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Synergistic Effect of Ciprofloxacin and Probiotics against Biofilm-Forming *Pseudomonas aeruginosa*

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Abstract

Background: the rise of biofilm- forming and antibiotic- resistant *Pseudomonas aeruginosa* has attempts initiatives to discover safe and natural alternative medicines, including probiotics. **Objective**: The current investigation sought to evaluate the antibacterial and antibiofilm properties producer derived from natural sources against clinical isolates of pathogenic *Pseudomonas aeruginosa* in Baghdad, Iraq. **Methodology:** This cross-sectional, laboratory-based in vitro study was conducted at the Department of Biology, College of Education for Pure Science, Diyala University, Iraq, from 30 October 2024 to 6 June 2026. All the patients who presented with different infectious diseases were selected as the study population. Clinical isolates of *Pseudomonas aeruginosa* were obtained from hospitals in Baghdad and Diyala, identified, and subsequently examined from their capacity to form biofilms and resist of antibiotics. Check the effectiveness of influence ciprofloxacin, probiotics, and their combination on biofilm formation then examination their influence on genotypic in two steps: the first step that detected biofilm genes (*pelA*) and quorum sensing gene (*lasI)*. The second step was using RT-RCR technique to exam effect of synergistic of ciprofloxacin and probiotics. **Results:** MIC and sub-MIC levels of ciprofloxacin were (1024-512 µg/ml) while probiotics had MIC and sub-MIC levels of (100 - 75 µg/ml), so gene expression was reduced to 0.3-fold compared to the control for the *pelA* gene, demonstrating significant downregulation under the effectiveness of CIP alone, while gene expression was reduced to 0.2-fold compared to the control for the *lasI* gene under the influence of a combination of ciprofloxacin and probiotics. **Conclusion**: The results suggest that probiotics may enhance the efficacy of ciprofloxacin against XDR *Pseudomonas aeruginosa* by significantly downregulating biofilm- and quorum-sensing-related genes (*pelA* to 0.3-fold and *lasI* to 0.2-fold), indicating a potential synergistic effect in controlling this pathogen. [*Bangladesh Journal of Infectious Diseases, December 2025;12(2):250-259*]

Keywords: *Pseudomonas aeruginosa*; *Lactobacillus plantarum*; probiotics; ciprofloxacin

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Introduction

Pseudomonas aeruginosa is an opportunistic bacterial pathogen affecting humans, particularly in immunocompromised patients. It is toxic and readily develops antibiotic resistance. This pathogenic bacterium possesses various virulence factors[1]. The World Health Organization (WHO) cautions that there is more morbidity and mortality from microbial infections because of the

Pseudomonas aeruginosa development of resistance to antibiotics[2-3]. There were global initiatives to create unique and stronger antibiotics as well as creative and effective administration and prevention strategies[4-5].

Ciprofloxacin is a fluoroquinolone antibiotic; it is used to treat a variety of pathogenic bacteria. One of the most popular antibiotics for *Pseudomonas aeruginosa* infections is used to treat infections by inhibiting bacterial DNA gyrase and topoisomerase IV, two crucial enzymes that regulate bacterial DNA supercoiling, replication, and repair. Ciprofloxacin acts as a bactericidal agent by inhibiting these enzymes, which stops bacteria from duplicating their DNA and triggers cell death. Treatment becomes more challenging when *Pseudomonas aeruginosa* has mutations that provide a ciprofloxacin resistance phenotype, which is influenced by mutations in many genes[6]. *Pseudomonas aeruginosa* has two main mechanisms of resistance: the gain of mutations in genes encoding the regulators of the efflux pumps that release these antibiotics and mutations in genes[6-7]. Discovering techniques that are effective or a new approach to treating XRD *Pseudomonas aeruginosa* is therefore essential, particularly given the severe effects of much use of antibiotics.

The Probiotics are active microorganisms that, when delivered in suitable amount, give beneficial health effects for the host. Probiotic microorganisms provide several advantages for the human digestive system. Probiotic bacteria include *Lactobacilli* [6-7].

Probiotics can prevent infections by competing with them for the few nutrients required for their metabolism, among other ways such as competing with other microbes. Certain probiotics prevent infections from adhering to the host cell. They act as bactericides by having H_2O_2, and organic acids are among metabolites with acids are among the metabolite's probiotics can release[7-8]. The benefits described above offer a lot of possibility for using probiotics as an antibiofilm agent[9]. The current investigation sought to evaluate the antibacterial and antibiofilm properties of producers derived from natural sources against clinical isolates of pathogenic *Pseudomonas aeruginosa* in Baghdad, Iraq.

Methodology

Study Settings and Population: This was a cross-sectional study. This study was conducted in the Department of Biology, College of Education for pure Science, Diyala University, Iraq, the study population consisted of 207 samples. All the patients who presented with different infectious diseases were selected as the study population. The clinical specimens were collected from both female and male, at different ages, from patients attending Baqubah Teaching Hospital, Iraq, the General Central Laboratories in Baqubah, Iraq, Nurse Private Hospital, Iraq and General Surgery Hospital in Baghdad, Iraq.

Bacterial Isolates: In the present study, out of a total of 207 clinical specimens (bronchial wash, burn swab, ear swab, endotracheal tube, sputum, urine, wound, and operation room), the clinical specimens were collected from both female and male gander, at different ages from patients attends Baqubah teaching hospital, the General Central Laboratories in Baqubah, Nurse Private Hospital and General Surgery Hospital in Baghdad. In addition, *Lactobacillus plantarum* ATCC 14917 was obtained from Cac Chemistry Analysis Center, Microbiology Department in Baghdad, and then approved identification by VITEK2 system.

Antibiotic Susceptibility Test (AST): In accordance with the guidelines provided by the Clinical Laboratory Standard Institute, an antibiotic susceptibility test was conducted using the disk diffusion method[10].

Detection of Biofilm Formation: Bacterial isolates MRD, *Pseudomonas aeruginosa* biofilm-forming potential was evaluated using a crystal violet staining-based 96-well microtiter plate assay[11].

Preparation of *Lactobacillus* Isolates Cell-Free Supernatants (CFCS): A single colony of pure culture of *Lactobacillus plantarum* was transferred to inoculate 5ml MRS broth. 1 ml of *Lactobacillus plantarum* was incubated at 35 °C for 24 hours under anaerobic conditions by using a candle jar[12]. And thereafter subjected to centrifugation at 4000 rpm for 10 min. At 4^0 C. The resultant supernatant was sterilized by filtration through a 0.22µm Millipore filter. The cell-free supernatant (CFU) of bacterial isolates was obtained for use as an inhibitory agent against *Pseudomonas aeruginosa*.

Determination of Minimum Inhibitory Concentration (MIC)

Ciprofloxacin: Ciprofloxacin serial dilutions ranging from 0.5 to 1024 µg/ml were prepared to perform this test. Three control tubes were used in

the experiment: one with sterile broth (sterility control), and one with antibiotic and sterile broth. The tubes were incubated at 37ºC for 18 to 24 hours before being checked for growth. Ciprofloxacin MICs were determined by using the lowest concentration at which no growth was seen. Additionally, each tube's contents were sub-cultured on Mueller-Hinton agar medium without any growth to search for any bacterial growth and calculate the minimum bacterial[13].

Probiotics: In Mueller Hinton broth, a few *Pseudomonas aeruginosa* colonies from fresh growth were diluted to 1.5 X 10^8 CFU/ml, suspension was evenly and gently dispersed over the MH agar medium. The plates were then left to dry. Five mm wells were cut out and filled with 100 µl of supernatant (100%,75%,50%), and then incubated for 24 hours at 35ºC. The inhibition zone surrounding the wells was measured according to Ali and Alqurayshi[14].

Effect of Probiotic Supernatant on Biofilm formation: One hundred µl of *Pseudomonas aeruginosa* in MHB at pH 7.0 as a positive control was added to the first well 100 as a standard, then 100 µl of supernatant *Lactobacillus plantarum* was added, and 50 µl of MHB was then mixed with surfactants from well one to 10 using a micropipette, 50 µl per well, then the standard *Lactobacillus plantarum* was diluted 100 times in MHB broth. 50 µl of bacterial solution adjusted with MacFarland was added to each surfactant well and the control wells. Less than fifteen minutes were needed to develop and inject the bacteria, incubated 24hours at 35 º C, finally, Resazurin (0.015%) was added to each well (30µl per well) and then incubated for an additional 2 to 4 hours to observe the color change. The result was calculated by watching the wells that showed no change in the blue resazurin color during the incubation time.

Genotyping Detection

DNA Template Preparation by the Boiling Method: The boiling procedure was used to create the DNA template. Five colonies of bacteria that had grown overnight were completely covered in 2ml of D.W followed by boiling for ten min. in a water bath. The supernatant was used as the template DNA for the PCR after centrifugation[15].

PCR Primers to Detection of Biofilm genes: The PCR was used to determine if biofilm genes were present; Table 1 lists the primer sequences. Tap Master Mix, which includes Taq polymerase, dNTPS, mgCl2, and the proper buffer, was used to conduct the PCR. 25 µl of reaction mixture, consisting of 12.5 µl of master mix, 1 µl of each forward and reverse primer solution, 1 µL of DNA, and nuclease-free water to complete the volume, were contained in each PCR tube. The PCR was conducted under the subsequent conditions: initial denaturation at 94^0 C for 3 min. followed by 30 cycles of denaturation at 94 ºC for 30 seconds. Annealing temperature for 30 seconds, extension at 72 ºC for 3.5 minutes (Table 1). The amplified DNA was subjected to separation using 1.0% agarose gel electrophoresis[16].

RNA Purification and extraction: In accordance with instructions, the TRIzol TM detector was used to extract RNA from two isolates (P10) that isolated from sputum and (P16) that were isolated from urine. By measuring absorbance using a Quants fluorometer, the RNA extract concentration was determined.

Table 1: Primer Sequences were Used In Study

Name Primers	Sequence 5-3		Annealing Tem. ºC	Product size (PB)	Reference
LasI	F	CACATCTGGGAACTCAGC	40 cycles at 95 C for 30 s, 56 C for 30 s, 72 C for 30 s	160	[17]
	R	ACGGATCATCATCTTCTCC			
pelA	F	CCTTCAGCCATCCGTTCTTCT	35 cycles at 94C for 30 sec, 52C for 40 sec, 72C for 50 sec	118	[18]
	R	TCGCGTACGAAGTCGACCTT			

Detection of Gene Expression by Quantitative Real Time PCR of *pelA* and *lasI* Genes: To determine and choose the isolates with the greatest and lowest resistance levels using gene expression, which is established through comparison of the mean CT values of the nor A gene and validated normalized by folding= 2AAct, where the ACT is the variation in CT threshold cycle between the target and reference genes as determined by quantitative real-time PCR for the reference gene (*Eub358*f TCCTACGGGTATCTAAT TATCTAATCCTG), *lasI*, and *PelA* gene in same sequence used in detection because the length of gene less than 200. 5 µL of the qPCR Master mix, 0.25 µL of each RT mix and $MgCl_2$, 0.5 µL of each Forword and reverse primer, 2.5 µL of nuclease-free water, and 1 µL of RNA were used for the RT-PCR. The following guidelines were followed for doing the RT-PCR: One cycle of RT enzyme activation at 37^0 C for 15 minutes the first denaturation (95^0 C, 10 min), and the 40^{th} cycle (95ºC, 20s), temperature for annealing: extensional 72^0 C for 20 seconds. Utilize the formula to determine gene expression.

Folding= 2^{-AACt}
AACT= ACT $_{treated}$ – ACT $_{control}$
ACT=CT $_{treated}$ –CT $_{reference\ gene}$

Statistical Analysis: The Statistical Packages of Social Sciences (SPSS 2019) program was used to detect the effect of different groups on study parameters (fold change of gene expression). Least significant difference (LSD) was used to significantly compare the means in this study. Using this test because we have more than two means for the fold change. A p-value less than 0.05 was statistically significant.

Ethical Consideration: The Institutional Review Board granted the study ethical approval. Since this was a cross-sectional study, each participant provided written informed consent. Every technique was carried out in compliance with the applicable rules and regulations, according to the ethical book numbered (CEPEC/04).

Results

Incidence of *Pseudomonas aeruginosa*: The *Pseudomonas aeruginosa* isolates were identified based on how they responded to the Gram stain dye, where the bacterial isolates were Gram-negative rods, and biochemical characteristics, as the results were positive for non-fermentation of lactose for 200 samples, where 118(59.0%) were positive, and 54(27.0%) were negative. Production of oxidase and peroxidase enzymes, along with characteristic greenish-yellow mucoid colonies on cetrimide agar, was observed and illustrated in Figure 1. The bacterial isolates also produced a distinctive fruity odor when grown on nutrient agar. Further identification using the VITEK 2 Compact system confirmed the isolates with 99% probability.

The prevalence of these isolates among different clinical specimens is in urinary tract infections: 14(18.1%) out of 77 individuals examined, wound 10(12.9%), sputum 7(9.0%), burn 23(29.8%), ear swab (10.8%), operation room 11(14.2%), and bronchial nasal 3(3.8%). *Pseudomonas aeruginosa* is now a common pathogen that has been successfully established in the hospital environment. In addition, *Lactobacillus plantarum* were small white colonies appeared in MRS agar after anaerobic incubation, and then confirmed identification by VITEK2 system.

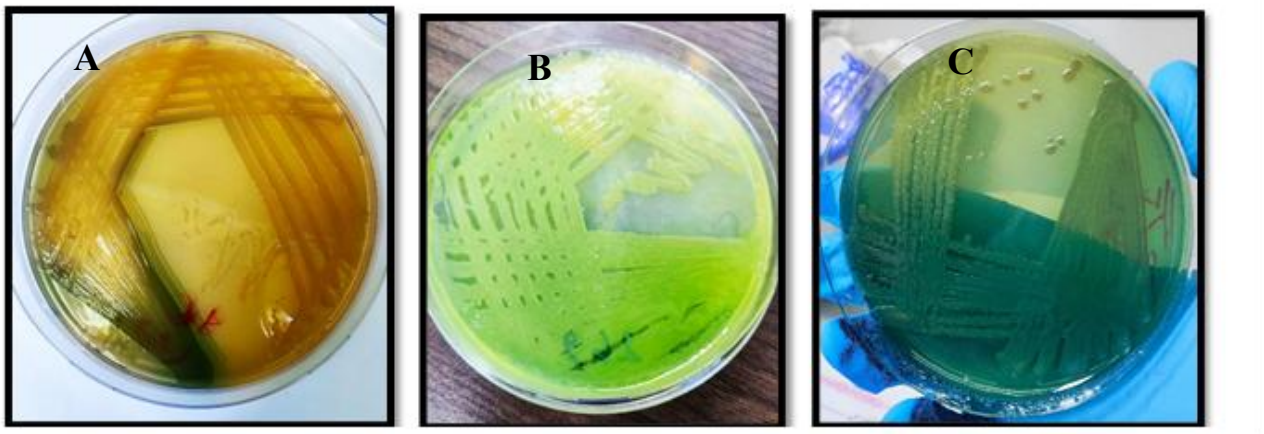

Figure I: Culture of *Pseudomonas aeruginosa* Bacteria on(a) MacConkey agar, (B) Cetrimide agar, (C) *Pseudomonas* Agar Media

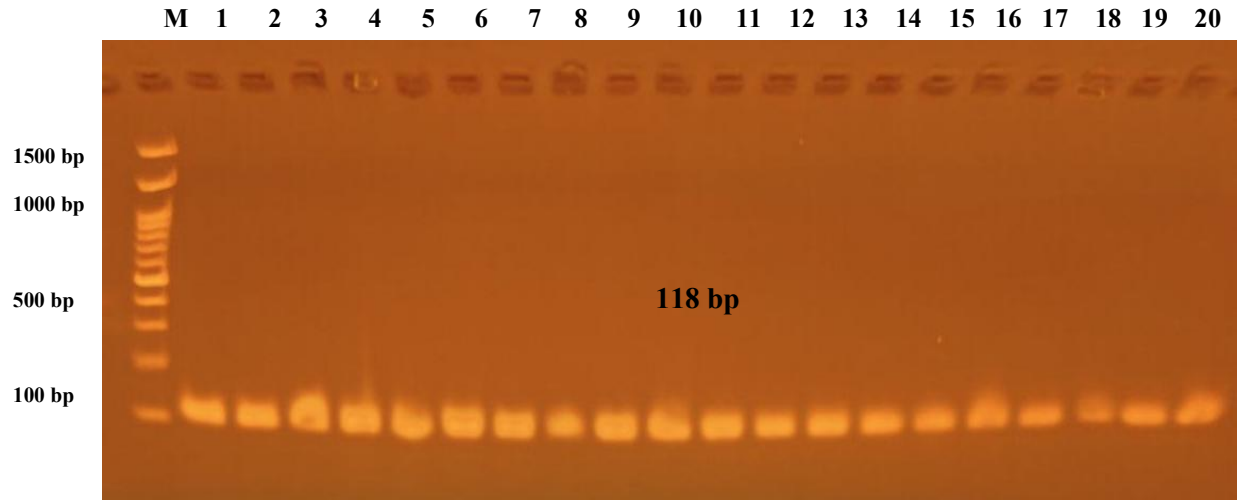

Figure II: Amplification products of *Pseudomonas aeruginosa pelA* after electrophoresis on 1.5% agarose gel for 60 min at 7v/cm2. (M): DNA ladder marker; Lanes (1-20): Bacterial isolates number

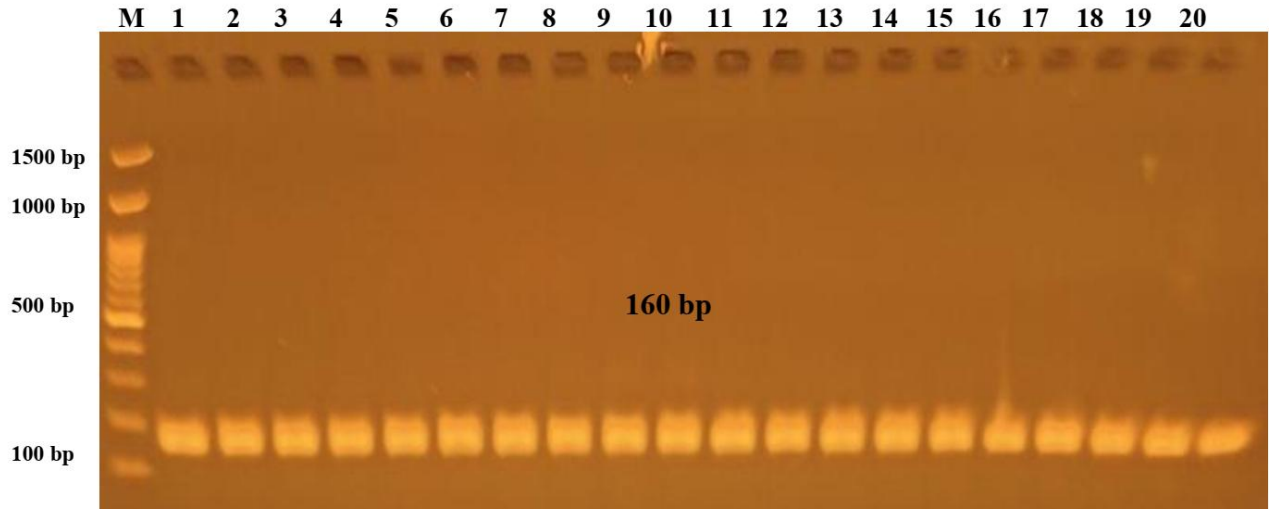

Figure III: Amplification products of *Pseudomonas aeruginosa lasI* gene after electrophoresis on 1.5% agarose gel 60 min at 70 volt/cm.(M): DNA ladder marker; Lanes (1-20): Bacterial isolate number

Molecular Study of *Pseudomonas aeruginosa* DNA: In this study, Twenty DNA -extracted samples were utilized to determine the presence of genes encoding biofilm and to ascertain the prevalence of each gene among pathogenic *Pseudomonas aeruginosa* clinical isolates using polymerase chain reaction (PCR) for each DNA sample. The RCR experiment comprised 20 isolates for the identification of biofilm (*PelA, LasI*). The PCR results have been validated using band analysis via gel electrophoresis. Because of the great biofilm-forming capacity of these isolates, the frequency of these genes was displayed for each clinical isolate. This gene was activated during biofilm formation[19], mentioned Shen et al[21] done in China that detected *pelA* in all urine isolates (Figure II).

A study done by Van et al[21] in Canada showed that the *pelA* gene is part of the essential genome of *Pseudomonas aeruginosa*. Agarose gel electrophoresis in this study agrees with a study done in the USA, Oregon State University, Corvallis, which treated integrated experiments with mathematical modelling to quantitatively analyses the *LasI*/*LasR* quorum sensing pathway in the opportunistic pathogen[22]. In addition, *LasI* gene was prevalent in all isolates (100%) from all sources (Figure III).

Antimicrobial Susceptibility Test and Biofilm Formation: Two XDR of *Pseudomonas aeruginosa* isolates were selected, *Pseudomonas aeruginosa* P10 was taken from urine specimen and P16 was taken from a wound specimen. Both of these isolates exhibited resistance to all antibiotics, such as Piperacillin, Ampicillin, ceftazidime, Cefepime, Meropenem, Imipenem, Gentamicin, Ciprofloxacin, Colistin, Polymycin B and Norfloxacin, were examined in this study. Both *Pseudomonas aeruginosa* XRD isolates (P10, P16) were strongly adherence biofilm formation therefore, they were chosen in this study among of twenty specimens.

Determinations of MIC

Ciprofloxacin: The goal of this experiment was to find the lowest dilution of the investigated Ciprofloxacin that would inhibit the growth of *Pseudomonas aeruginosa* isolates. Results showed that each two *Pseudomonas aeruginosa* P10 and P16 isolates had MIC and sub-MIC (1024-512 µg/ml), as proved by MBC.

Probiotics Produced by *Lactobacillus plantarum*: This experiment aimed to identify the lowest dilution of CFCS of the *Lactobacillus plantarum* isolates in examination. *Pseudomonas aeruginosa* P10 and P16 growth was shown to be significantly inhibited by *Lactobacillus plantarum* at MIC and sub-MIC levels of (100 - 75 µg/ml), respectively (Figure IV).

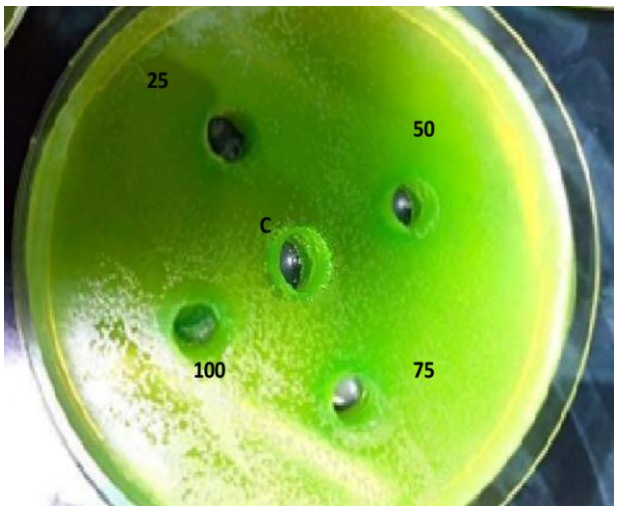

Figure IV: 100 µl supernatant of *Lactobacillus plantarum* in wells by different concentrations and their effected of *Pseudomonas aeruginosa* 10 cultured in MHA

Analysis of the potentially anti-biofilm activity of *Lactobacillus plantarum* species: The examined *Lactobacillus plantarum* isolates had a notable antibiofilm capacity when directly tested against *Pseudomonas aeruginosa* isolates P10 and P16; however, their MIC and SUB-MIC inhibited a weakly effect (Figure V).

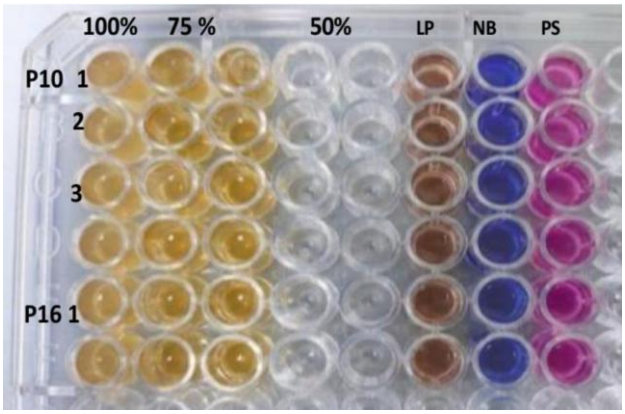

Figure V: Determination of treatment with three concentrations of *Lactobacillus plantarum* against *Pseudomonas aeruginosa* (P10: isolate 10, P16: isolated 16 repeated) cultured in nutrient broth with 1% glucose. LP: *Lactobacillus plantarum* isolates (Negative Control), NB: nutrient broth+1% glucose without growth (Blank), *Pseudomonas aeruginosa* culture in Normal saline (positive Control). The effect of supernatant probiotics was moderate to antibiofilm because the color of wells did change, while the control positive *Pseudomonas aeruginosa* didn't change the color of Resazurin

The direct effect of *Lactobacillus plantarum* showed a strong antibiofilm potential against in 100.0% concentration and a decay effect at a concentration 75.0% of supernatant probiotics, the 50% concentration, that non activated in this concentration on Two isolates of resistant *Pseudomonas aeruginosa* isolates, this effected of probiotics on biofilm formation (Figure VI) by ELISA device by comparing readings with optical density in 630 nm. *Pseudomonas aeruginosa* biofilm formation responded to the effect of three concentrations of probiotic extraction of *Lactobacillus plantarum* (100.0%, 75.0%, 50.0%).

It was shown that when comparing the biofilm formation by resistant *Pseudomonas aeruginosa* isolates at control with the same isolates under the effect of probiotics, the strongest effect on biofilm production was in under the effect of 100.0% at

(0.05-0.1 nm), then less the result under the effect at 75.0% (moderate) at (0.2-0.4 nm). Additionally, the non-effected (weak) in 50.0% concentration of probiotic at (0.4-0.5 nm).

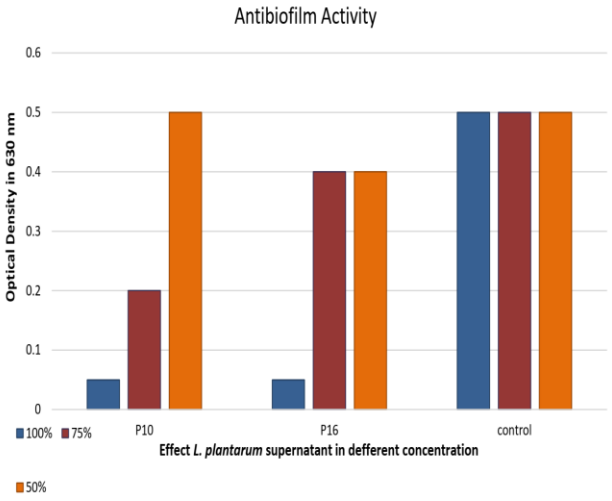

Figure VI: The inhibition by supernatant probiotics produced by *L. plantarum* isolate in three concentrations on *Pseudomonas aeruginosa* isolates compared with optical density at 630nm. the highest effect probiotic at 100%, the effect in 75% concentration to pro., the little effect of probiotic in 50% concentration

Gene Expression of *pelA* and *lasI* Genes of Biofilm: As *Pseudomonas aeruginosa* evolves, it develops a variety of complicated resistance mechanisms, such as drug extraction through the efflux pump, biofilm formation, and virulent factors, among others, to counteract the bactericidal action of antibiotics. Probiotics are a viable option to suppress antibacterial or biofilm microorganisms. These isolates originated from a variety of sources, including urine and wounds.

To evaluate the *pelA* gene's expression, it was contrasted with that of the *Eub358f* and *Eub806r* genes. Each sample was folded three times, and the average of the three results was used to determine the value of gene expression present in that sample. The *pel A*, which was substantially expressed before to Cip., Prob. treatment.

The results of gene expression for the *lasI* gene, which codes for the quorum-sensing system connecting with the biofilm of *Pseudomonas aeruginosa*. Furthermore, results indicated in figure (VIII) showed the significant influence ($P < 0.05$) combination of both probiotics and ciprofloxacin on the *lasI* gene of *Pseudomonas aeruginosa* compared with the mono effect of ciprofloxacin or probiotics.

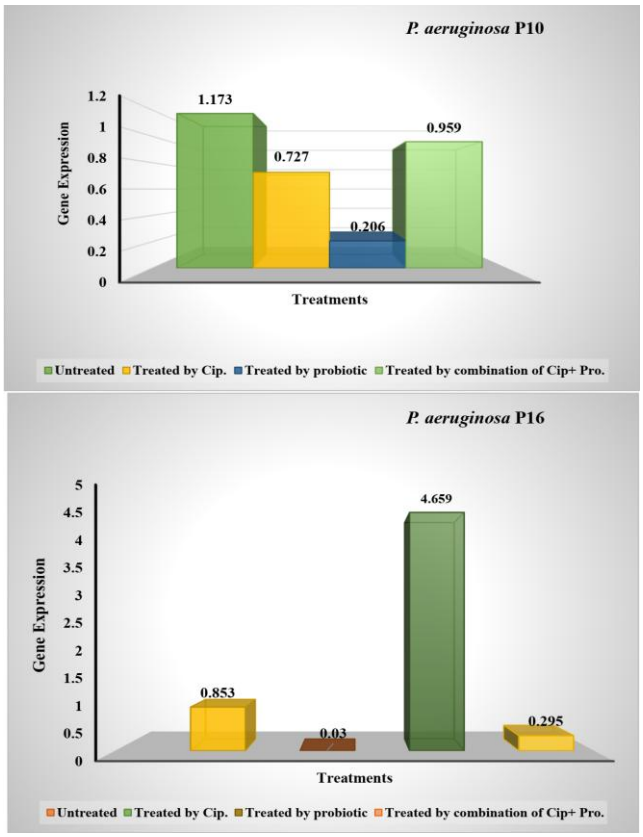

Figure VII: Gene Expression change of *pelA Pseudomonas aeruginosa* isolates in difference treatments

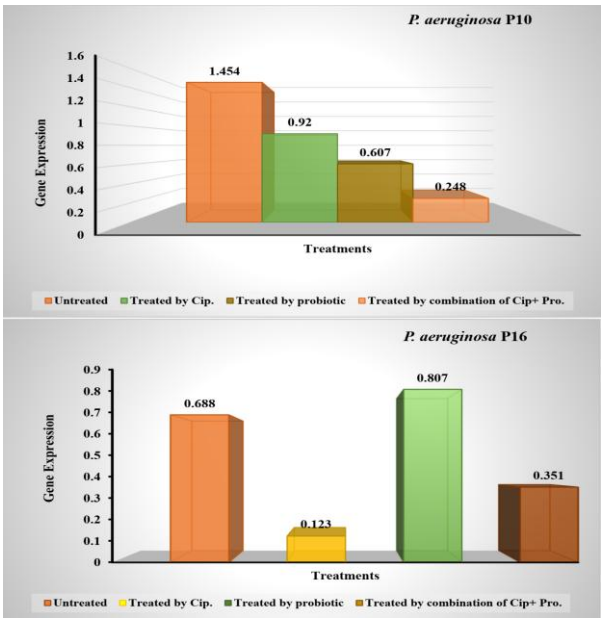

Figure VIII: Gene Expression change of *lasI Pseudomonas aeruginosa* isolates in different treatments

The results indicated the gene expression of the *lasI* gene in bacterial isolate P10 after 24 hours of incubation with the ciprofloxacin, probiotic, and combination of each other, after its expression decreased from 1 for the control sample to 0.920, 0.607and 0.248, respectively, in LSD 0.5917 at $P < 0.05$.

The *Pseudomonas aeruginosa* isolate was from a sputum source, and it was highly significant. To compare the effect of combination probiotics and ciprofloxacin on gene expression of *lasI* gene in P10, a highly significant (0.05) decrease in influence was 0.248 after treatment with the combination of probiotics and ciprofloxacin. The combination effect also reduced gene expression for isolate P16 at 0.351.

Discussion

A total of twenty *Pseudomonas aeruginosa* had *PelA* in their genome; this result was in agreement with the PCR, Shen et al[21] done in China that detected *pelA* in all urine isolates. A study done by Van Loon et al[22] in Canada showed that *pelA* genes were part of the essential genome of *Pseudomonas aeruginosa*. That agarose gel electrophoresis study agrees with a study done in USA, Oregon State University, Corvallis, which treated integrated experiments with mathematical modeling to quantitatively analyze the *LasI*/*LasR* quorum sensing pathway in the opportunistic pathogen[22].

The examined *Lactobacillus plantarum* isolates had a notable antibiofilm capacity when directly tested against *Pseudomonas aeruginosa* isolates P10 and P16; however, among the ways that probiotics supernatant from *Lactobacillus plantarum* strain demonstrates antimicrobial activity are the competitive eradication of bacterial adhesion and competition for nutrients and adhesion receptors, which results in vital and morphological metabolic alterations and the eventual death of bacteria[17,23]; co-aggregation was a mechanism by which microbial communities come together to form completely similar structures[24,12]; strong antimicrobial chemical synthesis, such as hydrogen peroxide (H2O2), lactic acid which lowers the pH of the reaction environment[9,25-26].

The inhibition by supernatant probiotics produced by *Lactobacillus plantarum* isolate in three concentrations on the biofilm of *Pseudomonas aeruginosa* isolates compared with optical density at 630 nm. The highest effect probiotic at 100.0%, the effect in 75.0% concentration to pro., the little effect of the probiotic in 50.0% concentration. This result agrees with the master's results conducted in the biotechnology college at Baghdad University by Yaaqoob[26], which found the biofilm formation of *Pseudomonas aeruginosa* inhibited under the effect of nanoparticles material extracted from *Lactobacillus plantarum* under different concentrations, when the result was confined between 1.82 and 1.76. In the same hand, Kadhim and Al-Hayanni[27] mentioned in a study published in the Babylon journals that *Lactobacillus plantarum* and *Lactobacillus acidophilus* were used as probiotics to decrease biofilm formation from MDR *Pseudomonas aeruginosa* isolates.

In the same hand, the gene expression of gene *Pel A* in P16 that was highly significant (0.01) from 0.03 after being treated by ciprofloxacin, towards the *pelA* gene was the untreated one. This study was agreed upon by Liu et al[28] who mentioned that ciprofloxacin was found to be the second most effective antibiotic against *Pseudomonas aeruginosa* because it targeted the replication of bacteria while interfering with topoisomerase work. This is responsible for uncoiling[29].

P10and P16 showed a highly significant (0.01) enhanced influence of 2.401829 after being treated by probiotics. This result was approved by the antibiofilm test mentioned above; these results agree with the study Huang and Hunag[30] in Taiwan, which found that patients with cancer should use probiotics with caution because of complications with gut-derived *Pseudomonas aeruginosa* sepsis in mice following chemotherapy. These changes made it easier for bacteria to go into the circulation, liver, and spleen[28]. On the other hand, a large number of studies have shown the effectiveness of probiotics to inhibit *Pseudomonas aeruginosa* isolates in various locations in patients[29-33]. The combined effect of Cip and Pro. approved with a study in Ahmed[31] showed a synergistic effect of *Lactobacillus* probiotics and antibiotic combinations against the most virulent bacteria.

The result of gene expression under stress is the combination of probiotics and ciprofloxacin on the *lasI* gene of *Pseudomonas aeruginosa* compared with the mono-effect of ciprofloxacin or probiotics.

As mentioned by Kart et al[34], ciprofloxacin at subinhibitory concentrations has been demonstrated to modify the expression of quorum-sensing genes in *Pseudomonas aeruginosa*. A study conducted by Patel et al[35] in Vadodara, India, agrees with this result. They were shown supernatant derived from *Lactobacillus plantarum* strains was found to interfere with the varied expression of *lasI* and *rhII.*

Maqbool et al[36] describe the probiotic culture, such as *Lactobacillus parasaci*, when used alongside traditional antibiotics, displays the most rapid inhibitory effect and prolonged efficacy against multidrug-resistant *Acinetobacter baumannii*.

Conclusions

Evaluation of treatment by ciprofloxacin at Sub-MIC showed inhibited biofilm, and expression of gene *pelA* was significantly higher at 0.05 than in the combination or probiotic alone. While the combination of ciprofloxacin had the appearance of a highly significant effect on gene expression, it was followed by ciprofloxacin and then probiotics, both separately, on gene expression of the *lasI* gene responsible for signaling in quorum sensing. Probiotics didn't replace antibiotics; instead, they augment their efficacy by disassembling the matrix or weakening the cells, so facilitating the antibiotics' access and action. *Lactobacillus* species and other probiotics were made substances that break down or deactivate signals (AHLs), which are the major QS signaling molecules in *Pseudomonas aeruginosa.* It also worked as signaling interference by producing AHL analogs, which were bound with the lasR receptor of QS in *Pseudomonas aeruginosa* instead of the *LasI* signal, or, as mentioned earlier, it produced antimicrobial compounds that inhibit QS. This study suggested that the combination of ciprofloxacin and probiotics to treat XDR *Pseudomonas aeruginosa* isolate production biofilm may enhance treatment effectiveness, decrease *Pseudomonas aeruginosa* isolates, and offer a unique complementary strategy against multidrug-resistant *Pseudomonas aeruginosa*.

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Conflict of Interest
The author has no conflicts of interest to disclose

Financial Disclosure
This study has been performed without any funding from outside else.

Authors' contributions
Conception and design by Hassan RK, Acquisition, analysis, and interpretation of data by Al-Zubaidi SJJ. Manuscript drafting and revising it critically by Hassan RK and Al-Zubaidi SJJ and Statistical sand approval of the final version of the manuscript was done by Hassan RK.

Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
The Institutional Review Board granted the study ethical approval. Since this was a prospective study, every study participant provided formal informed consent. Each method followed the appropriate rules and regulations. It was approved as research unsheathed from a Ph.D. thesis by Biology Department, College of Education for Pure Sciences University of Diyala, Diyala, Iraq.

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