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Prevalence of virulence factors among different phylogenetic groups of *Escherichia coli* isolated from patients with urinary tract infections

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Abstract

In this study, the biofilm formation, virulence factors and phylogenetic Groups of 42 *E. coli* isolates isolated from patients with UTI were determined. It was revealed that (16.7%) of the isolates were α-hemolytic, (21.4%) were β-hemolytic while , (61.9%) were γ-hemolytic.
Additionally, the percentage of biofilm formation was 50% for both weak and moderate biofilm formation. The results of molecular detection of virulence gene revealed that *afa*gene was found in (21.21%), *kps*gene was found in (84.84%), *pap* gene was found in (6.06%) and *sfa*gene in (60.60%) of multi drug resistance isolates. The phylogenetic group genes (*chuA*, *yjaA,* and TspE4.C2) were found in multi-drug resistance isolates in percentages (72.72%, 87.87% and 72.72%) respectively. Most isolates were included under the phylogeny group B2 (69.7%) of all multi drug resistance *E. coli* isolates while the percentages of the rest of the groups were A (21.21%), B1 (6.06%) and D (3.03%) of multi drug resistance isolates.

Keywords : biofilm , *E.coli*, UTI, phylogenetic **.**

Introduction.

E. coli is the most common cause of urinary tract infections (UTIs), including acute cystitis, pyelonephritis, and urosepsis, three common and clinically distinct UTI syndromes. It is widelyaccepted that uropathogenic*E. coli* (UPEC) originates from the distal gut microbiota [1]. Thebiological properties which may contribute to the pathogenicity of *E. coli* includes: adhesion toepithelial cells, production of enterotoxins, invasiveness and ability to multiply within the epithelialcells, insensitivity to complement lysis or inability to activate the

alternative pathway of complement,and resistance to phagocytic killing [2]. Virulence factors (VFs) associated with UPEC includes adhesins (P fimbriae, type 1 fimbriae, Sfimbriae and a fimbrialadhesin) to detection of these genes by specific primers in a polymerase chainreaction [3]. Determination of *E. coli* phylogenetic type is being of great epidemiological importancebecause there is a relation between the genetic background and the type of the extended spectrum β-lactamase [4]. *E. coli* strains are grouped into four major phylogenetic groups, designated A, B1, B2,and D and there is a relationship between the phylogeny and virulence [5].The virulent extra intestinalstrains belong mainly to B2 and to a lesser extent to D group, whereas most commensal strains belongto groups A and B1 [6], whilst the intestinal pathogenic strains belong to groups A, B1 and D [7]. Theaim of the present study was to determine the relationship between phylogenetic groups and virulencefactors of *E. coli* isolated from patients with UTI.

Material and Methods

2.1. Bacterial isolates

Forty-two *E. coli* isolates were recovered from urine samples of patients from two public hospitals inBabylon/ Iraq between November 2015 and February 2016. All *E. coli* isolates were identified bytypical morphology, routine biochemical tests and Vitek2 System as previously described [8]. Out ofthese 42 isolates, there were 33 (79%) that expressed mutli-drug resistance (MDR).

2.2. Hemolysin Production

The ability of the UPEC isolates to induce hemolysis on blood agar (Merck) was evaluated to detectthe hemolytic isolates. The bacteria were inoculated onto 5% sheep blood agar and incubatedovernight at 37°C. The appearance of a clear zone around the colony indicates β-hemolysis while thepresence of green-color indicates α-hemolysis [9].

2.3. Biofilm assay

Quantitative biofilm assay by spectrophotometric method as described [10, 11] used for detection ofbacterial ability for biofilm formation. This method included inoculation each of the tested isolatesinto 5ml of Brain Heart Infusion broth (Himedia, India) and overnight incubation at 37ºC, after that,the inoculation of into 96-microtiter wells plate containing 180µl of BHI broth with 2% glucose with20µl of *E. coli* of overnight cultures. Negative control wells contained the broth only. Plates wereIncubated for 24 hrs at 37ºC. Culture were removed and the wells were rinsed with PBS (pH 7.2), afterdrying at room temperature for 15 min. 200µl per well of crystal violet stain (1%) were added to eachwell and kept for 20 min. The stained biofilms were rinsed three times with PBS (pH 7.2), and allowed to dry at room temperature for 15 min., then extracted with 200 µl per well of 95% ethanol, and O.D at630 of each well was read using automatic microtiter plate reader. All assays were performed intriplicate. The average O.D values were calculated for all tested isolates and negative controls. **a)** Thecut-off value (O.Dc) was established, it was defined as a three standard deviations (SD) above the O.Dmean of the negative control. The final O.D values of a tested isolate was expressed as average O.Dvalue of the isolate reduced by O.Dc value. O.D = average O.D ofisolate – O.Dc. **b)** The biofilm results of isolates were divided into thefollowing: Non-biofilmproducer (O.D ≤ O.Dc); weak-biofilm producer (O.Dc< O.D ≤ 2xO.Dc); moderate-biofilm producer(2xO.Dc < O.D ≤ 4xO.Dc); strong biofilm producer (4xO.Dc < O.D).

2.4 Detection of virulence genes2.4.1. DNA extraction

Genomic DNA was extracted from the selected bacterial isolates by the Gentrapuregene Bact. /kit(Qiagen/ UAS) according to the instructions of the manufacturer. DNA purity and concentrations weremeasured by Nano Drop-spectrophotometer.

2.4.2. PCR amplification of virulence genes
Virulence genes characteristic were detected by PCR assays. The following genes were targeted; *pap*(P fimbriae), *sfa*(S fimbriae), *afa*(A fimbriae) and *kps*(Capsular polysaccharide). Determination of*pap*, *afa*, *sfa*and *kps*genes by uniplex-PCR technique. Primer sequences for amplification of theseindividual virulence genes are listed in Table (1). The reaction was carried out by using a 25µl reactionvolume, including, 12.5µl Taq Green master mix,1.5µl of each *pap*, *afa*, *sfa*and *kps*primers(10pmol),3µl of template DNA, and the volume was completed by nuclease free water.
The PCR was performed with a PCR thermal cycler (Cleaver Scientific/UK) under the followingconditions: to *pap* and *afa*genes: Initial denaturation for 1 minutes at 94°C; 30 cycles (1 minuteDenaturation at 94°C, and annealing for 30 sec at 63°C, 90 sec at 72°C), and a final extension cyclefor 5 minutes at 72°C. While *sfa*gene performed with a PCR thermal cycler under the followingconditions: Initial denaturation for 5 minutes at 94°C; 30 cycles (30 sec denaturation at 94°C, andannealing for 30 sec at 60°C, 30 sec at 72°C), and a final extension cycle for 7 minutes at 72°C. *kps*gene performed with a PCR thermal cycler under the following conditions: Initial denaturation for 4minutes at 95°C; 40 cycles (1 minutes denaturation at 95°C, and annealing for 1 minutes at 56°C, 1minutes at 72°C), and a final extension cycle for 8 minutes at 72°C. All PCR products were visualizedin a gel document system (Biometra/Germany).

Table 1. Sequence of primers for virulence genes and Phylogenetic Groups

PrimerPrimer Sequence 5′-3′Product References Name Size/bp
*pap*F-GCA ACA GCA ACG CTG GTT GCA TCAT336[12]R-AGA GAG AGC CAC TCT TAT ACG GAC A
*sfa*F-CTC CGG AGA ACT GGG TGC ATC TTA C 410[13]
R-CGG AGG AGT AAT TAC AAC CTG GCA
*afa*F-GCT GGG CAG CAA ACT GAT AAC TCT C750[12]
R-CAT CAA GCT GTT TGT TCG TCC GCC CG
*kps*F-CCA TCG ATA CGA TCA TTG CAC 40[14]R-ATT GCA AGG TAG TTC AGA CTC
*chuA*F-GAC GAA CCA ACG GTC AGG AT 279[15]
R-TGC CGC CAG TAC CAA AGA CA
yjaA F-TGA AGT GTC AGG AGA CGC TG211[15]
R-ATG GAG AAT GCG TTC CTC AAC
TspE4.C2 F-GAG TAA TGT CGG GGC ATT CA152[15]
R-CGC GCC AAC AAA GTA TTA CG

2.5. Phylogenetic Groups
Three markers were used according as described [15] in a rapid technique for determining the phylogenetic groups (A, B1, B2 and D) of *E. coli* strains based on PCR detection of the *chuA*and *yjaA*genes and DNA fragment TspE4.C2 table (1). The reaction was carried out using a 25µl mixtureincluding 12.5µl Taq Green master mix, 1.5µl of each *chuA ,yjaA*and TspE4.C2 primers (10 pmol),3µl template DNA and the volume was completed with nuclease free water. The PCR was performedwith a PCR thermal cycler under the following conditions, Initial denaturation for 5 minutes at 94°C;30 cycles (30 sce denaturation at 94°C, and annealing for 30 sec at 55°C, 30 sec at 72°C), and a finalextension cycle for 7 minutes at 72°C.

The results of triplex PCR product made it possible to establish a dichotomous decision tree forphylogenetic grouping (figure 1) as follows:

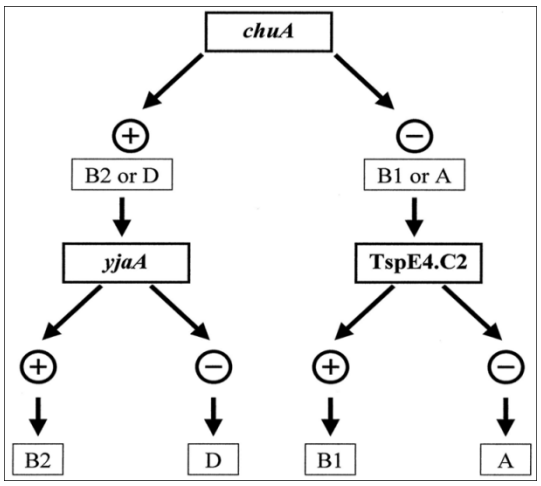

Figure 1. Dichotomous decision tree to determine the phylogenetic group of an *E. coli* strain by using the results of PCR amplification of the *chuA* and *yjaA* genes and DNA fragment TspE4.C2, [15].

2.6. Statistical analysis

SPSS version 23.0 statistical software package was used for statistical analysis by using Chi-squaretest and t-test for all obtained results and test of proportions, p value ≤ 0.05 and 95% confidenceinterval (CI) were considered statistically significant [16].

Results and Discussion.

3.1. Hemolysin Production

Hemolysin is a cytolytic toxin, a protein secreted by some *E. coli* isolates which can produce severaltypes of hemolysin, including an extracellular protein (α-hemolysin), a cell-bound protein (β-hemolysin) and a hemolysin expressed by nalidixic acid-resistant mutants (γ-hemolysin) [17]. γ-hemolysin cannot hemolyse human or rabbit red blood cells, unlike α- and β-hemolysins [18]. Destruction of red blood cells due to hemolysin production also is an importantcharacteristic of *E. coli* colonies on the surface of blood agar [19].

The results revealed that out of 42 *E. coli* isolates, 26 (61.90%) were γ-hemolytic which was the mostcommon, followed by 9 β-hemolytic (21.40%), and 7 α-hemolytic (16.7%) isolates as show in figure(2).

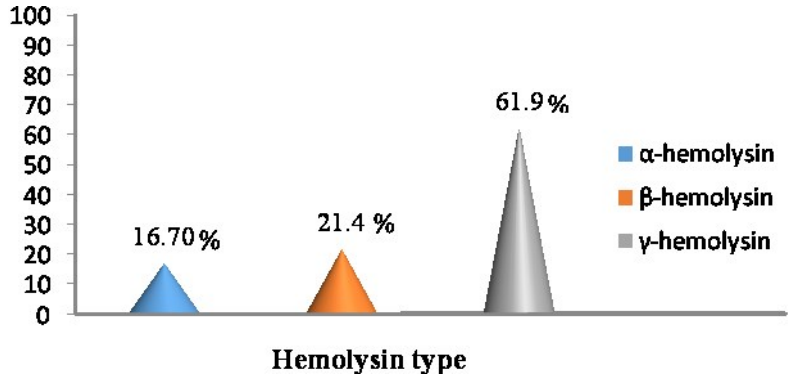

Figure 2.Types of hemolysin produced by *E.coli* isolates.

The frequency of hemolytic *E.coli*isolation from patient samples increases with the severity ofdisease it was, reported that 41.36 % strains showing hemolytic activity among isolates isolated fromextraintestinal infections, which is way too high when compared to our study [20, 21]. *E. coli* exotoxinalpha-hemolysin is thought to be a significant virulence factor in

No. (%) of *E.coli* Isolate	No. (%) of Genes Producer Isolates			
	pap	*Afa*	*sfa*	*Kps*
33 (100%)	2(6.06%)	7 (21.21%)	20 (60.60%)	28 (84.84%)

several clinical infections, such aspyelonephritis and septicemia [22]. It was reported that *E. coli* isolates hemolysin production as 38.5%, 12.8 %, and 48.5 % for α, ß, and γ respectively in Iraq. [23].

3.2. Biofilm Formation Test

A biofilm is a microbial derived sessile community characterized by cells that are irreversibly attachedto a substratum or interface or to each other, are embedded in a matrix of extracellular polymericsubstances that they have produced, and exhibit an altered phenotype with respect to growth rate andgene transcription. Bacterial biofilms are reported to be the most common cause of persistentinflammation [24].The results of this study revealed that quantification of biofilm formation by microtiter plate methodclassified 50% (n = 21) of the isolates as moderate biofilm producer, while 50% (n = 21) exhibitedweak biofilm formation. Al-Chalabi [25] found that 90% of *E.coli*isolated from UTI was producing biofilm. The biofilmformation is a two-step process in which the bacteria first adhere to a surface mediated by capsularantigen or flagellar antigen followed by multiplication to form a multi layered biofilm, which isassociated with production of exopolysaccharide matrix [26]. Bacteria that form biofilms have beenshown to be highly resistant to antimicrobial therapy [27]. Adherence and production of a biofilm by *E.coli*on different biomaterials and medical devices hasbeen documented [28]. The development of a biofilm is initiated when bacterial cells attached tosurface and begin to excrete slimy, glue-like substances, which serve to anchor the cells [29]. Bacteriathat adhere to implanted medical devices or damaged tissue can become the cause of persistentinfections. It has been estimated that 65% of microbial infections are associated with biofilms [30].

3.3. Determination of Genetic Virulence Profiles

The PCR assay results identified the adhesion systems 2 *pap* (6.06%), 7 *afa*(21.21%), 20 *sfa* (60.60%), and 28 *kps*(84.84%), table (2) and figures (3, 4, 5 and 6).

Table (2): Frequency of virulence factors of MDR isolates.

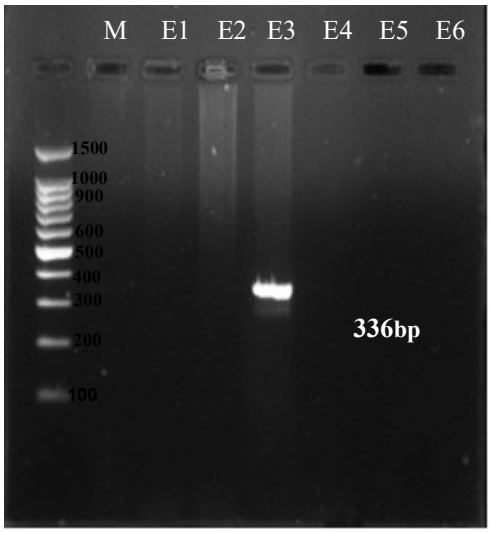

Figure 3.PCR amplification with specific primers for *pap* gene product. Lane M, 100-bp DNA marker. Lanes (E4)positive isolates,lanes (E1,2, 3, 5, 6 and 7) negative isolates, on 2 % agarose at 70 volt for2 hr

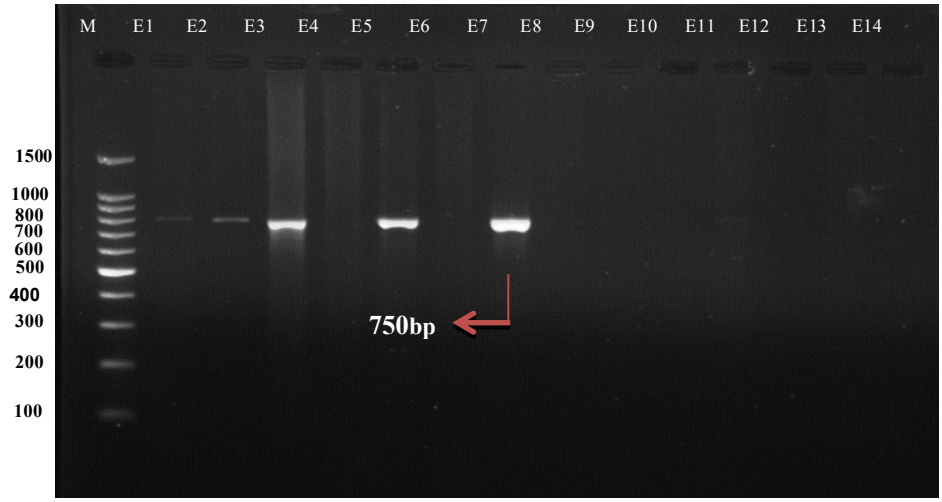

Figure 4.PCR amplification with specific primers for *afa*gene product. Lane M, 100-bp DNA marker.Lanes (E1,2,3, 5, 7, and 11) positive isolates, lanes (E4, 6, 8, 9, 10, 12, 13 and 14) negative isolates,on 2 % agarose at 70 volt for 2 hrs.

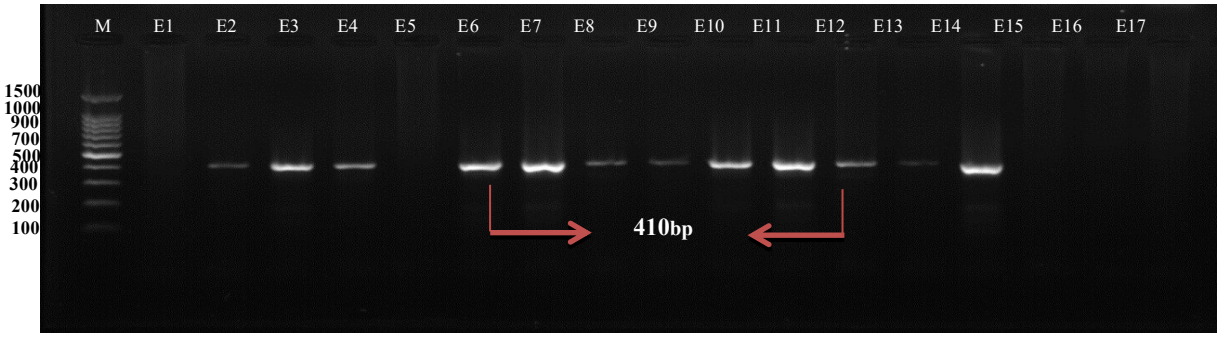

Figure 5.PCR amplification with specific primers for *sfa* gene product. Lane M, 100-bp DNA marker. Lanes (E2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13 and 14) positive isolates, lanes (E1, 5, 15, 16 and 17) negative isolates, on 1.5% agarose at 70 volt for 2 hrs.

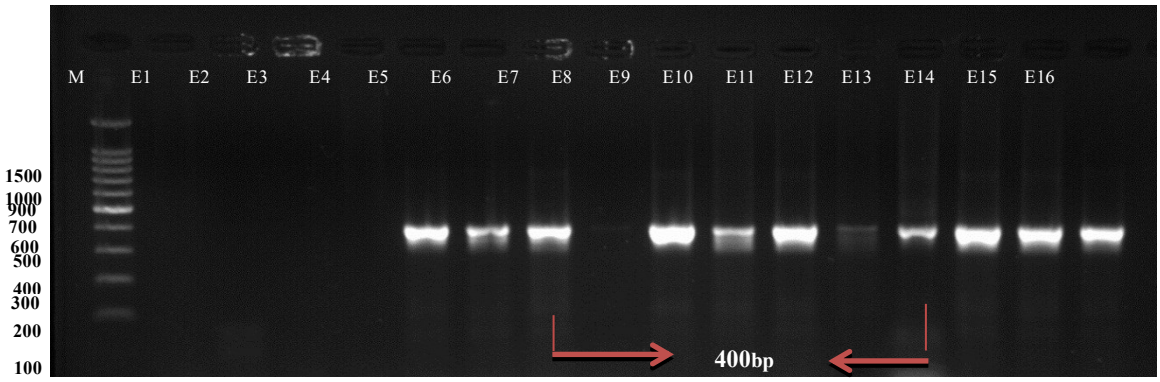

Figure 6.PCR amplification with specific primers for *kps*gene product. Lane M, 100-bp DNAmarker. Lanes (E5, 6, 7, 9, 10, 11, 12, 13, 14, 15 and 16) positive isolates, lanes (E1, 2, 3, 4 and 8)negative isolates, on 1.5% agarose at 70 volts for 2 hrs.

Virulence factors of UPEC are associated with colonization and survival in the normal urinary tract,and influence the pathogenicity of symptomatic or complicated UTIs [31]. These virulence factors areadhesion proteins (*pap, sfa,* and *afa*), toxins (e.g., hemolysin), and host defense-avoidancemechanisms (e.g., capsule (*kps*)) [32]. Bacterial adherence to urinary epithelia is a crucial step in thedevelopment of UTI, allowing the bacteria to persist in the urinary tract against flushing by urine flowand activation of host signaling pathways [33]. In the present study, the *kps*gene was the mostcommon virulence gene in *E. coli* isolates (84.84%). In contrast to other studies, the *sfa*was observedin 80% in UTIs isolates [34],[35]. Whereas Shetty [36], reported that the prevalence of *pap* gene washighest 60.87% (14/23) followed by *sfa*and *afa*39.1% (9/23) genes each.The results revealed the *afa*gene was detected in 21.21% of the isolates. Frequencies of *afa*rangingfrom 2 to 11% have been reported by Santo [37], and Abe [38].While the adhesions genes *pap* and *sfa*presented frequencies of 7.1 and 57%, respectively.

Previous studies, concerning the frequencies of *pap* and *sfa*varying from 0 to 54%, and from 0 to 64%respectively, have been isolated from different phylogenetic groups of UPEC [39],[40]. Also, it hasbeen found that the frequency of adhesion genes *fimH*(65.9%), *pap* (63.7%) and *sfa*(56%) genes inUPEC isolates in Egypt [41].Oliveira [32] reported that the frequency of virulence factor among UPEC strains were as follows:*sfaA*(26%), *pap* (25%) and *afa*(6%) genes in UPEC isolates in Brazil. While in a study on Iran, 30.2% and 18.75% of *E. coli* isolates possessed *pap* and *sfa*genes respectively [42].

Bacterial adherence to uroepithelial cells is an essential stage for the initiation and development ofUTI. The binding to the host cells is a key factor for development of infection, the expression ofdifferent types of adhesions, with affinity to distinct specific receptors, confers advantages topathogens [43].The adhesions such as S and P fimbriae play the major roles in the pathogenicity of *E. coli* strains bycolonization stage and overcoming host immunity [44]. Adhesion to uroepithelial cells by P fimbriaeis an important step for the beginning and expansion of UTI [45]. S fimbriae showed attachmentefficiency to epithelial and endothelial cells of the lower human urinary tract [46].

3.4. Phylogenetic Groups

Phylogenetic analysis was carried out to investigate the genetic backgrounds of the MBLs whether anyof the isolate belongs to pathogen group (B2+D) or commensal group (A+B1). In the present study,the phylogenetic analysis based on Clermont dichotomous tree has shown

that *E. coli* isolates dividedinto four main phylogentic groups: (A, B1, B2, and D) described by [15], (2000). The results indicatedthat the frequency of phylogeny genes *chuA*were (72.72%), *yjaA*(87.87%), and anonymous DNAfragment designated TspE4.C2 (72.72%), figure (7).

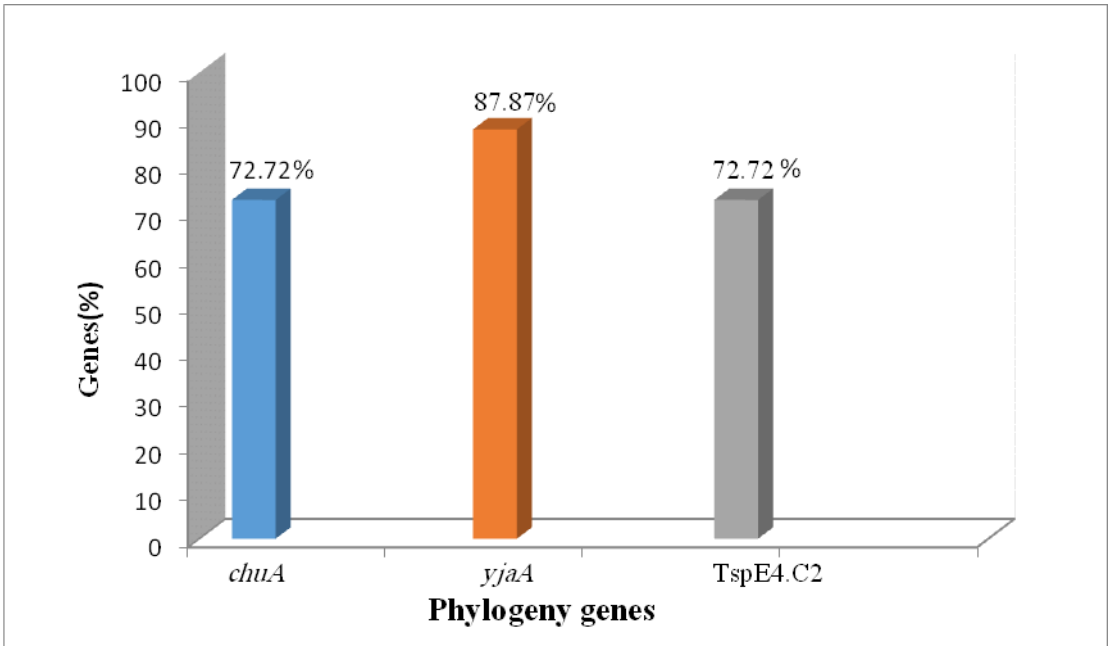

Figure 7. Distribution of phylogenetic genes *chuA, yjaA*andTspE4.C2 geneamong E. coli isolates.

All MDR *E. coli* tested to determine phylogenetic grouping that were performed using triplex PCR for*chuA*, *yjaA*genes and TspE4.C2 segment, in figure (8).

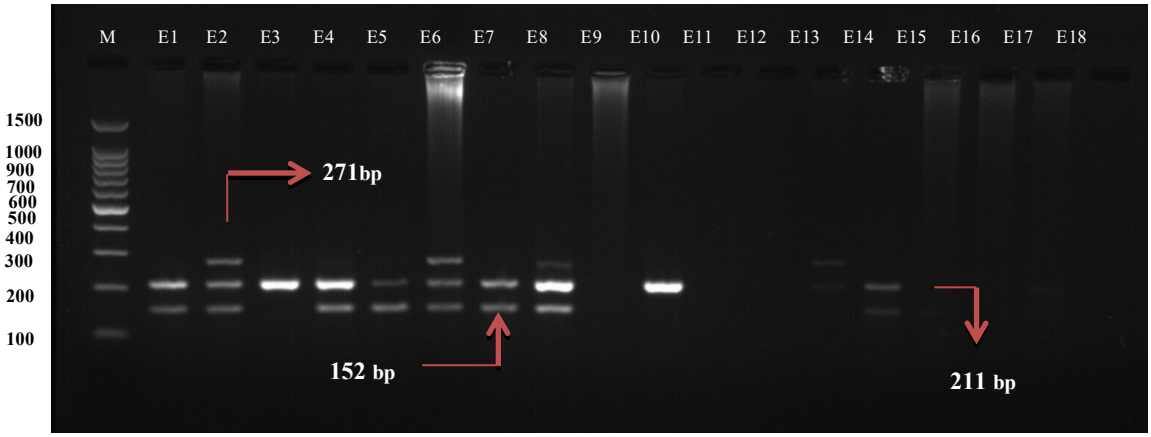

Figure 8. Triplex PCR based phylogenetic profile of *E. coli* isolates with specific primers for TspE4.C2*, yjaA,* and *chuA*genes product. Lane M, 100-bp DNA marker. Lanes (E2, 4, 5, 6, and 8)positive isolates with TspE4.C2*, yjaA,* and *chuA*genes, lanes (E1, 7, and 14) positive isolates withTspE4.C2 and *yjaA*genes, lanes (E3 and 10) positive isolates with *yjaA*gene, lane (E13) positiveisolate with *yjaA*and *chuA*genes. Lanes (E9, 11, 12, 15, 16, 17 and 18) negative isolates withTspE4.C2*, yjaA,* and *chuA*genes, on 1.5% agarose at 70 volts for 2 hrs.

Clermont[15] classified the phylogenetic grouping (A, B, B2 and D) of the *E. coli* isolates with a PCR methodbased on the combination of three DNA indicators that were *chuA*, *yjaA,* and anonymous DNAfragment designated TspE4.C2*,* as following:

- *chuA*-positive/*yjaA*-positive was *E. coli* isolate B2,
- *chuA*-positive/*yjaA*negative was isolate D,
- *chuA*-negative/TspE4.C2-positive was isolate B1, and
- *chuA*negative/TspE4.C2-negative was isolate A.

The results of phylogenetic group show B2 23(69.70%) was the most common, followed byphylogenetic groups A 7(21, 21%), B1 2(6.06%), and D 1(3.03%) isolates, table (3).

Table (3): Frequency of the different phylogenetic groups among *E. coli* isolates

Phylogenetic group	No.	Percentage %
A	7	21.21
B1	2	6.06
B2	23	69.70
D	1	3.03
Total isolates	33	100

Similarly, Bashir [47]*,* analyzed phylogenetic groups in 59 uropathogenic*E. coli* isolates and revealedthat group B2 was the most dominant followed by groups A, B1 and D. Moreover, the results of thisstudy agree with several studies by Ejrnaes [48], Lim [49], and López-Banda [50]*,* who reported thatthe most of the virulent extra intestinal strains are derived from group B2, and to a lesser extent to theD group. Both groups have a higher prevalence of extra intestinal virulence determinants than thestrains in the A and B1 groups.However, the current results differed from a previous study by Duriez [51], that determined thephylogenetic group of 168 commensal *E. coli* isolates isolated from the stool and showed that strains from phylogenetic groups A and B1 were the most common, followed by phylogenetic group Dstrains.

Rahdar [52], demonstrated that most of the genes needed for causing neonatal meningitis belonged tothe *E. coli* B2 phylogenetic group initially and horizontal transfer of these genes has occurred towardthe more genetically distant groups. Extra intestinal pathogenic strains usually fell into B2 and to alesser extent to group D that reported by Platell [53]. These differences in distribution of the phylogenetic groups among the isolates of geographicallydistinct populations in different studies may be due to the health status of the host, geographic climaticconditions, dietary factors, the use of antibiotics, or host genetic factors, in addition to the differencesarising from different sampling areas. Additionally, the results showed that the most of the virulence factors genes distributions were differsignificantly within the phylogenetic group at $p < 0.05$. However, they clustered among isolates ofgroup B2, while group D isolates did not carry any VFs gene as show in table (4).

Table (4): Relation among virulence factors genes and phylogenetic group.

Phylogenetic Groups	Virulence factorsgenes No. (%)			
	kps	*pap*	*sfa*	*afa*
A	5 (71.4)	0 (0)	4 (57.1)	1 (14.3)
B1	1 (50)	0 (0)	1 (50)	0 (0)
B2	22 (95.7)	1 (4.3)	15 (65.2)	6 (26.1)
D	0 (0)	0 (0)	0 (0)	0 (0)
p-value	0.000*			

*$\chi^2$was significant difference at ($p < 0.05$).

The predominant phylogenetic group was B2, widely associated with pathogenic strains may identifythis group as the highest pathogenic. Previous findings by [54] suggested that the highly pathogenicnature of the group B2 might be one of the reasons to cause UTI. So, the intestinal tract acts as thereservoir for the uropathogens of group A and B2. The pathogenic potential of *E. coli* strains isthought to be dependent on the presence of virulence factors [42]. Previous studies have indicated that phylogroups B2 and D possess more virulence properties such asbiofilm formation and hemolysin secretion when compared with phylogroups A and B1 isolates[55], [56]. However, the results revealed a significant difference between all pathogenic andcommensal groups as explain in table (5).

Table (5): Association among virulence factors genes and phylogenetic groups

Variable	Commensal groups (A+B1) No. (%)	Pathogenic groups (B2+D) No. (%)	Total	*P*- value
Virulence factor Genes				
afa	1(11.1)	6(25)	7	0.000*
kps	6(66.7)	22(91.7)	28	0.000*
pap	0(0)	2(8.3)	2	0.000*
sfa	5(55.5)	15(62.5)	20	0.000*

*$\chi^2$was significant difference at ($p < 0.05$).

The current study indicated that among all phylogenetic groups, all virulence genes: *pap*, *afa*, *sfa*, and*kps*which was more frequent in A and B1 isolates were present in significantly higher percentage inphylogenetic group B2 isolates at ($p < 0.05$).

Manoharan [57], reported that with increasing isolation of ESBL-producing isolates in the hospitalsettings necessitating the use of carbapenems which increases the problem of MBL production.The results of study showed high rates of MDR *E. coli* belong to the phylogenetic group B2 and in toa lesser extent to group D and displayed a great number of virulence genes and antibiotic resistancegenes among patients. These genes are present on mobile elements that could be transmitted todifferent pathogens, and multiple resistances to antimicrobial drugs among *E. coli* isolates complicatetherapeutic management of infections [8]. Moreover, the prevalence of isolates possessing severalresistance enzymes concomitantly has caused serious problems for treatment and diagnosis.

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Conflict of Interest
The authors have declared no conflict of interest .

References.

[1] Johnson J R and Russo, T A 2005 Molecular epidemiology of extraintestinal pathogenic(uropathogenic) Escherichia coli. *International Journal of Medical Microbiology*, **295**(6): 383-404.
[2] Agarwal J, Srivastava S and Singh M 2012 Pathogenomics of uropathogenic*Escherichia coli*.*Indian Journal of Medical Microbiology*, **30**(2), 141- 49.
[3] Arisoy M, Aysev D, Ekim M, Özel D, Köse S K, Özsoy E D and Akar N 2006 Detection of virulence factors of *Escherichia coli* from children by multiplex polymerase chain reaction. *International Journal of Clinical Practice*, **60**(2): 170-173.
[4] Yamamoto S 2007 Molecular Epidemiology of Uropathogenic*Escherichia coli*. *Journal Infection and Chemotherapy*, **13**(2): 68-73.
[5] Herzer P J, Inouye S , Inouye M and Whittam T S 1990 Phylogenetic distribution of branchedRNA-linked multicopy single-stranded DNA among natural isolates of *Escherichia coli*. *Journal of bacteriology*, **172**(11): 6175-6181.
[6] Johnson J R and 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.
[7] Pupo G M, Karaolis, D K, Lan R and Reeves P R 1997 Evolutionary relationships among pathogenic and nonpathogenic *Escherichia coli* strains inferred from multilocus enzyme electrophoresis and *mdh*sequence studies. *Infection and Immunity*, **65**(7):2685-2692.
[8] Almutairy A AA H, Alka'aby T AA and Abdulla A A 2016 Detection of Metallo-β-Lactamases and their association with integrons among Multidrug Resistant Clinical Isolates of *Escherichia coli. International Journal of PharmTech Research*, .**9** (12), pp 740-753**.**
[9] Baronet E J, Peterson L R and Finegold S M 1994 *Methods for testing antimicrobial effectiveness. In: Bailey and Scott's diagnostic microbiology*, 9th ed. (pp 168-193). Saint Louis: Mosby-Year Book, Inc.
[10] Stepanović S, Vuković D, Hola V, Bonaventura G D, Djukić S, Ćirković I and Ruzicka F 2007 Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. *Acta PathologicaMicrobiologicaetImmunologicaScandinavica,* **115**(8): 891-899.
[11] Lotfi G, Hafida H, Nihel K, Abdelmonaim K, Nadia A, Fatima N and Walter Z 2014 Detection of biofilm formation of a collection of fifty strains of *Staphylococcus aureus* isolated in Algeria at the University Hospital of Tlemcen. *African Journal of Bacteriology Research*, **6**(1):1-6.
[12] Karimian A, Momtaz H and Madani M 2012 Detection of uropathogenic*Escherichia coli*virulence factors in patients with urinary tract infections in Iran. *African Journal of Microbiology Research*, **6**(39): 6811-6816.
[13] Dezfulian H, Batisson I, Fairbrother J M, Lau P C, Nassar A, Szatmari G and Harel J 2003 Presence and characterization of extraintestinal pathogenic *Escherichia coli* virulence genes in F165-positive *E. coli* strains isolated from diseased calves and pigs. *Journal of Clinical Microbiology***, 41**(4):1375-1385.
[14] Park H K, Jung Y J, Chae H C, Shin Y J, Woo S Y, Park H S and Lee S J 2009 Comparison of *Escherichia coli* uropathogenic genes (*kps, usp*and *ireA*) and enteroaggregative genes (*aggR*and *aap*) via multiplex polymerase chain reaction from suprapubic urine specimens of young children with fever. *Scandinavian Journal of Urology and Nephrology,* **43**(1):51-57.

[15] Clermont O, Bonacorsi S and Bingen E 2000 Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Applied and environmental microbiology*, **66**(10):4555-4558.
[16] Paulson O S 2008 Biostatistics and Microbiology :ASurvival Manual. *Springer Sience and Business Media, LLC*.
[17] Cavalieri S J, Bohach G A and Snyder I S 1984 *Escherichia coli* α-hemolysin: characteristics and probable role in pathogenicity. *Microbiological Reviews*, **48**:326–343.
[18] Walton J R and Smith D H 1969 New hemolysin (γ) produced by *Escherichia coli. Journal of Bacteriology*, **98**:304–305.
[19] Harley J and Prescott H 2002 *Laboratory Exercises in Microbiology* (5th ed.). The McGraw-Hill Companies.
[20] Johnson J R, Moseley S L, Roberts P L and Stamm W E 1988Aerobactin and other virulence factor genes among strains of *Escherichia coli* causing urosepsis: association with patient characteristics. *Infection and Immunity*.**56**(2): 405–412.
[21] Raksha R, Srinivasa H and Macaden R S 2003 Occurrence and characterisation of uropathogenic*Escherichia coli* in urinary tract infections. *Indian Journal of Medical Microbiology*, **21**:102-107.
[22] O'Hanley P, Lalonde G and Ji G 1991 Alpha-hemolysin contributes to the pathogenicity of piliateddiagalactoside-binding *Escherichia coli* in the kidney: efficacy of an alpha hemolysin vaccine in preventing renal injury in the BALB/c mouse model of pyelonephritis. *Infection and Immunity*, **59**: 1153–1161.
[23] Nanakali Z and Ahmad Z F 2015 Antibiotic resistance study and detection of virulence gene among uropathogenic*Escherichia coli.kirkuk University Journal for Scientific Studies*, **10**(3): 205-229.
[24] Frye J G and Jackson C R 2013 Genetic mechanisms of antimicrobial resistance identified in *Salmonella enterica*, *Escherichia coli*, and *Enteroccocus*spp. isolated from US food animals. *Frontiers in Microbiology*, **23**(44): 135.
[25] Al-Chalabi R N 2004 Relation between hemolysin production and biofilm formation by uropathogenic*Escherichia coli*. M.Sc. Thesis, Al-Nahrain University, Iraq.
[26] Costerton I W, Stewart P S and Greenberg E P1999 Bacterial biofilms: a common cause of persistent infections. *Science*, **284**:1318-1322.
[27] Tyerman J G, Ponciano J M, Joyce P, Forney L J and Harmon L J 2013 The evolution of antibiotic susceptibility and resistance during the formation of *Escherichia coli* biofilms in the absence of antibiotics. *BMC Evolutionary Biology*, **13**(1): 22.
[28] Di Martino P, Cafferini N, Joly B and Darfeuille-Michaud A 2003 *Klebsiellapneumoniae*type 3 pili facilitate adherence and biofilm formation on abiotic surfaces. *Research in Microbiology*, **154**(1):9-16.
[29] O'Gara J P and Humphreys H 2001 *Staphylococcus epidermidis*biofilms: importance andimplications. *Journal of Medical Microbiology*, **50** (7):582-587.
[30] Teodósio J S, Simões M and Mergulhão F J 2012The influence of nonconjugative*Escherichia coli* plasmids on biofilm formation and resistance. *Journal of Applied Microbiology*, **113**(2):373-382.
[31] Chiou Y Y, Chen M J, Chiu N T, Lin C Y and Tseng C C 2010 Bacterial virulence factors areassociated with occurrence of acute pyelonephritis but not renal scarring. *The Journal of Urology*, **184**(5):2098-2102.
[32] Oliveira F A, Paludo K S, Arend L N, Farah S M, Pedrosa F O, Souza E M … *et al.* 2011 Virulence characteristics and antimicrobial susceptibility of uropathogenic*Escherichia coli* strains. *Genet of Moleclar Research*, **10**(4): 4114-25.
[33] Crépin S, Houle S, Charbonneau M È, Mourez M, HarelJ and Dozois C M 2012 Decreased expression of type 1 fimbriae by a *pst*mutant of uropathogenic*Escherichia coli* reduces urinary tract infection. *Infection and Immunity*, **80**(8): 2802-2815.
[34] Merçon M, Regua-Mangia A H, Teixeira L M, Irino K, Tuboi S H Goncalves R T and SantoroLopes G 2010 Urinary tract infections in renal transplant recipients: Virulence traits of Uropathogenic*Escherichia coli*. *In Transplantation proceedings*, **42** (2): 483-485.
[35] Tarchouna M, Ferjani A, Ben-Selma W and Boukadida J 2013 Distribution of

uropathogenicvirulence genes in *Escherichia coli* isolated from patients with urinary tract infection. *International Journal of Infectious Diseases*, **17**(6): e450-e453.
[36] Shetty A V, Kumar S H, Shekar M, Shetty A K and Karunasagar I 2014 Prevalence of adhesive genes among uropathogenic*Escherichia coli* strains isolated from patients with urinary tract infection in Mangalore. *Indian Journal of Medical Microbiology*, **32**(2): 175-178
[37] Santo E, Macedo C and Marin J M 2006 Virulence factors of uropathogenic*Escherichia coli* from a university hospital in RibeirãoPreto, São Paulo, Brazil. *Revista do Instituto de Medicina Tropical de São Paulo,* **48**(4):185-188.
[38] Abe C M, Salvador F A, Falsetti I N, Vieira M A, Blanco J, Blanco J E, *et al.* 2008 Uropathogenic*Escherichia coli* (UPEC) strains may carry virulence properties of diarrhoeagenic*E. coli*. FEMS *Immunology and Medical Microbiology*, **52**(3):397-406.
[39] Takahashi A, Kanamaru S, Kurazono H, Kunishima Y, Tsukamoto T, Ogawa O and Yamamoto S 2006 *Escherichia coli* isolates associated with uncomplicated and complicated cystitis and asymptomatic bacteriuria possess similar phylogenies, virulence genes, and Oserogroup profiles. *Journal of Clinical Microbiology*, **44**(12):4589-4592.
[40] Takahashi A, Muratani T, Yasuda M, Takahashi S, *et al.* 2009 Genetic profiles of fluoroquinolone-resistant *Escherichia coli* isolates obtained from patients with cystitis: phylogeny, virulence factors, PAIusp subtypes and mutation patterns. *Journal of Clinical Microbiology*, **47**(3):791-795.
[41] Zaki M E, and Elewa A 2015 Evaluation of Uropathogenic Virulence Genes in *Escherichia coli* Isolated from Children with Urinary Tract Infection. *International Journal of AdvanceResearch*,**3**(3):165-173.
[42] Farshad S, Emamghoraishi F and Japoni A 2010 Association of Virulent Genes *hly, sfa,cnf-1* and *pap* with Antibiotic Sensitivity in *Escherichia coli* Strains Isolated from Childrenwith Community-Acquired UTI. *Iranian Red Crescent Medical Journal*, **12**(1):33-37.
[43] Antão E M, Wieler L H and Ewers C 2009 Adhesive threads of extraintestinal pathogenic*Escherichia coli*. *Gut pathogens*, **1**(1): 22.
[44] Bahalo S, Tajbakhsh E, Tajbakhsh S, Momeni M and Tajbakhsh F 2013 Detection of somevirulence factors of *Escherichia coli* isolated from urinary tract infection isolated of childrenin Shahrekord Iran by Multiplex PCR. *Middle-East Journal of Scientific Research,***14**(1):29-32.
[45] Matiuzzi da Costa M, Drescher G, Maboni F, Weber S, Avila Botton S, Vainstein M.H, SchrankI S and Vargas A C 2008 Virulence factors and antimicrobial resistance of *Escherichia coli*isolated from urinary tract of swine in southern of Brazil. *Brazilian Journal ofMicrobiology*,**39**(4):741-743.
[46] Bien J, Sokolova O and Bozko P 2012 Role of uropathogenic*Escherichia coli* virulence factorsin development of urinary tract infection and kidney damage. *International Journal of Nephrology,***4**(3):469-476.
[47] Bashir S, Haque A, Sarwar Y A A and Anwar M I 2012 Virulence profile of different phylogenetic groups of locally isolated community acquired uropathogenic*Escherichia coli* from Faisalabad region of Pakistan. *Annals of Clinical Microbiology and Antimicrobials,* **11**(1):23.
[48] Ejrnaes K, Sandvang D, Lundgren B, Ferry S, Monsen T, Holm S, Lundholm R and FrimodtMoller N 2005 *Escherichia coli* causing recurrent urinary tract infections belong mainly tothe phylogenetic group B2. *Clinical Microbiology and Infection Supplement*,**11**:129.
[49] Lim K T, Yeo C C, Yasin R M, Balan G and Thong K L 2009 Characterization of multidrugresistant and extended-spectrum β-lactamase-producing *Klebsiellapneumoniae*strains fromMalaysian hospitals. *Journal of Medical Microbiology*, **58**(11):1463-1469.
[50] López-Banda D A, Carrillo-Casas E M, Leyva-Leyva M, Orozco-Hoyuela G, ManjarrezHernández Á H, Arroyo-Escalante S, …*et al.* 2014 Identification of virulence factors genes in*Escherichia coli* isolates from women with urinary tract infection in Mexico. *BioMedResearch International*, Vol.**20**, Article ID 959206, 10 pages.
[51] Duriez P, Clermont O, Bonacorsi S, Bingen E, Chaventré A, Elion J, Picard B and

Denamur E2001 Commensal *Escherichia coli* isolates are phylogenetically distributed amonggeographically distinct human populations. *Microbiology*, **147**(6):1671-1676.
[52] Rahdar M, Rashki A, Miri H R and Ghalehnoo M R 2015 Detection of *pap, sfa, afa, foc,* and*fim*Adhesion-Encoding Operons in Uropathogenic*Escherichia coli* Isolates Collected fromPatients with Urinary Tract Infection. *Jundishapur Journal of Microbiology*, **8**(8): e22647.
[53] Platell J L, Trott D J, Johnson J R, Heisig P, Heisig A, Clabots C R, Johnston B and Cobbold RN 2012 Prominence of an O75 clonal group (clonal complex 14) among non-ST131 fluoroquinolone resistant *Escherichia coli* causing extraintestinal infections in humans and dogs in Australia. *Antimicrobial Agents and Chemotherapy*, **56**(7): 3898-3904.
[54] Sabaté M, Moreno E, Perez T, Andreu A and Prats G 2006 Pathogenicity island markers incommensal and uropathogenic*Escherichia coli*isolates. *Clinical Microbiology and Infection*,**12**(9):880-886.
[55] Soto S M, Smithson A, Martinez J A, Horcajada J P, Mensa J. and Vila J 2007 Biofilm formationin uropathogenic*Escherichia coli* strains: relationship with prostatitis, urovirulence factorsand antimicrobial resistance. *The Journal of Urology*, **177**(1):365-368.
[56] JakobsenL,SpangholmDJ,PedersenK,JensenLB,EmborgHD,AgersøY,…*eta.*2010 Broiler chickens , broiler chicken meat, pigs and pork as sources of ExPEC related virulence genes and resistance in *Escherichia coli* isolates from community-dwelling humans and UTI patients. *International Journal of Food Microbiology*, **142**(1):264-272.
[57] Manoharan A, Chatterjee S, Mathai D and SARI Study Group 2010 Detectionand characterization of metallo beta lactamases producing *Pseudomonas aeruginosa*. *Indian Journal of Medical Microbiology*, **28**(3): 241-244.