



Molecular characterization of some new *E. coli* strains theoretically responsible for both intestinal and extraintestinal infections

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ABSTRACT

Strains of *E. coli* are divided into 3 major groups; commensal strains, diarrheagenic (intestinal) *E. coli* pathotypes and extraintestinal pathogenic *E. coli*. Extraintestinal pathogenic *E. coli* are unlike diarrheagenic pathotypes, they have not ability to cause intestinal disease in human, but they have normal ability for long-term colonization in the gut. This study aimed to spotlight on that intestinal and extraintestinal infections are not restricted to intestinal pathotypes and extraintestinal pathogenic *E. coli*, respectively. A total of 102 uropathogenic *E. coli* isolates were collected during 2012 and 2015. A multiplex PCR was used to detect phylogenetic groups, virulence factors for extraintestinal pathogenic *E. coli* and intestinal *E. coli* pathotypes genes. Results of this research showed that 12 (11.8%) uropathogenic *E. coli* isolates had genes that are theoretically responsible for intestinal diseases, were 10 of these isolates belonged to phylogenetic group D and 2 isolates to phylogenetic group A. We conclude from these results, this is the first report on the molecular characterization of *E. coli* that theoretically can cause both intestinal and extraintestinal infections simultaneously. The presence of these strains has a great impact on public health. More studies are necessary before definitive conclusions if these strains are a different clone that theoretically have ability to cause both intestinal and extraintestinal infections and belonged to phylogenetic groups other than A and D. Products of diarrheagenic genes in UPEC strains need further studies to detect their effects in intestinal infections.

Keywords: Extraintestinal pathogenic *E. coli*, diarrheagenic *E. coli* pathotypes, commensal *E. coli*.

INTRODUCTION

Escherichia coli (*E. coli*) is one of the most common isolates in medical microbiology laboratories and classified into three major groups: commensal strains, intestinal pathogenic (diarrheagenic) strains, and extraintestinal pathogenic strains.^[1] Intestinal pathotypes of this microorganism including: enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), diffusely adherent *E. coli* (DAEC), and enteroaggregative *E. coli* (EAEC), express potent toxins and cause severe gastroenteritis. In addition, in case of enterohaemorrhagic *E. coli* (EHEC) are also referred as a subset of verocytotoxigenic *E. coli* (VTEC) or shiga-like toxin producing *E. coli* (STEC) and enteropathogenic *E. coli* (EPEC) as, like VTEC, they produce verocytotoxins (VT) and have ability to form attaching and effacing lesions (A/E lesions) on epithelial cells, a characteristic of EPEC. EHEC strains may produce life-threatening systemic illness including hemorrhagic diarrhea, colitis, and hemolytic uremic syndrome (HUS).^[2] Extraintestinal pathogenic *E. coli* (ExPEC) strains, a definition which is based on the site of infection or the habitat, namely outside of the intestine, and the expression of certain virulence-associated genes. These strains are unlike diarrheagenic strains, they are unable to cause gastrointestinal disease in human, but they have normal ability for long-term colonization in the gut.^[3] Strains of ExPEC have been divided into two categories according to the site of infection: uropathogenic *E. coli* (UPEC) and meningitis/sepsis-associated *E. coli* (MNEC).^[2] Molecular epidemiology studies of ExPEC from around the world have identified several potential reservoirs, including the

human intestinal tract, and various nonhuman reservoirs.^[4,5] Extraintestinal *E. coli* infections are believed to arise from human fecal flora.^[6]

E. coli strains can be divided into four phylogenetic groups-A, B1, B2 and D using multiplex PCR method.^[7] Strains of ExPEC usually belong to groups B2 and D and harbor various VFs which allow them to induce diseases in hosts, the intestinal pathogenic strains belong to groups A, B1 and D, while the commensal strains are groups A and B1 in humans.^[8,9]

Strains of ExPEC are phylogenetically and epidemiologically distinct from commensal and diarrheagenic strains. Based on the prevalence of various VFs in clinical *E. coli* isolates, ExPEC were currently defined as *E. coli* isolates containing two or more of the following genes: papA (P fimbriae structural subunit) and/or papC (P fimbriae assembly), sfa/foc (S and F1C fimbriae subunits), afa/dra (Dr-antigenbinding adhesins), kpsMT II (group 2 capsular polysaccharide units), and iutA (aerobactin receptor).^[10] These VFs may be inherited vertically within the resulting virulent clones or transmitted horizontally to other lineages.^[11]

The current research aimed to spotlight on that intestinal infections and extraintestinal infections are not restricted to intestinal pathotypes and ExPEC, respectively.

MATERIALS AND METHODS

Sample Collection

A total 102 isolates of *E. coli* were isolated from inpatients and outpatients from hospitals and private labs from Jenin and Tulkarm governorates.^[12,13] These isolates were distributed as the following: The Martyar Dr. Khalil S. Hospital (n=23; 18 from urine samples, 5 from vaginal swabs), Al-Amal Hospital (n=5; all from urine samples), AL-Razi Hospital (n=14; 11 from urine samples, 3 from vaginal swabs), Al-Shamal Lab (n=7; all from urine samples) and Hi Lab (n=3; all from urine samples) and Thabet Hospital (n=50, all from urine samples). These samples were collected during 2012 and 2015.

DNA extraction

E. coli genome was prepared for PCR according to the method described previously.^[14] 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]), then the pellet was resuspended in 0.5 ml of sterile distilled water, and boiled for 10-15 min. The suspension then was 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 -20°C until use for further DNA analysis.

Detection of phylogenetic groups

Strains were assigned to one of the four *E. coli* phylogenetic groups (A, B1, B2 and D) using a multiplex PCR based on the presence or absence of three DNA fragments: chuA, yjaA, and TspE4C2.^[7] The primer pairs used were chuA.1 (5'-GAC GAA CCA ACG GTC AGG AT-3') and chuA.2 (5'-TGC CGC CAG TAC CAA AGA CA-3'), yjaA.1 (5'-TGA AGT GTC AGG AGA CGC TG-3') and yjaA.2 (5'-ATG GAG AAT GCG TTC CTC AAC-3'), and TspE4.C2.1 (5'-GAG TAA TGT CGG GGC ATT CA-3') and TspE4.C2.2 (5'-CGC GCC AAC AAA GTA TTA CG-3') giving amplification products of 279, 211 and 152 bp respectively. The combination of PCR products allowed phylogenetic group determination of *E. coli* strains. Each PCR reaction mix (25 µl) was performed using 12.5 µl of PCR premix with MgCl₂ (ReadyMixTM Taq PCR Reaction Mix with MgCl₂, 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 94°C followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s, ending with a final extension step at 72°C for 5 min. The PCR products (15 µl) were analyzed by electrophoresis on 1.5% agarose gel. The sizes of the amplicons were determined by comparing them with a 100-bp DNA ladder.

Detection of virulence factors (VFs) for ExPEC

The presence of 18 virulence genes was investigated using multiplex PCR divided into seven pools and their amplicon sizes are listed in Table 1. The genes detected were *fimH*, *afa*, *sfa/foc*, *papG* (three alleles), *cnf1*, *sat*, *hly*, *iutA*, *iroN*, *fyuA*, *iha*, *kpsMTIII*, *ompT*, *malX*, *traT* and *usp*. PCR reaction mix was performed as described in detection of phylogenetic groups. 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 94°C followed by 25 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 1 min, ending with a final extension step at 72°C for 5 min. The PCR products (15 µl) were analyzed by electrophoresis on 1.5% agarose gel. The sizes of the amplicons were determined by comparing them with a 100-bp DNA ladder.

Table 1: Virulence genes for PCR amplification, primer sequences, and amplicon size for extraintestinal *E. coli*^[15]

Virulence gene	Primer forward/reverse	Forward sequence 5'→3'	Reverse sequence 5'→3'	Product size (bp)	Primer mix
fimH malX	FimHf/FimHr malXf/malXr	TGCAGAACGGATAAGCCGTGG GGACATCTGTACAGCGCGCA	GCAGTCACCTGCCCTCCGGTA TCGCCACCAATCACAGCCGAAC	508 930	I
sfaI/foc iutA papGIII fyuA	sfaI/sfa2 AerJf/AerJr allele IIIf/allele IIIr yuaf/fyuar	CTCCGGAGAAGTGGGTGCATCTTAC GGCTGGACATCATGGAACTGG GGCCTGCAATGGATTTACCTGG TGATTAACCCCGCAGCGGAA	CGGAGGAGTAATTACAAACCTGGCA CGTCGGGAACGGGTAGAAATCG CCACCAAATGACCATGCCAGAC CGCAGTAGGCACGATGTTGTA	410 300 258 880	II
hlyA KpsT II papGI	hlyf/hlyr kpsIIf/kpsIIr allele If/allele Ir	AACAAGGATAAGCACTGTTCTGGCT GCGCATTGCTGATACTGTTG TCGTGCTCAGTCCGGAATTT	ACCATATAAGCGGTCATTCCCGTCA CATCCAGACGATAAGCATGAGCA TGGCATCCCCAACATTATCG	1117 272 461	III
traT papGII	TraTf/TraTr allele If/allele IIr	GGTGTGGTGCGATGAGCACAG GGGATGAGCGGCCTTTGAT	CACGGTTCAGCCATCCCTGAG CGGGCCCCAAGTAACTCG	290 190	IV
afa/dra cnfI	Afaf/Afar cnf1/cnf2	GGCAGAGGGCCGCAACAGGC AAGATGGAGTTTCTATGCAGGAG	CCCCTAACCGCCAGCATCTC CATTAGAGTCTGCCCTCATTATT	559 498	V
lha usp	IHAf/IHAr USP81f/USP695r	CTGGCGGAGGCTCTGAGATCA CGGCTCTACATCCGGTGCCTTG	TCCTTAAGCTCCCCGGGCTGA GACATATCCAGCCAGCGAGTTC	827 615	VI
ompT sat iroN	ompTf/ompTr sat 1/sat2 IRONECf/IRONECr	ATCTAGCCGAAGAAGGAGGC ACTGGCGGACTCATGTGT AAGTCAAAGCAGGGGTTGCCCG	CCCGGTCATAGTGTTCATC AACCCTGTAAGAAGACTGAGC0 GACGCCGACATTAAGACGCAG	559 387 665	VII

Table 2: Target genes for PCR amplification, location, amplicon size, primer sequences and annealing temperature for intestinal *E. coli* pathotypes^[16]

Organism	Target Gene	Location	Oligonucleotide sequence (5'→3')	Amplicon Size (bp)	Annealing temperature	Primer mix
EHEC	<i>VT</i>	Chromosome	GAGCGAAATAATTTATATGTG TGATGATGGCAATTCAGTAT	518	59°C	I
EHEC, EPEC	<i>eae</i>	Chromosome	CTGAACGGCGATTACGCGAA CGAGACGATACGATCCAG	917	59°C	I
EPEC	<i>bfpA</i>	Plasmid	AATGGTGTCTTGGCTTGTGC GCCGCTTTATCCAACCTGGTA	326	59°C	I
EAEC	<i>aggR</i>	Plasmid	GTATACAAAAAGAAGGAAGC ACAGAATCGTCAGCATCAGC	254	59°C	I
ETEC	<i>LT</i>	Plasmid	GCACACGGAGCTCCTCAGTC TCCTTCATCCTTTCAATGGCTTT	218	59°C	II
ETEC	<i>ST</i>	Plasmid	GCTAAACCAGTAGAG(C)TCTTCAAAA CCCCGTACAG(A)GCAGGATTACAACA	147	59°C	II
DAEC	<i>daaE</i>	Plasmid	GAACGTTGGTTAATGTGGGGTAA TATTCACCGGTCGGTTATCAGT	542	59°C	II
EIEC	<i>virF</i>	Chromosome	AGCTCAGGCAATGAACTTTGAC TGGGCTTGATATCCGATAAGTC	618	59°C	II
EIEC	<i>ipaH</i>	Plasmid	CTCGGCACGTTTTAATAGTCTGG GTGGAGAGCTGAAGTTTCTCTGC	933	59°C	II

Detection of intestinal *E. coli* pathotypes

The targeted genes for intestinal *E. coli* pathotypes included enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC) were amplified using oligonucleotide primer pairs listed in Table 2. PCR reaction mix was performed as described in detection of phylogenetic groups. DNA amplification was performed as follows: initial denaturation for 2 min at 94°C followed by 40 cycles at 92°C for 30 s for denaturation, annealing at 59°C for 30 s and extension at 72°C for 30 s. Final extension was carried out at 72°C for 5 min. The PCR products (15 µl) were analyzed by electrophoresis on 1.5% agarose gel. The sizes of the amplicons were determined by comparing them with a 100-bp DNA ladder.

Table 3: Characterization of 12 of UPEC strains showed that had genes responsible for intestinal infections

Virulence genes in UPEC	Diarrheagenic genes in UPEC strains												Gene prevalence n (%)
	EAEC (aggR)	EAEC (aggR)	ETEC (LT)	EPEC (eae and bfpA)	EAEC (aggR)	ETEC (ST)	EAEC (aggR)	ETEC (ST)	EAEC (aggR)	ETEC (LT)	ETEC (LT)	ETEC (LT)	
	Phylogenetic group												
	D	A	D	A	D	D	D	D	D	D	D	D	
<i>fimH</i>	1	1	1	1	1	1	1	1	0	1	1	1	11 (91.7)
<i>malX</i>	0	0	0	0	0	1	0	1	0	0	0	0	2 (16.7)
<i>sfa/foc</i>	0	0	0	0	1	0	0	0	0	0	0	0	1 (8.3)
<i>utaA</i>	1	1	1	0	0	1	1	1	1	1	0	1	9 (75)
<i>papGIII</i>	0	0	0	0	0	0	0	0	0	0	0	0	0 (0.0)
<i>fyuA</i>	1	1	1	0	1	1	1	1	1	1	1	0	10 (83.3)
<i>hlyA</i>	0	0	0	0	0	0	0	0	0	0	0	0	0 (0.0)
<i>kpstIII</i>	0	0	0	1	1	1	1	0	1	0	1	1	7 (58.3)
<i>papGI</i>	0	0	0	0	0	0	0	0	0	0	0	0	0 (0.0)
<i>traT</i>	1	1	1	0	1	1	0	1	1	1	1	1	10 (83.3)
<i>papGII</i>	0	0	0	0	0	0	0	1	0	0	0	1	2 (16.7)
<i>afa/dra</i>	1	0	1	0	1	0	0	1	0	0	0	0	4 (33.3)
<i>cnfI</i>	0	0	1	0	1	0	0	1	1	0	0	0	4 (33.3)
<i>iha</i>	1	1	0	0	1	1	1	1	1	0	0	0	9 (58.3)
<i>usp</i>	1	1	0	0	1	1	0	1	1	0	0	0	6 (50)
<i>ompT</i>	0	0	0	1	0	0	0	0	0	0	0	0	1 (8.3)
<i>sat</i>	1	0	0	1	0	1	0	0	0	0	0	0	3 (25)
<i>iroN</i>	1	0	0	1	1	0	0	0	0	1	0	0	4 (33.3)
Total genes/isolate (%)	9 (50)	6 (33.3)	6 (33.3)	5 (27.8)	10 (55.6)	9 (50)	5 (27.8)	10 (55.6)	7 (38.9)	5 (27.8)	4 (22.2)	5 (27.8)	

RESULTS

Results of this research showed that 12 (11.8%) isolates of ExPEC had genes that are theoretically responsible for intestinal diseases. Ten of these isolates belonged to phylogenetic group D and 2 strains belonged to phylogenetic group A. Results are presented in Table 3.

DISCUSSION

Extraintestinal pathogenic *E. coli* do not normally induce diarrhea, and those diarrheagenic strains usually do not cause extraintestinal diseases.^[17] Commensal strains are unable to cause gastrointestinal disease in human under normal immunological conditions, but they have normal ability for long-term colonization in the gut. The human commensal strains of phylogenetic groups A, B1 and D carried fewer VFs than virulent strains of the corresponding groups that caused extraintestinal infection. However, VF patterns for both pathogenic and commensal B2 phylogenetic group strains were the same.^[18] Both B2 and D phylogenetic groups are considered as the origin of many VFs within *E. coli* strains and are the major source for horizontal transfer of VFs into other phylogenetic groups.^[9] It has been reported that pathogenic *E. coli* may be emerged from fecal (commensal) strains by acquisition of specific VFs.^[19] The relationship between the *E. coli* genetic background and the acquisition of VFs is now better understood.^[20-22] The virulence genes in both intestinal and extraintestinal strains are primarily located within mobile genome elements including plasmids, transposons, bacteriophages, and pathogenicity islands.^[23] This phenomenon may contribute to spreading of VFs among related bacterial species.

These strains of this pathogenic group of *E. coli* which carry genes that responsible for causing intestinal and extraintestinal infections may be derived from commensal strains by the acquisition of different VFs by horizontal chromosomal or extra-chromosomal transfer during their presence in intestine, enabling them to become virulent.^[19] These findings have led us to suggest that these isolates have evolved certain characteristics that allow them to survive in the complex ecosystems of the human intestine and extraintestine, intestinal and extraintestinal pathogenicity of these isolates may be a side effect of *E. coli*'s adaptation to the intestinal milieu.

However, more studies are needed to drive definitive conclusions if these strains are a different clone that theoretically have ability to cause both intestinal and extraintestinal infections and belonged to phylogenetic groups other than A and D. Products of diarrheagenic genes in UPEC strains need further studies to detect their effects in intestinal infections. In our study, the molecular analysis of strains belonged to groups A and D in Palestine showed that group D had higher mean virulence score than group A and also more frequent among the strains obtained from

urinary tract infection.^[12,24] However, other study showed that 61% of *E. coli* urine isolates were belonged almost equally to phylogenetic groups B2 and D.^[25]

CONCLUSION

We conclude from these results, this is the first report on the molecular characterization of *E. coli* that theoretically can cause both intestinal and extraintestinal infections simultaneously. The presence of these strains has a great impact on public health.

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