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Antibacterial and molecular effects of povidone –iodine on pyocyanin-related gene expression in *Pseudomonas aeruginosa*

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Abstract

The objective of this study was to investigate the antibacterial and anti-pyocyanin effects of povidone–iodine against *pseudomonas aeruginosa.* Twelve isolates of *P. aeruginosa* were collected from wound samples and identified based on cetrmide agar and 16S rRNA gene analysis. PCR detection revealed that all isolates carried the *Phz*M, *phz*H, and *phz*S genes, which are responsible for pyocyanin pigment production. Pigment identification showed that showed that the isolates produced blue green pyocyanin pigment after 24, 48, and 72 hours of incubation. Two concentrations of povidone–iodine (4% and 10%) were tested for their inhibitory effects. The results indicated that povidone -iodines (4% and 10%)) inhibition of *P. aeruginosa* growth. RT-PCR analysis demonstrated that 4%and 10% povidone –iodine decresed the expression levels of *Phz*M, *phz*H, and *phz*S genes in the treated isolate (10), while increase the levels in the isolate (1). These findings suggest that povidone –iodine may influence pyocyanin-related gene expression in *P. aeruginosa*, although the response appears to be strain-dependent. Povidone–iodine exhibited significant antibacterial activity and influenced pyocyanin-related gene expression in *P. aeruginosa*. However, the molecular response differed between the tested isolates, suggesting that its effect on pyocyanin-associated virulence may be strain-specific.

Keywords: povidone–iodine, *Psedomonas aeruginos,* Pyocyanin, Antibacterial activity, Gene expression.

1. Introduction

Pseudomonas aeruginosa is an opportunistic bacterial pathogen responsible for many acute and chronic infections, particularly in individuals with compromised immunity (Li *et al.*,2015). *P. aeruginosa* is a Gram-negative, aerobic bacillus measuring approximately 0.5-0.8 μm in width and 1.5-3.0 μm in length. This organism can produce several types of phenazines, including pyoverdine (yellow and fluorescent), pyomelanin (light brown), pyroprene (red and brown), and pyocyanin (blue-green), which is the most abundant and biologivally active compound in this group (DeBritto *et al.*,2020; Jayaseelan *et al.*,2014). Pyocyanin plays a crucial role in the virulence of *P. aeruginosa* by generating reactive oxygen species (ROS), impairing ciliary function, and causing oxidative damage to host tissue, particularly within the lungs (Al-Thabhawee and Al-Dahmoshi, 2021).

The biosynthesis of pyocyanin originates from chorismic acid (CA), a key precursor compound. This process is regulated by two (*phz*A1B1C1D1E1F1G1 and *phz*A2B2C2D2E2F2G2), which control conversion of CA to phenazine-1-carboxylic acid (PCA). Subsequently, PCA is transformed into pyocyanin through the action of two genes, *phz*M and *phz*S. Specifically, PCA is converted to 5- methylphenazine-1-carboxylic acid betaine (MPCBA) by the phenazine-specific methyltransferase *Phz*M. MPCBA is then decarboxylated by flavin-dependent monooxygenase PhzS, ultimately producing pyocyanin (Marey et al.,2024). Given its central role in bacterial virulence and oxidative stress, inhibition of pyocyanin synthesis is considered a promising strategy for reducing the pathogenicity of *P. aeruginosa*. The global rise in bacterial resistance to antibiotics-including *P. aeruginosa,* methicillin-resistant *Staphylococcus aureus* (MRSA), and Enterococcus species- has renewed scientific interest in antiseptics and disinfectants as alternative or adjunct antimicribial agents. Among these, povidone-iodine (PVP-I) has drawn particular attention due to its broad-spectrum activity against bacteria, fungi, and viruses (Lachapelle *et al.*, 2013). PVP-I acts primarly by releasing free iodine,which penetrates microbial cell membranes and oxidizes essential biomolecules such as proteins, nucleotides, and fatty acids, ultimately leading to cell death(Kenesheva *et al.*,2023) , and it's found povidone iodine, silver lactate, chlorhexidine digluconate, and octenidine hydrochloride have also been observed to play a role in wound healing by reducing the activity of wound proteases because they act as antibacterial agents(Pavlík *et al.*,2019).

Recent studies have suggested that povidone-iodine not only exerts potent bactericidal effects but may also interfere with bacterial virulence mechanisms, including pigment production, quorum sensing, and biofilm formation. However, the specific influence of povidone-iodine on pyocyanin synthesis and regulation in *P. aeruginosa* remains poorly understood. Understanding this interaction could provide valuable insights into new therapeutic or preventive strategies aimed at attenuating bacterial virulence rather than solely relying on traditional antibiotics. Therefore, the present study aims to investigate the antibacterial and antipuocyanin effects of povidone-iodine against *P. aeruginosa*, with the goal of elucidating its potential role as both a disinfectant and a virulence-modulating agent.

2. Materials and Methods

Bacterial isolates and DNA extraction

Isolates of *P. aeruginosa* (12 isolates) were obtained from wound infections at the Burn Specialist Hospital, Medical city, Baghdad. Identification was confirmed by amplification of the 16S rRNA gene (Forbes *et al.*,2007). Genomic DNA was extracted using the Promega DNA extraction mini kit (USA) according to the manufacturer's instructions. PCR amplification of the 16S rRNA gene was performed using specific primers shown in table 1(Spilker *et*

al.,2004). The amplified products were electophoresed on 1% agarose gel stained with ethidium bromide and visualized under a UV Tranilluminator (figure 2). DNA was extracted using the Promega mini kit (USA). PCR amplification of the *phz*H, *phz*S and *phz*M genes was performed using specific primers under the conditions summarized in table 2. The PCR reactions were carried out in a Biorad thermocycler (USA) following the protocol described by (Spilker *et al.*,2004).

Table 1. Primer pairs used in the study

Primer		Sequence (5-3)	Product Size (bp)	Annealing Tempra-ture	References
Ps. Spp	F	GACGGGTGAGTAATGCCTA	956	61	Spilker *et al.*,2004
	R	CACTGGTGTTCCTTCCTATA			
*Phz*H	F	TGCGCGAGTTCAGCCACCTG	214	60	Li *et al.*,2015
	R	TCCGGGACATAGTCGGCGC			
*phz*S	F	CTGGTCGCCTATCCGATCTC	507	61	Al-Thabhawee, and Al-Dahmoshi, 2021.
	R	GCTCTTCTCGGTCTTCGGTC			
*Phz*M	F	GGATGGCCTTGGTCAATTCG	350	60	
	R	GATCTTCCAGGGCGATACCC			

Table (2) PCR thermo cycle conditions for *phz*H, *phz*S, *phz*M and 16S rRNA primer by PCR technique

Steps	Temprature C°	m: s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	60-61	00:30	
Extension	72	00:30	
Final extension	72	07:00	1
Hold	10	10:00	

Pyocyanin pigment production

P. aeruginosa isolates (12 isolates) were cultured on nutrient agar by the streaking method and incubated at 37 C° for 24, 48, and 72 hours for pyocyanin production. The antibacterial activity of povidone-iodine was evaluated using the broth microdilution method in sterile 96-well microtiter plates. Serial twofold dilutions of povidone-iodine were prepared in nutrient broth from the stock solutions (4% and 10%). Each well was inoculated with a standardized bacterial suspension of *P. aeruginosa* isolates. After incubation at 37 °C for 24 hours, 20 µL of reassuring solution was added to each well and the plates were incubated for an additional

2–4 hours. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of povidone–iodine that prevented the color change of reassuring from blue to pink, indicating nhibition of bacterial metabolic activity.

RNA extraction and *phz*M, *phz* H and *phz* S genes gene expression

Statistical analysis

Gene expression data obtained from RT-PCR were analyzed using the comparative Ct (2-ΔΔCt) method after normalization with the 16S rRNA housekeeping gene. All experiments were performed in triplicate to ensure reproducibility. The results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism software, and differences between treated and control samples were evaluated using Student's t-test. A p-value < 0.05 was considered statistically significant.

RNA was extracted using the Amini kit (Promega, USA). The 16SrRNA gene was used as a housekeeping gene, while the *phz*M, *phz* H, and *phz* S genes were selected for expression analysis. Specific primer sequences were showed for each gene in table 1, and quantitative PCR (qPCR) was performed under optimized conditions as described in table 3 for 16SsRNA and *phz*S genes (4), while *phz*M, *phz* H showned in table 4 (1

Table3. optimal condition to detection 16S rRNA (House keeping) and *phz* S genes expression

Stages	N. of cycles	Temprature	Times
Reverse Transcriptase enzyme activation	1	37Cº	15 minutes
Initial Denaturation		°95 C	5 minutes
Denaturation	40	°95 C	20 Seconds
Annealing		°61 C	20 seconds
Extension		°72 C	20 seconds
Melt on green		72-95 Cº	Cº/ second0.3

Table 4. optimal condition to detection *phz*M and *phz* H genes expression

Stages	N. of cycles	Temprature	Times
Initial Denaturation	1	°95 c	5 minutes
Denaturation	40	°95 c	20 Seconds
Annealing		°60 c	20 seconds
Extension		°72 c	20 seconds
Melt on green		72-95 cº	Cº/ second0.3

Povidone –Iodine Effects on *P. aeruginosa* Pigment Genes

The antibacterial activity of povidone–iodine was evaluated using the broth microdilution method in sterile 96-well microtiter plates. Serial twofold dilutions were prepared from the stock solutions of povidone–iodine (4% and 10%) in nutrient broth. Each well was inoculated with a standardized bacterial suspension of *P. aeruginosa*. The plates were incubated at 37 °C for 24 hours. After incubation, 20 µL of resazurin solution was added to each well and the plates were incubated for an additional 2–4 hours. Resazurin acts as an indicator of bacterial

metabolic activity; viable bacteria reduce the blue dye to pink resorufin. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of povidone–iodine that prevented the color change from blue to pink, indicating inhibition of bacterial metabolic activity.

3. Results

Microorgnism Identification

Twelve isolates of *Pseudomonas aeruginosa*were obtained on Cetrimide agar medium. This medium selectively supports the growth of *P. aeruginosa* while inhibiting other bacterial species. The colonies appeared pigmented, mostly blue-green in color, due to the production of the characteristic pigment(pyoverdine).

Molecular identification using 16S rRNAgene expression

Genomic DNA was extracted from all twelve clinical isolates of *p. aeruginosa.* The 16S rRNA gene was successfully amplified by PCR in all isolates, producing an amplicon of approximately 956 bp on a 1.5% agarose gel. The presence of this band confirmed the molecular identification of all isolates as *Pseudomonas aeruginosa* (figure1).

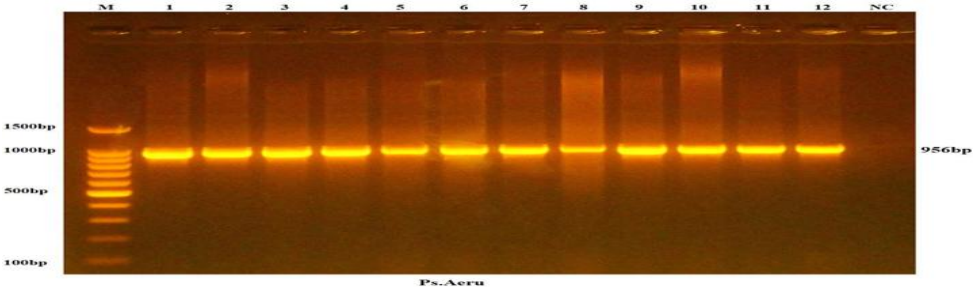

Figure 1. PCR amplification of the 16S rRNA gene *in Pseudomonas aeruginosa* isolates. DNA fragments were separated on 1.5% agarose gel and visualized under UV light. Lane M represents the DNA ladder (100 bp marker), while lanes 1–12 correspond to the clinical isolates showing the expected band size (~956 bp).

The total DNA of each clinical isolate (12 isolates) of *Pseudomonas aeruginos*a *was* used for amplification of *PhzM* , *phz*H, and *phz*S genes by PCR. The results revealed that all isolates processed these genes, with amplicon sizes of 350 bp for *Phz*M , 214 bp for *phz*H, and 507 pb for *phz*S as shown in Figure (2,3 and 4). This confirms the presence of phenazine biosynthetic genes in all tested isolates of *Pseudomonas aeruginosa.*

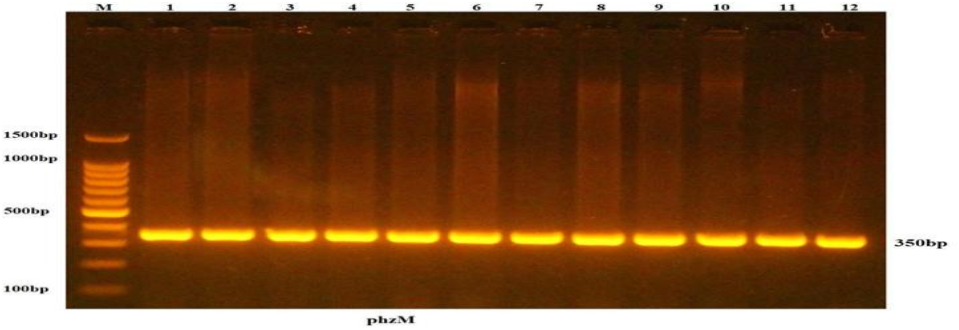

Figure 2. PCR amplification of the *phz*M gene (350 bp) in clinical isolates *Pseudomonas aeruginosa analyzed by* 1.5% agarose gel electrophoresis.

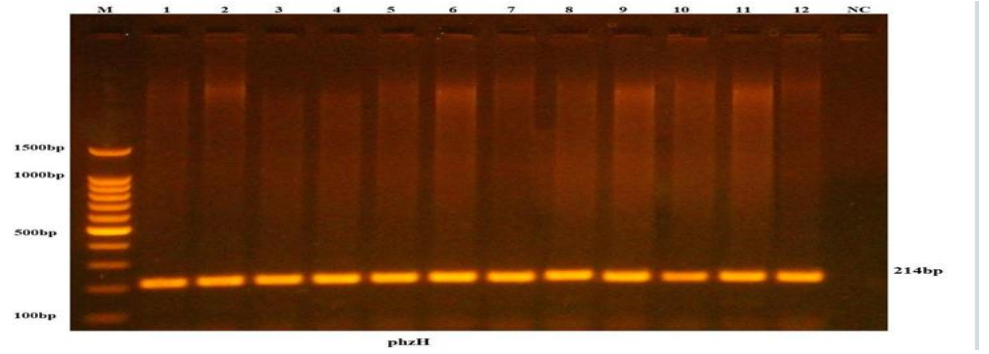

Figure 3. PCR amplification of the *phzH* gene (214 bp) in clinical isolates of *Pseudomonas aeruginosa analyzed* by 1.5% agarose gel electrophoresis.

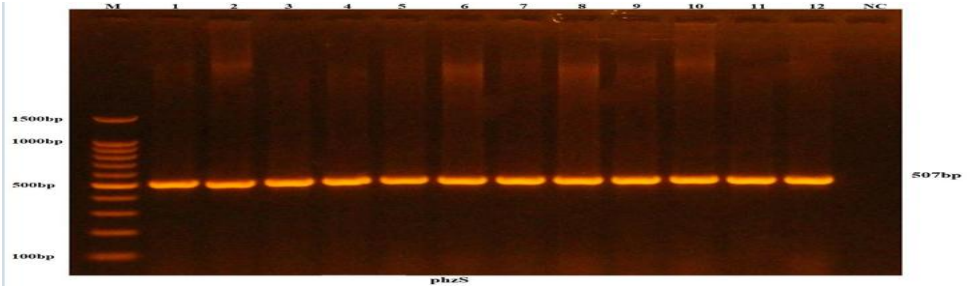

Figure 4: PCR amplification of the *phz* gene (507 bp) in clinical isolates of *Pseudomonas aeruginosa analyzed* by 1.5% agarose gel electrophoresis.

Pyocyanin pigment production

Table (5) demonstrates the effect of different incubation periods (24,48, and 72 hours) on the production of pyocyanin pigment by *Pseudomonas aeruginosa* isolates incubated at 37 C°. The results indicate that the intensity and color of the pigment varied among isolates and incubation times, ranging from yellow to green-yellow, reflecting differences in pyocyanin synthesis and metabolic activity during bacterial growth.

Table 5. Effect of incubation period on pyocyanin pigment production in *Pseudomonas aeruginosa* isolates.

Isolate	Pigment color (24h)	Pigment color (48 h)	Pigment color (72 h)
1	yellow	green	green
2	Green	Green -yellow	Green -yellow
3	yellow	Yellow	green
4	Green	Green -yellow	Green -yellow
5	Green	Green -yellow	Green -yellow
6	yellow	yellow	yellow
7	No. pigment	yellow	yellow
8	yellow	yellow	yellow
9	No. pigment	No. pigment	No.pigment
10	No. pigment	No. pigment	yellow
11	No. pigment	yellow	Yellow-green
12	No. color	yellow	yellow

Effect of Iodine on*Pseudomonas aeruginosa*growth

The minimum inhibitory concentrations (MICs) of the tested products (4% and 10%) povidone-iodine were measured using resazurin-based turbidimetric (TB) assay. The products were serially diluted twofold, ranging from 4% to 0.007% and 10% to 0.019% respectively. All sterility control wells for all tested bacteria remained blue after overnight incubation, followed by a 2–4-hour incubation with resazurin. In contrast, all wells in the growth control column (containing both bacteria and medium) showed a color change from blue to pink or pale pink, indicating bacterial growth. The minimum inhibitory concentration (MIC) of povidone–iodine against the testedPseudomonas aeruginosaisolates was determined using the resazurin-based microdilution assay. The MIC values were identified as the lowest concentrations that prevented the resazurin color change from blue to pink. Based on the serial dilution assay, the MIC values were approximately 1% for the 4% stock solution and 1.25% for the 10% stock solution, as shown in figure 5.

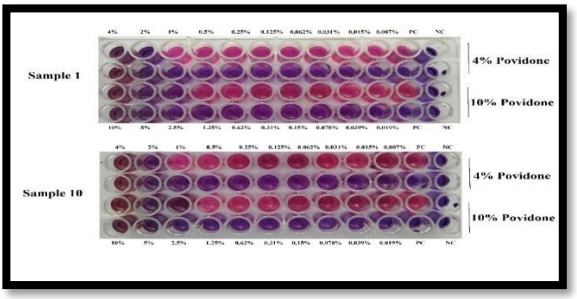

Figure 5. Effect of povidone –Iodines (4% and 10%) on *P. aeruginosai* solates (isolate 1 and 10) determined by resazurin- assay. MIC was defined as the lowest concentration that inhibited bacterial metabolic activity as indicated by the absence of resazurin color change.

Detection of the Effect of Povidone –Iodine (sub-MIC) on *phz*M, *phz* H and *phz* S Gene Expression on Pseudomonas aeruginosa using RT-PCR.

RT-PCR analysis (Figure 7) demonstrated that povidone-iodine treatment affected the expression of pyocyanin-related genes differently among the tested isolates. In isolate (1), the expression of the phzM gene increased after treatment with both 4% and 10 % povidone-iodine compared with the untreated control. In contrast, isolate (10) showed a clear reduction in phzM gene expression following treatment. These results indicate that the influence of povidone-iodine on pyocyanin biosynthesis genes is not uniform and may vary depending on characteristics of individual *Pseudomonas aeruginosa* isolates.

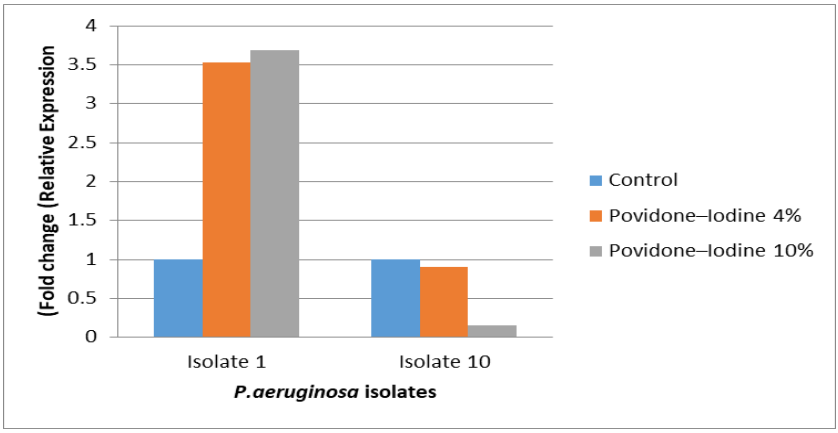

Figure 7. Effect of povidone–iodine (4% and 10%) on *phz*M gene expression in *P. aeruginosa* isolates (1 and 10). Data are presented as mean ± SD of three independent experiments.

RT-PCR analysis (Figure 8) demonstrated that treatment of *Pseudomonas aeruginosa* isolate (1) with povidone-iodine (4% and 10%) led to an increase in phzH gene expression to 2.70 and 2.16-fold compared to the control (1-fold). In contrast, *Pseudomonas aeruginosa* isolate (10) showed a reduction in phzH gene expression to 0.37 and 0.42- fold compared to control when treated with 10 ml and 1.25 ml of povidone-ioden (4% and 10%) respectively. These findings suggest that the response of pyocyanin-related genes to povidine-iodine treatment may be strain dependent.

The results shown in figure (9) demonstrated that povidone-iodine also affected *phz*S gene expression differently in the two tested isolates. In isolate (1), *phz*S expression increased after treatment, whereas in isolate (10) *phz*S expression decreased compared with the untreated control. This further supports that the influence of povidone-iodine on pyocyanin-related genes varies between isolates and may be strain-specific.

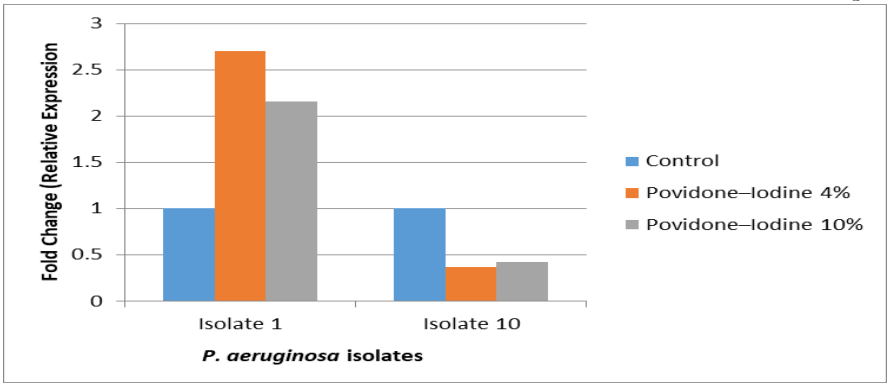

Figure 8. Effect of povidone –iodine (4% and 10%) on *phz*H gene expression in *P. aeruginosa isolates* (1 and 10). Data are presented as mean ± SD of three independent experiments.

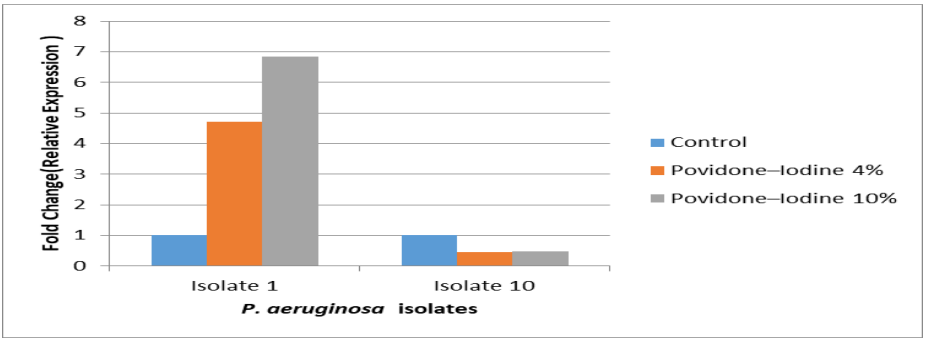

Figure 9. Effect of povidone –iodine (4% and 10%) on *phz*S gene expression in *P. aeruginosa isolates* (1 and 10). Data are presented as mean ± SD of three independent experiments.

4. Discussion

The diagnostic analysis using the 16S rRNA gene confirmed that all *P. aeruginosa* isolates were correctly identified. In the present study, PCR analysis confirmed that all tested isolates carried the *phz*M, phzH, and *phz*S genes, which are involved in pyocyanin biosynthesis. However, previous studies have reported variability in the distribution of these genes among clinical isolates of *P. aeruginosa,* with may reflect genetic diversity among strains isolated from different sources. Previous study suggesting genetic diversity in pyocyanin biosynthetic pathways among clinical isolates of *P. aeruginosa* (Marey *et al.*, 2024). These finding align with previous studies reporting variable distribution of phzM and phzS genes and their correlation with pyocyanin pigment production (Al-Thabhawee and Al-Dahmoshi, 2021).

The expression level of phzH, was increased by 9.5- fold indicating a strong actication of the quorum sensing (QS) system. These up regulations were statistically significant ($P < 0.05$) and are consistent with findings by Li *et al.* (2015), who demonstrated that exogenous AI-2 molecules can upregulate QS-associated genes in *P. aeruginosa* PAO1, thereby enhancing

virulence and pigment synthesis. These increases were statistically significant when normalized to a control gene ($P < 0.05$).

Furthermore, the current results indicate that certain MDRPA(multidrug-resistant *P. aeruginosa)* strains maintain their ability to produce pyocyanin despite the stress of antiseptic exposure.This pigment production is directly regulated by the *phzA-G* operon, primarily through *phzM, phzS*, and *phzH*, which catalyze the conversion of phenazine-1-carboxylic acid to *pyocyanin* (Al-Thabhawee and Al-Dahmoshi, 2021; Abd El-Baky *et al.*,2020). The present findings suggest that povidone–iodine may influence the regulation of pyocyanin biosynthesis genes in *Pseudomonas aeruginosa*. However, the observed responses differed between the tested isolates. While an increase in the expression of *phzM, phzH*, and *phzS* was observed in isolate (1), a decrease was detected in isolate (Spilker *et al.*,2004). This variation may reflect differences in genetic background, regulatory pathways, or stress responses among clinical isolates. Therefore, the influence of povidone–iodine on pyocyanin-associated virulence genes appears to be isolate-dependent.

The RT-PCR results obtained in the present study demonstrated that the effect of povidone–iodine on pyocyanin-related genes was not identical in the tested isolates. While the expression of phzM, phzH, and phzS genes increased in isolate (1), a reduction in gene expression was observed in isolate (10). This difference suggests that the molecular response to povidone–iodine may depend on the genetic background and physiological characteristics of individual *P. aeruginosa* strains. Therefore, the influence of povidone–iodine on virulence-associated genes should be interpreted cautiously and may be considered strain-dependent. There are several factors affecting the production of pyocyanin, including the type of medium used, the period of incubation, it was found King's A liquid medium was the best for pigment production compared to the nutrient broth medium. The shaking and incubation period (3-4 days) also improved the production of pyocyanin pigment (Elbargisy, 2021). It was noted that the production of pyocyanin pigment increased during the period between at 24-72 hours. and was also noted that a long incubation period may lead to a decrease in production (Gahlout *et al.*, 2017). It was also observed that approximately 43.3% of the *P. aeruginosa isolates were able* to produce pyocyanin, mainly those obtained from respiratory tract samples. The highest percentage of pigment-producing isolates (57%) was found in samples derived from skin and soft tissues, while isolates recovered from urine, blood and bone showed no ability to produce the characteristic green pigment. Notably, isolate PA25 obtained from sputum and PA10 isolated from wounds exhibited the highest pyocyanin production levels (Marey *et al.*,2024).These findings agreed with previous study who reported that 20% of *Pseudomonas aeruginosa* isolates carried both phzM and phzS genes, while 33.3% of the isolates lacked both genes, indicating genetic variation that may influence pigment biosynthesis and virulence potential(Abd El-Baky *et al.*,2020).

Results showed that 0.66% povidone-iodine (PI) was effective in treating corneal abscesses caused by *Pseudomonas aeruginosain* contact lens wearers (Castelnuovo, 2022). Similarly, Chlorhexidine gluconate (CHG)inhibited the growth of all *S. aureus* isolates and five out of six of *P. aeruginosa* isolates. In comparison, povidone-iodine inhibited the growth of all isolates and reduced biofilm formation at concentrations of 0.33% or lower. Meanwhile, CHG at a concentration as 0.0004% inhibited biofilm formation in all *S. aureus* isolates and in three out of six *Pseudomonas aeruginosa* isolates (Coles *et al.*,2024). Furthermore, an experimental study using a 0.6% ophthalmic solution containing PVP-I demonstrated high antimicrobial activity against *S. aureus* and *P. aeruginosa*. However, its fungicidal action was slower, requiring up to 30 minutes of light exposure for effective inhibition, particularly against Candida spp. (Abd El-Baky *et al.*, 2020). Several studies reported that Povidone –iodine (PVP-I) exhibits stronger antimicrobial activity than chlorhexidine gluconate (CHG), polyhex- anide (PHMB), and octenidine (OCT), due to its broad spectrum of action against Gram-negative bacteria and Gram-positive bacteria (Barreto *et al.*,2020). Previous study showed that 0.6% of povidone iodine was faster than of 5% in killing Gram-negative and Gram-positive bacteria

with a reduction rate of 3-log10 of the number of bacteria (Musumeci *et al.*,2019). Previous studies have indicated that a 1% PVP-I solution reduces bacterial loads in wounds ccompared to saline solution (Kanno *et al.*,2016). Another study reported that indicated when *Pseudomonas aeruginosa* was cultured in a medium containing cefotaxime, ampicillin or amoxiclav, the antibiotics enhanced the expression of pyocyanin-producing genes. This resulted in an increased expression of the *Phz*A1 gene by 235.56-fold and the *phz*M and *phz*S genes by 340.14 and 280.13-fold, respectively. Conversely, treatment with tetracycline led to reduced expression, showing a 1.44-fold change for *phz*A1 and 1.64- and 1.08- fold change for *phz*M and *phz*S, respectively (Faisal and Younis, 2024). The combined effect of PVP-I and hydrogen peroxide (H2O2) was found to downregulate the expression of genes responsible for biofilm formation (*ica*A, *ica*D, *ica*R *clf*A, *ica*B) in *S. aureus* (Wan *et al.*,2025). It has been suggested that iodine and its complex compounds influence bacterial metabolism through their various molecular forms-iodine ion, molecular iodine, and triiodide- which can penetrate cellular barriers and interact with amino acids. These complexes also affect bacterial efflux pumps and detoxification enzymes such as the copper transporter *cop*A and multicopper oxidase *cue*O in *E. coli* bacteria, the zinc transporter *zit*B, heavy metal ion efflux pump *znt*A, and the mercury ion reductase *mer*A2 *in S. aureus* (Kenesheva *et al.*,2023).

The use of Iodine-Containing nano-micelle drug FS-1 demonstrated a significant effect on typical microorganisms within five minutes of treatment against *E. coli* and *S. aureus*. The compound promoted a shift in bacterial metabolism toward anaerobic respiration, primarily due to the destruction of oxygen-dependent electron transfer systems by molecular iodine(I_2) (Korotetskiy *et al.*,2021). Furthermore, FS-1 influenced bacterial sensitivity antibiotics through genetic and metabolic alterations within the bacterial population. In *E. coli, FS-1 treatment induced a metabolic shift toward* anaerobic respiration, accompanied by inhibition of the tricarboxylic acid (TCA) cycle genes responsible for oxidative metabolism. This inhibition resulted in oxidative stress and enhanced expression of pathways involved in cell wall and cytoplasmic membrane repair. Additionally, FS-1 strongly inhibited several transmembrane peptide and amino acid transporters (Korotetskiy *et al.*,2020), suggesting a profound reprogramming of membrane-associated functions. A related study on *P. aeruginosa clinical isolates(n=143) revealed that pigment-producing strains-classified as* yellow, green, and non-pigmented- represented 30.1%, 39.8%, and 30.1% of the total population, respectively. Notably, yellow pigment production strains displayed the highest resistance to multiple antibiotic classes, including beta-lactams, aminoglycosides, and carbapenems, compared with the green and non-pigmented isolates (Kothari *et al.*, 2022). Although a small percentage of both non-pigmented and green strains (2.3% and 1.8%) exhibited resistance to colistin, the yellow variants frequently produced antibiotic-resistant enzymes such lactamase (ESBL, MBL, and AmpC). In contrast, green and non-pigmented strains produced fewer of these enzymes (Kothari *et al.*,2022). Morever, analysis of *P. aeruginosa* isolates showed a notable correlation between the concentration of pyocyanin pigment and the degree of antibiotic sensitivity, indicating that pigment and the degree of antibiotic sensitivity, indicating that pigment production may play a role in modulating bacterial resistance mechanisms (Chimi *et al.*,2024).

5. Conclusion

The present study demonstrated that povidone–iodine exhibited strong antibacterial activity against *Pseudomonas aeruginosa* clinical isolates. Molecular analysis confirmed the presence of *phzM*, *phzH*, and *phzS* genes involved in pyocyanin biosynthesis. However, the effect of povidone–iodine on gene expression was not uniform among the tested isolates. While increased expression of these genes was observed in isolate (1), a reduction was detected in isolate (10). These findings suggest that the influence of povidone–iodine on pyocyanin-asso-

ciated virulence genes may vary between strains. Therefore, povidone–iodine may have potential anti-virulence effects, although these effects appear to be isolate-dependent and require further investigation using a larger number of clinical isolates.

Acknowledgements

Conflict of interests

The authors declare that they have no conflicts of interest that could have influenced the conduct, analysis, or presentation of the research.

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