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IDENTIFICATION OF VIRULENCE FACTORS AMONG ESBL-PRODUCING ESCHERICHIA COLI CLINICAL ISOLATES FROM GAZA STRIP, PALESTINE

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ABSTRACT

This study was done to assess the occurrence of virulence factors and correlation of phylogenetic grouping in ESBLs producing *E. coli* isolates from Palestine.
Twenty-seven ESBLs producing *E. coli* isolates were collected between April and June 2013 from three hospitals in Gaza. Detection of genes encoding virulence factors, pathogenicity associated island marker (PAI) and phylogenetic groups were studied by PCR. The correlation of *E. coli* phylogenetic grouping with the virulence factors in the isolates was studied. The clonal relationships between the isolates were tested by pulsed-field gel electrophoresis (PFGE).
Overall, 44.4% of the 27 *E. coli* isolates belonged to phylogroup B2, 44.4% to D and 11.2% to A. Among the isolates, *fimH, traT* and *fimA* were the most frequent virulence genes and were found over 85% of the isolates. *PAIs* was found in 8 isolates while the *tcpC* gene was detected in one isolate. The overall prevalence of *PAI* was higher in group B2 as compared to groups A and D ($p<0.043$). A clonal diversity was confirmed among our isolates (27 unrelated PFGE profiles). We report for the first time the prevalence of *tcpC* as a new virulence marker and *PAI* in clinical ESBLs-producing *E. coli* in Palestine.
This study indicates that most of the ESBL- positive isolates showed virulence genes and most strains belonged to phylogenetic groups B2 and D. The study suggested that high number of virulence genes in studied strains may be important factors in the infections development.

Keywords: virulence factors; phylogenetic groups; pathogenicity islands *PAIs*; ESBL producers; antibiotic resistance; *E. coli*

INTRODUCTION

Escherichia coli are common inhabitant microorganism of the human intestinal and various animals. However, some *E. coli* strains can cause urinary tract and blood-stream infections **(Pitout, 2010)**' **(Manges, 2016)**. Extended-spectrum β-lactamases (ESBLs) are enzymes produced by Gram-negative bacteria, confer and increased resistance to β-lactam antibiotics such as ceftazidime, cefotaxime and aztreonam **(Paterson and Bonomo, 2005)**. The overall data on ESBL-containing *E. coli* in many countries of Middle East are worrying and this area may be one of the considerable epicenters of the ESBL pandemic globally **(Tayh *et al.*, 2016a)**.
The *E. coli* capacity enhances by many virulence factors (VFs) to cause infections; extraintestinal *E. coli* strains (ExPEC) carry genes encoding various VFs that can affect processes of host cell such as host defense avoidance mechanisms (capsule or O-specific antigen), combinations of adhesins (S and P fimbriae), toxins (cytonecrotizing and hemolysin factor), and iron acquisition systems (yersiniabactin and aerobactin) **(Ananias and Yano, 2008)**.VFs are important factors in the infections development by different mechanisms such as; help bacteria to attach and invade host cells, lyses host cells via toxins, evade host defenses and sequestering iron from the host which important for bacterial cellular activities. Genes coding for VFs are located with each other on large chromosomal regions recognized as pathogenicity islands (PAIs) **(Bingen-Bidois *et al.*, 2002)**.
Extraintestinal pathogenic *E. coli* differ with commensal according to phylogenetic attributes and virulence determinants **(Johnson and Stell, 2000)**. Recently, studies indicate that the commensal strains belong to phylogenetic groups B1 and A. However, ExPEC belong particularly to group B2 and, to a lower extent, phylogenetic group D **(Zhang *et al.*, 2002) (Duriez *et al.*, 2001)**.
Toll/interleukin-1 (IL-1) receptor (TIR) domain plays an important role in the mammalian to activate innate immunity. Pathogenic microorganisms improved mechanisms to inactivation the TLR dependent host defense and to raise the bacterial virulence for a host **(Nagarjuna *et al.*, 2015a)**. Recently, the TIR homologous protein TcpC has been detected in *E. coli* strains acts as a virulence factor by inhibiting innate host responses, enhancing persistence of bacteria and increase infection severity. In a previous study, they confirmed a role of *tcpC* in the urinary tract infection pathogenesis in *E. coli* but its role in sepsis pathogenesis is unknown **(Cirl *et al.*, 2008)**. In other bacteria, TIR domain contain protein (*BtpB*) is recognized as a virulence factor that control of local inflammatory responses in the enhancement of chronic brucellosis **(Salcedo *et al.*, 2013)**. *SaTlp1* and *SaTlp2* are virulence factor of *Staphylococcus aureus* that interact in mammalian cells with the innate immune signaling **(Spear *et al.*, 2012)**.
The virulence factors in ESBL-positive *E. coli* isolates were studied from different cases in different countries, from healthy school children in India **(Zhang *et al.*, 2002)**, from long-term hospitalized patients in China **(Zhao *et al.*, 2015)** and diarrheic cattle in France **(Wayne, 2014)**.
The objective of this work was to investigate phylogenetic group and major VFs among ESBLs producing *E. coli* isolates from Palestinian hospitals in Gaza Strip. This study is the first investigation of the prevalence of virulence factors, *PAI* and *tcpC* among ESBLs producing *E. coli* isolates and of the association of virulence factors with the phylogenetic group in ESBLs producing *E. coli* isolates in Palestine.

MATERIAL AND METHODS

Isolation and identification

The samples were obtained from patients visiting Palestinian hospitals in Gaza Strip and were transferred to the laboratory for processing. The isolated bacteria were cultured on plates of blood agar and MacConkey agar. Among the clinical isolates were obtained from urinary tract infection and wound infection, 69 were identified as *E. coli* by standard biochemical tests. These isolates were confirmed

by a species PCR of the *uidA* gene which encoding the β-glucuronidase **(Jouini *et al.*, 2010)**. *E. coli* strains were stored in 30% glycerol at -80 °C for further use.

Antibiotic susceptibility testing

Susceptibility testing was performed according to CLSI by the disc diffusion method for the following antimicrobial agents: amoxicillin-clavulanic acid, ampicillin, ceftazidime, cefoxitin, gentamicin, cefotaxime, amikacin, nalidixic acid, tobramycin, ciprofloxacin, kanamycin, imipenem, tetracycline, trimethoprim-sulfamethoxazole, and chloramphenicol (Bio-Rad, France). **(Wayne, 2014)**. The *E. coli* isolates were examined for ESBL production by double-disk synergy method by using a disk of amoxicillin/ clavulanic acid with two antimicrobial disks (ceftazidime and cefotaxime) **(Kaur *et al.*, 2013)**, the ESBL producing isolates were selected for more examinations. *E. coli* ATCC 25922 was used as a control strain.

Isolation of genomic DNA and PCR amplification

All isolates of *E. coli* confirmed by biochemical and molecular tests were sub-cultured on tryptone soy broth at 37° C for 18h. The extraction of the DNA was performed by the standard sodium acetate precipitation method. The concentration and the purity of DNA were determined using a NanoDrop™ spectrophotometer at A260/280 nm.

PCR amplification was performed in 25 µL reaction volume containing 2 µL DNA template, 1× buffer, 1.5 mM $MgCl_2$, 0.4 mM dNTPs, 0.6 µM forward and reverse primers and 1U Taq DNA polymerase (Biomer). PCR was done with a DNA thermal cycle (applied biosystems thermal cycler). The PCR reaction condition was as follows: initial denaturation at 94 °C for 4 min; 30 cycles of denaturation at 94 °C for 45 sec, annealing for 45 sec at specific temperature (Table 1), extension at 72°C for 45 sec; and a final extension (72 °C, 10 min).

Phylogenetic determination in ESBL-producing *E. coli*

PCR amplification was used to detect the presence of two genes (*chuA*, *yjaA*) and a fragment of DNA known as *TspE4C2* to detect phylogenetic grouping (A, B1, B2 and D) of *E.coli* isolates. Primers sequences are given in Table 1. The interpretation of results was done by dichotomous decision tree **(Zhang *et al.*, 2002)**.

Detection of Virulence factors

The *E. coli* pathogenicity is correlating with the VFs that may be encoded by chromosomal and plasmid genes, and in this study 13 genes encoding VFs were studied. The selected genes were *aer* (aerobactin iron uptake system), *cnf1* (cytotoxic necrotizing factor), *bfp* (bundle-forming pilus), *fimA* (encoding type 1 fimbriae), *hlyA* (haemolysin), *stx* (shiga toxin), *papC* (P fimbriae), *papG* allele III (adhesion PapG class III), *eae* (enteropathogenic attachment and effacement), s*fa* (S fimbriae), *afa* (A fimbriae), *traT* (serum resistance), *fimH* (encoding type 1 fimbriae), pathogenicity associated island marker (*PAI*) and the *tcpC* gene encoding VFs often found in pathogenic *E. coli* strains, were tested in the ESBL-positive isolates by PCR using specific primers (Table 1) **(Slama *et al.*, 2011)**.

The PCR was performed in our study including in all cases control positive and negative from the Ambedkar Centre for Biomedical Research (ACBR) and Universite´ Tunis-El Manar collection.

Table 1 Primers and polymerase chain reaction assay for virulence factors and phylogenetic genes

Functional category	Gene	Primer sequence (5'-3')	Size of amplicon (bp)	Annealing temperature (°C)	Reference
Phylogenetic genes					
	chuA	F: GACGAACCAACGGTCAGGAT R: TGCCGCCAGTACCAAAGACA	279	55	**(Slama *et al.*, 2011)**
	yjaA	F: TGAAGTGTCAGGAGACGCTG R: ATGGAGAATGCGTTCCTCAAC	211	55	**(Slama *et al.*, 2011)**
	TspE4C2	F: GAGTAATGTCGGGGCATTCA R: CGCGCCAACAAAGTATTACG	152	55	**(Slama *et al.*, 2011)**
Virulence factors					
Toxins					
Haemolysin	***Hly***	F : AACAAGGATAAGCACTGTTCTGGCT R : ACCATATAAGCGGTCATTCCCGTCA	1177 pb	63	**(Ruiz *et al.*, 2002)**
cytotoxic necrotizing factor	***cnf1***	F :AAGATGGAGTTTCCTATGCAGGAG R : CATTCAGAGTCCTGCCCTCATTATT	498 pb	63	**(Ruiz *et al.*, 2002)**
Shiga toxin	***Stx***	F: CTTCGGTATCCTATTCCCGG R: GGATGCATCTCTGGTCATTG	484 pb	63	**(Tahamtan *et al.*, 2010)**
Adhesins					
P fimbriae	***papC***	F : GACGGCTGTACTGCAGGGTGTGGCG R : ATATCCTTTCTGCAGGGATGCAATA	328 pb	63	**(Ruiz *et al.*, 2002)**
Bundle-forming pilus	***Bfp***	F : ACAAAGATACAACAAACAAAAA R :TTCAGCAGGAGTAAAAGCAGTC	260 pb	63	**(Ruiz *et al.*, 2002)**
P-fimbrialadhesin III	***papGIII***	F : CATTTATCGTCCTCCTCAACTTAG R : AAGAAGGGATTTTGTAGCGTC	482 pb	55	**(Ruiz *et al.*, 2002)**
encoding type 1 fimbriae	***fimA***	F : GTTGTTCTGTCGGCTCTGTC R : ATGGTGTTGGTTCCGTTATTC	447 pb	55	**(Ruiz *et al.*, 2002)**
Enteropathogenic attachment and effacement	***Eae***	F : CATTATGGAACGGCAGGT R : ATCTTCTGCGTACTGCGTTCA	760 pb	55	**(Jouini *et al.*, 2010)**
encoding type 1 fimbriae	***fimH***	F TGCAGAACGGATAAGCCGTGG R GCAGTCACCTGCCCTCCGGTA	506	55	**(Johnson and Stell, 2000)**
S fimbriae	***Sfa***	F CTCCGGAGAACTGGGTGCATCTTAC R CGGAGGAGTAATTACAAACCTGGCA	408	54	**(Birosova *et al.*, 2004)**
A fimbriae	***Afa***	F GCTGGGCAGCAAACTGATAACTCTC R CATCAAGCTGTTTGTTCGTCCGCCG	793	60	**(Birosova *et al.*, 2004)**
Invasion					
aerobactin iron uptake system	***Aer***	F : TACCGGATTGTCATATGCAGACCGT R : AATATCTTCCTCCAGTCCGGAGAAG	602 pb	63	**(Ruiz *et al.*, 2002)**
Serum resistance	***traT***	F GGTGTGGTGCGATGAGCACAG R CACGGTTCAGCCATCCCTGAG	288	57	**(Johnson and Stell, 2000)**
Others					
TIR domain-containing protein (*tcpC*)	***tcpC***	F GAGTGGAAGGAGGTTGAGGC- R GCAGTGCCATTTTATCCGCC	544	55	**(Erjavec *et al.*, 2010)**
Pathogenicity associated island	***PAI***	F GGACATCCTGTTACAGCGCGCA R TCGCCACCAATCACAGCCGAAC	922	57	**(Johnson and Stell, 2000)**

Data analysis

The data of phylogenetic grouping and VFs in ESBLs producing *E. coli* isolates were analyzed by the Statistical Package for the Social Sciences (SPSS) version 17 software (IBM Corporation, Somers, NY). Data comparison was achieved via analysis of Pearson's Chi-square. The level of statistical significance was set at $P< 0.05$.

Pulsed-field gel electrophoresis (PFGE) analysis

Genomic relatedness of the 27 ESBLs positive *E. coli* isolates was determined by PFGE using a restriction enzyme *XbaI*. The resulting restriction patterns of genomic DNA were analyzed with visual method and by GelCompar II software using the UPGMA algorithm and the Dice similarity coefficients **(Slama *et al.*, 2011)**.

RESULTS

In this study we screened 27 ESBL-containing strains from urine (n=19) and wound swabs (n=8) from three Palestinian hospitals for the determination of phylogenetic groups, and important VFs associated sequences responsible for extraintestinal pathogenesis.

Table 2 Virulence profile and phylogenetic groups of 27 ESBLs-producing *E. coli* isolates

Result code	*fimH*	*sfa*	*afa*	*traT*	*tcpC*	*PAI*	*fimA*	*pap C*	*papG III*	*aer*	Phylogenetic groups	Specimen
Ec414	+*	-*	-	+	-	-	+	-	-	-	A	Urine
Ec515	+	-	+	+	-	-	+	-	-	-	D	Wound
Ec621	+	-	-	+	-	-	+	-	-	-	D	Wound
Ec740	+	-	-	+	-	-	+	-	-	-	B2	Urine
Ec855	+	-	-	+	-	+	+	-	-	-	B2	Urine
Ec956	+	-	-	+	-	+	+	-	-	-	B2	Wound
Ec058	+	-	-	+	-	+	+	+	-	-	B2	Urine
Ec168	+	-	-	+	-	+	-	+	-	-	B2	Urine
Ec271	+	-	-	+	-	+	-	+	-	-	D	Urine
Ec375	+	-	-	+	-	-	+	-	-	-	D	Urine
Ec476	+	-	-	+	-	-	+	-	-	-	A	Wound
Ec579	+	+	-	+	-	-	-	-	+	-	D	Wound
Ec683	+	+	-	+	-	+	+	-	-	-	A	Wound
Ec784	+	+	-	+	-	+	+	-	+	-	B2	Urine
Ec892	+	+	-	+	+	+	+	-	-	-	B2	Urine
Ec998	+	+	-	+	-	+	+	+	-	+	D	Urine
Ec0108	+	+	+	+	-	-	+	-	-	-	D	Urine
Ec1116	+	-	+	+	-	-	+	-	-	-	D	Urine
Ec2117	+	+	-	+	-	-	+	-	-	-	D	Urine
Ec3121	+	-	+	+	-	-	+	-	-	-	D	Urine
Ec4126	+	+	+	+	-	+	+	-	-	+	B2	Urine
Ec5134	+	+	+	+	-	-	+	-	-	-	D	Urine
Ec6138	+	-	-	+	-	-	+	+	-	+	B2	Urine
Ec7141	+	-	+	+	-	-	+	+	-	+	D	Wound
Ec8142	+	+	-	+	-	-	+	+	-	+	B2	Wound
Ec147	-	-	-	-	-	-	+	-	-	+	B2	Urine
Ec9151	+	-	+	+	-	+	-	-	-	+	B2	Urine

***** +: Gene presence; ***** -: Gene absence

Antibiotic resistance among the isolates

All 27 *E. coli* isolates were tested to antimicrobial susceptibility against fifteen antibiotics. The percentage of resistance to antibiotics among ESBL positive isolates is shown in Figure 1. All isolates were resistance to cefotaxime and ampicillin. Resistant to sulfamethoxazole/trimethoprim, tetracycline, nalidixic acid, ciprofloxacin, ceftazidime and kanamycin were 77.8%, 74.1%, 66.7%, 59.3%, 51.9% and 51.9% respectively. Imipenem, amikacin and gentamicin were the most effective antibiotics with the studied isolates, where 85.2%, 81.5% and 74.1% were susceptible to imipenem, amikacin and gentamycin respectively. Sensitivity to amoxicillin-clavulanic acid, cefoxitin, tobramycin and chloramphenicol among ESBL producers were 74.1%, 74.1%, 63% and 63% respectively. Most of the isolates were found to be insensitivity to at least one agent in three or more antimicrobial classes, so they are considered as multidrug-resistant (MDR).

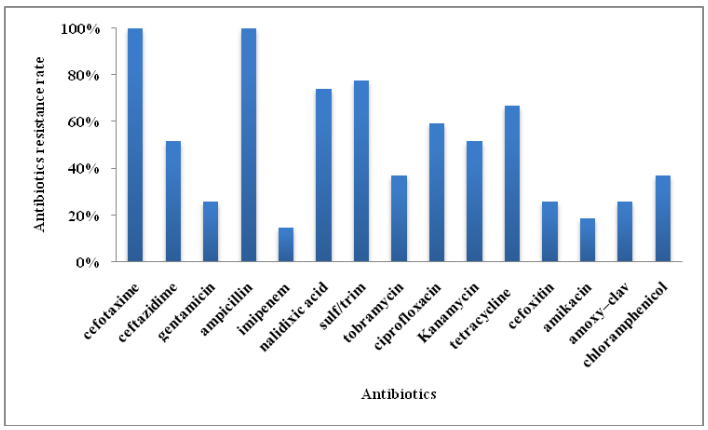

Figure 1 Antibiotics resistance of 27 ESBL-positive *E. coli* isolates

Phylogenetic groups and clonal relationship of isolates

The analysis of phylogenetic grouping was classified into four groups (A, B1, B2, and D). Our study observed that ESBLs positive *E. coli* isolates belonged to the phylogroups B2 (N=12;44.4%), D (N=12;44.4%), and A (N=3; 11%) whereas none of the isolates belonged to phylogroup B1. The PFGE analysis of the *E. coli* isolates demonstrated a wide genetic diversity.

Virulence profile

In this study, genes related to adhesins (*fimA*, *fimH*, *sfa*, *afa*, *papC*, *papGIII*, *bfp*, *eae*), toxins (*hly*, *cnf1*), invasion (*aer*) and protectins, like serum resistance (*traT*) and others (*PAI* and *tcpC*) were investigated. The prevalence of the virulence factors are reported in Table 2 and Figure 2. In regard to adhesins, the *fimH* was the most frequent virulence gene and was found in 96.3% (26/27) of the ESBLs producing *E. coli* isolates followed by *fimA* 85.2% (23/27). The frequency of *sfa* gene, *afa*, *papC*, and *papGIII* among the isolates was 33.3%, 29.6%, 25.9% and 7.4% respectively. *TraT* was detected in most of the isolates 96.3% (26/27). However, *aer* was found in 29.6% of the isolates. None of the isolates harbored *cnf1*, *hlyA*, *eae*, *bfp*, or *stx*. 40.7% of the isolates carried *PAI* associated sequence (11/27), while *tcpC* gene was detected only in one isolate (3.7%).

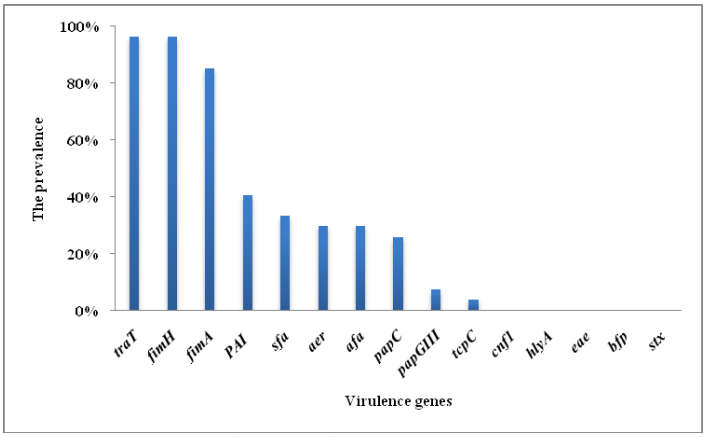

Figure 2 The prevalence of virulence factors among *E. coli* isolates

Correlation of phylogroups with VFs in ESBLs producing *E. coli*

The correlation of phylogenetic grouping with the virulence factors in different infections has been previously reported. The frequency of the virulence genes (*fimA*, *papG* III, *cnf1*, *hlyA*, *aer*, *papC*, *sfa*, *afa*,*traT*, *fimH*, *eae*, and *bfp*, *tcpC* and *PAI*) in the phylogenetic groups (A, B1, B2 and D) was studied. The prevalence of *PAI* in our isolates was higher in group B2 compared to A and D groups ($p<0.043$). However, no clear difference in the prevalence of *afa*, *fimH*, *sfa*, *traT* and *tcpC* in the *E. coli* isolates among various phylogenetic groups was seen. *TcpC* was found in one *E. coli* isolate and was restricted to phylogenetic group B2 (Figure 3). *afa* was found to be prevalent more in group D (N=6) than the group B2 (N=2). However, *aer* was found positive in 6 isolates for group B2 whereas 2 belong to group D (table 3 and figure 3).

Table 3 The frequency of the virulence genes in the phylogenetic groups

virulence genes	Phylogenetic grouping (n = 27)%				*P*-value
	A (%)	B2 (%)	D (%)	Total	
PAI	1 (3.7%)	8 (29.6%)	2 (7.4%)	11 (40.7%)	0.043
FimH	3 (11.1%)	11 (40.7%)	12 (44.4%)	26 (96.3%)	0.523
Sfa	0	4 (14.8%)	5 (18.5%)	9 (33.3%)	0.392
Afa	0	2 (7.4%)	6 (22.2%)	8 (29.6%)	0.099
TraT	3 (11.1%)	11 (40.7%)	12 (44.4%)	26 (96.3%)	0.523
TcpC	0	1 (3.7%)	0	1 (3.7%)	0.523
FimA	3	10 (37%)	10 (37%)	23 (85.2%)	0.746
PapC	0	4 (14.8%)	3 (11.1%)	7 (25.9%)	0.497
Aer	0	5 (18.5%)	2 (7.4%)	7 (25.9%)	0.209

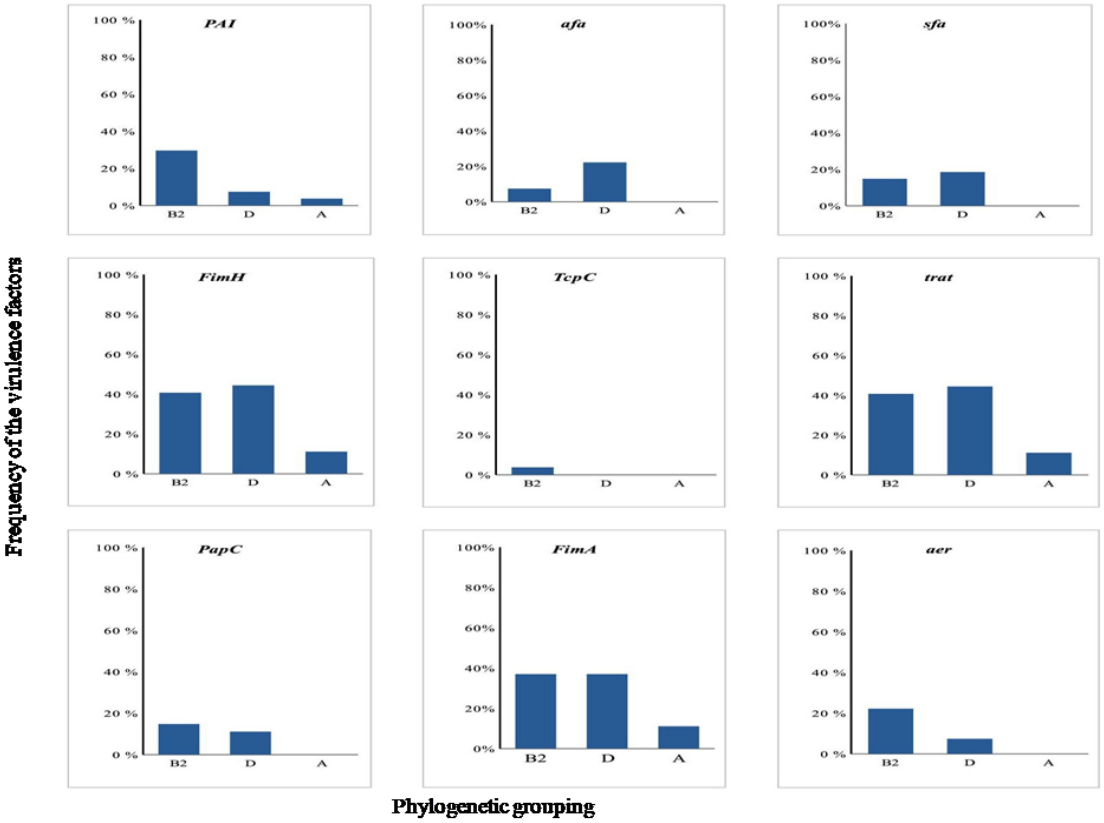

Figure 3 The prevalence of virulence factors among phylogenetic groups of *E. coli* isolates

DISCUSSION

E. coli is a normal microorganism inhabitant of the human intestines and can also cause severe human infections as an opportunistic pathogen. It is the most common Gram-negative bacillus causing infections in the urinary tract and blood-stream **(Pitout, 2010)**. ESBL-positive *E. coli* strains are a major worry problem in many hospitals worldwide, being implicated in many human sporadic infections and causing outbreaks **(Carattoli *et al.*, 2008)**.

Extraintestinal *E. coli* strains owing to a range of virulence factors, such as adhesins, hemolysin and fimbriae, which affect of host cell processes and contribute to bacterial pathogenesis **(Ananias and Yano, 2008)**. The genes encoding virulence factors are usually carried on pathogenicity islands, which have been studied in *E. coli* isolates previously. The pathogenicity of *E. coli* strains enhanced by several virulence factors **(Schmidt and Hensel, 2004)**. They are the products of different genes, which can be detected by the PCR method. In this study, we have studies the antibiotics resistance, phylogenetic grouping and VFs, in 27 ESBL containing *E. coli* isolated from urine and wound swabs from three Palestinian hospitals. The relationship between the presence of VFs and the phylogenetic groups was also analyzed.

The ESBL-containing *E. coli* isolates show co-resistance to many categories of commonly used antibiotics causing in limitation associated with therapeutic (antibiotics) options. Whereas imipenem was the most effective antibiotics with

the studied isolates. Similar reports of gram-negative bacteria were reported from burn units in Palestine **(Elmanama *et al.*, 2013; Tayh *et al.*, 2016b)**. The antibiotics resistance results are very significant for the physicians to choose an appropriate antimicrobial treatment. The higher proportion of resistance and ESBL rate in our study may be a result of the availability of antibiotics without a prescription. Reduction of antibiotic resistance can be by using antibiotics according antimicrobial stewardship guidelines, performing diagnostic testing and antimicrobial susceptibility testing, as well as via developments of new antibiotic **(Lee *et al.*, 2013)**.

Extraintestinal *E. coli* which cause infections have been shown to belong to phylogroups D and B2 **(Smith *et al.*, 2007)**. The results of this study indicated that most isolates belonged to phylogroups B2 (44.4%) and D (44.4%) which is in agreement with previous findings **(Slama *et al.*, 2011)**. Group A was the least frequently isolated phylogenetic group among our isolates, which is in accordance with similar studies **(Girardini *et al.*, 2012)**.

In a recent study in the West Bank, forty-one *E. coli* isolates were obtained from UTI hospitalized patients in three Palestinian hospitals and the results revealed that 13 isolates belonged to phylogroup B2, 12 isolates to group D, 11 isolates to A and five isolates to B1 **(Adwan *et al.*, 2014)**. In a Turkish study, ESBL-positive *E. coli* were distributed in groups D (35%), A (35%) and B2 (30%) **(Yumuk *et al.*, 2008)**. In the Canadian study, it was detected that the majority of ESBL-producing isolates were belonged to group D (63%), while 21% of isolates were derived from A and 13% from group B2 **(Pitout *et al.*, 2005)**.

PAI is highly prevalent among *E. coli* isolates causing extraintestinal infections and mostly belonged to phylogenetic groups D and B2 **(Östblom *et al.*, 2011)**. Our study was proved the prevalence of eleven PAI in *E .coli* strains isolated from the wound infection and urine with the high prevalence of PAI in group B2. Herzer et al. **(Herzer *et al.*, 1990)** reported that *E. coli* caused extraintestinal infections were enriched by PAIs. In a recent study in Estonia, among 432 isolates of phenotypically ESBL producing *E. coli* the PAI was detected in 215 (51%), this gene was identified to be more predominant in phylogenetic group B2 (186 isolates) **(Lillo *et al.*, 2014)**. Carattoli et al. found PAI in all ESBL containing *E. coli* isolates, and most of the isolates belonged to B2 phylogenetic group **(Carattoli *et al.*, 2008)**. In Brazil, 22 PAIs were found among *E. coli* strains and 15 of the total PAIs identified were present in group B2 **(Koga *et al.*, 2014)**. Our study for the first time identifies PAIs in *E. coli* isolates that produce ESBL in Palestine.

The pathogenicity of *E. coli* is a complex multi-factorial mechanism consisting of numerousVFs which vary among the different pathotypes. These factors include invasions, attachment functions, modifying factors of the host cell surface, and other VFs and many different toxins that affect in the target host cells **(Casadevall and Pirofski, 1999)**. The fimbriae factors allow *E. coli* to attach to the mucosa of the small gut where they can transfer their toxins straight to their target **(Kuhnert *et al.*, 2000)**. Genes coding for fimbrial Adhesins [*fimA, fimH, sfa* and *afa*] represent the most common virulence factors of our *E. coli* isolates. Our data showed the highest frequency of *fimH* and *fimA* (96.3% and 85.2%) compared with another fimbrial adensins genes, which may indicate an important role of the virulence genes in *E. coli* causing infection. The high prevalence of fim genes in our isolates is in accordance with other studies **(Ruiz *et al.*, 2002; Micenkova *et al.*, 2014; Nagarjuna *et al.*, 2015b)**. A study in Tunisia, among 18 ESBL positive *E. coli* isolates *fimA* was found in 11 isolates **(Jouini *et al.*, 2010)**.

Concerning P fimbriae (*pap*C), our findings are in agreement with the results of previous clinical studies, approximately 25-35% possess P fimbriae **(Koga *et al.*, 2014)**. In contrast to a study in America, PapC was high prevalent (77%) **(Johnson and Stell, 2000)**. The detection of P fimbriae (*pap*) in South Moravia region of the Czech Republic was reported in 2014 in a study evaluating the presence of virulence factors among 1181 *E. coli* isolates of human fecal origin in which *Pap* was detected in 33.3% (201/603) of extraintestinal pathogenic *E.coli* and 10.6% (19 /179) of Diarrhea-associated *E. coli*, whereas none of the commensal isolates harbored *Pap* **(Micenkova *et al.*, 2014)**.

The distribution of the S fimbriae encoding operons detected in *E. coli* isolates was also similar to previous data **(Micenkova *et al.*, 2014)**. In this study, we observed that 29.6% (n=8) of the *E. coli* isolates were carrying *afa* (afimbrial adhesins). Tarchouna et al. found *afa* gene in 20% of *E. coli* isolates from urine **(Tarchouna *et al.*, 2013)**. Another work revealed that the *afa* gene was found in 2% of *E. coli* strains from urine samples **(Birosova *et al.*, 2004)**. In our study, *afa* was found to be more prevalent in group D than the group B2. Thus our results were in agreement with the previous published data **(Johnson *et al.*, 2005)**.

TraT gene which encodes an outer membrane lipoprotein that participate to serum resistance **(Johnson and Russo, 2002)** was identified in high frequency in 96.3% of the isolates. The high predominance of the *traT* studied in *E. coli* strains may be indicating its function in the development of infection. In India, the results obtained were similar to our findings; the prevalence of *traT* in *E. coli* was 84.5% **(Nagarjuna *et al.*, 2015b)**. An American study, among 75 urosepsis isolates of *E. coli*, *traT* was found in 68% of the isolates **(Johnson and Stell, 2000)**. The invasion factor encoded by the *aer* gene (Iron uptake) was demonstrated in 29.6% of *E. coli* isolates. The high effectiveness of iron uptake system is mediated by the siderophore aerobactin. The existence of a siderophore may be a significant factor in the infection development **(Kuhnert *et al.*, 2000)**. The frequency of *aer* gene was detected in 30% of the *E. coli* isolates causing cystitis in a Spinach study, and this is in agreement with our findings **(Ruiz *et al.*, 2002)**. However, in a study in France, *aer* gene was higher than that of our study (80%) **(Bingen-Bidois *et al.*, 2002)**. In another study, that has been done in a Tunisian hospital, it was observed that *aer* gene was found in 85% of ESBL-containing *E. coli* strains **(Slama *et al.*, 2011)**. In our isolates most of *aer* positive isolates belongs to group B2 and this is in agreement with previous study **(Bingen-Bidois *et al.*, 2002)**.

The *TcpC* gene was detected in only one isolate. *TcpC* was first detected by Cirl et al. and they found that *tcpC* homologous sequences were confirmed in 40% and 21% of *E. coli* strains from pyelonephritis and cystitis respectively. Their findings proposed that *tcpC* increases the severity of urinary tract infection in humans and they provided the first proof that *E.coli* can survive and spread in the host by interfering with TLR signaling **(Cirl *et al.*, 2008)**. The rate of *tcpC* was lower among our isolates, in comparison with the finding of other studies; In Slovenia, they reported that they detected *tcpC* in 49 (23%) of the pathogenic *E. coli* isolates **(Erjavec *et al.*, 2010)**. In a recent study, they investigated the prevalence of the *tcpC* gene in blood and fecal *E. coli* strains from India. They found the prevalence of *tcpC* gene in the phylogenetic groups B2 and D was higher (40.3%) than the B1 and A groups (9.6%) **(Nagarjuna *et al.*, 2015a)**.

CONCLUSION

In conclusion, we investigated phylogenetic grouping and VFs profile in ESBLs-producing *E. coli* isolates from Palestine. To the best of our knowledge, this is the first report *tcpC* as new virulence marker and *PAI* in clinical ESBLs-positive *E. coli* in Palestine. The results indicate that *E. coli* from phylogenetic groups B2 and D were predominate in our isolates. Most of the ESBL- positive *E. coli* strains showed virulence genes. *FimH*, *traT* and *fimA* were highly prevalent among the isolates. This study exhibited that a high number of virulence factors in *E. coli* strains may be significant factors in the development of infection. The isolates show resistance to many classes of commonly used antibiotics causing in limitation associated with therapeutic options. A good recognition of the virulence factors and antimicrobial susceptibility of clinical ESBL-positive *E. coli* can contribute in early diagnosis and treatment of these infections. The findings are important in choice the most effective antibiotic based on antibiogram and application of strategies of infection prevention and control.

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Declarations:

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Ethical clearance: This research study was confirmed by the local Helsinki Committee in Gaza strip, an agreement was taken from patients to participate freely in this study.

REFERENCES

Adwan, K., Jarrar, N., Abu-Hijleh, A., Adwan, G. & Awwad, E. (2014). Molecular characterization of *Escherichia coli* isolates from patients with urinary tract infections in Palestine. Journal of medical microbiology, 63(2), 229-234. http://doi.org/10.1099/jmm.0.067140-0

Ananias, M., & Yano, T. (2008). Serogroups and virulence genotypes of *Escherichia coli* isolated from patients with sepsis. Brazilian Journal of Medical and Biological Research, 41(10), 877-883. http://doi.org/10.1590/S0100-879X2008001000008

Bingen-Bidois, M., Clermont, O., Bonacorsi, S. p., Terki, M., Brahimi, N. m., Loukil, C., Barraud, D. & Bingen, E. (2002). Phylogenetic analysis and prevalence of urosepsis strains of *Escherichia coli* bearing pathogenicity island-like domains. Infection and immunity, 70(6), 3216-3226. http://doi.org/10.1128/IAI.70.6.3216-3226.2002

Birosova, E., Siegfried, L., Kmet'ova, M., Makara, A., Ostro, A., Gresova, A., Urdzik, P., Liptakova, A., Molokacova, M. & Bartl, R. (2004). Detection of virulence factors in α-haemolytic *Escherichia coli* strains isolated from various clinical materials. Clinical microbiology and infection, 10(6), 569-573. http://doi.org/10.1111/j.1469-0691.2004.00922.x

Carattoli, A., Garcia-Fernandez, A., Varesi, P., Fortini, D., Gerardi, S., Penni, A., Mancini, C. & Giordano, A. (2008). Molecular epidemiology of *Escherichia coli* producing extended-spectrum β-lactamases isolated in Rome, Italy. Journal of clinical microbiology, 46(1), 103-108. http://doi.org/10.1128/JCM.01542-07

Casadevall, A., & Pirofski, L.-a. (1999). Host-pathogen interactions: redefining the basic concepts of virulence and pathogenicity. Infection and Immunity, 67(8), 3703-3713.

Cirl, C., Wieser, A., Yadav, M., Duerr, S., Schubert, S. r., Fischer, H., Stappert, D., Wantia, N., Rodriguez, N. & Wagner, H. (2008). Subversion of

Toll-like receptor signaling by a unique family of bacterial Toll/interleukin-1 receptor domain-containing proteins. Nature medicine, 14(4), 399-406. http://doi.org/10.1038/nm1734

Duriez, P., Clermont, O., Bonacorsi, S. p., Bingen, E., Chaventre, A., Elion, J., Picard, B. & Denamur, E. (2001). Commensal *Escherichia coli* isolates are phylogenetically distributed among geographically distinct human populations. Microbiology, 147(6), 1671-1676. http://doi.org/10.1099/00221287-147-6-1671

Elmanama, A. A., Al Laham, N. A. & Tayh, G. A. (2013). Antimicrobial susceptibility of bacterial isolates from burn units in Gaza. Burns, 39(8), 1612-1618. http://doi.org/10.1016/j.burns.2013.04.011

Erjavec, M. S., Jesenko, B., Petkovsek, Z. & Zgur-Bertok, D. (2010). Prevalence and associations of tcpC, a gene encoding a Toll/interleukin-1 receptor domain-containing protein, among *Escherichia coli* urinary tract infection, skin and soft tissue infection, and commensal isolates. Journal of clinical microbiology, 48(3), 966-968. http://doi.org/10.1128/JCM.01267-10

Girardini, L. K., Siqueira, F. M., Krewer, C. C., Krewer, C. C., Costa, M. M. d. & Vargas, A. C. d. (2012). Phylogenetic and pathotype analysis of *Escherichia coli* swine isolates from Southern Brazil. Pesquisa Veterinaria Brasileira, 32(5), 374-378. http://doi.org/10.1590/S0100-736X2012000500002

Herzer, P. J., Inouye, S., Inouye, M. & Whittam, T. S. (1990). Phylogenetic distribution of branched RNA-linked multicopy single-stranded DNA among natural isolates of *Escherichia coli*. Journal of bacteriology, 172(11), 6175-6181. http://doi.org/10.1128/jb.172.11.6175-6181.1990

Johnson, J. R., & Stell, A. L. (2000). Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. Journal of Infectious Diseases, 181(1), 261-272. http://doi.org/10.1086/315217

Johnson, J. R., & Russo, T. A. (2002). Uropathogenic *Escherichia coli* as agents of diverse nonâ€"urinary tract extraintestinal infections. Journal of Infectious Diseases, 186(6), 859-864. http://doi.org/10.1086/342490

Johnson, J. R., Kuskowski, M. A., O'Bryan, T. T., Colodner, R. & Raz, R. (2005). Virulence genotype and phylogenetic origin in relation to antibiotic resistance profile among *Escherichia coli* urine sample isolates from Israeli women with acute uncomplicated cystitis. Antimicrobial agents and chemotherapy, 49(1), 26-31. http://doi.org/10.1128/AAC.49.1.26-31.2005

Jouini, A., Ben Slama, K., Vinue, L., Ruiz, E., Saenz, Y., Somalo, S., Klibi, N., Zarazaga, M., Ben Moussa, M. & Boudabous, A. (2010). Detection of unrelated *Escherichia coli* strains harboring genes of CTX-M-15, OXA-1, and AAC (6')-Ib-cr enzymes in a Tunisian hospital and characterization of their integrons and virulence factors. Journal of Chemotherapy, 22(5), 318-323. http://doi.org/10.1179/joc.2010.22.5.318

Kaur, J., Chopra, S. & Sheevani, G. M. (2013). Modified double disc synergy test to detect ESBL production in urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae*. Journal of clinical and diagnostic research: JCDR, 7(2), 229. http://doi.org/10.7860/JCDR/2013/4619.2734

Koga, V. L., Tomazetto, G., Cyoia, P. S., Neves, M. S., Vidotto, M. C., Nakazato, G. & Kobayashi, R. K. T. (2014). Molecular screening of virulence genes in extraintestinal pathogenic *Escherichia coli* isolated from human blood culture in Brazil. BioMed research international, 2014. http://doi.org/10.1155/2014/465054

Kuhnert, P., Boerlin, P. & Frey, J. (2000). Target genes for virulence assessment of *Escherichia coli* isolates from water, food and the environment. FEMS microbiology reviews, 24(1), 107-117. http://doi.org/10.1111/j.1574-6976.2000.tb00535.x

Lee, C.-R., Cho, I. H., Jeong, B. C. & Lee, S. H. (2013). Strategies to minimize antibiotic resistance. International journal of environmental research and public health, 10(9), 4274-4305.

Lillo, J., Pai, K., Balode, A., Makarova, M., Huik, K., KÃµljalg, S., Ivanova, M., Kaftyreva, L., Miciuleviciene, J. & Naaber, P. (2014). Differences in extended-spectrum beta-lactamase producing *Escherichia coli* virulence factor genes in the Baltic Sea region. BioMed research international, 2014. http://doi.org/10.1155/2014/427254

Manges, A. (2016). *Escherichia coli* and urinary tract infections: the role of poultry-meat. Clinical Microbiology and Infection, 22(2), 122-129. http://doi.org/10.1016/j.cmi.2015.11.010

Micenkova, L., Staudova, B., Bosak, J., Mikalova, L., Littnerova, S., Vrba, M., Sevcikova, A., Woznicova, V. & Smajs, D. (2014). Bacteriocin-encoding genes and ExPEC virulence determinants are associated in human fecal *Escherichia coli* strains. BMC microbiology, 14(1), 1. http://doi.org/10.1186/1471-2180-14-109

Nagarjuna, D., Dhanda, R. S., Gaind, R. & Yadav, M. (2015a). tcpC as a prospective new virulence marker in blood *Escherichia coli* isolates from sepsis patients admitted to the intensive care unit. New microbes and new infections, 7, 28-30. http://doi.org/10.1016/j.nmni.2015.05.002

Nagarjuna, D., Mittal, G., Dhanda, R. S., Verma, P. K., Gaind, R. & Yadav, M. (2015b). Faecal *Escherichia coli* isolates show potential to cause endogenous infection in patients admitted to the ICU in a tertiary care hospital. New microbes and new infections, 7, 57-66. http://doi.org/10.1016/j.nmni.2015.05.006

Östblom, A., Adlerberth, I., Wold, A. E. & Nowrouzian, F. L. (2011). Pathogenicity island markers, virulence determinants malX and usp, and the capacity of *Escherichia coli* to persist in infants' commensal microbiotas. Applied and environmental microbiology, 77(7), 2303-2308. http://doi.org/10.1128/aem.02405-10

Paterson, D. L., & Bonomo, R. A. (2005). Extended-spectrum β-lactamases: a clinical update. Clinical microbiology reviews, 18(4), 657-686. http://doi.org/10.1128/CMR.18.4.657-686.2005

Pitout, J. D. D. (2010). Infections with extended-spectrum β-lactamase-producing Enterobacteriaceae. Drugs, 70(3), 313-333. http://doi.org/10.2165/11533040-000000000-00000

Pitout, J. D. D., Laupland, K. B., Church, D. L., Menard, M. L. & Johnson, J. R. (2005). Virulence factors of *Escherichia coli* isolates that produce CTX-M-type extended-spectrum β-lactamases. Antimicrobial agents and chemotherapy, 49(11), 4667-4670. http://doi.org/10.1128/AAC.49.11.4667-4670.2005

Ruiz, J., Simon, K., Horcajada, J. P., Velasco, M., Barranco, M., Roig, G., Moreno-Martanez, A., Martanez, J. A., de Anta, T. J. & Mensa, J. (2002). Differences in virulence factors among clinical isolates of *Escherichia coli* causing cystitis and pyelonephritis in women and prostatitis in men. Journal of clinical microbiology, 40(12), 4445-4449. http://doi.org/10.1128/jcm.40.12.4445-4449.2002

Salcedo, S., Marchesini, M. I., Degos, C., Terwagne, M., Von Bargen, K., Lepidi, H., Herrmann, C. K., Santos Lacerda, T. L., Imbert, P. & Pierre, P. (2013). BtpB, a novel Brucella TIR-containing effector protein with immune modulatory functions. Frontiers in cellular and infection microbiology, 3, 28.

Schmidt, H., & Hensel, M. (2004). Pathogenicity islands in bacterial pathogenesis. Clinical microbiology reviews, 17(1), 14-56. http://doi.org/10.1128/cmr.17.1.14-56.2004

Slama, K. B., Sallem, R. B., Jouini, A., Rachid, S., Moussa, L., Saenz, Y., Estepa, V., Somalo, S., Boudabous, A. & Torres, C. (2011). Diversity of genetic lineages among CTX-M-15 and CTX-M-14 producing *Escherichia coli* strains in a Tunisian hospital. Current microbiology, 62(6), 1794-1801. http://doi.org/10.1007/s00284-011-9930-4

Smith, J. L., Fratamico, P. M. & Gunther, N. W. (2007). Extraintestinal pathogenic *Escherichia* coli. Foodborne pathogens and disease, 4(2), 134-163. http://doi.org/10.1089/fpd.2007.0087

Spear, A. M., Rana, R. R., Jenner, D. C., Flick-Smith, H. C., Oyston, P. C., Simpson, P., Matthews, S. J., Byrne, B. & Atkins, H. S. (2012). A Toll/interleukin (IL)-1 receptor domain protein from Yersinia pestis interacts with mammalian IL-1/Toll-like receptor pathways but does not play a central role in the virulence of Y. pestis in a mouse model of bubonic plague. Microbiology, 158(6), 1593-1606.

Tahamtan, Y., Hayati, M. & Namavari, M. M. (2010). Prevalence and distribution of the stx1, stx2 genes in Shiga toxin producing *E. coli* (STEC) isolates from cattle. Iranian journal of microbiology, 2(1), 9-14.

Tarchouna, M., Ferjani, A., Ben-Selma, W. & Boukadida, J. (2013). Distribution of uropathogenic virulence genes in *Escherichia coli* isolated from patients with urinary tract infection. International Journal of Infectious Diseases, 17(6), e450-e453. http://doi.org/10.1016/j.ijid.2013.01.025

Tayh, G., Sallem, R. B., Yahia, H. B., Gharsa, H., Klibi, N., Boudabous, A. & Slama, K. B. (2016a). First report of extended-spectrum β-lactamases among clinical isolates of *Escherichia coli* in Gaza Strip, Palestine. Journal of Global Antimicrobial Resistance, 6, 17-21. http://doi.org/10.1016/j.jgar.2016.01.013

Tayh, G. A., Laham, A., Nahed, A., Elmanama, A. A. & Slama, K. B. (2016b). Occurrence and antimicrobial susceptibility pattern of ESBL among Gram-negative bacteria isolated from burn unit of Al Shifa hospital in Gaza, Palestine. The International Arabic Journal of Antimicrobial Agents, 5(3). http://dx.doi.org/10.3823/775

Wayne, P. (2014). CLSI performance standard of antimicrobial susceptibility testing: twenty-fourth international supplement. CLSI Document M100-S24, Clinical and Laboratory Standard Institute.

Yumuk, Z., Afacan, G., Nicolas-Chanoine, M.-H. l. n., Sotto, A. & Lavigne, J.-P. (2008). Turkey: a further country concerned by community-acquired *Escherichia coli* clone O25-ST131 producing CTX-M-15. Journal of antimicrobial chemotherapy, 62(2), 284-288. http://doi.org/10.1093/jac/dkn181

Zhang, L., Foxman, B. & Marrs, C. (2002). Both urinary and rectal *Escherichia coli* isolates are dominated by strains of phylogenetic group B2. Journal of clinical microbiology, 40(11), 3951-3955. http://doi.org/10.1128/JCM.40.11.3951-3955.2002

Zhao, R., Shi, J., Shen, Y., Li, Y., Han, Q., Zhang, X., Gu, G. & Xu, J. (2015). Phylogenetic distribution of virulence genes among ESBL-producing uropathogenic *Escherichia coli* isolated from long-term hospitalized patients. Journal of clinical and diagnostic research: JCDR, 9(7), DC01. http://doi.org/10.7860/JCDR/2015/13234.6157