Journal of King Saud University – Science 31 (2019) 648–652

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Uropathogenic *Escherichia coli* (UPEC) in Jordan: Prevalence of urovirulence genes and antibiotic resistance

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ARTICLE INFO

Article history:
Received 3 December 2017
Accepted 20 March 2018
Available online 21 March 2018

Keywords:
Escherichia coli
UPEC
Urovirulence genes
Urinary tract infections
Antimicrobial agent resistance

ABSTRACT

This study examined the prevalence of urovirulence genes in Uropathogenic *Escherichia coli* (UPEC) isolates obtained from Jordanian patients. In addition, antibiotic susceptibility profile of the isolates was also examined.

Isolates (n = 227) were subjected to PCR detection of *vat, fyuA, chuA, yfcV, sivH, shiA, sisA, sisB* and *eco274* genes. The UPEC virulence genes prevalence rates were *shiA* (92%), *sisA* (72%), *eco274* (44%), *sivH* (36%), *vat* (27%), *yfcV* (25%), *sisB* (25%), *chuA* (20%), and *fyuA* (18%). Approximately half and 82% of the isolates produced extended-spectrum beta-lactamases (ESBL) and were resistant to three or more antimicrobial classes, respectively. Among the isolates, highest resistance was for augmentin (83%) and nalidixic acid (78%). Modest resistance was for cefoxitin (21%) and cefixime (20%). Low resistance was for norfloxacin (5%), amikacin (3%), and ertapenem (0.4%).

In conclusion, The UPEC virulence genes *shiA* and *sisA* are highly prevalent among the isolates. In addition, high resistance of UPEC to augmentin and nalidixic acid was reported. This may help in elucidating UPEC pathogenesis, and facilitate better treatment strategies for urinary tract infection patients.

1. Introduction

Urinary tract infections (UTIs) frequently occur in humans. The uropathogenic *Escherichia coli* (UPEC) is significantly associated with etiologies of UTIs, including pyelonephritis and cystitis (Hannan et al., 2012). UPEC has many virulence factors that assist its colonization, invasion, and survival within the host urinary system (Behzadi et al., 2016; Bien et al., 2012; Mao et al., 2012). These factors include adhesins, siderophores, toxins, capsule production, and proteases. In addition, several autotransporter (AT) proteins, which are typically associated with phylogeny (Zude et al., 2014), are correlated with virulence and have been identified in UPEC (Restieri et al., 2007; Zalewska-Piatek et al., 2015). It is believed that AT proteins have structural features that permit their pass through biological membrane systems (Allsopp et al., 2010), in addition to adhesion, invasion, and biofilm formation during pathogenesis (Allsopp et al., 2012, 2010). Among the AT proteins is the serine protease toxin (vat), which is encoded by *vat*, and that is found in both UPEC and avian pathogenic *Escherichia coli* (APEC) isolates (Rossiter et al., 2015; Zhuge et al., 2013). *FyuA* encoding for a heme binding protein called yersiniabactin receptor, and *yfcV* encoding for one of the chaperone-usher fimbriae subunits that are associated with UPEC (Spurbeck et al., 2012). UPEC *chuA* gene is also encodes a heme binding protein that mediates heme uptake and transport an plays a role in UTIs (Torres et al., 2001).

The products of *shiA, sisA* and *sisB,* are involved in the pathogenesis of UPEC by down-regulating the innate inflammatory response during infection (Lloyd et al., 2009b; Mao et al., 2012). On the other hand, products of *eco274* and *sivH*, only demonstrated a correlation with UTIs, but their roles in pathogenesis remain undefined. (Cusumano et al., 2010; Lloyd et al., 2009a).

While *E. coli* archetypal UPEC virulence genes (*papG*, *cnfI*, *hlyA*, *iroN*, and *usp*) have been extensively studied (Farshad et al., 2012; Jahandeh et al., 2015), several non-archetypal UPEC virulence genes (*vat, fyuA, chuA, yfcV, sivH, shiA, sisA, sisB* and *eco274*) need further investigation among clinical isolates.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2018.03.009
1018-3647/

In the present study, urinary *E. coli* isolates from Jordan were characterized by molecular techniques to identify the incidence non-archetypal virulence genes of UPEC. In addition, antibiotic susceptibility profile of the isolates was also examined. UTI management has become increasingly difficult because of the emergence of resistance to first line antimicrobials used for empiric therapy (Qin et al., 2013). Furthermore, better understanding of virulence mechanisms and pathogenesis of UTI (UPEC virulence determinants) found in clinical UPEC isolates will help in providing new strategies to treat and prevent UPEC infections.

2. Materials and methods

2.1. Escherichia coli isolates

The study was approved by the institutional review board of Jordan University of Science and Technology. Two hundred and twenty-seven isolates of *E. coli* were isolated from urine samples having significant bacterial counts (>10^5 CFUs/mL), obtained from patients suffering from UTIs. Pure cultures were stored frozen at −80 °C in LB broth with 10% glycerol. Samples were collected from King Abdullah University Hospital (Irbid, Jordan), Jordan University Hospital (Amman, Jordan) and Prince Hamzah Hospital (Amman, Jordan).

2.1.1. Identification and antimicrobial susceptibility testing

E. coli were identified based on motility, ability to ferment glucose, lactose, and sucrose, and ability to utilize citrate and tryptophan. The Kirby-Bauer disc diffusion method, using a 0.5 McFarland-equivalent bacterial suspension spread over Mueller Hinton agar media, was utilized to determine isolates' resistance to 17 antibiotics used for the treatments of UTIs (Jenkins and Schuetz, 2012). Isolates with resistance or intermediate susceptibility were considered non-susceptible to the antimicrobial agent. Isolates demonstrating resistance or intermediate susceptibility to an antimicrobial agent were considered non-susceptible to that agent during statistical analysis. Extended-spectrum beta-lactamase (ESBL) producing isolates were identified using a phenotypic confirmatory test using ceftazidime (30 μg) versus ceftazidime (30 μg) with clavulanic acid (10 μg), and cefotaxime versus cefotaxime (30 μg) with clavulanic acid (10 μg). An isolate was identified as an ESBL producer if the difference in zone diameter was more than or equal to 5 mm for either or both combinations, according to Clinical and Laboratory Standards Institute [CLSI, 2015] recommendations.

2.1.2. Extraction of chromosomal and plasmid DNA

One mL of overnight LB broth bacterial culture was subjected to chromosomal DNA extraction using the Wizard® Genomic DNA Purification Kit (Promega, Madison, Wisconsin, United States), and 0.6 mL of bacterial culture was subjected to plasmid extraction using the Zyppy™ Plasmid Miniprep Kit (Zymo research, Irvine, California, United States). Isolated DNA preparations were stored frozen at −20 °C until used.

2.1.3. Molecular detection of UPEC virulence genes

The genes encoding several UPEC virulence factors were detected using conventional PCR technique as previously described (Mao et al., 2012; Spurbeck et al., 2012). Amplification of selected genes was done using nine sets of primers as shown in Table 1. *E. coli* ATCC 700928 strain (CFT073) was used as positive control for *sivH, shiA, sisA, sisB, vat, fyuA, chuA,* and *yfcV*. *Eco274* PCR product was confirmed by identifying the band size following electrophoretic separation (i.e., 207 bp). *E. coli* ATCC 700,926 strain (MG1655) was used as a negative control for all the genes. The PCR cycling protocol for all genes was 94 °C for ten minutes, 32 cycles of 94 °C for 60 s, 50 °C for 60 s, and 72 °C for 60 s. The cycles were followed by 10 min of 72 °C.

2.2. Statistical analysis

The IBM SPSS software version 21 (Armonk, NY, USA) was used for statistical analysis. The Pearson Chi-square test was used to compare frequency data. *P* value less than 0.05 was considered significant.

3. Results

Several *E. coli* isolates were highly resistant to several antimicrobial agents (Tables 2 and 3). Resistance ranged from 83% for augmentin to 0.4%, for ertapenem. Approximately half (49.8%) of the isolates were ESBL positive (data not shown). Among the isolates, 81.9% were resistant to three or more classes of antimicrobial agents (Table 3).

Table 1
Primers used in the amplification of *E. coli* genes.

Gene	Primer sequence 5′ to 3′		Product size (bp)	Origin	Refer-ence
sivH	F	TGCCGAAGTCTGGTTACCTT	185	Plasmid	Mao et al. (2012)
	R	TCCCCGCTTTATAGTCCGTC			
shiA	F	TCACCTTACTGGTATGAACTC	451	Plasmid	Mao et al. (2012)
	R	TCCAGGGCCAGACATATTCA			
sisA	F	TTGCCCGACAGGAGAATGAC	360	Chromosome	Mao et al. (2012)
	R	GCAGTATATGGCGTGCCTGT			
sisB	F	GAACGATAGATTATGCTTTG	518	Plasmid	Mao et al. (2012)
	R	TCAGTACACTGAAGGCTCGC			
eco274	F	TTGACAAAGCCTGCCTGACC	207	Chromosome	Mao et al. (2012)
	R	CCTCCAACCCGTGTTTTTGC			
vat	F	TGGAACGACAGTGGAATGGA	238	Chromosome	Vigil et al. (2011)
	R	ACCCCACACCGTAAAGCATA			
fyuA	F	GTAAACAATCTTCCCGCTCGGCAT	850	Chromosome	Vigil et al. (2011)
	R	TGACGATTAACGAACCGGAAGGA			
chuA	F	CTGAAACCATGACCGTTACG	652	Chromosome	Spurbeck et al. (2012)
	R	TTGTAGTAACGCACTAAACC			
yfcV	F	ACATGGAGACCACGTTCACC	292	Chromosome	Spurbeck et al. (2011)
	R	GTAATCTGGAATGTGGTCAGG			

Table 2
Antimicrobial susceptibility results of the isolates.

Antimicrobial agent	Intermediate; N (%)	Resistant; N (%)	Susceptible; N (%)
Amikacin	16 (7.1)	7 (3.1)	204 (89.9)
Augmentin	14 (6.2)	189 (83.2)	24 (10.6
Cefepime	20 (8.8)	88 (38.8)	119 (52.4)
Cefixime	38 (16.7)	45 (19.8)	144 (63.4)
Cefotaxime	2 (0.9)	129 (56.8)	96 (42.3)
Cefoxitin	42 (18.5)	48 (21.2)	137 (60.4)
Cefpodoxime	2 (0.9)	126 (55.5)	99 (43.6)
Ceftazidime	7 (3.1)	116 (51.2)	104 (45.8)
Ceftriaxone	1 (0.4)	125 (55.1)	101 (44.5)
Cefuroxime	21 (9.3)	140 (61.7)	66 (29.1
Ciprofloxacin	6 (2.6)	126 (55.5)	95 (41.9)
Ertapenem	1 (0.4)	1 (0.4)	225 (99.1)
Gentamycin	0 (0.0)	76 (33.5)	151 (66.5)
Nalidixic acid	4 (1.8)	177 (77.9)	46 (20.3)
Nitrofurantoin	28 (12.3)	29 (12.8)	170 (74.9)
Norfloxacin	45 (19.4)	12 (5.3)	170 (74.9)
Trimethoprim-sulfamethoxazole	1 (0.4)	166 (73.1)	60 (26.4)

Table 3
Isolates' resistance to antimicrobial agent classes.

Antimicrobial agent classes' resistance count	Frequency	Percent
0	10	4.4
1	9	4
2	22	9.7
3	19	8.4
4	16	7
5	41	18.1
6	48	21.1
7	47	20.7
8	15	6.6

Table 4
Prevalence of UPEC virulence genes among the isolates.

Gene	Present (%)
shiA	209 (92.1)
sisA	164 (72.2)
eco274	99 (43.6)
sivH	81 (35.7)
vat	63 (27.4)
yfcV	57 (25.1)
sisB	56 (24.7)
chuA	46 (20.3)
fyuA	41 (18.1)

Using PCR, urovirulence genes were detected at the following rates among the isolates: *shiA* (92%), *sisA* (72%), *eco274* (44%), *sivH* (36%), *vat* (27%), *yfcV* (25%), *sisB* (25%), *chuA* (20%), and *fyuA* (18%) (Table 4). Among the isolates, 67.4% were identified as UPEC, as they were positive for three or more of the urovirulence genes.

Finally, the profile of antibiotic resistance among the isolates was compared to that reported in other countries (Table 5). Overall, the study's isolates had higher or similar resistance rates compared to those reported elsewhere (Table 5).

4. Discussion

In the current study, *E. coli* isolates involved in human UTIs were characterized by PCR to determine prevalence of UPEC virulence genes. In addition, antibiotic susceptibility profile of the isolates was also examined.

UPEC strains are responsible for the majority of uncomplicated urinary tract infections. Many putative virulence genes of UPEC have been previously identified. However, UPEC strains differ in the number and expression levels of virulence genes, leading to variations in ability to grow and persist within the urinary tract (Bien et al., 2012).

Prevalence of non-archetypal urovirulence genes of UPEC strains is poorly defined among UTI patients. One of the aims of the present study was to identify the urovirulence genes of UPEC strains isolated from UTI symptomatic patients. Specifically, the presence of *sivH, shiA, sisA, sisB, eco274, chuA, yfcV, vat,* and *fyuA*, was investigated utilizing PCR-based analyses. We considered an isolate to be a UPEC strain, if it possessed three or more of the aforementioned genes.

Statistically significant associations were identified between multiple virulence genes, as some genes were more likely to coexist with other genes. This may suggest the co-carriage of multiple virulence genes on the same plasmid. In general, the prevalence of urovirulence genes presented in the current study is consistent with those from other related studies from the USA and Taiwan (Mao et al., 2012). *ShiA* and *sisA* were the most prevalent at 92% and 72%, respectively, while *fyuA* was the least prevalent at 18%. The other urovirulence genes were prevalent in 20–44% of the isolates. The higher prevalence of *sisA* in the current study (72%) compared to *sisB* (25%) is consistent with other studies that reported higher *sisA* prevalence than *sisB* among UPEC isolates (Lloyd et al., 2009a; Lloyd et al., 2009b; Mao et al., 2012). This difference

Table 5
Prevalence of antimicrobial resistance of isolates compared to other studies.

Antimicrobial agent	Resistance rates according to study (%)						
	This study	Jordan Nimri and Azaizeh (2012)	Ankara Aypak et al. (2009)	Pakistan Sabir et al. (2014)	Brazil Dias et al. (2009)	China Cao et al. (2011)	Iran Farshad et al. (2012)
Amikacin	3	3	–	13	3	7	–
Augmentin	83	33	33	63	19	25	–
Cefepime	39	–	–	–	–	–	–
Cefixime	20	54	–	–	–	–	20
Cefotaxime	57	50	–	90	2	–	–
Cefoxitin	21	–	–	–	8	–	–
Cefpodoxime	56	–	–	–	–	69	–
Ceftazidime	51	48	–	74	–	67	–
Ceftriaxone	55	–	16	43	–	46	–
Cefuroxime	62	56	23	58	4	67	–
Ciprofloxacin	56	40	–	54	–	75	9
Ertapenem	0.4	–	–	–	–	–	–
Gentamycin	34	37	6	60	3	63	15
Nalidixic acid	78	61	–	–	10	–	25
Nitrofurantoin	13	4	2	–	3	–	5
Norfloxacin	5	42	–	11	–	–	–
Trimethoprim-sulfamethoxazole	73	65	41	–	–	–	76
ESBL*	50	50	–	–	–	–	–

* ESBL is not an antimicrobial agent.

may be attributed to carriage of *sisA* mainly by extra-intestinal pathogenic *E. coli* strains and of *sisB* mainly by intestinal *E. coli* strains (Lloyd et al., 2009b).

Interestingly, SisA and SisB of CFT073 are homologs of ShiA of *Shigella flexneri*, and play a similar role in suppressing the host defense system during UTIs (Ingersoll and Zychlinsky, 2006; Lloyd et al., 2009b). Therefore, *sisA* and *sisB* which are located in pathogenicity islands, can be considered UPEC virulence factor genes. They are found in some but not all UPEC strains, which may explain the reported variability of inflammation among patients of UTIs (Lloyd et al., 2009b). The potential transcriptional regulator gene *eco274* which likely encodes a virulence factor involved in pyelonephritis and urosepsis, was prevalent among approximately 44% of the isolates. *SivH*, which encodes a putative invasive protein, had a prevalence rate of 36%. Although, the specific urovirulence mechanisms of the products of the *eco274* and *sivH* are yet to be identified, their association with UTIs highly suggests their identity as urovirulence genes.

FyuA, chuA, sisB, yfcV, and *vat*, were prevalent in 18–27% of the isolates. *E. coli* isolates carrying, *fyuA*, *chuA*, *yfcV*, and *vat,* were reported to colonize the urinary tract more efficiently than isolates lacking them (Spurbeck et al., 2012). In addition, the latter four genes are highly prevalent among UPEC (Mao et al., 2012; Spurbeck et al., 2012). The *fyuA* gene was absent from *E. coli* isolated from asymptomatic bacteriuria in healthy nonpregnant women (Srivastava et al., 2016). Expression of chuA gene was induced by growing UPEC strain CFT073 in urine (Alteri and Mobley, 2007). These findings confirmed the low prevalence *FyuA, chuA, sisB, yfcV*, and *vat* genes observed in the current study.

The products of *chuA* and *fyuA*, which were identified in a fifth of the isolates, are two receptors that contribute to UPEC pathogenesis during UTIs (Spurbeck et al., 2012). A study demonstrated that *chuA* and *fyuA*, contributed to systemic *E. coli* infections by functioning as heme and yersiniabactin receptors, respectively (Spurbeck et al., 2012).

A previous report has indicated a high correlation between *vat* (*tsh*) and UPEC infections but not to fecal *E. coli* strains (Heimer et al., 2004), and that *vat* contributed to development of systemic infections (Subashchandrabose et al., 2013). However, vat was not detected in 123 UPEC strains isolated from patients with UTIs (Momtaz et al., 2013). Therefore, *vat*, which was identified in 27% of this study's isolates, might be considered as a UPEC virulence factor. More studies are required to understand the contribution of *vat* to UPEC pathogenesis.

In Jordan and in other countries, the improper prescription of antimicrobials by physicians, purchasing antimicrobials without prescription, and the misuse of antimicrobials by individuals, likely contributes to increasing drug resistance by bacteria (Al-Bakri et al., 2005). In addition, drug resistance among the *Enterobacteriaceae* is increasing worldwide. A specific concern is the increasing prevalence of ESBL-producing *Enterobacteriaceae*, which are involved in several difficult-to-control infection outbreaks (Canton et al., 2008). Overall, the study's isolates had higher or similar resistance rates compared to those reported in Jordan (Abu Shaqra, 2001; Zatorski et al., 2015) and elsewhere (Aypak et al., 2009; Farshad et al., 2012; Sabir et al., 2014), which has the antimicrobial resistance profile of the isolates compared to that from other studies. The observed variations in resistance rates may be attributed to regional differences in strain prevalence and different standards and controls for prescription and use of antimicrobial agents.

According to the European antimicrobial resistance surveillance report which was published in 2011 (EARS-Net, 2012), *E. coli* resistance rates to aminopenicillins ranged from 39% in Finland, up to 71% in Bulgaria. Resistance to 3rd generation cephalosporins ranged from 4% in Lithuania, Sweden, and Norway, up to 30% in Slovakia, with a mean of 11%. Resistance to fluoroquinolones ranged from 11% in Finland, Sweden, and Norway, up to 42% in Cyprus and Italy. Resistance to aminoglycosides ranged from 3% in Iceland up to 24% in Romania, with a mean of 10%. All European countries had low resistant rates to carbapenems with a mean of <0.1% which is very close to our results for ertapenem at 0.4%. Our results demonstrated much higher resistance levels than those in Europe likely due to misuse and improper prescription of antimicrobials in Jordan. The rates of ESBL-producing isolates in Europe ranged from 68% in Slovakia up to 100% in Hungary, Lithuania, and Romania, with most other countries above 80% (EARS-Net, 2012). In contrast, our study isolates had a lower rate of ESBL phenotype, probably due to more prevalent use of 3rd generation cephalosporins in Europe, than in Jordan.

In conclusion, antimicrobial susceptibilities, and virulence factor profiles of *E. coli* isolates recovered from UTIs were determined. High rates of multi-drug resistance were reported.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgments

The study was supported by fund from Jordan University of Science and Technology (number: 149/2013).

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