCG	461
Pool IV	*traT*	TraTf/TraTr	GGTGTGGTGCGATGAGCACAG	CACGGTTCAGCCATCCCTGAG	290
	papGII	allelele If/allele IIr	GGGATGAGCGGGCCTTTGAT	CGGGCCCCCAAGTAACTCG	190
Pool V	*afa/dra*	Afaf/Afar	GGCAGAGGGCCGGCAACAGGC	CCCGTAACGCGCCAGCATCTC	559
	cnf1	cnf1/cnf2	AAGATGGAGTTTCCTATGCAGGAG	CATTCAGAGTCCTGCCCTCATTATT	498
Pool VI	*Iha*	IHAf/IHAr	CTGGCGGAGGCTCTGAGATCA	TCCTTAAGCTCCCGCGGCTGA	827
	usp	USP81f/USP695r	CGGCTCTTACATCGGTGCGTTG	GACATATCCAGCCAGCGAGTTC	615
Pool VII	*ompT*	ompTf/ompTr	ATCTAGCCGAAGAAGGAGGC	CCCGGGTCATAGTGTTCATC	559
	sat	sat1/sat2	ACTGGCGGACTCATGCTGT	AACCCTGTAAGAAGACTGAGC0	387
	iroN	IRONECf/IRONECr	AAGTCAAAGCAGGGGTTGCCCG	GACGCCGACATTAAGACGCAG	665

Table 2. Virulence genes, Virulence scores, prevalence of virulence genes and their distribution according the phylogenetic groups A and D

Virulence genes		Prevalence		Distribution of virulence factors according to phylogenetic group [a]		Virulence score (Mean)	
		No. of isolates	(%)	D (n=36)	A (n=13)	D 8.2 (294/36)	A 6.2 (80/13) *** [a]
Adhesin genes	*papGI*	0	0	0.0% (0/36)	0.0% (0/13)	0.0 (0/36)	0.0 (0/13)
	papGII	14	28	33.3% (12/36)	15.4% (2/13)	3.6 (128/36)	1.5 (19/13) **
	papGIII	2	4	5.6% (2/36)	0.0% (0/13)	0.3 (11/36)	0.0 (0/13)
	sfa/foc	20	40	41.7% (15/36)	30.3% (4/13)	4.1 (145/36)	2.8 (36/13)
	afa/dra	19	38	44.4% (16/36)	23.1% (3/13)	4.7 (168/36)	2.5 (33/13) *
	fimH	43	86	86.1% (31/36)	84.6% (11/13)	7.5 (269/36)	5.7 (74/13)
Toxin genes	*hlyA*	0	0	0.0% (0/36)	0.0% (0/13)	0.0 (0/36)	0.0 (0/13)
	cnfI	19	38	44.4% (16/36)	23.1% (3/13)	4.6 (166/36)	2.5 (33/13) *
	Sat	21	42	50% (18/36)	23.1% (3/13)	5 (179/36)	2.2 (28/13) ***
Iron uptake system genes	*fyuA*	26	52	58.3% (21/36)	38.5% (5/13)	5.5 (192/36)	3.2 (41/13) *
	utaA	37	74	75% (27/36)	69.2% (10/13)	6.9 (247/36)	4.9 (64/13)
	iroN	20	40	41.7% (15/36)	38.5% (5/13)	4.1 (145/36)	3 (39/13)
	Iha	32	64	61.1% (22/36)	76.9% (10/13)	6.2 (217/36)	5.3 (69/13) **
Cell protection genes	*kpsMTII*	32	64	72.2% (26/36)	46.2% (6/13)	6.6 (235/36)	3.2 (41/13) **
	traT	43	86	94.4% (34/36)	69.2% (9/13)	7.9 (282/36)	4.9 (64/13) ***
	ompT	15	30	33.3% (12/36)	23.1% (3/13)	3.3 (118/36)	2.3 (30/13)
Other genes	*MalX*	4	8	11.1% (4/36)	0.0% (0/13)	1.3 (46/36)	0.0 (0/13) ***
	Usp	29	58	63.9% (23/36)	46.2% (6/13)	6.3 (225/36)	3.8 (49/13) **

[a] *One isolate of group B1 is not included in distribution and virulence score mean*

* *significant difference at P<0.05,* ** *significant difference at P<0.01,* *** *significant difference at value P<0.001*

Table 3. Distribution of phylogenetic groups A and D and virulence genes among 49 *E. coli* isolates from UTIs according to fluoroquinolones and quinolones and resistance phenotypes

Virulence gene	No. (%) of *E. coli* isolates[a]					
	Phylogenetic group D n (%)		Phylogenetic group A n (%)		Phylogenetic groups D and A n (%)	Phylogenetic groups D and A n (%)
	resistant (n=27)	Susceptible (n=9)	resistant (n=8)	susceptible (n=5)	resistant (n=35)	susceptible (n=14)
papGI	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
papGII	8 (29.6)	4 (44.4)	0 (0.0)	2 (40.0)	8 (22.9)	6 (42.9)
papGIII	1 (3.7)	1 (11.0)	0 (0.0)	0 (0.0)	1 (2.9)	1 (7.1)
sfa/foc	10 (37.0)	5 (55.6)	2 (25.0)	2 (40.0)	12 (34.3)	7 (50.0)
afa/dra	11 (40.7)	5 (55.6)	2 (25.0)	1 (20.0)	13 (37.1)	6 (42.9)
fimH	22 (81.5)	9 (100.0)	8 (100.0)	3 (60.0)	30 (85.3)	12 (85.7)
hlyA	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
cnfI	11 (40.7)	5 (55.6)	2 (25.0)	1 (20.0)	13 (37.1)	6 (42.9)
Sat	14 (51.9)	4 (44.4)	1 (12.5)	2 (40.0)	15 (42.9)	6 (42.9)
fyuA	17 (63.0)	4 (44.4)	3 (37.5)	2 (40.0)	20 (57.1)	6 (42.9)
utaA	21 (77.8)	6 (66.7)	6 (75.0)	4 (80.0)	27 (77.1)	10(71.4)
iroN	9 (33.3)	6 (66.7)	3 (37.5)	2 (40.0)	12 (34.3)	8 (57.1)
Iha	17 (63.0)	5 (55.6)	6 (75.0)	4 (80.0)	23 (65.7)	9 (64.3)
kpsMTII	19 (70.4)	7 (77.8)	4 (50.0)	2 (40.0)	23 (65.7)	9 (64.3)
traT	26 (96.3)	8 (88.9)	6 (75.0)	3 (60.0)	32 (91.4)	11 (78.6)
ompT	7 (26.0)	5 (55.6)	1 (12.5)	2 (40.0)	8 (22.9)	7 (50.0)
MalX	2 (7.4)	0 (0.0)	0 (0.0)	0 (0.0)	2 (5.7)	0 (0.0)
Usp	17 (63.0)	6 (66.7)	4 (50.0)	2 (40.0)	21 (60.0)	8 (57.1)

[a]*All strains were susceptible to fluoroquinolones and quinolones or resistant to fluoroquinolones and/or quinolones*

UTI: Urinary tract infection

hlyA gene plays a major role in the damage of uroepithelium and all *hlyA* gene positive isolates hemolysed human erythrocytes [8]. Other frequently detected toxin genes were *cnfI* (38%) and *sat* (42%). These results indicate that *sat*, a vacuolating cytotoxin expressed by UPEC elicits defined damage to kidney epithelium during upper UTI [32]. It was proposed that *cnfI* production increases the capacity of UPEC strains to resist killing by neutrophils, which in turn permits these bacteria to infect deeper tissue and persist better in the lower urinary tract [33]. Both *IroN* and *iha* genes were reported to be putative uropathogenic virulence genes and were frequently associated with UPEC [34,35].

Association of other virulence factors, such as *sfa/foc*, *iutA*, *fyuA*, *ompT* and *traT* with UPEC strains has been reported previously [4,28,36]. Of these virulence factors, *OmpT* is considered as a strong predictor of bacteremia [37]. The product of *ompT* gene contributes to virulence by inactivating host defense proteins, cleaving host cell-surface peptide to expose them or create receptors for the pathogen and protamine inactivation [37,38].

No single virulence factor nor virulence profile that is entirely specific to UTI in general, but various combinations of these virulence factors have been found in isolated cultures of UPEC as the cause of pathogenicity [24,27,35]. This observation therefore, suggests synergistic action of virulence factors as the mechanism of UPEC invading host defense system to cause disease. Variation in the distribution of these virulence factors in isolated UPEC has been attributed to geographical differences, strain types, populations sampled and differences in association with host characteristics [28,35,39].

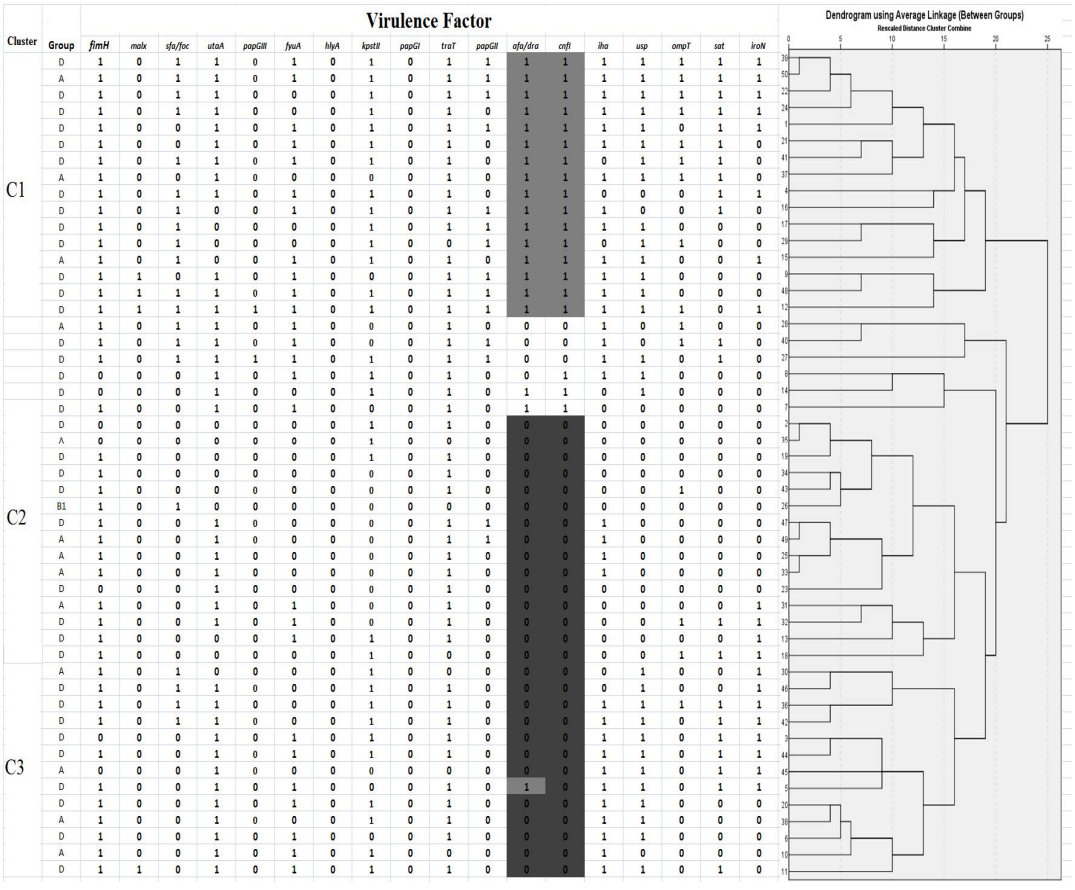

Fig. 1. Dendrogram of 50 uropathogenic *E. coli* strains isolated from urine samples based on the UPGMA method derived from analysis of the virulence factors and phylogenetic groups. C: Cluster; 1. presence of virulence factor and 0: absence of virulence factor

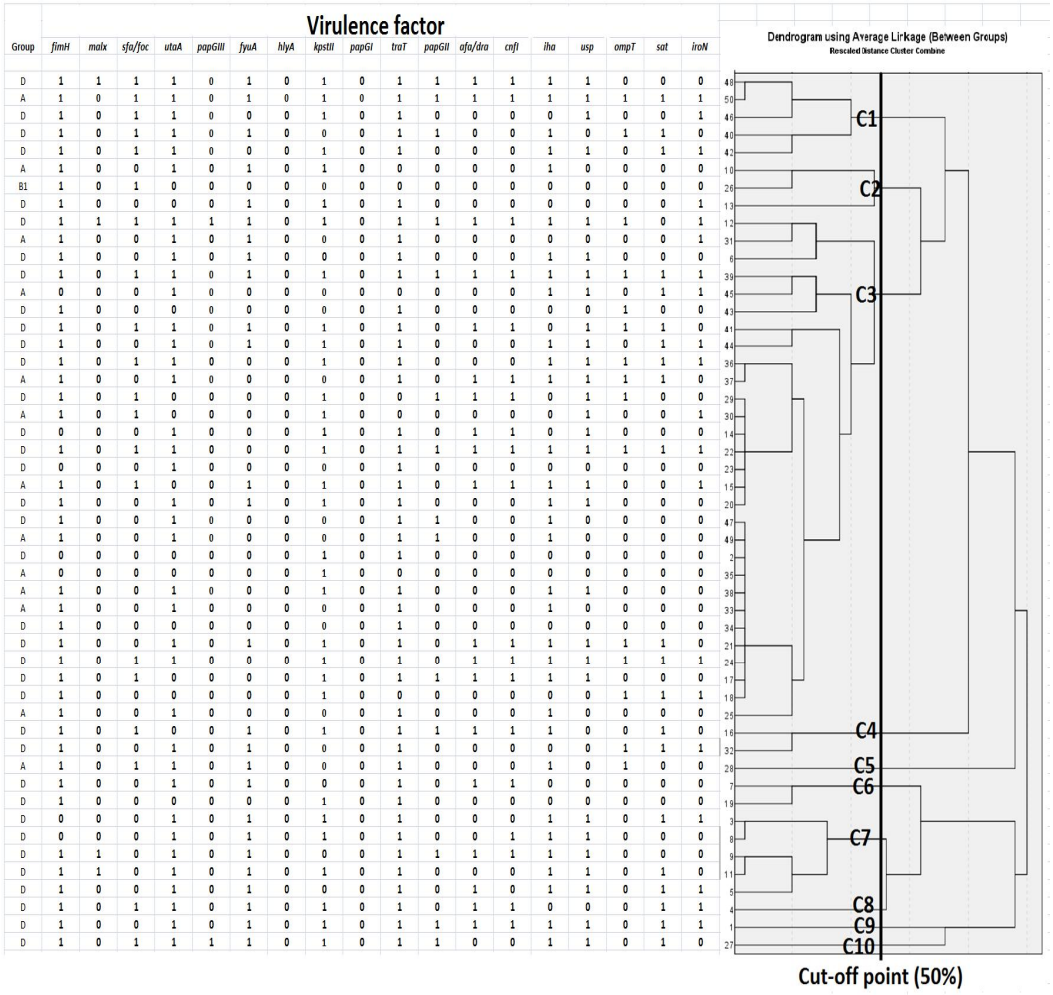

Fig. 2. Dendrogram of 50 uropathogenic *E. coli* isolates based on the UPGMA method derived from analysis of the ERIC-PCR-profiles, virulence factors and phylogenetic groups. C: Cluster; 1. presence of virulence factor and 0: absence of virulence factor

Results of current study are in agreement with other previous studies where they were found that virulent strains of *E. coli* mainly belong to phylogenetic group D were as more virulent than those belonged to phylogenetic group A. It was found that virulent strains of *E. coli* mainly belong to phylogenetic group B2 and D, where as less virulent of *E. coli* belong to phylogenetic groups B1 and A [40,41]. Other studies concerning virulence factors in UPEC [4,36,42], showed that *E. coli* strains belonging to phylogenetic group B2 carried a greater number of virulence factors than *E. coli* belonging to a non-B2 phylogenetic group. As reported in other studies, the differences observed between urinary tract syndromes were reflected in virulence scores, which were lowest among cystitis and highest among pyelonephritis isolates [4,13].

Data of current study about virulence scores showed that no significant difference between uropathogenic strains resistant to fluoroquinolones and/or quinolones and strains sensitive to both fluoroquinolones and quinolones. These results were in contrast to data reported previously [9,14,17], which suggested that quinolone-resistant ExPEC are less able to cause upper UTI and have fewer virulence factors than quinolone-susceptible *E. coli*. In addition, these results were not in agreement with other results [24], which showed that phylogenetic group B2 strains isolated from patients suffered from pyelonephritis exhibited high virulence scores and were associated with susceptibility to fluoroquinolones. This study demonstrated a relationship between

phylogenetic origin and high prevalence of virulence factors among strains belonged to phylogenetic group D. These results were in contrast to other studies [14,43], which suggested that quinolone resistance may be directly associated with virulence loss.

Many studies on this subject have been carried out in various parts of the world; such data is not available from Palestine where UTIs are very common diseases. This study was designed to analyze virulence factors and resistance phenotype to quinolones and fluoroquinolones in a collection of *E. coli* strains isolated from UTIs with previously known phylogenetic groups. Although our study is comprised of a relatively small number of samples and therefore faces limitations in statistical analysis, it provides an important epidemiological information about the virulence genes of UPEC isolated from Northern Palestine.

5. CONCLUSION

The molecular analysis of 50 *E. coli* urine isolates exhibited that strains of phylogenetic group D had higher virulence scores than group A. In addition, Data of current study about virulence scores showed that no significant difference between uropathogenic strains resistant to fluoroquinolones and/or quinolones and strains sensitive to both fluoroquinolones and quinolones. Further analysis of virulence factor profiles with regard to specific clinical symptoms and defined severity is recommended.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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