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Original article

Virulent *Escherichia coli* strains among Egyptian patients with acute diarrhoea versus urinary tract infection, and their antibiotic susceptibility

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ABSTRACT

Background and study aims: Diarrhoea and urinary tract infection (UTI) are common clinical problems. Meanwhile, *Escherichia coli* (*E. coli*), is the commonest bacterial pathogen reported in both of them. This study aimed to evaluate the pathogenic *E. coli* (PEC) in stool of acute diarrhoea and urine of UTI regarding their virulence genes and their influence on the susceptibility to routinely prescribed antibiotics.

Patients and methods: Twenty two stool and another 22 urine samples of patients with acute diarrhoea and UTI respectively were collected from patients admitted at Kasr Al-Ainy Hospital, Faculty of Medicine, Cairo University, Egypt. *E. coli* isolation, identification of their phyla; chuA, yjaA, and TspE4. C2, and further identification of 10 virulent genes; *fimH*, *papC*, papG//, papG///, papEF, afa, sfa, CNF1, *iroN* & hlyA was performed. Antibiotic susceptibility was studied against quinolones, gentamicin (GM), and trimethoprim-sulphamethoxazole (TMP-SMX).

Results: The studied virulence genes were comparably detected in both pathogenic samples. In diarrheogenic *E. coli* (DEC); phylum A was significantly related to both ciprofloxacin (CIP) and TMP-SMX resistance, and both of the virulence genes fimH and iroN were significantly related to all the studied antibiotics resistance, while afa was significantly related to nalidixic acid (NA) resistance. In uropathogenic *E. coli* (UEC); phylum D was significantly related to CIP and levofloxacin resistance, and both of the virulence genes fimH and iroN were significantly related to most of the studied antibiotics resistance.

Conclusion: The isolated PEC was evidently and broadly resistant to the studied antibiotics, with limited influence of their phyla and virulence genes (fimH and iroN).

Introduction

Diarrhoea is a worldwide major health problem. Mortality due to diarrhoeal infections may reach two million annually, mostly among children under 5 years in developing countries [1]. A wide range of infectious pathogens cause diarrhoea. Bacteria, amongst which is Escherichia coli, is the most common cause of diarrhoeal diseases in developing countries [2]. Also, urinary tract infection (UTI) is the second most common clinical symptom for experimental antimicrobial treatment in primary and secondary care [3], and *E. coli* is the most common uropathogenic bacteria causing it, particularly in females [4]. PCRs were specific and sensitive for rapid detection of target isolates in stools [5], and diarrheogenic *E. coli* were among the first strains for which molecular diagnostic techniques were applied [6]. This study aimed at assessing the prevalence of different virulent strains of *E. coli* in acute diarrhoea in comparison to urinary tract infection, and their susceptibility to antibiotics

Patients and methods

This was a laboratory based cohort study on *E. coli* isolates collected from stools and urine samples of adult patients admitted to the Kasr Al-Ainy Hospital, Faculty of Medicine, Cairo University, Egypt for diarrhoea and UTI, respectively before administration of antibiotics. The study was approved by the Ethical Committee of Microbiology and Tropical Medicine Departments, Faculty of Medicine, Cairo University

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All collected samples of pathogenic *E. coli* from hospitalised patients were subjected to the following. 1] ***Bacterial isolation.*** The collected samples were stored in brain heart infusion with 20% glycerol at −20 °C. 2] *E. coli bacterial isolation:* Samples were processed to isolate *E. coli* as described by the Clinical Laboratory Standard Institute (CLSI) [7]. The samples were inoculated into Mac Conkey (MAC) broth for enrichment at 37 °C for 24 h, the strains were biochemically identified based on their colony morphologies seen on MAC's agar plates and they were specialised by standard biochemical tests. The organisms revealed gram negative, pink coloured with rod shaped appearance and arranged in single or in pairs were suspected as *E. coli*. 3] PCR detection of pathogenicity islands (PAIs) in the diarrhoeagenic *E. coli* (DEC); a) Bacterial colonies from MacConkey (MAC) plates were inoculated into 5 ml phosphate-buffered saline (PBS) tube to a density of MacFarland 4 (109 5 × 109 bacterial/ml). The 5 ml bacterial suspension was boiled for 20 min and then centrifuged at 2500×*g* for 10 min to pellet cell debris. The supernatant was used for PCR assays. b) *Preparation of DNA template and primer:* The DNA templates were subjected to multiplex PCR for the detection of the virulence genes as shown in Table 1. *E. coli* ATCC 11,775 (negative control without virulence genes). Conventional PCR assay was used for detection of pathogenicity islands (PAIs) in the diarrhoeagenic *E. coli* isolates. *Isolation of Bacterial DNA:* DNA was isolated from *E. coli* by using boiling method [8]. A loop full of culture was mixed with 100 μl of sterile water in a micro centrifuge tube. This was followed by incubation at 100 °C using (Techne DRI-Block DB .2 A thermal cycler, finally, cellular debris was removed by centrifugation at (10,000 rpm for 15 min) and the supernatant was used in subsequent PCR, to amplify the different virulence gene. *Phylogenetic Classification. E. coli* strains belonged to four groups (A, B1, B2, or D) based on the presence of the *chu*A and *yja*A genes and the DNA fragment (TSPE4.C2). This PCR contained1.25U Taq DNA polymerase (Invitrogen) in 1x PCR buffer (Invitrogen), 20 pmol of each dNTP, 2.5 mM $MgCl_2$, and1 μM of each primer (Table 1). The programme of PCR consisted of 94 °C for 4 min, followed by 30 cycles of 94 °C for 5 seg and 54 °C for 10 seg, with a final extension step at 72 °C for 5 m [9]. TspE4.C2 and yjaA represent the phylogenetic marker for intestinal *E. coli* isolates, while chuA represents the phylogenetic marker for extraintestinal *E. coli* isolates [10,11]. Primers used in this study are shown in Table 1. 4] *Determination of Virulence Genes:* Thirteen virulent genes were detected by individual PCR. All PCR assays in this study were carried out in a 25 μl reaction volume containing 1 μ1 bacterial DNA, 12.5 μl My Taq Red Mix 2X, 1 μl of each primer (Sigma scientific, Egypt), 9.5 μl distilled water. The following distinct genes in particular islands were targeted *she* PAI (*pic* gene), HPI PAI (*irp2* and f*yu A* gene), LEE PAI (*eae* gene), TIA PAI (*tia* gene), SHI-2 PAI (*iutA gene*), EspC PAI (*espC gene*) and O-island PAI (*efa/lifA* gene and *pagC* gene). Once a PCR product for an individual virulence determined was confirmed using ultraviolet light and was recorded using gel documentation system. 5] *Antibiotic susceptibility testing*: it was performed by disk diffusion method obtained from Hi-Media labs, Mumbai, India. *E. coli* ATCC 25922 was used as a negative control strain and validate the disc diffusion test as well, as described by CLSI [7] [a. Select a pure culture plate of one of the organisms to be tested. b. Aseptically emulsify a colony from the plate in the sterile saline solution. Mix it thoroughly to ensure that no solid material from the colony is visible in the saline solution. c. Repeat until the turbidity of the saline solution visually match that of the

Table 1
Primers sequences for the virulence genes.

Genes	Primer sequences (5′–3′)	PCR condition (time)	No. of cycles	Amplicon size (bp)
fimH	F-TGCAGAACGGATAAGCCGTGG R-GCAGTCACCTGCCCTCCGGTA	95 °C (30 s) 60 °C (30 s) 72 °C (1 min)		508
papC	F-GACGGCTGTACTGCAGGGTGTGGC R-ATATCCTTTCTGCAGGGATGCAATA	95 °C (30 s) 63 °C (30 s) 72 °C (1 min)		328
papEF	F-GCAACAGCAACGCTGGTTGCATCAT R-AGAGAGAGCCACTCTTATACGGACA	95 °C (30 s) 55 °C (30 s) 72 °C (1 min)		336
papGII	F-GGAATGTGGTGATTACTCAAAGG R-TCCAGAGACTGTTCAAGAAGGAC	95 °C (30 s) 52 °C (30 s) 72 °C (1 min)	30	562
papGIII	F-CATGGCTGGTTGTTCCTAAACAT R-TCCAGAGACTGTGCAGAAGGAC	95 °C (30 s) 52 °C (30 s) 72 °C (1 min)		421
Afa	F-GCTGGGCAGCAAACTGATAACTCTC R-CATCAAGCTGTTTGTTCGTCCGCCG	95 °C (30 s) 60 °C (30 s) 72 °C (1 min)		559
Sfa	F-CGGAGGAGTAATTACAAACCTGGCA R-CTCCGGAGAACTGGGTGCATCTTAC	95 °C (30 s) 58 °C (30 s) 72 °C (30 s)		407
cnf1	F-AAGATGGAGTTTCCTATGCAG R-TCAGAGTCCTGCCCTCATTAT	95 °C (30 s) 54 °C (30 s) 72 °C (30 s)		498
hlyA	F-AACAAGGATAAGCACTGTTCTGGCT R-ACCATATAAGCGGTCATTCCCGTCA	95 °C (30 s) 63 °C (30 s) 72 °C (2 min)		1177
iroN	F-AAGTCAAAGCAGGGGTTGCC R-GACGCCGACATTAAGACGCAG	95 °C (30 s) 63 °C (30 s) 72 °C (1 min)		665
chuA	F-GAC GAA CCA ACG GTC AGG AT R-TGC CGC CAG TAC CAA AGA CA	94 °C (5 s)		279
YjaA	F-TGA AGT GTC AGG AGA CGC TG R-ATG GAG AAT GCG TTC CTC AAC	54 °C (10 s) 72 °C (5 min)		211
TSPE4.C2	F-GAG TAA TGT CGG GGC ATT CA R-CGC GCC AAC AAA GTA TTA CG			152

standard turbidity. d. Take a sterile swab and dip it into the broth culture of organism. e. Gently squeeze the swab against the inside of the tube in order to remove excess fluid in the swab. f. Take a sterile Mueller-Hinton agar (MHA) plate or a nutrient agar (NA) plate. g. Use the swab with the test organism to streak a MHA plate or a NA plate for a lawn of growth. h. After the streaking is complete, allow the plate to dry for 5 min. i. Antibiotic discs can be placed on the surface of the agar using sterilised forceps. j. Gently press the discs onto the surface of the agar using flame sterilised forceps or inoculation loop. k. Carefully invert the inoculated plates and incubate for 24 h at 37 °C. l. After incubation, use a metric ruler to measure the diameter of the zone of inhibition for each antibiotic used. m. Compare the measurement obtained from the individual antibiotics with the standard table to determine the sensitivity zone. n. Compare the measurement obtained from the individual antibiotics to the standard table to determine whether the tested bacterial species is sensitive or resistant to the tested antibiotic], such that discs of ciprofloxacin (CIP; 10 μg), norfloxacin (NOR), naldixic acid (NA; 30 μg), levofloxacin (LEV), ofloxacin (OFX), norfloxacin (NOR) for quinolones, and also for gentamicin (GM) and trimethoprim-sulphamethoxazole (TMP-SMX).

Table 2
Phyla of *E. coli* according to genetic sequencing.

Percent	Virulence genes			Phyla
	TSPE4.C2	yjaA	chuA	
13.63	–	–	–	A
13.63	–	+	–	
15.9	+	–	–	B1
11.36	–	+	+	B2
15.9	+	+	+	
15.9	–	–	+	D
13.63	+	–	+	

Table 3
Phyla of Pathogenic *E. coli* isolated in the studied samples.

Phyla of *E. coli* isolates	Pathogenic *E. coli* isolates n (%)		Total
	Diarrheogenic N = 22	Uropathogenic N = 22	N = 44 (%)
A	8 (36.4)	4 (18.2)	12 (27.3)
B1	5 (22.7)	2 (9.1)	7 (15.9)
B2	5 (22.7)	7 (31.8)	12 (27.3)
D	4 (18.2)	9 (40.9)	13 (29.5)

Table 4a
Virulence genes amongst the pathogenic *E. coli* isolates in the studied samples.

Virulence genes	*E. coli* isolates		p value	Total (%)
	Diarrheogenic N = 22	Uropathogenic N = 22		
fimH	19	19	1.000	38 (86.4)
papC	2	6	0.157	8 (18.2)
papG//	4	2	0.414	6 (13.6)
papG///	3	5	0.480	8 (18.2)
papEF	5	4	0.739	9 (20.5)
afa	9	4	0.166	13 (29.5)
sfa	1	2	0.564	3 (6.8)
CNF1	0	3	NS	3 (6.8)
iroN	12	13	0.841	25 (56.8)
hlyA	5	6	0.7630	11 (25)

Results

Pathogenic *E. coli* (PEC) strains were tested in 22 urine samples collected from Urology Department from admitted patients complaining of UTI, and another 22 stool samples of patients in Tropical Medicine and Hepatogastroenterology Departments from admitted patients complaining of acute diarrhoea.

Characterization of the phyla of the isolated pathogenic *E. coli* is shown in Table 2.

The distribution of the phyla of the PEC isolates is shown in Table 3.

The distribution of the 10 virulence genes in the PEC isolates, altogether and in terms of their phyla are shown in Tables 4a and 4b. The genes fimH, and iroN were the commonest virulence genes detected and comparable in both DEC and UEC samples (86.4% in both, and 54.5% and 59.1% respectively), while sfa (4.5% and 9% respectively) and CNF1 were the least detected, however CNF1 was not detected at all in DEC. The virulence genes were not related to either type of sample nor with the phyla of the *E. coli* isolates.

Antibiotic susceptibility of diarrheogenic *E. coli* (DEC) isolates in terms of their phyla is shown in Tables 5a and 5b, and in terms of the 10 studied virulence genes are shown in Tables 6a and 6b. All of them were resistant to the studied antibiotics in both samples, mostly in multiple folds yet insignificantly than susceptible ones.

Antibiotic susceptibility of uropathogenic *E. coli* (UEC) isolates in terms of their phyla is shown in Tables 7a and 7b, and in terms of the studied virulence genes in Tables 8a and 8b. Also, all of them were resistant to the studied antibiotics in both samples, mostly in multiple folds yet insignificantly than susceptible ones.

Discussion

Infectious diarrhoea is one of the most common causes of morbidity and mortality in developing countries, particularly in paediatrics [12]. *E. coli*, the predominant commensal of the human intestine, can be pathogenic causing diseases of the gastrointestinal, urinary and central nervous systems. It has been implicated

Table 4b
Distribution of virulence genes in phyla of *E. coli* phyla.

Virulence genes	Phyla of *E. coli* isolates N (%)				p value
	A (n = 12)	B1 (n = 7)	B2 (n = 12)	D (n = 13)	
1- fimH	9 (75)	7 (100)	11 (91.7)	9 (69.2)	0.828
2- papC	0	0	5 (41.7)	2 (15.4)	0.257
3- papG//	0	1 (14.3)	4 (33.3)	4 (30.8)	0.368
4- papG///	2 (16.7)	1 (14.3)	4 (33.3)	4 (30.8)	0.484
5- papEF	0	1 (14.3)	2 (16.7)	5 (38.5)	0.197
6- afa	7 (58.3)	1 (14.3)	3 (25)	3 (23)	0.143
7- sfa	0	1 (14.3)	1 (8.3)	1 (7.7)	1.000
8- CNF1	0	1 (14.3)	1 (8.3)	1 (7.7)	1.000
9-iroN	4 (33.3)	5 (71.4)	7 (58.3)	3 (23)	0.606
10- hlyA	3 (25)	2 (28.6)	2 (16.7)	2 (15.4)	0.954

Table 5a
Antibiotic Susceptibility in diarrheogenic *E. coli* isolates in terms of their phyla.

Phyla of *E. coli* isolates	Antibiotic susceptibility n (%)											
	NA		p value	CIP		p value	LEV		p value	OFX		p value
	R n = 18 (81.8)	S n = 4 (18.2)		R n = 18 (81.8)	S n = 4 (18.2)		R n = 16 (72.7)	S n = 6 27.3		R n = 16 (72.7)	S n = 6 (27.3)	
A(n = 8)	8(44.4)	0	NS	7(38.8)	1(25)	**0.034**	5(31.2)	3(50)	NS	8(50)	0	NS
B1(n = 5)	4(9)	1(25)	NS	4(22.2)	1(25)	NS	5(31.2)	0	NS	5(31.2)	0	NS
B(n = 5)	3(16.6)	2(50)	NS	5(27.7)	0	NS	5(31.2)	0	NS	5(31.2)	0	NS
D(n = 4)	4(9)	0	NS	2(11.1)	2(50)	NS	3(18.7)	1(16.7)	NS	5(18.7)	1(16.7)	NS

NA = Nalidixic acid, CIP = Ciprofloxacin, LEV = Levofloxacin, OFX = Ofloxacin, R = Resistant, S = Sensitive, NS = Non significant.

Table 5b
Antibiotic Susceptibility in diarrheogenic *E. coli* isolates in terms of their phyla.

Phyla of *E. coli* isolates	Antibiotic susceptibility n (%)								
	NOR		p value	GM		p value	TMP-SMX		p value
	R n = 16 (72.7)	S n = 6 (27.3)		R n = 19 (86.4)	S n = 3 (13.6)		R N = 18 (81.8)	S n = 4 (28.2)	
A (n = 8)	6(37.5)	2(33.3)	NS	5(26.3)	3(100)	NS	7(38.8)	1(25)	**0.034**
B1 (n = 5)	4(25)	1(16.6)	NS	3(15.7)	2(66.6)	NS	4(22.2)	1(25)	NS
B2 (n = 5)	2(12.5)	3(50)	NS	3(15.7)	2(66.6)	NS	5(27.7)	0	NS
D (n = 4)	3(18.7)	1(16.6)	NS	3(15.7)	1(33.3)	NS	3(16.6)	1(25)	NS

NOR = Norfloxacin, GM = Gentamicin, TMP-SMX = Trimethoprim-sulphamethoxazole, R = Resistant, S = Sensitive, NS = Non significant.

Table 6a
Antibiotic susceptibility in diarrheogenic *E. coli* isolates in terms of the studied virulence genes.

Virulence genes	Antibiotic susceptibility n (%)											
	CIP		p value	NA		p value	LEV		p value	OFX		p value
	R n = 18 (81.8)	S n = 4 (28.2)		R n = 18 (81.8)	S n = 4 (18.2)		R n = 16 (72.7)	S n = 6 (27.3)		R n = 16 (72.7)	S N = 6 (27.3)	
fimH (n = 19)	16(88.8)	3(75)	**0.003**	16(88.8)	3(75)	**0.003**	14(87.5)	5(83.3)	**0.039**	14(87.5)	5(83.3)	**0.039**
papC (n = 2)	1(5.5)	1(25)	NS	2(11.1)	0	NS	2(12.5)	0	NS	1(6.2)	1(16.6)	NS
papG// (n = 4)	3(16.6)	1(25)	NS	4(22.2)	0	NS	3(18.7)	1(16.6)	NS	2(12.5)	2(33.3)	NS
papG/// (n = 3)	1(5.5)	2(50)	NS	3(16.6)	0	NS	3(18.7)	0	NS	3(18.7)	0	NS
papEF (n = 5)	5(27.7)	0	NS	4(26.6)	1(25)	NS	5(31.2)	0	NS	5(31.2)	0	NS
afa (n = 9)	9(50)	0	NS	8(44.4)	1(25)	**0.020**	9(56.2)	0	NS	8(50)	1(16.6)	NS
sfa (n = 1)	1(5.5)	0	NS	1(5.5)	0	NS	1(6.2)	0	NS	0	1(16.6)	NS
CNF1 (n = 0)												
iroN (n = 12)	10(55.5)	1(25)	**0.007**	9(50)	2(50)	**0.035**	10(62.5)	1(16.6)	**0.007**	9(56.2)	2(33.3)	**0.035**
hlyA(n = 5)	4(22.2)	1(25)	NS	5(27.7)	0	NS	4(25)	1(16.6)	NS	3(18.7)	2(33.3)	NS

CCIP = Ciprofloxacin, NA = Nalidixic, LEV = Levofloxacin, OFX = Ofloxacin, R = resistant, S = sensitive, NS = Non significant.

Table 6b
Antibiotic susceptibility in diarrheogenic *E. coli* isolates in terms of the studied virulence genes.

Virulence genes	Antibiotic Susceptibility n (%)								
	NOR		p value	GM		p value	TMP-SMX		p value
	R n = 16 (72.7)	S N = 6 (27.3)		R n = 19 (86.4)	S N = 3 (23.7)		R n = 18 (81.8)	S n = 4 (28.2)	
fimH (n = 19)	14(87.5)	5(83.3)	**0.039**	17(89.4)	2(66.6)	**0.001**	17(94.4)	2(50)	**0.001**
papC (n = 2)	0	2(33.3)	NS	2(10.5)	0	NS	1(5.5)	1(25)	NS
papG// (n = 4)	3(18.7)	1(16.6)	NS	3(15.7)	1(33.3)	NS	3(16.6)	1(25)	NS
papG/// (n = 3)	3(18.7)	0	NS	2(10.5)	1(33.3)	NS	2(11.1)	1(25)	NS
papEF (n = 5)	4(28.5)	1(16.6)	NS	3(15.7)	2(66.6)	NS	4(22.2)	1(25)	NS
afa (n = 9)	7(43.7)	2(33.3)	NS	7(36.8)	2(66.6)	NS	6(33.3)	3(75)	NS
sfa (n = 1)	1(6.2)	0	NS	1(5.2)	0	NS	1(5.5%)	0)	NS
CNF1 (n = 0)									
iroN (n = 12)	9(56.2)	2(33.3)	**0.035**	10(52.6)	1(33.3)	**0.007**	9(50)	2(50)	**0.035**
hlyA(n = 5)	4(25)	1(16.6)	NS	4(21)	1(33.3)	NS	3(16.6)	2(50)	NS

NOR = Norfloxacin, GM = Gentamicin, TMP-SMX = Trimethoprim sulphamethoxazole, R = Resistant, S = sensitive, NS = Non significant.

Table 7a
Antibiotic Susceptibility in uropathogenic *E. coli* isolates in terms of their phyla.

Phyla of *E. coli* isolates	Antibiotic Susceptibility n (%)											
	NA		p value	CIP		p value	LEV		p value	OFX		p value
	R n = 19 (86.4)	S n = 3 (13.6)		R n = 20 (90.9)	S n = 2 (9.1)		R n = 17 (77.3)	S n = 5 (22.7)		R n = 19 (86.4)	S n = 3 (13.6)	
A (n = 4)	3(15.7)	1(33.3)	NS	3(15)	1(50)	NS	3(17.6)	1(20)	NS	3(15.7)	1(33.3)	NS
B1 (n = 2)	1(5.2%)	1(33.3)	NS	2(10)	0	NS	1(5.8)	1(20)	NS	2(10.5)	0	NS
B2 (n = 7)	7(36.8)	0	NS	5(25)	2(100)	NS	6(35.2)	1(20)	NS	5(26.3)	2(66.6)	NS
D (n = 9)	7(36.8)	2(66.6)	NS	8(40)	1(50)	**0.020**	8(47)	1(20)	**0.020**	7(36.8)	2(66.6)	NS

NA = Nalidixic acid, CIP = Ciprofloxacin, LEV = Levofloxacin, OFX = Ofloxacin, R = Resistant, S = Sensitive, NS = Non significant.

Table 7b
Antibiotic Susceptibility in uropathogenic *E. coli* isolates in terms of their phyla.

Phyla of *E. coli*	Antibiotic Susceptibility n (%)								
	NOR		p value	GM		p value	TMP-SMX		p value
	R n = 18 (81.8)	S n = 4 (18.2)		R n = 18 (81.8)	S n = 4 (18.2)		R N = 14 (63.6)	S n = 8 (36.4)	
A (n = 4)	4(22.2)	0	NS	2(11.1)	2(50)	NS	k	k	NS
B1 (n = 2)	1(5.5)	1(25)	NS	1(5.5)	1(25)	NS	2(14.2)	2(14.2)	NS
B2 (n = 7)	6(33.3)	1(25)	NS	4(22.2)	3(75)	NS	6(42.8)	6(42.8)	NS
D (n = 9)	7(38.8)	2(50)	NS	6(33.3)	3(75)	NS	9(64.2)	9(64.2)	NS

NOR = Norfloxacin, GM = Gentamicin, TMP-SMX = Trimethoprim-sulphamethoxazole, R = resistant, S = sensitive, NS = Non significant.

Table 8a
Antibiotic susceptibility in uropathogenic *E. coli* isolates in terms of the studied virulence genes.

Virule nce genes	Antibiotic susceptibility n (%)											
	NA		p value	CIP		p value	LEV		P value			p value
	R n = 19 (86.4)	S n = 3 (13.6)		R n = 20 (90.9)	S n = 5 (9.1)		R n = 17 (77.3)	S n = 5 (22.7)		R n = 19 (72.7)	S N = 3 (27.3)	
fimH (n = 19)	16(84.2)	3(100)	**0.003**	17(85)	2(40)	**0.001**	15(88.2)	4(80)	**0.012**	17(89.4)	2(66.6)	**0.001**
papC (n = 6)	4(21)	2(66.6)	0.414	5(25)	1(20)	0.102	5(29.4)	1(20)	0.102	6(31.5)	0	NS
papG// (n = 2)	1(5.2)	1(33.3)	1.000	1(5)	1(20)	1.000	2(11.7)	0	NS	2(10.5)	0	NS
papG/// (n = 5)	2(10.5)	2(66.6)	1.000	4(20)	1(20)	0.180	4(23.54)	1(20)	0.180	4(21)	1(33.3)	NS
papEF (n = 4)	4(21)	0	NS	3(15)	1(20)	0.317	2(11.7)	2(40)	1.000	3(15.7)	1(33.3)	NS
afa (n = 4)	4(21.0)	0	NS	3(15)	1(20)	0.317	3(17.6)	1(20)	0.317	3(15.7)	1(33.3)	NS
sfa (n = 2)	2(10.5)	0	NS	1(5)	1(20)	1.000	1(5.8)	1(20)	1.000	2(10.5)	0	NS
CNF1 (n = 3)	2(10.5)	1(33.3)	0.564	2(10)	1(20)	0.564	2(11.7)	1(20)	0.564	3(15.7)	0	NS
iroN (n = 1)	12(63.1)	1(33.3)	**0.002**	11(55)	2(20)	**0.013**	9(52.9)	4(80)	0.166	12(63.1)	1(33.3)	**0.002**
hlyA(n = 6)	4(21)	2(66.6)	0.414	5(25)	1(20)	0.102	5(29.4)	1(20)	0.102	5(26.3)	1(33.3)	NS

NA = Nalidixic acid, CIP = Ciprofloxacin, LEV = Levofloxacin, R = Resistant, S = Sensitive.

Table 8b
Antibiotic susceptibility in uropathogenic *E. coli* isolates in terms of the studied virulence genes.

Virulence genes	Antibiotic susceptibility n (%)								
	NOR		p value	GM		p value	TMP-SMX		p value
	R n = 18 (72.7)	S N = 4 (27.3)		R n = 18 (86.4)	S N = 4 (23.7)		R n = 14 (81.8)	S n = 8 (28.2)	
fimH (n = 19)	17(94.4)	2(50)	**0.001**	16(88.8)	3(75)	**0.003**	13(92.8)	6(75)	NS
papC (n = 6)	5(27.7)	1(25)	NS	4(22.2)	2(50)	NS	3(21.4)	3(37.5)	NS
papG// (n = 2)	2(11.1)	0	NS	1(5.5)	1(25)	NS	1(7.1)	1(12.5)	NS
papG/// (n = 5)	4(22.2)	1(25)	NS	4(22.2)	1(25)	NS	3(21.4)	2(25)	NS
papEF (n = 4)	3(16.6)	1(25)	NS	3(16.6)	1(25)	NS	3(21.4)	1(12.5)	NS
afa (n = 4)	3(16.6)	1(25)	NS	3(16.6)	1(25)	NS	2(14.2)	2(25)	NS
sfa (n = 2)	1(5.5)	1(25)	NS	1(5.5)	1(25)	NS	1(7.1)	1(12.5)	NS
CNF1 (n = 3)	2(11.1)	1(25)	NS	2(11.1)	1(25)	NS	3(21.4)	0	NS
iroN (n = 1)	8(44.4)	3(75)	NS	11(61.1)	2(50)	**0.013**	9(64.2)	4(50)	NS
hlyA(n = 6)	4(22.2)	2(50)	NS	5(27.7)	1(25)	NS	5(35.7)	1(12.5)	NS

NOR = Norfloxacin, GM = Gentamicin, TMP-SMX = Trimethoprim-sulphamethoxazole, R = Resistant, S = Sensitive, NS = Nonsignificant.

as an agent of diarrhoeal disease since the 1920s [13]. Prevalence of *E. coli* was almost 75% of diarrheogenic samples (75.7% among adults, and 74.2% among children) in Tunisia [14], while prevalence was 22.5% among Vietnamese children under 5 years of age and 132 different strains were identified [15]. Meanwhile, extraintestinal PEC has the ability to cause diverse and serious diseases, such as UTI [16,17].

Meanwhile, UTI is one of the most common infectious diseases that was extensively studied [18]. *E. coli* is the 1st bacteria causing UTI [4]. It constitutes 70–95% in upper and lower UTIs [19], along all year and significantly among female patients [4].

This study aimed at evaluating different phyla and virulent genes in DEC and UEC in patients admitted at Kasr Al-Ainy hospital including their influence on antibiotic susceptibility.

In this study, neither the phyla nor the virulence genes were significantly related to the type of sample. It is a fact that the expressions of different phyla and virulent genes vary geographically [11,20].

In this study, phylum D was the most commonly detected (29.5%), mainly among UEC (9/13; 69.2%), followed by phyla A and B2 (27.3% each), such that 2/3 of the former was detected in DEC, while the latter was comparably detected in both isolates. On the contrary, phylum B1was the least detected (15.9%), mainly (71.4%) detected in DEC.

On the other hand, regarding the virulence genes, hlyA gene was comparably detected in both DEC and UEC, and comparably detected among all the found phyla. hlyA gene was proved as a marker of more PEC i.e haemorrahgic and differentiating factor from other less and non PEC [21].

Regarding antibiotic susceptibility, all isolates from both samples were evidently and broadly resistant to the studied antibiotics. This is similar to the retrospective study done by Tadesse et al. who found the rise of multidrug resistance (≈3 antimicrobial drug classes) in *E. coli* from 7.2% during the 1950s to 63.6% during the 2000s [22].

This was significantly influenced by few virulence genes, while phyla had limited influence. Lately, major increases in emergence and spread of multidrug-resistant *E. coli* and increasing resistance to newer compounds, such as fluoroquinolones are documented [23], that was explained by Hooper, by the mutational resistances that restrict permeability, reduce target sensitivity or increase efflux [24]. Moreover, Batard et al., when studied 16 classes of antibacterial agents and the incidence of quinolone-resistant E. coli isolates, their results revealed that although the level of hospital use of quinolones influenced the incidence of quinolone resistance in E coli hospital isolates, the consumption of 2 other classes of antibiotics like cephalosporins and tetracyclines, was also associated with quinolone resistance [25].

As for DEC, resistance was highest against GM (86.4%), followed by CIP, NA and TMP-SMX (81.8%). That was higher than previous reports e.g Chomvarin et al. found that approximately 50% of the *E. coli* isolates were resistant to at least one antimicrobial agent, and generally high for nearly all antimicrobial agents [26], Also, Livermore a al. stated that the prevalence of ciprofloxacin resistance in E coli from bacteraemia particularly among older males [27]. Resistance to TMP-SMX (69%), and NA (44%). Poor efficacy of TMP ± SMX was also confirmed by Hien et al. 2008 [28].

Also, only the virulence genes fimH and iroN significantly influenced the resistance to all of the studied antibiotics, afa for NA, while phylum A significantly influenced CIP and TMP-SMX. That was similar to Hien et al. 2008 who found a high level of heterogeneity and meanwhile resistance to commonly used antimicrobials in their *E. coli* isolates [28], while Chomvarin et al. found that almost none of the isolates had virulence genes [26]. The African Okeke's. study have revealed the worrisome emergence of antimicrobial resistance and high asymptomatic carriage rates for DEC but bacterial and host factors that predispose to disease, as well as non-human reservoirs, are largely unknown [29].

While UEC in this study, resistance was highest against CIP (90.9%), that may be explained by its abuse [30], followed by NA and GM (86.4%). This is in partial agreement with Cranendonk et al. 2012 who found 97% of *E. coli* were resistant to CIP and 44% to GM in case of bacteraemia [31], and Livermore et al. 2002 who reported rise in the prevalence of CIP resistance in *E. coli* isolates from bacteraemias in England from 0.8% in 1990 to 3.7% in 1999 [32]. Accordingly, CIP was not recommended any more as empirical treatment in *E. coli* bacteraemia. Basu et al. reported higher resistance for NA (87.3%) than CIP (75.5%) [33]. This was against the proven efficacy of quinolones in UTI, particularly with their renal excretion [34]. Even resistance to TMP-SMX was high (81.8%) which was much more higher than that reported by Kahlmeter, et al. i.e ∼10% in both community and hospital settings in north America [30].

As regards UEC, also, only the virulence genes fimH and iroN significantly influenced the resistance to most of the studied antibiotics (fimH influenced all except TMP-SMX, iroN influenced all except LEV, NOR, and TMP-SMX). This partially agreed with Basu et al. 2013 who documented a highly significant and positive correlation between the studied 10 virulence genes and both NA and CIP resistances [33]. To the contrary, Vila et al 2002 stated that quinolones resistant UPEC strains showed overall reduced virulence [35]. On the other hand, phylum D significantly influenced CIP and LEV. This was different from previous results, for example those of Vila e al. [35], and Kawamura-Sato et al. who correlated phylum B2 with the quinolone resistance [36], while Johnson et al., and Johnson et al. found the opposite [37,38]. It is worth noting, as found in an Indian study, that cycling through foods is an important factor in the transmission and the spread of *E. coli* broad antibiotic resistance, (although less than ours; 14.7%, 27.2% of which were of extended spectrum), are easily transferred to other strains as well [39]. Similar findings were reported in a Canadian study [40]. This applies on UEC [41]. In this study, we concluded that the isolated PEC was evidently and broadly resistant to the studied antibiotics, with limited influence of their phyla and virulence genes (fimH and iroN).

Conflict of interest

Authors declare that there was No conflict of interest in this study whether personal or financial.

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