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Antibacterial and Antibiofilm effects of probiotic against Multidrug Resistant *Pseudomonas aeruginosa* bacteria from clinical isolates

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Abstract

Objective

Resistance to antibiotics represents a significant worldwide health issue that exposes the effective treatment of several bacterial illnesses, particularly those induced by multi-drug-resistant (MDR) *Pseudomonas aeruginosa*. Consequently, there's an imperative necessity to develop novel techniques to address resistant to antibiotics microorganisms. Probiotics which have demonstrated potential advantages in several disorders. Objective: The aim of study is to investigating the antibacterial and antibiofilm effects of probiotic against MDR *P. aeruginosa* clinical isolates.

Materials and methods

Twenty MDR P. *aeruginosa* isolates isolated from 165 samples obtained from different clinical specimen (ear, wound, burn, and urine). Antibiotics susceptibility test was performed using disc diffusion method. Lactobacilli species were isolated from 15 different natural samples (7 milk, 5 curd and 3 vagina), and identified by biochemical tests and confirmed using vitek 2 system. Cell free supernatant (CFS) had been utilized for evaluating both antibiofilm and antibacterials efficacy against multidrug-resistant *P. aeruginosa* isolates through the agar well diffusion method and microtiter plate assay to assess antibiofilm activity.

Results

Out of 15 different natural samples, 10 (3 milk, 5 curd and 2 vagina) samples were positive for Lactobacilli spp. Among 20 isolates of *P. aeruginosa* only seven isolates were strong biofilm producer. The findings indicated that the most potent inhibitory effects on the development for three isolates of P. aeruginosa (P7, P13, and P15) were observed at minimum inhibitory concentration (MIC) and sub-MIC levels (25 and 12.5 µg/mL, respectively, and four isolates (P3, P4, P11, and P18) exhibited MIC and sub-MIC (50 and 25 µg/mL), respectively. The inhibitory effect of CFS were done against only 7 MDR P. *aeruginosa* isolates which highest biofilm formation, The results showed antibacterial effect. In the susceptible isolates, the zone of inhibition varied from 3 mm to 12 mm in diameter.

Conclusions

Among 10 Lactobacillus spp. isolates, two isolates (from vaginal sources) exhibited no inhibitory activity against one or more of the multidrug-resistant *P. aeruginosa* strains. Lactobacilli spp. Isolated from (milk and curd) that have inhibitory effect on growth against *P. aeruginosa*, Findings ultimately corroborated the concept of employing probiotics as a substitute for antibiotics in the treatment of resistant to antibiotics pathogenic bacteria.

Keywords*:* biofilm, inhibitory effect, MDR, probiotics, *Pseudomonas aeruginosa*

Paper Type: Research Paper.

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Introduction

Pseudomonas aeruginosa constitutes an opportunistic pathogen affecting invertebrates, plants, and humans, particularly among immuno-compromised individuals. It's toxic and readily develops resistance to antibiotics (Jeong et al., 2023). This pathogen possesses different virulent

factors, which includes hemolysin, pyocyanin, and formation of biofilm (Abbas et al., 2017; Al-Charrakh et al., 2016). *Pseudomonas* strains generate several harmful components, such as adherence, lipopolysaccharides, flagella, endotoxin, exotoxin A, exoenzyme S, hemolysin, iron-binding proteins, type III secretion system, alginate, pyocyanin, and proteases (Alexandre et al., 2014; Salih et al., 2024; Shams Eldeen et al., 2021). These components facilitate the invasion, attachment, egress, and cell death of microbes. The bacterium *P. aeruginosa* has developed or acquired resistance to a wide variety of antibiotics (Shams Eldeen et al., 2021). The extensive utilize of antibiotics has augmented resistant to antibiotics in microorganisms globally. The rise of multi-drug-resistant (MDR), extensively drug-resistant (XDR), and particularly pandrug-resistant (PDR) infections poses a significant challenge in the management of hospitalized patients (Abootaleb et al., 2021). In *P. aeruginosa*, resistant to carbapenem was caused by modified penicillin-binding proteins, an augmented efflux mechanism, reduced permeability for outer membrane, or the carbapenemase enzymes that hydrolysis carbapenem (Shariati et al., 2019). *P. aeruginosa* that generates metallo-beta-lactamase (MBL) poses an increasing threat and represents a significant issue because to its emergence as one of the most dreaded mechanisms of resistance (Alavi Foumani et al., 2020; Horcajada et al., 2019). Their extensive substrates range, tendency for horizontal transmission, and resistance to inhibition by serine β-lactamase inhibitors that include clavulanic acid, sulbactam, and tazobactam distinguish them from other carbapenemase (Algammal et al., 2020). A biofilm consists of a meticulously structured assemblage of bacterial cells that adheres to external surfaces and has the potential to induce persistent infections in humans (Sharma et al., 2018; Valdéz et al., 2005). Shokri *et al.* identified a mechanism associated with biofilm production that contributes to resistance in *P. aeruginosa*, indicating that 77 (or 94%) of the examined strains of *P. aeruginosa* generated biofilms as a protective response to antibiotics (Shokri et al., 2018). Consequently, the architecture of biofilms may obstruct the penetration of antimicrobial agents, since some charges inhibitors may adhere to the opposite-charge polymers inside the biofilm's matrix (Marsh, 2009; Taraszkiewicz et al., 2013). Biofilms diminish the efficacy of antibiotics and induce resistance to the host's innate immune responses (Argenta et al., 2016; Valdéz et al., 2005). Consequently, more study is essential to formulate novel antibiotics and to ascertain alternative therapeutic approaches. Research is being conducted to determine the efficacy of probiotics, phages, or natural therapies in treating this infection without the use of antibiotics (Chatterjee et al., 2016). Probiotics are living microorganisms that, when provided in enough amounts, have a beneficial impact on the host organism (Sharma et al., 2018). One of the most intriguing aspects of probiotics is their potential to modulate the immune system. Research suggests that certain strains of probiotics could enhance both innate and adaptive immune responses. This modulation could have far-

reaching implications for conditions ranging from allergies to autoimmune disorders, offering a promising avenue for therapeutic intervention. (Chen et al., 2022) This microbe can alter the flora of their surrounding environment (Shams Eldeen et al., 2021). Moreover, probiotics may be efficacious in combating and avoiding infections associated with burn wounds (Argenta et al., 2016). Probiotic microorganisms exhibit anti-inflammation with regenerating properties in the treatment of skin injury (Abootaleb et al., 2021). The medicinal function of probiotics had been demonstrated through the synthesis of antimicrobial compounds, eradication of pathogens, and regulation of the immune system (Mazziotta et al., 2023). Probiotics have demonstrated antimicrobial action against pathogens (Kumar Bajaj et al., 2015). *L. acidophilus* serves as a microbiological barrier against many pathogens by competing for binding sites, enhancing the host's immune response, and producing antimicrobial chemicals such as acids, bactericides (Singh et al., 2020). At the same time, probiotics have been widely used as nutritional additives to improve intestinal health, increase nutrient absorption, and modulate immune responses in different organisms (Mohammadabadi et al., 2025a). Proposed mechanisms of action of probiotics include competition with pathogens, strengthening the intestinal epithelial barrier, production of beneficial metabolites, and stimulation of innate and adaptive immune pathways (Khezri et al., 2025; Mohammadabadi et al., 2025b). Meta-analyses and systematic reviews have indicated that probiotics can improve immune indices and various functional components in different conditions, although the consistency of results in individual studies is not complete and the efficacy depends on the microorganism species, dose, and environmental conditions (Mohammadabadi et al., 2025c). Probiotics have shown remarkable promise in managing various gastrointestinal disorders. Conditions such as irritable bowel syndrome, inflammatory bowel disease, gastric and colorectal cancers, and antibiotic-associated diarrhea have all been the subjects of extensive probiotic research (Fouché et al., 2023). Moreover, strains such as Bifidobacterium longum, Lactobacillus plantarum and Pediococcus acidilactici have shown to alleviate ETEC-induced diarrhea by regulating the immune response, rebalancing intestinal microbiota, and improving carbohydrate metabolism (Li et al., 2022) Therefore, probiotics have demonstrated a noteworthy anti-inflammatory effect, showcasing their potential as a valuable intervention in managing inflammatory conditions (Foey et al., 2022). The role of probiotics in metabolic health has garnered increasing attention, particularly in the context of obesity and hypercholesterolemia. For instance, oral administration of recombinant probiotics expressing lactoferrin could improve diet-induced lipid accumulation and inflammation in non-alcoholic fatty liver disease (Liu et al.,2022). Moreover, emerging evidence suggests that certain probiotic strains may influence metabolic pathways, leading to promotion of the intestinal transformation of ellagic acid, which leads to the upregulation of liver bile synthesis, thus preventing

hypercholesterolemia (Jin et al., 2023) These findings hold significant implications for the prevention and management of metabolic disorders. This bacterium may generate a significant category of antibiotic chemicals, including bacteriocins, which exhibit a broad spectrum of activities against infections. Probiotics may serve as a beneficial adjunct to antibiotics in the treatment of patients with *P. aeruginosa* infections (Abasgholizade & Fozouni, 2017). Certain probiotics inhibit the attachment of infections to host cells. Various probiotics may produce metabolic with antibacterial properties, including bacteriocins, hydrogen peroxide, and organic acids (Kumar Bajaj et al., 2015). Lactic acid bacteria (LAB), including Lactobacillus, are recognized probiotics that exhibit antiseptic qualities, enhance immunological function, facilitate repair of tissues, and promote angiogenesis (Shams Eldeen et al., 2021). Research indicates that a number of lactobacilli exhibit antibacterial properties against *P. aeruginosa*. The current work aims to isolate, describe, and examine the in-vitro anti-bacterials and anti-biofilms properties of Lactobacilli derived from several natural materials against multidrug-resistant P. aeruginosa clinical isolates.

Materials and methods

Bacterial isolation and identification: In the present study, a total of 165 different clinical samples including (urine, sputum, ear, wound, and burn), Twenty isolates of P. aeruginosa were submitted to initial diagnostics laboratory testing, including Gram staining, biochemical assays, and the VITEK2 system for bacterial identification. The study period was from October 2023 to April 2024. At Baquba Teaching Hospital and Al-Batool Hospital at Diyala city, Iraq.

Isolation and characterization of probiotics from natural samples: Ten isolates of Lactobacillus spp. were isolated from 15 different samples include (7 Milk, 5 Curd and 3 Vagina samples). All samples were cultured on De Man, Rogosa, and Sharpe (MRS) agar plates and incubated anaerobically at 37c to 48-72 hours in anaerobic jar with gas -pack kit, all isolates underwent initial diagnosis laboratory procedures, including Gram staining, biochemical assays, and the VITEK2 system for bacterial identification.

Antibiotics susceptibility test (AST): Antibiotics susceptibility test was done via disk diffusion method (Kirby-Bauer method) according to Clinical Laboratory Standard Institute recommendations (Alavi Foumani et al., 2020). The test was performed on all isolates of *P. aeruginosa*; the antibiotics chosen were Amikacin (30µg), Imipenem (10µg), Cefixime (5µg), Ceftriaxone (30µg), Ceftazidime (30µg), Amoxicillin- clavulanic acid (20/10µg), Ceftazidime (75µg), Streptomycin (15µg), Piperacilin (2µg), Ofloxacin (15µg), Cefotaxime (30µg), Meropenem (30µg), Azithromycin (15µg), and Ciprofloxacin (5µg) (Bioanalyse, Turkey) using the disc diffusion method. Isolate suspension was prepared at 0.5 McFarland, and 100 µl of suspension was spread onto plates with Mueller Hinton agar, followed by incubation at 37°C for

18 h. After incubation, the inhibition zone around the disc was measured in millimeters. The results were analyzed according to the recommended guidelines established by the Clinical and Laboratory Standards Institute (CLSI 2024) (Weinstein & Lewis, 2020). Detection of multidrug-resistant bacteria According to a new standardized international document presented by the Center for Disease Control and Prevention (CDC), MDR isolates were defined as acquired non-sensitivity to one or more antibiotics in ≥3 antibiotic categories.

Biofilm generation assay: The capability of multidrug-resistant P. aeruginosa isolates to produce biofilm was evaluated following the methodology established by O'Toole and Kolter (O'Toole, 2011). Isolates were cultivated in LB broth and maintained at 37°C overnight. Subsequently, the isolated bacteria were diluted 1:100 using Tryptic Soy Broth, thereafter, 200µl of the suspension was introduced into a 96-well microtiter plate (polyvinyl chloride) and incubating at 25°C. Following a 48-hour incubation, 150 µl of a 0.1% (w/v) aqueous solution of crystal violet was introduced into every well, that was subsequently rinsed thoroughly with double-distilled water (DDW). Following a 15-minute incubation, every test was conducted in triplicate, and the average OD 630 value of the examined wells was utilized to assess biofilm development capability. An uninoculated medium was utilized as a control for the calibration of optical density (OD). Table 1 categorizes the outcomes of biofilm development as biofilm-negative (-), weak (+), medium (++), and strong (+++).

Table 1. Categorization of Bacteria According to Biofilm Formation Strength on Microtiter Plates

Biofilm formation	Status	Result
$OD \leq OD_c$	Non-adherent	-
$OD_c < OD \leq 2 \times OD_c$	Weakly adherent	+
$2\times OD_c < OD \leq 4\times OD_c$	Mederatly adherent	++
$4 \times OD_c < OD$	Strongly adherent	+++

Preparation of lactobacillus isolates cell-free supernatants (CFCs): In accordance with the Sousa et al., (2008) study, the cell-free supernatants were prepared as follows: Bacteria were cultivated to the mid-exponential phase in MRS broth for 24h at 37°C in anaerobic conditions after being extracted off an agar plate (Sousa et al., 2008). The McFarland standard of 0.5 turbidity was employed to adjust the OD for standard solution of cell. The supernatant was prepared by added 0.1 mL from standard cell suspension to a tube containing MRS broth and incubating the combination for 24 hours at 37°C. Centrifugation at 10,000 × g for 15 minutes at 4°C, followed by filtering through a sterile 0.22 µm membrane and subsequent plating on MRS agar, yielded no growth of lactobacilli. The inhibitory activity of this recently made cell-free supernatant (stock solution) was tested.

Determination of minimum inhibitory concentration: Minimum inhibitory concentrations (MICs) are the lowest antimicrobial agent concentrations that would prevent the observable growth of a microbe following overnight incubation. The experiment was done according to the study of Elshikh *et al*., as follows: Each Lactobacillus isolate was grown in 100 µL of Muller Hinton broth (MHB) with P. aeruginosa at a pH of 7 in Column 1, while Columns 2-10 contained 50 µL of MHB broth alone (Elshikh et al., 2016). Column 12, which served as a control for sterility, contained 100 µL of diluted standardized inoculum, whereas column 11, which was visible on the processed plate, contained 100 µL of medium broth. Each well received 50 µL of surfactant after the surfactants were transferred and mixed utilizing a multichannel pipette from columns 1 to 10. Next, a hundred times as much MHB broth was added to the suspension of reference microbes. The control wells and all the wells treated with surfactant were supplemented with 50 liters from the bacterial solution with the adjusted OD600. 2.5×10^{1} CFU mL^{-1}. The whole time spent on making and dispensing the OD-adjusted bacteria was less than 15min. Following a 24-hour incubation at 37°C, resazurin (0.015%) was introduced to each well (30 µL per well), and the plates were then incubated for an additional 2 to 4 hours to facilitate the observation of color change. At the conclusion of the incubation time, columns exhibiting no change in the blue resazurin hue were deemed to be at or over the MIC value. Sub-MIC wells are subsequent to MIC wells.

Antimicrobial efficacy of probiotics against *P. aeruginosa* isolates-Agar diffusion assay: Thirty different P. aeruginosa isolates were used to investigate the antibacterial activity of each of the seven probiotic strains. The process began with the cultivation of P. aeruginosa on MHA media using a 0.5 McFarland concentration suspension, which was done in a spreadsheet. In the plates that were being grown, holes that were 6 mm in diameter were bored. Next, 50 µL of culture supernatants containing Lactobacillus isolates were added to the wells. Plates were incubated at 37°C for 24 hours, and then the inhibitory zone's width was determined (Fijan et al., 2022).

Assessing the development of biofilms: Strains of P. aeruginosa were cultured in 0.25% glucose-containing tryptic soy broth (TSB) for one night. Afterwards, a 0.5 McFarland (1:100) concentration was used to transfer this new culture to 96-well polystyrene microtiter plates. The wells were then incubated at 37°C for 24 hours after being supplemented with 100 microliters of diluted probiotic CFS. Each well's plankton bacterium was rinsed three times with distilled water that was sterile and then dried. The nonadherent, free-floating bacteria were removed after 150 µL of crystal violet (0.1%) was added to every well and allowed to settle for 15 minutes at room temperature. To get rid of the lost pigment, to wash it five times with distilled water. Distaining was accomplished by soaking the preparations in 200µL of 95% ethanol for 3min. Finally, each

sample was transferred in a volume of 150 µL to a new microtiter plate. At 600nm, an ELISA reader was utilized for measuring the OD of the ethanol dye solution. The formula was used to calculate the percentage of biofilm inhibition, as follows (Blando et al., 2019):

Biofilm reduction (%) = $OD_{control}$ /OD_{sample} X 100%.

Statistical analysis*: Every* test was conducted in duplicate. Every test included a negative control with a positive control. Statistical analysis was conducted using SPSS version 22 software, with a p value of less than 0.05 being statistical significance.

Results

Bacterial identification: Twenty isolates of *P. aeruginosa* were isolated from different specimens. The specimens comprised ear (6 isolates,30%), urine (7 isolates, 35%), burn (4 isolates,20%), and wound (3isolates,15%), Each of the twenty isolates were identified utilizing conventional biochemical assays, included both catalase and oxidase positive responses, as well as an alkaline/alkaline (ALK/ALK) reaction on Triple Sugar Iron (TSI) agar, gram stain negative and were positive growth on MacConkey agar.

Antibiotic sensitivity test: According to the sensitivity profile of multidrug-resistant isolates, 18 (90%) of *P. aeruginosa* isolates were resistance for both Ceftriaxone and Amoxicillin-clavulanic acid, while the lowest resistance against imipenem was 45%, Resistance classification for all 20 MDR *P. aeruginosa* isolates were 5(25%) XDR and 15 (75%) MDR, as shown in figure 1. and 2.

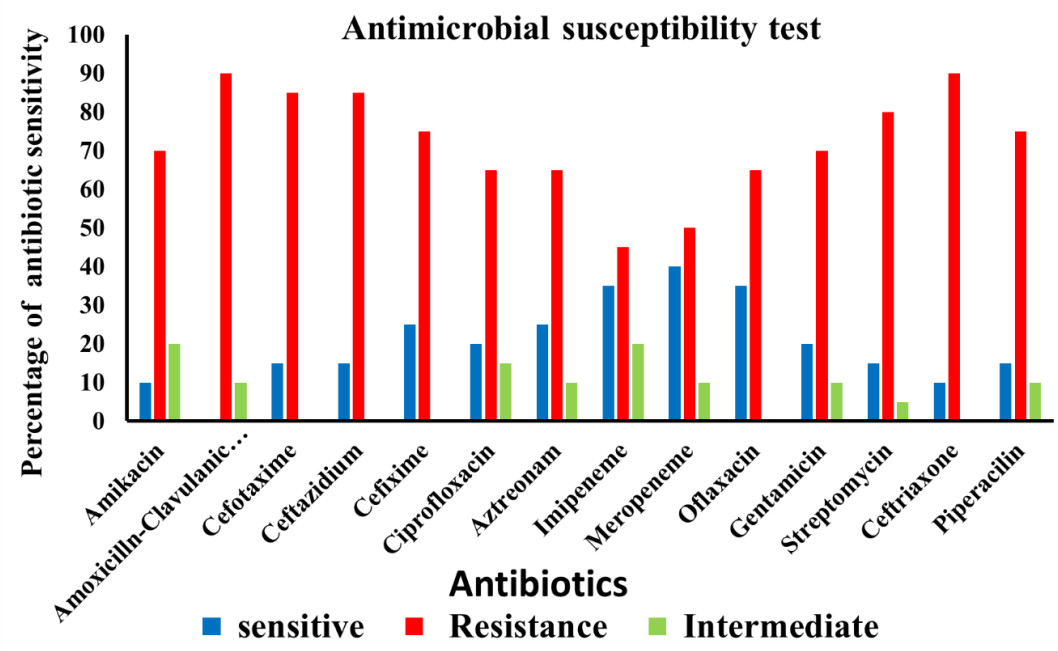

Figure 1. Antimicrobial sensitivity tests against *P. aeruginosa* isolates (n=20)

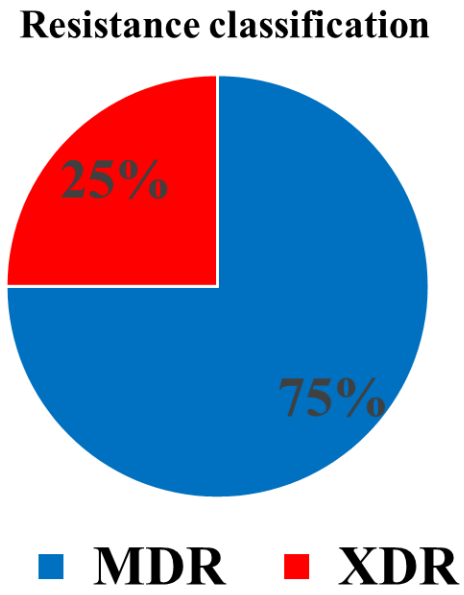

Figure 2. Resistance classification for each isolate

Measuring of biofilm formation: Results showed 20 MDR *P. aeruginosa* isolates were tested using microtiter plate assay that 7 (35 %) isolates were strongly producer for biofilm and 13(65 %) isolates were moderately, as shown in Table 2. This classification was performed according to measuring of absorbency for every well at 630 nm utilizing ELISA reader and by extracted the OD value for the control well from all the test OD values.

Table 2. Status of the Level of Biofilm Formation on *P. aeruginosa* Isolates

Level of biofilm production	No. & % of isolates
Strongly producer	7 (35%)
Moderately producer	13(65%)
Weak or non-producer	0 %
Total	20 (100%)

Antibacterial efficacy of CFS against *P. aeruginosa:* Out of 10 *lactobacillus spp.* isolates, two isolate (vaginal source) did not show inhibition effect against any of the MDR *P. aeruginosa* and the CFS for eight probiotic isolates exhibited antipseudomonal efficacy. Probiotic isolates (L3) had the most significant inhibitory activity against P. aeruginosa. This isolate has yielded an average inhibition of 12 mm against pathogen isolates using the well-diffusion method. The minimal inhibitory zone was associated with strain (L8) (3 mm).

Antimicrobial susceptibility test and biofilm formation: Seven MDR *P. aeruginosa* isolates highly biofilm producers were selected, namely (P3, P4, P7, P11, P13, P15 and P18) for measuring inhibition effect of Lactobacillus spp. Assessment the MIC for cell-free culture supernatants from Lactobacillus isolates to identify the lowest dilution of CFCs that inhibits the growth for P. aeruginosa isolates. The findings indicated that the most potent inhibitory impacts on the growth of three isolates of *P. aeruginosa* (P7, P13, and P15) were observed at MIC and sub-MIC concentrations of 25 and 12.5 µg/mL, respectively. In contrast, four isolates (P3, P4, P11, and P18) exhibited MIC and sub-MIC values of 50 and 25 µg/mL, respectively, of Lactobacillus spp., as shown in Table 3.

Table 3. MIC Values of Lactobacillus spp. Against MDR *P. aeruginosa*

NO. of P. aeruginosa	Concentration of MIC of Lactobacillus spp.			
	100	50	25	12.5
P3	-	MIC	Sub-MIC	
P4	-	MIC	Sub-MIC	
P7	-		MIC	Sub-MIC
P11	-	MIC	Sub-MIC	
P13	-		MIC	Sub-MIC
P15	-		MIC	Sub-MIC
P18	-	MIC	Sub-MIC	

Assessment of Lactobacillus spp. efficacy in anti-biofilm activities: Lactobacillus spp. isolates were exhibited varying degree of biofilm -disrupting activity against *P. aeruginosa* isolates. The findings indicate that lactobacilli have a significant antibiofilm capability against seven isolates of P. aeruginosa, with four isolates demonstrating substantial MIC effects and three showing weak effects, While the effect of sub-MIC show strong effect against 3 isolates and moderate against 4 isolates, as shown in table 4 and figure 3.

Table 4. Antibiofilm activity of Lactobacillus spp. against P. aeruginosa isolates

Effect of L. acidophilus	Direct	MIC	Sub-MIC
P3	Non adherent	Moderate	Strong
P4	Non adherent	Moderate	Moderate
P7	Non adherent	Moderate	Strong
P11	Non adherent	Weak	Moderate
P13	Non adherent	Weak	Moderate
P15	Non adherent	Weak	Moderate
P18	Non adherent	Moderate	Strong

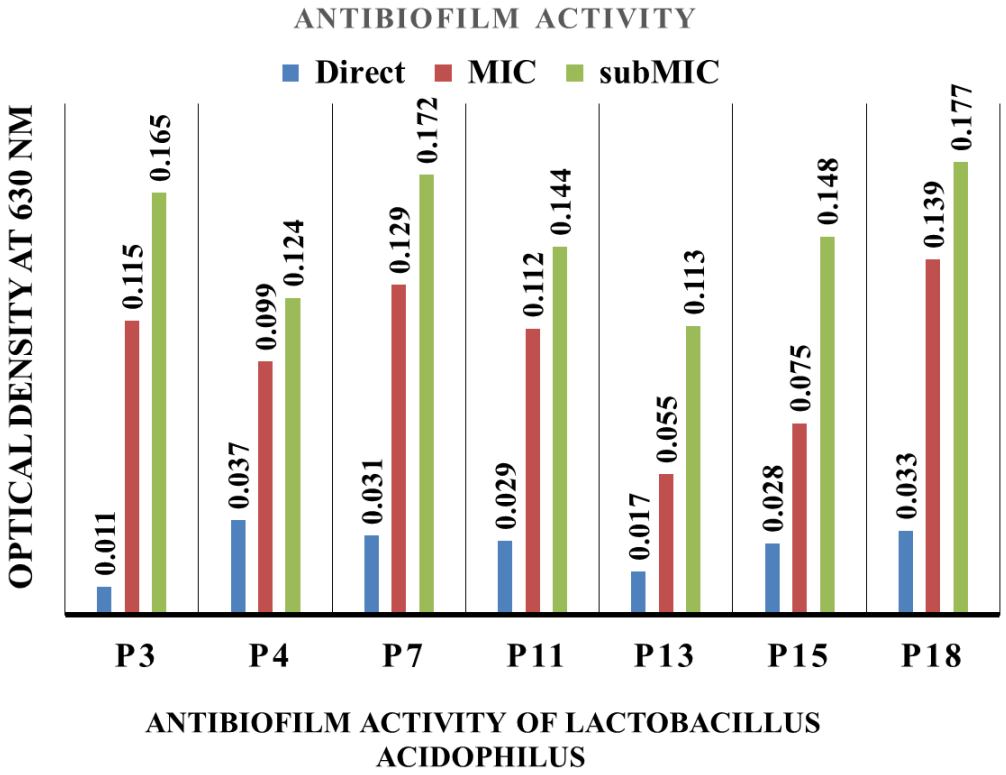

Figure 3. The antibiofilm potential of Lactobacillus *acidophilus* against seven isolates of MDR *P. aeruginosa* (P3, P4, P7, P11, P13, P15, P18)

Discussion

The persistent application of traditional methods for treating microbial illnesses has led to the emergence of resistant isolates, rendering diseases increasingly challenging to manage. Research is presently concentrated on the development of novel antibiotics and other therapeutic approaches that do not include antibiotics (Kiymaci et al., 2018). Moreover, probiotics are regarded as a non-toxic and accessible alternative to antibiotics (Shokri et al., 2018). Probiotic impacts are strain-specific; hence, their efficacy varies, and not every strain were suggested for the same health advantages (Alexandre et al., 2014). Of the 20 isolates of *P. aeruginosa*, 10 were classified as multidrug-resistant, exhibiting resistance to three classes of antimicrobials, complicating the selection of adequate antimicrobial treatment, with the overuse of antibiotics has resulted in a rise in P. aeruginosa's resistance to several antibiotics, which in turn has led to an increase in the prevalence of MDR strains of the bacteria (Yayan et al., 2015). As a result of this study, Lactobacilli spp. exhibit significant antibiofilm efficacy against each of the seven isolates of *P. aeruginosa* upon direct application. Gaspar *et al.* observed that L. acidophilus inhibited *S. agalactiae* and *P. aeruginosa*, while did not affect the *E. coli*, *S. aureus*, or *C. albicans* strains examined (Gaspar et al., 2018). Numerous studies have concentrated on utilizing *L. acidophilus*

and *L. plantarum* as efficacious probiotics to combat MDR *P. aeruginosa*, therefore addressing problems linked to this pathogenic bacterium, including nosocomial infections. *Lactobacillus spp*. may serve as an effective anti-virulence agent (antibiofilm). Findings indicated that natively isolated lactobacilli possess superior probiotic qualities compared to those that are commercially isolated (Díaz et al., 2020). Because it may prevent *P. aeruginosa* biofilm development, *L. acidophilus* may be a useful tool in the fight against this infection (Asadzadegan et al., 2023). The study conducted by Asadzadegan *et al.* (2023) demonstrated that the application of probiotics bacteria in treatment strategies can be highly successful in avoiding or mitigating hospital-acquired illnesses (Asadzadegan et al., 2023). The supernatant for isolated lactobacilli exhibits a substantial inhibitory impact on the activities of the primary pathogenic components of *P. aeruginosa* strains (Jiang et al., 2023). The study finding is supported by Mehboudi's et al which confirmed the impact of probiotic cell-free metabolites on multidrug-resistant (MDR) P. aeruginosa strains indicating that probiotic metabolites demonstrated antimicrobial activity against MDR P. aeruginosa while also down regulating expression of antibiotic resistance genes (Ghanbarzadeh Corehtash et al., 2015). P. aeruginosa has been shown to be capable of biofilm formation. An infection's persistence and resistance are largely attributable to biofilm, a virulence component (Ghanbarzadeh Corehtash et al., 2015). Many studies have concentrated on the use of Lactobacillus spp. as an alternative agent in the treatment of biofilm-associated illnesses because of their antibiofilm activities against antibiotic-resistant strains of S. aureus and P. aeruginosa (Brunel & Guery, 2017). P. aeruginosa biofilm development it prevented by L. acidophilus this may be a useful tool in the fight against this infection (Kar & Devnath, 2021).

Conclusion: Conclusion The results showed the possibility of using L. acidophilus and L. plantarum as effective probiotics to deal with multidrug-resistant P. aeruginosa, and hence treat some diseases associated with this pathogenic bacterium such as nosocomial infections. Also, Lactobacillus spp. can be a good anti-virulence agent (antibiofilm). In addition, the results showed that naturally isolated lactobacilli have more excellent probiotic properties than commercially isolated ones.

Author Contributions

Conceptualization: JIM; project administration: JIM and HAY; Methodology: JIM and JGM; Data analysis: JGM; Resources: JIM and JGM; Writing-original draft preparation: JIM, JGM, and HAY; Writing-review and editing: JIM and HAY; Visualization: JIM, JGM, and HAY. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

Available on request.

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Ethical Considerations

The present study registered in the University of Diyala (Approval letter session number on 25-ASJ-Sci165 on 22 Oct 2024)**.**

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Conflict of Interest

The authors declare no conflict of interest.

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اثرات آنتی‌باکتریال و آنتی‌بیوفیلم پروبیوتیک‌ها علیه باکتری *Pseudomonas aeruginosa* مقاوم به چند دارو از جدایه‌های بالینی

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چکیده

هدف: مقاومت آنتی‌بیوتیکی یک مشکل عمده سلامت جهانی محسوب می‌شود که درمان مؤثر بسیاری از عفونت‌های باکتریایی، به‌ویژه عفونت‌های ناشی از *Pseudomonas aeruginosa* مقاوم به چند دارو (MDR)، را با چالش مواجه کرده است. از این‌رو، نیاز مبرمی به توسعه راهکارهای نوین برای مقابله با میکروارگانیسم‌های مقاوم به آنتی‌بیوتیک وجود دارد. پروبیوتیک‌ها مزایای بالقوه‌ای را در درمان اختلالات مختلف نشان داده‌اند. هدف این مطالعه بررسی اثرات آنتی‌باکتریال و آنتی‌بیوفیلم پروبیوتیک‌ها علیه جدایه‌های بالینی *P. aeruginosa* مقاوم به چند دارو بود.

مواد و روش‌ها: در این مطالعه، ۲۰ جدایه *P. aeruginosa* مقاوم به چند دارو از مجموع ۱۶۵ نمونه بالینی شامل نمونه‌های گوش، زخم، سوختگی و ادرار جداسازی شد. آزمون حساسیت آنتی‌بیوتیکی با استفاده از روش انتشار دیسک انجام گرفت. گونه‌های *Lactobacillus* از ۱۵ نمونه طبیعی مختلف (۷ نمونه شیر، ۵ نمونه ماست و ۳ نمونه واژینال) جدا شده و با استفاده از آزمون‌های بیوشیمیایی شناسایی و به‌وسیله سیستم Vitek 2 تأیید شدند. سوپرناتانت بدون سلول (CFS) برای ارزیابی فعالیت‌های آنتی‌باکتریال

و آنتی‌بیوفیلم علیه جدایه‌های *P. aeruginosa* مقاوم به چند دارو، با استفاده از روش انتشار در چاهک آگار و آزمون میکروپلیت برای سنجش فعالیت ضد بیوفیلم به‌کار گرفته شد.

نتایج: از میان ۱۵ نمونه طبیعی، ۱۰ نمونه (۳ شیر، ۵ ماست و ۲ واژینال) از نظر حضور گونه‌های *Lactobacillus* مثبت بودند. از میان ۲۰ جدایه *P. aeruginosa*، تنها ۷ جدایه تولیدکننده قوی بیوفیلم بودند. نتایج نشان داد که قوی‌ترین اثرات مهاری بر رشد سه جدایه *P. aeruginosa* (P7، P13 و P15) در غلظت حداقل مهارکنندگی (MIC) و زیر MIC (به‌ترتیب ۲۵ و ۱۲/۵ میکروگرم بر میلی‌لیتر) مشاهده شد، در حالی‌که چهار جدایه دیگر (P3، P4، P11 و P18) و MIC و زیر MIC برابر با ۵۰ و ۲۵ میکروگرم بر میلی‌لیتر را نشان دادند. اثر مهاری CFS تنها علیه ۷ جدایه *P. aeruginosa* مقاوم به چند دارو با بیشترین میزان تشکیل بیوفیلم بررسی شد و نتایج، اثر آنتی‌باکتریال قابل توجهی را نشان داد. در جدایه‌های حساس، قطر هاله عدم رشد بین ۳ تا ۱۲ میلی‌متر متغیر بود.

نتیجه‌گیری: از میان ۱۰ جدایه *Lactobacillus spp.*، دو جدایه (با منشأ واژینال) هیچ‌گونه فعالیت مهاری علیه یک یا چند سویه *P. aeruginosa* مقاوم به چند دارو نشان ندادند. گونه‌های *Lactobacillus* جداشده از شیر و ماست دارای اثر مهاری بر رشد *P. aeruginosa* بودند. یافته‌ها در نهایت این مفهوم را تأیید می‌کنند که می‌توان از پروبیوتیک‌ها به‌عنوان جایگزینی برای آنتی‌بیوتیک‌ها در درمان باکتری‌های بیماری‌زای مقاوم به آنتی‌بیوتیک استفاده کرد.

کلمات کلیدی: اثر مهاری، بیوفیلم، پروبیوتیک‌ها، MDR، *Pseudomonas aeruginosa*

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