Evaluation of Biocides Susceptibility and Expression of Qace-Δ1 And Suge1 Genes of Pseudomonas Aeruginosa in Exposure to Biocides and Meropenem

Narjis Hadi shghaitand[1], Kais Kassim Ghaima[2]

[1,2]*Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq. Email: Kaisskasim22@ige.uobaghdad.edu.iq*

KEYWORDS

Biocides, Gene expression, Pseudomonas aeruginosa, qacE -Δ1 and sugE1.

ABSTRACT

Pseudomonas aeruginosa is a species of medical significance that plays a role in nosocomial infections, particularly those resulting from burns and wounds. Bacterial antibiotic resistance may have developed as a result of the abuse of biocides in hospitals. This study aimed to determine the minimum inhibitory concentrations (MICs) of carbapenem-resistant P. aeruginosa isolates in relation to ethylenediaminetetraacetic acid (EDTA), chlorohexidine (CHX), and resazurin dye using a microtiter plate assay. Additionally, it evaluated the expression of the efflux pump genes (qacE-Δ1 and sugE1) at sub-inhibitory concentrations of the biocides using RT-qPCR (delta delta Ct method) to improve their efflux pump system. The MICs of 24 P. aeruginosa isolates (14 isolates were resist to carbapenems and 10 isolates sensitive to carbapenems) against Chloroxylenol (Dettol) revealed that the range of MICs were (0. 125-2%). The results of MICs of Chlorohexidine (CHX) revealed that the range of inhibitory activity was (0.8-102.4 µg/ml), also the findings of MICs of EDTA demonstrated that the range of inhibitory activity was (125-2000 µg/ml). The genes involved in efflux pump (qacE -Δ1 and sugE1) were found to be significantly up-regulated in the local isolates, according to our findings.. The four isolates' sugE1 gene expression folds ranged from 1.81 to 5.39; this result was more than the fold of the control (the fold change is 1), with the meropenem isolate having the highest fold value (5.39) and sub-MIC value (8 µg/ml). Furthermore, the four isolates' qacE-Δ1 gene expression folds ranged from 2.11 to 5.01, which was higher than the fold of the control, where the highest fold value was recorded for the EDTA (5.01) with the sub-MIC value (125-500 µg/ml). Also, a high value of upregulation of the gene qacE –Δ1, during the treatment with dettol with sub-MIC (0.25-0.5%), and the fold change was 4.19. In conclusion, the present findings indicated to the significant relationship between the treatments with meropenem and the high values of gene expression of the gene sugE1 in comparison with the control and other agents, Furthermore, compared to the antibiotic and control, there was a strong correlation found between the high fold changes of the gene qacE -Δ1 with the presence of dettol and EDTA.

1. Introduction

Numerous reports of hospital outbreaks related with *Pseudomonas aeruginosa*, especially the burn infections. Moreover, there has been a notable rise in the prevalence of *P. aeruginosa* strains that are resistant to drugs (MDR). These multidrug resistant isolates are ubiquitous in the hospital setting. Hospital-acquired infections (HAIs) must therefore be prevented and controlled, and this is why hospital disinfection policies are so important (Bhardwaj *et al*., 2021). Chemicals known as biocides include disinfectants and antiseptics. Hospitals frequently use these substances to manage, get rid of, or lessen environmental bioburden. Particularly important in reducing these infections and stopping the spread of infectious pathogens in hospitals are disinfectants (Jones and Joshi, 2021). The decreased susceptibility to biocides could result from genetic elements acquiring or from chromosomal gene mutations. It could also be an inherited trait. The efflux pumps (qacEΔ1, and qacE) are examples of biocide resistance genes that are important in this process. Transposons or plasmids are two possible carriers of these genes. Reduced bacterial susceptibility to biocides has become a significant cause for worry. This phenomenon can lead to the spread of pathogens resistant to biocide and deteriorate hospital environmental surfaces that need to be disinfected (Verdial *et al*, 2023). Many local studies investigated the role of efflux pumps of *P. aeruginosa* in the resistance of antibiotics and other antimicrobial agents (Friyah. and Rasheed, 2018; AL-Ameen and Ghareeb, 2022).Numerous small multidrug resistance (SMR) proteins have been detected in bacterial pathogens, and resistance to clinically prescribed antibiotics like aminoglycosides, β-lactams, inhibitors of dihydrofolate, and different antiseptics has been discovered (Gaurav *et al*., 2023). Antibiotic-inactivating enzyme production and decreased outer membrane permeability caused by overexpression of efflux pumps are the primary causes of *P. aeruginosa's* intrinsic resistance. The primary efflux pumps of *P. aeruginosa* that contribute to resistance to numerous antibiotics and biocides are those of the SMR family (Lorusso *et al*., 2022).

The current study aimed to determine the gene expression levels of the efflux pump genes (*qacEΔ1*

and *sugE1*) in P. aeruginosa isolates as well as their patterns of sensitivity to biocides. The results of this study will undoubtedly aid in the management of these bacteria in hospitals and the prevention of nosocomial infections, as will our understanding of P. aeruginosa's susceptibility to biocides and how it is connected to efflux pump genes.

2. Materials And Methods

P. aeruginosa's Minimum Inhibitory Concentrations (MICs) against antiseptics Dettol (chloroxylenol)

The Clinical and Laboratory Standards Institute's microdilution method (Microtiter Plate Assay with Resazurin Dye) was used to determine MIC (CLSI, 2021). Chloroxylenol (Dettol) is a disinfectant and bacteriocide by distructing the cell membrane of bacteria used to test the susceptibility of pseudomonas aeruginosa against it . A concentration of Chloroxylenol (Dettol) was used is 4%. The P. aeruginosa cultures in Brain Heart Agar was used to create the bacterial inoculum. It was cultured for 18–24 hours at 37 °C before the test. To get a turbidity of 0.5 on the McFarland scale, the bacterial sample was diluted to 1x108 CFU/mL. The material was then diluted in MHB 1:2 till 5x105 CFU/mL was the final in order to reach the McFarland scale's turbidity of 0.5. The material was then diluted in MHB 1:2 till 5x105 CFU/mL was the final concentration. The diluted bacterial suspension was placed in a 96-well plate, and 4% Chloroxylenol (Dettol) was added. A final amount of 200 µL each well was composed of 100 µL of diluted bacteria solution and 100 µL of chemical. P. aeruginosa with MHB was added to one line of the wells for the negative growth control, while only MHB was added to the other line for the positive growth control. Resazurin (0.015%) was added to each well (20 µl per well) and incubated for 20 minutes at 37 °C after the initial 24-hour incubation period to examine color change. After incubation, columns that showed no color change (blue resazurin color stayed constant) were rated as being higher than the MIC value. At the conclusion of the incubation period, the lowest chemical concentration at which no bacterial growth was seen was identified as the MIC.

Chlorohexidine (CHX)

Using a 96-well microtiter plate, to calculate the (MIC) of the chlorohexidine , Mostly used for mouthwash, hand hygiene, alcohol-based antiseptics, and hospital surgery, chlorhexidine is a broad-spectrum antiseptic agent that has been used extensively in both human and veterinary healthcare for many years (Kampf, 2016). Its antibacterial method of action is characterized by the rupture of bacterial membranes, which allows cytoplasmic components to flow out (Gilbert and Moore, 2005). The P. aeruginosa cultures in Brain Heart Agar was used to create the bacterial inoculum. It was cultured for 18–24 hours at 37 °C before the test. To get a turbidity of 0.5 on the McFarland scale, the bacterial sample was diluted to 1x108 CFU/mL. The material was then diluted in MHB 1:2 till 5x105 CFU/mL was the final concentration. The Chlorohexidine 1% were added to the bacterial sample that has been diluted in a 96-well plate. A final amount of 200 µL each well was composed of 100 µL of diluted bacteria solution and 100 µL of chemical. Only MHB was added to the growth controls, both positive and negative to one line of the wells and P. aeruginosa with MHB to the other line, respectively. After 24 hours resazurin (0.015%) was applied to each well (20 µl per well) at 37 °C, and the color change was observed after 20 minutes of incubation. After incubation, columns that showed no color change (blue resazurin color stayed constant) were rated as being higher than the MIC value. At the conclusion of the incubation period, the lowest chemical concentration at which no bacterial growth was seen was identified as the MIC.

Ethylenediaminetetraacetic acid (EDTA)

The 96-well microtiter plate was utilized to calculate the (MIC) of the EDTA. A metal chelator called EDTA can alter the permeability of Gram-negative bacteria's outer membrane by disrupting its lipopolysaccharide structure. This makes the membrane more permeable and increases the biocidal activity of antimicrobial drugs.To preparation the EDTA before the MIC , was dissolved 0.4mg in 10ml of distilled water to become EDTA 4% . The P. aeruginosa cultures in Brain Heart Agar was

used to create the bacterial inoculum. It was cultured for 18–24 hours at 37 °C before the test. To get a turbidity of 0.5 on the McFarland scale, the bacterial sample was diluted to 1x108 CFU/mL. The material was then diluted in MHB 1:2 till 5x105 CFU/mL was the final concentration. The 96-well plate with the diluted bacterial sample was filled with 4% EDTA. A final amount of 200 µL each well was composed of 100 µL of diluted bacteria solution and 100 µL of chemical. *P. aeruginosa* and MHB were added to one line of the wells for the negative growth control, while only MHB was added to the other line for the positive growth control. Resazurin (0.015%) was added to each well (20 µl per well) and incubated for 20 minutes at 37 °C after the initial 24-hour incubation period to examine color change. After incubation, columns that showed no color change (blue resazurin color stayed constant) were rated as being higher than the MIC value. At the conclusion of the incubation period, the lowest chemical concentration at which no bacterial growth was seen was identified as the MIC.

MOLECULAR ASSAY

Gene expression Analysis Using qRT PCR Technique

The evaluation of the gene expression of the two genes was done before and after the treatment with the antimicrobial agents to determine the impact of the Dettol, EDTA and Meropenem in combination with bacteria on the gene expression of *qacE-Δ1* and *sugE1* linked to efflux pump system. The Dettol and EDTA were employed at sub MIC doses to promote bacterial growth.

Table (1): Primers used in this Study

Target gene	Nucleotide sequence (5'—3')	Length	Size product	The reference
***qacE* -F**	**TGCGTTCCTGGATCTATCTG**	**20**	**206**	**(Bakht, *et al.*, 2022)**
***qacE*-R**	**GACGATGCCAATGCCTTC**	**18**		
***qacE-Δ1*-F**	**TTGTTATCGCAATAGTTG**	**18**	**202**	
***qacE-Δ1*-R**	**AATGGCTGTAATTATGAC**	**18**		
***sugE1*-F**	**CCGTTGGTCTGAAATACAC**	**19**	**196**	
***sugE1*-R**	**ATGGATTCGCCGAACAGG**	**18**		
***16S rRNA*-F**	**CCGTGTCTCAGTTCCAGT**	**18**	**104**	**(Joshi, *et al.*, 2014)**
***16S rRNA*-R**	**TGAGCCTAGGTCGGATTA**	**18**		

Table (2): Component of qPCR reaction on this study

Master mix components	Stock	Unit	Final	Unit	Volume
					1 sample
qPCR Master Mix	2	X	1	X	5
RT mix	50	x	1	x	0.25
MgCl2					0.25
Forward primer	10	µM	0.5	µM	0.5
Reverse primer	10	µM	0.5	µM	0.5
Nuclease Free Water					2.5

RNA					1
Total volume		ng/µl		ng/µl	10
Aliquot per single rxn	**9 µl of Master mix per tube and add 1µl of Template**				

Table (3): Real Time PCR Program

Steps	**Temperature**	**Time**	**Cycle No.**
RT.Enzyme Activation	**37°C**	**15:00**	**1**
Initial Denaturation	**95 °C**	**05:00**	
Denaturation	**95 °C**	**00:20**	**40**
Annealing	***qacE-Δ1* 51°C**	**00:20**	
	***SugE1* 53 °C**		
	***16sRNA* 55°C**		
Extension	**72 °C**	**00:20**	

After adding the Antimicrobial agents

1- To track the development of bacteria in media, Muller-Hinton broth tubes were produced with the proper amounts of Dettol, EDTA, and meropenem and incubated for 24 hours at 37°C.

2- After growth, RNA extraction was done by the same steps by TRIzol™ Reagent kit.

3- The same primers, RT master mix and programs were used as before add the Dettol , EDTA and meropenem.

3. Results And Discussion

Minimum Inhibitory Concentrations (MICs) of *P. aeruginosa* against antiseptics Chloroxylenol (Dettol)

The minimum inhibitory concentrations of 24 *P. aeruginosa* isolates (14 isolates were resist to carbapenems and 10 isolates sensitive to carbapenems) against Chloroxylenol (Dettol) were evaluated by microtiter plate assay with resazurin dye. The results revealed that the range of minimum inhibitory concentrations were (0. 125-2%) and The isolates' MICs varied from one another (Figure 1 and table 4). Additionally, the sensitive carbapenems' lowest inhibitory concentration findings *P. aeruginosa* demonstrated that the range of inhibitory activity was 0.0156-0.125%, where these concentrations were lower than the concentrations of the carbapenems-resistant isolates.

Table (4). The minimum inhibitory concentrations (MICs) of Chloroxylenol (Dettol) against carbapenems-resistant *pseudomonas aeruginosa* at concentrations (0.0039-2%).

Dettol Conc. (%)	The isolate code														Positive control	Negative control
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14		
2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
1	+	+	–	–	+	–	–	+	+	+	–	+	–	–	+	–
0.5	+	+	–	–	+	–	–	+	+	+	+	+	+	–	+	–
0.25	+	+	–	+	+	+	–	+	+	+	+	+	+	–	+	–
0.125	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–
0.0625	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
0.0312	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
0.0156	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
0.0078	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
0.0039	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–

- =No growth, + =Growth

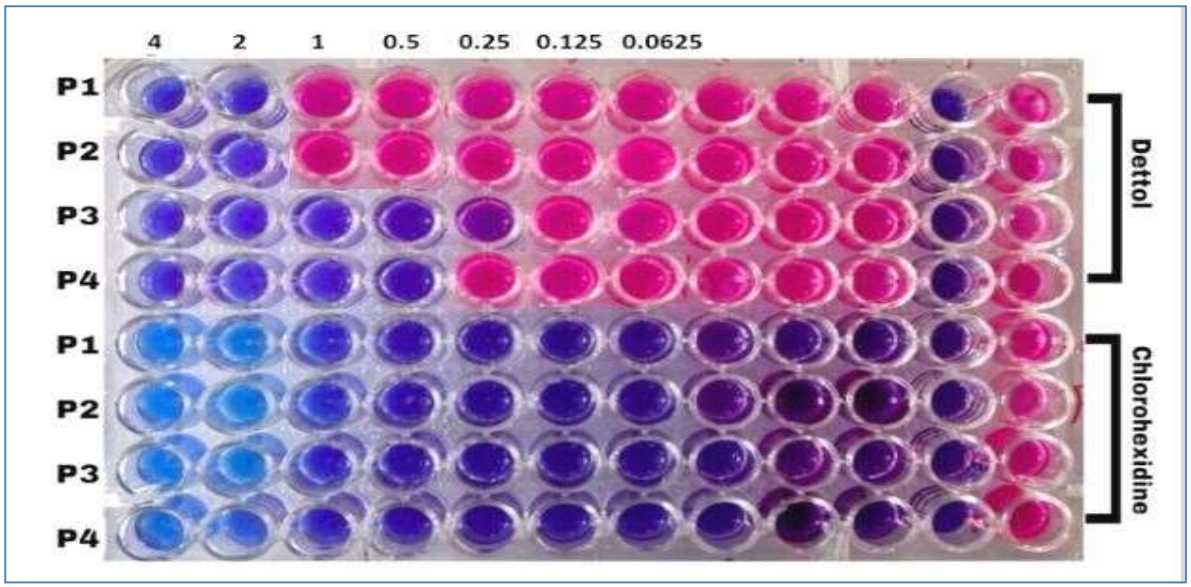

Figure (1). The minimum inhibitory concentrations (MICs) of Chloroxylenol (Dettol) against carbapenems-resistant *pseudomonas aeruginosa* at concentrations (0.0039-2%) by Microtiter Plate Assay with Resazurin Dye**.**

Chlorohexidine (CHX)

Results of Chlorohexidine's (CHX) minimum inhibitory concentrations against 24 isolates of *P. aeruginosa* (14 isolates were resist to carbapenems and 10 isolates sensitive to carbapenems) against Chlorohexidine (CHX) were evaluated by Resazurin dye microtiter plate experimentation. The findings showed that the isolates' MICs varied and that the inhibitory activity ranged from 0.8 to 102.4 µg/ml (Table 5). Additionally, the outcomes of the lowest inhibitory doses of sensitive-carbapenems *P. aeruginosa* demonstrated that the range of inhibitory activity was 0.8-6.4 µg/ml, where these concentrations were lower than the concentrations of the carbapenems-resistant isolates.

Table (5): The minimum inhibitory concentrations (MICs) of Chlorohexidine (CHX) against carbapenems-resistant *pseudomonas aeruginosa* at concentrations (0.8-1024 µg/ml).

Chlorohexidine Conc. (µg/ml)	The isolate code														Positive control	Negative control
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14		
102.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
51.2	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-
25.6	+	-	-	-	+	-	-	+	+	+	-	+	-	-	+	-
12.8	+	+	-	+	+	+	-	+	+	+	+	+	-	-	+	-
6.4	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	-
3.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
1.6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
0.8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
51.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-

- =No growth, + =Growth

Ethylenediaminetetraacetic acid (EDTA)

By using resazurin dye in a microtiter plate test, the minimum inhibitory doses of EDTA against 24 P. aeruginosa isolates (ten isolates sensitive to carbapenems and 14 isolates resistant to them) were assessed. The range of inhibitory action, according to the results, was (125-2000 µg/ml) and the isolates' MICs varied from one another (Table 3). Additionally, the results of the lowest inhibitory concentrations of P. aeruginosa that was sensitive to carbapenems showed that the range of inhibitory activity was 3.9–62.5 µg/ml, and that these concentrations were lower than the isolates that were resistant to the antibiotics.

Table (6): The minimum inhibitory concentrations (MICs) of EDTA against carbapenems-resistant *pseudomonas aeruginosa* at concentrations (3. 9-2000 µg/ml).

EDTA Conc. (µg/ml)	The isolate code														Positive control	Negative control
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14		
2000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
1000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
500	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-
250	+	+	-	+	+	-	-	-	+	-	-	+	-	-	+	-
125	+	+	+	+	+	+	-	-	+	+	+	+	+	-	+	-
62.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
31.25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
15.62	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
7.81	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-

- =No growth, + =Growth

Paterson, (2021), using EDTA, or ethylenediaminetetraacetic acid, to assess how well they worked

against both gram-positive and gram-negative bacteria. also determined the chelating agent's minimum inhibitory concentration using the microdilution method. According to Bakht *et al.* (2021), there is genotypic and phenotypic resistance to the common disinfectants chlorohexidine 2% and chloroxylenol (Dettol) 4.8%. They determined the minimal bactericidal concentration (MBC) and minimal inhibitory concentration (MIC) of disinfectants using the micro titter method, and they discovered that chlorohexidine and chloroxylenol (Dettol) were the most efficient disinfectants.

The gene Expression by Quantitative Real Time-PCR

RNA extraction

RNA was extracted from the isolates before and after treating with Chloroxylenol (Dettol), Ethylenediaminetetraacetic acid (EDTA) and Meropenem. Total RNA was extracted using TRIzol™ Reagent.

Effect of Chloroxylenol (Dettol), Ethylenediaminetetraacetic acid (EDTA), and Meropenem on gene expression of efflux pump genes.

In order to improve the role of these antimicrobial agents on the efflux pump system by affecting the expression of these genes, particularly with carbapenems-resistant strains, the main goal of this step was to measure the gene expression of the efflux pump genes (qacE -Δ1 and sugE1) of *P. aeruginosa* isolates and compare the gene expression in the presence and absence of these antimicrobial agents. One way that reverse transcription quantitative PCR (RT-qPCR) differs from other gene expression techniques is that, in a relative quantification analysis, the tests often compare the expression level of a given gene across various samples (Sanders *et al.*, 2014). The cycle threshold, or Ct value, was used to record the amplification. The 16s rRNA gene was the housekeeping gene employed in this investigation. This gene's consistent expression in the cells or tissues under study under various situations is why it is used in molecular investigations. This study used a quantitative real-time polymerase chain reaction (RT-PCR) assay to assess the mRNA expression of efflux pump genes by comparing samples of bacterial growth that were treated and untreated with antimicrobial drugs using concentrations below the minimum inhibitory concentration (MIC) for each sample. The quantitative real-time PCR program was used to record the Ct values of amplified genes.

Using relative quantification (RQ) from delta delta Ct value, the gene expression fold change was calculated. In table 4 and figures (4) and (5), the folds of gene expression for the two genes were compiled.The genes involved in efflux pump (*qacE -Δ1* and *sugE1*) were found to be significantly up-regulated in the local isolates, according to our findings. The four isolates' sugE1 gene expression folds ranged from 1.81 to 5.39, which is higher than the fold of the control (the fold change is 1), where the highest fold value was recorded for the meropenem (5.39) with the sub-MIC value (8 µg/ml), where the lowest fold value was recorded for the EDTA (1.81) with the sub-MIC value (125-500 µg/ml). Also, The four isolates' qacE-Δ1 gene expression folds varied from 2.11 to 5.01, which was more than the fold of the control (the fold change is 1), where the highest fold value was recorded for the EDTA (5.01) with the sub-MIC value (125-500 µg/ml), where the lowest fold value was recorded for the meropenem (2.11) with the sub-MIC value (8 µg/ml). Also, a high value of upregulation of the gene *qacE –Δ1,* during the treatment with dettol with sub-MIC (0.25-0.5%), and the fold change was 4.19.

Table (7). The fold of Gene expression of efflux pump genes (*qacE -Δ1* and *sugE1*) before and after treatment with biocides and the antibiotic (Meropenem) by sub-MIC for each agent of *P. aeruginosa.*

	***Sug-E1* gene**	***qacE-Δ1* gene**
The sub-inhibitory dose of antibacterial agent	**The Fold Change (Average of four isolates)**	**The Fold Change (Average of four isolates)**
Control	**1.00 ±0.00 c**	**1.00 ±0.00 c**

Dettol (0.25-0.5%)	**2.55 ±0.27 b**	**4.19 ±0.32 a**
EDTA (125-500 µg/ml)	**1.81 ±0.16 bc**	**5.01 ±0.39 a**
Meropenem (8 µg/ml)	**5.39 ±0.45 a**	**2.11 ±0.18 b**
L.S.D.	**0.863 ****	**1.028 ****
(P-value)	**(0.0037)**	**(0.0008)**
Means having with the different letters in same column differed significantly. ** (P≤0.01).		

The present findings indicated to the significant relationship between the treatments with meropenem and the high values of gene expression of the gene *sugE1* in comparison with the control and other agents, also there was a significant association between the high fold changes of the gene *qacE -Δ1* and the presence of dettol and EDTA in comparison with control and the antibiotic.

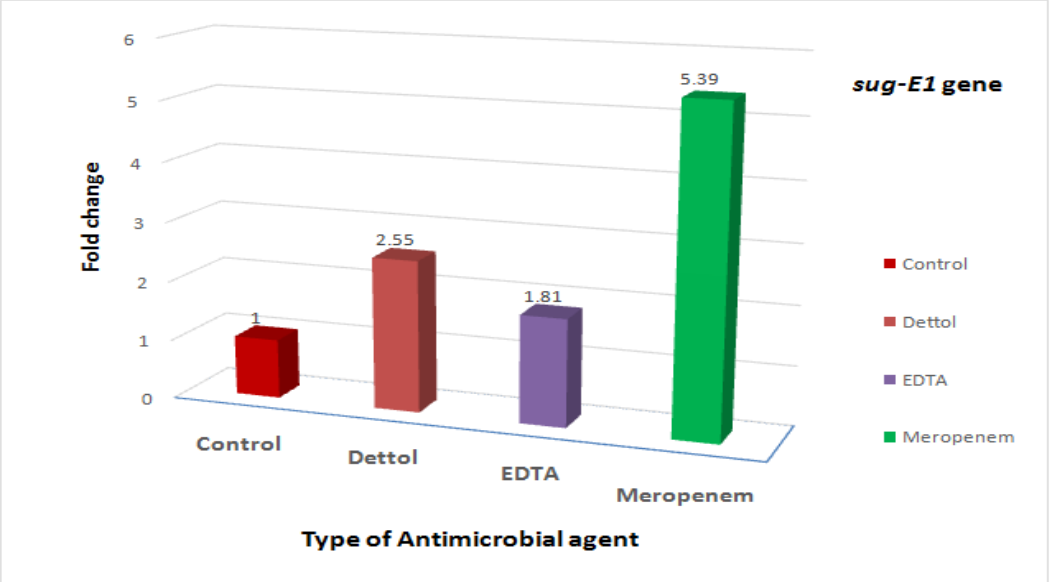

Figure (4). Fold of gene expression of *sugE1* gene for different *P. aeruginosa* isolates at the sub-MICs of biocides and Meropenem depending on ΔΔCt method.

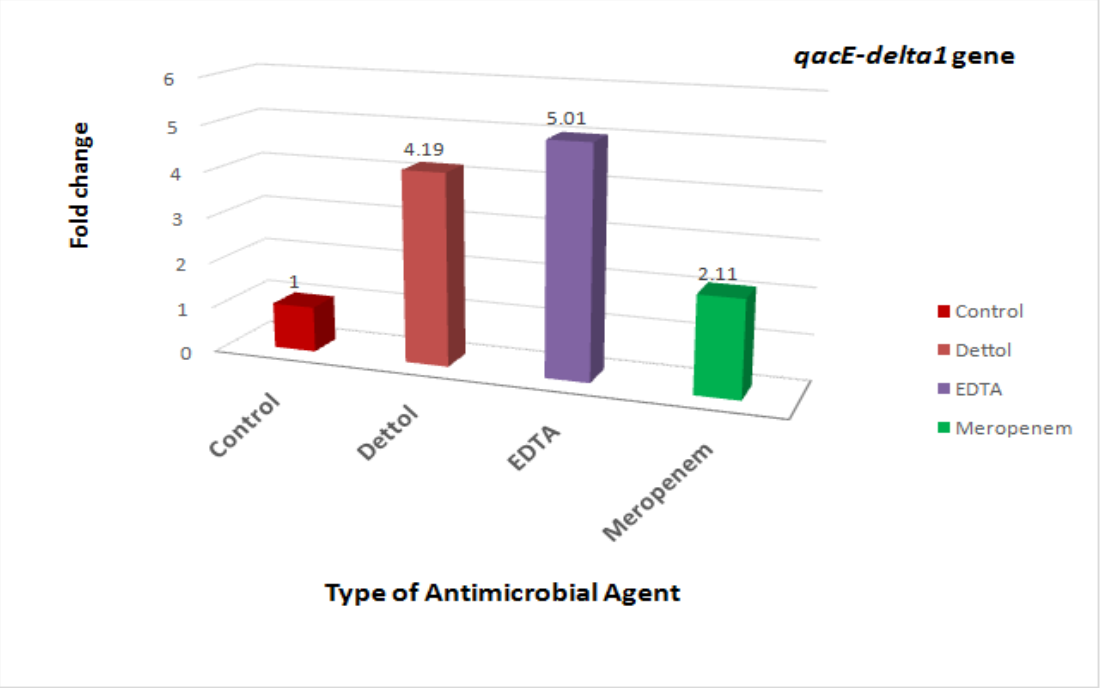

Figure (5). Fold of gene expression of *qacEdelta1* gene for different *P. aeruginosa* isolates at the sub-MICs of biocides and Meropenem depending on ΔΔCt method**.**

Quaternary ammonium compound (QAC) resistance determinant is another term for the *sugE1* gene, which, together with the *qacE* and *qacEΔ1* genes, is a member of the small multidrug resistance (SMR) protein. QacE is a 115 amino acid protein that was initially identified in E. Coli. Other bacterial species have functionally active deletion versions of QacE, or QacEΔ. The broad-host plasmids that replicate in Gram-negative bacteria provided the sequences for their genes (Wassenaar *et al*., 2015).In hospitals in Baghdad, the local study of Abdullah and Al-Azzawi (2022), included 69 *P. aeruginosa* isolates from samples of burns and wounds. The *qacE* gene was examined in 97.1% of *P. aeruginosa* responses to quaternary ammonium compounds in the study. After the *QacE* genes were sequenced, a number of mutations were found, including missense, insertion, and several silent mutations. Some of the mutations had an impact on the translation of proteins, while others had no effect at all.
The present investigation suggested that the *qacEΔ1* gene plays a part in the resistance to EDTA and dettol. However, these findings disagreed with those of Romão *et al.* (2011), who found that 48% of the isolates had the *qacEΔ1* gene. A significant correlation was observed between the presence of the *qacEΔ1* gene, which was carried by 88% of the multiresistant isolates. Out of all the isolates that were tested for the disinfectant, 70% of the isolates that were susceptible to the biocide had the *qacEΔ1* gene, while 90% of the less susceptible strains carried this gene. Decreased susceptibility to the disinfectant occurred regardless of the presence of *qacEΔ1*, indicating that it is not a significant factor in *P. aeruginosa's* resistance to biocide.The function of SUG proteins in *P. aeruginosa* disinfectant resistance may be connected to biocide resistance. Antibiotic resistance genes are often carried by integrons, which are linked to the *qacEdelata1*. Antibiotic resistance is therefore frequently associated with the presence of *qacEdelata1*. Nevertheless, no correlation was discovered between quaternary ammonium compound resistance in Gram-negative bacteria, such as *P. aeruginosa,* and the presence of *qacE/qacEdelata1*(Subedi *et al*., 2018). One of the studies showed that Of the 72 Pseudomonas aeruginosa clinical isolates, 67 (93.05%) and 25 (34.72%) carried the qacEΔ1 and qacE genes, respectively. Furthermore, *qacEΔ1* gene detection differs significantly across MDR and non-MDR *P. aeruginosa* isolates. In 50 out of 54 (92.59%) MDR isolates and 7 out of 18 (38.89%) non-MDR bacteria, the *qacEΔ1* was found. While no *qacEΔ1* gene was found in non-MDR isolates, gene *qacE* was detected in 25 (46.29%) MDR isolates. The results showed that the genes *qacE* and qacΔE1 are more common in MDR strains of *P. aeruginosa* than in non-MDR strains. (Chowdhury et al., 2021).

Numerous research studies have demonstrated the function of efflux pump inhibitors in mitigating antibiotic resistance in *P. aeruginosa* clinical isolates (Al-Jumaily and Abd, 2017;Odaa and Al-Mathkhury, 2023).

Many studies revealed that The co-administration of antibacterial agents, including antibiotics and other antimicrobial agents, together with EDTA has garnered attention and has been extensively researched due to its potential to provide synergistic effects and potentially mitigate resistance development issues. Our findings showed that EDTA has a strong inhibitory effect and can boost the biocides' inhibitory potency. Because biocides and EDTA have been shown to work in concert or synergize, using these agents is advised and can go on (Senna *et al.*, 2018; Anari *et al*., 2022). Anari *et al*., (2022) The findings showed that the biocides under study were far more effective when EDTA was added. Additionally, they discovered that resistance to the tested biocides (higher MICs and MBCs) against strains of Stenotrophomonas maltophilia did not correlate with the high prevalence of the sugE1 gene. Because of this, it is recommended to use EDTA in conjunction with antiseptic and disinfection to maximize their potency and effectiveness. The study's findings Banin *et al*., (2006), imply that the chelation of various divalent cations necessary to stabilize the biofilm matrix mediates the action of EDTA against biofilm cells, and the findings suggest that magnesium, calcium, and iron EDTA chelation can promote cell detachment from the biofilm. Because EDTA chelates the magnesium linked to LPS, it also makes biofilm cell death easier.

Conclusion

It has been observed that the Chloroxylenol (Dettol) antiseptic is ineffective against *Pseudomonas aeruginosa*. The Ethylenediaminetetraacetic acid (EDTA) is a good antibacterial agent and represent as enhancer for biocides inhibitor activity. It has been observed that the Chlorohexidine (CHX) antiseptic is effective more than the Chloroxylenol (dettol) against *P. aeruginosa*. The efflux pump genes (*qacE-Δ1*, and *sugE1*) in *P. aeruginosa* isolates play a crucial role in the development of resistance against these antibiotics and biocides. The present findings revealed a significant relationship between the treatments with meropenem and the high values of gene expression of the gene *sugE1*, while high fold changes of the gene *qacE -Δ1* were related with dettol and EDTA treatments.

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