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Multidrug Resistance in Pseudomonas Aeruginosa and Evaluation of Probiotics as Alternative Therapeutic Candidates

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Abstract: Pseudomonas aeruginosa is a critical opportunistic pathogen and a major cause of hospital-acquired infections. Its remarkable ability to develop multidrug resistance (MDR) has become a serious global health concern, especially in burn patients and immunocompromised individuals (Wang et al., 2024; Ramatla et al., 2025). This study aimed to investigate the prevalence and resistance profiles of P. aeruginosa isolates from clinical specimens and to evaluate the inhibitory potential of Lactobacillus rhamnosus and Lactobacillus acidophilus (Artini et al., 2024; Al-Janabi et al., 2025).

A total of 150 clinical samples (burn wounds, pus, urine, and sputum) were analyzed. Sixty isolates (40.0%) were confirmed as P. aeruginosa. Resistance rates were highest against ceftazidime (83.3%)

and ciprofloxacin (78.3%), while imipenem (40.0%) and amikacin (36.7%) retained partial effectiveness (Kim et al., 2024). Both probiotics inhibited MDR isolates, with L. rhamnosus producing significantly larger inhibition zones (17.8 ± 2.1 mm) compared with L. acidophilus (13.2 ± 1.7 mm; $p < 0.05$).

In conclusion, the study confirms the alarming resistance levels of P. aeruginosa and highlights the promising potential of probiotics as adjunctive antimicrobial strategies.

Keywords: Pseudomonas aeruginosa , burn wounds , Multidrug Resistance

Introduction

Pseudomonas aeruginosa is an opportunistic Gram-negative pathogen of critical clinical importance. It is frequently associated with burn wound infections, urinary tract infections, pneumonia, and bacteremia. Its clinical impact is largely due to intrinsic and acquired resistance mechanisms, including efflux pumps, β-lactamases, porin alterations, and biofilm formation (Wang et al., 2024).

The World Health Organization (WHO, 2024) placed carbapenem-resistant P. aeruginosa among the critical category of bacterial priority pathogens (WHO, 2024a; WHO, 2024b). Several systematic reviews have confirmed the rising prevalence of MDR strains, especially in burn centers, where skin barrier disruption and antibiotic pressure create a high-risk environment (Asefa et al., 2023; Kim et al., 2024; Jebali et al., 2025).

Conventional antibiotic options are becoming increasingly ineffective, driving research into non-antibiotic alternatives. Probiotics, particularly Lactobacillus spp., can produce antimicrobial compounds such as bacteriocins and biosurfactants, reduce pH, and inhibit pathogen adhesion and biofilm formation (Bober et al., 2020; Artini et al., 2024; Abdel-Rahman et al., 2024). Nevertheless, evidence against Gram-negative MDR pathogens is still limited.

This study therefore aimed to assess the prevalence of P. aeruginosa in clinical samples, analyze its resistance patterns, and evaluate the in vitro inhibitory activity of L. rhamnosus and L. acidophilus.

Materials and Methods

Study design and setting. This analytical cross-sectional laboratory study was conducted on consecutively collected clinical specimens from hospitalized patients in multiple wards, including burn units, surgical wards, intensive care units, and medical wards. All samples were processed in the bacteriology laboratory according to standard biosafety procedures (CLSI, 2024a, 2024b; American Society for Microbiology, 2023).

Sample collection and transport. A total of 150 non-duplicate clinical specimens were obtained under aseptic technique and included burn wound swabs, pus aspirates, urine, and sputum. Swabs were placed in sterile transport media and delivered to the laboratory within two hours to

minimize overgrowth and drying artifacts (CLSI, 2024a; Kim et al., 2024).

Isolation and identification of P. aeruginosa. Specimens were inoculated onto cetrimide agar and blood agar and incubated at 37°C for 24–48 hours. Colonies displaying characteristic morphology (grape-like odor, β-hemolysis in some strains, and pyocyanin pigmentation) were screened by oxidase test and confirmed via standard biochemical reactions (oxidase-positive, non-fermentative Gram-negative rods) (Wang et al., 2024).

Antimicrobial susceptibility testing (AST). AST was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar plates following CLSI M02 and M100 documents (CLSI, 2024a, 2024b). The following agents were tested: ceftazidime, ciprofloxacin, imipenem, amikacin, piperacillin–tazobactam, and gentamicin. Zone diameters were interpreted per CLSI (2024). Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as quality control strains (Ransom, 2023).

Definitions. Multidrug resistance (MDR) was defined as non-susceptibility to at least one agent in three or more antimicrobial categories, in accordance with international expert proposals (Magiorakos et al., 2012; Kadri et al., 2023).

Probiotic strains and preparation. Lactobacillus rhamnosus and Lactobacillus acidophilus were revived from lyophilized stocks and subcultured in De Man, Rogosa and Sharpe (MRS) broth at 37°C under microaerophilic conditions. For antimicrobial testing, cell-free supernatants (CFS) were prepared by centrifugation at 10,000 rpm for 10 minutes followed by filtration through 0.22 µm membranes. To exclude acid and peroxide effects, the CFS were adjusted to pH 7.0 and treated with catalase (Bober et al., 2020; Artini et al., 2024).

Agar well diffusion assay. Standardized inocula of MDR P. aeruginosa (0.5 McFarland) were spread on Mueller–Hinton plates. Wells (6 mm) were bored and filled with 100 µL of neutralized, catalase-treated CFS of each probiotic. Plates were incubated at 37°C for 24 hours and inhibition zones were measured in millimeters. Each experiment was performed in triplicate, and results are reported as mean ± standard deviation (Abdel-Rahman et al., 2024; Al-Janabi et al., 2025).

Statistical analysis. Data were entered into spreadsheets and checked for completeness. Continuous data are presented as mean ± SD. Two-tailed Student's t-test was used to compare mean inhibition zones between probiotics, with significance set at $p < .05$. Where appropriate, 95% confidence intervals were calculated. Results were interpreted in the context of recent regional and global evidence (Asefa et al., 2023; Ramatla et al., 2025).

Ethical considerations. The study followed institutional ethical guidelines for research on clinical specimens with de-identification and no patient-level intervention. The protocol adhered to the principles of the Declaration of Helsinki. No identifiable patient data are reported.

Results

Prevalence of P. aeruginosa. Out of 150 clinical specimens, 60 (40.0%) yielded P. aeruginosa. Burn wound swabs had the highest isolation frequency (48.3%), followed by pus specimens (41.7%), while urine (6.7%) and sputum (3.3%) were less frequent sources, mirroring patterns described in the regional literature (Asefa et al., 2023; Kim et al., 2024).

Antibiotic resistance patterns. Resistance was highest to ceftazidime (83.3%) and ciprofloxacin (78.3%). Imipenem (40.0%) and amikacin (36.7%) retained partial effectiveness. Piperacillin–tazobactam (55.0%) and gentamicin (61.7%) showed intermediate resistance levels. These findings are consistent with global trends of rising fluoroquinolone and cephalosporin resistance and concerning carbapenem resistance pressures (Wang et al., 2024; Ramatla et al., 2025).

Table 1: Antibiotic resistance patterns of P. aeruginosa isolates (n = 60)

Antibiotic	Resistant (%)	Intermediate (%)	Sensitive (%)
Ceftazidime	83.3	10.0	6.7
Ciprofloxacin	78.3	11.7	10.0
Imipenem	40.0	15.0	45.0
Amikacin	36.7	13.3	50.0
Piperacillin–Tazobactam	55.0	18.3	26.7
Gentamicin	61.7	15.0	23.3

Probiotic inhibitory activity. Both probiotics exhibited measurable inhibitory activity against MDR isolates. L. rhamnosus produced significantly larger inhibition zones (17.8 ± 2.1 mm) than L. acidophilus (13.2 ± 1.7 mm; $p < .05$), consistent with literature attributing stronger activity to L. rhamnosus due to bacteriocin and biosurfactant production (Artini et al., 2024; Abdel-Rahman et al., 2024; Al-Janabi et al., 2025).

Table 2 : Inhibition zones of probiotic strains against MDR P. aeruginosa

Probiotic strain	Inhibition zone (mm, mean ± SD)
Lactobacillus rhamnosus	17.8 ± 2.1
Lactobacillus acidophilus	13.2 ± 1.7

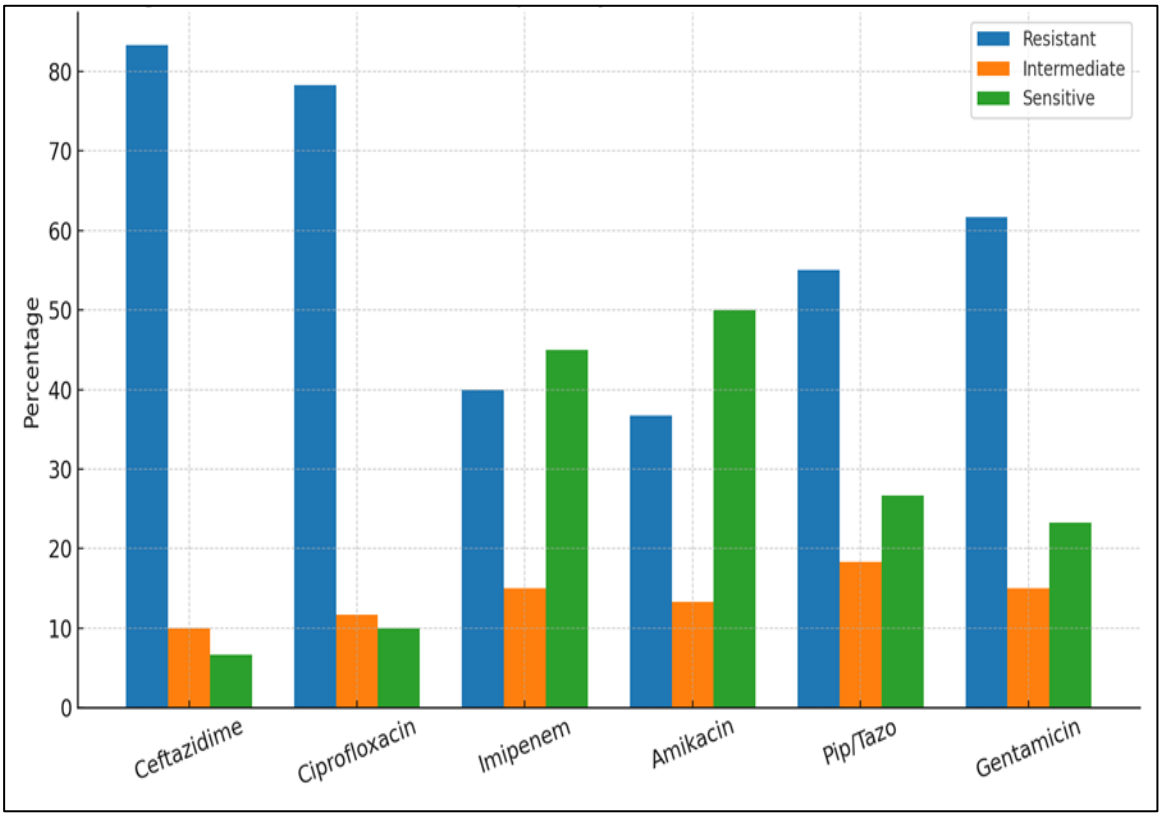

Figure 1. Resistance and susceptibility distribution across antibiotics (n = 60).

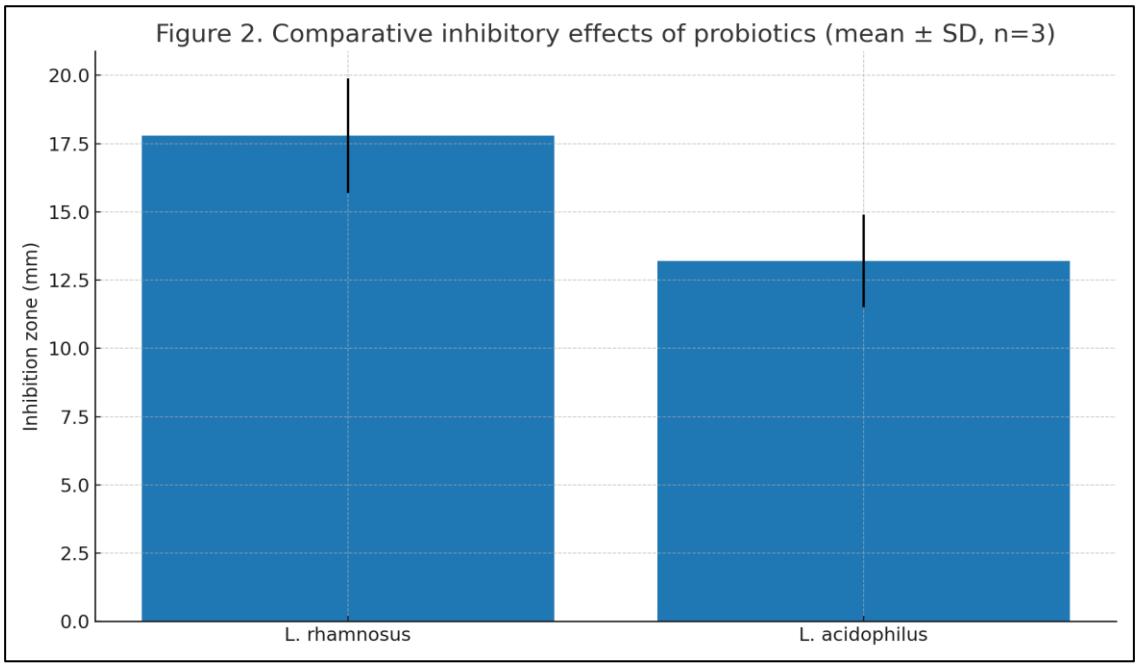

Figure 2. Comparative inhibitory effects of probiotics against MDR P. aeruginosa (mean ± SD, n = 3).

Discussion

The prevalence of P. aeruginosa (40%) in this cohort aligns with findings from tertiary care settings where burn wounds are a dominant source of infection (Asefa et al., 2023; Kim et al., 2024). High resistance to ceftazidime (83.3%) and ciprofloxacin (78.3%) mirrors global reports of diminishing efficacy for third-generation cephalosporins and fluoroquinolones, while the partial effectiveness of imipenem (40.0% resistance) and amikacin (36.7% resistance) suggests their role as important, albeit increasingly threatened, therapeutic options (Wang et al., 2024; Ramatla et al., 2025).

Geographical comparisons indicate that ceftazidime resistance often ranges between 70–90% and ciprofloxacin resistance between 65–85% in studies from Asia and the Middle East, consistent with our observations (Asefa et al., 2023). The sustained pressure on carbapenems, reflected by 30–50% resistance in several reports, underscores the narrowing antibiotic pipeline and the need for stewardship and infection control reinforcement (WHO, 2024a, 2024b; Wang et al., 2024).

Our probiotic data are notable: L. rhamnosus demonstrated significantly greater inhibition than L. acidophilus. Prior studies attribute such differences to strain-specific production of bacteriocins, organic acids, and biosurfactants, as well as interference with quorum sensing and biofilms (Bober et al., 2020; Artini et al., 2024; Abdel-Rahman et al., 2024; Al-Janabi et al., 2025). While in vitro effects are promising, translation to clinical benefit requires rigorous pharmacodynamic and safety evaluation in animal models and controlled clinical trials.

Strengths of this work include standardized CLSI-based AST, explicit MDR definitions, and head-to-head testing of two widely accessible probiotic species. Limitations include the lack of molecular resistance gene profiling, absence of MIC testing for probiotic metabolites, and an in vitro design that may not recapitulate host immunity, microbiome interactions, and pharmacokinetics. Future studies should incorporate genomic surveillance, metabolomic characterization of probiotic products, and synergy testing with priority antibiotics.

Conclusion

Pseudomonas aeruginosa remains a formidable MDR pathogen in hospital settings, particularly among burn patients. The high resistance to cephalosporins and fluoroquinolones and the concerning pressure on carbapenems emphasize the urgency of stewardship and infection

prevention programs. In vitro, L. rhamnosus exhibited stronger inhibitory activity than L. acidophilus against MDR isolates, supporting exploration of probiotics as adjunctive strategies. Translation to clinical practice will require integrated molecular studies, animal models, and well-designed clinical trials.

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