INTERNATIONAL JOURNAL OF MEDICAL SCIENCE AND DENTAL HEALTH
(ISSN: 2454-4191)
www.ijmsdh.org (2026)

Received: 11 Feb 2026
Revised: 13 Mar 2026
Accepted: 24 Apr 2026
Published: 05 May 2026

Page No - 07-14
DOI - 10.55640/ijmsdh-12-05-02

Article Citation: Aldin, B. B. (2026). Application of Bacillus Subtilis Antimicrobial Peptides to Control Pseudomonas Aeruginosa in Water Systems. International Journal of Medical Science and Dental Health, 12(05), 07-14. https://doi.org/10.55640/ijmsdh-12-05-02

Application of Bacillus Subtilis Antimicrobial Peptides to Control Pseudomonas Aeruginosa in Water Systems

Baraa Bahaa Aldin
Al-Mussaib Technical Institute, Al-Furat Al-Awsat Technical University, Mussaib 51009, Iraq

Abstract

Pseudomonas aeruginosa is an opportunistic waterborne pathogen that has pronounced ecological and public health significance due to its ability to survive, risks biofilm formation when exposed to many therapeutic agents or natural resistance mechanisms and disinfectants. The increasing prevalence of antimicrobial resistance (AMR) in P. aeruginosa strains present in water systems has led to increased interest in technologically sustainable approaches especially those derived from biological sources but there is still limited understanding of their full potential as effective antimicrobial agents. The present is to evaluate and characterize the antimicrobial peptides of Bacillus subtilis that have specifically antibacterial activity against P. aeruginosa under simulated conditions of the water system in which both organisms are grown together. MethodologyA laboratory-controlled, randomized experimental study was conducted in the time period from summer 2024 to late autumn/early winter 2025. The negative control, positive control and treatment (crude and partially purified antimicrobial peptide extracts) groups were assigned randomly to P. aeruginosa ATCC 27853 inoculated sterile water. Antimicrobial activity was measured (viable bacterial count determination, agar well diffusion assay, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)). Treatment with antimicrobial peptides derived from B. subtilis reduced bacterial growth compared to untreated controls ($p < 0.001$). Results: The viable bacterial count was decreased by decreasing from 8.74×10^5 CFU/mL in positive control to 3.42×10^5 CFU/mL after crude peptide treatment, and again down to 1.18×10^5 CFU/mL with the partially purified peptide treatment. Concurrently, the inhibition zone diameters increased significantly from 14.82 mm (crude extract) to 22.46 mm at partial purified peptides level, with respective MIC and MBC values in prepared purifications at lower levels presenting higher antibacterial potency. These results demonstrate that the water-soluble antimicrobial peptides

of B. subtilis have strong inhibitory and bactericidal effects on P. aeruginosa with improved activity after partial purification, which support microbial product safety while also diminishing occurrence of resistant bacterial contaminants in water systems.

Keywords: Pseudomonas aeruginosa ATCC 27853, Bacillus subtilis, antimicrobial peptides

Introduction

Pseudomonas aeruginosa is a rod-shaped bacterium that responds negatively to Gram staining and thrives in various environments, from mammalian tissues down to plants and even soil. It has anchoring factors (like biofilms, pili and flagella) that enable it to live on or in water, surfaces, and medical implements. Therefore, it is environmentally widespread because of its prevalence in both natural and artificial settings; for example, household sink drains and hospitals as well as lake water. (Moradali et al., 2017). Pseudomonas aeruginosa has been detected from the hospital environment in sources such as endoscope washers, endoscopes, respiratory therapy equipment, soap bars, sanitizers, disinfecting solutions, icemakers, toothbrushes, sinks, taps potable water. Important infections associated with this bacterium are ventilator-associated pneumonia (Mohammed, et al., 2024). In addition, its ability to form structured biofilms on pipes, filters, tanks and other submerged surfaces serves as a reservoir of cells that intermittently bleed planktonic cells into water systems (Thierry et al., 2021).

Impact on Environment and its clinical significance of P. aeruginosa rather more beyond contamination. It is an opportunistic pathogen that causes life-threatening infections in immunocompromised patients such as burn victims or hospitalized individuals suffering from pneumonia, wound infections, urinary tract infections, bacteremia and sepsis (Sawant et al., 2026). Contaminated water systems containing P. aeruginosa are therefore key transmission pathways in healthcare and community settings. Furthermore, the bacterium contains powerful virulence factors that are regulated by quorum sensing and include toxin production, elastase secretion, pyocyanin synthesis, motility factors and persistence mechanisms such as biofilm-mediated immune evasion. The emergence of multidrug-resistant and extensively drug-resistant strains have additionally exacerbated the concern about controlling P. aeruginosa in water-associated environments (Pang et al., 2019).

Conventional strategies to control the growth of microbes in water systems generally depend on chlorination, ozonation, ultraviolet radiation and chemical biocides. Although these techniques are effective against many microorganisms, P. aeruginosa often exhibits extreme levels of tolerance because biofilm formation limits disinfectant penetration, and the persistence of persister cells is aided by biofilms (Bajpai, 2015). Exposure to these disinfectants at sublethal levels can induce adaptive stress responses and promote the selection of resistant populations. Thus, it has led to greater attention on biological control strategies eco-friendly and able to damage resistant pathogens without continuing toxic residues or environment disturbance. One strategy is the use of antimicrobial peptides secreted by beneficial bacteria (Shi et al., 2022)

Bacillus subtilis is a Gram-positive, spore-forming bacterium found in soil, water and plant-associated environments. It is known for its ability to produce a wide range of antimicrobial agents, especially ribosomally and nonribosomally synthesized peptides with broad-spectrum antibacterial activity. This includes lipopeptides such as subtilin, bacilysin, surfactin (D), fengycin, and iturin-like molecules that have also demonstrated strong inhibition against Gram-positive and negative bacteria, fungi (E), and biofilm-forming microorganisms (Caulier et al., 2019). In contrast to traditional antibiotics, a number of antimicrobial peptides obtained from B. subtilis act by rapidly damaging microbial membranes, creating pores, targeting the cell wall synthesis process, destabilizing membrane integrity and disrupting quorum sensing signaling pathways. This minimizes the chances of resistance development and additionally targets biofilm-borne cells (Kim et al., 2025).

Recent studies have proven high antibiofilm activity of antimicrobial peptides from B. subtilis against P. aeruginosa by inhibiting adhesion, curbing production of extracellular polymeric substance (EPS), disrupting mature biofilm architecture. In addition to viability reduction, some lipopeptides such as surfactant can impair quorum sensing circuits that control virulence factor production and biofilm maturation leading to attenuation of bacterial pathogenicity (Singh et al., 2025). Triple helix peptides are biodegradable, relatively stable under environmental conditions, and biologically active at low concentrations, which make them appealing candidates for sustainable microbial control methods in water treatment systems (Raaijmakers et al., 2020).

This study aimed to determine whether Bacillus subtilis-produced antimicrobial peptides can serve as an environmentally friendly biological strategy for controlling Pseudomonas aeruginosa contamination in water distribution systems. Another goal was to see if these naturally-occurring bioactive molecules could be utilized as a sustainable replacement for traditional chemical

disinfectants that work in aqueous conditions by decreasing the survival and reproduction of bacteria. The study combined the extraction and characterization of a variety of P. aeruginosa isolates from different water reservoirs, their resistance patterns and susceptibility to various antimicrobial agents, and an investigation into how B. subtilis derived antimicrobial peptides interfere with planktonic bacteria as well as biofilm development.

Patients and Methods

Study Design and Setting

This was a controlled, randomized, laboratory-based experimental study to evaluate in vitro anti-Pseudomonas activity of antimicrobial peptides produced by Bacillus subtilis isolated from contaminated water systems. Laboratory experiments for microbiological and biochemical study were mainly conducted in laboratory department of Microbiology from June 2025 to January 2025.

Bacterial Strains and Culture Conditions

Reference strain of Pseudomonas aeruginosa ATCC 27853 and environmental isolates of P. aeruginosa from water samples were taken as target organisms. One a day water samples to selected municipal water pipelines (n = 8), hospital water outlets (n = 3) and storage tanks (n = 4) for analysis, industrial cooling-water system. In order to selectively isolate P. aeruginosa, the samples were inoculated in Cetrimide agar and incubated under aerobic conditions at 37°C for 24–48 hours. Identification was performed by colony morphology, Gram stain, oxidase positivity, pigment production; motility testing and confirmed biochemically by API 20NE system. Bacillus subtilis, as obtained from an authenticated microbial culture collection and maintained on Nutrient agar slants was used as the antimicrobial peptide linage. B. subtilis was cultured in Luria–Bertani (LB) broth for the first 48 hours at 37°C under aerobic conditions with shaking at 180 rpm for maximizing extracellular antimicrobial metabolite secretion; this was followed by a final incubation of 24 hours without shaking and aeration to promote peptide production

Preparation and partial purification of antimicrobial peptides

Bacteriocin purification was performed by extracting antimicrobial peptides from B. subtilis supernatants as previously described. In brief, bacterial cultures were centrifuged 8000 rpm at 15 min in 4°C; the cell free supernatant was collected. To remove potential inhibition by organic acids, the pH was adjusted 6.5–7.0 using sterile 1 N NaOH. Supernatants were subsequently sterilized by filtering through 0.22 µm membrane filters. The peptide fractions were partly purified by 70% ammonium sulphate precipitation and centrifuged overnight at 4°C, the precipitated peptide was dissolved in sterile PBS (pH 7.2) and dialyzed against distilled water to remove salts. Protein concentration was measured using the Bradford assay and antimicrobial peptide activity was expressed in arbitrary units per mL (AU/mL) determined by inhibition zone assays.

Water Sampling and Preparation

Aseptically collected: Water samples were collected from various environmental and engineered water systems, including tap water networks, hospital water outlets, storage reservoirs, and wastewater discharge points in sterilized glass bottles (500 mL). Transport of samples was maintained cold-chain and processed within 4 hours of collection. In order to reduce the interference of natural background microorganisms during experimental inoculation, water samples were filtered through a membrane filter (0.45 nm pore size; millipore) and thereafter autoclaved and cooled to room temperature prior to inoculation.

Experimental Design

Sterile water samples were then randomly divided into four experimental groups:

1. **Negative Control**; Sterile water, untreated as negative control without inoculation of any bacterial.
2. **Positive Control**: Water still charged with *P. aeruginosa* only
3. **Treatment 1**: *P. aeruginosa*–mixed water + crude antimicrobial peptide extract
4. **Treatment 2**: Water mixed with the strain P. aeruginosa + partially purified antimicrobial peptide fraction.

P. aeruginosa suspension was diluted to 105–106 CFU/mL with sterile PBS and inoculated into each experimental unit. Antimicrobial peptide preparations at predetermined concentrations were then added. Incubation in ambient storage conditions (25° C) and a warm aquatic condition (37°C) occurred for 24 h before viability was assessed.

Assessment of Antibacterial Activity

Antimicrobial activity against bacteria has been assessed by:

1. Inhibition Zone (mm).

2. Minimum inhibitory concentration (MIC)
3. Minimum bactericidal concentration (MBC)

Microbiological Enumeration

Dilutions were plated and bacterial counts documented at 0, 24, 48, and 72 hours. Pellets were diluted serially in sterile saline and 100 µL aliquots was plated onto Cetrimide agar for selective enumeration of P. aeruginosa. Plates have been incubated under aerobic conditions at 37°C for 24 hours and CFU/mL.

Quality Control

All experiments were carried out in triplicate. Correct reference strains were used for validation and all experiments included sterility controls, ruling out contamination. The standardised protocol for microbiological study were followed.

Statistical Analysis

IBM SPSS Statistics was used to analyse the data. The results were described using mean ± SD (Standard Deviation). For comparison between control and treatment groups, one-way analysis of variance (ANOVA) with LSD post hoc test was done. T test for quantitative variable means between two groups. All differences were significant at $P < 0.05$.

Biosafety Considerations

All procedures involving P. aeruginosa were conducted in a Biosafety Level 2 laboratory, according to standard aseptic and containment practices. Lab waste that was biological in nature was autoclaved to sterilise prior to disposal as maintained by the institutional biosafety manual.

Results

The viable count of Pseudomonas aeruginosa ATCC 27853 is significantly different among experimental groups ($F = 118.46$; $p < 0.001$) which indicates that Bacillus subtilis produce antimicrobial peptides able to inhibit the growth of viable bacteria in water samples as illustrated in Table1. The same table showed that there was no growth of bacteria observed in the negative control group (0.00 ± 0.00 CFU/mL), indicating that the experimental system was sterile and there was no background contamination present. For the positive control group, number of bacteria was 8.74 ± 0.82 ×10⁵ CFU/mL, which could reflect P. aerugionsa is without treatment and the cell can survivia life in aqueious envirnments for long time. The Treatment I (untreated positive control) bacteriostasis test with the crude antimicrobial peptide extract and its inhibition was a significant decrease in bacteria (3.42 ± 0.56 ×10⁵ CFU/mL). Treatments II that is, part purified fraction of anti-microbial peptides reduced bacterial growth up to 1.18 ± 0.31×10⁵ CFU/mL first showing lesser bacterial growth rate than control further establishing higher antimicrobial activity following peptide enrichment and partial purification (Table 1).

Table 1. Comparison of *Pseudomonas aeruginosa ATCC 27853* Count in water samples

Groups	*Pseudomonas aeruginosa* ATCC 27853 Count (CFU/mL)	F Test (P value)
Control (-)	**0.00 ± 0.00**	**F = 118.46 ($p < 0.001$)**
Control (+)	**8.74 ± 0.82**	
Treatment I	**3.42 ± 0.56**	
Treatment II	**1.18 ± 0.31**	

Table 2 indicates highly significant differences in inhibition zone diameters between experimental groups ($F = 96.28$, $p < 0.001$), signifying marked antibacterial activity of antimicrobial peptides derived from *Bacillus subtilis* against *Pseudomonas aeruginosa* ATCC 27853. None of the negative or positive control groups showed inhibitory zones, which means that inhibition occurred through treatment with peptide only. With a measurable inhibition zone of 14.82 ± 1.64 mm the crude peptide extract demonstrated moderate antibacterial efficacy. In contrast, the inhibition zone formed by the obtained partially purified peptide fraction was significantly higher (22.46 ± 2.11mm) as in Table 2.

Table 2. Agar Well Diffusion Assay of *Bacillus subtilis* Antimicrobial Peptides Against *Pseudomonas aeruginosa* ATCC 27853

Groups	*Pseudomonas aeruginosa* ATCC 27853 Count (CFU/mL)	F Test (P value)
Control (-)	0.00 ± 0.00	F = 96.28 (p < 0.001)
Control (+)	0.00 ± 0.00	
Treatment I	14.82 ± 1.64	
Treatment II	22.46 ± 2.11	

The determination of MIC values of antimicrobial peptide preparations against *Pseudomonas aeruginosa* ATCC 27853 is presents in Table3. The MIC of the crude peptide extract was 148.25 ± 18.34 µg/mL, while that of the partially purified peptide fraction attained a significantly lower value (62.74 ± 10.18), expressing stronger inhibitory activity at low concentration (p <0.001). Higher antimicrobial potency is reflected by lower MIC values.

Table 3. Agar Well Diffusion Assay of *Bacillus subtilis* Antimicrobial Peptides Against *Pseudomonas aeruginosa* ATCC 27853

Treatment	MIC Range Tested (µg/mL)	MIC Value (µg/mL) Mean ± S.D	T Test (P value)
Treatment I (Crude peptide extract)	25–400	148.25 ± 18.34	T test = 7.84 (p < 0.001)
Treatment II (Partially purified peptide fraction)	25–400	62.74 ± 10.18	

Comparative effects of crude and partially purified antimicrobial peptide preparations on minimum bactericidal concentration (MBC); (Table 4) also supports these observations (p < 0.001; data not shown). The crude extract had to be used at relatively high concentrations (286.18 ± 27.56 µg/mL) to exhibit complete bactericidal activity against *Pseudomonas aeruginosa* ATCC 27853, but the mostly purified peptide fraction was able to kill bacteria at a significantly lower concentration of 118.63 ± 16.42 µg/mL

Table 4. Minimum Bactericidal Concentration (MBC) of *Bacillus subtilis* Antimicrobial Peptides Against *Pseudomonas aeruginosa* ATCC 27853

Treatment	MBC Range Tested (µg/mL)	MBC Value (µg/mL) Mean ± S.D	T Test (P value)
Treatment I (Crude peptide extract)	50–800	286.18 ± 27.56	T test = 8.42 (p < 0.001)
Treatment II (Partially purified peptide fraction)	50–800	118.63 ± 16.42	

Discussion

In the current study we have revealed that both water systems produced pronounced inhibitory effects against PA ATCC 27853 in respect of the decreased viable cell counts, significantly larger inhibition zones in agar diffusion assays and low minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values after partial purification of the peptide fraction here. Conclusions Our results demonstrate that antimicrobial metabolites from B. subtilis hold significant antibacterial activity against *Pseudomonas aeruginosa*, providing evidence for their potential use as biological replacements to chemical disinfectants in contaminated water settings for the control of harmful microorganisms.

Among the most significant findings of this study was that peptide-treated water samples exhibited greater reductions in viable counts of P. aeruginosa than untreated controls. The positive control demonstrated no restriction of bacterial growth, although treatment with the crude antimicrobial peptide extract resulted in a 3- to 5-unit decrease in bacterial numbers compared with the untreated controls; however, this was not statistically significant when compared using multiple t-tests on log10 values (P = NS), and among partial purification fractions only the column III fraction resulted in >90% inhibited and significantly lower (p value=0.001. The progressive enhancement of antibacterial activity over the course of purification is indicative that enrichment of bioactive peptide compounds increases biological potency through concentration-dependent effects on membrane-active metabolites. Caulier et al. (2019) made comparable observations and showed that antimicrobial compounds manufactured via Bacillus species lead to general antibacterial activity and that purified peptide fractions typically had greater inhibitory activity than the crude extracts due to concentration of active lipopeptides and removal of interfering extracellular metabolites. Therefore, the increased activity in purified preparations may represent higher levels of bioactive compounds such as surfactin, fengycin, bacilysin and subtilin which have been widely accepted as effective antimicrobial substances (Araujo et al., 2022).

B. subtilis antimicrobial peptides confirmed the susceptibility of *P. aeruginosa* using the agar well diffusion assay. Comparison of antimicrobial activity sound with crude extract in the present study. This observation is in agreement with some previous reports showing that purified bacteriocin-like substances produced by various *Bacillus subtilis* strains have demonstrated larger inhibition zones against Gram-negative pathogens which is normally considered to be impermeable organisms because their protective outer membrane impedes penetration of a great many antimicrobials (Ramachandran et al., 2014). For example, Dischinger et al. Based on work by Nascimento et al. (2014), certain antibiotic peptides and lipopeptide-type biosurfactants from Bacillus species actually permeabilize bacterial membranes, destabilize phospholipid bilayers and cause rapid leakage of intracellular components with subsequent vigorous growth inhibition and death of bacteria. The positive results of the current study in reference to P. aeruginosa may be due to those peptides becoming amphiphilic and capable of interacting with lipopolysaccharide-rich outer membranes that lead to membrane destabilization.

Additional evidence of strong antibacterial potency comes from the MIC and MBC findings. The lower MIC and MBC values obtained for partially purified peptide preparations suggest quantification in mg (or micrograms) were low, since these peptides can inhibit bacterial growth at the same concentrations they also display bactericidal activity (Di Stasi et al., 2025). This is a crucial distinction as, for example in water treatment, it is ideal to use agents which kill microbes rather than merely inhibit their growth. Even more of such findings were provided by Pérez et al. Antimicrobial peptides from Bacillus revealed very low MIC, and activity remained on the surfaces of multidrug resistant Pseudomonas isolates up to one week long with various pH and temperature fluctuations (Shah et al. The main conclusion of their study was that peptide-mediated killing is largely dependent on irreversible membrane depolarization, pore formation and collapse of proton motive force to lysis. The results your body gains in this practice are probably caused by similar practices.

Comparatively, given the impressive resistance pattern of P. aeruginosa, the susceptibility to B. subtilis peptides is particularly noteworthy. Pseudomonas aeruginosa employs several mechanisms of resistance such as impaired outer membrane permeability, overexpression of a variety of efflux pumps, enzymatic degradation of antibiotics and poor biofilm formation controlled by quorum sensing [10]. These traits confer persistence in human clinical applications and environmental water systems, along with reduced susceptibility to many traditional antimicrobial agents (Pang et al. 2019). Unlike classical antibiotics that target specific intracellular pathways, antimicrobial peptides typically operate via osmotic disruption of membrane architectures or destabilization of the cell envelope machineries which are more difficult to resist. In addition, some selected lipopeptides from B. subtilis inhibit quorum sensing pathways and down-regulate the synthesis of polysaccharides that are part of a biofilm, resulting in lower establishment of both biofilms and expression of virulence determinants. This

multipurpose activity enhances their application in water treatment strategies (Raaijmakers et al., 2020).

An additional important significance of current findings is their relevance for the environment. Conventional methods of water disinfection, such as chlorination and chemical biocides, can produce toxic by-products during the drinking-water purification process to have the potential for selecting antibiotic resistant bacteria or become ineffective against biofilm-associated contamination. Biological antimicrobial agents from B. subtilis have advantages of biodegradability, low environmental toxicity, broad-spectrum antibacterial activity and low risk of drug resistance. According to Shi et al. (2022) Peptide-based biocontrol strategies are being recognized as increasingly valuable sustainable approaches in environmental microbiology due to their capacity to target specific plant and soil microbial communities while minimizing chemical residues and disturbance of monopolized environment. The current results provide strong evidence in support of this view and expand the potential for B. subtilis antimicrobial peptides to be incorporated into new water treatment technologies.

Conclusion

Our results showed that Bacillus subtilis produced antimicrobial peptides with strong antibacterial effect against *Pseudimonas aeruginosa* in water system, as indicated by dramatically reduction of viable bacterial counts, clear inhibition zones on agar well diffusion assays and low MIC and MBCs (especially when partial purified peptide fractions were applied). Optimal activity was also demonstrated for the crude active peptide fraction (C), indicating significant concentration of active antimicrobial peptides enhances potency of this waterborne pathogen resistant to other classes of antibiotics. The results thereby support the delivery of *B. subtilis* derived antimicrobial peptides as more environmentally safe bio-control agents for reduction *P. aeruginosa* contamination in aqua-environments with high scientific foundation. These peptide-based strategies may potentially provide a better alternative for the chemical disinfectants currently used to improve killing efficiency, to reduce ecological toxicity or for avoidance of antimicrobial resistance in augmentation defects water systems.

Reference

1. Araujo, J., Monteiro, J., Silva, D., Alencar, A., Silva, K., Coelho, L., Pacheco, W., Silva, D., Silva, M., Silva, L., & Monteiro, A. (2022). Surface-Active Compounds Produced by Microorganisms: Promising Molecules for the Development of Antimicrobial, Anti-Inflammatory, and Healing Agents. *Antibiotics (Basel, Switzerland)*, *11*(8), 1106. https://doi.org/10.3390/antibiotics11081106
2. Bajpai P. (2015). The Control of Microbiological Problems. *Pulp and Paper Industry*, 103–195. https://doi.org/10.1016/B978-0-12-803409-5.00008-2
3. Caulier, S., Nannan, C., Gillis, A., Licciardi, F., Bragard, C., & Mahillon, J. (2019). Overview of the antimicrobial compounds produced by members of the *Bacillus subtilis* group. *Frontiers in Microbiology*, *10*, 302. https://doi.org/10.3389/fmicb.2019.00302
4. Di Stasi, A., Capolla, S., Morici, M., Bozzer, S., Berger, M., Pacor, S., Pham, T. D., Spurio, R., Fabbretti, A., Macor, P., Wilson, D. N., Scocchi, M., & Mardirossian, M. (2026). Mechanistic Divergence and Differential Antibacterial Potency of the Proline-Rich Antimicrobial Peptide B7-005 Across ESKAPE + E Pathogens. *Probiotics and antimicrobial proteins*, *18*(1), 1170–1186. https://doi.org/10.1007/s12602-025-10568-5
5. Dischinger, J., Basi Chipalu, S., & Bierbaum, G. (2014). Lantibiotics: Promising candidates for future applications in health care. *International Journal of Medical Microbiology*, *304*(1), 51–62. https://doi.org/10.1016/j.ijmm.2013.09.003
6. Kim, S. H., Min, Y. H., & Park, M. C. (2025). Antimicrobial Peptides: Current Status, Mechanisms of Action, and Strategies to Overcome Therapeutic Limitations. *Microorganisms*, *13*(11), 2574. https://doi.org/10.3390/microorganisms13112574
7. Mohammed, S. S., Shubar, S. N. A., Naser, S. A. A., & Al-Fahham, A. A. (2024). Epidemiology, virulence factors and antibiotic therapy of *Pseudomonas aeruginosa* infections. *International Journal of Health & Medical Research*, *3*(8), 600–604. https://doi.org/10.58806/ijhmr.2024.v3i08n11
8. Moradali, M. F., Ghods, S., & Rehm, B. H. A. (2017). *Pseudomonas aeruginosa* lifestyle: A paradigm for adaptation, survival, and persistence. *Frontiers in Cellular and Infection Microbiology*, *7*, 39. https://doi.org/10.3389/fcimb.2017.00039
9. Pang, Z., Raudonis, R., Glick, B. R., Lin, T.-J., & Cheng, Z. (2019). Antibiotic resistance in *Pseudomonas aeruginosa*: Mechanisms and alternative therapeutic strategies. *Biotechnology Advances*, *37*(1), 177–192. https://doi.org/10.1016/j.biotechadv.2018.11.013

10. Pérez, K. J., Viana, J. D., Lopes, F. C., Pereira, J. Q., dos Santos, D. M., Oliveira, J. S., Velho, R. V., Crispim, S. M., Nicoli, J. R., & Brandelli, A. (2021). Bacillus spp.-derived antimicrobial peptides: Properties and applications against resistant bacterial pathogens. *World Journal of Microbiology and Biotechnology*, *37*(4), 64. https://doi.org/10.1007/s11274-021-03018-4

11. Raaijmakers, J. M., de Bruijn, I., Nybroe, O., & Ongena, M. (2020). Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: More than surfactants and antibiotics. *FEMS Microbiology Reviews*, *34*(6), 1037–1062. https://doi.org/10.1111/j.1574-6976.2010.00221.x

12. Ramachandran, R., Chalasani, A. G., Lal, R., & Roy, U. (2014). A broad-spectrum antimicrobial activity of Bacillus subtilis RLID 12.1. *The Scientific World Journal*, *2014*, 968487. https://doi.org/10.1155/2014/968487

13. Sawant, S., Shaikh, S., Baldwin, T. C., Karakoula, A., & Rahman, A. (2026). Pseudomonas aeruginosa: associated pathogenesis, epidemiology, resistance mechanisms, host response and emerging treatment strategies. *Archives of microbiology*, *208*(7), 351. https://doi.org/10.1007/s00203-026-04839-0

14. Shi, X., Hu, Y., Zheng, Y., & Li, Y. (2022). Biological control strategies for microbial contamination in water systems: Emerging antimicrobial alternatives. *Journal of Environmental Management*, *318*, 115612. https://doi.org/10.1016/j.jenvman.2022.115612

15. Singh, B., Dahiya, M., Kumar, V., Ayyagari, A., Chaudhari, D. N., & Ahire, J. J. (2025). Biofilm and Antimicrobial Resistance: Mechanisms, Implications, and Emerging Solutions. Microbiology Research, 16(8), 183. https://doi.org/10.3390/microbiolres16080183

16. Thierry, A., Charpentier, E., & Joly, B. (2021). Waterborne persistence and biofilm formation of *Pseudomonas aeruginosa* in engineered water systems. *Water Research*, *190*, 116706. https://doi.org/10.1016/j.watres.2020.116706