

Prevalence of Virulence Genes and Their Association with Antimicrobial Resistance Among Pathogenic *E. coli* Isolated from Egyptian Patients with Different Clinical Infections


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Introduction: *Escherichia (E.) coli* can cause intestinal and extra-intestinal infections which ranged from mild to life-threatening infections. The severity of infection is a product of many factors including virulence properties and antimicrobial resistance.

Objectives: To determine the antibiotic resistance pattern, the distribution of virulence factors and their association with one another and with some selected resistance genes.

Methods: Virulence properties were analyzed phenotypically while antimicrobial susceptibility was tested by Kirby-Bauer agar disc diffusion method. In addition, 64 *E. coli* isolates were tested for 6 colicin genes, *fimH*, *hlyA*, *traT*, *csgA*, *crl* virulence genes and *bla*-*CTX-M-15*, *bla*-*oxa-2*, and *bla*-*oxa-10* resistance genes by polymerase chain reaction (PCR).

Results: Extra-intestinal pathogenic *E. coli* isolated from urine and blood samples represented a battery of virulence factors and resistance genes with a great ability to produce biofilm. Also, a significant association ($P < 0.05$) among most of the tested colicin, virulence and resistance genes was observed. The observed associations indicate the importance and contribution of the tested factors in the establishment and the progress of infection especially with *Extra-intestinal E. coli* (ExPEC) which is considered a great challenging health problem.

Conclusion: There is a need for studying how to control these factors to decrease the rate and the severity of infections. The relationship between virulence factors and resistance genes is complex and needs more studies that should be specific for each area.

Keywords: *E. coli*, virulence, resistance, colicin genes, ESBL, *bla*-*CTX-M-15*, *bla*-*oxa-2*, *bla*-*oxa-10*

Introduction

The acquisition of virulence and resistance genes is believed to increase the pathogenicity of microorganisms and the severity of infection with the great possibility of therapy failure. *Escherichia coli* is an opportunistic pathogen, commensal bacteria that can be found as normal flora in humans and animals. It can be classified according to the site of its existence into commensal, intestinal and extra-intestinal *E. coli*. Commensal *E. coli* may acquire many virulence genes that may, in combination, result in intestinal and extra-intestinal *E. coli* infections. Also, diarrheagenic *E. coli* strains can be classified according to their virulence into different pathotypes which are enterotoxigenic (ETEC), enteropathogenic (EPEC),

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enterohemorrhagic (EHEC), enteroinvasive (EIEC), and enteroaggregative (EaggEC). Each one of these pathotypes has some virulence factors that in combination are responsible for their pathogenicity of each type and have an important role in infection. For instance, toxin production, hemolysins, proteases, cell surface hydrophobicity, colicins, etc.^{1,2} *E. coli* produces bacteriocins such as colicins and microcins which are bactericidal peptides and considered as virulence factors in different *E. coli* strains or pathotypes.³ Colicins produced by *E. coli* under stress; prohibiting the colonization of other *E. coli* strains without affecting their producing strains. Also, colicins increase the host colonization by their producing strain. So, the resistance of other strains to colicins is considered as a cause of their pathogenicity.⁴

Over 25 different colicins have been specified and categorized on the base of their pathways. Colicins E1 inhibit the synthesis of all macromolecular without respiration arrest.⁵ Genes encoding colicins E1 were found in ExPEC⁴ especially uropathogenic *E. coli* (UPEC). Colicins E2, E7, E8 and E9 cause cleavage of DNA,⁶ colicins E3, E4 and E6 lead to rRNA hydrolysis,⁷ while colicins E5 cleave tRNA.⁸ Microcins V are urovirulence,⁹ while colicins B are highly identified in bowel diseases caused by *E. coli*.¹⁰

Furthermore, virulence factors in UPEC are mainly genes encoding adhesion like fimbriae which facilitate invasion and colonization of epithelial cells¹¹ and thin flexible aggregative protein filaments which are known as curli and their encoding genes are *crl* and *csgA*.¹² Adhesion increases the expression of bacterial toxins, iron acquisition and eschewal the host defense mechanisms.¹³ The adhesion genes which are mostly prevalent are *iha* and *fimH*;¹⁴ while toxin gene is *hlyA*.¹⁵ ExPEC in bloodstream is able to overcome the serum bactericidal effect encoded by *traT* gene.¹⁶

In addition to the role of acquisition of virulence genes and its effect on the pathogenicity, the acquisition of resistance genes plays an important role in therapeutic failure and the increase of mortality rate. The inability to control the emergence of multi-drug (MDR), extensive drug (XDR) and pandrug resistance will increase the mortality rates to 10 million people by 2050. Misuse and overuse of antibiotics in therapeutic purposes for human and therapeutic purposes and as growth promoters in livestock lead to the emergence of resistance to many antimicrobial classes as penicillins, cephalosporines, tetracyclines, sulfonamides, macrolides and polymyxins.¹⁷

One of the most prevalent resistance factors is extended spectrum β -lactamase (ESBL) produced by

Enterobacteriaceae which first identified in the 1980s.¹⁸ The most prevalent ESBL is CTX-M type (cefotaxinase)¹⁹ and *E. coli* having CTX-M ESBL was multidrug-resistant to most drugs.²⁰ OXA β -lactamase (oxacillinase) is another type which was identified as chromosomally mediated enzyme and revealed a high resistance to penicillin and showed more carbapenems resistance activity.²¹ Variation of OXA β -lactamases takes place by alteration of their amino acids and some *E. coli* isolates may have many variants of OXA β -lactamases. So, the single strain may have variable types of ESBLs.²²

The aim of this study was to determine the antibiotic resistance pattern, the distribution of different virulence factors of *E. coli* isolated from different sources of infections and their association with one another and with some selected resistance genes.

Methods

Patients, Samples and Identification of Isolates

Our study comprised 200 patients, who were admitted to Minia university hospital during the period from January to July 2019. In brief, 125 males and 75 females were included with a median age of 46 (range 23–56). Samples were collected from patients with urinary tract infections (80 urine samples), gastroenteritis (50 stool samples), septicemia (30 blood samples) and wound infections (40 wound swab samples) as part of the routine hospital laboratory procedures. Samples were obtained after 2 days of their hospitalization. The following antibiotics were used until the results of cultures were available: two doses of ciprofloxacin (urinary tract infections), one dose of cefotaxime (wound infections), two doses of metronidazole and ciprofloxacin (diarrhea) and one dose of imipenem and vancomycin (septicemia).

All samples were cultured and identified using the conventional microbiological procedures. Samples positive for *E. coli* showed pink colonies on MacConkey agar (Oxoid, UK). *E. coli* positive cultures were confirmed by different biochemical tests (catalase, sugar fermentation, indole and nitrate reduction tests) and the formation of metallic sheen on Eosin methylene blue agar (EMB) (Himedia, India).

Phenotypic Identification of Virulence Properties of *E. coli* Isolates

Virulence properties were determined phenotypically as follows: Hemolysis was tested by inoculating isolates into 5%

sheep blood agar plates. Clear zones around colonies indicate hemolysin production. Biofilm formation was tested using the tissue culture plate method (TCP).²³ Cell surface hydrophobicity was determined by the salt aggregation test (SAT). Protease test was detected by culturing the tested isolates on skim milk agar and the formation of clear zones around colonies is considered positive. Mannose resistant and mannose sensitive haemagglutination test was done by mixing one drop of blood group "O" with a drop of bacterial cultures on a slide, followed by rotating the slide for 5 min at 37°C. Clumps formed were indicated as haemagglutination. Mannose sensitive haemagglutination was indicated by the addition of 2% w/v of d-mannose. The absence of haemagglutination indicates Mannose sensitivity while the presence of Haemagglutination indicates Mannose resistance. Curli fimbriae expression was determined by growing the tested isolates on agar plates containing 0.1% tryptone, 0.05% yeast extract, 0.002% Coomassie brilliant blue, 0.004% Congo red and 1.5% agar. Curli production can be detected by the presence of red colonies while white colonies were negative for curli. Colicin production: fresh *E. coli* cultures were cultured on the surface of nutrient agar for 24 hrs at 37°C. Plates were exposed for chloroform vapors for 2 hrs, and then left for 30 mins to evaporate chloroform. Isolates were inoculated perpendicular to the original cultures. The tested isolates were examined for inhibition of growth.^{24–29}

Antimicrobial Susceptibility Testing and Phenotypic Detection of ESBL

Antimicrobial susceptibility testing of *E. coli* isolates was performed on Mueller Hinton agar plates using the Kirby–Bauer agar disc diffusion method.³⁰ Antibiotic discs used in this study were obtained from Bioanalyse (Turkey). Isolates were defined as sensitive or resistant depending on the measurement of inhibition zone diameters following the criteria of Clinical Laboratory standards Institute (CLSI). The incidence of resistance to each antibiotic was calculated by the number of resistant isolates to specific antibiotics/total number of isolates Multiplied by 100. Detection of ESBL production was performed by a double-disk synergy test using *E. coli* ATCC 25922 as a control.^{31,32}

DNA Extraction

The DNA template was prepared by the boiling of the suspensions of bacterial pellets for 10 min and using the supernatant directly in the PCR assay.³³

PCR Primers and Testing Conditions

PCR was used to amplify the targeted virulence and resistance genes. PCR reaction mixture was done in 25 µL reaction volumes containing 12.5 µL master mix (Bioline, USA), 1 µL of each 10 µmol⁻¹ forward and reverse primers (Laboratories Midland Certified Reagent Company Inc.), 2 µL DNA template and 8.5 µL pyrogen-free water. PCR cycling was performed using the conditions summarized in Table 1 according to Yamamoto et al,³⁴ Johnson et al,³⁵ Schamberger et al,³⁶ Schamberger et al,³⁷ Bhattacharjee et al,³⁸ Pal and Singh,¹² Lin et al,³⁹ and Tahamtan et al⁴⁰ PCR products were analyzed using 1.5% agarose gel electrophoresis containing ethidium bromide at 8 V/cm. Then, the reaction product was visualized under Gel doc/UV transilluminator.⁴¹

Statistical Analysis

Statistical analysis was performed using SPSS, 17 statistical software (SPSS Inc., Chicago, IL). In order to compare the frequencies obtained for phenotypic properties, virulence genes and antibiotic resistance, chi-square (X^2) and Fisher's exact test were used. Correlations were established using Pearson's correlation coefficient (r^2) in bivariate linear correlations ($P < 0.05$). P-value is significant if it is ≤ 0.05 .

Results

Out of 200 samples, 96 (48%) and 88 (44%) samples were positive for Gram-negative and Gram-positive bacteria, respectively, while 16 samples (8%) were negative for growth.

Out of 96 Gram-negative bacteria, 64 isolates (66.66%) were confirmed as *E. coli* (10 (15.6%) from wound samples, 22 (34.4%) from urine samples, 23 (35.9%) from stool samples and 9 (14%) from blood samples).

Phenotypic Characteristics

E. coli isolated from urine samples showed greater hemolytic activity (19/22, 86.3%), MRHA (14/22, 63.6%) (Adherence mediated by non-type I pili) and curli fimbriae production (20/22, 90.9%) compared to *E. coli* isolated from other sources. On the other hand, about 88.8% (8/9 isolates) and 66.6% (6/9 isolates) of *E. coli* isolated from blood samples showed high cell surface hydrophobicity and mannose sensitivity (adherence mediated by type I pili). A high incidence of strong biofilm production was observed among *E. coli* isolated from urine (5/22, 22.7%)

Table 1 List of Primers Used in This Study

Name of Gene	Primer Sequence	Amplicon Size	PCR Condition	Reference
Colicin V	F:CACACACAAACGGGAGCTGTT R:CTTCCCGCAGCATAGTTCCAT	680 bp	35 cycles of 94 °C for 30s, 55 °C for 30 s, 72 °C for 1 min	(Johnson and Stell, 2000) [35]
Colicin Ia/Ib	F:ACGTATTACAAATCCCGGTGC R:CTTTTCTTCAACAGGGCA	1250 bp	35 cycles of 94 °C for 30s, 55 °C for 30 s, 72 °C for 1 min	(Schamberger and Diez-Gonzalez, 2004) [36]
Colicin B	F:AAGAAAATGACGAGAAGACG R:GAAAGACCAAAGGCTATAAGG	493 bp	35 cycles of 94 °C for 30s, 55 °C for 30 s, 72 °C for 1 min	(Schamberger and Diez-Gonzalez, 2004) [36]
Colicin M	F:CATCACCATCAACTAATTACC R:CTCTTTACCAGAAAACATCG	737 bp	35 cycles of 94 °C for 30s, 55 °C for 30 s, 72 °C for 1 min	(Schamberger and Diez-Gonzalez, 2004) [36]
Colicin E1	F:TTTGAATGGTACTCCTGACGG R:GTTCCAGCAAGCAAGCTAAA	1398 bp	35 cycles of 94 °C for 30s, 55 °C for 30 s, 72 °C for 1 min	(Schamberger and Diez-Gonzalez, 2004) [36]
Colicin E2-E9	F:CGACAGGCTAAAGCTGTTTCAGGT R:TGCAGCAGCATCAATGCAGCCT	219 bp	35 cycles of 94 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min	(Tahamtan et al, 2012) [40]
crl	F:TTTCGATTGTCTGGCTGTAT R:CTTCAGATTCAGCGTCGTC	250 bp	30 cycles of 94 °C for 40 s, 52°C for 40 s, 72°C for 45 s	(Pal and Singh, 2007) [12]
csgA	F:ACTCTGACTTGACTATTACC R:AGATGCAGTCTGGTCAAC	200 bp	30 cycles of 94 °C for 1 min, 48°C for 1min, 72°C for 1 min	(Pal and Singh, 2007) [12]
fimH	F:TGCAGAACGGATAAGCCGTGG R:GCAGTCACCTGCCCTCCGGTA	508 bp	25 cycles of 94°C for 30s, 63 °C for 30 s, 68 °C for 3min	(Johnson and Stell, 2000) [35]
hlyA	F:AACAAGGATAAGCACTGTTCTGGCT R:ACCATATAAGCGGTCATTCCCGTCA	1177 bp	25 cycles of 94°C for 30s, 63 °C for 30 s, 68 °C for 3min	(Yamamoto et al, 1995) [34]
traT	F:GGTGTGGTGCGATGAGCACAG R:CACGGTTCAGCCATCCCTGAG	290 bp	25 cycles of 94°C for 30s, 63 °C for 30 s, 68 °C for 3min	(Johnson and Stell, 2000) [35]
bla_{-OXA-2}	F: AAGAA ACGCTACTCGCCTGC R: CCACTCAACCCATC CTACCC'	478 bp	40 cycles of 94 °C for 1 min, 55°C for 1min, 72°C for 1 min	(Bhattacharjee et al, 2007) [38]
bla_{-OXA-10}	F:TCTTTTCGAGTACGGCATTAGC R:CCA ATGATGCCCTCACTTTCC	760bp	35 cycles of 96 °C for 1 min, 56°C for 1min, 72°C for 1 min	(Lin et al, 2012) [39]
bla_{-CTX-M-5}	F:CGCTTTGCGATGTGCAG R:ACCGCGATATCGTTGGT	550 bp	40 cycles of 94 °C for 1 min, 55°C for 1min, 72°C for 1 min	(Bhattacharjee et al, 2007) [38]

followed by those isolated from blood samples (2/9, 22.2%) (Table 2).

Prevalence of Virulence Genes Among the Tested Isolates

Irrespective of the source of *E. coli* isolates, the most common virulence genes among all isolates were *csgA* and *crl* (50/64 (78.1%) and 49/64 (76.5%), respectively) and *fimH* (48/64, 75%). The distribution of virulence factors among *E. coli* isolates isolated from different sources is represented in Table 3 which showed that all eight virulence genes tested appeared in the tested urine and stool samples.

On the other hand, *col la-lb* was determined among *E. coli* isolated from different sources with high prevalence among *E. coli* isolated from stool (19/23, 82.6%) while *colV* was more common among fecal and blood isolates (14/23, 60.8% and 5/9, 55.5%, respectively) (Table 3).

High prevalence of *colE1* genes was observed among *E. coli* isolated from stool and urine samples (18/23, 78.3% and 8/22, 36.4%, respectively). *colM* and *colB* genes were not identified in any isolates obtained from blood and stool samples but low prevalence for both genes was observed among *E. coli* isolated from wound swab samples (20% for *colM* and 10% for *colB*, respectively) and uropathogenic

Table 2 Distribution of Virulence Properties Among *E. coli* Clinical Isolates Collected from Different Infections

Virulence Properties	Samples (N= 64)				P-value	Total
	Urine (n=22) N (%)*	Stool (n=23) N (%)*	Blood (n=9) N (%)*	Wound Swabs (n=10) N (%)*		N (%)**
Hemolysis	19 (86.3)	2 (8.6)	4 (44.4)	2 (20)	<0.001*	27 (42.1)
Colicin production	17 (77.2)	20 (86.9)	5 (50)	6 (60)	0.185	58 (90.6)
MRHA test	14 (63.6)	12 (52.2)	5 (55.5)	5 (50)	0.849	36 (56.2)
Mannose sensitivity	13 (59)	8 (34.7)	6 (66.6)	6 (60)	0.241	33 (51.5)
Curli fimbriae production	20 (90.9)	19 (82.6)	6 (66.6)	6 (60)	0.160	51 (79.6)
Cell surface hydrophobicity	18 (81.8)	20 (86.9)	8 (88.8)	6 (60)	0.283	52 (81.2)
Protease production	7 (31.8)	9 (39.1)	2 (22.2)	3 (30)	0.821	21 (32.8)
Biofilm	9 (40.9)	10 (43.4)	3 (33.3)	2 (20)	0.978	24 (37.5)
• Weak/None	8 (36.3)	8 (34.7)	4 (44.4)	3 (30)		23 (35.9)
• Moderate	5 (22.7)	5 (21.7)	2 (22.2)	1 (10)		13 (20.3)
• High						

Notes: *Percentages were correlated to the total number of each type of samples. **Percentages were correlated to the total number of *E. coli* isolate. P values are significant at <0.05.

Abbreviation: MRHA, Mannose Resistant Hemagglutination test.

E. coli (4/22, 18.2% each) was observed. Also, all *E. coli* isolates obtained from blood samples were negative for *colE1*, *colE2-E9*. On the other hand, *fimH* (90.9%), *csgA* (90.9%), *HlyA* (81.8%) and *crl* (86.4%) genes were the most common virulence genes in UPEC while *crl* and *csgA* (88.8% each) genes were the most common virulence genes in *E. coli* isolated from blood samples (Table 3). The difference in the distribution and frequency of virulence genes among *E. coli* isolates with respect to the source of samples is illustrated in Tornado Figure 1.

Antimicrobial Resistance of the Tested Isolates

No significant differences (P-value >0.05) among *E. coli* isolates of different sources in the antibiotic resistance pattern were observed. Figure 2 shows that Meropenem and imipenem were the most effective antibiotics. *E. coli* isolated from stool samples showed the lowest resistance to meropenem (13%) while those isolated from wound samples showed the lowest resistance to imipenem (20%).

Prevalence of ESBL Production and the Tested Resistance Genes Among Isolates

No significant differences (P-value >0.05) among the distribution of the resistance genes and the source of samples were reported in this study. Table 4 shows that ESBL production was common among *E. coli* isolates isolated from blood and urine samples (5/9, 55.5 and 12/22, 54.5%, respectively). Also, *bla*_{-CTX-M-15} was more common among blood isolates

followed by those isolated from urine (4/9, 44.4 and 8/22, 36.3%, respectively). The co-existence of *bla*_{-CTX-M-15} with *bla*_{-OXA-10} (1/9, 11.1%) and *bla*_{-OXA-2} (2/9, 22.2%) were more common among *E. coli* isolated from blood in comparison to those obtained from other sources. In addition, no association between *bla*_{-OXA-2} and *bla*_{-OXA-10} was observed among the tested isolates (Table 4).

Associations Among Virulence Factors

There were distinctive, complex associations and relationships among the tested virulence factors and with one another (Tables 5–8). Colicin genes were found to be common among *E. coli* isolated from urine and stool samples. In UPEC, a significant strong positive association between *colM* with *colE1*, *colB*, *traT* and *csgA* was reported. *colB* was positively associated with *colV*, *colE1*, *colIa-Ib* and *crl* genes, while *colV* showed a positive association with *colE1*, *colIa-Ib*, *fimH*, *traT* and *crl* genes. *colE2-E9* showed a positive association with *colIa-Ib* and *csgA* genes, while *colIa-Ib* showed a positive association with *hlyA*, *traT*, *csgA* and *crl* genes (Table 5). A moderate positive association was reported among the tested genes in *E. coli* isolated from wound samples (Table 6).

For *E. coli* isolated from stool samples, *colM* showed positive association with *colE2-E9*, *colIa-Ib* and *fimH*. *colV* showed a moderate association with *colE2-E9*, *colIa-Ib* (Table 7). A forceful positive correlation between *colV* and *fimH*, *hlyA*, *traT*, *csgA* and *crl* genes was observed in *E. coli* isolated from blood samples (Table 8).

Table 3 Distribution of Virulence Genes Among *E. coli* Clinical Isolates Collected from Different Infections

Virulence Genes	Samples				P-value	Total (n = 64) N (%)**
	Urine (n=22) N (%)*	Stool (n=23) N (%)*	Blood (n=9) N (%)*	Wound Swabs (n=10) N (%) *		
<i>Colicin genes:</i>						
<i>Col M</i>	4 (18.2)	0 (0)	0 (0)	2 (20)	0.109	5 (7.8)
<i>Col B</i>	4 (18.2)	0 (0)	0 (0)	1 (10)	0.084	6 (9.3)
<i>Col V</i>	4 (18.2)	14 (60.8)	5 (55.5)	4 (40)	0.027*	27 (42.1)
<i>Col E1</i>	8 (36.4)	18 (78.3)	0 (0)	3 (30)	0.001*	29 (45.3)
<i>Col E2-E9</i>	4 (18.2)	15 (65.2)	0 (0)	1 (10)	<0.001*	20 (31.25)
<i>Col Ia-Ib</i>	8 (36.4)	19 (82.6)	2 (22.2)	5 (50)	0.003*	34 (53.1)
<i>Other virulence genes</i>						
<i>fimH</i>	20 (90.9)	18 (78.3)	6 (66.6)	4 (40)	0.019*	48 (75)
<i>HlyA</i>	18 (81.8)	4 (17.4)	5 (55.5)	2 (20)	<0.001*	29 (45.3)
<i>traT</i>	13 (59.1)	7 (30.4)	5 (55.5)	4 (40)	0.237	29 (45.3)
<i>CsgA</i>	20 (90.9)	18 (78.3)	8 (88.8)	4 (40)	0.011*	50 (78.1)
<i>Crl</i>	19 (86.4)	17 (73.9)	8 (88.8)	5 (50)	0.114	49 (76.5)
<i>No virulence genes found in each isolate</i>						
0	2 (9)	3 (13)	1 (11.1)	3 (30)	0.451	9 (14)
1	0 (0)	2 (8.6)	2 (22.2)	2 (20)	0.147	6 (9.3)
2	2 (9)	3 (13)	1 (11.1)	2 (20)	0.857	8 (12.5)
3	10 (45.4)	7 (30.4)	2 (22.2)	0 (0)	0.069	19 (29.6)
4	1 (4.5)	1 (4.3)	0 (0)	0 (0)	0.999	2 (3.1)
5	1 (4.5)	2 (8.6)	0 (0)	0 (0)	0.999	3 (4.6)
6	0 (0)	0 (0)	1 (11.1)	0 (0)	0.999	1 (1.5)
7	1 (4.5)	2 (8.6)	2 (22.2)	1 (10)	0.499	6 (9.3)
8	1 (4.5)	1 (4.3)	0 (0)	0 (0)	0.499	2 (3.1)

Notes: *Percentages were correlated to the total number of *E. coli* isolated from each type of samples. **Percentages were correlated to the total number of *E. coli* isolates. P values are significant at <0.05.

fimH gene showed significant strong association only with *traT* gene in uropathogenic *E. coli* (UPEC) isolates and those isolated from wound infections but showed a significant strong positive association with *hlyA*, *traT*, *csgA* and *crl* genes in *E. coli* isolated from stool and blood samples. On the other hand, *hlyA* showed a strong positive association with *csgA* gene in UPEC isolates. Furthermore, *hlyA* showed a strong correlation with *fimH*, *traT*, *csgA* in blood and stool isolates. In fecal isolates, a strong association between *hlyA*, *traT*, *csgA* and *crl* genes was observed (Tables 5–8).

Association of Virulence Factors and Resistance Genes

It was found that *bla*_{-CTX-M-15} gene showed significant (P<0.01) positive association with *colV*, *colE2-E9*, *col Ia-lb*, *hlyA* and *csgA* in UPEC isolates but showed a negative association with the tested virulence genes in *E. coli*

isolated from stool. Also, *bla*_{-CTX-M-15} showed no significant correlation with *colV*, *fimH*, *traT* genes in *E. coli* isolated from blood samples and *colV*, *fimH*, *hlyA* and *traT* in *E. coli* isolated from Wound samples. *bla*_{-OXA-2} showed strong correlations with *colM*, *colB*, *colE* and *crl* genes in UPEC but showed moderate correlations with *colM* in wound isolates. *bla*_{-OXA-10} was mostly associated with *bla*_{-CTX-M-15} in case of *E. coli* isolated from stool and blood samples, with *traT*, *hlyA* and *bla*_{-CTX-M-15} in case of *E. coli* isolated from wound samples and with *colM*, *colB*, *crl* and *bla*_{-CTX-M-15} genes in case of uropathogenic *E. coli* (Tables 5–8).

Discussion

Having many bacterial virulence factors was reported to affect the severity and the extent of infection of any pathogenic microorganisms. In addition, the ability of a microorganism to cause diseases depends not only on

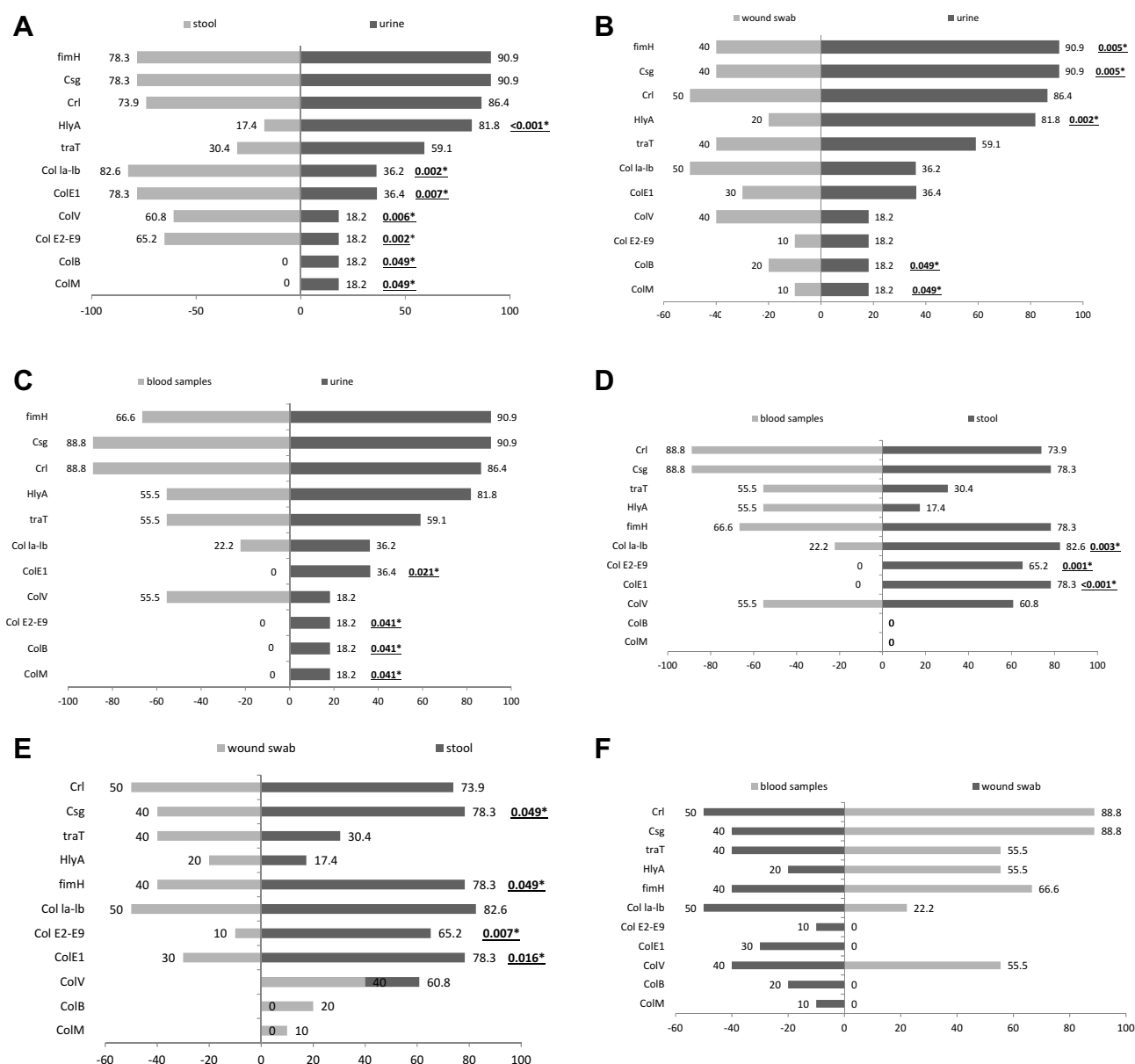


Figure 1 Virulence genotypes of the tested *E. coli* isolates based on the type of samples. (A): Stool and urine samples. (B): Wound and urine samples. (C): Blood and urine samples. (D): Blood and stool samples. (E): Wound and stool samples. (F): Blood and wound samples.

their virulence factors but also due to the patient underlying diseases and the other host determinants.^{42–44}

Fimbriae and pili have a role in the hydrophobic characters of bacterial cells and in the adhesion. Hemolytic activity plays a role in tissue damage and the interference with the local immune response. Also, MRHA are adhesive factors which are essential in the well establishment of *E. coli* to various tissues. Cell surface hydrophobicity of the bacterial cell surface promotes the adherence of the bacteria to various surfaces like the mucosal epithelial cells. Our results showed that the hemolytic activity, mannose resistant haemagglutination

and curli fimbriae production were more common in the urinary tract infection isolates in comparison to other extra-intestinal and fecal isolates which were in agreement with that reported by Fakruddin et al⁴⁵ and Najjar et al²⁵ Furthermore, we found that hydrophobic *E. coli* were common among blood isolates that were different from that reported by Fakruddin et al⁴⁵ who found that urinary isolates were more hydrophobic than isolates from other sources but blood isolates showed low hydrophobicity with high values of salt aggregation test (SAT) suggesting that surface hydrophobicity has the minor role in the pathogenesis of septicemia. Shruthi et al⁴⁶ reported

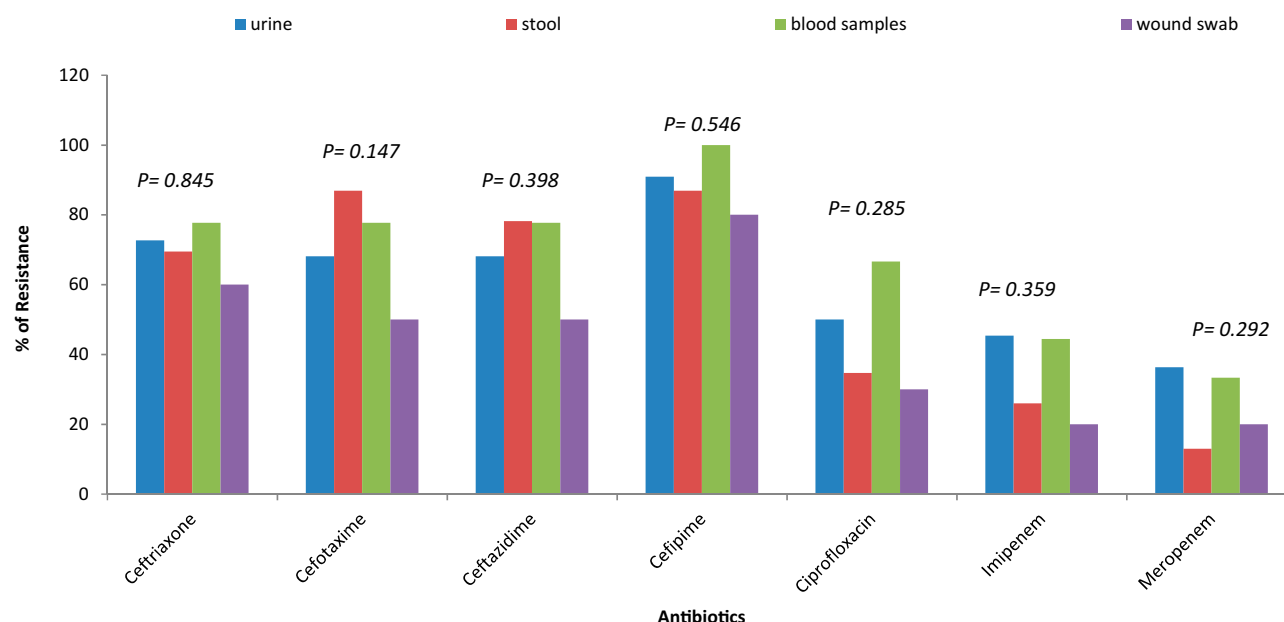


Figure 2 Distribution of antibiotic resistance among *E. coli* clinical isolates of different sources.

that MRHA was more in fecal isolates than in urine isolates.

Biofilm formation is a crucial step that facilitates the initial adhesion, exopolysaccharide production and subsequent dispersion and spread. Our results showed no significant difference among the tested *E. coli* isolates isolated from different sources but it was observed that biofilm production was most common among isolates obtained from blood and urine samples. Reisner et al⁴⁷ reported that biofilm production is not dependent on the *E. coli* origin but it is affected by the composition of growth media, environmental conditions and the expression of some biofilm promoting factors such as curli and nonconjugative pili which can increase biofilm production. In addition, most *E. coli* isolates were moderate biofilm producers (urine (45.4%), stool (34.7), blood (44.4%) and wound swabs (30%)). Prevalence of moderate production of biofilm by urine isolates was also reported by Samie and Nkgau⁴⁸ Protease production was common in fecal and urine isolates that may indicate the ability of these isolates to cause damage to urinary and the intestinal cells. Fujishige et al⁴⁹ and Vermelho et al⁵⁰ reported the importance of detecting the proteolytic activity of the microorganisms that help in the understanding of their role in the pathogenesis and tissue damage.

Colicin production is an important character that is observed in both pathogenic and commensal *E. coli*. Colicins have mainly three cytotoxic functions which

are: nuclease activity causing degradation of DNA or RNA of the target cells (colicins E2-E9), pore-forming colicins which can form channels and depolarize cytoplasmic membranes (*colB*, *col Ia-Ib*, K) and the inhibition of peptidoglycan synthesis that only represented by colicin M.⁵¹

Irrespective to *E. coli* isolates from stool samples, Uropathogenic *E. coli* showed a high prevalence of *colM* and *colE1* in comparison to other ExPEC that was in agreement with Azpiroz et al⁵² and Rijavec et al⁵³ *ColV* (microcin V) was common among ExPEC isolated from blood which agreed with that reported by Davies et al,⁵⁴ Fakruddin et al⁴⁵ and Micenková et al⁵⁵ *E. coli* isolated from wound samples showed a high prevalence of *colM* in comparison to the other ExPEC while 50% of isolates were positive for *Col Ia-Ib* which is in agreement with that reported by Micenková et al.⁵⁵

There was a positive correlation among colicin production and the expression of other virulence factors. Many researchers showed the high frequency and the positive correlation of bacteriocin production and the expression of many virulence factors indicating the possibility of their contribution to *E. coli* pathogenicity.^{9,55-57} Ozanne et al⁵⁸ and Smith⁵⁹ showed that mice injected by *colV* showed 100% death for macrophages in the peritoneal fluids. Also, mice showed symptoms resemble that observed in endotoxin shock. These findings suggested the contribution of *colV* in *E. coli* pathogenesis.

Table 4 Distribution of ESBL Production and Resistance Genes Among the Isolated *E. coli* from Different Sources

	Source of Isolates				P-value
	Urine (n=22) N (%)*	Stool (n=23) N (%)*	Blood (n=9) N (%)*	Wound Swabs (n=10) N (%) *	
ESBL Producers	12 (54.5)	10 (43.4)	5 (55.5)	3 (30)	0.672
Resistance Genes					
bla_{-CTX-M-15}	8 (36.3)	6 (26)	4 (44.4)	3 (30)	0.756
bla_{-OXA-2} like	3 (13.6)	0 (0)	2 (22.2)	1 (10)	0.203
bla_{-OXA-10} like	2 (9)	1 (4.3)	2 (22.2)	0 (0)	0.999
bla_{-CTX-M-15} + bla_{-OXA-2} like	2 (9)	0 (0)	2 (22.2)	1 (10)	0.999
bla_{-CTX-M-15} + bla_{-OXA-10} like	1 (4.5)	0 (0)	1 (11.1)	1 (10)	0.999
bla_{-OXA-2} like + bla_{-OXA-10} like	0 (0)	0 (0)	0 (0)	0 (0)	0.999

Notes: *Percentages were correlated to the total number of *E. coli* isolated from each type of samples. P values are significant at <0.05.

In this study we tried to determine the most frequently occurring virulence factors among *E. coli* isolates isolated from different origins. *fimH* gene is the gene encoding type 1 fimbriae and is important in the establishment of infections. It was found that *fimH*, *hlyA*, *traT*, *csgA*, and *crl* genes were more common among Uropathogenic *E. coli* compared to isolates of other ExPEC and intestinal isolates indicating that uropathogenic isolates were more virulent than other tested isolates. Cergole-Novella et al⁶⁰ showed that there was an association among *fimH*, *crl*, *csg*, *traT* and *colV* with *bla_{-CTX-M-15}* in *E. coli* isolated from gastroenteritis which was in agreement with our results.

The high prevalence and association of *fimH* (90.9%) with uropathogenic isolates were reported by many studies.^{61–63} On the other hand, some studies showed a lower incidence of *fimH* among uropathogenic isolates such as Tabasi et al⁶⁴ and Paniagua-Contreras et al¹⁴ *HlyA* gene was more frequently common among uropathogenic *E. coli* and those isolated from blood (81.8% and 55.5%). Prevalence of hemolysin protein contributes to virulence of both *E. coli* of urine or blood origin as it is a pore-forming protein. Also, it was found associated with isolates of urinary tract infections that may give rise to bacteremia.^{65,66} This study showed that *traT* was more common among *E. coli* isolated from urine and blood samples (59.1% and 55.5%, respectively). *E. coli* with serum resistance were highly virulent, as they can escape the complement system and promote serum survival and increase the risk of developing septic shock and the increase in mortality.^{3,16} In our study, Multi-drug resistance (MDR) (resistance to ≥ 3 antimicrobials of different classes) to most of the tested antibiotics was more common among urine and blood *E. coli* isolates especially to

cefepime (100% resistance) and the other tested cephalosporins. Ciprofloxacin resistance was observed mostly among urine and blood isolates (50% and 66.6%, respectively) which in agreement with Raeispour and Ranjbar,⁶⁷ Abdi et al⁶⁸ and Hashemizadeh et al⁶⁹ Cergole-Novella et al⁶⁰ showed that *fimH*, *crl*, *csg*, *traT* and *colV* were common among all tested *E. coli* obtained from different sources (urinary tract infection, septicemia, respiratory infection and gastroenteritis) with 100% prevalence of *fimH*, *crl*, *csg* genes among all isolates followed by *traT* (83.3%). Also, they showed that biofilm production was observed among isolates from gastroenteritis, sepsis and UTI.

ESBL production was found to be more common among *E. coli* isolated from blood and urine samples. Also, all tested resistance genes (*bla_{-CTX-M-15}*, *bla_{-oxa-2}*, and *bla_{-oxa-10}*) were found to be more prevalent among *E. coli* isolates obtained from urine and blood.⁷⁰ OXA-type β -lactamases have high hydrolytic activity against oxacillin and cloxacillin but they are poorly inhibited by clavulanic acid. OXA-2 and OXA-10 have recently reported to have extended hydrolytic spectrum to include oxyimino cephalosporins. OXA-2 was first reported in pseudomonas spp. then in *E. coli* from Israel in 2005. Many studies reported that the increase in the expression of different virulence factors results in the increase of the microbial pathogenicity. Also, antibiotic resistance genes expression was found to increase the microbial pathogenicity. So, antibiotic resistance genes were considered as a subtype of virulence factors. By using biofilm as an example, the presence of pili, fimbriae, flagella promotes the adhesion of microbes to biotic or abiotic surfaces.^{71,72} In addition, these factors promote the formation of biofilm and the

Table 5 Relationships Between Virulence Factors Genes and Resistance Genes in Uropathogenic *E. coli* Isolates

	Correlations										
	colM	colB	colV	colEI	colE2-E9	colIa-Ib	fimH	hlyA	traT	CsgA	Crl
colM	NA	0.681**	0.550*	0.719**	0.586*	0.540*	0.568*	0.559*	0.678**	0.658*	0.477
colB		NA	0.747**	0.851**	0.609*	0.767**	0.466	0.611*	0.572*	0.671**	0.797**
colV			NA	0.774**	0.677**	0.788**	0.673**	0.515	0.690**	0.479	0.746*
colEI				NA	0.672**	0.821**	0.549*	0.591*	0.743**	0.595*	0.722**
colE2-E9					NA	0.665**	0.422	0.445	0.518	0.667**	0.584*
colIa-Ib						NA	0.289	0.691**	0.667**	0.767**	0.668*
fimH							NA	0.309	0.715**	0.187	0.350*
hlyA								NA	0.545*	0.749**	0.520*
traT									NA	0.511	0.254
CsgA										NA	0.430
Crl											NA
bla-CTX-M-15											0.683
bla-Oxa-2											NA
bla-Oxa-10											0.635
											NA
											0.750
											NA

Notes: Statistical analysis of associations between virulence factors (VFs). P values were calculated by Fisher's exact test. *Correlation is significant at 0.05 level (2-tailed). **Correlation is significant at 0.01 level (2-tailed). Abbreviation: NA, not applicable.

Table 6 Relationships Between Virulence Factors Genes and Resistance Genes in *E. coli* Isolated from Wound Samples

	Correlations													
	colM	colB	colV	colEI	colE2-E9	colla-lb	fimH	hlyA	traT	CsgA	CrI	bla _{-CTX-M-15}	bla _{-oxa-2}	bla _{-oxa-10}
ColM	NA	0.147	0.505	-0.048	0.222	-0.184	0.471	0.167	0.427	0.059	-0.050	0.615	0.577	0.417
ColB		NA	0.387	0.576*	0.079	0.040	0.531	0.059	0.151	0.458	0.354	0.165	0.000	0.079*
ColV			NA	0.569*	0.014	-0.293	0.499	-0.063	0.121	0.099	-0.025	0.461	0.438	0.309*
Col EI				NA	0.176	-0.081	0.314	0.216	0.177	0.610*	0.489	0.348*	-0.083*	0.288
Col E2-E9					NA	0.642*	0.609*	0.556*	0.552*	0.380	0.378	0.368	0.192	0.481
Col Ia-lb						NA	0.450	0.184	0.154	0.287	0.430	-0.115	-0.294	-0.151
fimH							NA		0.88 **	0.491	0.265	0.518	0.408	0.471
hlyA								NA	0.88 **	0.576*	0.400	0.722	0.289	0.750
traT									NA	0.465	0.352	0.850	0.462	0.801
CsgA										NA	0.636	0.430	-0.136	0.288
CrI											NA	0.289	-0.231	0.144
bla _{-CTX-M-15}												NA	0.662	0.863
bla _{-Oxa-2}													NA	0.770
bla _{-Oxa-10}														NA

Notes: Statistical analysis of associations between virulence factors (VFs). *P* values were calculated by Fisher's exact test. *Correlation is significant at 0.05 level (2-tailed). **Correlation is significant at 0.01 level (2-tailed).
Abbreviation: NA, not applicable.

Table 7 Relationships Between Virulence Factors Genes and Resistance Genes in Fecal *E. coli* Isolates

	Correlations													
	colM	colB	colV	colEI	colE2-E9	colla-lb	fimH	hlyA	traT	CsgA	Crl	bla _{-CTX-M-15}	bla _{-oxa-2}	bla _{-oxa-10}
ColM	NA	–	0.487	0.552*	0.636*	0.616*	0.574*	0.208	0.132	0.366	0.371	0.018 ^a	.	–0.245*
ColB	–	NA	–	–	–	–	–	–	–	–	–	–	–	–
ColV			NA	0.423	0.600*	0.680**	0.458	0.269	0.221	0.434	0.545	–0.144 ^a	–	0.063
Col EI				NA	0.260	0.384	0.298	0.057	–0.007	0.206	0.212*	0.090 ^a	–	0.134
Col E2-E9					NA	0.972**	0.226	0.037	–0.016	0.187	0.129*	–0.215 ^a	–	–0.394
Col la-lb						NA	0.196	–0.013	–0.019	0.182	0.158*	–0.251 ^a	–	–0.380
fimH							NA	0.719**	0.740**	0.825**	0.857*	–0.057*	–	0.224
hlyA								NA	0.859**	0.843**	0.847	–0.288 ^a	–	0.168
traT									NA	0.833	0.905	–0.265	–	0.155
CsgA										NA	0.918	–0.234	–	0.116
Crl											NA	–0.252	–	0.160
bla _{-CTX-M-15}												NA	–	0.714
bla _{-Oxa-2}													NA	–
bla _{-Oxa-10}														NA

Notes: Statistical analysis of associations between virulence factors (VFs). P values were calculated by Fisher's exact test. *Correlation is significant at 0.05 level (2-tailed). **Correlation is significant at 0.01 level (2-tailed). ^aCannot be computed because at least one variable is constant.

Abbreviation: NA, not applicable.

Table 8 Relationships Between Virulence Factors Genes and Resistance Genes in *E. coli* Isolated from Blood Samples

	Correlations									
	colM	colB	colV	colEI	colE2-E9	colla-lb	fimH	hlyA	traT	CsgA
ColM	NA	—	—	—	—	—	—	—	—	—
ColB		NA	—	—	—	—	—	—	—	—
ColV			NA	—	—	0.695**	0.944**	0.892**	0.814**	0.866**
Col EI				NA	—	—	—	—	—	—
Col E2-E9				NA	NA	—	—	—	—	—
Col Ia-lb						NA	0.699**	0.678**	0.854**	0.817**
fimH							NA	0.939**	0.823**	0.912**
hlyA								NA	0.918**	0.885**
traT									NA	0.878
CsgA									NA	NA
CrI									NA	0.968
bla _{-CTX-M-15}									NA	0.578
bla _{-Oxa-2}									NA	0.515
bla _{-Oxa-10}									NA	0.376
									NA	0.408
									NA	0.815
									NA	0.666
									NA	0.770
									NA	NA

Notes: Statistical analysis of associations between virulence factors (VFs). P values were calculated by Fisher's exact test. *Correlation is significant at 0.05 level (2-tailed). **Correlation is significant at 0.01 level (2-tailed). ^aCannot be computed because at least one variable is constant.

Abbreviation: NA, not applicable.

expression of quorum sensing signals (regulates cellular functions depending on the cellular density) resulting in the increase of pathogenicity and resistance to antibiotics.⁷³ Furthermore, it was found that there are large plasmids that carry many virulence genes in association with antibiotic resistance genes (hybrid plasmids) which means that selection of these plasmids by antibiotics may select for some virulence characteristics (horizontal gene transfer) as an adverse effect to the antibiotic therapy.⁷² In another study done by Escudeiro et al,⁷⁴ the authors reported that there is a strong correlation among virulence factors and antibiotic resistance and the acquisition of new virulence genes is followed by the acquisition of new resistance genes. From the previous findings, there are a widespread of virulence genes in association with resistance genes which increase the need for enhanced surveillance and the emergence of new antimicrobials with anti-virulence ability.

Conclusion

The relationship between virulence factors and resistance genes is complex and needs more studies that should be specific for each area. There is a significant association among colicin, virulence and resistance genes indicating their contribution in the establishment and the progress of infection, especially with ExPEC. *Extra-intestinal E. coli* isolated from urine and blood samples represent a battery of virulence factors and resistance genes with a great ability to produce biofilm which is considered a great challenging health problem. So, there is a need for studying how to control these factors to decrease the rate and the severity of infections by the emergence of new antimicrobials with anti-virulence ability.

Informed Consent

Informed consent is not required as samples were obtained from the laboratory of hospitals as part of the routine hospital laboratory procedure.

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