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Research Article

Downregulation of the Gene Expression of Efflux Pump Gene (*acrA*) and its Regulator (*ramA*) in *Klebsiella pneumoniae* by Quaternary Ammonium Compounds

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I N F O

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A B S T R A C T

Introduction: Biocides are commonly used for disinfection in a variety of contexts. They are generally used to avoid infection by controlling biofilm on medical equipment. However, the literature lacks information on the effect of biocide on efflux pump gene expression.

Objective: To determine the influence of biocide on biofilm development and efflux pump *acrA* and *ramA* gene expression.

Methodology: The microtiter plate method was used to identify biofilm development in 80 isolates of *K. pneumoniae*. The minimal inhibitory concentrations (MIC) of three biocides (quaternary ammonium compound (QAC), chlorohexidine digluconate, and chloroxylenol) were estimated. The effect of QAC on the intensity and viability of biofilms was investigated as well. Its influence on biofilm was evaluated using scanning electron microscopy. For each isolate, gene expression of the *acrA* and *ramA* was evaluated before and after treatment with the biocide (at ½MIC).

Results: The study revealed that 60% of isolates formed moderate biofilms. Biofilms of all isolates were inhibited by QAC at high concentrations. Moreover, QAC at ½MIC downregulated the gene expression of the efflux pump gene (*acrA*) and its regulator (*ramA*).

Conclusion: Biofilms of all isolates were prevented by QAC at high doses. Furthermore, QAC at ½MIC decreased the expression of the efflux pump gene (*acrA*) and its regulator (*ramA*).

Keywords: *Klebsiella pneumoniae*, Efflux pump, Biocide, Biofilm, *acrA* gene, *ramA* gene

Introduction

Klebsiella pneumoniae belongs to the Enterobacteriaceae family. It is gram-negative rod-shaped, encapsulated, and non-motile,[1] and may cause severe and life-threatening infections, including pneumonia, urinary tract infections, soft tissue infections, intravascular line infections, intraabdominal infections, and bacteraemia.[2]

The capacity of *K. pneumonia* to produce biofilms protects it from the human immune system's reactions and antimicrobial agents, promoting its persistence on epithelial tissues and the exterior surfaces of indwelling devices.[3]

K. pneumoniae developed different strategies to resist the antibiotics; some of these strategies include the expulsion of the antibiotic using the efflux pump.[4] AcrAB and OqxAB are two important RND pumps in *K. pneumoniae*.[5] These two efflux pumps are encoded by the genes *acrAB* and *oqxAB*, and *ramA* acts as their transcription activator.[6]

Biocides are chemical agents that kill microorganisms and are widely used for disinfection in various settings, including homes, industries, and healthcare environments.[7] They are widely used to control biofilm on medical equipment to prevent contamination.[8]

Given that the literature lacks information about the impact of biocide on the gene expression of efflux pump genes, the current study aimed to detect the effect of biocide on biofilm formation and the gene expression of efflux pump *acrA* and *ramA* genes.

Materials and Methods

Klebsiella pneumoniae Isolates

A total of 80 *K. pneumoniae* isolates were obtained from the Microbiology laboratory at the Department of Biology, College of Science, University of Baghdad from September to November 2022. These isolates were originally collected from different clinical sources, including blood, mid-stream urine, sputum, wound, fluids, pus, abscess, skin, tissue, swabs, nasal wash, bronchial wash, central venous line, expressed prostatic secretion, endotracheal tube, and Foley tips from patients admitted to different hospitals including Baghdad Teaching Hospital, Ghazi Al- Hariri Hospital for Surgical Specialties, Nursing Home Hospital, and Teaching Laboratories at Baghdad Medical City, Baghdad, Iraq.

Biofilm Formation Assay

Biofilm production was detected using the microtiter plate technique, as reported by Jaffar et al.[9] 20 µl of a bacterial suspension, overnight cultured in brain heart infusion broth (adjusted to 0.5 McFarland standard), was inoculated into 96-well polystyrene microtiter plates containing 180 µl of brain heart infusion broth (Himedia, India) containing 1% glucose. After 24 hours at 37 °C, the plates were washed three times with distilled water and dried. Biofilm fixation was done by adding 200 µl of absolute methanol (Alphachemika, India) to each well for 15 minutes, then washing and air-drying. The plates were stained with 0.5% crystal violet solution (CDH, India) for 15 minutes, washed three times in water, dried for 30 minutes at 37 °C, and resolubilised with 200 µl of absolute ethanol (Thomas Baker, India) and glacial acetic acid (Himedia, India) (1:1) for 10 minutes. The optical density (OD) of each well was measured at 630 nm using a microtiter plate reader. The cut-off, also known as ODc, was determined to be three standard deviations higher than the mean OD of the negative control. Based on the ODc value, all isolates were divided into four groups: non-producer (OD ≤ ODc), weak biofilm (ODc < OD ≤ 2X ODc), moderate biofilm (2X ODc < OD ≤ 4X ODc), and strong biofilm (OD > 4X ODc).

Minimum Inhibitory Concentration of Biocide

The minimal inhibitory concentrations (MIC) of the quaternary ammonium compound (QAC) (SteriTech, Lebanon), chlorohexidine digluconate (CERKAMED, Poland), and chloroxylenol (Dettol, United Arab Emirates) were determined using a turbidometric test based on resazurin as described by Teh et al.[10] Double serial concentration of these biocides was used in Mueller Hinton broth and after overnight incubation at 37 °C, 5 µl of resazurin (6.75 mg/ml) (BDH, England) was added to each well and incubated for another 4 hours at 37 °C. Colour changes (from blue to pink) were noticed and recorded, and the MIC was identified as the lowest concentration before the colour change.

Determination of the Effect of QAC on Biofilm

The impact of QAC concentrations on biofilm intensity and viability is as follows:[11]

A volume of 180 µl of brain heart infusion broth (Himedia, India) containing 1% glucose was transferred into the wells of a 96-well plate, then 20 µl of overnight diluted and adjusted to 0.5 MacFarland turbidity standard bacterial suspension was added to each plate well. After incubation for 24 hours at 37 ºC, the plate was washed with water and was air-dried. 200 µl of Muller Hinton Broth (Solarbio, China) containing 2500, 5000, 10000, and 20000 µg/ml of QAC (SteriTech, Lebanon) was added and incubated at the same condition. It was gently washed with distilled water and air-dried. After drying, steps of fixation, staining, and measuring at 630 nm of the biofilm formation assay were performed.

In order to test the effect of QAC on the viability of biofilm cells, a similar protocol mentioned above was followed except that after incubation, all plates were gently washed with distilled water and air-dried. Subsequently, 200 µl of normal saline was added and a sterile loop was used to scrape the bottom of the well. The suspension was serially diluted, plated onto nutrient agar, and incubated overnight

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at 37 °C. After incubation, colonies were enumerated and the number of colony-forming units per millilitre (CFU/ml) was calculated.

Determination of the Conformation of Biofilm by Scanning Electron Microscope

In the biocide-free state, a piece of polystyrene was inserted into a container containing brain heart infusion broth with 1% glucose, and 10 µl of overnight diluted bacterial suspension of adjusted turbidity to a 0.5 MacFarland standard was added. In the Biocide treatment condition, similar contents were presented in the normal state container, except that the medium was supplemented with 10000 µg/ml QAC as a final concentration. Both containers were incubated at 37 ºC for 24 hours; after incubation, the polystyrene pieces were washed, air-dried, and fixed by immersing the two polystyrene pieces in absolute methanol (Alphachemika, India) for 10 minutes, washed, and air-dried. Scanning electron microscopy (Thermo Fisher Inspect F50, USA) was performed.

Molecular Study

PCR Primers

In the current study, the reference genes (*acrA*, *ramA*, and *rpoB*) were downloaded from GenBank as FASTA files, and the Geneious Prime was used as a bioinformatics software to verify the right annealing temperature and the binding site for each set of primers. Additionally, the primers were designed using the same software and tested by various online tools, mainly OligoAnalyzer from Integrated DNA Technology.

All primers were dissolved in nuclease-free water to obtain a stock solution of 100 picomol/µl. This stock was diluted by adding 10 µl of primer stock solution to 90 µl of nuclease-free water to yield a working primer solution of 10 picomol/µl. Table 1 shows the primer sequences.

Table 1.Primers used in the Current Study

Gene	Primer Sequence (5`- 3`)	Product Size (bp)
acrA	F-GCGTCATGGTAGTGGGTGAAG	160
	R-GCTTTTACCTGCACACCTGGT	
ramA	F-CGGGTAAAGGTCTGTTGCGAA	139
	R- AGTACAAAGGGGAGAGCCTGG	
rpoB	F-AGCGGTGCAGAATAAGTCACG	140
	R-GAAGCTGCTTTCCGTTCCGTA	

DNA Extraction

All isolates' DNA was extracted using the EasyPure® Genomic DNA Kit (Transgene®, China) manufacturing process, and the concentration and purity were measured using the Qubit 4 (ThermoFisher®, USA).

Molecular Detection of *acrA*, *ramA*, and *ropB*

PCR was done using a thermocycler (ThermoFisher, USA), adding 12.5 µl of OneTaq® 2X Master Mix (NEB®, England), 5 µl of the DNA sample, 1.5 µl of each primer at a concentration of 10 pmol/µl, and 4.5 µl of free-nuclease water. The reaction was carried out under ideal PCR conditions for the gene as listed in Table 2. Then 10 µl PCR product and DNA ladder were transferred onto a 1.5% agarose gel, and at the end of the run, gel documentation with a high-resolution camera was utilised to acquire images and evaluate the bands.

Table 2.PCR Conditions for Efflux Pump Genes and *rpoB*

Step	Temperature (ºC)	Time (Minutes : Seconds)	Number of Cycles
Initial denaturation	94	05:00	1
Denaturation	94	00:30	35
Annealing	56[a], 55[b], 55[c]	00:45	
Extension	72	00:45	
Final extension	72	07:00	1

[a]*acrA*, [b]*ramA*, [c]*rpoB*

Gene Expression of *acrA* and *ramA* Genes by RT-qPCR

Preparation of Bacterial Suspension

The gene expression of *acrA* and *ramA* genes was measured before and after each isolate's treatment with the biocide (at ½MIC).

RNA Extraction

The RNA was extracted with TRIzol™ reagent (Invitrogen, USA) according to the manufacturer's instructions and the concentration and purity were measured using the Qubit 4 (ThermoFisher®, USA). cDNA was synthesised using ProtoScript® First Strand cDNA Synthesis Kit (NEB, UK). The resultant cDNA was quantified using Qubit™ dsDNA HS Assay Kit (ThermoFisher®, USA).

Quantitative RT-qPCR

Quantitative detection was based on SyberGreen's fluorescence power. The reaction mixture was composed of the following components, with their quantities as shown in Table 3.

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Table 3.RT-q PCR Components

Reactants	Volume (µl)
Luna Universal qPCR Master Mix	10
Forward primer (10 µM)	1
Reverse primer (10 µM)	1
Template cDNA (ng/µl)	5
Nuclease-free water	3

After this, the real-time PCR programme was set up using the specified thermocycling protocol as shown in Table 4.

Table 4.Thermocycling Protocol and Conditions

Step	Temperature (°C)	Time	Number of Cycles
Initial denaturation	95	60 seconds	1
Denaturation	95	15 seconds	45
Extension	60	30 seconds	
Melt curve	60-95	40 minutes	1

The analysis of Gene Expression follows the Livak formula.[12] These results were normalised to the expression of the housekeeping gene (*rpoB*), as shown below:

Fold change = $2^{-\Delta\Delta Ct}$----------(1)

ΔΔCt = ΔCt (Treatment) - ΔCt (Control)----------(2)

ΔCt = Ct of target gene - Ct of a housekeeping gene----(3)

Statistical Methods

Kolmogorov-Smirnov test was performed to test the normality distribution of data. The study variables were expressed as mean ± standard deviation. T test and one way ANOVA followed by the Tukey test were employed to assess significant differences among means of study variables. The differences were considered significant when p value ≤ 0.05. The statistical analysis was performed using GraphPad Prism version 9.5.0.[13]

Ethical Statement

All participants agreed to provide the investigator with the specimens. The Ethics Committee of the College of Science, University of Baghdad approved this work (Ref. CSEC/0922/0067). Informed consent according to the Declaration of Helsinki was obtained from all participants.

Results

Biofilm-forming Capacity of *K. pneumoniae*

Microtiter plate results for *K. pneumoniae* biofilm production showed that out of 80 isolates, 22 (27.5%) isolates were non-biofilm producers, while 58 (72.5%) were biofilm producers, including 10 (12.5%) moderate and 48 (60%) weak biofilm producers as shown in Figure 1.

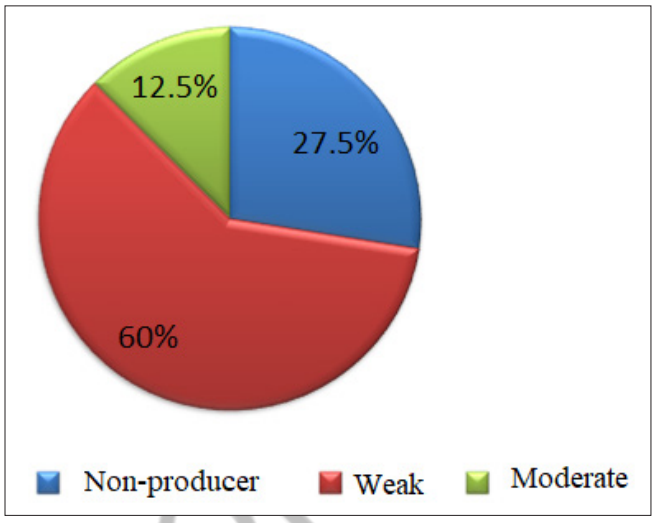

Figure 1.*K. pneumoniae* Biofilm Formation

Minimum Inhibitory Concentration (MIC) of Biocides

The biocide's MIC grouped in Table 5 demonstrates that the MIC of QAC was 2500 µg/ml for all isolates. However, chloroxylenol revealed a difference in MIC values among the isolates. Regarding the MIC of chlorohexidine, the present findings indicated that all *K. pneumoniae* isolates had a MIC value of 19.5 µg/ml except K19 and K101, for which the MIC was 39.06 µg/ml.

Table 5.MIC of Biocides against *K. pneumoniae* Isolates

Isolates Code	MIC of Biocide (µg/ml)		
	QAC (µg/ml)	Chloroxylenol (µg/ml)	Chlorohexidine (µg/ml)
K10	2500	175	19.5
K19	2500	175	39.06
K26	2500	2812	19.5
K28	2500	2812	19.5
K36	2500	351	19.5
K42	2500	351	19.5
K79	2500	351	19.5
K96	2500	175	19.5
K101	2500	351	39.06
K105	2500	1406	19.5

The Effect of QAC on Biofilm

The results in Table 6 displayed the antibiofilm action of

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QAC against identified *K. pneumoniae*. All isolates' biofilm was inhibited by concentrations of 20000 and 10000 µg/ml of biocide. The isolates K36, K79, and K101 were inhibited by all biocide concentrations (20000, 10000, 5000, and 2500 µg/ml). No viable cells were detected and the biofilm showed decreased intensity. The isolates K10, K19, K26, K28, and K105 demonstrated viable cells in a concentration of 5000 and 2500 µg/ml of biocide, and the biofilm intensity decreased. In contrast, the isolates K42 and K96 showed viable cells only in a biocide concentration of 2500 µg/ml with decreased biofilm intensity.

Table 6.Effect of QAC on Biofilm

Isolates Code	QAC Concentration (µg/ml)	OD ± SD	Biofilm Intensity	Bacterial Count ± SD (× 10^5 CFU/ml)
K10	0	0.449^a ± 0.021	Moderate	125^a ± 2.32
	2500	0.129^b ± 0.004	Weak	8^b ± 0.52
	5000	0.124^b ± 0.002	NP	6^b ± 0.17
	10000	0.115^b ± 0.005	NP	ND
	20000	0.128^b ± 0.002	Weak	ND
K19	0	0.680^a ± 0.012	Moderate	5^a ± 0.98
	2500	0.103^b ± 0.001	NP	0.088^b ± 0.006
	5000	0.124^b ± 0.006	NP	0.076^b ± 0.004
	10000	0.126^b ± 0.007	Weak	ND
	20000	0.116^b ± 0.004	NP	ND
K26	0	0.26^a ± 0.019	Moderate	112^a ± 3.05
	2500	0.118^b ± 0.004	NP	12.5^b ± 1.55
	5000	0.125^b ± 0.014	NP	7.8^b ± 0.73
	10000	0.125^b ± 0.002	NP	ND
	20000	0.116^b ± 0.005	NP	ND
K28	0	0.272^a ± 0.009	Moderate	58^a ± 2.06
	2500	0.113^b ± 0.001	NP	1.2^b ± 0.22
	5000	0.114^b ± 0.004	NP	0.36^c ± 0.031
	10000	0.123^b ± 0.003	NP	ND
	20000	0.157^b ± 0.015	Weak	ND
K36	0	0.306^a ± 0.021	Moderate	99 ± 3.34
	2500	0.120^b ± 0.005	NP	ND
	5000	0.119^b ± 0.005	NP	ND
	10000	0.117^b ± 0.006	NP	ND
	20000	0.126^b ± 0.010	Weak	ND
K42	0	0.272^a ± 0.050	Moderate	175^a ± 3.81
	2500	0.132^b ± 0.008	Weak	0.475^b ± 0.062
	5000	0.136^b ± 0.011	Weak	ND
	10000	0.128^b ± 0.014	NP	ND
	20000	0.117^b ± 0.010	NP	ND
K79	0	0.341^a ± 0.047	Moderate	765 ± 3.28
	2500	0.199^b ± 0.004	Weak	ND

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	5000	$0.191^{b} \pm 0.042$	Weak	ND
	10000	$0.171^{b} \pm 0.041$	Weak	ND
	20000	$0.196^{b} \pm 0.016$	Weak	ND
K96	0	$0.241^{a} \pm 0.020$	Moderate	134^{a}
	2500	$0.110^{b} \pm 0.034$	NP	$78^{b} \pm 2.09$
	5000	$0.116^{b} \pm 0.038$	NP	ND
	10000	$0.116^{b} \pm 0.040$	NP	ND
	20000	$0.117^{b} \pm 0.005$	NP	ND
K101	0	$0.245^{a} \pm 0.011$	Moderate	14 ± 1.38
	2500	$0.114^{b} \pm 0.005$	NP	ND
	5000	$0.118^{b} \pm 0.005$	NP	ND
	10000	$0.119^{b} \pm 0.003$	NP	ND
	20000	$0.113^{b} \pm 0.004$	NP	ND
K105	0	$0.254^{a} \pm 0.006$	Moderate	$73.5^{a} \pm 3.76$
	2500	$0.113^{b} \pm 0.004$	NP	$0.465^{b} \pm 0.041$
	5000	$0.111^{b} \pm 0.005$	NP	$0.0092^{c} \pm 0.00022$
	10000	$0.117^{b} \pm 0.003$	NP	ND
	20000	$0.115^{b} \pm 0.004$	NP	ND

NP: non-producer, ND: not detected. The means with different letters (a, b, and c) have significant differences.

Determination of the Conformation of Biofilm by Scanning Electron Microscope

Scanning electron microscope (SEM) image revealed the untreated *K. pneumoniae* biofilm as large aggregations of cells in which the structures identified were made up of microcolonies of bacterial cells embedded in an exopolysaccharide (EPS) matrix and separated from each other by water channels (Figure 2a). Comparatively, scarce *K. pneumoniae* cell distribution was seen after exposure to biocides (Figure 2b).

Molecular Detection of *acrA, ramA, ropB* Gene

acrA gene was found in nine out of 10 (90%) isolates, while *ramA* and *rpoB* genes were detected in all isolates, as shown in Figure 3.

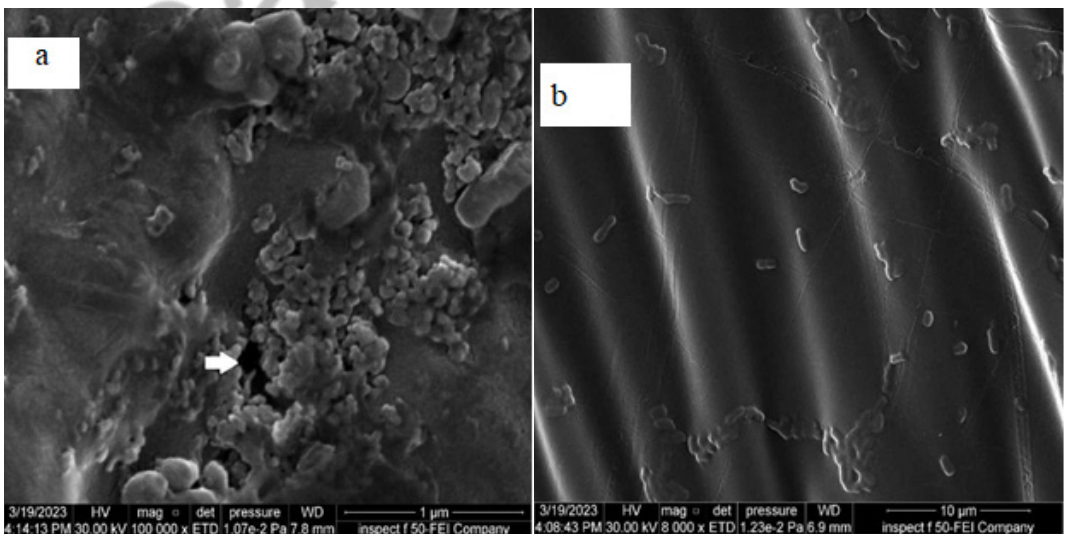

Figure 2.Scanning Electron Microscopy for the Effect of Biocides on Biofilm Conformation (Arrow denotes a Water Channel)

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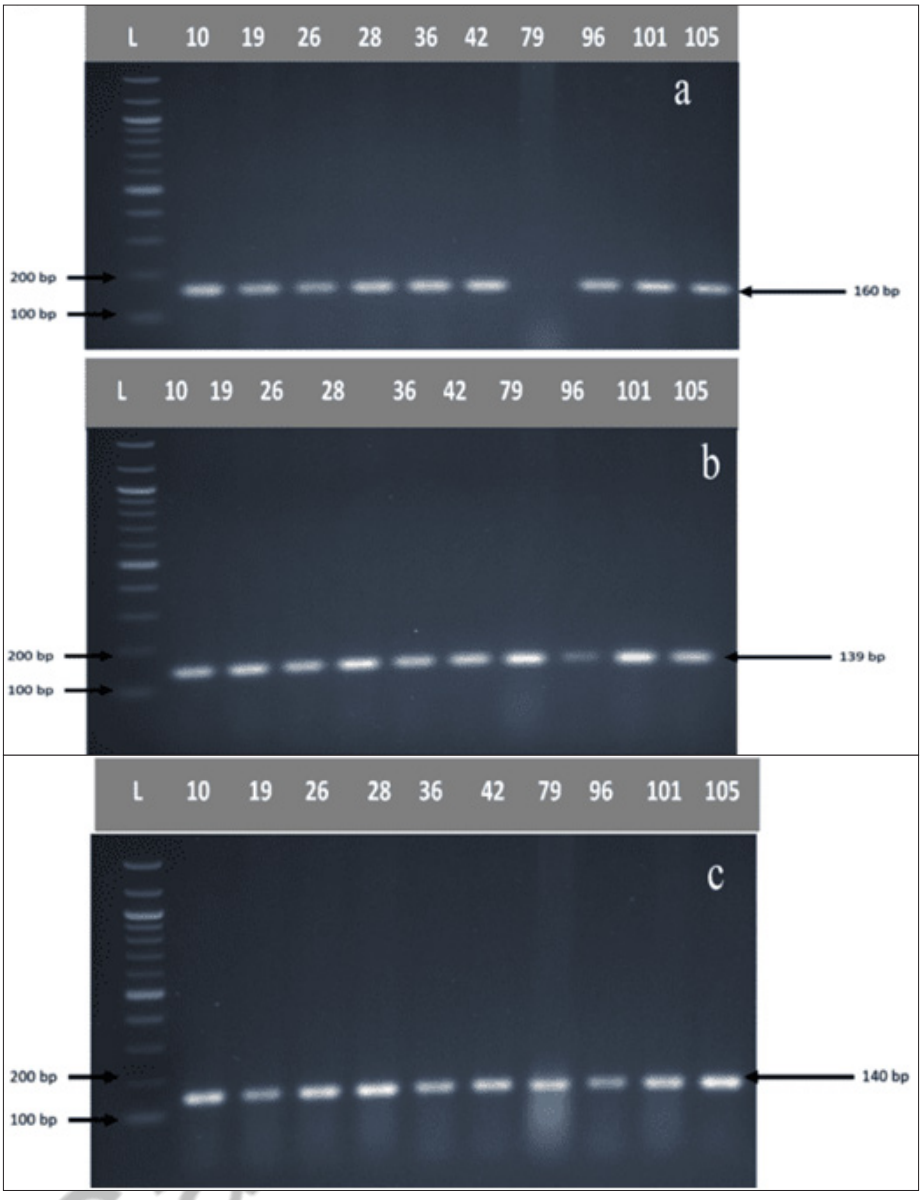

Figure 3.Agarose Gel Electrophoresis of PCR Product of (a) *acrA*, (b) *ramA*, and (c) *rpoB* Genes run on 2% Agarose (80 min at 80 volts) Stained with Ethidium Bromide, Lanes 10, 19, 26, 36, 42, 79, 96, 101, and 105 represent *K. pneumoniae* Isolates; L: DNA ladder (100 bp)

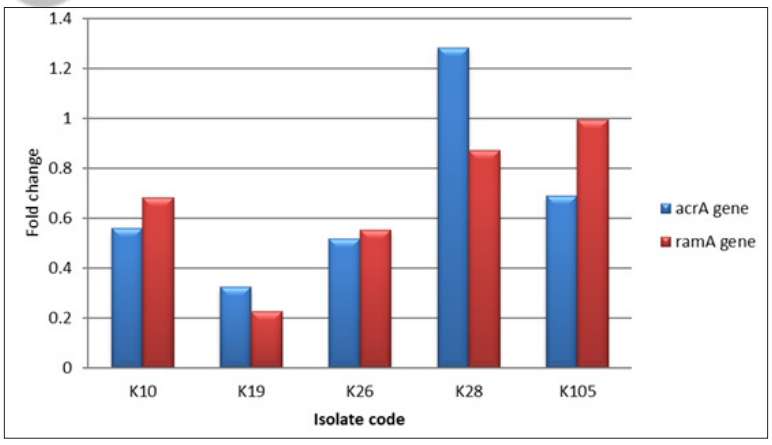

Figure 4.Gene Expression of Efflux Pump Genes *acrA* and *ramA* in *K. pneumoniae* Isolates

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Gene Expression of *acrA* and *ramA* Gene under the Effect of Biocide by RT-qPCR

The chosen isolates represented those that were most affected by biocide and carried *acrA* and *ramA* genes (Table 6). Figure 4 shows the effects of biocides on *acrA* and *ramA* gene expression using *rpoB* as the housekeeping gene. Exposure to biocide downregulated the expression of the efflux pump gene, *acrA*, and its regulator, *ramA*, as compared to untreated control.

Discussion

K. pneumoniae can form a biofilm (an aggregation of cells embedded within a self-produced matrix consisting of exopolysaccharides, proteins, DNA, and lipopeptides of extracellular polymeric substance) that can adhere to each other and to a surface.[14]

Microtiter plate results for *K. pneumoniae* biofilm production demonstrated that most isolates formed biofilm and 60% of them developed weak biofilm. These results agreed with a local study in Baghdad city done by Ali et al.,[15] in which it was observed that out of 39 *K. pneumoniae* isolates, 1 (2.56%) was a moderate biofilm producer, 21 (53.85%) were weak biofilm producers, and 17 (43.59%) were non-producers. On the contrary, a local study by Jabar et al.[16] found that all *K. pneumoniae* isolates were strong biofilm producers, apart from their origin. Also, the present findings were in disagreement with the study by Makhrmash et al.[3] as they revealed that among 56 isolates of *K. pneumoniae,* 10 (18%) formed weak biofilm, 14 (25%) formed moderate biofilm, and 32 (57%) formed strong biofilm.

The MIC of the QAC against all identified *K. pneumoniae* isolates was 2500 µg/ml which agrees with the results of a study done by Frolov et al.[17] They demonstrated that all QACs that were tested possessed a high level of antibacterial activity against *K. pneumoniae* planktonic bacteria. Also, our results came in parallel with a study from Iran by Monirzadeh et al.[18] which utilised two quaternary ammonium biocides and found that *K. pneumoniae* strains were highly sensitive to these biocides.

Quaternary ammonium-based disinfectants are excellent antimicrobials because they kill microbes effectively, last a long time, and are suitable for the environment. However, their improper use could cause bacteria to adapt to these disinfectants and accelerate the emergence of resistant strains by encouraging the development of *qac* genes (resistance to QACs), which results in significant problems.[19]

The minimum inhibitory concentrations of chloroxylenol exhibited a variation among isolates. These results are consistent with a study from Egypt by Samir et al.,[20] which used chloroxylenol (Dettol 4.8%) against *K. pneumoniae* and revealed that all isolates were inhibited by 1.6 mg/ml, while the study by AlBany et al.[21] in Duhok City does not agree with the results as it revealed that *K. pneumoniae* isolates resist all concentrations of chloroxylenol.

All *K. pneumoniae* isolates had a chlorohexidine MIC of 19.5 µg/ml except two isolates (for which the MIC was 39.06 µg/ml), which is in agreement with the study from the Kingdom of Saudi Arabia by Vijayakumar et al.[22] It revealed that the chlorohexidine MIC range was 16-64 µg/m and there was no evidence of a decreased sensitivity of *K. pneumoniae* to the biocides. Also, Afshar-Yavari et al.[23] indicated that chlorhexidine's MIC values ranged from 32 µg/ml to 128 µg/ml against *K. pneumoniae* isolated from a clinical specimen.

In the experiment on the effect of biocide on biofilm, the QAC displayed antibiofilm action against all identified *K. pneumoniae* isolates; the substantial reduction in biofilm was accompanied by a decrease in bacterial count in the biofilm. The quantitative assessment of mature biofilms formed by *K. pneumoniae* isolates using a colourimetric microtiter plate test demonstrated that the biofilm of isolates became weak following biocide treatment due to significantly decreased biofilm thickness. The decrease in OD after treatment indicated that these concentrations prevented bacteria from adhering to the polystyrene surface of the microtiter plate, which resulted in the separation of the pre-formed biofilm.[24]

A study by Elekhnawy et al.[25] indicated that adaptation to benzalkonium chloride (BAC) biocide (a QAC biocide) increased the proportion of *K. pneumoniae* isolates that form biofilms from 30% to 56%. Also, the viability of *K. pneumoniae* in the biofilm population was studied before and after BAC adaptation. The viability of the bacterial cells did not change much after BAC adaptation.

The present results match with the study findings of Frolov et al.[17] about the biocidal action of QACs against clinical pathogens, which showed that QACs were effective against *K. pneumoniae* biofilm. Also, a study by Machuca et al.[26] of polyhexanide (polyhexamethylene biguanide)-betaine (PHMB-B) compared to 2% chlorhexidine against *K. pneumoniae* biofilms, showed biocides' effectiveness.

A study of Dettol's effect on the formation of biofilm by pathogenic microorganisms conducted by Najim[27] revealed that the biofilm inhibition appeared at the dilution of 1:10 (MIC) of the detergent for most bacterial isolates when compared with the positive control, and the highest dilution of detergent (more than 1:10) lost the ability to eradicate biofilm growth.

The inhibition of biofilm by biocide was confirmed by SEM, and the current study matches a local study conducted by Aziz and Al-Jubori [28] who used SEM analysis to understand the biofilm development among *K. pneumoniae* isolates collected from different clinical samples. Also, the study by

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Adeosun et al.[29] employed SEM micrographs to examine the biofilms formed by *K. pneumoniae* strains after exposure to phytol and glycitein as compared to untreated biofilms.

The results of conventional PCR for *acrA* gene indicated that the frequency of these genes was 90%. These results are consistent with the local study by Jabar and Hassoon[4] which demonstrated that the efflux pump *acrA* gene was present in all selected *K. pneumoniae* isolates. The study by Khalid and Ghaima[30] also agrees with our results as the study reveals that *acrA* gene was found in all tested *K. pneumoniae* isolates.

The result of *ramA* gene detection revealed that the frequency of these genes was 100%, which agrees with a local study by Al Saadi and Al-Dreaghi[31] as this study indicated that the *ramA* gene was 100% positive for *K. pneumoniae*. Also, our results agree with those of Al-Bayati and Samarasinghe[32] as they detected the *ramA* gene in *K. pneumoniae* isolates. The use of the *rpoB* gene as a housekeeping gene in the current study is consistent with the study of Hammoudi and Hussein[33] and that of Al-Zubaidi and Al-Taai[34] both of which used the *rpoB* gene as a housekeeping gene.

The RT-qPCR analysis for *acrA* and *ramA* gene expression showed that biocide downregulates the efflux pumps *acrA* and *ramA* gene expression in the *Klebsiella pneumoniae* biofilm.

Our results contradict the findings of Wand et al.[35] who found that exposure to a biocide like chlorhexidine led to an increased expression of *acrA* and its activator *ramA* in *K. pneumoniae*.

Conclusion

QAC was able to reduce the intensity and viable count of *K. pneumoniae* biofilms. Moreover, QAC at ½MIC downregulated the gene expression of the efflux pump gene (*acrA*) and its regulator (*ramA*).

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Conflict of Interest: None

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