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Genetic relation and virulence factors of carbapenemase-producing Uropathogenic *Escherichia coli* from urinary tract infections in Iraq

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Abstract

Background: Uropathogenic *Escherichia coli* (*E. coli)* or UPEC is among major nosocomial pathogens causing urinary tract infections (UTIs). The emergence of carbapenem- resistant strains is a major concern regarding the UTI treatment. The subjective of this study included assessment of genetic relation and screening of virulence factors among carbapenemase-producing *E. coli* from UTI.

Methods: Three-hundred *E. coli* isolates were collected from cystitis and pyelonephritis. Antibiotic susceptibility test was conducted by disk diffusion as provided by clinical and laboratory standards institute (CLSI) version 2017. Carbapenemase production and related genes and virulence factors were screened by polymerase chain reaction (PCR) technique. Genetic relation of isolates was evaluated using phylogrouping and serogrouping.

Results: Of 300 isolates, 11 (3.66%) of them were carbapenemase-producing *E. coli* (CP-*E. coli*). Imipenem minimum inhibitory concentration ranged 4-128µg/ml. The *bla* $_{\text{OXA-48}}$ and *bla*$_{\text{IMP}}$ genes were co-existed in three (1%) isolates (imipenem MIC: 64-128µg/ml), but the *bla* $_{\text{KPC}}$, *bla* $_{\text{NDM}}$ and *bla* $_{\text{VIM}}$ were not amplified. Predominant virulence genes included *iutA* (n=293, 97.66%), *fyuA* (n=256, 85.33%), *inh* (n=249, 83%), *traT* (n=247, 82.33%), *papII* (n=96, 32%), *fimH* (n=93, 31%), *csgA* (n=92, 30.66%). All the CP-*E. coli* contained the *iutA, fyuA, traT, papII, fimH* and *csgA* genes. O1 (32%), O16 (15%) and O25 (7%) serogroups were predominant and 6/11, 4/11 and 1/11 of CP-*E. coli* belonged to O1, O25 and O75 serogroups, respectively. Among eleven CP-*E. coli* isolates, nine of them were belonged to the B2

phylogroup and two isolates were belonged to B1 phylogroup.

Conclusion: CP-*E. coli* (or CP-UPEC) contained the bla_{IMP} and bla_{OXA-48} genes and belonged to O25/B2. High rate of virulence factors among CP-*E. coli* from UTI is a concern in Baghdad hospitals. The spread of isolates with resistance to last-line antibiotics must be controlled.
Keywords: *Escherichia coli*, carbapenemase, phylogroups, serogrouping, virulence typing

1. Introduction

Uropathogenic Escherichia coli (UPEC) is a substantial agent of urinary tract infections (UTIs) (75–90%) alongside some other infections (Nojoomi & Ghasemian, 2019). Evolution of antibiotic resistance mostly against β-lactams by producing extended spectrum β-lactamases and carbapenems is a worldwide concern as these enzymes confer wide-spectrum of activity (Iredell, Brown, & Tagg, 2016; Ma et al., 2018; Peirano, Hung Ying Sang, Pitondo-Silva, Laupland, & Pitout, 2012). Carbapenems as choices for elimination of ESBL-producing bacterial infections has recently been faced with resistance by serious infections among patients with high mortality rate (Jayol et al., 2017). Resistance to carbapenems occurs through a variety of mechanisms such as efflux pumps and various carbapenemase enzymes. These enzymes are encoded by bla_{IMP}, $bla_{OXA-48-like}$, bla_{VIM}, bla_{SIM}, bla_{KPC} and bla_{NDM} genes (Ageevets et al., 2014). Another mechanism by which clinical isolates employ for resistance to antibiotics and harsh environmental conditions includes biofilm formation. It has been revealed that multidrug-resistant (MDR) or CP-*E. coli* isolates can produce biofilms by means of various strategies such as expression of bacterial adhesins. It has been also supposed that some major *E. coli* virulent clonal groups with special phylogroups/serogroups cause severe and drug-resistant infections (Dormanesh et al., 2014; Iguchi et al., 2015). Among them, O25b-B2-ST131, clonal group A (CGA) and O15:K52:H1-D-ST393 are notable (Prakapaite et al., 2019; Rehman et al., 2017). Strains from extra-intestinal infections such as UTI have been mostly belonged to the B2 and D phylogroups. Noticeably, these strains belong to various serogroups and express numerous virulence factors (VFs) such as adhesins and toxins (Abe et al., 2008; Agarwal, Mishra, Srivastava, Srivastava, & Pandey, 2014; Ali et al., 2016; da Silva, de Mello Santos, & Silva, 2017). Hence disclosure of drug resistance mechanisms and virulence properties of MDR strains is crucial opening new avenues towards eradication of related infections and/or their control and prevention.

Our subjective was determination of genetic relation and virulence factors of carbapenemase-producing *E. coli* from UTI in Baghdad, Iraq.

2. Materials and methods

2-1. Bacterial isolates

A total of 300 *E. coli* isolates were obtained from cystitis (n=200) and pyelonephritis (n=100) among patients suffering from UTI symptoms, referred to two Baghdad hospitals during Jan 2017-Dec 2018. The isolates were identified through culture of urine samples onto Chrom Agar medium and using biochemical tests. Samples with $\geq 10^5$ CFU/mL were considered as UTI.

Subsequently, they were kept at -70 °C in trypticase soy broth containing 30% (v/v) glycerol for further studies.

2-2. Antibiotic susceptibility testing

Susceptibility to various antimicrobials was measured using disc diffusion method on Mueller-Hinton agar (Merk, Germany) and was interpreted considering the Clinical and Laboratory Standards Institute (CLSI) version 2017 instructions. ciprofloxacin (CIP:5µg), pepracillin-tazobactam (PTZ:110µg), amoxicillin (AMX:30 µg), erythromycin (ERY:30µg), co-amoxiclav (AMC:30µg), ceftazidime (CAZ:30µg), cotrimoxazole (SXT:25µg), cefazolin (CZ:30µg), cefotaxime (CTX:30µg), imipenem (IMI:10µg), meropenem (MEN:10µg), gentamycin (GM:10µg), fosfomycin (FO:30µg), nitrophorantoien (FM:300µg) and tetracycline (TE:30µg) were tested. *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC25923 and *Klebsiella oxytoca* ATCC 13182 were cultured for the quality assessment of disks (Alkhudhairy, Alshadeedi, Mahmood, Al-Bustan, & Ghasemian, 2019; Patel, 2017).

2.3. Phenotypic AmpC, ESBLs and carbapenemase production

The ESBLs production was confirmed using E-test, synergy test and the combine disk by cefoxitin disk +/- benzo-boronic acid (AmpC expression). In order to determine carbapenemase production, the combine disk consisting imipenem /meropenem +/- EDTA (0.5 molar) and the Carba-NP test were carried out considering protocol by CLSI (Patel, 2017).

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of imipenem was determined using twofold broth dilution according to CLSI version 2017 guidelines. The stock was prepared using phosphate buffer, pH 7.2, 0.01 mol/L. Firstly, double 10x stocks were prepared and diluted twofold to prepare optional ranges. Antibiotic concentration range included 0.25-256µg/mL (Patel, 2017).

2-3. Biofilm formation

Phenotypic evaluation of biofilm production was carried out using microtiter tissue plate (Mtp) assay as previously mentioned (Barati et al., 2016; Novais et al., 2013). Briefly, the isolates were cultured into Luria-Bertani broth with 1% glucose and incubated at 37°C for 24h. Next, fixation using methanol and dying using 10% crystal violet (blue color) performed and absorbance rate was assessed using ELISA reader at 590 nm. Negative control included wells with culture medium and without bacterial isolates. The cut-off ODc

was considered as three standard deviations above the mean OD of the negative control (culture medium), and strains were classified as non-adherent (OD ≤ ODc), weakly adherent (ODc < OD ≤2 × ODc), moderately adherent (2 × ODc < OD ≤ 4 × ODc), or strongly adherent (OD > 4 × ODc) (Novais, et al., 2013).

2.4. Cytotoxicity

The cell cytotoxicity of virulent and CR-*E. coli* isolates was implemented by treatment of Hep-2 cells with bacterial supernatants using the cell culture (DMEM medium). *E. coli* ATCC25922 and a cytotoxin-producing strain were used as the negative and positive control, respectively. A change of cellular shape from normal to round cells was considered as cytotoxicity effect as observe using Neobar lam.

2-5. PCR amplification for carbapenemases genes

Carbapenemases genes were detected with specific primers for *bla* $_{OXA-48}$, *bla* $_{KPC}$, *bla* $_{IMP}$, *bla* $_{VIM}$ and *bla* $_{NDM}$ (Table 1). The total DNA was extracted from by boiling Method. PCR was performed using commercially available PCR Master Mix (AMPLIQON, Denmark) according to the manufacturer's instructions. Briefly, 2 µl template DNA (~100 ng/µl), 1 µl of each primer (10 pmoles/µl), and 6µl DNase-free distilled water were added to 6 µl of Master Mix in a final volume of 15 µl.

Table 1. Primers used for detection of carbapenemase genes in this study

Primer	Sequence: 5'-3'	Product (bp)	reference
bla$_{IMP}$	F: GGGTGGGGCGTTCTTCCTA R: TCTATTCCGCCCCTGCTGTC	198 (62°C)	(Ghasemian et al., 2019; Ghasemian et al., 2018)
Bla$_{OXA-48-like}$	F: CGCCCGCTCGACGTTCAAGAT R: TCGGCCAGCAGCGGATAGGACAC	484 (64°C)	
bla$_{NDM}$	F: CGCACCTCATGTTTGAATTCGCC R: GTCGCAAAGCCCAGCTTCGC	1015 (62°C)	
bla$_{KPC}$	F: TTGCCGGTCGTGTTTCCCTTTAGC R: GGCCGCCGTGCAATACAGTGATA	282 (64°C)	
bla$_{VIM}$	F: CATTGTCCGTGATGGTGATGAGT R: GCGTGTCGACGGTGATGC	205 (62°C)	

Amplification involved an initial denaturation at 94°C for 5 min followed by 32 cycles of denaturation (94°C, 50 s), annealing (62°C, 1 min for *bla* $_{NDM}$, *bla* $_{IMP}$, 45 s for *bla* $_{VIM}$ and

64°C, 50 s for *bla* $_{KPC}$ and *bla* $_{OXA-48}$) and extension (72°C, 1 min for all), with a final extension step (72°C, 8 min for all). The amplified DNA was separated by gel electrophoresis on 1% agarose, and visualized under UV transillumination.

2-4. **Amplification of *bla* $_{CTX-M1}$ group**

The *bla* $_{CTX-M1}$ was detected among CP-*E. coli* with specific primers including F: 5'-CGCTTTGCGATGTGCAG -3' and R: 5'- ACCGCGATATCGTTGGT-3'. Amplification involved an initial denaturation at 94°C for 5 min followed by 34 cycles of denaturation (93°C, 1 min), annealing (54°C, 45 s) and extension (72°C, 1 min), with a final extension step (72°C, 8 min).

2-5. Serogrouping and phylogrouping for carbapenemase genes positive isolates

Serotypes were detected by the amplification of antigen O1-O15 types (table2). PCR was performed with a Bio-Rad thermal cycler T100 under the following conditions: denaturation for 5 min at 94 °C; 30 cycles of 1 min at 94 °C, 1 min at 61 °C, and 50 s at 72 °C; and a final extension for 10 min at 72 °C.

Phylogrouping was performed based on the presence or absence of three genomic DNA fragments (*chuA* and *yjaA* genes and the DNA fragment TspE4.C2), Primers used are listed in Table1. The PCR was performed with Bio-Rad thermal cycler T100 under the following conditions: denaturation for 4 min at 94 °C; 33 cycles of 45 s at 94 °C, 45 s at 55 °C, and 45 s at 72 °C; and a final extension for 10 min at 72 °C (Amarsy et al., 2019).

2-6. Virulence typing of isolates containing carbapenemase genes

Specific primers for detection of virulence genes in this study have been depicted in Table 1. Virulence genes including receptor toll/interleukin 1 (*tcpC*), capsular polysaccharide synthesis K1 (*kpsMTII*), cytotoxic necrotizing factor 1 (*cnf1*), cytolethal distending toxin (*cdt*), serum survival (*traT*), secretion autoinducer toxin (sat), α-hemolysin (*hlyA*), vaculating autoinducer toxin (*vat*), invasion of brain endothelium (*ibeA*), ferric aerobactin receptor (iron uptake/transport) (*iutA*), curli fimbriae (*csgA*) and serine protease autoinducer (*pic*) were studied by PCR. Primer sequences and amplification conditions have been mentioned previously (Demir & Kaleli, 2004; Grude et al., 2007; Müller, Stephan, & Nüesch-Inderbinen, 2016; Rehman, et al., 2017; Turchi et al., 2019).

Table 2. Primers and annealing temperatures used for the phylogroups and serogroups genes

Target gene	Sequence (5' to 3')	Amplicon Size, bp	Optimal annealing temperature (°C)
chuA	F: GACGAACCAACGGTCAGGAT	279	55

	R: TGCCGCCAGTACCAAAGACA		
yjaA	F: TGAAGTGTCAGGAGACGCTG R: ATGGAGAATGCGTTCCTCAAC	211	55
TspE4.C2	F: GAGTAATGTCGGGGCATTCA R: CGCGCCAACAAAGTATTACG	152	55
rfbO1	F: ATACCGACGACGCCGATCTG R: CCAGAAATACACTTGGAGAC	189	59
rfbO2	F: ATACCGACGACGCCGATCTG R: GTGACTATTTCGTTACAAGC	274	59
rfbO18	F: ATACCGACGACGCCGATCTG R: GAAGATGGCTATAATGGTTG	360	59
rfbO16	F: ATACCGACGACGCCGATCTG R: GGATCATTTATGCTGGTACG	450	5[illegible]
rfbO6	F: ATACCGACGACGCCGATCTG R: AAATGAGCGCCCACCATTAC	5[illegible]4	59
rfbO7	F: ATACCGACGACGCCGATCTG R: CGAAGATCATCCACGATCCG	722	59
rfbO4	F: ATACCGACGACGCCGATCTC R: AGGGGCCATTTGACCCAC[illegible]	193	67
rfbO12	F: ATACCGACGACGCCCAT[illegible]TG R: GTGTCAAATG[illegible]TG[illegible]ACCG	239	59
rfbO25	F: ATACCGACGACGCCGATCTG R: GAGATCCAAAAACAGTTTGTG	313	59
rfbO75	F: ATACCGACGACGCCGATCTG R: GTAATAATGCTTGCGAAACC	419	58
rfbO15	F: ATACCGACGACGCCGATCTG R: TGATAATGACCAACTCGACG	536	59
rfbO157	F: ATACCGACGACGCCGATCTG R: TACGACAGAGAGTGTCTGAG	672	59

Table3. Primers used for amplification of virulence genes

Primers		PCR product size (bp)	Reference
cdt	FP: 5′-AAATCACCAAGAATCATCCAGTTA-3′ RP: 5′-AAATCTCCTGCAATCATCCAGTTTA-3′	430	(J. R. Johnson & Stell, 2000)
kpsMTII	FP: 5′-GCGCATTTGCTGATACTGTTG-3′ RP: 5′-CATCCAGACGATAAGCATGAGCA-3′	272	(J. R. Johnson & Stell, 2000)
TcpC	FP: 5′-GAGTGGAAGGAGGTTGAGGC-3′ RP: 5′-GCAGTGCCATTTTATCCGCC-3′	544	(Nagarjuna, Dhanda, Gaind, & Yadav, 2015)
iutA	FP: 5′-GGCTGGACATCATGGGAACTGG-3′ RP: 5′-CGTCGGGAACGGGTAGAATCG-3′	302	(T. J. Johnson et al., 2008)
traT	FP: 5′-GGTGTGGTGCGATGAGCACAG-3′ RP: 5′-CACGGTTCAGCCATCCCTGAG-3′	290	(T. J. Johnson, et al., 2008)
hlyA	FP: 5′- GCATCATCAAGCGTAC[illegible]C-3′ RP: 5′- AATGAGCCAAGCTG[illegible]AAGCT-3′	534	(Wani, Samanta, Munshi, Bhat, & Nishikawa, 2006)
cnf1	FP: 5′- AAGATGG[illegible]GTTTCCTATGCAGGAG-3′ RP: 5′- CATTCAGAGTCCTGCCCTCATTAT-3′	498	(Tapader et al., 2014)
ibeA	FP: 5′- AGGCAGGTGTGCGCCGCGTAC-3′ RP: 5′-TGGTGCTCCGGCAAACCATGC-3′	171	(Tapader, et al., 2014)
vat	FP: 5′- AACGGTTGGTGGCAACAATCC-3′ RP: 5′- AGCCCTGTAGAATGGCGAGTA-3′	420	(Tapader, et al., 2014)
sat	FP: 5′- TCAGAAGCTCAGCGAATCATTG-3′ RP: 5′- CCATTATCACCAGTAAAACGCACC-3′	930	(Tapader, et al.,

			2014)
pic	FP: 5′- ACTGGATCTTAAGGCTCAGGAT-3′ RP: 5′- GACTTAATGTCACTGTTCAGCG-3′	572	(Tapader, et al., 2014)
CsgA	FP: 5′- GGCGGAAATGGTTCAGATGTTG-3′ RP: 5′- CGTATTCATAAGCTTCTCCCGA-3′	295	(Domingos et al., 2016)

2.7. Statistical analysis

The data was analyzed using IBM SPSS (IBM, Armonk, NY, USA) version 20, where Chi-Square (between two groups) and analysis of variance (ANOVA) were employed. The relation of virulence rate among CP-*E. coli* was analyzed using multinomial logistic regression test. A 95% confidence interval and p value<0.05 were considered as significance level.

2.8. Ethical statement

This study was ethically approved by University of Al-Qadisiyah/College of Science/Biology Department, Iraq.

3. Results

3.1. Demographic data

The age range of patients was 22-73 years with mean±SD of 53.22±7. The age range of >60 years (n=116, 38.66%, 78 of which were infected with MDR *E. coli*) was higher infected with multidrug-resistant (MDR) *E. coli*. Previous two months of hospitalization (75%, p=0.001) and consumption of cephalosporins (71.33%, p=0.002), fluoroquinolones (23%), amikacin (12%) and carbapenems (23%) were also significant risk factors for development of MDR and CP-*E. coli*. Among underlying diseases (cardiovascular diseases, kidney or liver disorders and cancer), no significant correlation was detected neither with UTI nor infection with CP-*E. coli*.

3.2. Cytotoxicity

We observed that none of 11 CP-*E. coli* supernatant contents had cytotoxic effect against Hep-2 cells compared to negative and positive control (fig.1).

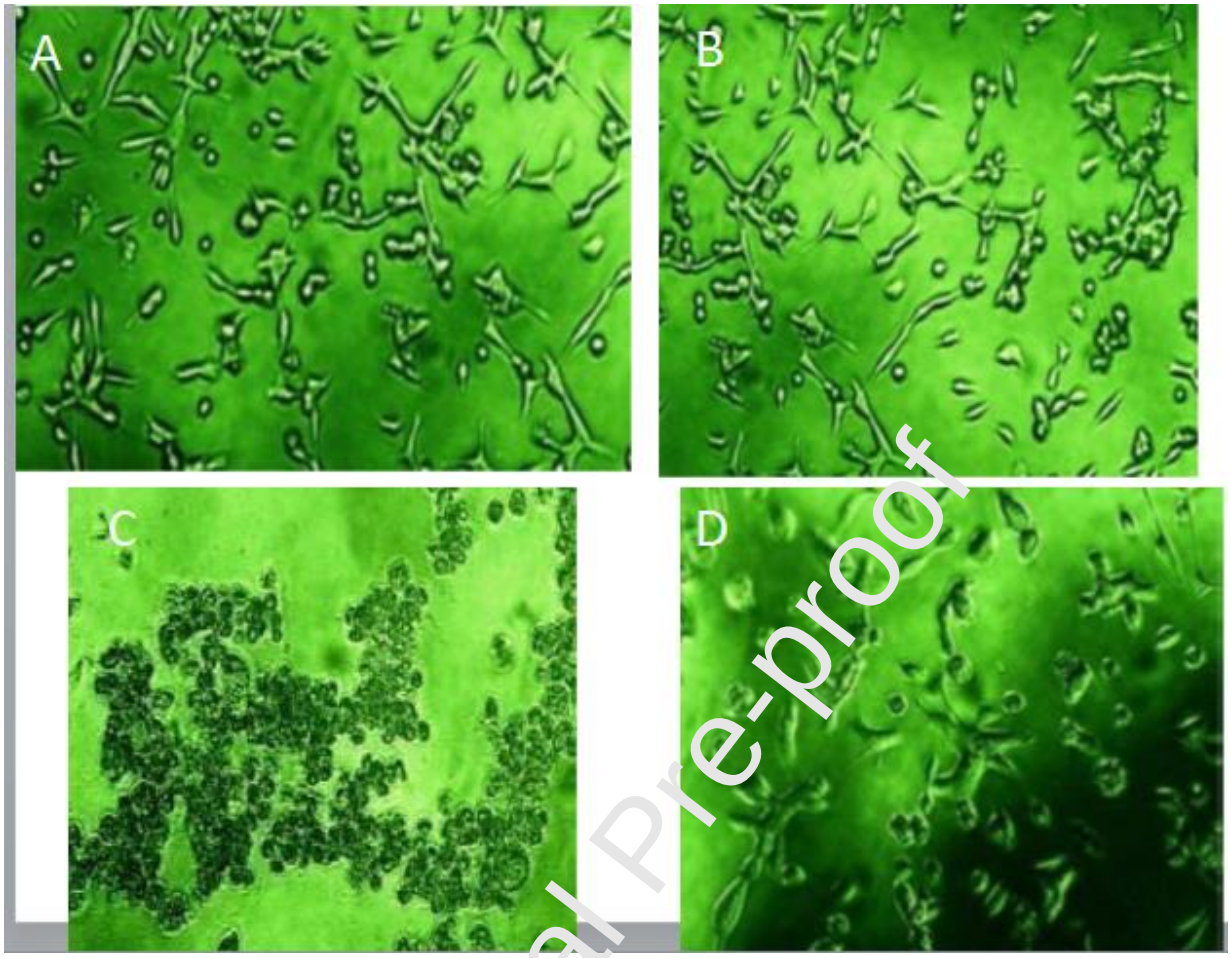

Fig.1. The cytotoxicity assessment of CP-*E. coli*; A: effect of negative control, B and D: effect by two of test CP-*E. coli*, C: lethal effect of positive control leaving rounding dead cells

Hence, we observed that these CP-*E. coli* isolates lacked cytotoxic ability against Hep-2 cells.

3-3. susceptibility and biofilm formation

All the isolates were susceptible to nitrofurantoin and fosfomycin disks. Additionally, all were resistant to amoxicillin, cefazolin, tetracycline and cefotaxime. Resistance to erythromycin, ciprofloxacin, ceftazidime, piperacillin- tazobactam, co-amoxiclav, cotrimoxazole, gentamicin, meropenem and imipenem included 70%, 70%, 66%, 46.7%, 46.7%, 23%, 19.4%, 3.66% and 3.66%, respectively. The rate of MDR-*E. coli* from UTI included 66%. The rate of ESBL-producing and CP-*E. coli* included 46.7% and 3.66%, respectively. Carbapenemase-producing strains were resistant to other classes except nitrofurantoin and fosfomycin. As depicted in table 4, seven and four CP-*E. coli* (or UPEC) isolates were from cystitis and pyelonephritis, respectively. Although most of CP-*E. coli* contained O1 serogroup, three of them which had the bla_{IMP} and $bla_{\text{oxa-48}}$ belonged to O25/B2.

Table 4. Characteristics of patients and CP-*E. coli*

Isolate	Gender (age)	Specimen	ESBL	IMI_{MIC} (µg/mL)	Biofilm	*bla*$_{CTXM1}$	*bla*$_{IMP}$	*bla*$_{OXA-48}$	Phyl/Sero
1	M (52)	C	Yes	64	M	Yes	Yes	Yes	B2/O25
2	M (49)	C	Yes	16	M	Yes			B2/O75
3	F (56)	P	Yes	8	W	Yes			B1/O1
4	F (51)	P	Yes	8	W	Yes			B2/O25
5	M (48)	C	Yes	8	S	Yes			B1/O1
6	F (63)	C	Yes	128	S	Yes	Yes	Yes	B2/O25
7	M (49)	P	Yes	4	M	Yes			B2/O1
8	F (65)	P	Yes	128	S	Yes	Yes	Yes	B2/O25
9	F (62)	C	Yes	4	M	Yes			B2/O1
10	M (59)	C	Yes	4	S	Yes			B2/O1
11	F (61)	C	Yes	4	S	Yes			B2/O1

ESBL: extended-spectrum β-lactamase, C: cystitis, P: pyelonephritis, IMI_{MIC}: imipenem minimum inhibitory concentration, M: male, F: female, S: strong, M: moderate, W: weak, Phyl/Sero: phylogroup/serogroup

The imipenem MIC ranged 4-128µg/ml. higher MICs was associated with the existence of bla_{IMP} and bla_{OXA-48} genes. All the carbapenemase-producers were also ESBL-producers which carried the bla_{CTXM1} gene. In addition, half of the 300 isolates (n=150) were moderate-level biofilm producers, while 11% and 39% were strong and weak biofilm producers, respectively.

Carbapenemases- and virulence factors-encoding genes

The *bla*$_{OXA-48}$ and *bla*$_{IMP}$ genes were co-existed in three (1%) isolates (imipenem MIC: 64-128µg/ml), but the *bla* $_{KPC}$, *bla* $_{NDM}$ and *bla* $_{VIM}$ were not amplified.

Predominant virulence genes included *iutA* (n=293, 97.66%), *fyuA* (n=256, 85.33%), *inh* (n=249, 83%), *traT* (n=247, 82.33%), *papII* (n=96, 32%), *fimH* (n=93, 31%), *csgA* (n=92, 30.66%). The prevalence rate of *jfmA*, *ompT*, *iucD*, *sat*, *usp*, *sfa/foc*, *hly*, *cnf-1*, *afa* and *iroN* included 88 (29.33%), 84 (28%), 84 (28%), 61 (20.33%), 51 (17%), 43 (14.33%), 40 (13.33%), 40 (13.33%), 31 (10.33%) and 28 (9.33%), respectively. Notably, *ibeA*, *tcpC* and *pic* genes were not amplified. All the CP-*E. coli* contained the *iutA*, *fyuA*, *traT*, *papII*, *fimH* and *csgA* genes and there was high rate of *fimA* (63.7%, n=7), *ompT* (54.55%, n=6) and *iucD* (54.55%, n=6) genes. Noticeably, the O1 (32%), O16 (15%) and O25 (7%) serogroups were predominant.

The virulence features of CP-*E. coli* from cystitis and pyelonephritis has been represented in table5.

Table5. Virulence features of CP-*E. coli* from cystitis and pyelonephritis

Strains	Carbapenemase genes	Virulence genes
C1	*bla*$_{OXA-48}$ and *bla*$_{IMP}$	*iutA, fyuA, traT, papII, fimH, csgA, inh,*

		fimA, ompT, iucD
C2		*iutA, fyuA, traT, papII, fimH, csgA, fimA, iucD*
C3		*iutA, fyuA, traT, papII, fimH, csgA, fimA, ompT, iucD*
C4	*bla*$_{\text{OXA-48}}$ and *bla*$_{\text{IMP}}$	*iutA, fyuA, traT, papII, fimH, csgA, inh, fimA, ompT, iucD*
C5		*iutA, fyuA, traT, papII, fimH, csgA, ompT*
C6		*iutA, fyuA, traT, papII, fimH, csgA*
C7		*iutA, fyuA, traT, papII, fimH, csgA, fimA, ompT, iucD, fimA, ompT, iucD*
P1		*iutA, fyuA, traT, papII, fimH, csgA, inh*
P2		*iutA, fyuA, traT, papII, fimH, csgA*
P3	*bla*$_{\text{OXA-48}}$ and *bla*$_{\text{IMP}}$	*iutA, fyuA, traT, papII, fimH, csgA, inh, fimA, ompT, iucD*
P4		*iutA, fyuA, traT, papII, fimH, csgA, fimA*

C: cystitis, P: pyelonephritis

As revealed in table 4, the CP-*E. coli* from cystitis and pyelonephritis carried high rate of virulence genes though sample size was small

3-4. **Serogrouping and phylogrouping of carbapenemase producers**

Among eleven CP-*E. coli* isolates, nine of them were belonged to the B2 phylogroup and two isolates were belonged to B1 phylogroup. Additionally, 6/11, 4/11 and 1/11 of CP-*E. coli* were belonged to O1, O25 and O75 serogroups, respectively. Those three strains carrying *bla*$_{\text{IMP}}$ and *bla*$_{\text{OXA-48}}$ genes belonged to B2 phylogroup and O25 serogroup (table3).

Discussion

Carbapenemase producing Gram-negative bacteria are associated with serious threat to public health with higher costs and mortality rate worldwide (Alkhudhairy, et al., 2019; Kanaan, Al-Shadeedi, Al-Massody, & Ghasemian, 2020). The spread and evolution of carbapenem resistance among pathogenic strains is a great concern. Studies have revealed that among major carbapenemases from *E. coli* collected in a neighboring country among patients with UTI, the *bla* $_{\text{OXA-48}}$ gene was predominant carbapenemase, reaching to 53% among of UPEC strains South East Iran (KhadijeRezaieKeikhaie, 2018), likely due to geographic location and the borderlines of Zabul city, while in our study it's the prevalence was 1%. Type of samples maybe also influence on the evolution of carbapenemases (Solgi et al., 2017; Uskudar-Guclu, Guney, Sig, Kilic, & Baysallar, 2019). Moreover, the presence of insertion elements or transposons facilitate its transmission into chromosome or plasmid (Beyrouthy et al., 2014).

In Spain, Germany and Egypt it was included 72%, 58.3% and 33%, respectively among

carbapenem-resistant strains which was different from our results (Ghaith, Mohamed, Farahat, Aboulkasem Shahin, & Mohamed, 2019; Kaase et al., 2016; Ortega et al., 2016) .

Our three *bla* $_{\text{OXA-48}}$ and *bla*$_{\text{IMP}}$-bearing isolates (two from cystitis and one from pyelonephritis, imipenem MIC: 64-128µg/ml) were resistant to most of antibiotics except for nitrofurantoin, fosfomycin and gentamicin.

In a study by Hojabri among *bla* $_{\text{OXA-48}}$/CTX-M-positive O25b/O16-ST131 isolates, O25 serogroup was mostly common, while O16 serogroup showed higher resistance to β-lactam. In our study, those strains belonging to O16 and O25 serogroups were resistant to cephalosporins, amoxicillin and amoxiclav, erythromycin and tetracycline. In addition, O25 serogroup was resistant to ciprofloxacin, carbapenems and gentamicin indicating O25 serogroup strains being more potential for the spread of CP- *E. coli* (Hojabri et al., 2017; Mohamed et al., 2020; Prakapaite, et al., 2019).

In this study, *iutA* (97.66%), *fyuA* (85.33%), *inh* (83%) and *traT* (82.33%) were predominant virulence genes. All the CP-*E. coli* contained the *iutA, fyuA, traT, papII, fimH* and *csgA* genes. Noticeably, the O1 (32%), O16 (15%) and O25 (7%) serogroups were predominant.

Among eleven CP-*E. coli* isolates, nine of them were belonged to the B2 phylogroup which is dominant phylogroup in the initiation of extra-intestinal infections and exhibit higher virulence rate. We observed no difference between the phylogroups and virulence factors of CP-*E. coli* strains.

The prevalence of metallo-beta-lactamase (MBL) genes was low and the rate of *bla* $_{\text{KPC}}$ was 0% by PCR among 300 UPEC isolates. A study by Tavakoli and Gheitani did not found the *bla* $_{\text{KPC}}$ similar to our study (Gheitani, Razeli, Moghim, & Nasr Isfahani, 2018; Tavakoly, Jamali, Mojtahedi, Khan Mirzaei, & Sherafagari, 2018), while its frequency in Spain and Germany was respectively 1.7% and 23.8% among CP- *E. coli* (Kaase, et al., 2016; Ortega, et al., 2016).

In a study by Zeighami, similar to our study, they didn't detect this gene (Zeighami, Haghi, & Hajiahmadi, 2015). Additionally, in a study by Adam in China, MBL genes included 27% and 45% respectively among CP- and carbapenem-susceptible *E. coli*, indicating a significant association between the antibiotic resistance and the presence of MBL genes (Adam & Elhag, 2018).

Previous studies regarding *E. coli* carrying *bla* $_{\text{NDM}}$ in our country are scarce (Hussein, 2018), and our study did not detect this gene.

We determined CP- *E. coli* from UTI which carried *bla*$_{\text{IMP}}$ and *bla*$_{\text{OXA-48}}$ carbapenemases and had IMI$_{\text{MIC}}$ of 64-128µg/ml. These strains were belonged to B2 phylogroup and O25 serogroup and carried several virulence genes with the ability to form biofilms but not cytotoxic effects. The prevalence of carbapenemases is various among countries, thus, guidelines and appropriate infection control measures are needed to prevent such infections among patients.

Conflict of interest

None to declare

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Highlights

Of 300 isolates, 11 (3.66%) of them were CP-*E. coli*.

Imipenem minimum inhibitory concentration ranged 4-128µg/ml.

The *bla* $_{\text{OXA-48}}$ and *bla*$_{\text{IMP}}$ genes were co-existed in three (1%) isolates (imipenem MIC: 64-128µg/ml), but the *bla* $_{\text{KPC}}$, *bla* $_{\text{NDM}}$ and *bla* $_{\text{VIM}}$ were not amplified.

Predominant virulence genes included *iutA* (n=293, 97.66%), *fyuA* (n=256, 85.33%), *inh* (n=249, 83%), *traT* (n=247, 82.33%) and *papII* (n=96, 32%).

All the CP-*E. coli* contained the *iutA*, *fyuA*, *traT*, *papII*, *fimH* and *csgA* genes.

O1 (32%), O16 (15%) and O25 (7%) serogroups were predominant and 6/11, 4/11 and 1/11 of CP-*E. coli* belonged to O1, O25 and O75 serogroups, respectively.

Among eleven CP-*E. coli* isolates, nine of them were belonged to the B2 phylogroup and two isolates were belonged to B1 phylogroup.