Correlated between Sera Levels of Interleukins(IL-6, IL-17 and IL-23) with Virulence Genes Detected in Carbapenem-Resistant *E.coli*

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Abstract

Current study demonstrated that 14.58% of isolated *E. coli which* positive for each of *Omp -A, IMP- A, NDM- A* and *Fim- H* genes were resistant to carbapenem and the study showed that 30.51% of *E. coli* isolates with KPC were resistant to carbapenem while other *E. coli* isolates with negative all genes were resistant to carbapenem , so the Percentage as 72.92% of isolated *E. coli* were positive for each of *Omp – A, IMP – A, NDM- A* and *Fim – H* genes were occurred in females compared with 27.02% occurred in males and 40% of isolated *E. coli* with positive for *KPC* gene were in females with UTI. as well as 10, 50% of isolated *E. coli* were positive for each of *Omp- A, IMP - A, NDM- A* and *Fim - H* genes were occurred in the age group 15-24 year and no isolate were form patients above 54 year.

the highest mean levels IL-6 and IL-17 was recorded in UTI patients infected with *E. coli* positive for each of *Omp- A , IMP- A , NDM- A* and *Fim- H* comparing with patients infected with *E. coli* negative to these genes. Conclusion Carbapenem-resistant *E. coli correlated with founded* each of genes (Omp -A, IMP- A, NDM- A and Fim- H) , whilst highest correlated between Carbapenem-resistant *E. coli* with KPC.so sera levels of both interleukins (IL-6 and IL-17) were recorded in UTI patients infected with *E. coli* positive for each genes (Omp- A , IMP- A , NDM- A and Fim- H) comparing with patients infected with *E. coli* negative to these genes.

Keywords *: sera levels of interleukins(IL-6, IL-17 and IL-23) ; virulence genes ; carbapenem-resistant E. coli*

Introduction

E. coli belongs to the Enterobacteriaceae family and is present in the natural microbiota of humans and other homoeothermic animals. The *E. coli* associated with UTIs are denoted uropathogenic *E. coli*(UPEC) **(1)** ,With the widespread use of antibiotics, the carbapenem-resistant strains have become a serious public health issue in the worldwide and are usually resistant to almost antibiotics**(2)** .Carbapenem-resistant Enterobacteriaceae (CRE), specially included three species of the Enterobacteriaceae family, the *Klebsiella, Enterobacter* and *Escherichia.coli* have developed resistance to a group of antibiotics called "Carbapenems", which are often used as the last line of treatment when other antibiotics are not effective in treating infections caused by them**(3)** . Carbapenem-resistant *E. coli* strains is a main risk for global public health, but little is known of carbapenemase producing *E. coli* in Iraq.

E.coli strains have some gene regions responsible for virulence factors which may encode adhesins, toxins, siderophores and haemolysin. Outer membrane protein A (OmpA) is a major protein in the *Escherichia coli* outer membrane , it is the abundant outer membrane proteins (OMPs) , with typically 100,000 copies per cell**(4)**

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**(5)** . 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**(6)** .

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 [(7)] (Plos *et al.,*1991).*Fim*A, associated with ancillary proteins *Fim*F, *Fim*G, and the adhesin protein *Fim*H, encoded by the *fim* gene cluster [(8)] . This type of fimbria is common among Enterobacteriaceae, also several variants have been strongly associated with UPEC [(9)] . Their role in infection is unclear, although it has been suggested that they may be involved in the initial stages of colonizing in the upper respiratory tract [(9;10)] .

Interleukin (IL)-6 is a pro-inflammatory cytokine, the concentration of which increases in the early stage of bacterial infection. Interleukin-6 has regenerative activities, which, when absent, aggravated the development of the inflammatory process.

So IL-23 is believed to be important in the expansion and survival of these IL-17-producing cells(naïve T cells) [(11)] . In addition, an intact IL-23-IL-17 axis seems to be essential for host protection against infections, as well as in the pathogenesis of certain autoimmune diseases [(12)] .aim of current study was evaluate the possible correlations between carbapenem-resistant *Escherichia coli* and frequency of virulence gene in UTI*s* infections, and correlated between carbapenem-resistant *E.coli* and level of IL-6; IL-17& IL- 23.

Materials and Methods

Primers Used in Current Study: All the primers used in this study listed in table (1).

Table (1): All Primers used in this study

Gene		Sequence of forward and reverse Primer(5´- 3´)	Product bp	Origin
Fim (h) (5 ' – 3 ')	F	TGC AGA ACG GAT AAG CCG TGG	508	Alpha DNA Co.(Canada)
	R	GCA GTC ACC TGC CCT CCG GTA		
OmpA	F	ACCCTGGTTGTAAGCGTCAG	419	
	R	GTAAAAACCACGACACCGGC		
KPC	F R	CGTCTAGTTCTGCTGTCTTG CTTGTCATCCTTGTTAGGCG	798	
NDM-1	F	ACCCTGGTTGTAAGCGTCAG GTAAAAACCACGACACCGGC	621	
	R			
IMP-1	F R	CGTCTAGTTCTGCTGTCTTG CTTGTCATCCTTGTTAGGCG	232	
	F			

Note: All primers have the following universal tail which is used as a sequencing primer:
oF : GTT TTC CCA GTC ACG ACG TTG TA
oR: TTG TGA GCG GAT AAC AAT TTC
Type 1 fimbriae in commensal e.coli derived from healthy by pawel pus z….ect

Specimens collection

All specimens were collected during the beginning of September 2018 to the end of April 2019,One hundred and eighty midstream urine specimens were collected from patients attending hospitals (Baghdad Teaching Hospital / Medical city ; Abn Al Baladi hospital ; Imam Ali hospital and Al-Numman hospital, Iskan) hospitals .

The Specimens were collected according to[13] . Urine cultured immediately after collection (from hospital laboratory) by streaking 0.01 ml of urine on Blood agar, MacConkey agar, and Eosin Methylene Blue agarin order to isolate *Escherichia coli* only.

Estimation of IL-6 ; IL-17 and IL-23 in Serum:according to commercially available kit , using the quantitative sandwich enzyme.

Extraction of DNA from *E.coli* and Each bacterium was isolated in this study was subjected to molecular screening study using PCR amplification.

To determine the phylogenetic groups of all isolates in the current study, five primers were used which are IMP ; NDM-1; KPS ; Omp A and *Fim h* gens by PCR

Statistical Analysis;Data analysis was done by descriptive statistics and using SPSS version 20. values of $p \leq 0.05$ were regarded as statistically significant relationships.

Findings

The study demonstrated that 14.58% of isolated *E. coli which* positive for each of Omp -A, IMP- A, NDM-A and Fim- H genes were resistant to carbapenem and the study showed that 30.51% of *E. coli* isolates with KPC were resistant to carbapenem while other *E. coli* isolates with negative all genes were resistant to carbapenem (Table 1).

Table (1): Distributions of virulence genes according to resistance of carbapenem

Genes		Total No. No.	Carbapenem sensitive		Carbapenem resistance		P. value
			%	No.	%		
Omp - A	Positive	48	41	85.42	7	14.58	P<0.01 HS
	Negative	12	0	0	12	100	
IMP - A	Positive	48	41	85.42	7	14.58	P<0.01 HS
	Negative	12	0	0	12	100	
NDM- A	Positive	48	41	85.42	7	14.58	P<0.01
	Negative	12	0	0	12	100	
Fim - H	Positive	48	41	85.42	7	14.58	P<0.01
	Negative	12	0	0	12	100	
KPC	Positive	59	41	69.49	19	30.51	P>0.5
	Negative	1	0	0	1	100	

In table 2 the Percentage as 72.92% of isolated *E. coli* were positive for each of Omp – A, IMP - A, NDM- A and Fim – H genes were occurred in females compared with 27.02% occurred in males and 40% of isolated *E. coli* with positive for KPC gene were in females with UTI.

Table (2): Distributions of virulence genes according to gender of patients

Genes		Total No. No.	Female		Male		P. value
			%	No.	%		
Omp - A	Positive	48	35	72.92	13	27.02	P>0.5 NS
	Negative	12	6	50	6	50	
IMP - A	Positive	48	35	72.92	13	27.02	P>0.5 NS
	Negative	12	6	50	6	50	
NDM- A	Positive	48	35	72.92	13	27.02	P>0.5 NS
	Negative	12	6	50	6	50	
Fim - H	Positive	48	35	72.92	13	27.02	P>0.5 NS
	Negative	12	6	50	6	50	
KPC	Positive	59	40	83.33	19	16.67	P>0.5 NS
	Negative	1	1	100	0	0	

In table showed that 10, 50% of isolated *E. coli* were positive for each of Omp- A, IMP - A, NDM- A and Fim -– H genes were occurred in the age group 15-24 year and no isolate were form patients above 54 year.

Table (3): Distributions of virulence genes according to age of patients

Age groups	Omp – A, Fim – H, NDM-1 and IMP,				KPC,			
	Positive		Negative		Positive		Negative	
	No.	%	No.	%	No.	%	No.	%
15-24	6	50	14	29.17	1	100	19	32.21
25-34	3	25	11	22.92	0	0	13	22.03
35-44	2	16.67	14	29.17	0	0	16	27.12
45-54	1	8.33	7	14.58	0	0	7	11.86
>54	0	0	2	4.16	0	0	4	6.78
Total	12	100	48	100	1	100	59	100
P. value	>0.05 (NS)				>0.05 (NS)			

In table 4, the highest mean levels IL-6 and IL-17 was recorded in UTI patients infected with *E. coli* positive for each of Omp- A , IMP- A , NDM- A and Fim- H comparing with patients infected with *E. coli* negative to these genes.

Table (4): Relation of virulence genes with interleukin IL-6, IL-17 and IL-23 levels

GENE Patients		**Interleukins levels (IL)**	**N**	**(Mean ± S.D)**	**P. value**
Omp - A	-	IL- 6	48	112.9 ±96.2	N.S
	+		12	178.1±128.3	
	-	IL- 17	48	405.6±340.2	N.S
	+		12	614.0 ±533.1	
	-	IL- 23	48	361.0±239.9	S
	+		12	111.1±92.4	
Fim - H	-	IL- 6	48	112.9 ±96.2	N.S
	+		12	178.1±128.3	
	-	IL- 17	48	405.6±340.2	N.S
	+		12	614.0 ±533.1	
	-	IL- 23	48	361.0±239.9	S
	+		12	111.1±92.4	
KPC	-	IL- 6	59	127.4 ±120.8	N.S
	+		1	41.8 ±none	
	-	IL- 17	59	452.3 ±391.6	N.S
	+		1	151.2 ± none	
	-	IL- 23	59	510.4 ±1028.8	N.S
	+		1	572.143 ±none	
IMP	-	IL- 6	48	112.9 ±96.2	N.S
	+		12	178.1±128.3	
	-	IL- 17	48	405.6±340.2	N.S
	+		12	614.0 ±533.1	
	-	IL- 23	48	361.0±239.9	N.S
	+		12	111.1±92.4	
NDM-1	-	IL- 6	48	112.9 ±96.2	N.S
	+		12	178.1±128.3	
	-	IL- 17	48	405.6±340.2	N.S
	+		12	614.0 ±533.1	
	-	IL- 23	48	361.0±239.9	S
	+		12	111.1±92.4	

Discussion

Carbapenem-resistant *Enterobacteriaceae* (CRE) is an urgent public health problem worldwide. These multidrug-resistant organisms exhibit resistance to most, if not all, available antibiotics today and are associated with considerable mortality[14] . In one study, approximately 32% of patients with bloodstream infections caused by carbapenemase-producing CRE died within 14 days[15] . SO [16] Explain the rapid spread of CRE is due to the clonal and plasmid-mediated dissemination of clinical carbapenem-resistant strains[17], Whilst [18] found that 32.2% of UTI patients with carbapenem resistant *E. coli* were females.

Cytokines IL-6 and IL-8 are mediators of inflammation in response to bacterial infection, and when measured in plasma or serum these may be used as early biomarkers of infection[19] IL-6 and IL-8 levels are also known to be elevated in the urine of patients with UTI, whereas reportedly none are measurable in the urine of healthy controls. The high levels of interleukin 6 in women with acute UTI contribute to increase body temperature and stimulate the production of the C-reactive protein and indicating systemic response of the body toward infection[18].Moreover, [20] reported higher levels of IL-6 and IL-8 in urine in patients with UTI caused by fimbriated *E. coli* compared to patients with UTI caused by non-fimbriated *E. coli*. On the other hand, elevated level of IL-17 in addition to IL-6 in patients with UTI as early innate reaction. Additionally, in murine model that both IL-17 and IL-23 enhance the body to eradicate utopathogenic *E. coil*[21]. The early innate responses include bacterial expulsion, urothelial exfoliation, and bladder inflammation that is characterized by the production of the pro-inflammatory cytokine interleukin 6 (IL-6), granulocyte chemotactic cytokines such as IL-8, the hormone granulocyte colony stimulating factor (G-CSF), and the T cell-associated, pro-inflammatory cytokine IL-17A [22] .

Carbapenem resistance may also be due to AmpC type enzymes or ESBLs along with imperme ability of the membrane [23].Membrane imperme ability can be linked to modifications or absence of OmpC and/or OmpF porin channels or presence of drug efflux pumps [24] .

Conclusion

1- levels of interleukin(IL-23) were increased significantly ($P<0.05$) in UTI patients who positive with CRP test compared with CRP negative , also IL-17 elevated moderately in patients with CRP positive while no difference in level of IL-6 between the two groups.

2- Carbapenem-resistant *E. coli correlated with founded* each of genes (Omp -A, IMP- A, NDM-A and Fim- H) , whilst highest correlated between Carbapenem-resistant *E. coli* with KPC.

Conflict of Interest: Non

Source of Findings: Non

Ethical Clearance: Non

References

1) Mulvey M.A., Schilling J.D., Martinez J.J. Bad bugs and beleaguered bladders: interplay between uropathogenic *Escherichia coli* and innate host defenses. Proc Natl Acad Sci U S A. 2000;97:8829–8835.

2) Nordmann,P.Carbapenemase-producing Enterobacteriaceae: overview of a major public health challengeMed Mal Infect, 44 (2014), pp. 51-56.

3) Shraddha Prasad Gunjal, Prasad Niranjan Gunjal, Nagaraju Vanaparthi, Kher Sudheer.Carbapenem resistance profie amongst *Escherichia coli* and klebsiella pneumoniae in a tertiary care hospital in ahmedagar , Maharashtra.. Int J Med Res Health Sci. 2014;3(2):424-427.

4) Tektook ,Nihad Khalawe; Khalid Ibrahim Al-Lehibi; Raad K.Al-Husseinei,2017.Prevalence Some Pathogenic Bacteria Causing UTI in Diabetic Patients In / Specialized Center For Endocrinology and Diabetes of Baghdad City–Iraq.Medical Journal of Babylon 14 (2), 260 – 266

5) 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. 4- Koebnik R, Locher KP, Van Gelder P. Structure and function of bacterial outer membrane proteins: barrels in a nutshell. Mol Microbiol. 2000;37:239–253.

6) 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.

7) 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.

8) Feria C, Machado J, Correia JD, Goncalves J, Gaastra W. Virulence genes and P fimbriae PapA subunit diversity in canine and feline uropathogenic Escherichia coli. Vet Microbiol. 2001;82(1):81–9.

9) Simms AN, Mobley HL. Multiple genes repress motility in uropathogenic Escherichia coli constitutively expressing type 1 fimbriae. J Bacteriol. 2008;190(10):3747–56.

10) Kibret M, Abera B (2014) Prevalence and antibiogram of bacterial isolates from urinary tract infections at dessie health research laboratory, Ethiopia. Asian Pac J Trop Biomed 4: 164-168

11) Veldhoen, M., and B. Stockinger. 2006. a 'Jack of all trades': the link with pro-inflammatory IL-17-producing T cells. Trends Immunol. 27:358-361

12) Mangan, P. R., L. E. Harrington, D. B. O'Quinn, W. S. Helms, D. C. Bullard, C. O. Elson, R. D. Hatton, S. M. Wahl, T. R. Schoeb, and C. T. Weaver. 2006. Transforming growth factor-beta induces development of the T(H)17 lineage. Nature 441:231-234.

13) Van de Veerdonk FL, Kullberg BJ, Verschueren IC, Hendriks T, van der Meer JW, Joosten LA, Netea MG. Differential effects of IL-17 pathway in disseminated candidiasis and zymosan-induced multiple organ failure. Shock. 2010;34(4):407–411 .

14) Centers for Disease Control and Prevention (CDC). 2013. Vital signs: carbapenem-resistant *Enterobacteriaceae*. MMWR Morb Mortal Wkly Rep 62:165–170.

15) Tamma PD, Goodman KE, Harris AD, Tekle T, Roberts A, Taiwo A, Simner PJ. 2017. Comparing the outcomes of patients with carbapenemase-producing and non-carbapenemase-producing carbapenem-resistant *Enterobacteriaceae* bacteremia. Clin Infect Dis 64:257–264

16) Darunee Chotiprasitsakul, Sirawat Srichatrapimuk, Suppachok Kirdlarp, Alexander D Pyden, andPitak Santanirand.Epidemiology of carbapenem-resistant Enterobacteriaceae: a 5-year experience at a tertiary care hospital .Infect Drug Resist. 2019; 12: 461–468

17) Overbeek, R., Olson, R., Pusch, G. D., Olsen, G. J., Davis, J. J., Disz, T., et al. (2014). The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res*. 42, D206–D214.

18) Zhang.Y; Qi Wang, Yuyao Yin, Hongbin Chen, Longyang Jin. Epidemiology of Carbapenem-Resistant *Enterobacteriaceae* Infections: Report from the China CRE Network. Antimicrob Agents Chemother. 2018 ; 62(2): e01882-17.

19) Sheu JN, Chen MC, Lue KH, Cheng SL, Lee IC, Chen SM, et al. Serum and urine levels of interleukin-6 and interleukin-8 in children with acute pyelonephritis. Cytokine. 2006;36: 276–282.

20) Benson, M.; U. Jodal, W. Agace . "Interleukin (IL)-6 and IL-8 in children with febrile urinary tract infection and asymptomatic bacteriuria," Journal of Infectious Diseases, vol. 174, no. 5, pp. 1080–1084, 1996.

21) Sivick KE , Schaller MA; Smith SN ; Mobley HL (2010).The innate immune response to uropathogenic *Escherichia coli* involves IL-17A in a murine model of urinary tract infection *J Immunol* 184(4): 2065-2075 vol. 184, no. 4, pp. 2065–2075, 2010.

22) Van de Veerdonk FL, Kullberg BJ, Verschueren IC, Hendriks T, van der Meer JW, Joosten LA, Netea MG. Differential effects of IL-17 pathway in disseminated candidiasis and zymosan-induced multiple organ failure. Shock. 2010;34(4):407–411.

23) Pirko , E. Y; Salman, H.K. and Tektook , N.K.(2016).Study of some virulence factors of *Coagulase* negative staphylococci (CONS) isolated from urinary tract infections (UTI).World Journal of Pharmaceutical Research,5(11);68-78.

24) Sugawara E, Nestorovich EM, Bezrukov SM, Nikaido H. Pseudomonas aeruginosa porin OprF exists in two different conformations. J Biol Chem. 2006;281:16220–16229. 23-Araj GF, Avedissian AZ, Ayyash NS, Bey HA, El RA, Hammoud RZ. A reflection on bacterial resistance to antimicrobial agents at a major tertiary care center in Lebanon over a decade. J Med Liban. 2012;60: 125–135. pmid:23198452.