DOI : 10.35124/bca.2019.19.2.4259

Biochem. Cell. Arch. Vol. 19, No. 2, pp. 4259-4264, 2019 | www.connectjournals.com/bca | *ISSN 0972-5075*

ROLE OF PATHOGENICITY ISLANDS AND INTEGRONS WITH HORIZONTAL TRANSFER MULTIPLE DRUG RESISTANCE OF UPEC ISOLATES : A MOLECULAR STUDY

Noor M. Faisal*, Ahmed D. Jabbar, Aya A. Hussein and Zainab A. Bachay

Wasit University, Iraq.

*e-mail: noormuhanad941@gmail.com

(Received 17 April 2019, Revised 28 June 2019, Accepted 25 July 2019)

ABSTRACT : Sixty UPEC isolates were collected from female suffered from recurrent urinary tract infections. Antibiotic resistance profile was determined by VITEK2. Results showed ampicillin resistance was most prevalent 83.3% (41/60), followed by ceftriaxone, 71.7% for, while cefoxitine relative, cefazolin shows moderate resistant since 60% of UPEC isolates. This work demonstrates that 48% of isolates were positive for integron (Int I). 90.9% of UPEC were *intI* positive isolates, which accounts of MDR-UPEC isolates. From the *IntI* positive mentioned above, 35(87.5%) of MDR-UPEC isolates were positive for type 1 integron signified by *intI1*, whereas a little lower results obtained with integrin II represented by *intI2*, 30 (75%) isolates. About 51.3% of isolates have PAI markers. The most prevalent PAI among MDR-UPEC was PAI IV 58(96.6%), followed by PAI III 42 (70%), PAI II563 3(5%), PAI IIJ96 5(8.3%) and PAI I6352(3.3%). PAIs are considered to be a subclass of genomic islands that are acquired by horizontal gene transfer via transduction, conjugation and transformation, and provide 'quantum leaps' in microbial evolution. The MDR-UPEC isolate were screen for replication regions of IncFI and IncFII plasmid. The present study established a high incidence of repFIA plasmid was found statistically associated with papC, sfaA and fimH. No obvious role found for CRISPR's associated genes (for both class 1 and class 2) in association with antibiotic resistance.

Key words : Pathogenicity island (I, II, III, IV), integrons (I, II), MDR-UPEC, microRNA.

INTRODUCTION

The spread and appearance of antimicrobial resistance caused desirous an increasing and concern health problem. *E. coli* strains are part of the normal flora in the gastrointestinal tract in the diverse animal and humans (Pitout, 2012). However, they are also the major cause of extra intestinal infection like meningitis, UTIs, and infection relate "with intravascular devices" (Chamberlain, 2009). As extraintestinal pathogenic *E. coli* (ExPEC), including UPEC, yearly affects a large proportion of the population, being responsible of as many as 80% of UTI in otherwise healthy people, so they are a major target of antimicrobial therapy (Poey *et al*, 2012). The management of infections caused by ExPEC has been complicated by the emergence of antimicrobial resistance. Resistance genes are disseminated by plasmids or by transposons and can be integrated into DNA elements designated integrons (Cambray *et al*, 2011).

Integrons are compose of a site-specific recombination system capable of integrating and expressing genes in cassettes. Both antibiotic resistances and virulence factors can be encoded by mobile elements. If co-integration occurs, antibiotic pressure also selects for virulence factors, which in turn could lead to more virulent antibiotic-resistant strains (Rijavec *et al*, 2006). Certainly, plasmids belonging to the IncF incompatibility group encoding both antibiotic resistance and virulence factors have to be examined (Carattoli, 2009).

CRISPR or clustered regularly interspersed short palindromic repeats, is quit new field of study and the possible link between this system and pathogencity of bacteria is not understood.

We try to find the involvement of the type of PAI, CRISPR system and integrons in the pathogencity of UPEC and antibiotic resistance.

MATERIALS AND METHODS

Antibiotic sensetivity test

Antibiotic sensitivity was determined by Automated VITEK2 system, Biomerieux.

2.2 Molecular study

2.2.1 Bacterial Nucleic Acid Extraction:

Total genomic bacterial nucleic acid was extracted as recommended by the manufacturer.Cat. # GBB100

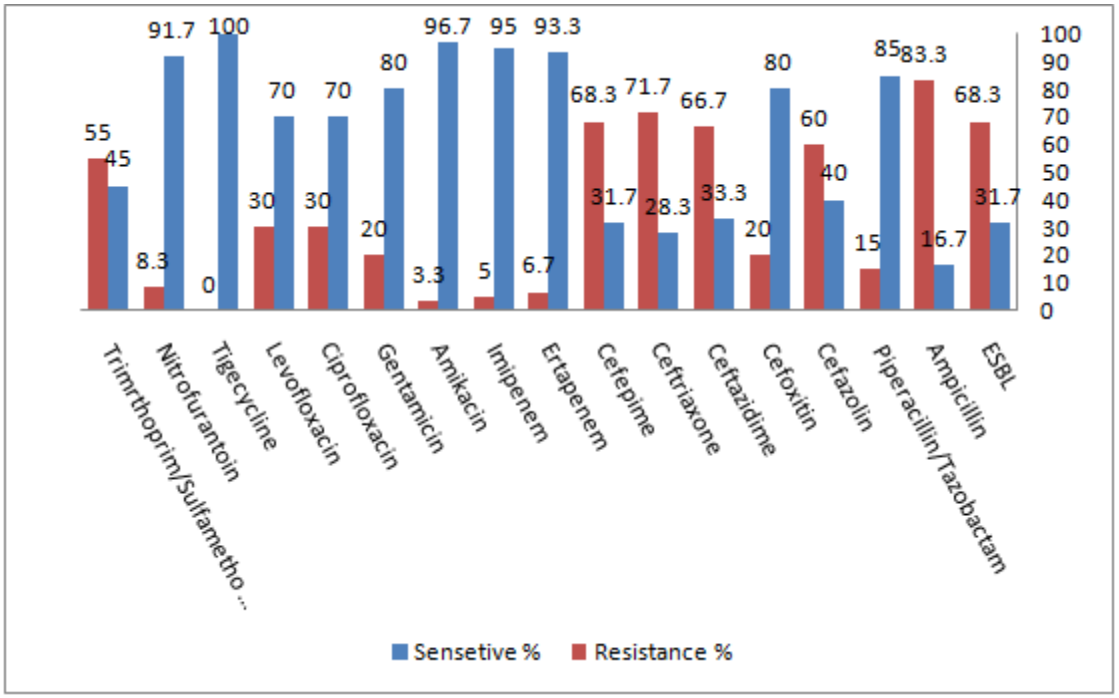

Fig. 1 : Frequency of antibiotics resistance in 60 UPEC isolates.

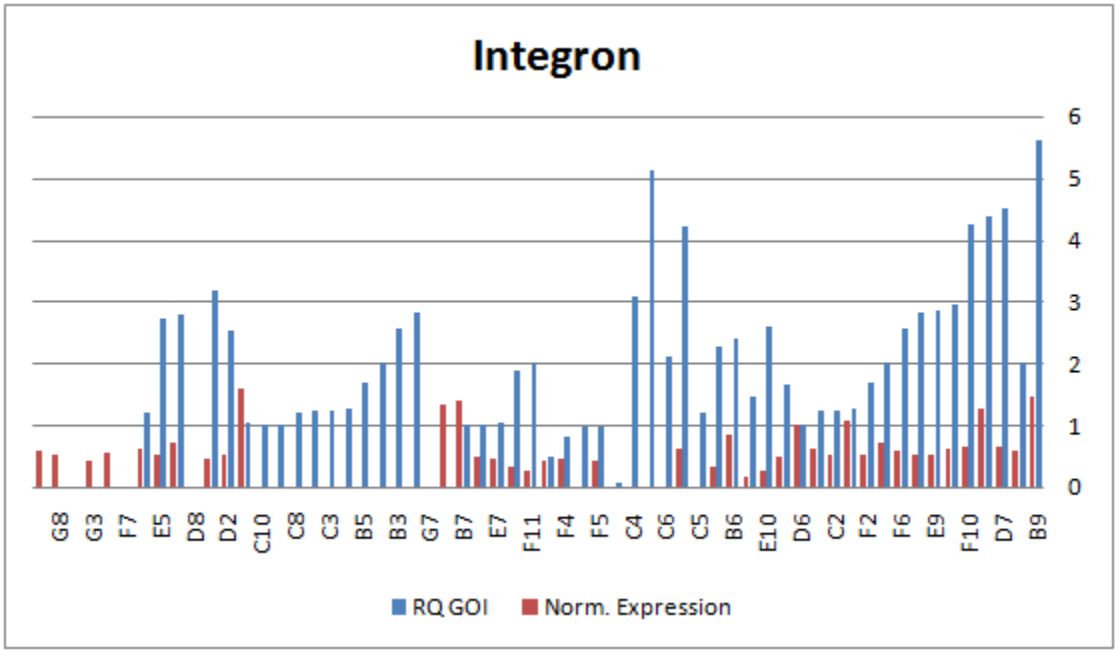

Fig. 2 : Compare with normal level expression in corresponding sensitive isolates.

supplied by Genaid, Canada. RNA was extracted as recommended by kit manufacturer, TransGen Biotech, Catalog Number : ER101-01

cDNA synthesis

Extracted RNA were converted immediately to cDNA using MessageAmp™ II-Bacteria Kit and stored at –20^{0}C.

Primers

Primers were purchased from BIONEER company as lyophilized powder in Eppendorf tubes (1.5 ml) (Table 1).

Real Time PCR amplification

PCR was carried out in 20 µl and as follows 1-2 µl of primers, 5µl of DNA sample and DEPC-distilled water was adjusted to 20 µl final volume. All this components was added to master mix tube and mixed thoroughly by pipetting and followed by short spin. The components of PCR program were summarized in Table 2.

Table 1 : Primers used in this syudy.

Target	Primer name	Primer sequence (5´-3´)	Size(bp)	References
Cas1	Cas1-F	CGC CTG CAT TAT GCT CGA AC	783	Ruiz-Garbajosa *et al* (2006)
	Cas1 -R	CAT TTT GCG CACCAC CTT CA		
Cas2	Cas 2-F	ATG AGC ATG GTC GTG GTT GT	285	Ruiz-Garbajosa *et al* (2006)
	Cas 2-R	CCC ATCCAA ATC CAC CGG AA		
intI1	Int1-F	CAG TGG ACA TAA GCC TGT TC	160	Rowe-Magnus *et al* (2002)
	Int1-R	CCC GAG GCA TAG ACT GTA		
intI2	intI2-F	CAC GGA GCG ACA AAA AGG T	789	Rowe-Magnus *et al* (2002)
	intI2-R	GTA GCA AAC GAG TGA CGA AAT G		
Class2 integron variable region	hep74	CGG GAT CCC GGA CGG CAT GCA CGA TTT GTA	variable	White *et al* (2001)
	hep51	GAT GCC ATC GCA AGT ACG AG		
blaSHV	Fwd	GGG TTA TTC TTA TTT GTC GC	713	Dallenne *et al* (2010)
	Rev	TTAGCGTTGCCAGTGGTC		
blaOXA	Fwd	GGCACCAGATTCAACTTTCAAG	564	Dallenne *et al* (2010)
	Rev	GAC CCC AAG TTT CCT GTA AGT G		
blaAmpC	Fwd	CCC CGC TTA TAG AGC AAC AA	634	Mendonça *et al* (2007)
	Rev	TCA ATG GTC GAC TTC ACA CC		
blaCTX-M	Fwd	SCS ATG TGC AGY ACC AGT AA	688	Saladin *et al* (2002)
	Rev	CCG CRA TAT GRT TGG TGG TG		
PAI III 536	sfaAI.1	CGG GCA TGC AAT TAT CTT TG	200	Sabate´ *et al* (2006)
	sf aAI.2	TGT GTA GAT GCA GTC ACT CCG		
PAI IV 536	IRP2 FP	AAG GAT TCG CTG TTA CCG GAC	300	Sabate´ *et al* (2006)
	IRP2 RP	TCG TCG GGC AGC GTT TCT TCT		
PAIIICFT073	cft073.2Ent1	ATGGATGTTGTATCGCGC	400	Sabate´ *et al* (2006)
	cft073.2Ent2	ACGAGCATGTGGATCTGC		
PAI I J96	papGIf	TCGTGCTCAGGTCCGGAATTT	400	Sabate´ *et al* (2006)
	p apGIr	TGGCATCCCACATTATCG		
papC	pap1	GACGGCTGTACTGCAGGGTGTGGCG	228	Le bouguenec *et al* (1992)
	p ap2	ATATCCTTTCTGCAGGGATGCAATA		
sfaA	sfa1	CTCCGGAGAACTGGGTGCATCTTAC	410	Le bouguenec *et al* (1992)
	sf a2	CGGAGGAGTAATTACAAACCTGGCA		
fimH	fimH f	TGCAGAACGGATAAGCCGTGG	508	Obata-Yasuoka *et al* (2002)
	f imH r	GCAGTCACCTGCCCTCCGGTA		
GAPDH	Fwd	AGG TCG GTGTGA ACG GAT TTG	741	Barber *et al* (2005)
	Rev	TGT AGA CCA TGT AGT TGA GGT CA		
mir3p-393	Common Fwd	TGTGGGCACTCGAAGATACGGAT	Variable, depends on isolates	Kang *et al* (2013)
	Rev	TTTGCTCTTTAAAAATC		
miR-5p-756	Rev	TGTGGGCACTCGAAGATACGGAT		
mir3p-40	Rev	GTTGTGAGGTTAAGCGACT		
mir5p-79	Rev	CTCGAAGATACGGATTCTTAAC		

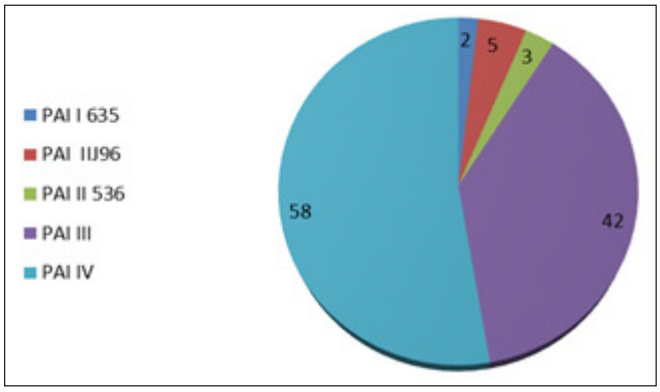

Fig. 3 : Distribution of PAI in 60 isolate.

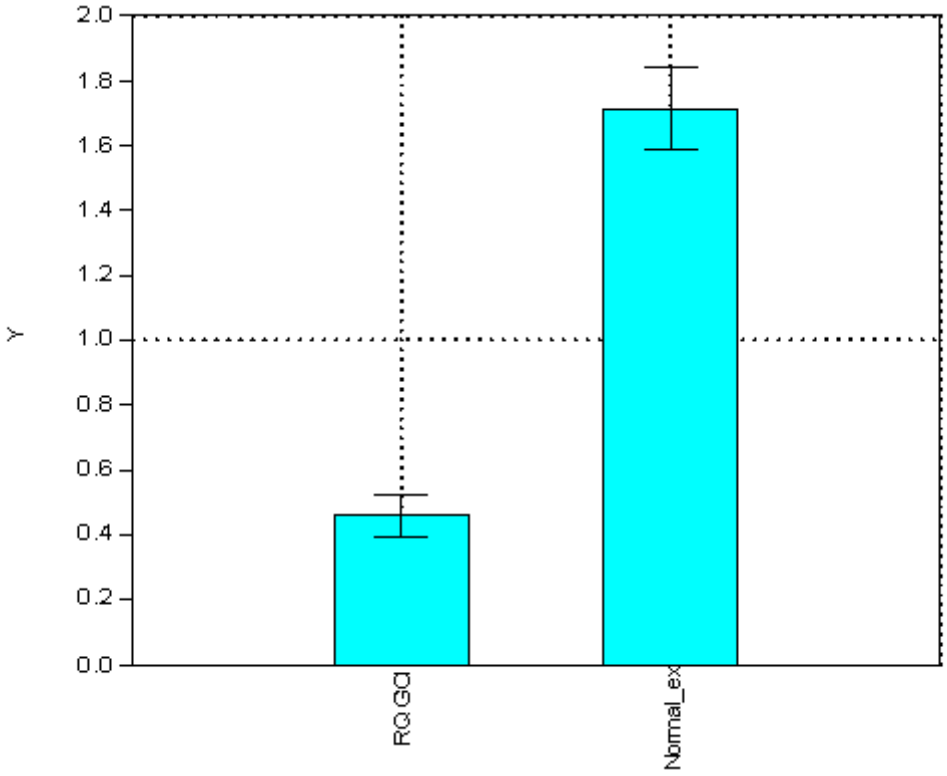

Fig. 4 : Cas 1 relative quantification in normal and MDR UPEC.

Table 2 : PCR program.

Step	Temperature	Time	Cycle
Pre-denaturation	95 ºC	10 minuets	1 cycle
Denaturation	95 ºC	5-30 second	40 cycle
Annealing	54ºC	30 Second	
Extension			
Hold	25 ºC	1minuets	1 cycle

RESULTS

This study adopted VITEK 2, which is highly precise in determination of automated and use micro liter quantities of antibiotics and employs repetitive turbid metric monitoring of bacterial growth during an abbreviated incubation period. Ampicillin resistance was most prevalent 83.3% (41/60). The prevalence of resistance to ceftriaxone, which is one of thirdgeneration cephalosporin, was 71.7% for, 60%(40/60), but on contrary cefoxitine are very effective since it shows sensitivity that reach 80%, even though it's one of the first generation cephalosporine. Another cefoxitine relative, cefazolin shows moderate resistant since 60% of UPEC isolates. Fluoroquinolones tested in this study shows same resistance frequency 30% which is shows good affectivity (18/60). Some carbapenems antibiotics involved in this study, Ertapenem and Imipenem shows outstanding results 93.3% and 95% sensitivity,

Table 3 : Multiple antibiotic resistance phenotypes of 60 UPEC isolates.

No. of antibiotic	No. of isolates (%)	Resistance pattern
0	8 (13.3)	Non MDR
1	4 (6.7)	Non MDR
2	4 (6.7)	Non MDR
3	2 (3.3)	MDR
4	0 (0)	MDR
5	9 (15)	MDR
6	12 (20)	MDR
7	7 (11.7)	MDR
8	3 (5)	MDR
9	5 (8.3)	MDR
10	3 (5)	MDR
11	1 (1.7)	MDR
12	2 (3.3)	MDR

Table 4 : Antibiotic susceptibility of integron 1 positive and integron 1negativee MDR-UPEC isolates.

Antibiotic	Integron +		Integron -		P value
	Resistant	Sensitive	Resistant	Sensitive	
Ampicillin	48	3	3	6	0.0001*
Piperacillin/Tazobactam	9	39	0	12	0.1823
Cefazolin	32	16	1	11	0.0006*
Cefoxitin	13	35	1	11	0.2617
Ceftazidime	37	9	3	11	0.0001*
Ceftriaxone	40	8	1	11	0.0004*
Cefepime	40	8	1	11	
Ertapenem	4	44	0	12	0.5744
Imipenem	4	45	0	11	1.000
Amikacin	11	37	1	11	0.4278
Gentamicin	11	37	1	11	
Ciprofloxacin	19	28	3	10	0.3377
Levofloxacin	17	31	1	11	0.1464
Tigecycline	12	36	1	11	0.4263
Nitrofurantoin	13	35	0	12	0.0526
Trimrthoprim/sulpho	31	17	2	10	0.0038*

* significant association.

respectively. Nitrofurantoin antibiotics shows good. Ceftazidem resistant isolate shows frequency of 66.7% (40/60). UPEC isolates shows moderate resistance to Trimethoprim/sulfomethoxazole combination 55% (33/60). Tigecycline displays wonderful activity since it is diminishes all UPEC under test. Results summarized in Fig. 1.

According to antibiotic sensitivity scan, the studied UPEC isolates were categorized into three groups depending on resistance pattern, results as following: 8 (13.3%) isolates were susceptible to all of the tested antibiotics; 4 (6.7%) isolates expressed resistant against one and two antibiotics of the tested classes of antibiotics; and 44 (75%) isolates were resistant to three antibiotic categories and were considered as MDR (Table 3).

Detection of Integrons among UPEC

This work demonstrate that 48(n_{60}) UPEC isolates were positive for integron (Int I), which represents 80% form all isolates, also there are 40 MDR-UPEC positive *intI* genes isolates, which accounts 90.9% of MDR-UPEC isolates. From the *IntI* positive mentioned above, 35(87.5%) of MDR-UPEC isolates were positive for type 1 integron signified by *intI1*, whereas, a little lower results obtained with integrin II represented by *intI2*, 30 (75%) isolates (Fig. 2).

The level of gene expression of integron has been determined to check the efficiency of integrons in establishing antibiotic resistance status. Results indicate that gene expression was up to 5 folds (which maximum level in all scanned isolates) in compare with normal level expression in corresponding sensitive isolates. Relative gene expression was normalized with GAPDH housekeeping gene. Results are shown in Table 4.

Distributions of PAI in "MDR-UPEC isolate"

Generally all 60 isolates used in this study demonstrated the 51.3 (85%) of isolates have PAI markers. The most prevalent PAI among MDR-UPEC was PAI IV 58(96.6%), followed by PAI III 42 (70%), PAI II563 3(5%), PAI IIJ96 5(8.3%) and PAII6352 (3.3%). Pathogenicity islands (PAIs) are distinct genetic elements on the chromosomes of a large number of bacterial pathogens. PAIs encode various virulence factors and are normally absent from non-pathogenic strains of the same or closely related species. PAIs are considered to be a subclass of genomic islands that are acquired by horizontal gene transfer via transduction, conjugation and transformation, and provide 'quantum leaps' in microbial evolution (Fig. 3).

CRISPRs genes

Clustered, regularly interspaced, short palindromic repeats (CRISPRs) are involved defense against foreign DNA in bacterial species. They have also been associated with a slower spread of antibiotic resistance. Thus, in this work, CRISPRs expression level between susceptible and resistant strains, assuming that the observed resistances were largely due to plasmid-borne determinants (Fig. 4).

CONCLUSION

1. Multiple drug resistance is widely spreads among UPEC.
2. Antibiotics being used incorrectly even by physicians also being distributed among peoples.
3. Integrons I and II are expressed in about 5 folds and its statically correlated with Ampicillin, Cefazolin, Ceftazidime, Ceftriaxone, Cefepime, Trimrthoprim/ sulphoresistance status.
4. Almost all EPEC contains PAI, especially PAI IV which is exist in about 97% of total isolates.
5. From the papC and sfaA and fimH virulence factors; the last is widely spread factor among UPEC isolates.
6. Expression level of CRISPR associated enzyme is lowered significantly in MDR UPEC.

REFERENCES

Brdar I, Rijavec M and Loncaric D (2006) Goal orientations, coping with school failure and school achievement. *European Journal of Psychology of Education* **21**(1), 53-70.

Cambray G, Sanchez-Alberola N, Campoy S, Guerin É, Da Re S, González-Zorn B and Erill I (2011) Prevalence of SOS-mediated control of integron integrase expression as an adaptive trait of chromosomal and mobile integrons. *Mobile DNA* **2**(1), 6.

Carattoli A (2011) Plasmids in Gram negatives: molecular typing of resistance plasmids. *Int. J. Med. Microbiol.* **301**(8), 654-658.

Hayes B J, Bowman P J, Chamberlain A J and Goddard M E (2009) Invited review: Genomic selection in dairy cattle: Progress and challenges. *J. Dairy Sci.* **92**(2), 433-443.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira M A, Bender D and Sham P C (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *The American Journal of Human Genetics* **81**(3), 559-575.

Rubin J E and Pitout J D (2014) Extended-spectrum â-lactamase, carbapenemase and AmpC producing Enterobacteriaceae in companion animals. *Vet. Microbiol.* **170**(1-2), 10-18.

Tong W T, Low W Y, Wong Y L, Choong S P and Jegasothy R (2012) Exploring pregnancy termination experiences and needs among Malaysian women: A qualitative study. *BMC Public Health* **12**(1), 743.