Phylogrouping, Serogrouping, Virulence Factors and Carbapenemase Genes among Carbapenemase-Producing *Escherichia Coli* From Urinary Tract Infections

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Abstract

Introduction: *Escherichia coli* 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.

Materials and methods: Three-Hundred *E. coli* isolates were enrolled. Antibiotic susceptibility test was conducted by disk diffusion as provided by clinical and laboratory standards institute (CLSI). Carbapenemase production and presence of *bla* $_{\text{OXA-48}}$, *bla* $_{\text{KPC}}$, *bla* $_{\text{NDM}}$, *bla* $_{\text{VIM}}$ and *bla* $_{\text{IMP}}$ genes were evaluated by PCR technique. Virulence factors genes were also screened by PCR technique. Genetic relation of isolates was implemented using phylogrouping and serogrouping.

Results: Of 300 isolates, 11 (3.66%) of them were resistant to carbapenems (CR-*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 CR-*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 CR-*E. coli* belonged to O1, O25 and O75 serogroups, respectively. Among eleven CR-*E. coli* isolates, nine of them were belonged to the B2 phylogroup and two isolates were belonged to B1 phylogroup.

Conclusion: CR-*E. coli* contained the *bla*$_{\text{IMP}}$ and *bla*$_{\text{OXA-48}}$ genes and predominantly O1 serogroup. High rate of virulence factors among CR-*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*

Introduction

Escherichia coli is a substantial agent of urinary tract infections (UTIs) (75–90%) alongside some other infections [(1)].

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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 [(2)(3, 4)].

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 [(5)]. Resistance to carbapenems

occurs through a variety of mechanisms such as efflux pumps and various carbapenemase enzymes. These enzymes are encoded by bla_{IMP}, $bla_{OXA\text{-}48\text{-}like}$, bla_{VIM}, bla_{SIM}, bla_{KPC} and bla_{NDM} genes. 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) isolates can produce biofilms by means of various strategies such as expression of bacterial adhesins. It has been also supposed that some major virulent *E. coli* clonal groups with special phylogroups/ serogroups cause severe and drug-resistant infections. Among them, O25b-B2-ST131, clonal group A (CGA) and O15:K52:H1-D-ST393 are notable [6,7]. Strains from UTI infections mostly belong to the B2 and D phylogroups [8]. Noticeably, these strains belong to various serogroups [9,10] and express numerous virulence factors (VFs) such as adhesins and toxins [11,12].

Materials and Methods

An aggregate on 300 *E. coli* isolates have been present beyond countless origins amongst hospitalized patients during Jan 2017-Dec 2018. The isolates were identified using Chrom Agar medium and biochemical tests.

Subsequently, they were stored at -70 °C in trypticase soy broth containing thirty percntage (v/v) glycerol for other studies.

Antibiotic susceptibility testing

Various antimicrobials were measured using disc diffusion method on Mueller-Hinton agar (Merk, Germany) and were explicated considering the Clinical and Laboratory Standards Institute (CLSI) 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 and *Klebsiella oxytoca* ATCC 13182 were cultured for the quality assessment of disks

AmpC and ESBLs phenotypic and carbapenemase production

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

Biofilm formation

Phenotypic evaluation of biofilm production was carried out using microtiter tissue plate (Mtp) assay as previously mentioned [13]. 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 performed and absorbance rate was assessed using ELISA reader at 490nm. Negative control included wells with culture medium and without bacterial isolates.

PCR amplification for carbapenemases genes

Carbapenemases genes were detected with specific primers for $bla_{OXA\text{-}48}$, bla_{KPC}, bla_{IMP}, bla_{VIM} and bla_{NDM} (Table 1). The total DNA was extracted from by boiling Method. PCR was executed the usage of commercially accessible PCR Master Mix (AMPLIQON, Denmark) in accordance in conformity with the manufacturer's instructions. Briefly, 2 µl template DNA (~100 ng/µl), 1 µl concerning every primer (10 pmoles/µl), then 6µl DNase-free distilled cloud were delivered according to 6 µl over Master Mix into a final quantity regarding 15 µl.

Amplification involved an initial denaturation at 94°C for 5 min followed by 32 cycles of denaturation (94°C, 50 s), annealing (56°C, 1 min for $bla_{OXA\text{-}48}$, bla_{KPC}, bla_{IMP}, 50°C, 45 s for bla_{VIM}, 57°C, 1 min and 62°C, 50 s for bla_{NDM}) and extension (72°C, 1 min), with a final extension step (72°C, 8 minutes). The amplified DNA was separated by gel electrophoresis on 1% agarose, and visualized under UV transillumination.

Results

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 determined. Among underlying diseases (cardiovascular diseases, kidney or liver disorders and cancer), no significant correlation was detected with UTI.

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, gentamycin, 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%. Carbapenemase-producing isolates and Extended-spectrum β-lactamase- (ESBL-) included 46.7% and 3.66%, respectively. Carbapenemase-producing strains were resistant to other classes except nitrofurantoin and fosfomycin (table1and 2).

Table1. Characteristics of carbapenemase-producing isolates

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

ESBL: extended-spectrum β-lactamase, 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.

Table 2. The antibiogram for isolates containing *bla* $_{OXA-48}$

Isolate	FO	CAZ	PTZ	CTX	AMX	CZ	AMC	IMI	FM	GM	CIP	MEN	SXT	ERY	TE
S 1	S	R	S	R	R	R	R	S	S	S	S	S	R	R	R
S 2	S	R	S	R	R	R	R	S	S	R	R	S	S	I	I
S 3	S	R	S	R	R	R	R	S	S	S	S	S	S	R	R

CIP:ciprofloxacin, AMC: co-amoxiclav, AMX:amoxicillin, CTX:cefotaxime, CAZ:ceftazidime, CZ:cefazolin, IMI:imipenem, MEN:meropenem, GM:gentamycin, SXT:cotrimoxazole, FO:fosfomycin, PTZ:pepracillin_ tazobactam, ERY:erythromycin, FM:nitrophorantoien TE:tetracycline

Amplification of carbapenemase genes

Among the 300 *Escherichia coli* strains, three isolates contained bla_{OXA-48} carbapenemase enzyme gene (3%), and *bla* $_{KPC}$, *bla* $_{NDM}$, *bla* $_{VIM}$ and *bla* $_{IMP}$ were not found.

Determination of *bla* $_{CTX-M1}$ enzyme gene of isolates containing *bla* $_{OXA-48}$

The three isolates containing *bla* $_{OXA-48}$, *bla* $_{CTX-M1}$ was found as shown in (Table 3).

Table3: Results of resistance genes for isolates containing *bla* $_{OXA-48}$

	bla CTX-M1	bla OXA-48	bla KPC	bla NDM	bla VIM	bla IMP
S1	+	+	-	-	-	-
S2	+	+	-	-	-	-
S3	+	+	-	-	-	-

Serogrouping, philogrouping and virulence factors of isolates containing *bla* $_{OXA-48}$

Among the three isolates containing *bla* $_{OXA-48}$, two of them belonged to the phylogroup B2 and one isolate was B1. Serogroups included O25, O16 and one of them were non-type able with this method. All the three isolates contained *papΠ, inh, iutA, csgA, traT, fyuA and fimH* virulence genes. Also, none of the three isolates *cdt, ibe*, *vat, sat*, *tcp* virulence genes were not found as shown in (Table 4).

Table 4. The phylogroups, serogroups and virulence factors for isolates containing *bla* $_{OXA-48}$

Isolate	Philogroups	Serogroups	papII	inh	ompT	iucD	fyuA	fimH	Cnf	pic	traT	kpsM	csgA	hlyA	iut	biofilm
S 1	B2	NI	+	+	-	-	+	+	-	+	+	-	+	-	+	N
S 2	B2	O16	+	+	+	+	+	+	+	-	+	+	+	+	+	W
S 3	B1	O25	+	+	+	-	+	+	-	-	+	-	+	+	+	W

In the biofilm assay, one isolate produced no biofilm, two isolates produced weak biofilm (Table 4).

Discussion

The studies depicted that among of five Carbapenemase examined of the *E. coli* in collected from patients with UTI, *bla* $_{OXA-48}$ were identified as dominant Carbapenemase, the study of rezaei in Zabul, the prevalence of this gene was 53% among of UPEC strains in 2018[14], probably the difference was leaded in geographic location and the borderlines of Zabul. also in the study of Eslami of *E. coli* in Collection from septic, *bla* $_{OXA-48}$ was 3%[15], while in our study the prevalence of *bla* $_{OXA-48}$ was 1%, the reason of difference was in the type of sample (blood), and Solgi of 47 carbapnemase producing enterobacteriaceae in Collection from rectal swabs of hospitalized patients, only 10 *E. coli* isolates was positive [16], which is different in the type of sample and the non-sensitive to Carbapenems of the isolates .other countries Spain, Germany and Egypt were reports 72%, 58.3% and 33% to respectively that was different from our results[17,13] Because all samples were non sensitive to carbapenems.

Also, three isolates containing *bla* $_{OXA-48}$ to ceftazidime, cefotaxime, and cefazolin was resistant due to the presence bla $_{CTX-M1}$. Therefore, we cannot use wide range of cephalosporins in treatment. these isolates were Sensitive to fosfomycin and carbapenems and we use these antibiotics in treating patients.

In the study of phylogrouping of three isolates contain *bla* $_{OXA-48}$, two isolates are belonged to B2 phylogroup, which is dominant phylogroup in the initiate extra-intestinal infections, which has a higher virulence, and the virulence factors of pup Π, inh, fyuA, fimH, traT, csgA, iut were common in the two isolates of B2 phylogroup.

Accoring to that virulence factors cnf, kpsM, hlyA has significant role in pathogens, and all are negative in TMU1 isolate. Comparison of Pathogenicity depicted that the TMU1 to TMU2 is lower Pathogenicity which both of them were belonged B2 phylogroup. the virulence factors cnf, kpsM, hlyA were positive in TMU2, and its

weak biofilm while the TMU1 biofilm was negative.

TMU3 is belonged B1 phylogroup, we expected to exist fewer virulence factors in TMU3, but many virulence factors were expressed by TMU3 similar to TMU1and TMU2, therefore in our study there were no variety between the phylogroups and factors of virulence.

The serogrouping results showed that three isolates contain *bla* $_{OXA-48}$, O16 and O25 serogroups were identified, one of the isolates was non-typing by this method.

the significant relation nonexistence between phylogroups and serogroups. the difference in prevalence of virulence factors mainly is due to differences in epidemiology, clinical samples, ages, clonal groups and various strains of *E. coli*.

The prevalence of MBL genes and *bla* $_{KPC}$ by PCR assays among 300 *E. coli* clinical isolates was 0%. the study of Tavakoli and Gheitani Similar to our study *bla* $_{KPC}$ not found while its frequency in Spain and Germany was 1.7% and 28.8% respectively in carbapenem non-sensitive *E. coli* [13,17].

In the study of Zayghamia, similar to our study, metallo-beta-lactamases was not found[18]. and Eslami *bla* $_{VIM}$ and *bla* $_{IMP}$ were reported 3% and 2% respectively, difference observed perhapas was due to the type of sample (blood)[15]. Additionally, in the study Adama in China in 2018, MBL genes positive among sensitive and resistant isolates to carbapenems were 27% and 45% respectively, which indicates a significant association between the antibiotic susceptibility and the presence of MBL genes.

The studies are low in *E.coli* contain *bla* $_{NDM}$ in our country, and first report pertain was of our study, which the frequency was 0%, in the study of Fazeli *bla* $_{NDM}$ 12.2% on *Klebsiella pneumoniae* in 2015, and in the study of Solgi among 47 CREs isolates only one *E. coli* isolate was positive[16]. while the frequency in Spain and Germany was 1.7% and 8.3% respectively in non-sensitive carbapenem *E. coli* [13, 17].

The prevalence of carbapenemases is various in countries, Thus, guidelines and appropriate infection control measures are needed to prevent such infections among patients.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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