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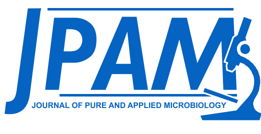

RESEARCH ARTICLE OPEN ACCESS

Ag/F Tio2 Nanoparticles activity against *algD* and *plcH* Genes of *Pseudomonas aeruginosa* Isolated from Patients with Cystic Fibrosis in Al Muthanna City

Hawraa F. Wali*, Yasir Adil Jabbar Alabdali and Mohammed Qasim Waheeb

Department of Biology, College of Science, Al Muthanna University, AL-Muthanna, Iraq.

Abstract

Silver nanoparticles (AgNPs), have been widely used as antibacterial therapy for any microorganisms that are multidrug resistance to antibiotics. *Pseudomonas aeruginosa* is the most common respiratory pathogen in patients with cystic fibrosis (CF), was collected from Al-Muthanna hospitals. These isolates were drug-resistant against tetracycline, chloramphenicol, erythromycin, streptomycin, azithromycin and trimethoprim, while they were sensitive to imipenem. The conventional PCR was used to screen for many different virulent genes and eventually, the only *algD* and *plcH* were detected among 11 *Pseudomonas aeruginosa* strains over of twenty isolates of *P. aeruginosa* isolated from patients with cystic fibrosis (CF) disease. The Ag/F Tio2 NPs was used as antibacterial to test the AgNPs activity against the expression of *algD* and *plcH* genes that are screened as a common complication of gene virulence in the cystic fibrosis (CF) disease. The results showed a significant effect on *algD* and *plcH* genes expression of *P. aeruginosa*.

Keywords: *Pseudomonas aeruginosa*, Ag/F Tio2, Cystic Fibrosis, Nanoparticles

***Correspondence:** houralaen2015@gmail.com

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INTRODUCTION

Silver nanoparticles are considered antimicrobial,potency agents to treat and inhibit the bacterial infection. It has been utilizedas antibacterial against multidrug resistant bacteria such as *P. aeruginosa*[1]. Silver nanoparticles have shown a strong effect on microorganisms such as bacteria, viruses and fungi compared to other minerals[2]. Silver nanoparticles have been used in the field of health products such as medical devices antimicrobials and applications[3]. Cystic fibrosis (CF) is the most common respiratory disease that is caused by a primary pathogen *Pseudomonas aeruginosa* in addition to *Staphylococcus aureus*[4,5,7].

The infection with Cystic fibrosis (CF) is caused by many microorganisms such as pathogenic bacteria and fungal bacteria or fungi causes the inflammation leading to destruction of the lungs and ultimately death. Patients infected with Cystic fibrosis are more susceptible to failure in the respiratory system which is eventually cause the death[6]. Moreover, the mutation into conductance gene (CFTR), encoding for a membrane protein, involved in electrolytes transport. This type of mutation is leading to the destruction of epithelial cell function as the production of a result of sticky and bronchial secretions[6,22,23]. *Pseudomonas aeruginosa* is a major cause of chronic bronchial infection in cystic fibrosis[8]. In the CF lung, the *P. aeruginosa* is genetically adapt to cause infection in the lung. This modification is caused that *P. aeruginosa* is loss of quorum sensing (QS), which is the mechanism for productive the bacterial infection[9]. The *P. aeruginosa* is often the nosocomial bacteria as well as is multidrug resistance bacteria against many antibiotics such as fluoroquinolones, aminoglycosides and cephalosporin due to acquisition of different mechanisms for antibiotics resistance[10,11].

Pseudomonas aeruginosa colonizes the lung, causing cystic fibrosis and patients infected with this disease have mucosal lesions due to the production of polysaccharide genes[18]. This also helps bacteria confirm bacterial biofilms that are important in bacterial infection of the lung[26]. Biofilm is required for both regulators which are AlgB and AlgR controlled by *algD*, coding for the hydrogenase GDP, which has a major role in the biosynthesis of genes[18,26].

The *P. aeruginosa* causes many diseases upon on the virulence genes factors leading to cause the infection such as cystic fibrosis (CF). The *plcH* gene activity is phosphatidylcholine (PC) and sphingomyelin. This lipid makes the cellular membrane which is important in bacterial biofilm[27]. Many studies have been reported that the *plcH* gene (phospholipase C/sphingomyelinase) becomes expressed during the lung infection of *P. aeruginosa*[28].

The aim of this study was to detect most virulence genes in *P. aeruginosa* isolated from patients with Cystic fibrosis (CF) and test the antibacterial activity of Ag/F Tio2 nanoparticles against these genes expressions in *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Samples collection

In this study, 100 clinical samples of sputum were collected from patients with cystic fibrosis infection from AL-Hussein Teaching Hospital in Al Muthanna city between the period from September-2018 to April-2019.

Isolation and identification

The isolation and differentiation of the *Pseudomonas aeruginosa* strains were confirmed by growing the samples onto CHROMagar™ media and then were confirmed using VITEK- 2 Compact system (Biomerieux/ France)[14].

DNA extraction

Bacterial genomic DNAs were extracted using the Favor Prep Tissue Genomic DNA Extraction Mini Kit (Favorgen, Taiwan) following the manufacturer's protocol.

PCR method

The multiplex PCR was performed, after gDNA extraction from the *P. aeruginosa* isolates in a total volume of 25 µl using Maxime™ PCR PreMix (i-Taq) (iNtRON Biotechnology), including primers see oligo Table (1), following the manufacturer's protocol. PCR programme for Tag was normally consisted as in Table (2).

Once the PCR reactions were completed, separated on a 0.8% agarose gel at 100V for 25 min and then the DNA samples were visualised on a UV transilluminator.

Nanoparticles preparation

The Ag/F Tio_2 nanoparticles were collected from College of Science for Women-University of Babylon at final concentration of 10 mg/ml and we diluted to 1 mg/ml as final concentration with bacterial broth.

Antibacterial activity of Ag/F Tio2 nanoparticles

The antimicrobial activity of Ag/F Tio2 was evaluated by growing the suspension of culturing bacteria to a final concentration of 1 mg/ml of Ag/F Tio2. Subsequently, the gDNA was extraction from the samples before and after nanoparticles treatment then the PCR was performed to test the effect of the nanoparticles of both *algD* and *plcH* genes expression.

Antibiotic Susceptibility Test

The antibiotic susceptibility test for *P. aeruginosa* was done using disc diffusion on Mueller-Hinton agar (CM0337-OXOID), following[15]. Briefly, the standard of *P. aeruginosa* in oculum of $1.5*10^8$ cell was cultured on Mueller Hintonagar. Subsequently, the antibiotics discs were placed on contaminated plates. Finally,the plates were incubated at 37°C for 24-48 h, then the diameter of the inhibition zone was measured by mm[15].

Statistical Methods

The antibiotic resistance patterns were tested by the measured using ANOVA, GraphPad Prism software.

RESULTS AND DISCUSSION

In the study, clinical and resistant isolates of *P. aeruginosa* were isolated from cystic fibrosis (CF)and screened for different virulence genes that are important in causing *P. aeruginosa* pathogenesis. One hundred clinical sputum were collected, followed by culturing onto CHROMagar™ media to isolated *P. aeruginosa* strains. Eventually, twenty strains were isolated and confirmed by using VITEK- 2 Compact6system. Interestingly, the PCR screen was shown the detection of both

Table 1. Primers used in this paper

Oligo	Sequence	Use	PCR product (size) (bp)
YA1	CGTCTGCCGCGAGATCGGCT	Forward to screen for algD gene	313
YA1	GACCTCGACGGTCTTGCGGA	Reverse to screen for algD gene	
YA4	GCACGTGGTCATCCTGATGC	Forward to screen for plcH gene	608
YA4	TCCGTAGGCGTCGACGTAC	Reverse to screen for plcH gene	

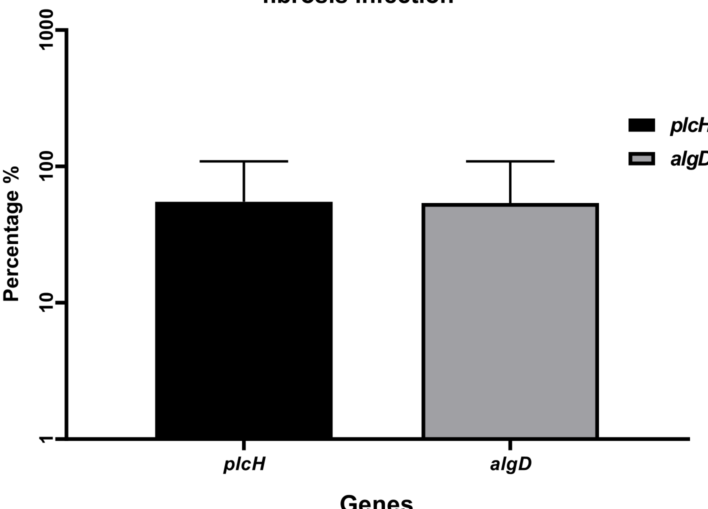

Fig. 1. The prevalence rate of virulence gene of *P. aeruginosa* isolates from cystic fibrosis (CF). The gene *algD* and *plcH* were detected in *P. aeruginosa* strains at a rate of 55%.

algD and plcH genes at prevalence rate 55% of *P. aeruginosa* strains isolated from cystic fibrosis (CF) Fig. (1).

The antibiotics sensitivity for the P. aeruginosa isolates against different antibiotics was shown the highest resistance percentage for tetracycline, chloramphenicol, erythromycin, streptomycin, azithromycin, trimethoprim, while the lowest resistance against the imipenem at 20% at the p-value of 0.0212 (Fig. 2).

The results of PCR amplification to specific *algD* and *plcH* genes was detected in all *P. aeruginosa* isolates from cystic fibrosis (CF) infections. The PCR was gave the expected product of 313 bp for *algD* gene, Fig. (3-A1), and the PCR amplification showed the expected product of 608 bp (Fig. 3-B1).

Table 2. PCR programme used for genes screen

Steps	Temperature	Time	Number Cycles
Initial denaturation	94°C	3 min	1
Denaturation	94°C	30 sec	32
Annealing	55°C	30 sec	
Extension	72°C	1 min per kb	
Final extension	72°C	10 min	1
Hold	12°C	∞	

The *P. aeruginosa* strains that were given positive results for *algD* and *plcH* genes detection were then treated with Ag/F Tio2 nanoparticles. The *P. aeruginosa* strains were grown up with Ag/F Tio2 nanoparticles using brain heart infusion broth and incubated at 37°C for 24 h. The gDNA was extracted from treated samples. The PCR amplification showed that disappearance of gene expression for both *algD* and plcH genes (Figs. 3-A2 & 3-B2). This result suggested that the Ag/F Tio2 nanoparticles have an effect on down-regulation of both *algD* and *plcH* genes in the *P. aeruginosa*.

Pseudomonas aeruginosa is a common complication for many infections such as cystic fibrosis (CF) and wound, associated with resistance for multi-drug antibiotics resistance, leading to the increase of the rate of both morbidity and mortality[16,19].

Pseudomonas aeruginosa is Gram-negative bacteria have been recently treated with AgNPs showing a strong effect on gene expression by up-regulated 27 genes and 32 down-regulated genes[17]. The actual mechanism of AgNPs is the interference with the function of the bacterial cell membrane leading to generate reactive oxygen species (ROS). Moreover, the AgNPs have a similar role to silver ions in the regulation of the membrane transporter proteins. These mechanisms lead to an effect on bacterial

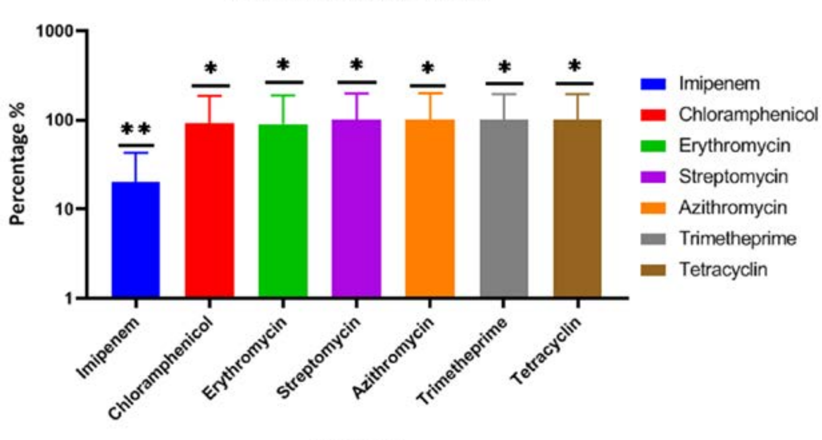

Fig. 2. The percentage of antibiotic resistance of *P. aeruginosa* strains isolated from cystic fibrosis (CF) in al Muthanna hospitals shows the highest percentage of the resistance for all antibiotics were tested, while the strains were shown the lowest resistance level against imipenem. The p-value of antibiotics resistance is 0.0212 that measured using ANOVA, GraphPad Prism software. **: the population means are significantly different at the p-value= 0.05 level.

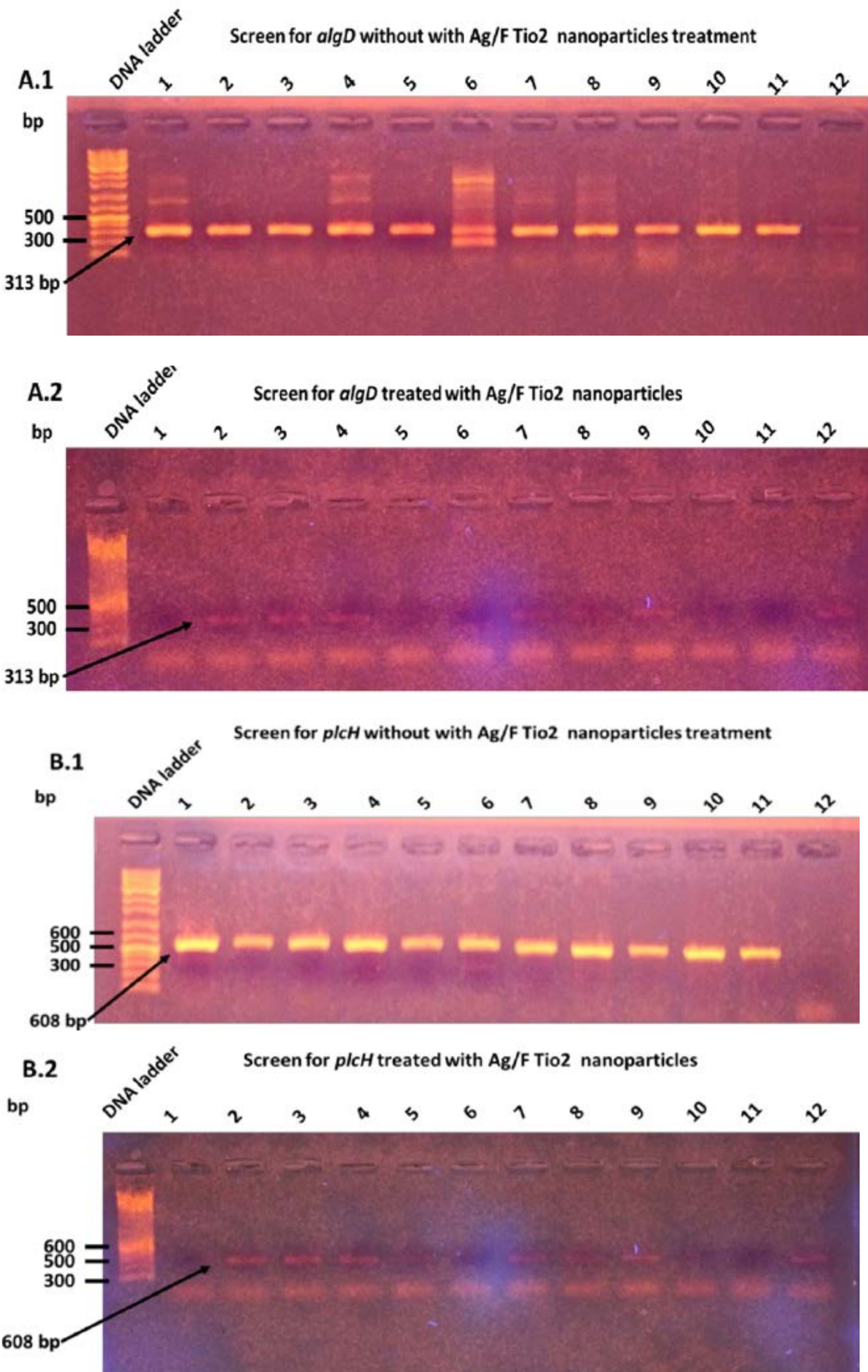

Fig. 3. PCR amplification of both *algD* and *plcH* genes *P. aeruginosa* isolated from patients with Cystic Fibrosis. before and after Ag/F Tio2 treatment. A.1: PCR amplification of *algD*, showing amplified the predicted product of 313 bp. A.2: The *algD* gene amplification after Ag/F Tio2 treatment showing the Ag/F Tio2 nanoparticles effect on *algD* gene amplification. B.1: The *plcH* gene amplification showing amplified the predicted product of 608 bp. B.2 The *plcH* gene amplification after Ag/F Tio2 treatment showing the Ag/F Tio2 nanoparticles effect on *plcH* gene amplification. DNA ladder: molecular weight: 100–10000 bp.

growth and inhibition of multi drugs resistance bacteria[17]. Likewise, the silver nanoparticles have been tested against different bacteria such as *E. coli*, *S. aureus* methicillin-resistant, *K. pneumonia* and *P. aeruginosa* showing a promising activity but the activity against *P. aeruginosa* was the highest at a rate of 90% compared with others bacteria[21].

The PCR screen results showed the detection for both *algD* and *plcH* genes and this is agreed with others studied for *Pseudomonas aeruginosa* strains isolated from the lungs with cystic fibrosis disease[24]. The transcription of *algD* gene, which encodes to GDP-mannose dehydrogenase, causing for production of exopolysaccharide alginate that is important in the infection of *Pseudomonas aeruginosa* in the lung[18]. However, the *plcH* gene expression is responsible for biofilm formation due to making lipid in the bacterial cell membrane and the mutant of this gene have been shown a defective in colonizing in the lung of a model animal with no biofilm formation[20].

In conclusion, this study has demonstrated that the Ag/F Tio2 nanoparticles can affect the expression of the most important virulence genes (*algD* and *plcH*) in *P. aeruginosa* during the lung infection. Our results suggest that the silver nanoparticles can be used as alternative antibacterial therapy against cystic fibrosis disease.

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CONFLICT OF INTEREST

The authors declare that there is no conflicts of interest

FUNDING

None.

AUTHORS' CONTRIBUTION

All authors designed and performed the experiments. YA analyzed the data, YA and MQ wrote the manuscript. All authors read and approved the manuscript.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript and/or the Supplementary Files.

ETHICS STATEMENT

Not applicable.

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