Available online at **www.ijmrhs.com**

ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2016, 5, 6:158-163

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

[*]Ghaleb Adwan, Kamel Adwan and Hiba Bourinee

Department of Biology and Biotechnology, An-Najah National University, P.O. Box (7)-Nablus, Palestine
** Corresponding e-mail: adwang@najah.edu*

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 phylogentic group D and 2 isolates to phylogentic 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 s 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 f*imH*, *afa*, *sfa/foc*, *papG* (three alleles), *cnf1*, *sat*, *hly*, *iutA*, *iroN*, *fyuA*, *iha*, *kpsMTII*, *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	FimHf/FimHr	TGCAGAACGGATAAGCCGTGG	GCAGTCACCTGCCCTCCGGTA	508	I
malX	malXf/malXr	GGACATCCTGTTACAGCGCGCA	TCGCCACCAATCACAGCCGAAC	930	
sfa/foc	sfa1/sfa2	CTCCGGAGAACTGGGTGCATCTTAC	CGGAGGAGTAATTACAAACCTGGCA	410	II
iutA	AerJf/AerJr	GGCTGGACATCATGGGAACTGG	CGTCGGGAACGGGTAGAATCG	300	
papGIII	allele IIIf/allele IIIr	GGCCTGCAATGGATTTACCTGG	CCACCAAATGACCATGCCAGAC	258	
fyuA	yuaf/fyuar	TGATTAACCCCGCGACGGGAA	CGCAGTAGGCACGATGTTGTA	880	
hlyA	hlyf/hlyr	AACAAGGATAAGCACTGTTCTGGCT	ACCATATAAGCGGTCATTCCCGTCA	1117	III
KpsT II	kpsIIf/kpsIIr	GCGCATTTGCTGATACTGTTG	CATCCAGACGATAAGCATGAGCA	272	
papGI	allele If/allele Ir	TCGTGCTCAGGTCCGGAATTT	TGGCATCCCCCAACATTATCG	461	
traT	TraTf/TraTr	GGTGTGGTGCGATGAGCACAG	CACGGTTCAGCCATCCCTGAG	290	IV
papGII	allelele If/allele IIr	GGGATGAGCGGGCCTTTGAT	CGGGCCCCCAAGTAACTCG	190	
afa/dra	Afaf/Afar	GGCAGAGGGCCGGCAACAGGC	CCCGTAACGCGCCAGCATCTC	559	V
cnf1	cnf1/cnf2	AAGATGGAGTTTCCTATGCAGGAG	CATTCAGAGTCCTGCCCTCATTATT	498	
Iha	IHAf/IHAr	CTGGCGGAGGCTCTGAGATCA	TCCTTAAGCTCCCGCGGCTGA	827	VI
usp	USP81f/USP695r	CGGCTCTTACATCGGTGCGTTG	GACATATCCAGCCAGCGAGTTC	615	
ompT	ompTf/ompTr	ATCTAGCCGAAGAAGGAGGC	CCCGGGTCATAGTGTTCATC	559	VII
sat	sat1/sat2	ACTGGCGGACTCATGCTGT	AACCCTGTAAGAAGACTGAGC0	387	
iroN	IRONECf/IRONECr	AAGTCAAAGCAGGGGTTGCCCG	GACGCCGACATTAAGACGCAG	665	

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	*VT*	Chromosome	GAGCGAAATAATTTATATGTG TGATGATGGCAATTCAGTAT	518	59°C	I
EHEC, EPEC	eae	Chromosome	CTGAACGGCGATTACGCGAA CGAGACGATACGATCCAG	917	59°C	I
EPEC	*bfpA*	Plasmid	AATGGTGCTTGCGCTTGCTGC GCCGCTTTATCCAACCTGGTA	326	59°C	I
EAEC	*aggR*	Plasmid	GTATACACAAAAGAAGGAAGC ACAGAATCGTCAGCATCAGC	254	59°C	I
ETEC	*LT*	Plasmid	GCACACGGAGCTCCTCAGTC TCCTTCATCCTTTCAATGGCTTT	218	59°C	II
ETEC	*ST*	Plasmid	GCTAAACCAGTAGAG(C)TCTTCAAAA CCCGGTACAG(A)GCAGGATTACAACA	147	59°C	II
DAEC	*daaE*	Plasmid	GAACGTTGGTTAATGTGGGGTAA TATTCACCGGTCGGTTATCAGT	542	59°C	II
EIEC	virF	Chromosome	AGCTCAGGCAATGAAACTTTGAC TGGGCTTGATATTCCGATAAGTC	618	59°C	II
EIEC	ipaH	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 *bfp*A)	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	
fimH	1	1	1	1	1	1	1	1	0	1	1	1	11 (91.7)
malx	0	0	0	0	0	1	0	1	0	0	0	0	2 (16.7)
sfa/foc	0	0	0	0	1	0	0	0	0	0	0	0	1 (8.3)
utaA	1	1	1	0	0	1	1	1	1	1	0	1	9 (75)
papGIII	0	0	0	0	0	0	0	0	0	0	0	0	0 (0.0)
fyuA	1	1	1	0	1	1	1	1	1	1	1	0	10 (83.3)
hlyA	0	0	0	0	0	0	0	0	0	0	0	0	0 (0.0)
kpstII	0	0	0	1	1	1	1	0	1	0	1	1	7 (58.3)
papGI	0	0	0	0	0	0	0	0	0	0	0	0	0 (0.0)
traT	1	1	1	0	1	1	0	1	1	1	1	1	10 (83.3)
papGII	0	0	0	0	0	0	0	1	0	0	0	1	2 (16.7)
afa/dra	1	0	1	0	1	0	0	1	0	0	0	0	4 (33.3)
cnfI	0	0	1	0	1	0	0	1	1	0	0	0	4 (33.3)
iha	1	1	0	0	1	1	1	1	1	0	0	0	9 (58.3)
usp	1	1	0	0	1	1	0	1	1	0	0	0	6 (50)
ompT	0	0	0	1	0	0	0	0	0	0	0	0	1 (8.3)
sat	1	0	0	1	0	1	0	0	0	0	0	0	3 (25)
iroN	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 phylogentic group D and 2 strains belonged to phylogentic 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.

REFERENCES

[1] Russo TA, Johnson JR. Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli*: ExPEC J Infect Dis. 2000;181:1753-4.
[2] Bekal S, Brousseau R, Masson L, Prefontaine G, Fairbrother J, Harel J. Rapid identification of *Escherichia coli* pathotypes by virulence gene detection with DNA microarrays. J Clin Microbiol. 2003;41(5):2113-25.
[3] Johnson J R, Russo TA. Uropathogenic *Escherichia coli* as agents of diverse non-urinary tract extraintestinal infections. J Infect Dis. 2002;186:859-64.
[4] Smith JL, Fratamico PM, Gunther NW. Extraintestinal pathogenic *Escherichia coli*. Foodborne Pathog Dis. 2007;4(2):134-63.
[5] Manges AR, Johnson JR (2012) Food-borne origins of *Escherichia coli* causing extraintestinal infections. Clin Infect Dis 55(5): 712-719.
[6] Bailey JK, Pinyon JL, Anantham S, Hall RM. Distribution of human commensal Escherichia coli phylogenetic groups. J Clin Microbiol. 2010;48(9):3455-6.
[7] Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol. 2000;66:4555-8.
[8] Pupo GM, Karaolis DKR, Lan R, Reeves PR. Evolutionary relationships among pathogenic and nonpathogenic *Escherichia coli* strains inferred from multilocus enzyme electrophoresis and *mdh* sequence studies. Infect Immun. 1997;65:2685-92.
[9] Johnson JR, Delavari P, Kuskowski M, Stell AL. Phylogenetic distribution of extraintestinal virulence-associated traits in *Escherichia coli*. J Infect Dis. 2001;183:78-88.
[10] Johnson JR, Russo TA. Molecular epidemiology of extraintestinal pathogenic (uropathogenic) *Escherichia coli*. Int J Med Microbiol. 2005;295:383-404.
[11] Johnson JR, O'Bryan TT. Improved repetitive-element PCR fingerprinting for resolving pathogenic and nonpathogenic phylogenetic groups within *Escherichia coli*. Clin Diagn Lab Immunol. 2000;7(2):265-73.
[12] Adwan G, Issa B, Adwan K. Virulence profile, fluoroquinolone and quinolone resistance of uropathogenic *Escherichia coli* isolates recovered from Thabet Hospital-Tulkarm, Palestine. Br Microbiol Res J. 2015;5(5):412-23.
[13] Adwan G, Abu Jaber A. Frequency and molecular characterization of β-lactamases producing *Escherichia coli* isolated from North of Palestine. Br Microbiol Res J. 2016;11(5):1-13, Article no.BMRJ.22631.
[14] Adwan G, Adwan K, Jarrar N, Salama Y, Barakat A (2013) Prevalence of *seg, seh* and *sei* genes among clinical and nasal *Staphylococcus aureus* isolates. Br Microbiol Res J 3(2): 139-149.
[15] Ferjani S, Saidani M, Ennigrou S, Hsairi M, Ben Redjeb S. Virulence determinants, phylogenetic groups and fluoroquinolone resistance in *Escherichia coli* isolated from cystitis and pyelonephritis. Pathol Biol (Paris). 2012;60(5):270-274.
[16] Gómez-Duarte OG, Bai J, Newell E. Detection of *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*, *Vibrio cholerae*, and *Campylobacte*r spp. enteropathogens by 3-reaction multiplex polymerase chain reaction. Diagn Microbiol Infect Dis. 2009;63(1):1-9.
[17] Russo TA, Johnson JR. Medical and economic impact of extraintestinal infections due to *Escherichia coli:* focus on an increasingly important endemic problem. Microbes Infect. 2003;5:449-56.
[18] Duriez P, Clermont O, Bonacorsi S, Bingen E, Chaventré A, Elion J, Picard B, Denamur E. Commensal *Escherichia coli* isolates are phylogenetically distributed among geographically distinct human populations. Microbiol. 2001;147(Pt 6):1671-6.
[19] Ochman H, Lawrence JG, Groisman EA. Lateral gene transfer and the nature of bacterial innovation. Nature. 2000;405:299-304.
[20] Bidet P, Bonacorsi S, Clermont O, De Montille C, Brahimi N, Bingen E. Multiple insertional events, restricted by the genetic background, have led to acquisition of pathogenicity island IIJ96-like domains among *Escherichia coli* strains of different clinical origins. Infect Immun. 2005;73:4081-7.

[21] Escobar-Paramo P, Clermont O, Blanc-Potard AB, Bui H, Le Bouguénec C, Denamur E (2004) A specific genetic background is required for acquisition and expression of virulence factors in *Escherichia coli*. Mol Biol Evol. 2004;21:1085-94.
[22] Li B, Sun JY, Han LZ, Huang XH, Fu Q, Ni YX. Phylogenetic groups and pathogenicity island markers in fecal *Escherichia coli* isolates from asymptomatic humans in China. Appl Environ Microbiol. 2010;76(19): 6698-700.
[23] Kaper JB. Pathogenic *Escherichia coli*. Int J Med Microbial. 2005;295:355-6.
[24] Adwan G, Issa B. Characterization of *Escherichia coli* isolates from patients with urinary tract infections from Thabet Hospital-Tulkarm, Palestine. An-Najah Univ J Res - Nat Sci. 2016;30:31-52.
[25] Adwan K, Jarrar N, Abu-Hijleh A, Adwan G, Awwad, E. Molecular characterization of *Escherichia coli* isolates from patients with urinary tract infections in Palestine. J Med Microbiol. 2014;63:229-34.