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A N I N T E R N A T I O N A L J O U R N A L

Escherichia Coli: Characteristics of Carbapenem Resistance and Virulence Factors

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ABSTRACT

In this study, fifty Escherichia coli strains were analyzed by multiplex polymerase chain reaction for the genes expressed carbapenemase and virulence factors in order to determine the presence of carbapenemase and nine virulence factors and investigate the association between these two characteristics. When carbapenemase susceptibility was taken into consideration, OXA-48 type carbapenemase *was determined for 22% of the total strains. Also, the frequency of virulence gene regions in E.coli infections and virulence gene profiles of these isolates were examined and the frequency of pap, afa, sfa, fimA, iroN, aer, iutA, hly and cnf-1 genes were 24, 38, 20, 84, 28, 90, 92, 10 and 34% respectively. A significant correlation was found between the presence of fimA and afa gene regions and carbapenem susceptibility (P< 0.05). Based on the combination of carbapenemase and virulence factor genes, 24 different gene profiles were determined for all strains. The results of the study appear to indicate that fimA and afa genes correlate with carbapenem susceptibility, the relations of fimA with urinary tract infections and pap with complicated urinary tract infections. It also indicates that sfa and afa genes correlate with other infections except urinary tract infections.*

Key words: Carbapenemase; *Escherichia coli*; multiplex polymerase chain reaction; virulence factor.

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INTRODUCTION

Escherichia coli causes urinary tract infections, neonatal meningitis, sepsis and intestinal infections more frequently than other members of the *Enterobacteriaceae* and it is responsible for 80% of community-acquired urinary tract infections.[1]
Extended-spectrum beta-lactamases (ESBL) which hydrolyze penicillins, cephalosporins, monobactams and generally inhibited by beta-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam can be produced by the members of *Enterobacteriaceae*.[2] The increasing usage of carbapenems, the first treatment option in ESBL producing *E. coli* infections, has brought out the problem of carbapenem resistance.[3] Carbapenems was firstly identified in clinical isolate of *Enterobacter cloacae*[4] and then carbapenem-resistant *Enterobacteriaceae* was reported from around the world.[5] Serine carbapenemases (KPC and OXA-48) and metallo-beta-lactamases (VIM, IMP and NDM) are the most commonly seen among carbapenemases.[5]
E.coli strains have some gene regions responsible for virulence factors which may encode adhesins, toxins, siderophores and haemolysin. Type 1 fimbriae, coded by plasmid-mediated *fimA* gene and commonly found in these strains from lower urinary system infections, enable *E.coli* to adhere to human ureteral mucosa epithelial cells.[6] P fimbriae that is expressed by plasmid-mediated *pap* (pyelonephritis-associated pili) gene, is produced by *E.coli* colonization of upper urinary system.[7] Afimbrial adhesin encoded by plasmid- or chromosom- mediated *afa* and S fimbriae encoded by plasmid *sfa* gene regions are commonly found in urinary system infection originated in isolates as well as sepsis and meningitis.[8,9]
Siderophores commonly found in *E.coli* isolates are aerobactin and ferric aerobactin, encoded by *aer* and *iutA* genes.[10] Also, salmochelin type siderophore encoded by *iroN* gene in recent years has become important.[11] These genes are expressed as plasmid-mediated and they are especially related to necrotoxigenic, uropathogenic and septicemic *E.coli* isolates causing urinary tract infections, septicemia, bacteremia and systemic infections. [6,12] Also, cytotoxic necrotizing factor 1 (*cnf-1*) and haemolysin (*hly*) toxins are important for diversity of *E.coli* infections[13,14] and these gene regions expressed by plasmids are mostly produced by uropathogenic *E.coli* strains.[15]
The aim of the study is to determine the presence of carbapenemase and virulence factors of *E.coli* clinical specimens and evaluate the possible correlations between carbapenem resistance and virulence factors. Also, the frequency of virulence gene regions in *E.coli* infections and virulence genotypes of these isolates were determined.

MATERIAL AND METHODS

Bacterial strains and identification

In this study, *E.coli* strains (n=50), obtained from clinical specimens including abscess, bronchial, urea, blood, plevral efusion, tracheal, wound, catheter, body and ear fluid, were collected from three different hospitals in Ankara. Conventional methodology (Gram staining, hemolysis of blood agar, string test, IMViC tests, lactose fermentation, ornithine decarboxylase and motility tests), automatized system (Vitek-32 System, bioMerrieux, France) and CHROMagar Orientation (CHROMagar Company, Paris, France) were used for identification of the strains.

Antimicrobial susceptibility testing
Susceptibility to extended spectrum beta lactamases (ESBL) and carbapenems were determined with CHROMagar ESBL (CHROMagar Company, Paris, France)[16] and CHROMagar KPC (CHROMagar Company, Paris, France)[17] respectively.

DNA isolation
Genomic and plasmid DNA were isolated using NucleoSpin®Tissue (Macherey-Nagel, Germany) and NucleoSpin®Plasmid (Macherey-Nagel, Germany) and were stored in -20ºC.

Multiplex PCR analysis of carbapenemase gene regions
The reaction conditions were modified from Poirel et al. [18]. The Multiplex PCR mixture consisted of 1XPCR buffer (20mM Tris HCl, 10mM $(NH_4)_2SO_4$, 10mM KCl, 2mM $MgSO_4$, 0,1% Triton X-100), 0,05mM dNTP, 2U Taq polymerase (NEB, Beverly, MA), 50 µmol/L each of primers (NEB, Beverly, MA) and 2µl DNA. The primers used in this study are listed in Table 1.
Amplification was carried out with the following thermal cycling conditions: 5 minutes of pre-denaturation at 95°C, followed by 35 cycles: 1 minute at 95°C, 1 minute at 52°C, 1 minute at 72°C and 10 minutes of final elongation at 72°C (Sensoquest Labcycler, Germany).

Table 1. Carbapenemase gene regions[18]

Primer	**Sequence* (5'-3')**	**Gene**	**Product size (bp)**
KPC-F	CGTCTAGTTCTGCTGTCTTG	bla_{KPC}	798
KPC-R	CTTGTCATCCTTGTTAGGCG		
NDM-1-F	GGTTTGGCGATCTGGTTTTC	bla_{NDM-1}	621
NDM-1-R	CGGAATGGCTCATCACGATC		
OXA-48-F	GCGTGGTTAAGGATGAACAC	bla_{OXA-48}	438
OXA-48-R	CATCAAGTTCAACCCAACCG		
IMP-F	GGAATAGAGTGGCTTAAYTCTC	bla_{IMP}	232
IMP-R	GGTTTAAYAAAACAACCACC		
VIM-F	GATGGTGTTTGGTCGCATA	bla_{VIM}	390
VIM-R	CGAATGCGCAGCACCAG		

**Y=C or T*
IMP, imipenem metallo-β-lactamase; KPC, Klebsiella pneumoniae carbapenemase; NDM, New Delhi metallo-β-lactamase; OXA, oxacillinase; VIM, verona integron-encoded metallo-β-lactamase.

Multiplex PCR analysis of virulence gene regions

The mix for the detection of *pap* genes consisted of 2XPCR buffer (40mM Tris HCl, 20mM $(NH_4)_2SO_4$, 20mM KCl, 4mM $MgSO_4$, 0,2% Triton X-100), 0,2mM dNTP, 2U Taq polymerase (NEB, Beverly, MA), 2,5 µmol/L each of primers (NEB, Beverly, MA) and 2,5µl DNA. The mix for the detection of other groups (*afa-sfa-fimA, hly-iroN-aer-cnf1-iutA)* was at the same concentrations. The primers used in this study are listed in Table 2.

Table 2. Virulence gene regions of *E.coli*[13,14,19]

Primer	Sequence (5'-3')	Product size (bp)
fimA		
fimA-F	GTTGTTCTGTCGGCTCTGTC	447
fimA-R	ATGGTGTTGGTTCCGTTATTC	
pap		
pap1	GACGGCTGTACTGCAGGGTGTGGCG	328
pap2	ATATCCTTTCTGCAGGGATGCAATA	
pap3	GCAACAGCAACGCTGGTTGCATCAT	336
pap4	AGAGAGAGCCACTCTTATACGGACA	
sfa		
sfa-F	CTCCGGAGAACTGGGTGCATCTTAC	410
sfa-R	CGGAGGAGTAATTACAAACCTGGCA	
aer		
aer-F	TACCGGATTGTCATATGCAGACCG	602
aer-R	AATATCTTCCTCCAGTCCGGAGAAG	
cnf-1		
cnf-F	AAGATGGAGTTTCCTATGCAGGAG	498
cnf-R	CATTCAGAGTCCTGCCCTCATTATT	
hly		
hly-F	AACAAGGATAAGCACTGTTCTGGCT	1177
hly-R	ACCATATAAGCGGTCATTCCCGTCA	
afa		
afa-F	GCTGGGCAGCAAACTGATAACTCTC	750
afa-R	CATCAAGCTGTTTGTTCGTCCGCCG	
iutA		
iutA-F	GGCTGGACATCATGGGAACTGG	300
iutA-R	CGTCGGGAACGGGTAGAATCG	
iroN		
iroN-F	AAGTCAAAGCAGGGGTTGCCCG	665
iroN-R	GACGCCGACATTAAGACGCAG	

Amplification was carried out with the following thermal cycling conditions: 5 minutes of pre-denaturation at 95°C, followed by 30 cycles: 1 minute at 94°C, 1 minute at 58°C, 1 minute at 72°C and 10 minutes of final elongation at 72°C (Sensoquest Labcycler, Germany).

PCR products were analyzed by electrophoresis in a 1.8% agarose gel at 150 V for 2 h in 1 × TBE (89 mM Tris, 89 mM Boric Acid and 2 mM EDTA) containing 0.05 mg/L ethidium bromide and images were captured by Gel Logic 200 Molecular Imaging System (Kodak; Rochester).

Data analysis
The Fisher's Exact Test were performed for all clinical data. P value of <0.05 was considered statistically significant.

RESULTS

In total, 50 *Escherichia coli* strains were identified by microbiology standard methods and chromogenic medium.

Analysis of the carbapenemase gene regions
Among *E.coli* isolates, plasmid mediated oxacillinase (OXA-48) gene was determined in 11 strains (22%) (Fig. 1). Carbapenem gene regions of these isolates are given in Figure 1. Similarly, carbapenem resistance in these strains was determined phenotypically, using CHROMagar KPC.

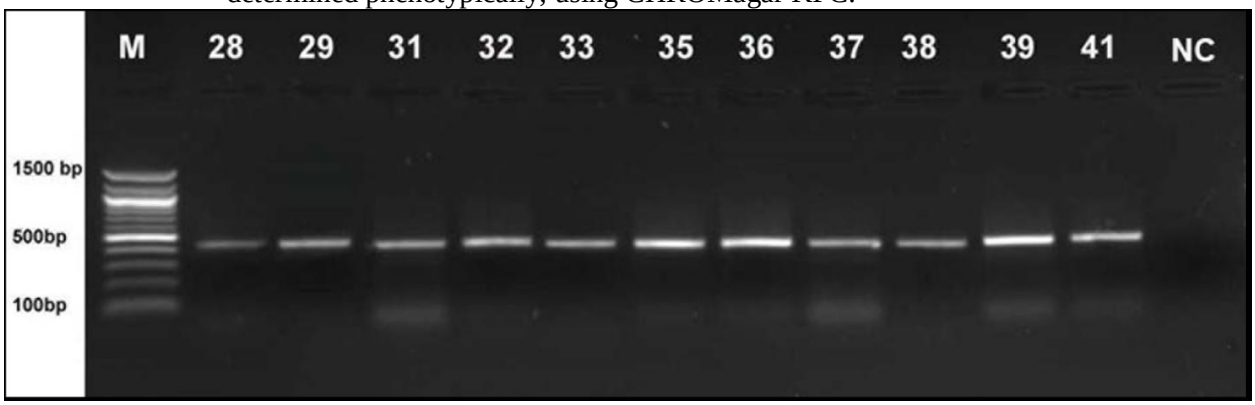

Figure 1. OXA-48 type carbapenemase of *E.coli* strains (28-41; E.coli, NC; Negative control, M; 100 bp DNA molecular marker).

Analysis of the virulence gene regions
In this study, nine virulence gene regions (*pap, afa, fimA, sfa, hly, iroN, aer, cnf-1* and *iutA*) for *E.coli* isolates were analyzed by Multiplex PCR. These gene regions are shown in Figure 2.

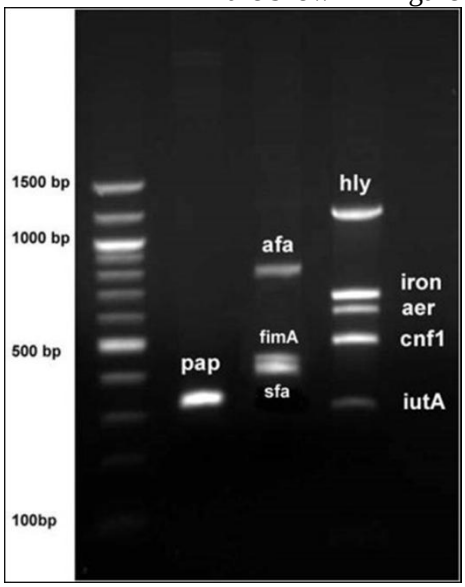

Figure 2. Determined virulence gene regions in *E.coli* (pap; 328-336 bp, afa; 750 bp, fimA; 447 bp, sfa; 410 bp, hly; 1177 bp, iroN; 665 bp, aer; 602 bp, cnf-1; 498 bp, iutA; 300 bp).

The distribution of the virulence gene regions in *E.coli* strains are shown in Figure 3. A high prevalence of virulence genes expressing siderophores (*iutA*; 92%, *aer*; 90%) in *E.coli* strains was observed. The Multiplex PCR results showed that among the strains, 84% were positive for *fimA*, 38 for *afa*, 34 for *cnf-1*, 28 for *iroN*, 24 for *pap*, 20 for *afa* and 10 for *hly*. The least common virulence gene region among strains is *hly* gene region responsible for hemolysis.

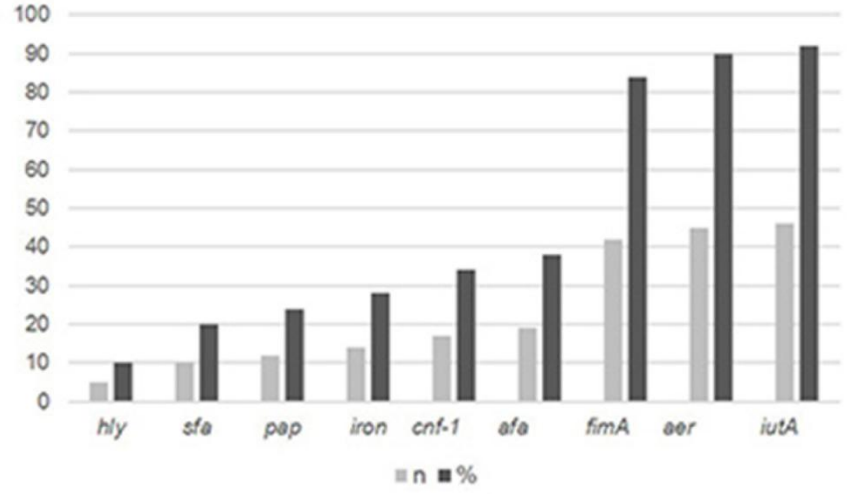

Figure 3. Distribution of virulence gene regions in E.coli strains. "n" is the number of isolates that were found to possess a given gene; "%" represents n as the percentage of the 50 strains studied.

Distribution (%) of virulence factors in different clinical *E.coli* strains, as distribution of gene regions related with fimbria types (*afa*, *fimA*, *sfa* and *pap*), siderophore formation (*iroN, iutA* and *aer*) and other virulence factors (*cnf-1* and *hly)*, are shown in Figure 4. Great diversity of virulence in urine isolates were observed, whereas any virulence gene is not found in catheter.

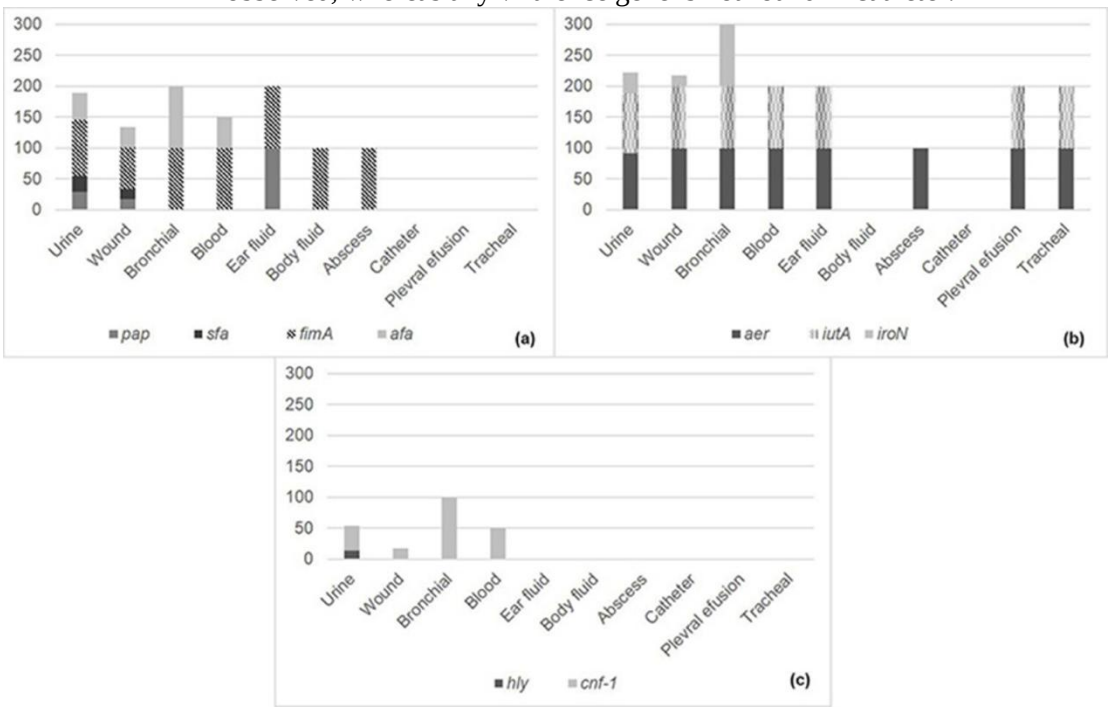

Figure 4. Distribution (%) of virulence genes (a; fimbriae, b; siderophore, c; others) in different clinical sources (urine; 35, wound; 6, blood; 2, bronchial; 1, ear fluid; 1, body fluid; 1, abscess; 1, catheter; 1, plevral efusion; 1, tracheal; 1 strain).

Virulence factor gene profiles of *E. coli* isolates

In this study, when the carbapenem and virulence gene profiles are evaluated and 24 types of virulence profiles are identified (Table 3). In total, 98% of the strains carry at least one virulence gene.

Table 3. Carbapenem resistance and virulence gene profiles of *E.coli* strains

Strains	Clinical source	Carbapenem resistance	Virulence gene profiles
29	Catheter	OXA-48	*not determined*
44	Body fluid	susceptible	*fimA*
48	Urine	susceptible	
41	Urine	OXA-48	*fimA, iutA*
1	Abscess	susceptible	*fimA, aer*
28	Plevral efusion	OXA-48	*aer, iutA*
32	Wound	OXA-48	
35	Wound	OXA-48	
36	Tracheal	OXA-48	
30	Urine	susceptible	
49	Urine	susceptible	
27	Urine	susceptible	*aer, iutA, cnf-1*
7	Urine	susceptible	*fimA, aer, iutA*
19	Urine	susceptible	
40	Urine	susceptible	
43	Wound	susceptible	
33	Urine	OXA-48	*fimA, sfa, aer, iutA*
18	Urine	susceptible	*fimA, pap, aer, iutA*
45	Urine	susceptible	
46	Ear fluid	susceptible	
37	Blood	OXA-48	*fimA, afa, aer, iutA*
5	Urine	susceptible	
8	Urine	susceptible	
9	Urine	susceptible	
24	Wound	susceptible	
25	Wound	susceptible	
50	Urine	susceptible	
31	Urine	OXA-48	*fimA, aer, iutA, cnf-1*
4	Urine	susceptible	
6	Urine	susceptible	
17	Blood	susceptible	
34	Urine	susceptible	*fimA, sfa, aer, iutA, iroN*
38	Urine	OXA-48	*fimA, pap, aer, iutA, hly*
16	Urine	susceptible	*fimA, pap, aer, iutA, iroN*
21	Urine	susceptible	*fimA, pap, afa, aer, iutA*
26	Urine	susceptible	

3	Urine	susceptible	*fimA, afa, aer, iutA, cnf-1*
20	Urine	susceptible	
12	Urine	susceptible	*fimA, pap, afa, aer, iutA, iroN*
15	Urine	susceptible	
23	Urine	susceptible	*fimA, sfa, afa, iutA, iroN, cnf-1*
2	Bronchial	susceptible	*fimA, afa, aer, iutA, iroN, cnf-1*
13	Urine	susceptible	
22	Urine	susceptible	*fimA, sfa, aer, iutA, iroN, hly, cnf-1*
47	Urine	susceptible	
11	Urine	susceptible	*fimA, sfa, afa, aer, iutA, iroN, cnf-1*
42	Wound	susceptible	*fimA, pap, sfa, aer, iutA, iroN, cnf-1*
39	Urine	OXA-48	*fimA, pap, sfa, aer, iutA, iroN, hly, cnf-1*
10	Urine	susceptible	*fimA, sfa, afa, aer, iutA, iroN, hly, cnf-1*
14	Urine	susceptible	*fimA, pap, sfa, afa, aer, iutA, iroN, cnf-1*

When the virulence profiles in Table 3 are examined, carbapenem resistant *E.coli* isolated from catheter (strain 29) was not included any virulence gene regions. On the other hand, carbapenem resistant *E.coli* (strain 39) isolated from urine has eight virulence gene regions and the strain is the most virulent isolate among other.

Correlation between virulence genes and carbapenem resistance

The overall virulence factor productions between carbapenem resistant (n=11) and carbapenem susceptible (n= 39) *E.coli* strains are shown in Table 4. Correlation between the presence of the *fimA* or *afa* gene regions and carbapenem resistance was statistically significant (*P< 0.05)*. Most of *E.coli* strains carrying these genes were susceptible to carbapenem.

Table 4. Distribution of carbapenem resistant and susceptible *E.coli* strains

Virulence factors	Carbapenem resistant strains (n=11) (22%)	Carbapenem susceptible strains (n=39) (78%)	*P value*
pap	2 (18)	10 (26)	>0.05
sfa	2 (18)	8 (21)	>0.05
fimA	6 (55)	36 (92)	**<0.05**
afa	1 (9)	18 (46)	**<0.05**
hly	2 (18)	3 (8)	>0.05
cnf-1	2 (18)	15 (38)	>0.05
iutA	10 (91)	36 (92)	>0.05
aer	9 (82)	36 (92)	>0.05
iroN	1 (9)	13 (33)	>0.05

DISCUSSION

Serine carbapenemases (KPC and OXA-48) and metallo-beta-lactamases (VIM, IMP and NDM) are frequently found in the members of the *Enterobacteriaceae.* [20] OXA-48 type carbapenemases are commonly reported in *E.coli*[21] and recently NDM[22], IMP[23] and KPC[24] type carbapenemases have also been determined. In our study,

only OXA-48 type resistance is found in *E.coli* isolates. This plasmid-based resistance has been reported in Turkey and around the world and it is more common than other types of resistance.[5,25,26]

Siderophores are the most important virulence factor supporting bacterial infection in tissues and blood, in case of there is iron deficiency. Aerobactin and ferric aerobactin expressed by a*er* and *iutA* gene regions are important siderophores in *E.coli* virulence and commonly found in many isolates.[10,11] In this study, it is found that the most common regions for these genes were related with siderophores.

aer gene was reported to have the highest ratio among the virulence factor genes.[13,27] Besides, a study with extrainstestinal *E.coli* isolates, the highest *iutA* gene region within virulence gene regions was found and this gene region was not determined in the control isolates.[12] In our study, another siderophore related gene region is *iroN*. This gene region is responsible of salmochelin and causes invasion in urothelial cells.[11] And also, this gene region was reported to have the highest ratio after *iutA*.[12]

Another virulence gene region found with high incidence in our study was *fimA* which is related to Type I fimbriae. This gene, commonly found most of the *E.coli* isolates, is observed both pathogenic and commensal isolates despite being an important virulence factor.[6,8] Similarly, it was reported in many studies that this gene region was more than other fimbria types.[12,27,28]

Urinary tract infections (cystitis, pyelonephritis etc.) which are considerably common and seen by many different symptoms[8,29], the virulence factor diversity is mostly found in urine samples in this study. The most important virulence factors for urinary tract infections are fimbriae. It was reported that no important difference in presence frequency of type I fimbria between low and high virulence isolates in the urinary tract.[30] In our study, *fimA* was found in most of the urine samples. Similarly, in Johnson et al., this gene region, despite commonly found in most *E.coli* isolates, is thought to be specific gene region for urinary tract infection. [28]

pap gene region, particularly with pyelonephritis, plays an important role in pathogenesis of uropathogenic *E.coli*, and this gene region is highly found in the isolates of urinary tract infection[29] and kidneys.[31] Thus, this gene region is found almost three-fold lower than the results reported before. On the other hand similar result were reported in our study.[13] For all the studies, even for the ones with high or low percentage, it is clear that this gene region is mostly related to complicated urinary tract infections.

The decay acceleration factor (DEF) in humans, which is a glycoprotein found in hemopoietic, endothelial, intestinal and urinary cells, is a receptor for afimbrial adhesin expressed with *afa* gene region.[6] In our study, this gene was found in urine samples as well as blood and bronchial samples. *Sfa* gene region related with S fimbria is found in urinary tract infections as well as infections such as sepsis, meningitis like *afa* gene region.[8] These gene regions were reported in none[29] or low percentage[28] of the samples isolated complicated urinary tract infection and there are no specific gene regions for these infections.

Siderophores in urinary tract infections are virulence factors frequently found in most pathogenic types of *E.coli* after fimbria. Even though, *iutA* gene region was reported in high incidence in complicated urinary tract infection compared to control group and acute cholangite.[29] Landgraf et al. reported that relation between *iutA* gene and uropathogenics was low.[32] Also, salmochelin (*iroN*) was found higher than ferric aerobactin (*iutA*) in several types' urinary tract infections.[11,28] In our study, presence of *aer* and *iutA* gene regions in many types of clinical strains and *iroN* gene in urine shows that this gene is a siderophore specific to urinary tract infection.

Hemolysin (*hly*) is produced by various pathogenic types of *E.coli* causing extraintestinal and intestinal infections, but its effect on virulence is not completely clarified.[6,8] *hly* gen region being related with complicated urinary tract infections

such as pyelonephritis and cystitis is reported by many researchers.[8,27,29] Similarly, *cnf-1* gene region is produced in one third of the isolates causing pyelonephritis and kidney invasion.[8] These gene regions are especially important in development of inflammation in urinary tract infections.[33] Also in our study, *cnf-1* gene region was highly found in urine and *hly* gene region was only found in urine.

Although no statistically important relation found between carbapenem resistance and virulence factors in this study, the effect of beta-lactam resistance in virulence is known. The relation between the virulence factors and ESBL in *E.coli* were reported by many researchers.[34,35] Also, Arısoy et al. reported that increase of virulence genes were related with resistance to some antibiotics or sensitivity to others.[13] In recent studies, a few mechanisms were focused for the relation and one of the mechanism is the plasmids carrying antibiotic resistance [36] and others are porin loss[37], modifications in penicillin binding proteins and efflux pumps mechanism.[38] Efflux pumps are responsible for discharging of molecules containing virulence factors regulated by quorum sensing which has a positive effect on antibiotics resistance and virulence.[39,40] *E.coli* strain 28 which previously reported with porin loss and OXA-48 resistance[25], was determined to have gene regions related with siderophore (*aer, iutA)* in this study. Similarly, virulence factors were highly found in carbapenem susceptible isolates, shows that other mechanisms may have an effect on the relation between carbapenem resistance and virulence. Therefore, determining of beta-lactam group resistance, these mechanisms should be taken into consideration.

CONCLUSIONS

Consequently, results demonstrated that virulence factors, antibiotic resistance, porin loss, multi drug efflux pump and quorum sensing molecules should be considered collective manner in the further studies about bacterial pathogenesis for developing effective treatments.

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REFERENCES

1. Blondeau JM. Current issues in the management of urinary tract infections: Extended-release ciprofloxacin as a novel treatment option. Drugs 2004;6: 611-28.
2. Falagas ME, Karageorgopoulos DE. Extended-spectrum beta-lactamase-producing organisms. Journal of Hospital Infection 2009;73:345-54.
3. McLaughlin M, Advincula MR, Malczynski M, Qi C, Bolon M, Scheetz MC. Correlations of Antibiotic Use and Carbapenem Resistance in *Enterobacteriaceae*, Antimicrobial Agents and Chemotherapy 2013;57:5131-3.
4. Naas T, Nordmann P. Analysis of a carbapenem hydrolyzing class A b-lactamase from *Enterobacter cloacae* and of its LysR-type regulatory protein. Proceedings of the National Academy of Sciences U.S.A. 1994,91:7693-7.
5. Nordmann P, Naas T, Poirel L. Global Spread of Carbapenemase-producing *Enterobacteriaceae.* Emerging Infectious Diseases 2011;17:1791-8.

6. Mainil J. *Escherichia coli* virulence factors. Veterinary Immunology and Immunopathology 2013;152:2-12.
7. Wang MC, Tseng CC, Chen CY, Wu JJ, Huang JJ. The role of bacterial virulence and host factors in patients with Escherichia coli bacteremia who have acute cholangitis or upper urinary tract infection. Clinical Infectious Diseases 2002;15:1161-6.
8. Bien J, Sokolova O, Bozko P. Role of Uropathogenic Escherichia coli Virulence Factors in Development of Urinary Tract Infection and Kidney Damage. International Journal of Nephrology 2012;681473.
9. Dobrindt U, Blum-Oehler G, Hartsch T. S-fimbria-encoding determinant sfaI is located on pathogenicity island III536 of uropathogenic *Escherichia coli* strain 53. Infection and Immunity 2001;69:4248–42.
10. Demir M, Kaleli I. Production by Escherichia coli isolates of siderophore and other virulence factors and their pathogenic role in a cutaneous infection model. Clinical Microbiology and Infection 2004;10:1011-4.
11. Henderson JP, Crowley JR, Pinkner JS, Walker JN, Tsukayama P, Stamm WE et al. Quantitative Metabolomics Reveals an Epigenetic Blueprint for Iron Acquisition in Uropathogenic *Escherichia coli*. PLoS Pathogens. 2009;5:e1000305.
12. Koga VL, Tomazetto G, Cyoia PS, Neves MS, Vidotto MC, Nakazato G, et al. Molecular Screening of Virulence Genes in Extraintestinal Pathogenic *Escherichia coli* Isolated from Human Blood Culture in Brazil. BioMed Research International, 2014;15:2014:465054.
13. Arısoy M, Yousefi Rad A, Akın A, Akar N. Relationship between susceptibility to antimicrobials and virulence factors in pediatric *Escherichia coli* isolates. International Journal of Antimicrobial Agents 2008;31,4–8.
14. Guiral E, Bosch J, Vila J, Soto SM. Prevalence of *Escherichia coli* among samples collected from the genital tract in pregnant and nonpregnan two men: relationship with virulence. FEMS, Microbiology Letters 2011;314:170-3.
15. Welinder-Olsson C, Eriksson E, Kaijser B. Virulence genes in verocytotoxigenic *Escherichia coli* strains isolated from humans and cattle. APMIS 2005;113:577-85.
16. Saito R, Koyano S, Nagai R, Okamura N, Moriya K, Koike K. Evaluation of a chromogenic agar medium for the detection of extended-spectrum beta-lactamase-producing *Enterobacteriaceae*. Letters in Applied Microbiology 2010;51:704-6.
17. Panagea T, Galani I, Souli M, Adamou P, Antoniadou A, Giamarellou H. Evaluation of CHROMagarTM KPC for the detection of carbapenemase-producing Enterobacteriaceae in rectal surveillance cultures. International Journal of Antimicrobial Agents 2011;37:124-8.
18. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagnostic Microbiology and Infectious Disease 2011;70:119-123.
19. Ruiz J, Simon K, Horcajada JP, Velasco M, Barranco M, Roig G et al. Differences in Virulence Factors among Clinical Isolates of *Escherichia coli* Causing Cystitis and Pyelonephritis in Women and Prostatitis in Men. J. Clin. Microbiol. 2002;40:4445-9.
20. Walsh TR. Emerging carbapenemases: A global perspective. Int. J. of Antimicrob. Agents 2010;36:8-14.
21. Poirel L, Yilmaz M, Istanbullu A, Arslan F, Mert A. Spread of NDM-1-producing Enterobacteriaceae in a neonatal intensive care unit, Istanbul, Turkey. Antimicrobial Agents and Chemotherapy 2014;58:2929-33.
22. Mushtaq S, Irfan S, Sarma JB, Doumith M, Pike R, Pitout J et al. Phylogenetic diversity of *Escherichia coli* strains producing NDM-type carbapenemases. J. Antimicrob. Chemother. 2011;66:2002-5.
23. Aktas Z, Satana D, Kayacan C, Ozbek B, Gurler N, Somer A et al. Carbapenem resistance in Turkey: Repeat report on OXA-48 in *Klebsiella pneumoniae* and first report on IMP-1 beta-lactamase in *Escherichia coli*. African Journal of Microbiology Research 2012;6:3874-8.
24. Morris D, Boyle F, Ludden C, Condon I, Hale J, O'Connell N et al. Production of KPC-2 Carbapenemase by an *Escherichia coli* Clinical Isolate Belonging to the International ST131 Clone. Antimicrob. Agents Chemother. 2011;55:4935-6.
25. Gülmez D, Woodford N, Palepou Marie-France I, Mushtaq S, Metan G, Yakupogullari Y et al. Carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates from

Turkey with OXA-48-like carbapenemases and outer membrane protein loss. International Journal of Antimicrobial Agents 2008;31:523-6.
26. Carrer A, Poirel L, Yılmaz M, Arikan Akan Ö, Feriha C, Cuzon G et al. Spread of OXA-48-Encoding Plasmid in Turkey and Beyond. Antimicrobial Agents and Chemotherapy 2010;54:1369-73.
27. Tarchouna M, Ferjani A, Ben-Selma W, Boukadida J. Distribution of uropathogenic virulence genes in *Escherichia coli* isolated from patients with urinary tract infection. International Journal of Infectious Diseases 2013;17:e450-3.
28. Johnson JR, Kuskowski MA, Gajewski A, Soto S, Horcajada JP, Jimenez de Anta MT et al. Extended Virulence Genotypes and Phylogenetic Background of *Escherichia coli* Isolates from Patients with Cystitis, Pyelonephritis, or Prostatitis. The Journal of Infectious Diseases 2005;191:46–50.
29. Wang MC, Tseng CC, Chen CY, Wu JJ, Huang JJ. The role of bacterial virulence and host factors in patients with *Escherichia coli* bacteremia who have acute cholangitis or upper urinary tract infection. Clinical Infectious Diseases 2002;15:1161-6.
30. Plos K, Lomberg H, Hull S, Johansson I, Svanborg C. *Escherichia coli* in patients with renal scarring: genotype and phenotype of Gal alpha 1-4Gal beta-, Forssman- and mannose-specific adhesins. Pediatric Infectious Disease Journal 1991;10:15-9.
31. Roberts J, Marklund B, Ilver D, Haslam D, Kaak M, Baskin B et al. The Gal(alpha 1-4)Gal-specific tip adhesin of *Escherichia coli* P-fimbriae is needed for pyelonephritis to occur in the normal urinary tract, Proceedings of the National Academy of Sciences U.S.A. 1994;91:11889-93.
32. Landgraf TN, Berlese A, Fernandes FF, Milanezi ML, Martinez R, Panunto-Castelo A. The ferric aerobactin receptor iutA, a protein isolated on agarose column, is not essential for uropathogenic *Escherichia coli* infection. Revista Latino-Americana de Enfermagem 2012;20:340-5.
33. Garcia TA, Ventura CL, Smith MA, Merrell DS, O'Brien AD. Cytotoxic Necrotizing Factor 1 and Hemolysin from Uropathogenic *Escherichia coli* Elicit Different Host Responses in the Murine Bladder. Infection and Immunity 2013;81:99–109.
34. Pitout JDD, Laupland KB, Church DL, Menard ML, Johnson JR. Virulence Factors of *Escherichia coli* Isolates That Produce CTX-M-Type Extended-Spectrum β-Lactamases. Antimicrob. Agents Chemother. 2005;49:4667-70.
35. Sharma S, Bhat GK, Shenoy S. Virulence factors and drug resistance in *Escherichia coli* isolated from extraintestinal infections. Indian Journal of Medical Microbiology 2007;25:369-73.
36. Fekete PZ, Brzuszkiewicz E, Blum-Oehler G, Olasz F, Szabó M, Gottschalk G et al. DNA sequence analysis of the composite plasmid pTC conferring virulence and antimicrobial resistance for porcine enterotoxigenic *Escherichia coli*. International Journal of Medical Microbiology 2012;302:4-9.
37. Rolhion N, Carvalho FA, Darfeuille-Michaud A. OmpC and the sigma(E) regulatory pathway are involved in adhesion and invasion of the Crohn's disease-associated *Escherichia coli* strain LF82. Molecular Microbiology 2007;63:1684-700.
38. Beceiro A, Tomás M, Bou G. Antimicrobial Resistance and Virulence: a Successful or Deleterious Association in the Bacterial World? Clinical Microbiology Reviews 2013;26:185–230.
39. Yang YX, Xu ZH, Zhang YQ, Tian J, Weng LX, Wang LH. A new quorum-sensing inhibitor attenuates virulence and decreases antibiotic resistance in *Pseudomonas aeruginosa*. Journal of Microbiology 2012;50:987-93.
40. Wang H, Tu F, Gui Z, Lu X, Chu W. Antibiotic resistance profiles and quorum sensing-dependent virulence factors in clinical isolates of *Pseudomonas aeruginosa*. Indian Journal of Microbiology 2013;53:163-7.

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