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The Role of Mutation and Gene Expression Level of marRAB operon in Multi Antibiotic Resistance uropathic *Escherichia coli* and 0157:H7 isolates from patients in Baghdad

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Abstract

Multiple antibiotic resistance operon *(marRAB)* is a pattern of chromosomally encoded multiple antibiotic resistance, exerts its effect by encoding repressor called multiple antibiotic resistance R (*marR*) and transcription factor called multiple antibiotic resistance A (*marA*) that modulate efflux pumps and porin expression in the bacterial cell membrane**.** The current study is a prospective and cross-section study included a total of 170 specimens (100 urine specimens and70 stool specimens, collected from admitted and outpatients have signs and symptoms of urinary tract infection and gastro-intestnal tract infection, respectively) in period from December 2018 to April 2019. Molecular detection of *pap EF* was done using conventional PCR**.** Mutations in *marR* were detected using sequencing. Gene expression levels of *marA* , *acrA* and *acrB* was quantified using real-time quantitative polymerase chain reaction. The results shown that (29/100) isolates were identified as UPEC and (19/70) isolates were identified as *E. coli* O157:H7. Results of *marR* mutation detection shown that 3/42 *E.coli* isolates shown nucleotide changes (substitution-transition and substitution- transversion) in *marR* .Association between mutations in *marR* and expression levels of *marA* , *acrA* and *acrB* shown that overexpression of these genes was detected in two isolates have a nucleotide change in *marR*. Also, it was found that a highly statistically significant correlation between the level of gene expression of *acrA* and *acrB* (p-value ≤ 0.01).

In conclusion Tyrosin-Histidin mutation in *marR* may have a role in increased expression of *marA, acrA and acrB*, and may be result in the initiation of multidrug resistance phenotype.

Key words: Antibiotic susceptibility, *E. coli*, *mar R*, Multiple antibiotic resistance operon.

INTRODUCTION

Uropathogenic *Escherichia coli* (UPEC) responsible for urinary tract infection(UTI) and recurrent diseases worldwide, especially in Middle East countries [1,2]. Entero-hemorrhagic *E.coli* serotype O157:H7 considered as the most common causes of acute diarrhea in children under five years in developing countries [3]. Multiple antibiotic resistance operon (mar RAB) is a regulatory locus operating under positive and negative feedback loop in *E.coli*. [4]. The chromosomal *mar* locus of *E.coli* consist from two transcribed operons, marC and marRAB operon. marC operon encodes membrane protein with unknown function in antibiotic resistance phenotypes. marRAB operon encodes genes for three proteins (MarR, MarA, and MarB) and its negatively regulated by MarR [5].

Multiple antibiotic resistance R have a conserved helix turn helix DNA binding style and binds directly to palindromic sequences (TTGCC and GGCAA) on two sites in the marRAB promoter, resulting in repression of marRAB operon [6]. Multiple antibiotic resistance A act as transcriptional activator of many genes on chromosome of *E.coli* and suppressed by MarR. MarA protein related to AraC family of transcriptional activators and it is activates its own transcription and regulon genes of *mar* by binding to mar boxes (twenty base pair asymmetric sequences) with DNA [7,8]. Mar boxes are located upstream of thirty five sites of the promoters of the target genes. From these target genes are *micF*, that down regulates the expression of *ompF* (coded for OmpF, the outré membrane protein). OmpF is the site of a drug entry such as fluoroquinolones (e.g. ciprofolaxin) [9,10]. Another type of MarA target genes are *acrA* ,*acrB* and *Tolc* which encodes for three components of membrane efflux transporter (AcrAB-Tolc) that extrudes the drugs (like ciprofloxacin and tetracycline) out of the cells [11].

Acriflavine protein (AcrAB-TolC) is triple system related to resistance nodulation division (RND) transporters family [11]. It is consist from three structures(*acrA* ,*acrB* and *Tolc*). AcrB, an inner membrane efflux transporter, have a function in removing antibiotics from cytoplasm to the periplasm. The second part from this pupm system is AcrA, the linker protein guides the inner membrane to transport the antibiotic via the third part, outer membrane channel (TolC), to the environment [12,13]. Acriflavine R(AcrR) encoded by *acrR*, act as repressor to acrAB operon, located in the upstream of acrAB operon [14]. AcrR attached to operator site of acrAB operon through its DNA binding helix turn helix style and causes operon repression [15]. AcrAB-TolC efflux system confers resistance to different type of antimicrobial like detergents, dyes, fluoroquinolones and other lipophilic antibiotics such as chloramphenicol, β-lactams, erythromycin and tetracycline [16].

The chromosomal *marR* mutants of *E.coli* express elevated levels of resistance to a wide range of structurally unrelated antibiotics, such as tetracycline and fluoroquinolones,[4] and its inactivation lead to increased level of MarA, up regulation of acrAB-tolc expression and resulting in increased number of activated AcrAB-TolC

efflux pumps that cause drug resistance phenotype [4,17,18].

The current study aimed to evaluate the role of mutation in marRAB-operon in resistance to antibiotics, in order to determine drug of choice for resistant bacteria. Also, it aimed to determine the expression levels of *marA* and *acrAB* to assist their roles in responsiveness to antibiotics.

MATERIALS AND METHODS

Patients and sampling

The current study is a prospective descriptive (cross-sectional) study included a total of 170 specimens collected from outpatients and admitted patients recruited to Al-Imamein Medical City Hospital, Ibn AL-Balady Hospital and the Medical City Hospital, Baghdad, Iraq from December 2018 to April 2019. These specimens were included 100 urine specimens (64 from female and 36 from male) and 70 stool specimens (36 from female and 34 from male). Data collected from included patients involved (name, age, gender, previous antibiotic treatment, previous hospital adhesion).Any patients take antibiotics before one month was excluded. This study was approved by the ethical committee of the College of Medicine, Al-Nahrain University.

Identification of *Escherichia coli*

Urine specimens were inoculated on MacConkey agar using a sterile cotton swab. Stool specimens were inoculated on Hi CHROM EC0157:H7 agar using a sterile loop, then incubated at 37°C for18 hr.

Single colonies selected from pure culture and used to prepare bacterial suspension used in Vitek2 system for identification of bacteria, antibiotic sensitivity test and for bacterial cell pellet used in the molecular study.

Vitek2 system identification

A sterile swab was used to transfer a sufficient number of colonies from pure culture and suspended in 3 mL of sterile saline. The turbidity was adjusted to 0.5 MacFarland turbidity range and measured using visible spectrophotometer DensiChekTM Plus. Bacterial suspension used for the inoculation of identification card of Vitek2 system (bioMérieux/France).

Antibiotic susceptibility

Antibiotic susceptibility test was done using Kirby-Bauer Disk Diffusion method [19]. The diameter of inhibition zone was matured and the results were recorded as sensitive, resist or intermediate according to CLSI 2016 [20].

The isolate was typed as MDR if shown resistance to at least one agent in three or more antimicrobial categories,while considered as XDR if bacterial isolates remain susceptible to only one or two antimicrobial categories [21]. Isolates that show resistance to all agents in all antimicrobial categories considered as PDR.

Identification of uropathic *E.coli* by detection of *pap EF* using Conventional PCR

Extraction of DNA was done using Wizard® Genomic DNA Purification Kit following manufacturer instructions [22].PCR was performed using a specific primer set for the detection of *pap EF* in bacterial extracted DNA [23].

Amplification of *mar R* using Conventional PCR

PCR was performed using a specific primer set for amplification of *mar R* gene [24].PCR Master mix was prepared in a total volume 100µl per reaction, as following: 1X of PCR Buffer (5X), 200 µM of dNTP mixture, 50 pMol of each forward and reverse primers, 3.5U of Taq DNA polymerase(5U/µl).DNA template (2µl of 50ng) was added to the reaction tube and no template control(NTC) reaction tube was prepared contain all PCR master mix components but ddH2O(2 μl) was added instead of DNA. PCR reaction tubes were transferred to the thermal cycler (Eppendorf,Germany) programmed as following: 94 ºC for 4 minutes as initial denaturation followed by 30 cycle of 94 ºC for1 minute, 55ºC for 1 minutes, 72ºC for 2 minutes and final extension 72ºC for10 minutes. PCR products were electrophoresed in 1.5% agarose gel. The appearance of a band with a molecular size 568 bp referred to the amplification of *marR.*

DNA sequencing of *marR*

The amplicons of *mar R* from 42 selected isolates, 29/ 42(69.0%) UPEC and 13/ 42(30.9%) *E. coli O157:H7*, were sent for Sanger sequencing using automated DNA sequencer ABI 3730XL (Macrogen Corporation, Korea). The results were analyzed using Genious software. Sequences were compared with standard strains(NCBI) that arranged in five groups depending on the similarity between each other using online BLAST software(http: //www.ncbi.nlm.nih.gov/BLAST/). Appendix no.2

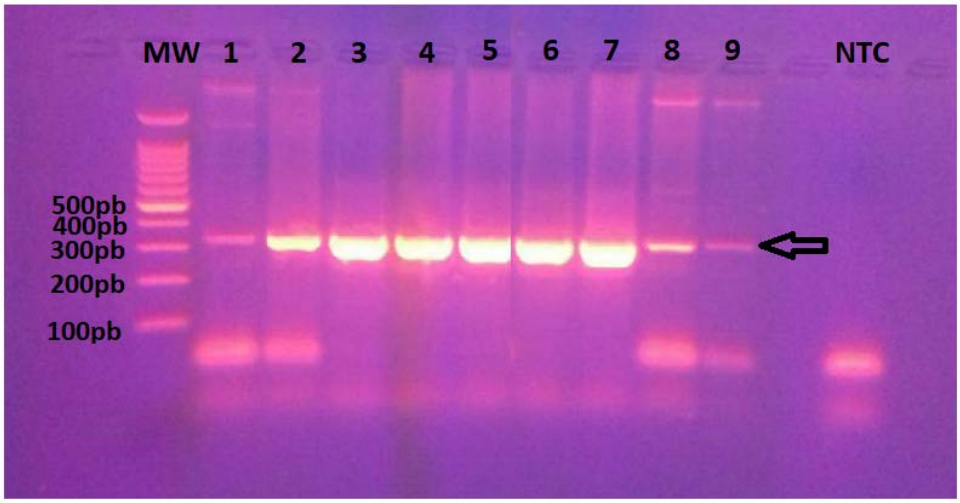
Quantification of gene expression level of *marA, acrA* and *acrB* using RT Q-PCR

Total RNA was extracted from bacterial pellet using SV Total RNA Isolation System following manufacturer instructions (Promega, USA,Cat. no. Z3100) [25].Concentration and purity of extracted DNA, RNA and cDNA were measured using the Nano-drop instrument(Act Gene NAS99).

Expression levels of *mar* A, *acr* A and *acr* B were measured in 23 cDNA selected samples of *E.coli* isolates, 16 / 23 (69.5%) UPEC and 7/ 23(30.4%) *E.coli O157:H7* using GoTaq® 2-Step RT-qPCR System (Promega, USA, Cat.no. A6010) [26]. From these, 14/23 isolates were resistant to tetracycline and ciprofloxacin, and 9/23 isolates were sensitive to tetracycline and ciprofloxacin. Primer sets were used mentioned in Table 1.

Table 1. Primer sets used in RT-QPCR:

Gene	Sequence of forward primer(5´- 3´)	Sequence of reverse primer (5´- 3´)	Product size(bp)
marA	CATAGCATTTTGGACTGGAT	TACTTTCCTTCAGCTTTTGC	187
acrA	CTCTCAGGCAGCTTAGCCCTAA	TGCAGAGGTTCAGTTTTGACTGTT	107
acrB	GGTCGATTCCGTTCTCCGTTA	CTACCTGGAAGTAAACGTCATTGGT	107
rpsL	GCAAAAACGTGGCGTATGTACTC	TTCGAAACCGTTAGTCAGACGAA	104



Figar 1. Agarose gel electrophoresis of PCR amplified products of *papEF* .
Lane 1-9: amplified products of *papEF* (326 bp empty arrow).MW: molecular weight ladder of 100bp. NTC: no template control. Electrophoresis was done in 2% agarose gel at (5V/cm) for 60 min.

Statistical analysis:

Data were analyzed based on SPSS version 23 and Microsoft Office Excel 2010. Categorical variables were expressed as number and percentage. Quantitative variables were first analyzed for normality distribution using the Kolmogorov-Smirnov test, then they were expressed as a median and inter-quartile range because of being non-normally distributed in addition to minimum and maximum values. Mann Whitney U test was used to study difference mean rank of non-normally distributed data between any two groups. The level of significance was set at $P \leq 0.05$ and the level of high significance was considered at $P \leq 0.01$.

RESULTS

Identification of *Escherichia coli* from urine and stool specimens

Identification of bacteria in 100 urine specimens using culturing on MacConkey agar and Vitek2 system were showed the following bacteria: 45(45%) isolates of *E.coli*, 5(5%) isolates of *Pseudomonas aeroginosa* , 3(3%) isolates of *Klebsiella pneumonie* and 47(47%) specimens shown no growth of bacteria.

Culturing of 70 stool specimens on Hi CHROM ECO157:H7 agar was showed growth of the following bacteria: 19 (27.1%) isolates of *E. coli O157:H7* , 20(28.5%) isolates of *Klebsiella pneumonie*, 16(22.8%) isolates of *Pseudomonas aeroginosa* and 15(21.4%) isolates showed mix growth of *Pseudomonas aeroginosa and Klebsiella pneumonie.* Also, specimens positive for *E.coli O157:H7* were tested using Vitek2 system for confirmation identification and the results showed the same sensitivity (100%).

Detection of *pap EF*

Only 29/45 (64.4%) *E. coli* isolates from urine were positive to *pap EF* and considered as UPEC.The result of agarose gel electrophoresis of PCR amplified products of *papEF* shown in fig. 1.

Table 2. Antibiotic Susceptibility for UPEC

Antibiotic	Sensitive		Intermediate		Resistant	
	n	%	*n*	%	*n*	%
IMP	23	79.3	0	0.0	3	10.3
AM	0	0.0	0	0.0	29	100.0
P	0	0.0	0	0.0	29	100.0
CRO	1	3.4	0	0.0	28	96.5
NA	3	10.3	0	0.0	26	89.6
CIP	7	24.1	0	0.0	22	75.8
TMP	9	31.0	0	0.0	20	68.9
AZM	19	65.5	0	0.0	10	34.4
AK	4	13.7	5	17.2	20	68.9
CN	15	51.7	1	3.4	13	44.8
TE	7	24.1	0	0.0	22	75.8
RA	0	0.0	0	0.0	29	100.0

|* (IMP): imipenem, ampicillin (AM), penicillin G (P), ceftriaxone(CRO),naldixic acid (NA) , Ciprofloxacin (CIP) , trimethoprim(TMP),azithromycin (AZM) , amicacin (AK) , gentamicin (CN), tetracycline(TE), rifampin(RA)

Table 3. Antibiotic Susceptibility for *E.coliO157:H7*

Antibiotic	Sensitive		Intermediate		Resistant	
	n	%	*n*	%	*n*	%
IMP	18	94.7	1	5.2	0	0.0
AM	1	5.2	0	0.0	18	94.7
P	0	0.0	0	0.0	19	100.0
CRO	1	5.2	0	0.0	18	94.7
NA	6	31.5	0	0.0	13	68.4
CIP	14	73.6	0	0.0	5	26.3
TMP	3	15.7	0	0.0	16	84.2
AZM	7	36.8	0	0.0	12	63.1
AK	8	42.1	2	10.5	10	52.6
CN	13	68.4	1	5.2	5	26.3
TE	8	42.1	0	0.0	11	57.8
RA	0	0.0	1	5.2	18	94.7

|* (IMP): imipenem, ampicillin (AM), penicillin G (P), ceftriaxone(CRO),naldixic acid (NA) , Ciprofloxacin (CIP) , trimethoprim(TMP),azithromycin (AZM) , amicacin (AK) , gentamicin (CN), tetracycline(TE), rifampin(RA)

Table 4. Type of resistance according to the type of bacteria.

		UPEC *n* = 29		*E.coliO157:H7* *n* = 19		Total
		n	**%**	***N***	**%**	
Type of resistance	**MDR**	5	17.24	4	21.1	9(18.75%)
	XDR	23	79.31	15	78.9	38(97.16%)
	PAN	1	3.4	0	0.0	1(2.0%)

*MDR : multi drug resistant , XDR : extensively drug resistant , PAN **:** pan drug-resistant

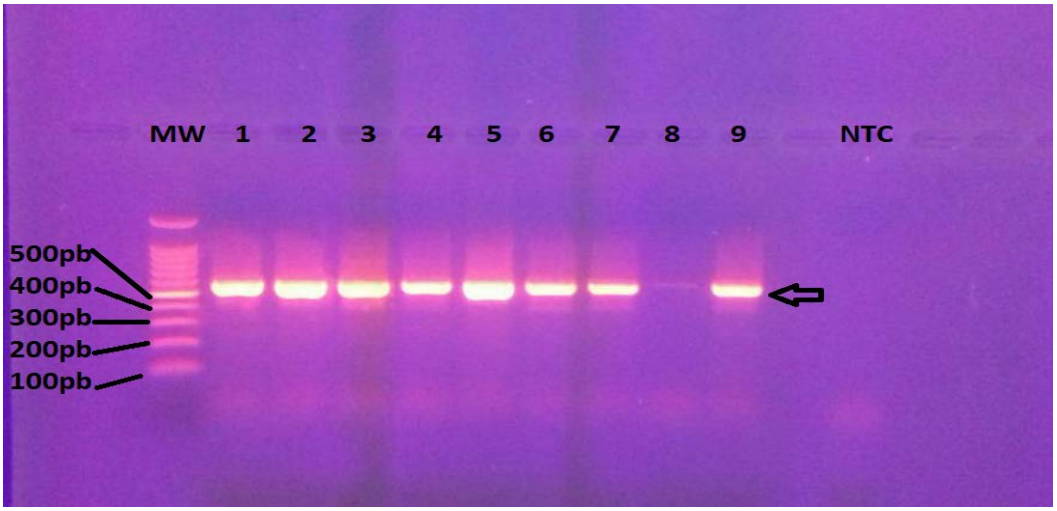


Figure 2. Agarose gel electrophoresis of PCR amplified products of *marR* gene of *E.coli*.
Lane 1-9: amplified products of *marR* gene(568 bp empty arrow).MW: molecular weight ladder of 100bp. NTC: no template control. Electrophoresis was done in 2% agarose gel at (5V/cm) for 60 min.

Antibiotic Susceptibility Test

Antibiotic susceptibility of 29 isolates of UPEC was tested for 12 antibiotics, Table 2. The results showed that all isolates were completely resistant to penicillin G(P), ampicillin (AM) and rifampin(RA) were 29/29(100%) and the lower percentage of resistance was for imipenem 3/29(10.3%) isolates.

Antibiotic susceptibility of 19 isolates of *E.coli O157:H7* was tested for 12 antibiotics, Table 3. The results showed that all isolates were completely resistant to penicillin G(P) and were completely sensitive for imipenem.

The percentage of bacteria that showed XDR was 23/29 (79.31%) isolates of UPEC and 15/19 (87.9%) isolates of *E.coliO157:H7*,Table 4.

Amplification of *marR* :

From 48 *E. coli* isolates, only 42/48 (87.5%) isolates (29 UPEC , 13*E.coliO157:H7*) were positive for *marR*. The result of agarose gel electrophoresis of PCR amplified products of *marR* shown in fig. 2.

Quantification of gene expression level of *mar A, acr A* and *acr B* by Reverse Transcription Real-Time PCR

Twenty-six strains were chosen according to the resistance or sensitivity to antibiotics tetracycline and ciprofloxacin for quantification the level of gene expression of *marA* , *acrA* and *acrB*. Only 23/26(88.46%) strains (16 UPEC, 7 *E.coliO157:H7)*give results for gene expression of *marA* , *acrA* and *acrB* , while 3 strains were showed failure reactions.

The results shown that the maximum increase in gene expression level of *marA* and *acrB* was seen in *E.coli* O157:H7 strains while the maximum increase in gene expression level of *acrA* was in UPEC strains. The correlation between gene expression level and type of specimen was statistically not significant.

Studying the relation between gene expression level of *marA* (fold change) and type of resistance showed that two XDR isolates (NO.21 and NO.34) have a high level of gene expression for *marA*(8.139 fold change) out of the median(0.87),while one XDR isolate (NO 4) have very high gene expression level (12.64 fold change) out of the median (0.87). MDR was only one isolate.

Studying the relation between gene expression level (fold change) of *acrA* and type of resistance showed that two XDR isolates(NO.18 and NO.4) have a high level of gene expression for *acrA*(13.88 and 9.754 fold change, respectively) out of the median(0.71).MDR was only one isolate.

Studying the relation between gene expression level (fold change)of *acrB* and type of resistance showed that three XDR isolates (NO.18,4 and 30) have a high level of gene expression level for *acrB* (11.851, 69.84, 3.936 fold change, respectively) out of the median(0.38). MDR was only one isolate.

Studying the correlation among levels of gene expression of *mar A*, *acrA* and *acrB* showed that no significant correlation between the level of gene expression of *marA* and *acrA*,and *marA* and *acrB*, *p*-value =0.4 and 0.7, respectively,Table 5.

Table 5. Correlation between level of gene expression of *marA, acrA* and *acrB.*

		acrA	*acrB*
marA	*r*	0.238	0.092
	p	0.413	0.753
	n	14	14

* r: Spearman rank coefficient; p: *p*-value; n: number of strains.

A highly statistically significant correlation between level of gene expression of *acrA* and *acrB*, *p*-value ≤ 0.01,Table 6.

Table 6. Correlation between level of gene expression of *acrA* and *acrB.*

		acrB
acrA	r	0.666
	p	0.009*
	n	14

* r: spearman rank coefficient; p: p value ; n: number of strains.

Sequencing analysis of *marR* in association with level of genes expression of *marA, acrA* and *acrB*

Results of the analysis of *marR* sequencing of 42 PCR amplified products related to 42 *E.coli* isolates was shown that there were three clades of isolates according to mutations in *marR* sequences in compere with standard strains and according to compare between studied strains, fig. 3 and table 7. Three strains have shown nucleotide changes (substitution-transition and substitution-transversion) in *marR* consider as unique nucleotide changes. One UPEC strain (NO.30), located in clade 3, two *E.coli* O157:H7 strains isolated from stool, one strain (NO.4) located in clad 3, and NO.26 located in clad 2. Strain (NO.34)UPEC located in clad 2, considered as XDR and strains (NO. 30, 33 and 14) located in clad 3, considered as XDR showed overexpressed in *marA* and strain (NO.18) located in clad 1, considered as XDR showed overexpressed in *acrA* and *acrB*.

Regarding to sequencing of *marR*, fig. (3) shown that isolate NO.2*(E.coliO157:H7)* and isolate NO.18 (UPEC) from the same clad (clad1).

In clad 2, isolate NO.10 (UPEC)and isolate NO.37 *(E.coliO157:H7)* consider as sister taxa . Isolate NO.6(UPEC) and isolate NO.27 *(E.coliO157:H7)* consider as sister taxa and the genetic between them were (0). Isolate NO.15(UPEC) and isolate NO.39 *(E.coliO157:H7)* consider as sister taxa. In clad 3, isolate NO.1 (UPEC) and isolate NO.4 *(E.coliO157:H7)* consider as sister taxa .

DISCUSSION

In this study, 29/45 (64.4%) UPEC isolates were identified as positive to *papEF* using conventional PCR. Iraqi study by (Salih *et al*.,2015) found that 58/112 (51.785%) UPEC isolated from patients with UTI recruited to seven Iraqi hospitals have *pap* virulence gene identified using multiplex PCR [27]. Iranian study by (Ebadi *et al* .,2017) identified *papEF* in 34/107 (31.7%) UPEC isolates using PCR [28]. Another Iraqi study in Wasit by (Mohammed *et al*.,2019) found that 12/16 (75.0%) *E. coli* isolated from UTIs have *pap* gene identified using conventional PCR[29]. Pakistan study by (Ali *et al*.,2019) found that *papEF* was detected in 37/155 (24%) UPEC isolates using PCR [30].

The results of this study showed that the percentage of *UPEC* isolates and *E.coliO157:H7* isolates exhibited XDR pattern was a higher than that exhibited MDR.Iraqi study in Erbil by (Ahmed *et al*., 2019) found that percentage of MDR *E.coli* was 36/74(48.6%)isolates, while XDR was 19/74(25.6%)isolates and PDR was 3/74(4.0%) [31].

In this study,22/29 (75.8%) isolates of UPEC were showed resistance to ciprofloxacin and tetracycline. While in *E.coli O157:H7*, the percentage of resistance to ciprofloxacin was 5/19 (26.3%) isolates and for tetracycline was 11/19(57.8%), UPEC and *E.coli O157:H7* were highly sensitive to Imipenem (IMP).Carbapenem antibiotics are considered to be the most potent group of antimicrobial agents with proven efficacy in the treatment against severe bacterial infections like imipenem that inhibits bacterial cell wall synthesis [32]. Iraqi study in

Erbil by (Mohammad *et al*.,2018) found that 22/25 (88%) isolates were resist to tetracycline and 10/25(40%) isolates were resist to ciprofloxacin using disk diffusion method [33]. Iraqi study in Al-Najaf by (Majeed *et al*.,2019) found that most *E.coli* isolated from urine specimens were highly resistant to amoxicillin 39/49(79.6%) isolates, while imipenem was the best antimicrobial agent against bacterial isolates and showed complete sensitivity,49/49 (100%) isolates. Resistance to ciprofloxacin was 15/49 (30.6%) isolates and tetracycline was 19/49 (38.7%) isolates and the percentage of MDR *E.coli* was 34/49(69.3%) while XDR *E.coli* was 1/49(2.0%) [34]**.**

Iranian study by (Joshua *et al*.,2018) found that 60 *E. coli* strains isolated from urine specimens identified as MDR, were resistant to tetracycline (50%), ciprofloxacin (34%) and to imipenem (0%). Because of the unreasonable use of antibiotics, bacterial resistance has been raised [35].

Regarding antibiotic susceptibility of *E. coli O157:H7,* Iraqi study in Baghdad by (Hassan *et al*.,2015) found that 17/37 (45.9%) *E. coli O157:H7* isolated from human stool samples were resistant to ciprofloxacin and 5/37 (13.5%) isolates were resist to imipenem that detected using disc-diffusion method [36].

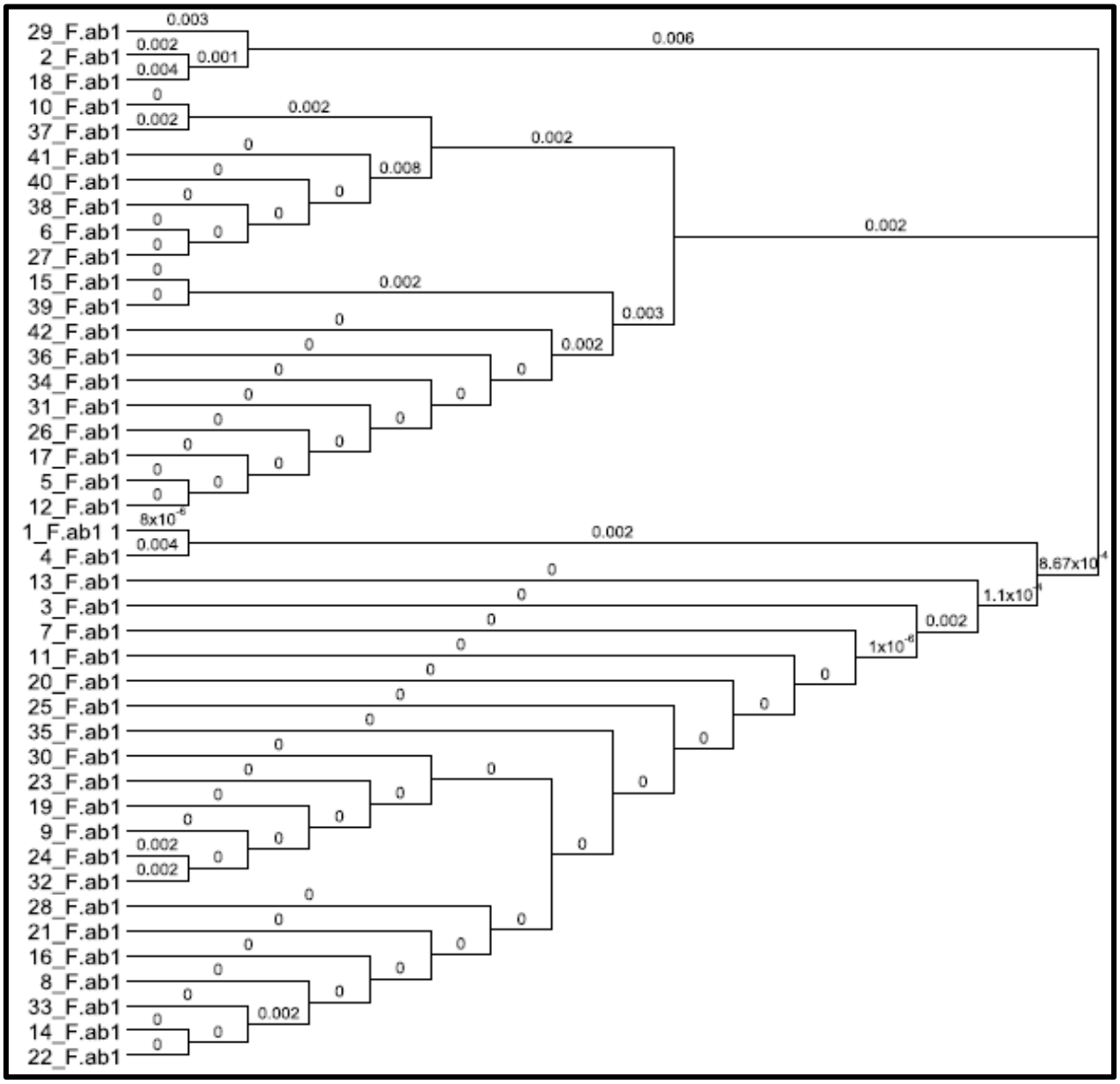


Figure 3. Partial sequencing tree of *marR* in UPEC and *E. coli O157:H7.*The analysis involved 42 isolates sequences and 98 standard strains sequences from the NCBI GenBank database, Appendix NO.2.

Table 7. Correlation between *marR* mutated and un-mutated strains with level of gene expression for *marA*, *acrA* and *acrB*.

	Nucleotide change	Amino acid change	Type of sample	NO. of isolates	Type of resistance	Level of gene expression (fold change)		
						marA	*acrA*	*acrB*
a d	No change	No change	Urine	18	XDR	0.086	13.88	11.851
Clade 2	C–A transversion GA – AG transition G –A transition A –G transition	Alanine – Glutamate Arginine – Lysine Valine – Valine Leucine - Leucine	Stool	26	XDR	0.293	0.044	0.911
	No change	No change	Urine	6	XDR	0.512	0.294	0.296
	No change	No change	Urine	34	XDR	8.139	0.949	0.565
Clade3	No change	No change	Urine	8	XDR	0.037	0.91	0.246
	No change	No change	Urine	11	XDR	0.867	1.859	0.97
	No change	No change	Urine	33	XDR	2.488	0.714	0.227
	No change	No change	Urine	14	XDR	3.084	0.43	0.191
	No change	No change	Urine	35	XDR	0.234	0.396	0.378
	No change	No change	urine	21	XDR	8.139	0.488	0.26
	No change	No change	Urine	23	XDR	0.216	0.219	0.221
	No change	No change	Urine	25	MDR	0.714	1.193	0.7
	G - A transition	Alanine – Alanine	Urine	30	XDR	2.453	3.771	3.936
	G -T transversion T-C transition	Serine - Serine Tyrosin - Histidin	Stool	4	XDR	12.64	9.754	69.84

*Fold change =2^- ΔΔCt

Nigerian study by (Igwe *et al.,* 2016) found that 33/50 (60%) *E. coli O157: H7* isolated from stool samples, 60% of them were resistant to tetracycline, 20% were resistant to ciprofloxacin. The percentage ofMDR was 54.5% and of XDR was 18.2% [37]. Ethiopia study by (Shewa *et al*.,2017) found that 4/34(11.7%) *E. coliO157:H7* isolated from human stool specimens were identified by conventional biochemical methods, 4/4(100%) of isolates were resistant to Ampicillin while all isolates were sensitive to nalidixic acid, Ciprofloxacin and Ceftriaxone that detected using disk diffusion method [38].

Iraqi study in Basrah found that from 36/83(43.3%) *E. coli O157: H7* isolated from children suffering from diarrhea, 27/36 (75%) of isolates were resistance to ciprofloxacin, 9/36 (25%) of isolates were resistant to tetracycline,32/36 (90%)of isolates were resistant to pencilin G and 18/36 (50%) of isolates were resistance to ceftriaxone [39]. South Africa study by (Bolukaoto *et al*.,2019) found that from 12/520 (2.3%)of *E.coli O157:H7* isolated from human stool specimens, 7/12 (58.3%) of isolates were resistant to ampicillin, 3/12(25%) of isolates were resistant to amoxicillin and also for trimethoprim and 2/12(16.6%)of isolates were resistant to Imipenem that detected using disk diffusion method [40].

In the Asia-Pacific region, nearly half of *E. coli* urinary isolates were resistant to ciprofloxacin [41].

The cause of the variation in the percentages of antibiotics resistance and MDR of *E. coli* between different studies may be related to the types of patients involved in each study such as geographical distribution, the severity of infection, age and prior antibiotic use [42]. Also, increased level of resistance to fluroroquinolones, such as ciprofloxacin and other structurally unrelated antibiotics, like tetracycline may be attributed to over activation of multidrug efflux pumps, mainly AcrAB-TolC pump in *E. coli* as a result of overexpression of *marA* and thereby over-activation of *acrAB* and *tolC* genes [14,43].

The result of DNA sequence analysis of *marR* showed that there were three strains have a mutation in *marR*. In this study, isolate NO.26 have a mutation in *marR*(Alanine → Glutamate) that did not result in overexpression of *marA*, *acrA* and *acrB* (0.293, 0.044 and 0.911 fold change, respectively). Germany's study by (Mcmurry *et al*., 1998) found that mutations in *marR***,** Alanine → Glutamate (1602C→A) and Glycine → Serine

(1751G→A), considered as genotypic variations that did not result in loss of repressor activity of *marR* [44]. While Sweden study by (Alzrigat *et al*.,2017) found that in *E.coli* isolated from UTIs, mutations in *marR* Alanine 133 → Glutamate(C→ A) associated with reduced susceptibility to ciprofloxacin and correlates with increased expression of the positive regulator *marA*, and of the components of the AcrAB-TolC efflux pump [45].

In this study, isolate NO.4 have a mutation in *marR* (Tyrosin → Histidine) result in overexpressed of *marA* ,*acrA* and *acrB*. Spanish study by (Sa´enz *et al*., 2004) found that *marR* mutation in 15 ampicillin resistant *E.coli* isolates resulted in amino acid change Glycine 103 → Serine, Tyrosin 137→ Histidine and Leucine 36 → Glutamate that detected using PCR and sequencing, and these mutations contributed to MDR phenotype [46]. While Germany's study by (Mcmurry *et al*., 1998) found that mutations in *marR* Tyrosin 137→ Histidine considered as genotypic variations that did not result in loss of repressor activity of *marR* [44].

Previous studies were referred to other types of mutations in *marR* of *E.coli* result in the development of the MDR phenotype. England study by (Cohen Seth *et al*.,1993) found that *E.coli* strains have mutation in *marR* as (G→A transversion at position 1447) lead to change in amino acid (Methionine →Valine) and (G →T single base change at position 1674) lead to change in amino acid (Arginine → Leucine). These mutations result in transcriptional activation of the marRAB operon and increase the rate of resistance to tetracycline, chloramphenicol, and norfloxacin [47]. A study by (Keeney *et al*., 2008) identified frameshift mutation (insertion of a cytosine at position 355), using sequencing, in *marR* gene in tigecycline resistance *E.coli* isolates whereas tigecycline susceptible strains lacked this type of mutation and this mutation resulted in loss of repressor function of *marR* which result in overexpression of *marA* and *acrAB* [48].

Iranian study by (Jaktaji *et al*., 2013) found that two strains of *E. coli* have resistance to ciprofloxacin and tetracycline, one strain had a mutation in operator site of *marR*AB operon and overexpression of *marA* did not associate with overexpression in *acrA*. A second strain had *marR* mutation did not associate with overexpression in *marA* [49].

In this study, it was found that two *E. coli O157:H7* isolates (isolates NO. 26 and NO.4) have a mutation in *marR* while only one UPEC isolates (isolate NO.30) have a mutation in *marR*. England study by **(**Carone *et al*.,2014) found that 54/63(85.7%)isolates of *E. coli O157:H7* resistant to chloramphenicol, tetracycline and nalidixic acid have transition mutation(AT → GC at position1,944,842) and a transversion mutation (A→ C at position 1,944,707), detected using real-time PCR and sequencing, resulted in amino acid change (Leucine78 → Arginine and Leucine 33 → Proline, respectively) in *marR* which lead to loss of MarR repressor activity and increased *marA* expression level, but chloramphenicol sensitive strains have not like these mutations. Also, they found that *marR* mutations occur at a higher frequency in *E. coli O157:H7* than *E.coli K-12* [50].

In this study, no statically significant association between gene expression level of *marA* and *acrA*(p = 0.4),and between *marA* and *acrB* (p = 0.7). While there was a highly significant association between the level of gene expression of *acrA* and *acrB*, (r= 0.666, p= 0.009). In tetracycline and ciprofloxacin resistance strains, the expression level of *marA* was (2.8 fold change) and the expression of *acrB* (3.21 fold change) was higher than the expression level of *acrA* (1.98 fold change) compared with tetracycline and ciprofloxacin sensitive strains. A study by (Han *et al*.,2012) found that 44/89(49.4%) isolates of *E.coli* with fluoroquinolone-resistant showed overexpressed of *acrAB* [51].

Egyptian study by (Helaly *et al*., 2016) found that 21/40 (52.5%) *E. coli* isolated from urine typed as MDR has expression level of *acrA* (3.9 ± 1.58) fold higher than that of *acrB* (2.6 ± 1.09) fold, by using real-time PCR and there was a moderate correlation between genes expression levels of these genes (r=0.593, p<0.001) [52]. Other Egyptian study by (Abdelhamid *et al*., 2017) found that 22/28 (78.6%) *E.coli* isolates resist to levofloxacin isolated from patients with UTI showed overexpression of *acrA* ranging from (2-9.52) folds while 6/28 (21.4%) isolates of the levofloxacin resistance did not show overexpression of *acrA* identified using reverse transcription real-time-PCR and they concluded that fluoroquinolone resistance may be contributed to other mechanisms such as gyrase or topoisomerase IV mutations [53].

In this study the results of the sequencing of *marR* shown that some strains from UPEC and *E.coliO157:H7* return to the same clade and consider as sister taxa.Iraqi study by (Al-Dawmy *et al*.,2013) found that 11/126(8.7%) *E.coli O157:H7* isolates were identified from stool specimens and 3/98(3.0%) *E.coli O157:H7* isolates were identified from urine specimens using culturing on Sorbitol MacConkey agar plus cefixime potassium tellurite (SMA-CT) and CHROM agar *E .coli O157:H7* and Potassium Cyanide (KCN) Test as specific biochemical test and Latex agglutination test for detection of serotyping *E.coli O157: H7*. Also, they found that the highest rate of infection was in age group from (1month to 5 years) [54].

Iraqi study by (Al-wgaa *et al*.,2017) found that 8/228 (3.50%) *E.coli O157: H7* isolated from urine specimens from patients suffering from UTIs, identified using(blood and MacConkey agar) and selective media (Cefixime Tellurite - Sorbitol MacConkey agar (CT-SMAC) and CHROME agar O157 and biochemical tests like IMViC [55]. China study by (Xie *et al*., 2018) found that 23/285(8.07%) *E.coli O157: H7* isolated from UTI patients[56].

The host's own fecal flora is the primary source of UPEC [57]. Urinary tract infections (UTI) caused by enterohemorrhagic *Escherichia coli* (EHEC) especially in infants and children because most UTIs result from fecal-perineal-urethral retrograde ascent of uropathogens, fecal and perineal flora are important factors in the development of a UTI. In addition, early identification is very essential because if uncorrected, they may serve as a reservoir for bacterial persistence and result in recurrent UTI [54].

CONCLUSIONS

In *E. coli* studed isolates, mutation (Tyrosin>Histidin) in *marR* may have a role in inducing overexpression of *marA*.

Conflict of Interests

The author declare that there is no conflict of interest.

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