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Article

Study Gene Expression of *OXA-48*and *CTX-M-1* Genes Cephalosporin resistance in *Escherichia coli* isolated from urinary tract infections in Baghdad hospitals

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Abstract

Escherichia coli are a normal flora in the human. It is pathogenic in patients with immune system disorders and is the leading cause of enteritis, urinary tract infection, septicemia and other medical infections. E. coli is the most common cause of community and hospital-acquired urinary tract infections (UTIs). It is responsible for >80% of all cases of UTI. These study samples were taken from Al-Karama Teaching Hospital and Al-Yarmouk Teaching Hospital in Baghdad. This study aimed to determine the Minimum Inhibition concentration (MIC) of Ceftazidim. As well as comparing the effect of antibiotics and without antibiotics on beta-lactam genes (OXA-48 –CTXM-1) resistance to Cephalosporin where there is an overexpression of gene expression in the antibiotic between (125-64) µg/ml. Bacteria produce beta-lactam enzymes that break down the beta-lactam ring in the antibiotic, which reduces the effectiveness of antibiotics. This mechanism is one of the mechanisms of resistance in bacteria. The presence of both genes increases the resistance of this species to Cephalosporin. The results of gene expression when treated with antibiotics for the OXA-48 gene in his sample were 1.4, and the highest value was 14.5, as well as for the CTX-M-1 gene, where the results ranged between the lowest value of 1.3 and the highest value of 7.3 when compared to the control samples, we notice an overexpression of gene. It was concluded that the resistance of E. coli to Ceftazidim was related to the genes blaOXA45 and CTXM-1, but the primary role may be due to blaOXA45.

Keywords: Gene Expression, OXA-48 Gene, CTX-M-1 Gene, Cephalosporin, *Escherichia coli.*

Introduction

Widespread diseases caused by multidrug-resistant bacteria accompany global population growth. Urinary tract infection (UTI) is the most common community-acquired and hospital-acquired[1] Bacterial infection typically characterized by a high treatment failure rate and recurrence of infection[2]. They cause urinary tract infections, including Escherichia coli, Staphylococcus saprophyticus, Klebsiella

spp, Proteus spp, and Enterococcus spp[3,4]. B-lactam antibiotics, which constitute 60% of worldwide antibiotic usage, are among the most influential and common ly used agents in Treating infectious diseases[5]. Escherichia coli is employ several mechanisms of resistance against different groups of antibiotics, including target modification, alteration of cell membrane permeability, production of enzymes, alteration of metabolic pathways and efflux pumps[6]. One of the most common mechanisms of resistance is the production of extended-release beta-lactamases (ESBL) and carbapenemase that degrade all penicillins, cephalosporins, ximino, cephalosporins, and monomers[7]. Carbapenemase genes, mediated by mobile genetic elements carrying additional resistance elements, confer resistance to various groups of antibiotics, resulting in multidrug resistance (MDR)[8].OXA-48, a class D carbapenemase, is of significant concern owing to its difficulty in detection and its association with treatment failure. Moreover, OXA-48-like enzyme variants are plasmid-coded and associated with rapid dissemination in community settings[9]. The CTX-M-type β-lactamases represent a group with a typical extended-spectrum β-lactamase (ESBL)-resistance phenotype[10]. Transferable plasmids encode these enzymes. The enzyme responsible for this particular ESBL phenotype not affecting ceftazidime was named CTX-M 1 because of its preferential hydrolytic activity against cefotaxime (CTX -M)[11].

Materials and Methods

Collection of samples

One hundred eighty midstream urine samples (MSU) were collected from patients of all ages and sexes with urinary tract infections (UTIs). Samples were collected from Al-Karama Teaching Hospital and Al-Yarmouk Teaching Hospital from the beginning of November 2021 until the end of February 2022. From 180 urine samples, preliminary results showed growth in 112 (62.3%) specimens and 68 samples (37.7%).

Quantitative MIC

1. Using the micropipette, dispense 100µl of the medium into all microtiter plate wells.

2. Pipette 100 µl of appropriate antibiotic solutions into the wells in column A (far left of the plate).

3. Using the micropipette set at 100 µl, mix the antibiotics into the wells in column A by sucking up and down 6-8 times. Do not splash.

4. Withdraw 100 µl from column A and add this to column B. This makes column B a tenfold dilution of column A, transfer 100 µl to column C, and repeat the procedure to column H only. The same set of tips can be used for the entire dilution series.

5. Discard 100 µl from column H.

6. With the similar micropipette set to 100 µl, dispense bacteria into all wells.

7. Incubate the plates at 37C.

8. When satisfactory growth is obtained (24 hours).

9. After incubation, 20 µl of resazurin dye was added to all the wells and incubated for 30 minutes to observe any color changes. The Minimum Inhibitory Concentrations were determined visually in broth micro dilutions as the lowest concentrations of the extracts at which no color changed from blue to pink in the resazurin broth assay[12,13].

Quantitative Real Time-PCR

RNA extraction

RNA was extracted from the isolates before and after treatment with the antibiotic. Total RNA was extracted using TRIzol™Reagen.

Preparation of primers

Specific primers were obtained (Table 1) according to the previous studies to detect the gene expression.

Primer	Sequence 5-3	Product size	Reference
16S rRNA	**F-**CCATACGCTTTGCTGTTCGG	122	**(Mezaal et al., 2007)**
	R-ATCTCCACGCCGTTGATGTT		
OXA-48	**F**-TTGGTGGCATCGATTATCGG	136	**(Fils et al.,2019)**
	R- GAGCACTTCTTTTGTGATGGC		
CTX-M-1	**F-**CAGCGCTTTTGCCGTCTAAG	744	**(Fils et al.,2019)**
	R- TTTGCGATGTGCAGTCCAGTAAG		

Table 1: Primer sequences used qRT-PCR in this study.

Quantitative Real-time PCR Assay (QRT- PCR)

By using the Qubit® 1-Step RT-qPCR System (Qubit®- USA), the Amplification of a fragment of mRNA was performed with the following master amplification reaction with the Qubit of two ----Step RT-PCR list in Table (2) and the (Qubit®- USA) in Table (3). Several experiments were done for more appropriate synthesis of cDNA and annealing temperature.

Component	20 ul Reaction
Luna Universal qPCR Master Mix	10 ul
Forward primer (10 µM)	1 ul
Reverse primer (10 µM)	1 ul
Template DNA	5 ul
Nuclease-free Water	3 ul
Total	20

Table 2: Quantitative RT-PCR Reaction Mix.

Cycle Step	Stage	Temperature	Time
1	Initial Denaturation	95 ºC	60 seconds
40-45	Denaturation Extension	95ºC 60 ºC	15 seconds 30seconds (+plate read)
1	Melt Curve	60-95 ºC	40 minutes

Table 3: Quantitative RT-PCR Reaction Mix.

Delta delta Ct method (14)

Delta Ct (ΔΔCt) method is the simplest one, as it is a direct Ct values comparison between the target gene and the reference gene relative quantification involving the choice of a calibrator sample. The calibrator sample can be the untreated sample, with an optimum temperature of 37 ºC, or any sample that wants to compare the unknown samples. Firstly, the ΔCt between the target gene and the reference gene is calculated for each sample (for the unknown and calibrator samples), as shown in the following equation.

$$\Delta Ct = Ct\ target\ gene - Ct\ reference\ gene$$

Then, the difference between the ΔCt of the unknown and the ΔCt of the calibrator is calculated, giving the ΔΔCt value.

$$\Delta\Delta Ct = (Ct\ target - Ct\ reference)\ sample - (Ct\ target - Ct\ reference)\ control$$

The normalized target amount in the sample is then equal to $2^{-\Delta\Delta Ct}$, and this value can be used to compare expression levels in samples[14]. The relative changes in mRNA expression levels were determined using a comparative threshold cycle (C.T.) method ($2^{-\Delta\Delta Ct}$). The result was collected and analyzed by the Livak formula.

Results

Results of quantitative Real-time PCR

Real-time PCR quantification in the present experiment utilized the SYBR green, a fluorescent dye that recognizes and intercalates every double-stranded DNA, including cDNA. The Amplification was recorded as a Ct value (cycle threshold).

Real time PCR Quantification of 16S rRNA Expression

The Ct value of 16S rRNA, the housekeeping gene used in the present study, is shown in Table (4). The range of Ct value for 16S rRNA in the control group was (11.08-16.51) with a mean ±SD (14.10 ±1.99). The resistant isolates before treatment with the antibiotic group ranged from (12.06-15.14) with a mean ± SD (13.4 ±1.07), and in resistant isolates after treatment.

Sample	CT *16S RNA*	CT *OXA48*	ΔCT	ΔΔCT	Fold
1 B.T.	11.08	11.6	0.52	0	1
2 B.T.	13.98	37.75	23.77	0	1
3 B.T.	16.51	38.14	21.63	0	1
4 B.T.	15.88	42.48	26.6	0	1
5 B.T.	14.36	34.89	20.53	0	1
6 B.T.	12.82	34.91	22.09	0	1
1 A.T. (A.b)	12.06	37.94	25.88	25.36	2.32209
2A.T (A.b)	13.46	32.95	19.49	69.14	1.5374
3 A.T. (A.b)	13.54	34.63	21.09	-0.54	1.453972517
4 A.T. (A.b)	12.21	34.71	22.5	-4.1	17.1483754
5 A.T. (A.b)	14.21	33.36	19.15	-1.38	2.602683711
6 A.T. (A.b)	15.14	33.37	18.23	-3.86	14.52030649
LSD (5.13) P-value 0.05					

Table 4: Gene expression of OXA-48 comparison between antibiotic and control.

Real time PCR quantification of OXA-48 Expression

The range of Ct value for OXA-48 in isolates before treatment with group was (11.6- 42.48) with a means Ct value (33.2 ±10.9). The range of Ct value for oxo-48 in isolates after treatment with the antibiotic group was (32.95-34.71) with mean ±SD—ct values (34.4±1.83). There was a significant difference in the mean Ct values between the different study groups, as shown in Table (4). The mean Ct values in isolates after treatment with antibiotics were higher than the mean Ct value of isolates those in isolates, which reflects that the genes are present in mRNA samples.

Real-time PCR quantification of CTX-M-1 Expression

The range of Ct value for ctx-m-1 in resistant isolates before treatment with the group was (10.78-17.89) with a mean ± S.D. value (15.0 ± 2.5). The range of Ct value for CTX-M-1 in isolates after treatment with the antibiotic group was (13.8-41.61) with mean ± S.D. Ct values (22.2 ±12.0).

The mean Ct values in isolates after antibiotic treatment were higher than those of isolates before and after treatment antibiotics. This, in turn, was slightly higher than those in isolates, which reflects that the genes are present in mRNA samples. It is evident from these results that the antibiotic group is associated with the highest copy number of target gene on mRNAs, reflecting its higher expression.

Sample	*CT 16S RNA*	*CT CTXM1*	ΔCT	ΔΔCT	Fold
1 B.T.	11.08	10.78	-0.3	0	1
2 B.T.	13.98	14.84	0.86	0	1
3 B.T.	16.51	16.27	-0.24	0	1

4 B.T.	15.88	17.89	2.01	0	1
5 B.T.	14.36	16.75	2.39	0	1
6 B.T.	12.82	13.88	1.06	0	1
1 A.T. (A.b)	14.74	41.67	26.93	27.23	6.35
2 A.T. (A.b)	13.02	32.95	19.93	19.07	1.82
3 A.T. (A.b)	13.46	13.88	0.42	33.72	7.07
4 A.T. (A.b)	12.21	13.8	1.59	-0.42	1.337928
5 A.T. (A.b)	17.21	16.72	-0.49	-2.88	7.361501
6 A.T. (A.b)	15.14	14.69	-0.45	-1.51	2.8481
LSD (2.77) P-Value 0.001					

Table 5: Gene expression comparison between antibiotic and control of the CTX-M-1 gene.

Fold gene expiation OXA-48 and CTX-M-1

The results of the research showed gene OXA-48 in figure (1) that the bacteria in the samples resulted in untreated mean ±SD (1± 0) with an antibiotic from the cephalosporin group were mean ± SD (6.5 ±7.2). The sub-MIC antibiotic for samples (1, 2, 4 and 5) at 125µg/ ml and for samples (3 and 7) at a 64 µg/ml concentration.

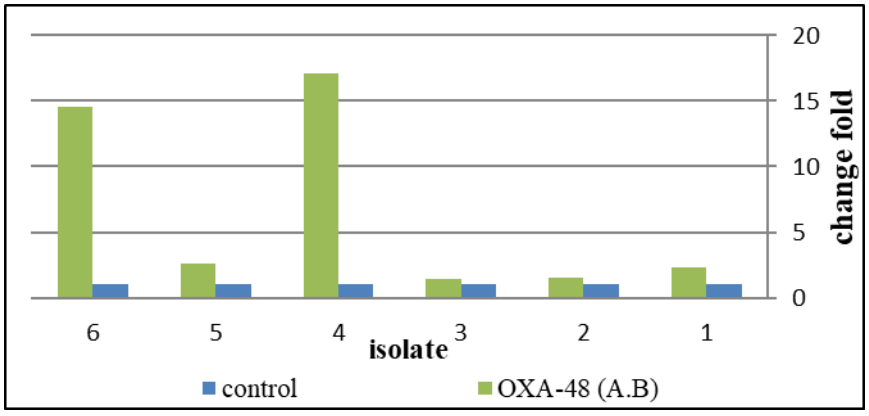

Figure 1: Fold of gene expression of OXA-48 gene.

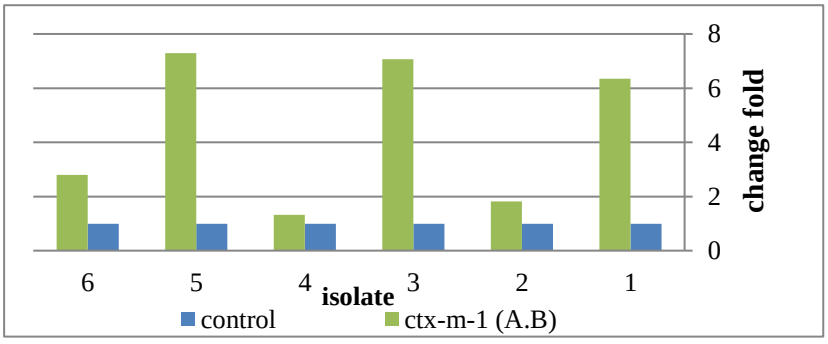

Figure 2: Fold of gene expression of CTX-M-1 gene.

Discussion

The lower Ct value indicates the presence of higher copies of the target and vice versa. Regarding gene expression, high Ct values indicate low gene expression,

and low Ct values indicate high gene expression14,15. The primary purpose of this step was to quantify gene expression of the CTX-M-1 and OXA-48 genes and compare the quantity of gene expression in the presence of the antibiotic in its absence to identify the role of these genes in the resistance of E.coli. The 16SrRNA gene, as the housekeeping gene, was used in the present experiment because its expression remains constant in the investigated cells under different conditions16. The present experiment of quantitative PCR was conducted by us-ing 6 cephalosporin-resistant isolates of E.coli that have CTX-M-1 and OXA-48 genes together. In the present experiment, quantitative R.T.- PCR assay analyzed the mRNA expression of CTX-M-1 and OXA-48 genes by comparing the un-treated and treated groups of samples of resistant bacteria grown with antibiotics. The Ct values of Amplification of the genes were recorded from quantitative R.T.- PCR software. The fold change of gene expression was calculated using relative quantification from the delta Ct value (details shown in materials and methods). In CTX-M-1 and OXA-48 genes, when the MIC antibiotic mean value was lower (32 µg/ml) in isolates after antibiotic treatment, the mean gene ex-pression fold was slightly higher from1.

It is evident from these results that the antibiotic group is associated with the highest copy number of target gene on mRNAs, reflecting its higher expression and least copy number of target gene carried on mRNA in isolates17.

In isolates, which reflects that the genes are present in mRNAs samples. It is ev-ident from these results that the antibiotic group is associated with the highest copy number of target gene on mRNAs, reflecting its higher expression, and the least copy number of target gene carried on mRNA in isolates. There was a sig-nificant difference in the mean Ct values between the different study groups, as shown in Table 5.

His study findings are consistent with those of Denmark, provided an expe-ri-ment within E.coli isolates under cefotaxime pressure and measured the ex-pres-sion of genes CTX-M-1, revealing increased expression in level from con-centra-tion cefotaxime18.

The results of the research showed that the bacteria in the samples were un-treat-ed mean ±SD (1±0) with antibiotics from the cephalosporin groups were mean ±SD (4.4 ± 2.7), the results were sub-MIC antibiotics for samples (1, 2, 4 and 5) at a concentration of 125µg/ ml, and for samples (3 and 7) at a concen-tra-tion of 64 µg/ml in the figure (2) shows fold of gene expression of CTX-M-1 gene where this study proved that by using gene expression using real-time PCR, there was an overexpression of gene by comparing it with isolates that were not treated with antibiotics19. 20 documented that the fold of gene expression of OXA-48 gene bla(CTX-M-1)-group β-lactamase genes of Cephalosporin's re-sistance because of β-lactamase genes in Different sequence variants were de-tected in some isolates of E. coli.

Conclusion

It was concluded that Sub MIC antibiotic for samples (1, 2, 4 and 5) at 125µg/ ml and for samples (3 and 7) at a concentration of 64 µg/ml. The percentage of the presence of a gene OXA-48 (23%) while was CTX-M-1 (100%). Gene ex-pression was measured in real-time Time-PCR (qPCR), and it was found that the genes OXA-48 and CTX-M-1 over gene expression after treat with antibiot-ics.

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