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Therapeutic efficiency of probiotic lactobacillus acidophilus, lactobacillus rhamnosus and antibiotic against isolates of pathogenic bacterial from skin wound infections

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***Abstract*---**Background: Probiotic refer to harmless live normal flora micro-organisms (M.O), which are mostly strains of *Lactobacillus* spp., *Bifidobacterium* spp., and others. These microorganisms (M.O) help the host's health when given in the right dosages. Aims: To identify bacterial species isolated from wound infections, and also to evaluate *in-vitro* the antimicrobial activity of selected probiotic species and antibiotic against some pathogenic bacteria. Methods: Records of wound swabs collected from 102 patients with high suspicion of wound infections. Bacterial colonies were Gram stained and microscopically examined. Biochemical tests were done to identify pathogen species. The microtitre plate method (MTP) was used to detect biofilm formation by bacteria. The Kirby-Bauer disk diffusion method was used for antibiotic testing. The well diffusion method and agar spot method was used for detection probiotic affectivity to a group of bacteria isolated (totally 38 isolates). Results: Prevalence of wound infections was 81.4% (CI: 60.869). A total of 10 species were isolated from 83 infected wounds. The most common bacterial species detected was *Staphylococcus aureus* (26.5%), followed by *Pseudomonas aeruginosa* 14.4%, *Proteus mirabilis* (12%), *Escherichia coli* 10.8%, and *Enterobacter cloacae* (8.4%). Polymicrobial infection was found in 25(30.1%) of the samples and was mainly constituted with two species. The most common association was *P. aeruginosa / P. mirabilis*. The results demonstrated that all 83 of isolates biofilm production with varied titer and highly statistical differences *(P*<0.0001; CI = 70.057). In susceptibility testing using well diffusion method, *L. acidophilus* had effect against 32(84.2%) of 38 isolates (*P* = 0.004) and *L. rhamnosus* had coverage against 35(92.1%) of 38 isolates (*P* = 0.003). While in agar spot method, *L. acidophilus* had

effect against 33(86.8%) of 38 isolates (P = 0.005) and *L. rhamnosus* had coverage against 36(94.7%) of 38 isolates (P = 0.004). The activity (resistance or sensitivity) of the remaining 15 antibacterial agents utilized varied in their coverage, which ranged from 41.7% to 100%. Conclusion: This study indicates the antibacterial efficacy properties of *Lactobacillus* spp., to inhibit the growth *in-vitro* of pathogenic bacteria, which cause wound. But probiotic cannot inhibit strong biofilm formation for all isolates.

***Keywords*---**Lactobacillus acidophilus, Lactobacillus rhamnosus, probiotic, biofilm formation, antimicrobial agents, skin wound infections.

Introduction

A wound is defined as an interruption of the integrity or dysfunction of the skin layers [1, 2]. Wounds are classified as acute or chronic in the most cases. The first type includes burns, scratches, abrasions, and bug bites are examples of acute ulcers that are expected to heal in an expected time. Patients' quality of life is drastically reduced when their skin is damaged by trauma or burn injury [3]. However, depending on the degree of the injury, therapeutic. There are ways to quicken the healing process [4]. Moreover, in the majority of cases, serious burn or gunshot wounds necessitate surgery, debridement, and antibiotic therapy. Processes related accompanied by an underlying risk factor that is diabetes mellitus (D.M) or immunological weakness, on other hand, are the most common cause of chronic wounds. Leg and artery ulcers, non-healing surgical site, and Diabetic foot ulcers are examples of chronic wounds [5]. Another risk agents for chronic wounds include foreign objects, advanced age, chronic conditions, such as diabetic mellitus, obesity, immune-deficient diseases, and infection with microbes [6, 7]. Common pathogenic microbes that induce wound infections include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Acinetobacter baumannii* [8, 9]. By employing numerous virulence factors, these bacteria can cause harm and diseases [10]. Wounds, particularly burn injuries, are inhabited by Gram-positive Micro-organism (M.O), including *S. aureus* in first week, and Gram-negative bacteria penetrate the wound in the second week [11]. As bacteria multiply, they begin to invade deeper tissues, and sepsis develops when pathogenic bacteria penetrate the lymphatic system and capillaries. One of the most dangerous clinical forms of infection in patients with inflamed wounds is sepsis, which requires prompt treatment, especially in patients in burn units, due to the presence of the extremely poisonous lipopolysaccharides (LPS) in their cell walls, dangerous and harmful bacteria include *P. aeruginosa* and *A. baumannii* [12].

Probiotics are live M.O that provides health benefits to the host when consumed in adequate amounts by restoring the microbial balance in the system [13]. Scientists have been attempting to clarify and define the probiotic effect ever since [14]. Furthermore, lactic acid bacteria (LAB) create part to the autochthonous micro-biota of a variety of foods, such as yogurt, sour cream, sausages, olives, and others [15, 16]. They are defined as a set of lactic-acid-producing, low G+C-

percentage organisms. Gram-positive cocci or rods, non-spore-forming bacteria, and catalase-negative bacteria share many biochemical, physiological, and genetic properties [17]. They must be able to interact with or send signals to the immune system in order to moderate immune system activity. They must also be able to stimulate local metabolic action [18]. Furthermore, probiotics should be consistent, safe, active, and capable of remaining usable for extended periods of time under storage conditions. It must be capable of restoring and replacing intestinal micro-flora [19].

Material and Method

Study design

This research was cross-sectional study during the period from 9st November, 2021 to 15th February, 2022. A wound swab samples taken from patients with suspected skin wound infections, including burn, surgery, diabetic foot, and trauma, between the ages of 17 and 70. Patients were admitted to Al-Zahra Teaching Hospital, Al Karma Hospital, Fairuz hospital and private clinics in Wasit province. Samples were taken by sterile disposable cotton swabs and transport swab. They were, then, cultured onto (MacConkey agar, Blood agar, and Chocolate agar/ Liofilchem) plates before incubating aerobically and (anaerobically with Co_2) at 37°C for (24h to 48h). After incubation, identified based on colony morphology, microscopic Gram stain investigation, and standard biochemical tests [20].

Exclusion and inclusion criteria

In order to include all patients in the study who had suspected wound infections, the records were carefully examined. Patients were considered to have a wound infections if they displayed any of the following symptoms: fever, erythema, localized warmth, foul smell, and darkening of granulation tissue. Patients who were really unwell and those who had had antibiotic treatment one to two weeks before to the research were excluded. The patients' age, sex, and wound type were recorded. After superficial pre-cleansing of wounds with normal saline, Excess saline was removed carefully by using sterile gauze; each specimen was collected by rotating a sterile swab during the wound surface of a $1cm^2$ area in a zig-zag motion, from the center of the wound to outside.

Detection of biofilm formation

To detect biofilm formation, preparation of biofilm solution using glacial acetic acid (33%) by adding 33ml of glacial acetic acid to 67 ml of D.W, and used phosphate buffer saline via suspended 9.86g in 1liter of D.W, and autoclaved at 121°C/15 pounds/inch2 for 15mnts. Then, preserved in refrigerator till used. Finally, this process was used ethanol 96% via mixed 96ml of ethanol (100%) with 4ml D.W [21, 22].

Microtitre plate method

The isolates' capacity to form biofilm was examined on 96-well flat-bottomed

micro-titer polystyrene plates. In three wells of a microtitre plate, add 200μL of bacterial suspensions in brain heart infusion broth for each isolate. All microtitre plates were then incubated for 24 hrs at 37°C. As controls, wells with brain heart infusion were filled. After that, the contents of each well were discarded and the planktonic bacteria were removed by washing with phosphate-buffered saline three times. 96% of ethanol was used to fix the adhesive bacteria for 5 minutes, after which the plates were emptied and left to dry. The plates were then stained with 100μL of crystal violet solution (1%) per well (w/v) and left to sit for 5 minutes. A sterile solution of distilled water was used to remove the excess discoloration. Then, 200μL of glacial acetic acid (33%) per well (v/v) was added to carry out the quantitative analysis of the biofilm. Using a BioTek Reader, microtitre plates were incubated for 15 minutes before the absorbance at 570_{nm} was measured, as labelled that biofilm formation was considered as highly positive ($OD_{570} \geq 0.24$), weak positive ($0.12 \leq OD_{570} < 0.24$), or negative ($OD_{570} < 0.12$) [21].

Antibiotic Susceptibility

Susceptibility to antibiotic chemotherapy was determined by the Kirby-Bauer disc diffusion method, and results interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2022). The antibiotics tested were amikacin 30µg, amoxicillin-clavulanate 30µg, azithromycin 15µg, ceftazidime 30µg, ciprofloxacin 5µg, gentamicin 10µg, ceftriaxone 30µg, nitrofurantoin 30 µg, imipenem 10µg, levofloxacin 5µg, meropenem 10µg, nalidixic acid 30µg, piperacillin 100µg, trimethoprim sulfamethoxazole 25µg, and tobomycin 30µg.

Methods for detection probiotic affectivity

Well diffusion method

In this method, all *Lactobacillus* spp. were cultured in MRSB (10^7CFU/ml) at 37°C with 5% CO_2 for 24hrs, and used as the broth-culture-bacteria (BCB). In addition, staphylococci isolates were grown in broth culture and incubated at 37°C for 24hrs. In addition, cell free supernatant (CFS) for *Lactobacillus* spp. was obtained by centrifuging the culture at 10000 rpm/15mnts., then clarified the supernatant through a filter paper of 0.22µm pore size [23].

Agar spot method

Spots of *Lactobacillus* spp. from an overnight culture in MRS broth were placed on the surface of MRS agar, and colonies were then allowed to grow for 24 hrs. at 37°C. The pathogenic bacteria were put into semi-solid nutritional medium (1.3g nutrient broth + 0.75g nutrient agar in 100ml D.W) and then poured over MRS agar after 24 hrs. of culture. The plates were incubated at 37°C for 24 hrs, and an inhibitory zone was then examined. The areas of inhibition were categorized as (−) for no visible inhibition, (+) for inhibition between 0.5 and 6 mm, (++) for inhibition between 7 and 12 mm, and (+++) for inhibition of more than 12 mm [24].

Statistical analyses

All data were statistically analyzed using the Statistical-Package-for-Social-Science (SPSS, version 25.0) for Windows program. Chi Square was used to assess all results that had a significant level ($P \leq 0.05$).

Result

Prevalence of wound infections

The current study was conducted on 102 specimens from skin wound infections of suspected patients, 57 (55.9%) male and 45 (44.1%) female subject. The results were distributed according to the patient's age between 17-70 years old with highly statistical differences, as showed in the (Table 1).

Table (1): The distribution of patients according to age groups and gender

Variable		Frequency	%	*P*-value
Gender	Male	57.0	55.9	0.013
	Female	45.0	44.1	
Age (years)	17-20	6	5.9	
	21-30	22	21.5	
	31-40	19	18.6	
	41-50	28	27.4	
	51-60	15	14.7	
	61-70	12	11.8	

P<0.05, Chi-square= 14.443

Type of wound

One hundred and two swab specimens from skin wound infections were collected in this study which include burn (n=30, 29.4%), diabetic foot (n=27, 26.4%), surgery (n=25, 24.5%), and trauma (n=20, 19.6%). about 83 swab (81.4%) show positive culture of bacterial growth from different skin wound sites versus 19 swab (18.6%) show negative results for culturing, as showed in the (Table 2).

Table 2: The distribution of sample collection according to site of wound and gender

Type of wound	No. of swab	Patient's with infected wounds		No. of infected swabs	Patient with infected wounds		*P*-value
		Males	Females		Males	Females	
Burn	30(29.4%)	17	13	25(83.3%)	14	11	0.285
Diabetic foot	27(26.4%)	17	10	21(77.8%)	15	6	
Surgery	25(24.5%)	10	15	20(80.0%)	7	13	
Trauma	20(19.6%)	13	7	17(85.0%)	11	6	
Total	102	57	45	83(81.3%)	47	36	

P<0.05, Chi-square= 3.788

Age and sex

A higher incidence of wound infections in the 17 to 70 year age group, but age did not significantly correlate with the incidence of wound infections. Both the type of wound and the type of organism that was isolated did not significantly correlate with the subject's sex (*P* = 0.28 and *P* = 0.57, respectively).

Isolation of bacteria

A culture study based on morphological and biochemical tests revealed a high incidence of Gram negative bacteria 46(55.5%), that includes *Pseudomonas aeruginosa* showed that a high percent 12(14.4%), followed *Proteus mirabilis* 10(12.0%). Moreover, *Escherichia coli* 9(10.8%), *Enterobacter cloacae* 7(8.4%), *Klebsiella Pneumoniae* 7(8.4%), , while *Enterococcus facieum* was the least detected isolate 1(1.2%). Whereas Gram-positive bacteria recorded 37(44.5%), that includes *S. aureus* had the highest percentage of isolated M.O in this investigation 22(26.5%), followed by *S. epidermidis* 7(8.4%), *S. hominis* 4(4.8%) and *S. haemolyticus* 4(4.8%). *S. aureus* was the predominant Gram positive M.O isolated, accounting for almost quarter of the isolates. *P. aeruginosa* was the predominant Gram negative M.O isolated from the wound swabs. However, there was significant associated between organism and wound types (*P* = 0.0002; CI = 60.869), (Figure 1).

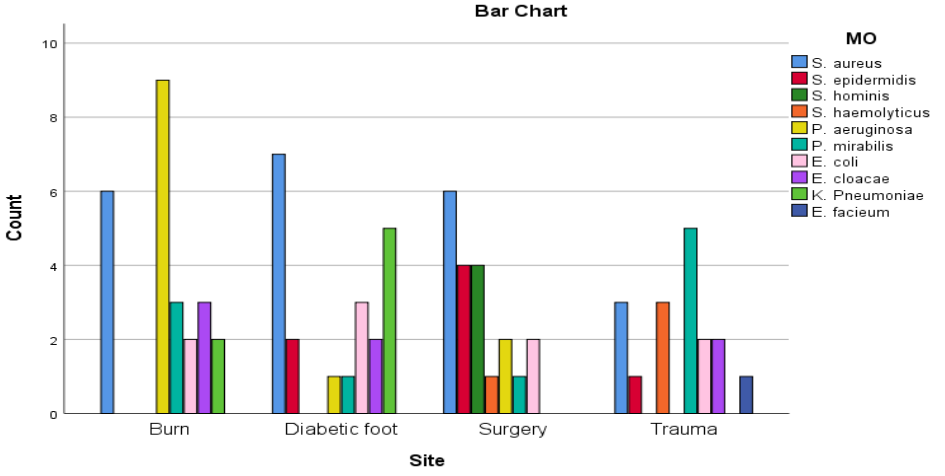
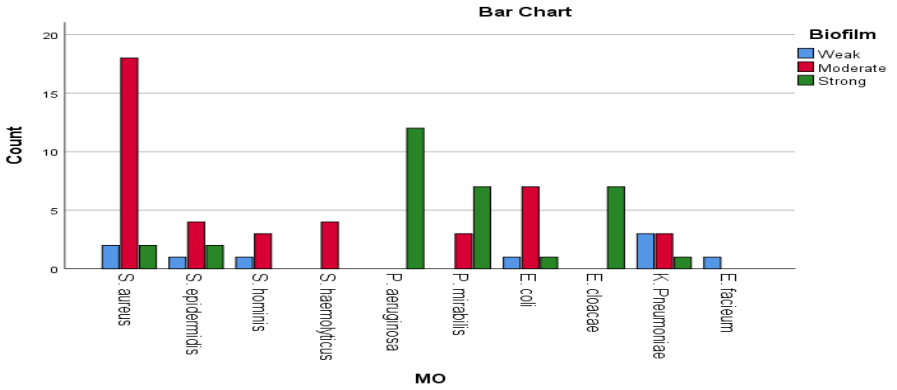


Figure 1: Frequency of organisms based on wound types

Biofilm production by using microtitre plate (MTP)

In present study, a total 83 isolates evaluated using MTP. MTP is the most sensitive, accurate, and trustworthy screening approach for identifying the development of biofilms was found. MTP was a quantitative technique that reflected the industry standard for identifying biofilms (Al-Dahmoshi, 2013). In MTP method for detection of biofilm production, the results demonstrated that all 83 of isolates biofilm production, with varied titer and highly statistical differences (*P*<o.ooo1; CI = 70.057), as showed in (Figure 2).



(Figure 2): MTP Method that used for biofilm production and percent of each type

Effect of probiotic on the skin wound bacteria

Antimicrobial effect of *Lactobacillus* spp., against some bacterial skin wound infections using well diffusion methods and agar spot method. The synthesis of several anti-bacterial chemicals, such as organic acids, bacteriocins, CO_2, H_2O_2, or other substances like antibiotics, was a sign of the antimicrobial activity of probiotics in different *Lactobacillus* spp. The results in the present study with probiotic bacteria against different bacteria isolated from skin wound infections showed variance of inhibition ability ranging in case of bacterial culture broth (BCB) from 11-24 mm and in case of cell free supernatant (CFS) from 9-15 mm by using well diffusion method, while in agar spot method, the inhibition ability ranging from 12-26. The *L. rhamnosus* showed high significant (P = 0.003) ability to inhibit growth of *P. aeruginosa*, an *P. mirabilis*, *E. coli* and *E. cloacae* more than *L. acidophilus* (P = 0.004) by using well diffusion method, as illustrated in the (Table 3).

Table 3: Antimicrobial effect *Lactobacillus* spp., against different skin wound bacteria isolated using well diffusion method

Bacteria	*L acidophilus*	*P-value*	*L. rhamnosus*	*P-value*
	No.(%)	0.004	No.(%)	0.003
P. aeruginosa	9(75.0%)		10(83.3%)	
P. mirabilis	9(90.0%)		10(100%)	
E. coli	9(100%)		9(100%)	
E. cloacae	5(71.4%)		6(85.7%)	

P<0.05, Chi-square= 1.691

The highest inhibition zone of (BCB) by *L. rhamnosus* was 24 mm; meanwhile, highest inhibition zone by *L. acidophilus* was 19 mm using well diffusion method. In addition, the highest inhibition zone of (CFS) in both *L. rhamnosus* and *L. acidophilus* was 15 mm, and 11 mm, respectively, using well diffusion method, as illustrated in (Table 4).

Table 4: Antimicrobial effect of *Lactobacillus* spp. in millimeters using well diffusion method by BCB and CFS.

Bacteria	BCB					
	L. acidophilus			*L. rhamnosus*		
	Active No.(%) ≥ 15mm	Moderate active No.(%) 11-14mm	Less active No.(%) ≤ 10mm	Active No.(%) ≥ 15mm	Moderate active No.(%) 11-14mm	Less active No.(%) ≤ 10mm
P. aeruginosa	4(33.3%)	5(41.7%)		6(54.5%)	4(36.3%)	
P. mirabilis	6(60%)	3(30%)		8(80%)	2(20%)	
E. coli	4(44.4%)	5(55.6%)		6(66.7%)	3(33.3%)	
E. cloacae	2(28.5%)	3(42.8%)		4(57.1%)	2(28.5%)	
	CFS					
Bacteria	*L. acidophilus*			*L. rhamnosus*		
	Active No.(%) ≥ 15mm	Moderate active No.(%) 11-14mm	Less active No.(%) ≤ 10mm	Active No.(%) ≥ 15mm	Moderate active No.(%) 11-14mm	Less active No.(%) ≤ 10mm
P. aeruginosa		3(25%)	6(50%)		4(36.3)	6(54.6%)
P. mirabilis		4(40%)	5(50%)		6(60%)	4(40%)
E. coli		5(55.6%)	4(44.4%)		7(77.8%)	2(22.2%)
E. cloacae		2(28.5%)	3(42.8%)		3(42.8%)	3(42.8%)

P<0.05

Agar spot method are another method of antimicrobial effect of *L. acidophilus* and *L. rhamnosus* against different skin wound bacteria isolated., as showed in (Table 5).

Table 5: Antimicrobial effect *Lactobacillus* spp., against different skin wound bacteria isolated using agar spot method

Bacteria	*L acidophilus*	*P-value*	*L. rhamnosus*	*P-value*
	No.(%)	0.005	No.(%)	0.004
P. aeruginosa	10(83.3%)		11(91.7%)	
P. mirabilis	9(90.0%)		10(100%)	
E. coli	9(100%)		9(100%)	
E. cloacae	5(71.4%)		6(85.7%)	

P<0.05, Chi-square= 1.765

According to Zone diameter of inhibition, the highest inhibition zone with *L. rhamnosus* was 26 mm; meanwhile-highest inhibition zone with *L. acidophilus* was 22 mm using agar spot method, as showed in (Table 6).

Table 6: Antimicrobial effect of *Lactobacillus* spp. in millimeters using agar spot method

Bacteria	Agar spot method			
	L. acidophilus		*L. rhamnosus*	
	Active No.(%) > 12mm (+ + +)	Moderate active No.(%) 7-12mm (++)	Active No.(%) > 12mm (+ + +)	Moderate active No.(%) 7-12mm (++)
P. aeruginosa	9(75%)	1(8.3%)	11(91.7%)	
P. mirabilis	8(80%)	1(10%)	10(100%)	
E. coli	5(55.6%)	4(44.4%)	9(100%)	
E. cloacae	3(42.8%)	2(28.5%)	4(57.1%)	2(28.5%)

P<0.05

Comparison of the effect of antibiotics and probiotics on biofilm formation by some pathogenic skin wound bacteria isolates

In this current study the effect of *L. rhamnosus* on biofilm formation for pathogenic skin wound bacteria showed in well diffusion method and agar spot method by statically analysis (P = 0.003, P = 0.004) respectively, that means it had ability to prevent biofilm formation. In addition, *L. acidophilus* also showed a relationship with inhibition of biofilm formation for pathogenic skin wound bacteria in well diffusion method and agar spot method by statically analysis (P = 0.004, P = 0.005) respectively, but slightly less than *L. rhamnosus.* The effect of both *lactobacillus* spp., bacteria on these bacteria by statically analysis indicated there are relationship of probiotic and inhibition of strong biofilm formation, as in (Table 7).

Table 7: Effect of antibiotics and probiotics on pathogenic skin wound infections

Antibacterial agent	*E. coli*	*P. mirabilis*	*E. cloacae*	*P. aeruginosa*
Strong biofilm	1(11.1%)	7(70%)	7(100%)	10(100%)
L. acidophilus	(100%)	(90%)	(71.4%)	(83.3%)
L. rhamnosus	(100%)	(100%)	(85.7%)	(91.7%)
AK 30µg	S(77.8%)	S(80%)	R(71.4%)	R(41.7%)
AUG 30µg	R(100%)	R(90%)	R(100%)	R(100%)
AZM 15µg	R(55.6%)	S(70%)	R(100%)	-
CRO 30µg	R(77.8%)	R(70%)	R(71.4%)	-
F 30 µg	S(77.8%)	R(60%)	R(71.4%)	-
LEV 5µg	S(66.7%)	R(60%)	S(57.1%)	R(50%)
MRO 10µg	S(66.7%)	S(80%)	R(71.4%)	S(91.7%)
NA 30µg	R(66.7%)	R(70%)	R(100%)	R(100%)
CN 10µg	R(56.6%)	R(90%)	R(100%)	S(58.3%)
SXT 25µg	R(44.4%)	R(80%)	R(100%)	-
CAZ 30µg	R(44.4%)	R(50%)	R(85.7%)	R(58.3%)
CIP 5µg	R(44.4%)	R(50%)	R(71.4%)	R(50%)
IMI 10µg	R(77.8%)	I(60%)	R(71.4%)	S(41.7%)
PRL 100µg	R(77.8%)	S(80%)	R(100%)	S(41.7%)

TOB30µg	R(55.6%)	R(50%)	R(100%)	R(66.7%)

Significant difference (P<0.001); Chi square= 38.487; S= sensitive; R= resistant

Discussion

The M.O that were most often isolated in this study were *S. aureus* 6(30.0%), followed by *S. epidermidis* 4(20.0%), *S. hominis* 4(20.0%), *S. haemolyticus* 3(15.0%), *P. aeruginosa* 2(10.0%), and then *P. mirabilis* 1(5.0%). This shows their ability to infect the skin with a variety of virulence factors that encourage host tissue adhesion, such as coagulase, protein A, leukocidins, haemolysins, and superantigens [25, 26, 27, 28]. The causes of the increased incidence of *P. aeruginosa* isolates from burn wound more than other types of wounds may be related to factors such as nosocomial pathogen acquisition with frequent, prolonged hospitalization that exacerbates illnesses, prior or Random antibacterial drug treatment and the immunosuppressive effects of burn wound infection [29, 30]. *E. coli, E. cloacae, K. pneumoniae, and P. mirabilis* isolates were segregated with low frequencies and different percents due to the development of nosocomial properties, their migration from the gastrointestinal, urinary, and respiratory tracts to wounds, and their immunosuppressive activities [25, 31, 32]. Detection of biofilm formation by MTP showed that *S. aureus* 2(9.0%) of isolate produce weak biofilm, 18(82.0%) isolate as moderate biofilm and 2(9.0%) isolates strong biofilm, In *S. epidermidis* 1(16.7%) of isolate produce weak biofilm, 1(14.2%) isolate as moderate biofilm and 4(66.7%) isolates strong biofilm. similar to that revealed in Baghdad was result showed that the OD values obtained by *S. epidermidis* were higher than the OD values obtained by *S. aureus* [33]. Also agreed with another study reported [34]. In *P. aeruginosa* 12(100%) isolates strong biofilm. In this study, the percentage of strong biofilm formation close to the result in Baghdad was result showed that the percentage was (100%) [34]. In *P. mirabilis* 3(30%) isolate as moderate biofilm and 7(70%) isolates strong biofilm. In this study, the percentage of strong biofilm formation close to the result that reported *P. mirabilis* is one of the strongest biofilm production isolated from skin wound [35]. In *E. coli* 1(11.1%) of isolate produce weak biofilm, 7(77.8%) isolate as moderate biofilm and 1(11.1%) isolates strong biofilm. In this study, the percentage of strong biofilm formation agreed to the result in Egypt was (10.0%) [36].

In *E. cloacae* 7(100%) isolates strong biofilm. In this study, Similar result that reported *Enterobacter* spp., isolates from wound infections have more capacity to produce biofilms than isolates from Urinary system [37]. Furthermore, these result are disagreed with other study reported in Baghdad was showed moderate percentage was (40.0%) and strong percentage was (60.0%) for *Enterobacter* spp. [38]. According to susceptibility testing, the study tested *L. acidophilus* and *L. rhamnosus* to a wide range of antibacterial drugs that might be applied locally or systemically in cases of skin wounds. Table 7 displayed the susceptibility of bacterial isolates with multidrug resistance to several antibacterial compounds when applied in varying amounts [39]. All isolates displayed high sensitivity to *L. acidophilus* and *L. rhamnosus*. These result of probiotic come in accordant to another study reported that, the probiotic *L. rhamnosus* enhancement of inhibitory zone diameters in well diffusion method higher than *L. acidophilus* against *P. aeruginosa* [39]. In addition, these result agreed with another study

reported that showed the effect of *L. rhamnosus* on *P. aeruginosa* was (72.2%), While the effect of *L. acidophilus* on *P. aeruginosa* was (61.1%) [19]. Moreover, another study in Baghdad found that *L acidophilus* has ability to inhibit against (100%) of *P. aeruginosa*, an *P. mirabilis*, *E. coli* and *E. cloacae* [40]. When comparing diameters to (CFS), the antibacterial action of (BCB) utilizing the well diffusion method showed the largest inhibitory zone. This may be because the (BCB) contains higher concentrations of inhibitory chemicals, such as those linked to intact bacteria and antimicrobial compounds. The existence of various secondary metabolites by *Lactobacillus* spp., such as lactic acids, biosurfactant, and other fermentation products, as well as bacteriocins, may play a significant role in the greatest removal between the harmful bacteria [41]. The result in current study showed that the agar spot method slightly more effective method compared to well diffusion method. In comparison to the well diffusion method, the agar spot method was generally highly effective. This may be due to *Lactobacillus* spp., grown on agar medium is able to synthesize additional inhibitory substance-bacteriocins in a significantly greater amount than *Lactobacillus* spp., cultivated on liquid culture [19]. Furthermore, the results concluded that *L. rhamnosus* exhibited a strong ability to stop bacteria from growing *in-vitro* for most harmful Gram-negative bacteria, which cause infections in skin wounds. This demonstrates that *L. rhamnosus* bacteria's more effectiveness as a treatment than *L. acidophilus*. Most of isolates showed highly sensitive percent to *L. acidophilus* and *L. rhamnosus*. The antibacterial activity of *Lactobacilli* spp., is explained by these results through interaction with Toll-like receptors 2 (TLR-2) It is capable of recognizing lipoteichoic acid (LTA), bacterial lipoproteins, and other ways, synthesis of a variety of antimicrobial chemicals, including antibiotics, lactic acid, and hydrogen peroxide [42]. Moreover, through competing with other pathogenic M.O for nutrients, Pathogenic growth and proliferation can be effectively suppressed by lactobacilli. In accordance with Lactobacilli's antibacterial activity, the innate immune system's macrophages and natural killer cells (NKCs) were said to exhibit stimulatory qualities *in-vitro* [43]. Systemic inflammatory reactions to pathogenic bacterial DNA can be reduced by probiotic Lactobacilli DNA [44]. These immunological effects of lactobacilli were crucial for inhibiting or reducing bacterial complications that could result in the patient's death. Probiotic cannot inhibit strong biofilm formation for all isolates, while results showed that there was no resistance to some antibiotics such as amikacin and nitrofurantoin, the biofilm-forming bacteria have high ability to be resistant to antibiotics, and this may be due to reduced capacity of antibiotics penetration into biofilm core because of physical barrier that made of polymeric substances on the external surface [45].

Conclusion

This study shows that *Lactobacillus* spp., has antibacterial effectiveness and immunological characteristics against pathogenic wound infections.

References

1. Abd El-Baky, R. M., Ibrahim, R. A., Mohamed, D. S., Ahmed, E. F., and Hashem, Z. S. (2020). Prevalence of virulence genes and their association with antimicrobial resistance among pathogenic E. coli isolated from

Egyptian patients with different clinical infections. *Infection and Drug Resistance*, *13*, 1221.
2. Abriouel, H., Benomar, N., Cobo, A., Caballero, N., Fuentes, M. Á. F., Pérez-Pulido, R., and Gálvez, A. (2012). Characterization of lactic acid bacteria from naturally-fermented Manzanilla Aloreña green table olives. *Food microbiology*, 32(2), 308-316.
3. Al-Azzawi, M. K., Makharmash, J. H., and Al-Malkey, N. K. (2020). The effect of *Lactobacillus* species on the *Pseudomonas aeruginosa*. *Drug Invention Today*, 14(2).
4. al-Husni, Halah Muhammad Husayn. (2011). Comparative study between methicillin-resistant coagulase positive and negative Staphylococci. (Master's theses Theses and Dissertations Master). University of Baghdad, Iraq.
5. Al-Malkey, M. K., Ismeeal, M. C., Al-Hur, F. J. A., Mohammed, S. W., and Nayyef, H. J. (2017). Antimicrobial effect of probiotic Lactobacillus spp. On *Pseudomonas aeruginosa*. *Journal of Contemporary Medical Sciences*, 3(10), 218-223.
6. Atiyeh, B. S., and Al-Amm, C. A. (2001). Immunology of burn injury-an overview. *Annals of Burns and Fire Disasters*, *14*(2), 78-84.
7. Avila-Novoa, M. G., Iñíguez-Moreno, M., Solís-Velázquez, O. A., González-Gomez, J. P., Guerrero-Medina, P. J., and Gutiérrez-Lomelí, M. (2018). Biofilm formation by Staphylococcus aureus isolated from food contact surfaces in the dairy industry of Jalisco, Mexico. *Journal of Food Quality*, 2018.
8. Bahramian, A., Khoshnood, S., Shariati, A., Doustdar, F., Chirani, A. S., and Heidary, M. (2019). Molecular characterization of the pilS2 gene and its association with the frequency of *Pseudomonas aeruginosa* plasmid pKLC102 and PAPI-1 pathogenicity island. *Infection and Drug Resistance*, 12, 221.
9. Bowler, P. G., Duerden, B. I., and Armstrong, D. G. (2001). Wound microbiology and associated approaches to wound management. *Clinical microbiology reviews*, 14(2), 244-269.
10. Bowler, P. G., Duerden, B. I., and Armstrong, D. G. (2001). Wound microbiology and associated approaches to wound management. *Clinical microbiology reviews*, 14(2), 244-269.
11. Burnham, J. P., Kirby, J. P., and Kollef, M. H. (2016). Diagnosis and management of skin and soft tissue infections in the intensive care unit: a review. *Intensive care medicine*, 42(12), 1899-1911.
12. Cardona, A. F., and Wilson, S. E. (2015). Skin and soft-tissue infections: a critical review and the role of telavancin in their treatment. *Clinical Infectious Diseases*, 61(suppl_2), S69-S78.
13. Christensen, G. D., Simpson, W. A., Younger, J. J., Baddour, L. M., Barrett, F. F., Melton, D. M., and Beachey, E. H. (1985). Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. *Journal of clinical microbiology*, *22*(6), 996-1006.
14. Fijan, S., Frauwallner, A., Langerholc, T., Krebs, B., ter Haar née Younes, J. A., Heschl, A., ... and Rogelj, I. (2019). Efficacy of using probiotics with antagonistic activity against pathogens of wound infections: an integrative review of literature. *BioMed research international*.
15. Flayyih, May and Abu-Risha, Rasmyia and Hasan, Jinan. (2012). The Prophylactic Role of Lipopolysaccharide of *Pseudomonas aeruginosa* Against

Corneal. *Infection Journal of Biotechnology Research Center*. 6. 96-101.
16. Foster, T. J. (2002). Staphylococcus aureus. *Molecular Medical Microbiology*, 839-888.
17. Glik, J., Kawecki, M., Gaździk, T., and Nowak, M. (2012). The impact of the types of microorganisms isolated from blood and wounds on the results of treatment in burn patients with sepsis. *Polski Przeglad Chirurgiczny*, 84(1), 6-16.
18. Guo, B., Dong, R., Liang, Y., and Li, M. (2021). Haemostatic materials for wound healing applications. *Nature Reviews Chemistry*, 5(11), 773-791.
19. Herek, O., OZTURK, H., Ozyurt, M., Albay, A., and Cetinkursun, S. (2000). Effects of treatment with immunoglobulin on bacterial translocation in burn wound infection. *Annals of Burns and Fire Disasters*, *13*(1), 13-17.
20. Holzapfel, W. H. N., and Wood, B. J. (2012). The genera of lactic acid bacteria (Vol. 2). *Springer Science and Business Media*.
21. James, C., and Natalie, S. (2014). Microbiology. *A laboratory manual. Pearson Education*.
22. Jebur, M. S. (2010). Therapeutic efficacy of *Lactobacillus acidophilus* against bacterial isolates from burn wounds. *North American journal of medical sciences*, 2(12), 586.
23. Jijon H, Backer J, Diaz H, et al. (2004). DNA from probiotic bacteria modulates murine and human epithelial and immune function. *Gastroenterology*; 126:1358- 1373.
24. Kazou, M., Grafakou, A., Tsakalidou, E., and Georgalaki, M. (2021). Zooming into the microbiota of home-made and industrial kefir produced in Greece using classical microbiological and amplicon-based metagenomics analyses. *Frontiers in Microbiology*, 12, 621069.
25. Komolafe, O. O., James, J., Kalongolera, L., and Makoka, M. (2003). Bacteriology of burns at the queen elizabeth central hospital, Blantyre, Malawi. *Burns*, *29*(3), 235-238.
26. Kusumawati, A. H., Wulan, I. R., & Ridwanuloh, D. (2020). Formulation and physical evaluation sheet mask from red rice (Oryza Nivara) and virgin coconut oil (Cocos Nucifera L). *International Journal of Health & Medical Sciences*, *3*(1), 60-64. https://doi.org/10.31295/ijhms.v3n1.148
27. Lari, A. R., Alaghehbandan, R., and Akhlaghi, L. (2005). Burn wound infections and antimicrobial resistance in Tehran, Iran: an increasing problem. *Annals of burns and fire disasters*, *18*(2), 68.
28. Macedo, J. L. S. D., Rosa, S. C., and Castro, C. (2003). Sepsis in burned patients. *Revista da Sociedade Brasileira de Medicina Tropical*, *36*, 647-652.
29. Marquès, C., Tasse, J., Pracros, A., Collin, V., Franceschi, C., Laurent, F., ... and Forestier, C. (2015). Effects of antibiotics on biofilm and unattached cells of a clinical *Staphylococcus aureus* isolate from bone and joint infection. *Journal of medical microbiology*, 64(9), 1021-1026.
30. Mc-Cracken BJ, Gaskins HR. (1999). Probiotics and the immune system. In: Tannok GW(ed.): probiotics: a critical review. Norfolk, Va: *Horizon Scientific Press*; 85-111.
31. Modaresifar, K., Azizian, S., Ganjian, M., Fratila-Apachitei, L. E., and Zadpoor, A. A. (2019). Bactericidal effects of Nano patterns: A systematic review. *Acta Biomaterialia*, 83, 29-36.
32. Murray, P. M., and Finegold, S. M. (1984). Anaerobes in burn-wound infections. *Reviews of infectious diseases*, 6(Supplement_1), S184-S186.

33. Nowroozi, J., Mirzaii, M., and Norouzi, M. (2004). Study of Lactobacillus as probiotic bacteria. *Iranian Journal of Public Health*, *33*(2), 1-7.
34. Oleiwi, Sanaa and Suhail, Shaymaa and Radif, Hala and Doi,. (2021). Morphological and Molecular Study of Biofilm Formation by *Enterobacter cloacae*. *Annals of Tropical Medicine and Public Health*. 24. 176-186 .
35. Paongphan, P., and Taweechotipatr, M. (2019). Characterization of probiotic lactic acid bacteria producing bile-salt hydrolase for development of fermented milk product (Doctoral dissertation, Srinakharinwirot University), 8-22.
36. Salas-Jara, M. J., Ilabaca, A., Vega, M., and García, A. (2016). Biofilm forming *Lactobacillus*: new challenges for the development of probiotics. *Microorganisms*, 4(3), 35.
37. Shariati, A., Asadian, E., Fallah, F., Azimi, T., Hashemi, A., Sharahi, J. Y., and Moghadam, M. T. (2019). Evaluation of Nano-curcumin effects on expression levels of virulence genes and biofilm production of multidrug-resistant *Pseudomonas aeruginosa* isolated from burn wound infections in Tehran, Iran. *Infection and Drug Resistance*, 12, 2223.
38. Sharifi-Rad, J., Rodrigues, C. F., Stojanović-Radić, Z., Dimitrijević, M., Aleksić, A., Neffe-Skocińska, K., ... and Calina, D. (2020). Probiotics: versatile bioactive components in promoting human health. *Medicina*, *56*(9), 433.
39. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, *5*(1), i-v. https://doi.org/10.53730/ijhs.v5n1.2864
40. Vallor, A. C., Antonio, M. A., Hawes, S. E., and Hillier, S. L. (2001). Factors associated with acquisition of, or persistent colonization by, vaginal lactobacilli: role of hydrogen peroxide production. *The Journal of infectious diseases*, *184*(11), 1431-1436.
41. Vélez, M. P., Hermans, K., Verhoeven, T. L. A., Lebeer, S. E., Vanderleyden, J., and De Keersmaecker, S. C. J. (2007). Identification and characterization of starter lactic acid bacteria and probiotics from Columbian dairy products. *Journal of Applied Microbiology*, *103*(3), 666-674.
42. Vindenes, H., and Bjerknes, R. (1995). Microbial colonization of large wounds. *Burns*, *21*(8), 575-579.
43. Wasfi, R., Mansour, L., Hashem, A., Abd El-Rahman, O., Hanora, A., and Ashour, M. (2012). Antimicrobial activities against biofilm formed by *Proteus mirabilis* isolates from wound and urinary tract infections. *Indian Journal of Medical Microbiology*, 30(1), 76.
44. Wasfi, R., Mansour, L., Hashem, A., Abd El-Rahman, O., Hanora, A., and Ashour, M. (2012). Antimicrobial activities against biofilm formed by *Proteus mirabilis* isolates from wound and urinary tract infections. *Indian Journal of Medical Microbiology*, 30(1), 76.
45. Wu, X. D., Liu, M. M., Liang, X., Hu, N., and Huang, W. (2018). Effects of perioperative supplementation with pro-/synbiotics on clinical outcomes in surgical patients: a meta-analysis with trial sequential analysis of randomized controlled trials. *Clinical Nutrition*, 37(2), 505-515.
46. Wu, X. Y. (2006). Studies on the impact of probiotic bacteria on enteric microbial diversity and immune response.
47. Yu, R., Zhang, H., and Guo, B. (2022). Conductive biomaterials as bioactive wound dressing for wound healing and skin tissue engineering. *Nano-micro letters*, 14(1), 1-46.