Egyptian Journal of Medical Microbiology Volume 34 / No.1 / January 2025 131-140 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Evaluation of the Antagonistic Activity of *Lactobacillus strains against* Pathogenic Organisms isolated from Various Infected Wounds: An in Vitro Study

[1]Eman E. Hegazy*, [1]Mona A. Gadallah, [2]Tarek G. Shoukr, [1]Marwa G. Bahey
[1]Department of Microbiology and Immunology, Faculty of Medicine, Tanta University, Egypt
[2]Department of Plastic and Reconstructive Surgery, Faculty of Medicine, Tanta University

Key words:
Lactobacillus, Fructooligosaccharides, probiotic, prebiotic

Corresponding Author:
Eman Elsayed Ali Hegazy, assistant professor of Medical Microbiology and Immunology, Faculty of medicine, Tanta University, Egypt.
Tel.: +2 01099008274
dr_emanhegazy2010@yahoo.com

ABSTRACT

Background: *With the serious global threat of antimicrobial resistance, finding therapeutic alternatives has become a critical priority. Probiotics appear to be promising treatments due to their known natural inhibitory effects on various pathogens.* ***Objectives:*** *To evaluate the potential antagonistic effects of the probiotic Lactobacillus plantarum and Lactobacillus rhamnosus as well as their cell-free supernatant (CFS) and the synergistic interaction with commercial prebiotics like Fructooligosaccharides (FOS) on different pathogens isolated from infected wounds.* ***Methodology:*** *Identification of the pathogenic microorganisms was done. The tissue culture plate method evaluated the biofilm formation ability among the isolated pathogens. The antimicrobial activity of L. plantarum and L. rhamnosus and their CFS were investigated by radial streak technique and agar well diffusion. The anti-biofilm effect of CFS of lactobacilli was evaluated using tissue culture plate assay.* ***Results:*** *Seventy-two isolates were identified, 73.6% were Gram-negative, and the most prevalent was Klebsiella pneumoniae. Biofilm formation was identified in 69.5 % of the isolates, among them E. coli, Acinetobacter baumannii, and Proteus mirabilis were the most frequent biofilm-forming organisms. L. plantarum, L. rhamnosus and their CFS exhibited antimicrobial and antibiofilm activity against all tested pathogens with variable degrees. The addition of FOS enhanced both lactobacilli's antimicrobial and antibiofilm properties.* ***Conclusion:*** *The probiotics used showed significant antagonistic activity against various pathogens paving the way to be used as a new treatment option.*

INTRODUCTION

Skin serves as an important barrier against microbial invasions. However, if the normal skin anatomy is compromised, the skin's particular immune function deteriorates, imposing the administration of additional protection to the injured skin [1].

Wound exudates expose wounded skin to the danger of being colonized by pathogenic microorganisms, which will interfere with wound healing and may possibly cause septicemia. Moreover, inadequate wound management results in significant healthcare costs due to prolonged hospital stays and enormous doses of antibiotics[2].

Bacterial persistence in wound sites is linked to biofilm production, which are famously resistant to conventional antibiotics [3]. As a result of wide spread of antimicrobial resistance, the need for alternative antimicrobial agents is critical. The most recent approach to wound dressings is the use of novel therapeutic agents such as probiotics[4]. The word "probiotics" describes bacteria that, when given in proper amounts to hosts, improve their health. Probiotic topical treatment has been investigated in models of cutaneous damage in humans, where the main objective is to lower infection, boost immune response, and improve wound healing [5].

Probiotics prevent wound infections via several antimicrobial mechanisms. They secrete antimicrobial substances like bacteriocins, organic acids, and biosurfactants. Also, they prevent the adherence of harmful microorganisms to epithelial cells, preventing bacterial invasion, disrupt the quorum-sensing mechanism of the pathogens, and inhibit biofilm formation[6]. Additionally, they produce lactic and acetic acids during the metabolism of carbohydrates, which promotes a lower pH of the medium and prevents the development of some pathogens [7].

Prebiotic substances like polysaccharides and oligosaccharides enhance the growth of probiotics. Synbiotics, that incorporate probiotics and prebiotics, have received awareness for their beneficial effects in preventing polymicrobial infections in wound environments. Probiotics and prebiotics are examples of

active ingredients co-encapsulated in topical drugs which represents a novel and promising therapeutic strategy for wound healing and recovery. Their synergistic properties, offering a new perspective on developing the ideal solution for wound care [8].Therefore, the current study aimed to analyze the microbiological profile of various infected wounds and to evaluate the potential antagonistic effects of the probiotic *L. plantarum* and *L. rhamnosus* as well as their cell free supernatant (CFS) and the synergistic interaction with commercial prebiotics like Fructooligosaccharides (FOS) on the isolated pathogens.

METHODOLOGY

Study Design

This cross-sectional study was carried out at Tanta University's Medical Microbiology and Immunology Department, Faculty of Medicine, from October 2023 to April 2024. All patients suspected of wound infections admitted to the Surgery Department of Tanta University Hospitals during the study period, who were willing to give samples and agreed with consent to the study were involved. Patients not fulfilling the criteria of infected wounds were not included in this study. Ethical approval was provided by the Tanta Faculty of Medicine's Ethics and Research Committee (Approval code 36264PR498/1/24).

Identification of pathogenic organisms.

Wound swab samples were gathered from ninety patients with wound infections and immediately transferred under aseptic conditions to the Microbiology laboratory. Samples were cultivated on nutrient, blood agar, and MacConkey agar (Oxoid UK) then incubated aerobically at 37◦ C for 24- 48 hours. Colonies were identified by colonial morphology, Gram staining, and Biochemical reactions[9].

Determination of antimicrobial activity of probiotics:

Preparation of the probiotic strains:

L. plantarum and *L. rhamnosus* strains were acquired from the Faculty of Agriculture, Microbiological Resources Center, Ain Shams University, Egypt. They were cultivated on deMan, Rogosa and Sharpe (MRS) broth (**Hi-Media**). at 37°C in candle jar for 24 hours, then subculture done on MRS agar. Confirmation of the colonies was carried out by colony morphology, Gram reaction, catalase test, and oxidase test [10].

Preparation of the probiotic's cell-free supernatant (CFS):

Every probiotic strain was grown as a monoculture in the MRS broth to create CFS. Centrifuging the strain grown for 48 hours at 37 °C at 6000 rpm for 10 minutes at 4 °C, and the supernatant was filtered with a sterile Millipore filter, and further used [11].

Radial streak method:

The Radial streak procedure was performed as described by Coman et al.[11] using a medium containing MRS agar and Trypticase soy agar. The plates were spread with 0.5 McFarland of Lactobacillus strain covering a circular area in the center of the plate and then incubated at 37°c for 48 hrs. After that the plates were inoculated with the pathogenic strains in radial lines extending from the periphery to the center of the plate, incubated for 24 hrs at 37°c. Interpretation by measurement of the inhibition zone size. The circle diameter (CD, cm) of the probiotic strain spreading zone was subtracted from the observed inhibition zone diameter (IZD, cm) to determine the growth inhibitory activity (GI, cm), which was then calculated as GI = (IZD-CD)/ 2.

Agar well diffusion method:

Müller-Hinton agar plates (Oxoid UK) were swabbed with the cultures of various pathogens. Wells with 8 mm diameter were created in the agar plate, and then CFS of probiotic and synbiotic were added in the wells. The plates were then incubated at 37°C for 24 hours. The inhibition was detected by measuring the diameter of the zone of inhibition (ZOI) surrounding the well[11].

Determination of anti-biofilm activity of CFS of probiotic and synbiotics using the microtiter plate assay:

Biofilm formation was evaluated according to Park et al., [12]. The anti-biofilm effect of CFS of probiotic alone and synbiotic was assessed against the biofilm producing pathogens. Briefly**,** Overnight cultures of the biofilm producing pathogens were diluted to 1:100 in 15 ml brain heart infusion then,100 µL of each isolated pathogen was added to each well, 100 µL of the probiotics and synbiotics CFSs were added to each well to adjust the volume to 200 µL per each well. Control wells were prepared without adding the CFS. After incubation at 37C for 24 h, two washes with 200 µL of distilled water were done, the biofilm that had formed in each well was allowed to dry for 45 minutes. Then, 100 µL of 0.4% crystal violet was applied to each well. Then, 200 µL of 95% ethanol was added to the wells to cause instant discoloration, and they were then cleaned four times with distilled water. Lastly, 100 µL of the discolored solution was moved to a fresh plate's well, and the crystal violet was measured at 570 nm using a microplate reader. The biofilm formation was quantified by comparing the absorbance values of wells treated with CFS to those of untreated control wells [13].

Statistical analysis

IBM SPSS software, version 20.0 (Armonk, NY: IBM Corp.), was used to analyze the data. Utilizing the Shapiro-Wilk test, continuous data was examined for normality. Quantitative data were expressed as range (minimum and maximum), mean, standard deviation for normally distributed quantitative variables **F-test**

(ANOVA) was used to compare between more than two groups, and **Post Hoc test (Tukey)** for pairwise comparisons**.** In contrast, for not normally distributed quantitative variables when comparing more than two examined groups, the Kruskal Wallis test was employed, and for pairwise comparisons, Dunn's multiple comparisons test. The 5% level was used to assess the results' significance.

RESULTS

Regarding the culture result, no growth was detected in 28.9% while 64.3% showed monomicrobial growth and 7.8% showed polymicrobial growth. and a total of 72 isolates were identified .73.6% of the organisms were Gram negative ,17% Gram positive, and 9.9% were candida spp. The most prevalent isolate was *K. pneumoniae* 25%, while Enterococcus is the least prevalent representing 4.2% as demonstrated in table 1.

Table 1: Demographic data of the studied cases and culture results (n = 90).

	No. (%)
Sex	
Male	54 (60.0%)
Female	36 (40.0%)
Age (years)	
Mean ± SD.	41.46 ± 14.50
Median (Min. – Max.)	44 (16 – 75)
Culture result	
No growth	26 (28.9%)
Monomicrobial growth	58 (64.4%)
Polymicrobial growth	7 (7.8%)
Gram negative isolates	53(73.6%)
Gram positive isolates	12(17%)
Fungi	7(9.7%)
Organism (No. 72)	
Klebsiella pneumoniae	18 (25 %)
Pseudomonas aeruginosa	15(20.8%)
E. coli	12 (16.7%)
Staphylococcus aureus	9 (12.5%)
Candida spp	7(9.7%)
Proteus mirabilis	4 (5.6%)
Acinetobacter baumannii	4 (5.6%)
Enterococcus spp	3 (4.2%)

Regarding the biofilm formation among the isolated organisms, 69.5 % were biofilm producers. *E. coli*. *Acinetobacter baumannii* and *Proteus mirabilis* were the most frequently biofilm forming organisms (75%), followed by *P. aeruginosa*, *K. pneumoniae*, Candida, *Staphylococcus aureus*, and Enterococcus 73.3%, 72.2%,71.4,55.6%, and 33.3% respectively as shown in figure 1.

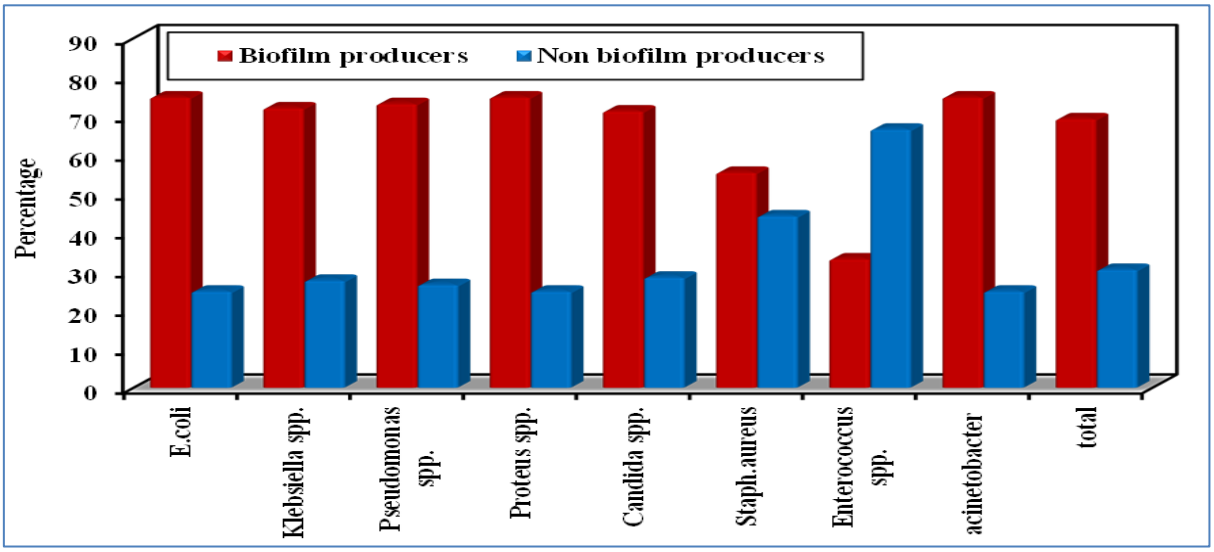

Fig. 1: Biofilm formation among isolated pathogens

The CFS of *L. rhamnosus* and *L. plantarum* and the CFS of their combination showed the highest antibiofilm activity against *Proteus mirabilis*, enterococcus. The addition of FOS to *L. rhamnosus* enhanced its antibiofilm activity against *Proteus mirabilis*, enterococcus, pseudomonas, *Staph. aureus* and Acinetobacter. Addition of FOS to *L. plantarum* enhanced antibiofilm activity of its CFS against Pseudomonas, *E. coli* and Klebsiella. The addition of FOS to the combination of *L. plantarum* and *L. rhamnosus* enhanced the antibiofilm activity of their CFS against Pseudomonas, *E. coli*, klebsiella and *Staph. aureus* as demonstrated in figure 2.

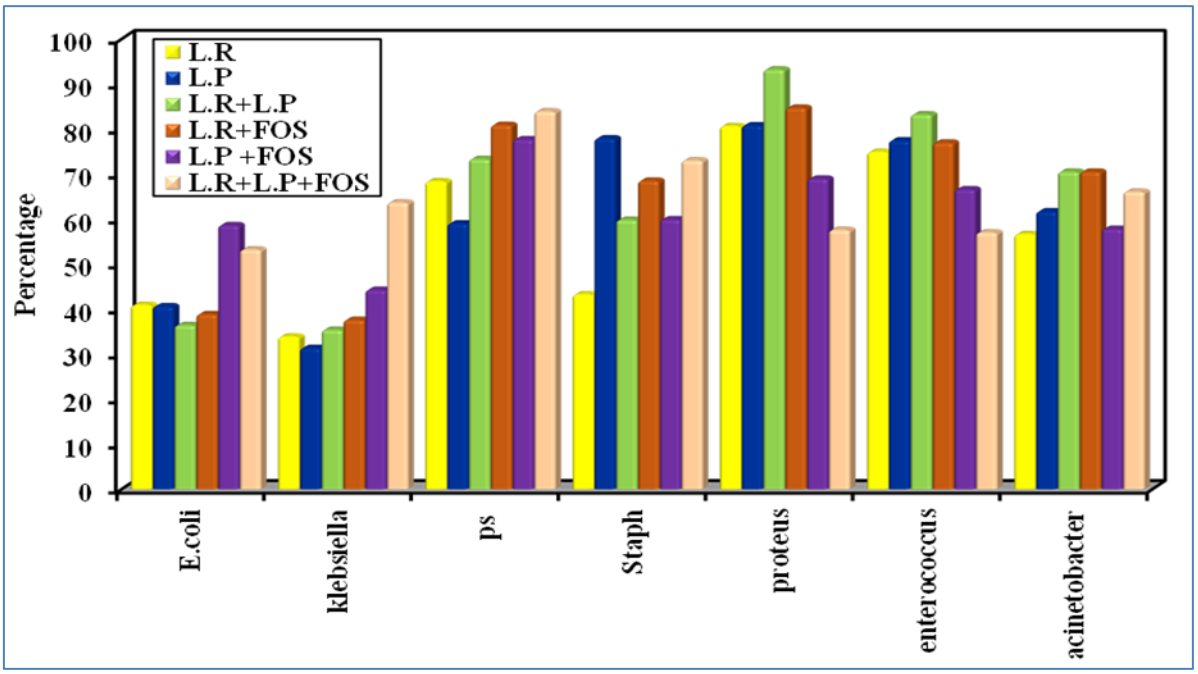

Fig. 2: Antibiofilm activity of CFS of lactobacillus strains with and without FOS against isolated pathogens.

The antimicrobial activity *L. rhamnosus, L. plantarum* and their combination was determined by radial streak method, all showed intermediate inhibition of the involved isolated pathogenic organisms, with statistically significant difference between them against *E. coli, klebsiella, staphylococcus aureus*, Candida *spp., and Acinetobacter*. The diameter of the zone of inhibition of the tested *L. rhamnosus, L. plantarum* and their combination was found to range from (6.50 – 23mm), (8-20 mm), and (10-25 mm) respectively.

Lactobacillus rhamnosus showed the highest growth inhibitory activity against Enterococcus spp. with mean diameter of zone of inhibition (15 ± 2 mm), followed by pseudomonas with mean diameter of (14.5 ± 4.57 mm). *L. plantarum* showed the highest growth inhibitory activity against pseudomonas spp. and enterococcus spp with mean diameter of (15.1 ± 2.13 mm), and (15 ± 1 mm), respectively. The least growth inhibitory activity was against Candida. The combination of both *L. rhamnosus and L. plantarum* showed the highest growth inhibitory activity against pseudomonas spp. with mean diameter of (17.2 ± 2.64). followed by candida spp with mean diameter of (16.9 ± 1.21) as demonstrated in table 2.

Table 2: Inhibition zone diameters (mm) generated by lactobacillus strains using the radial streak method

Organism	*L. Rhamnosus*	*L. Plantarum*	Combination	Test of Sig.	p
E-coli					
Min. – Max.	7.50 – 13	10 – 15	10 – 18	F=	<0.001*
Mean ± SD.	9.50 ± 1.76	11.8[a] ± 1.57	14.3[ab] ± 2.64	16.198*	
Klebsiella spp					
Min. – Max.	7 – 18	8 – 20	11 – 20	H=	0.006*
Mean ± SD.	11.6 ± 4.21	11.8 ± 3.10	14.9[ab] ± 2.66	10.270*	
Pseudomonas spp					
Min. – Max.	6.50 – 23	12 – 18	14 – 25	F=	0.074
Mean ± SD.	14.5 ± 4.57	15.1 ± 2.13	17.2 ± 2.64	2.766	
Proteus spp					
Min. – Max.	7.50 – 15	10 – 15	11 – 15	F=	0.762
Mean ± SD.	11.9 ± 3.17	13 ± 2.16	13 ± 1.83	0.280	
Staphylococcus aureus					
Min. – Max.	7.50 – 16	10 – 20	10 – 20	F=	0.027*
Mean ± SD.	9.72 ± 2.59	12.9 ± 3.10	13.8[a] ± 3.60	4.195*	
Candida spp					
Min. – Max.	10 – 12	8 – 12	15 – 18	F=	<0.001*
Mean ± SD.	10.9 ± 1.07	9.86 ± 1.35	16.9[ab] ± 1.21	67.968*	
Enterococcus					
Min. – Max.	13 – 17	14 – 16	10 – 15	F=	0.232
Mean ± SD.	15 ± 2	15 ± 1	12.3 ± 2.52	1.882	
Acinetobacter					
Min. – Max.	12 – 15	12 – 14	14 – 17	F=	0.022*
Mean ± SD.	13 ± 1.41	12.8 ± 0.96	15.5[ab] ± 1.29	6.055*	

SD: **Standard deviation**
F: F for One way ANOVA test
p: p value for comparing the three studied groups.
*: Statistically significant at $p \leq 0.05$
a: Significant with ***L. Rhamnosus***
b: Significant with ***L. Plantarum***

Regarding the antimicrobial effect of CFS of the tested probiotics, when using the *L. rhamnosus* CFS, the diameter of the zone of inhibition was found to range from 11 to 19 mm. Maximum inhibition was detected against enterococcus, Acinetobacter. When using the *L. plantarum* CFS, the diameter of the zone of inhibition was found to range from 13 to 20 mm. Maximum inhibition was detected against enterococcus, and pseudomonas. When using the CFS of both probiotics, the diameter of the zone of inhibition was found to range from 11 to 20 mm. Maximum inhibition was detected against Acinetobacter and Pseudomonas. There was a statistically significant difference between them, against *Klebsiella, Proteus, Staph. aureus, Candida spp. and Acinetobacter spp* as shown in table 3.

Table 3: Inhibition Zone Diameters (mm) produced by CSF using agar well diffusion method.

Organism	*L. rhamnosus*	*L. plantarum*	Combination	F	p
E-coli					
Min. – Max.	12 – 17	13 – 18	11 – 18	2.355	0.111
Mean ± SD.	13.9 ± 1.93	15.6 ± 1.68	14.3 ± 2.23		
Klebsiella					
Min. – Max.	12 – 16	14 – 18	12 – 19	6.451*	0.003*
Mean ± SD.	13.6 ± 1.20	15.6[a] ± 0.98	14.7 ± 2.45		
Pseudomonas					
Min. – Max.	13 – 19	14 – 20	13 – 20	2.303	0.112
Mean ± SD.	15.4 ± 2.13	16.7 ± 1.94	15.4 ± 1.80		
Proteus					
Min. – Max.	12 – 15	15 – 18	13 – 15	6.763*	0.016*
Mean ± SD.	13 ± 1.41	16.3[a] ± 1.50	14 ± 0.82		
Staphylococcus					
Min. – Max.	11 – 15	13 – 17	13 – 16	11.114*	<0.001*
Mean ± SD.	12.8 ± 1.20	15.3[a] ± 1.32	14.8[a] ± 1.09		
Candida					
Min. – Max.	13 – 15	14 – 16	14 – 16	1.293	0.299
Mean ± SD.	14.1 ± 0.90	14.9 ± 0.90	14.9 ± 1.07		
Enterococcus					
Min. – Max.	16 – 18	15 – 18	14 – 16	2.385	0.173
Mean ± SD.	17 ± 1	16.7 ± 1.53	15 ± 1		
Acinetobacter					
Min. – Max.	15 – 17	13 – 14	16 – 18	13.0*	0.002*
Mean ± SD.	16 ± 1.15	13.5[a] ± 0.58	17[b] ± 1.15		

SD: **Standard deviation**
F: F for One way ANOVA test. p: p value for comparing between the three studied groups
*: Statistically significant at $p \leq 0.05$
a: Significant with **L.R**
b: Significant with **L.P**

As regards the Comparison between antimicrobial effect of CFS with Vs without FOS using agar well diffusion method. Addition of FOS to *L. rhamnosus* enhances the antimicrobial activity of its CFS against all tested pathogens with statistically significant difference against *E. coli, klebsiella, proteus, staph. aureus*, and *Candida* spp. Also, the addition of FOS to *L. plantarum* enhanced the antimicrobial activity of its CFS against all tested pathogens with statistically significant difference against *E. coli, klebsiella, Staph. aureus*, and *Candida* spp. Also, the addition of FOS to *L. plantarum and L. rhamnosus* combination enhanced the antimicrobial activity of its CFS against all tested pathogens with statistically significant differences against *E. coli, Klebsiella, Proteus, Staph. aureus*, Candida spp, and Enterococcus as shown in table 4

.

Table 4: Comparison between antimicrobial effect of CFS of with Vs without FOS using agar well diffusion method

Organism	L.R	L.R+FOS	L.P	L.P+FOS	L.R + L.P	L.R+L.P+FOS
E-coli						
Min. – Max.	12 – 17	15 – 21	13 – 18	13 – 22	11 – 18	17 – 23
Mean ± SD.	13.9 ± 1.93	17.8 ± 1.53	15.6 ± 1.68	18 ± 2.49	14.3 ± 2.23	19.4 ± 1.98
t(p)	5.515* (<0.001*)		2.792* (0.011*)		5.912* (<0.001*)	
Klebsiella						
Min. – Max.	12 – 16	15 – 21	14 – 18	17 – 26	12 – 19	18 – 26
Mean ± SD.	13.6 ± 1.20	17.6 ± 2.25	15.6 ± 0.98	21.3 ± 3.23	14.7 ± 2.45	21 ± 2.20
t(p)	6.645* (<0.001*)		7.186* (<0.001*)		8.167* (<0.001*)	
Pseudomonas						
Min. – Max.	13 – 19	15 – 18	14 – 20	14 – 20	13 – 20	15 – 20
Mean ± SD.	15.4 ± 2.13	16.5 ± 1.06	16.7 ± 1.94	15.9 ± 1.88	15.4 ± 1.80	16.3 ± 1.59
t(p)	1.735 (0.098)		1.240 (0.225)		1.503 (0.144)	
Proteus						
Min. – Max.	12 – 15	15 – 18	15 – 18	16 – 18	13 – 15	15 – 17
Mean ± SD.	13 ± 1.41	16.5 ± 1.29	16.3 ± 1.50	16.8 ± 0.96	14 ± 0.82	16.3 ± 0.96
t(p)	3.656* (0.011*)		0.562 (0.595)		3.576* (0.012*)	
Staphylococcus						
Min. – Max.	11 – 15	14 – 20	13 – 17	17 – 21	13 – 16	16 – 22
Mean ± SD.	12.8 ± 1.20	16.9 ± 1.90	15.3 ± 1.32	19.1 ± 1.45	14.8 ± 1.09	19.1 ± 1.62
t(p)	5.485* (<0.001*)		5.768* (<0.001*)		6.664* (<0.001*)	
Candida						
Min. – Max.	13 – 15	15 – 17	14 – 16	15 – 20	14 – 16	15 – 22
Mean ± SD.	14.1 ± 0.90	16 ± 0.82	14.9 ± 0.90	17 ± 1.63	14.9 ± 1.07	18.6 ± 2.44
t(p)	4.044* (0.002*)		3.041* (0.010*)		3.689* (0.003*)	
Enterococcus						
Min. – Max.	16 – 18	16 – 18	15 – 18	17 – 21	14 – 16	19 – 22
Mean ± SD.	17 ± 1	17 ± 1	16.7 ± 1.53	19 ± 2	15 ± 1	20.3 ± 1.53
t(p)	0.00 (1.000)		1.606 (0.184)		5.060* (0.007*)	
Acinetobacter						
Min. – Max.	15 – 17	14 – 17	13 – 14	17 – 20	16 – 18	17 – 21
Mean ± SD.	16 ± 1.15	15.5 ± 1.29	13.5 ± 0.58	18.8 ± 1.26	17 ± 1.15	19.5 ± 1.73
t(p)	0.577 (0.585)		7.584* (<0.001*)		2.402 (0.053)	

SD: **Standard deviation** **t: Student t-test**
p: p value for comparing the two studied groups
*: Statistically significant at $p \leq 0.05$

DISCUSSION

Considering the rising prevalence and spread of antibiotic-resistant bacteria, probiotics provide a possible therapeutic substitute for topical antibiotic usage[14].

In the current study, the culture growth rate was 71.1%, which was higher than that reported by Upreti et al [15]. However, a high culture growth rate of 82.2% was also observed by Ahmed et al[16].

In this study monomicrobial growth was higher than polymicrobial growth. which agreed with the results reported Hassan et al and Mohammed et al [17,18]. Conversely, Ahmed et al [16] and Yeong et al [19] observed a higher prevalence of polymicrobial growth than monomicrobial growth.

In our research, Gram-negative bacteria were isolated more frequently than Gram-positive bacteria. These findings were consistent with various research that also found a higher prevalence of Gram-negative isolates [17,20,21]. Conversely, Khanam, et al [22] showed high percentage of Gram-positive bacterial isolation.

In the current study, *Klebsiella pneumoniae* was the dominant pathogen, which agrees with the study carried out by Fahim [23] who revealed a predominance of *Klebsiella* species. While Mahat, et al[24] showed predominance of *Pseudomonas* species. However, Ahmed, et al[16] found that the dominant isolate was *S. aureus*.

In this research, the antimicrobial effects of *L. rhamnosus*, *L. plantarum*, and their combination were investigated. All of them demonstrated intermediate

inhibition against isolated pathogens. *L. rhamnosus* and *L. plantarum* demonstrated the highest growth inhibitory activity against *Enterococcus* spp., and *Pseudomonas*. the least growth inhibitory activity against *candida* spp. The combination of both *L. rhamnosus and L. plantarum* showed the highest growth inhibitory activity against *pseudomonas* spp. followed by *candida* spp.

Consistent with our findings Coman, et al[11] reported that probiotic strains exhibited a significant inhibition of Gram-positive and Gram-negative pathogens. *L. Plantarum* is well accepted for topical administration due to its anti-microbial activity [25,26] Moreover, Moraffah, et al [27] has proven the efficient role of *L. plantarum* in managing wound infection. *L. rhamnosus* showed antimicrobial effect on various pathogens, embracing carbapenem-resistant *Acinetobacter baumannii* and methicillin-resistant *Staph. aureus* [28]. Moreover, Chen, et al [29] revealed that carbapenem-resistant *Enterobacteriaceae* can be inhibited by Lactobacillus strains.The CFS of *lactobacilli* exhibited diverse antibacterial effects, and this might be explained by the release of various antibacterial agents or metabolites[30].

Regarding the antimicrobial effect of CFS of the tested probiotics. When using the *L. rhamnosus* CFS or *L. plantarum* CFS, maximum inhibition was detected against *Enterococcus* spp. When using the CFS of both probiotics, maximum inhibition was detected against *Acinetobacter* and *pseudomonas.* Similarly, High anti-ESBL activity was demonstrated by CFS of the tested Lactobacillus strains against *Pseudomonas aeruginosa* and Extended-Spectrum β-Lactamase Producing *Klebsiella pneumoniae* as reported by El-Mokhtar, et al [31]. Moreover, Singh, et al[32] reported that the CFS of *L. rhamnosus* displayed strong inhibition against *Pseudomonas spp* and moderate inhibition of *staph. aureus.*

Regarding the Comparison between antimicrobial effect of CFS with Vs without FOS using agar well diffusion method. Addition of FOS to *L. rhamnosus* and *L. plantarum* enhanced the antimicrobial activity of their CFS against all tested pathogens.

Similarly, Lim, et al [33] reported that the addition of FOS to *L. acidophilus* or *L. paracasei* had great potential as a synbiotic. Moreover, Tulumoğlu et al [34]found that FOS promoted the antimicrobial activity of *L. casei* against pathogenic bacteria

In this study, concerning the antibiofilm activity of CFS of lactobacillus strains, they showed antibiofilm effect against all biofilm producing organisms, but their biofilm inhibition effect was variable. The addition of FOS to *L. rhamnosus* enhanced the antibiofilm activity of its CFS against *proteus, enterococcus, pseudomonas, staphylococcus aureus and Acinetobacter*. The addition of FOS to *L. plantarum* enhanced the antibiofilm activity of its CFS against *pseudomonas, E. coli and klebsiella*. The addition of FOS to the combination of *L. plantarum* and *L. rhamnosus* enhanced the antibiofilm activity of their CFS against *pseudomonas, E. coli, Klebsiella and Staphylococcus aureus*

El-Mokhtar, et al [31]reported that the biofilm formation by *K. pneumoniae* and *P. aeruginosa* were inhabited by the CFS of the used lactobacillus strains.

Similarly, Rezaei, et al [35] reported that the CFS of *L. plantarum and L. rhamnosus*, inhibit the biofilm formation by *L. monocytogenes* and *P. aeruginosa*. Also, Kim,et al[36] reported that *L. acidophilus* could remove biofilms formed by enterohemorrhagic *E. coli*.

Probiotics have anti-biofilm properties through the release of anti-microbial chemicals, eliminating pathogens, disrupting proteins and cell membranes, producing biosurfactants, and inhibiting the expression of genes linked to biofilm formation [37].

CONCLUSIONS

The probiotic *L. rhamnosus* and *L. plantarum have* significant antimicrobial as well as antibiofilm activity against various pathogens causing wound infections. Furthermore, their antagonistic activity was enhanced through synergy with Fructooligosaccharides. This probiotic-based therapy strategy has the potential to improve the care of long-term wounds and can also be used to treat other biofilm associated infections.

Declarations:
Consent for publication: Not applicable
Material and data accessibility: Data are available upon request.
Funding: No grants from funding organizations were given.
The author(s) declare no potential conflicts of interest

REFERENCES

1. Tamer TM, Sabet MM, Omer AM, Abbas E, Eid AI, *et al.* Hemostatic and antibacterial PVA/Kaolin composite sponges loaded with penicillin–streptomycin for wound dressing applications. Scientific Reports. 2021 ;11(1):3428.
2. Puca V, Marulli RZ, Grande R, Vitale I, Niro A, *et al*. Microbial species isolated from infected wounds and antimicrobial resistance analysis: Data emerging from a three-years retrospective study. Antibiotics. 2021 ;10(10):1162.
3. Morgan SJ, Lippman SI, Bautista GE, Harrison JJ, Harding CL, *et al.* Bacterial fitness in chronic wounds appears to be mediated by the capacity for high-density growth, not virulence or biofilm functions. PLoS pathogens. 2019 ;15(3):e1007511.

4. Aghamohammad S, Rohani M. Antibiotic resistance and the alternatives to conventional antibiotics: The role of probiotics and microbiota in combating antimicrobial resistance. Microbiological Research. 2023; 267:127275.
5. Knackstedt R, Knackstedt T, Gatherwright J. The role of topical probiotics on wound healing: A review of animal and human studies. Int Wound J. 2020 ;17(6):1687-1694.
6. Singh A, Devi A, Mandal UK. Role of probiotics in wound healing. Wound Healing Research: Current Trends and Future Directions. 2021:285-99.
7. Hager CL, Isham N, Schrom KP, Chandra J, McCormick T, et al. Effects of a novel probiotic combination on pathogenic bacterial-fungal polymicrobial biofilms. MBio. 2019 ;10(2):10-128.
8. Nezamdoost-Sani N, Khaledabad MA, Amiri S, Khaneghah AM. Alginate, and derivatives hydrogels in encapsulation of probiotic bacteria: An updated review. Food Bioscience. 2023; 52:102433.
9. Cheesbrough, M. Microbiological tests in district laboratory practice in tropical countries. 2nd ed. Cambridge University Press,2006; 2: 62-127.
10. Kajal A, Ankur G, Jagriti S. Isolation and identification of Lactobacilli bacteria from raw cow milk in local region of Agra. Int J Adv Res Biol Sci. 2017;4(11):98-102.
11. Coman MM, Verdenelli MC, Cecchini C, Silvi S, Orpianesi C, et al. In vitro evaluation of antimicrobial activity of Lactobacillus rhamnosus IMC 501®, Lactobacillus paracasei IMC 502® and SYNBIO® against pathogens. Journal of applied microbiology. 2014;117(2):518-27.
12. Park YJ, Kim YJ, Yu HH, Lee NK, Paik HD. Cell-free supernatants of Bacillus subtilis and Bacillus polyfermenticus inhibit Listeria monocytogenes biofilm formation. Food Control. 2023; 144:109387.
13. Stefania DM, Miranda P, Diana M, Claudia Z, Rita P, Donatella P. Antibiofilm and antiadhesive activities of different synbiotics. Journal of Probiotics and Health. 2017;5(3):182-91.
14. Fijan S, Frauwallner A, Langerholc T, Krebs B, ter Haar JA, et al. Efficacy of using probiotics with antagonistic activity against pathogens of wound infections: an integrative review of literature. BioMed research international. 2019;2019(1):7585486.
15. Upreti N, Rayamajhee B, Sherchan SP, Choudhari MK, Banjara MR. Prevalence of methicillin resistant Staphylococcus aureus, multidrug resistant and extended spectrum β-lactamase producing gram negative bacilli causing wound infections at a tertiary care hospital of Nepal. Antimicrobial Resistance & Infection Control. 2018; 7:1-0.
16. Ahmed EF, Rasmi AH, Darwish AM, Gad GF. Prevalence and resistance profile of bacteria isolated from wound infections among a group of patients in upper Egypt: a descriptive cross-sectional study. BMC Research Notes. 2023;16(1):106.
17. Hassan MA, Abd El-Aziz S, Elbadry HM, Samy A, Tamer TM. Prevalence, antimicrobial resistance profile, and characterization of multi-drug-resistant bacteria from various infected wounds in North Egypt. Saudi Journal of Biological Sciences. 2022;29(4):2978-88.
18. Mohammed A, Seid ME, Gebrecherkos T, Tiruneh M, Moges F. Bacterial isolates and their antimicrobial susceptibility patterns of wound infections among inpatients and outpatients attending the University of Gondar Referral Hospital, Northwest Ethiopia. International journal of microbiology. 2017;2017(1):8953829.
19. Yeong EK, Sheng WH, Hsueh PR, Hsieh SM, Huang HF, et al. The wound microbiology and the outcomes of the systemic antibiotic prophylaxis in a mass burn casualty incident. Journal of Burn Care & Research. 2020;41(1):95-103.
20. Maharjan N, Mahawal BS. Bacteriological profile of wound infection and antibiotic susceptibility pattern of various isolates in a tertiary care center. Journal of Lumbini Medical College. 2020;8(2):218-24.
21. Radwan AG, Shaheen AM, Ghonaim NF, Amer WH, Elmahdy HS, Eissa RA. Klebsiella pneumoniae in Neonatal Sepsis: A Growing Challenge of Multidrug Resistance in a Tertiary Care Setting. Egyptian Journal of Medical Microbiology. 2024 Oct 1;33(4).
22. Khanam RA, Islam MR, Sharif A, Parveen R, Sharmin I, Yusuf MA. Bacteriological profiles of pus with antimicrobial sensitivity pattern at a teaching hospital in Dhaka City. Bangladesh J. 2018;5(1).
23. Fahim NA. Prevalence and antimicrobial susceptibility profile of multidrug-resistant bacteria among intensive care units patients at Ain Shams University Hospitals in Egypt—a retrospective study. Journal of the Egyptian Public Health Association. 2021; 96:1-0.
24. Mahat P, Manandhar S, Baidya R. Bacteriological profile of wound infection and antibiotic susceptibility pattern of the isolates. J Microbiol Exp. 2017;4(5):126-33.
25. Huang T, Li Z, Qu X, Yao G, Kwok LY, et al. Preliminary purification and partial characterization of a functional bacteriocin of Lacticaseibacillus

paracasei Zhang and mining for its gene cluster. Probiotics and Antimicrobial Proteins. 2024:1-3.

26. Peral MC, Huaman Martinez MA, Valdez JC. Bacteriotherapy with Lactobacillus plantarum in burns. International wound journal. 2009;6(1):73-81.
27. Moraffah F, Kiani M, Abdollahi M, Yoosefi S, Vatanara A, Samadi N. In vitro-in vivo correlation for the antibacterial effect of Lactiplantibacillus plantarum as a topical healer for infected burn wound. Probiotics and Antimicrobial Proteins. 2022;14(4):675-89.
28. Rodriguez C, Ramlaoui D, Gasca B, Azis A, Leal C, Lopez C, et al. Lacticaseibacillus rhamnosus CRL 2244 secreted metabolites display killing and antibiotic synergistic activity against multi-drug resistant pathogens. Plos one. 2024;19(6):e0306273.
29. Chen CC, Lai CC, Huang HL, Huang WY, Toh HS, et al. Antimicrobial activity of Lactobacillus species against carbapenem-resistant Enterobacteriaceae. Frontiers in microbiology. 2019; 10:789.
30. de Lira FM, Tanaka FY, Rios EA, Carrilho SM, de Abreu SS, et al. Identification of lactic acid bacteria with anti-listeria activity. Characterization and application of a bacteriocinogenic strain in the control of Listeria monocytogenes in cheese. Journal of Dairy Research. 2023;90(3):318-23.
31. El-Mokhtar MA, Hassanein KM, Ahmed AS, Gad GF, Amin MM, Hassanein OF. Antagonistic activities of cell-free supernatants of lactobacilli against extended-spectrum β-lactamase producing Klebsiella pneumoniae and Pseudomonas aeruginosa. Infect Drug Resist. 2020; 17: 543-52. S235603.
32. Singh AK, Hertzberger RY, Knaus UG. Hydrogen peroxide production by lactobacilli promotes epithelial restitution during colitis. Redox biology. 2018; 16:11-20.
33. Lim SM, Jeong KS, Lee NG, Park SM, Ahn DH. Synergy effects by combination with lactic acid bacteria and frutooligosaccharides on the cell growth and antimicrobial activity. Food science and biotechnology. 2011 Nov; 20:1389-97.
34. Tulumoğlu Ş, Erdem B, Şimşek Ö. The effects of inulin and fructo-oligosaccharide on the probiotic properties of Lactobacillus spp. isolated from human milk. Zeitschrift für Naturforschung C. 2018;73(9-10):367-73.
35. Rezaei Z, Khanzadi S, Salari A. Biofilm formation and antagonistic activity of Lacticaseibacillus rhamnosus (PTCC1712) and Lactiplantibacillus plantarum (PTCC1745). Amb Express. 2021; 11:1-7.
36. Kim Y, Oh S, Kim SH. Released exopolysaccharide (r-EPS) pro duced from probiotic bacteria reduce biofilm formation of enterohe morrhagic Escherichia coli O157: H7. Biochem Biophys Res Commun. 2009;379(2):324–329.
37. Nasreen S, Ali S, Andleeb S, Summer M, Hussain T, et al. Mechanisms of medicinal, pharmaceutical, and immunomodulatory action of probiotics bacteria and their secondary metabolites against disease management: an overview. Folia Microbiologica. 2024;69(3):549-65.