Detection of The Quaternary Ammonium Compound Antiseptic Resistance Gene (qac A/B qac C and qac E gene) In Pseudomonas Aeruginosa Recovered from Topical Wound Infection

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Abstracts: Background: Quaternary ammonium compounds (QACs) have represented one of the most visible and effective classes of antiseptics and disinfectants substances that used in different parts of life including food production, water treatment and healthcare such as hospitals to prevent infections and intoxications. The increased use of disinfectants containing quaternary ammonium compounds (QACs) has raised concerns about the development of resistance. Aim of the study: this study pursues to determination frequency of antiseptic resistant Quaternary Ammonium Compound (qacA/B, qacC and qacE gene) in Pseudomonas aeruginosa, recovered from topical wound infection. Methods: Two swab samples were collected from 200 patients complain from burn, traumatic injury and wound after surgery, Suspected isolates of P. aeruginosa which showed Beta hemolysis on Blood agar media while occur colorless colonies (non-lactose fermenter) on MacConkey. Furthermore, identified using VITEK-2 System. The efficacy of antiseptics chlorhexidine (CLX), benzalkonium (BZK) and cetrimide (CET) was tested against P. aeruginosa by Well diffusion assay test and MIC procedure. The confirmed P. aeruginosa isolates have been used for Genomic DNA extraction from a fresh overnight culture, after that DNA templates was used to target (qacA/B, qacC and qacE gene) by using specific Primers sequences. Results: Out of 56 P. aeruginosa a considerable number of isolates 48 (85.7%) of them were resistance to chlorhexidine and 8 (14.3%) were sensitive, moreover substantial number of isolates 42 (75.0%) of them were resistance to cetrimide and 14 (25.0%) were sensitive; No sensitive rate to benzalkonium were observed in all isolates, qacA/B gene were identified in 32(57.1%) were positive for this gene while qacC gene were detected in 46(82.1%) were positive for this gene, Furthermore, qacE gene were detected in 50 (89.2%) were positive for this gene in 56 P. aeruginosa isolates. Conclusion: the current study, shows that the qacA/B, qacC and qacE genes which harbored resistance to quaternary ammonium compound antiseptics are widespread in Pseudomonas aeruginosa isolates in wound and burn patients.

Keywords: Quaternary ammonium compound, Antiseptic Resistance Gene, Chlorhexidine (CLX), Benzalkonium (BZK), Cetrimide (CET).

1. INTRODUCTION

The use of disinfectants is widespread in various industries, including healthcare, food production, and water treatment, to prevent infections and intoxications. Antimicrobial resistance, which is a concern, can be caused by the use of disinfectants. Resistance to disinfectants has been relatively overlooked, despite the significant attention given to antibiotic resistance. This may be due to the lack of a widely accepted definition of disinfectant resistance and the modest increases in susceptibility typically observed **(1).**

Quaternary ammonium compounds (QACs) which is belong to a cationic surfactant group of disinfectants it's an important antiseptic and disinfectants in a range of industries and healthcare facilities because they offer several benefits over other regularly used disinfectants. Possessing qualities like low toxicity, high surface activity, and no corrosion **(2).**

Quaternary ammonium compounds (QACs) tolerance or resistance mechanisms include changes in bacterial cell wall structure, changes in cell membrane function, efflux pumps, biofilm formation, The development of tolerance or resistance to QACs and antibiotics has been demonstrated by laboratory studies. As well, tolerance to benzalkonium chloride (BAC) was correlated with clinically defined antibiotic resistance **(3).**

The qac genes are a family of multidrug resistance genes found in bacteria. These genes encode for efflux pumps that are capable of pumping out a variety of toxic compounds from the bacterial cell, thereby conferring resistance to multiple antibiotic classes. Furthermore, Qac genes code for antiseptic resistance **(4).** There have been reports of a number of qacs genes, including *qacA/B* genes, *qacC/D*, *qacE/F, qacG, qacH, qacJ,* and *qacZ*. Gram positive bacteria frequently have *qacA/B* genes followed by *qacC/D* genes, whereas Gram negative bacteria typically have *qacE* genes **(4).** These genes have been found in various bacterial species, and they can confer resistance to a range of compounds, including antibiotics, disinfectants, and dyes. The Qac genes are named after the first member of the family to be discovered like *qacA* gene which is found in *Pseudomonas aeruginosa* **(5).** This study strives for Determination frequency of antiseptic resistant Quaternary Ammonium Compound (*qacA/B, qacC* and *qacE* gene) in *Pseudomonas aeruginosa* isolates recovered from topical wound infection.

2. MATERIAL AND METHODS

The current cross-sectional investigation involved wound and burn swab samples which were collected from different hospitals in Baghdad, Iraq, during the period from August 2022 to November 2022; it's carried out in a total of 200 patients (male and female) who were clinically suspected to have a bacterial infection of burn, traumatic injury and wound after surgery. Two swab samples from each participant were collected aseptically, by rotating the sterile cotton swab stick over the selected area as deep as possible for 10 seconds before wound cleaning and dressing one of the swab was inserted into sterile transport tube media, while the second one used for direct detection by using the Gram stain procedure **(6).**

The collected swabs were inoculated on blood agar, then incubated at 37ºC for 24 hours at aerobic condition for cultivation the isolated bacteria, Suspected isolates of *p. aeruginosa* which showed Beta hemolysis on Blood agar media further tested using Gram stain in which *Pseudomonas* species appeared as Gram-negative and then inoculated on MacConkey agar and incubated at 37 ºC for 24 hour, bacteria that showed the characteristic of pigment production and fruity odor and its ability to grow at 42°C were furthermore identified using VITEK-2 System according to the manufacturer instructions (bioMérieux company).

2.1. Antiseptic Susceptibility

2.1.1. Well Diffusion Assay Test And Minimum Inhibitory Concentration.

The efficacy of antiseptics chlorhexidine (CLX), benzalkonium (BZK) and Cetrimide was tested against *p. aeruginosa*, the bacteria were cultured in test tubes containing 2 ml of Brain heart infusion broth. The tubes were incubated at 37°C for 24 hours. The inoculum density of selected bacteria was adjusted by using 0.5 McFarland standard tubes then plated onto Muller-Hinton agar in three directions by dipped sterile swabs in suspension. Wells (6 mm diameter) were punched in the plates using a sterile stainless-steel borer. 50 microliters of each antiseptics mentioned above were placed with concentrations ranging from 125 mg/ml to 31.25 mg/ml. Distilled water was used as a negative control; culture plates were incubated at 35°C for 72 hours; when the incubation was complete, the diameter of the inhibition zone around the well was measured and compared with a clinical laboratory institute (CLSI) **(7).** In addition to that Minimum inhibitory concentration of antiseptics (chlorhexidine (CLX), benzalkonium (BZK) and cetrimide (CET)) was determined by a standard agar dilution method according to the Clinical and Laboratory Standards Institute **(8).**

2.1.2. Molecular Assay for Detection of *qacA/B, qacC* and *qacE* genes in *p. aeruginosa* isolates:

The confirmed *p. aeruginosa* isolates have been used for Genomic DNA extraction from a fresh overnight culture by using Geneaid Presto™ Mini gDNA Bacteria Kit (Taiwan) After that DNA templates was used to target (*qacA/B, qacC* and *qacE gene*) by using specific Primers sequences which was listed in table (1)

Table (1): Primer sequences used for detection of Quaternary ammonium compounds (QACs) genes

Name of the gene	Seq.	Annea Temp.	Size	Referenc
qacA/B	F- 5' CTATGGCAATAGGAGATATGGTGT 3' R-5-CCACTACAGATTCTTCAGCTACATG 3'	**55**	**321bp**	**(9)**
qac C	5'-GGCTTTTCAAAATTTATACCATCCT-3' 5'-ATGCGATGTTCCGAAAATGT-3'	**56**	**249 bp**	**(10)**
Qac E	**F-**5´**TAGCGAGGGCTTTACTAA GC**3´ **R-**5´**CCCATACCTACAAAGCCCCA**3´	**58**	**207bp**	**(11)**

Conventional monoplex PCR technique was carried out to amplify fragments of *qacA/B* (321bp), *qacC* (249bp) and *qacE* (207bp) genes separately. PCR cycling program parameters used in this reaction for (*qacA/B, qacC* and *qacE)* genes detection and amplification protocol are initiated according to describe by ….

2.2. Statistical Analysis

To detect the effect of different factors in study parameters, the Statistical Analysis System- SPSS program was used. To make a significant comparison between means, the T-test was used. In this study, the Chi-square test was used to compare percentages (0.05 and 0.01 probability).

3. RESULTS

3.1. Pseudomonas Aeruginosa Isolates Distribution According to Type of Injury

In this study, out of 159 positive bacterial cultures from patients; in as many as 98(61.6%) of them was recovered from wound injury; of them 17 (17.3%) was *p. aeruginosa*, in addition to that 61 (38.4%) of the total isolates were regained from Burn injury; in which *p. aeruginosa* was 39 (63.9%). Table (2)

Table (2) : *Pseudomonas aeruginosa* isolates distribution according to type of injury

Type of injury	Number of *p. aeruginosa*
Wound	17
%Within type of Bacteria	17.3%
% Of Total isolates	10.7%
Burn	39
%Within type of Bacteria	63.9%
% Of Total isolates	24.5%
Total *p. aeruginosa*	56 (35.2%)

3.2. Antiseptic Resistance of *Pseudomonas Aeruginosa* Isolates By Well-Diffusion Method

By the well-diffusion method, *Pseudomonas aeruginosa* isolates were tested for their susceptibility to chlorhexidine (CLX), benzalkonium (BZK), and cetrimide (CET). Of 65 *Pseudomonas aeruginosa* a considerable number of isolates 48 (85.7%) resistance to Chlorhexidine (CLX) and only 8 (14.3%) isolates were sensitive, moreover substantial number of isolates 42 (75.0%) resistance to cetrimide and only 14 (25.0%) isolates were sensitive; No sensitive rate to Benzalkonium were observed in all isolates of *Pseudomonas aeruginosa* Table (3).

Table (3) : Antiseptic resistance of Staphylococcus *aureus* isolates by Well-diffusion method;

Antiseptic agents	***Pseudomonas aeruginosa***		Total
	Sensitive	Resistant	
Chlorohexidine	8	48	65
% Within type of bacteria	14.3%	85.7%	100 %
Benzalkonium	0	65	65
% Within type of bacteria	0	100%	100%
Cetrimide	14	42	56
% Within type of bacteria	25.0%	75.0%	100%

3.3. Minimum Inhibitory Concentration (MIC) of *Pseudomonas Aeruginosa* Isolates To Antiseptic

The minimal inhibitory concentrations (MIC) of chlorhexidine (CLX), benzalkonium (BZK), and cetrimide (CET) were determined by a broth dilution method for all *Pseudomonas aeruginosa* isolates. The MIC is the concentration of the greater dilution tube where there was no bacterial growth. The MIC results of 56 *Pseudomonas aeruginosa* isolates were highly resistant to Chlorhexidine (CLX), and Cetrimide (MIC 512 µg/mL to 256 µg/mL). Almost all bacterial isolates revealed that high level resistance with MIC 512 µg/mL to Benzalkonium.

3.4. Molecular Detection of *qacA/B, qacC* and *qacE* Genes in *Pseudomonas Aeruginosa* Isolates.

All 56 *Pseudomonas aeruginosa* isolates were subjected to conventional PCR amplification studies for detection of chlorhexidine (CLX), benzalkonium (BZK), and cetrimide (CET) resistance genes. The results of the current study showed that *qacA/B* gene were identified in 32 (57.1%) of 56 *Pseudomonas aeruginosa*; this gene was frequently amplified with products size 321 bp figure (1); while *qacC* gene were detected in 46 (82.1%) out of 56 *Pseudomonas aeruginosa*, which was frequently amplified with products size 249 bp figure (2). Furthermore, *qacE* gene were detected in 50 (89.2%) of 56 *Pseudomonas aeruginosa*, this gene was frequently amplified with products size 207 bp figure (3).

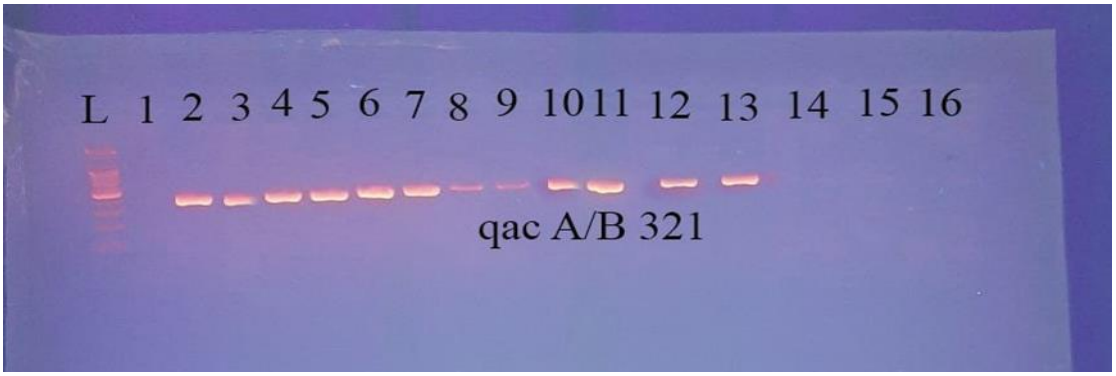

Figure (1): Gel electrophoresis (1.5% agarose,7v/cm^2,) of the PCR products, lane (1)100bp DNA ladder; (2-13): Positive sample for ***qacA/B*** **gene** (321bp) lane (1,14,15 and 16) Negative samples.

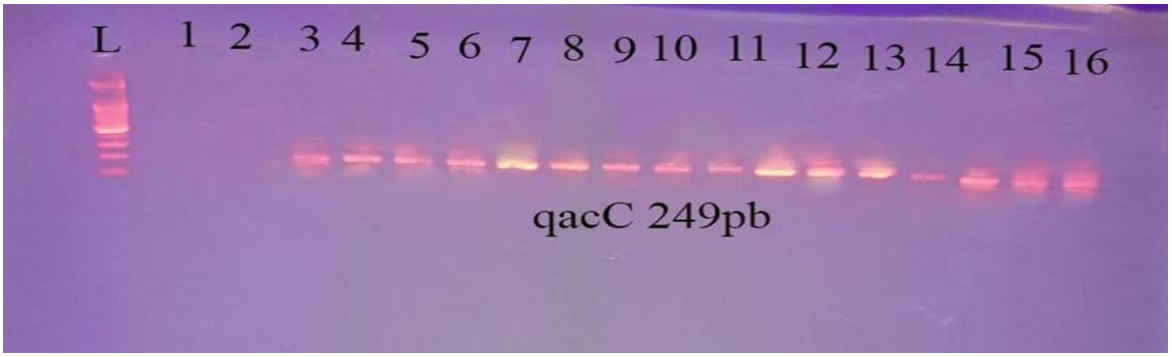

Figure (2): Gel electrophoresis (1.5% agarose,7v/cm^2,) of the PCR products, lane (1)100bp DNA ladder; (3-16): Positive sample for ***qacC* gene** (249 bp) lane (1 and 2) Negative samples.

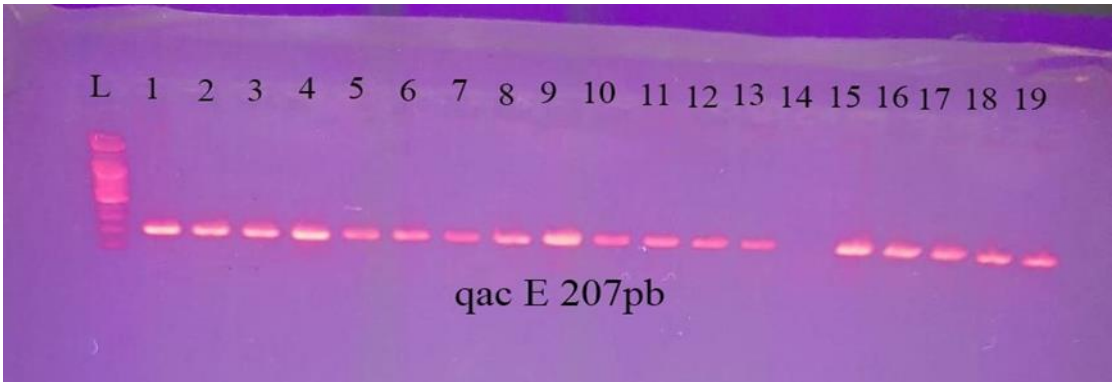

Figure (3): Gel electrophoresis (1.5% agarose,7v/cm^2,) of the PCR products, lane (1)100bp DNA ladder; (1-13 and 15-18): Positive sample for ***qacE* gene** (207 bp) lane (14) Negative samples

4. Discussion

Previous studies mentioned that, Biocide that used is far less regulated than antibiotic use, leading to concerns regarding the development of biocide tolerance and the possible role these agents play in driving the emergence of multidrug-resistant (MDR) pathogens however the un-responses to antiseptic was highly increased in Gram positive as well as other Gram-negative species in last years, and the natural resistance mechanism hypothesized to be promoted by mostly driven by the acquisition of genes that provide such resistance **(12,13).**

As with antibiotics, bacteria also have mechanisms for disinfectants resistance, and one of the problematic situations can occur is replace one set of problems (increasing antibiotic resistance) with another (increasing resistance to disinfectants) **(14).**

4.1. Pseudomonas Aeruginosa Isolates Distribution According to Type Of Injury

In this study, out of 159 positive bacterial cultures from patients presented signs of wound and Burn injures infection; the *Pseudomonas aeruginosa* comprises 56 isolates (35.2%). *Pseudomonas aeruginosa* is a one of the common pathogens that has been identified as a cause of various nosocomial infections contracted in the community **(15).**

According to last investigation the distribution of *Pseudomonas aeruginosa* isolated from clinical skin samples varied from country to country and from hospital to another **(16).**

Moreover, Wong SY et al and Serra R et al reported that; pseudomonas bacterial species induce more aggressive damage in injuries healing due to the ability to express virulence factors and surface proteins which can

interfere with wound healing **(17,18).** For what concerns type of injuries; the most frequent isolated bacteria from wound and Burn injures was *Pseudomonas aeruginosa* **(19,20).**

4.2. Antiseptic Resistance of *Pseudomonas Aeruginosa* Isolates by Well-Diffusion Method

Cetrimide resistance in *Pseudomonas aeruginosa* was (94.4%), interestingly in this study no sensitive rate to benzalkonium were observed in all isolates of *Pseudomonas aeruginosa.* Noteworthy, present results reported that the isolated bacteria of *Pseudomonas aeruginosa* that showed resistance to antibiotics were also tolerate to antiseptic; in line with these results there are numerous reports on antimicrobial resistance to antiseptic and antibiotics, at the same time **(21,22).**

4.3. Minimum Inhibitory Concentration (MIC) Of *Staphylococcus Aureus* Isolates to Antiseptic

Antiseptics are crucial for stopping the spread the infection of pathogenic microorganisms when administered properly. Higher MICs of biological agents and the formation of strains resistant to antiseptics and many medications may be the result of improper usage and the dosage of antiseptic that used in hospitals for infection control **(23).**

Many practical methods have been used to determine MIC, but all of them based to the same principle. In current research, the MIC values of study isolates for antiseptic were found to be very high ranged from 512 µg/mL to 256 µg/mL.

In this study, the susceptibility and MIC to chlorhexidine (CLX), benzalkonium (BZK), and Cetrimide was determined because they are widely used as antiseptic and disinfectants in healthcare facilities in our country, this study reported that the high rate of resistance to chlorhexidine (85.7%) out of 56 isolates; cetrimide resistance in *Pseudomonas aeruginosa* was (75.0%), while; no sensitive rate to benzalkonium were observed in all isolates of *Pseudomonas aeruginosa.*

Concurrent resistance to an antiseptic was noted in many local studies such as Hamad BD 2019 **(24)** who noted that, increase rate of resistance to Dettol (septal), Hepatine, Povidone-iodine to the *Pseudomonas aeruginosa* isolated from wound and burn infection **(25).**

The highest proportion of resistant to antiseptic have been reported in other studies globally **(26,27).** From other hand Khudair AN and Mahmood SS indicate that P. aeruginosa isolates showed a high resistance to biocides **(28).**

Decreased susceptibility and acquired resistance to disinfectants has been documented through the world primarily against quaternary ammonium compounds (QAC), and phenols component and one of the most reason to such dilemma is the misuse of antiseptic in human medicine, food industry, agriculture and animal production **(26,29,30).**

Cross-resistance among antibiotics and antiseptic may arise through a variety of shared pathways, involving efflux pump systems, permeability alterations, and biofilm development **(31).**

Indeed cross- and co-resistance between various type of antiseptic and antibiotic resistant bacteria have even been reported **(32,33).** Russell declares that there is cross-resistance between biocides and antibiotics and the use of biocides selects for antibiotic resistance **(34).** On the basis of this concept, a lot of research has been conducted that confirmed such hypothesis **(27,29,35)**.

Cross-resistance to antiseptics like QACs and other antibiotics may be explained in part by the presence of antimicrobial resistance genes on mobile genetic elements like plasmids and transposons, microorganisms may acquire the resistance determinants on conjugative plasmids or genetic mobile elements **(36).**

Plasmid-mediated efflux pumps are crucial defense mechanisms against many antibiotics and antiseptics. Regular use of antibiotics and antiseptics to kill and stop the spread of microorganisms may, in turn, put selective pressure on the retention of resistance genes in microorganisms, including genes or clusters of genes encoding resistance to both antibiotics and disinfectants **(37).**

4.4. Molecular Detection Of *Qaca/B, Qacc* And *Qace* Genes In *Pseudomonas Aeuroginosa* Isolates.

In this study, high occurrence rate for quaternary ammonium compounds (QAC) genes including *qacA/B, qacC* and *qacE* was detected in *Pseudomonas aeuroginosa.* In which *qacA/B* were identified in 32 (57.1%) of 56 *Pseudomonas aeuroginosa* while *qacC* were detected in 46 (82.1%) furthermore, *qacE* were detected in 50 (89.2%). Such results in line with other broad studies that mentioned increase detection rate to quaternary ammonium compounds (QAC) genes in Gram positive bacteria isolated from burns and wound infection **(38,39).** The raise in identification rate to quaternary ammonium compounds (QAC) genes may evident to reduced susceptibility to such product of antiseptic. Moreover, expanded and more indiscriminate use of antiseptic in healthcare facilities could ride the emergence of genetic elements that accountable for reduced susceptibility, with unreliable consequences for human safety. This may be attributed to the extensive use of these types of disinfectants in the routine infection control **(40).** Although the *qac* genes are widely spread among clinical and environmental bacteria, it is obvious that their distribution is generally linked with a particular bacterial species. The *qacE* gene is widely spread in Gram negative bacteria, mainly in *Enterobacteriaceae* and *Pseudomonas* spp **(41).**

Furthermore, in this study, the *qacC* and *qacE* gene provide resistance to quaternary ammonium compounds (QAC) more than *qacA/B* such result disagreement with study in the United States **(42)**, European countries **(43)** and Iran **(44).** That mentioned *qacA/B* genes predominant resistance genes while our results in part in line with study by Ignak S et al; who detected the *qacA/B* genes in (10.3%) **(45).**

The discrimination in such results may be reflect difference in the specimen source and type of bacteria in each study and may be consider that the antiseptic resistant is a local problem which differed from hospital to another.

The increasing usage of quaternary ammonium compounds (QAC) in healthcare settings has both benefits and potential drawbacks. Quaternary ammonium compounds (QAC) are commonly used as a disinfectant and hand sanitizer in healthcare facilities due to its antimicrobial properties.

For strains that are resistant to antibiotics, antiseptic application may be employed but the hard scientific fact is that these bacteria have the ability to gain resistance in order to maintain itself in medical facilities

The survival of Staphylococcus species in hospital settings may be aided by the link between antibiotic resistance and qac genes. These Staphylococci's endurance in areas with little in the way of antiseptic residues makes them potentially dangerous for infection management.

Conclusion:

The current study, shows that the qacA*/B, qacC* and *qacE genes* which harbored resistance to quaternary ammonium compound antiseptics are widespread in *Pseudomonas aeruginosa* isolates in wound and burn patients.

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