The Journal of Medical Research

Research Article

JMR 2024; 10(1):20-26
January- February
ISSN:2395-7565

www.medicinearticle.com
Received:27-12-2023
Accepted:26-02-2024
DOI: 10.31254/jmr.2024.10105

Anti-*Staphylococcus aureus* activity of *Lactobacillus rhamnosus* and *Lactobacillus acidophilus* on clinical isolates- an *in vitro* study

Ananthu Raj[1], Harish Kumar K S[2], Anju Anil[1], Azhar M M[1], Nithyamol Kuriakose[1]

[1] Department of Medical Microbiology, School of Medical Education, Centre for Professional and Advanced Studies, Kottayam, Kerala, India

[2] Professor and Head of the Department, Department of Medical Microbiology, School of Medical Education, Centre for Professional and Advanced Studies, Kottayam, Kerala, India

Abstract

Background: This study evaluated the *in vitro* antimicrobial and antagonistic activity of both neat and pH-adjusted whole-cell suspensions of *L. rhamnosus* and *L. acidophilus* towards *S. aureus*, as an alternative to control the spread of MRSA, which is endemic in India. Materials and Methods: The study was conducted at School of Medical Education, Kerala, India, between October 2022 and May 2023. Anti-*Staphylococcus aureus* activity of *Lactobacillus rhamnosus* MTCC 1408 and *Lactobacillus acidophilus* MTCC 10307 grown on MRS agar, was evaluated on a total of 76 clinical isolates of *S. aureus* from Kerala, India, by the Agar overlay method. The study involved a comparison of probiotic activities by both neat and pH-adjusted whole-cell suspensions of *L. rhamnosus* and *L. acidophilus*. Results: The study results indicate that the mean value of inhibition zones produced by *L. rhamnosus* was greater than that of *L. acidophilus*, and statistically significant data was obtained in which the pH-adjusted whole cell suspensions of *L. rhamnosus* exhibited larger inhibition zones than that of neat suspensions of the same. Also, the mean value of activity by pH-adjusted *L. acidophilus* suspension was slightly greater than its neat suspension. Conclusions: *L. rhamnosus* and *L. acidophilus* were found to possess *in vitro* anti-*Staphylococcus aureus* activity. The antimicrobial and antagonistic activity of pH-adjusted live suspensions suggests the possible use of *L. acidophilus* and *L. rhamnosus* as probiotic treatment options in sites other than the gastrointestinal tract, supporting their use as local administration and topical applications, in particular for the treatment and decolonization of *S. aureus*.

Keywords: Probiotics, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Staphylococcus aureus*/MRSA, Antimicrobial and antagonistic activity.

***Corresponding author:**
Dr. Harish Kumar K S
Professor and Head of the Department, Department of Medical Microbiology, School of Medical Education, Centre for Professional and Advanced Studies, Kottayam, Kerala, India
Email:
drharishkumarks@gmail.com

INTRODUCTION

Staphylococcus aureus is a Gram-positive commensal that colonizes the skin and mucosae of approximately 30% of the human population persistently and another 60% transiently. Although generally mild, antibiotic resistance amplifies the capacity of *S. aureus* to establish itself as a serious human pathogen, being the leading cause of endocarditis, bacteraemia, osteomyelitis and skin and soft tissue infections [1,2]. With the advent of hospital-based medicine, *S. aureus* quickly emerged as a leading cause of healthcare-associated infections [2]. Due to its high morbidity and mortality rates in conjunction with the ability to resist most antibiotics on the market, it was defined as a "superbug" [3]. MRSA strains express an additional penicillin binding protein (PBP), known as PBP2a, which has been hypothesized to have originated from *Staphylococcus sciuri* [4]. Methicillin resistance is mediated by the gene *mecA*, acquired by horizontal transfer of the mobile genetic element staphylococcal cassette chromosome *mec* (SCC*mec*), which manifests as resistance to virtually all β-lactams except the 5th generation cephalosporin β-lactams, ceftaroline and ceftobiprole [5-7]. Biofilm production capabilities of MRSA on biotic and abiotic surfaces also contributes to its antibiotic resistance and pervasiveness [3]. Antimicrobial agents used are ineffective against biofilm-forming bacteria, since they induce a selective pressure on the pathogens, triggering the development of resistance to certain agents [8]. Prolonged administration of antibiotics may result in microbial dysbiosis in the gut by promoting the selection of drug-resistant superbugs, which increases the risk of horizontal gene transfer and thereby turning the gut into a hub of multidrug-resistant genes [9]. As the current therapeutic approaches are not entirely effective, new complementary strategies should be prioritized, considering the increasing prevalence of MRSA strains.

Probiotics defined by WHO are, 'the live microorganisms which when administered in adequate amounts confer a health benefit on the host.' Probiotics can substantially improve the function of the immune, digestive, and respiratory systems and have a significant effect on the alleviation of infectious diseases in

children and other high-risk groups [10]. Lactic Acid Bacteria are generally members of human gastrointestinal, oral, and vaginal microbiota with probiotic activity, playing a beneficial role in the ecosystem with their probiotic spectrum of activity including nutritional, physiological, and antimicrobial effects [11]. Probiotic bacteria can produce various antimicrobial substances such as lactic acid, hydrogen peroxide, bacteriocins, and bacteriocin-like substances, which can directly inhibit pathogens. The indirect effects against pathogens can originate from competitive exclusion mechanisms with which the bacteria compete for essential nutrients or chemicals and passively occupy a niche previously occupied by a pathogen or by actively restricting the adhesion of pathogens to the surfaces [12]. These observations have led to the development of various foods and feeds containing LAB cells for probiotic use in man and animals [11].

Lactobacillus probiotic strains have been shown to possess inhibitory activity against the growth of pathogenic bacteria and positive effects on host health [13]. Lactobacilli are frequently selected as probiotics due to the expression of many crucial properties such as: high tolerance to acid and bile, capability to adhere to intestinal surfaces, ability to withstand low pH and gastric juice, antimicrobial activity, resistance to antibiotics, production of exopolysaccharides and removal of cholesterol. Lactobacilli such as *Lactobacillus acidophilus*, *L. casei*, *L. paracasei*, *L. rhamnosus*, *L. delbrueckii* subsp. *bulgaricus*, *L. brevis*, *L. johnsonii*, *L. plantarum* and *L. fermentum* are commonly used as probiotic products [14].

Lacticaseibacillus rhamnosus, formerly known as *Lactobacillus rhamnosus* [21], has been widely studied for its probiotic applications of which several strains are extensively used in food formulations, health, and functional foods as probiotics [15,16]. *L. rhamnosus* can survive and thrive through the acidic environment of the gastrointestinal tract while adhering to the intestinal epithelial cells and displays an excellent mucus-adhering *property* compared to related *Lactobacillus* strains [17]. *L. acidophilus* has better resistance to both acid and bile salt in comparison with many other probiotics, which enables the survival and proliferation of *L. acidophilus* in the harsh environment of the gastrointestinal tract, providing further opportunities for its products to effectively function within the human body. *L. acidophilus* has multiple effects on the human body, including nutritional effects, regulation of intestinal flora balance, enhancement of immunity, age-delaying and anti-cancer effects, and support of cholesterol reduction [18,19].

MATERIALS AND METHODS

Microorganisms and growth conditions

The present cross-sectional study was conducted at School of Medical Education, Kerala, India, between October 2022, and May 2023. Clinical isolates of *S. aureus*, a total of 76 were collected from diagnostic microbiology laboratories in central Kerala, India. *S. aureus* isolates obtained from clinical samples were sub-cultured onto Mueller Hinton Agar (MHA), identified using Gram staining, and biochemical tests and confirmed by performing a coagulase test. Two probiotic strains of lactobacilli; *Lactobacillus rhamnosus* MTCC 1408 and *Lactobacillus acidophilus* MTCC 10307, were grown on MRS (deMan, Rogosa and Sharpe agar) (Hi-Media Labs) medium by incubating at 37°C for 48 hours, anaerobically.

Agar overlay method

Detection of antimicrobial and antagonistic activities indicating the probiotic activity of *L. rhamnosus* and *L. acidophilus* on *S. aureus* was performed by the Agar overlay method as described by Fleming et al., with modifications [20]. MRS agar was spot inoculated with 5µL neat overnight culture of *L. rhamnosus* and *L. acidophilus* in BHI broth. A part of the overnight culture of *Lactobacillus spp.* was adjusted to 6.5–7.0 pH using 1N NaOH and also was spot inoculated on MRS agar. The plates were incubated overnight at 37˚C in the presence of 5 – 10% CO_2. Post incubation, growth of both neat and pH-adjusted *L. rhamnosus* and *L. acidophilus* appear on the surface of MRS medium as visible spots. Clinical isolates of *S. aureus* to be tested were transferred to BHI broth and incubated overnight at 37°C. MRS agar with the spot culture of *L. rhamnosus* and *L. acidophilus* was further overlaid with 7mL of molten BHI soft agar (0.75%) cooled to 40-45˚C, which was seeded with 100µL (corresponding to approximately 7 log CFU) of *S. aureus* isolates in BHI broth. After overnight incubation at 37˚C, inhibition zones were formed around *Lactobacillus* spots (Figure: 1) which were diametrically measured in millimetres and interpreted as described by Shokryazdan et al. with modifications [21]. The interpretation criteria are as follows: diameter of zone of inhibition ≥ 12mm: strong inhibition, 8-11mm: intermediate, 4-7mm: weak and < 4mm: no inhibition.

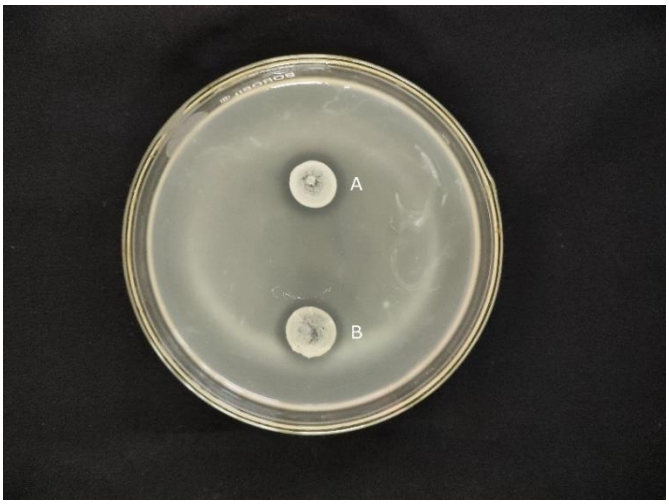

Figure 1: Antimicrobial and antagonistic activity of *L. rhamnosus* (A) and *L. acidophilus* (B) on *S. aureus*

Antimicrobial susceptibility testing of *S. aureus*

Antibiotic susceptibility profiles of *S. aureus* isolates were determined by the Kirby-Bauer disk diffusion method as prescribed by CLSI M02, performed on Mueller Hinton Agar and analysed according to interpretive standards of CLSI M100-S33 and were classified into MRSA and MSSA [22,23]. Antimicrobials used were; Cefoxitin (30µg), Mupirocin (200µg), Gentamicin (10 µg), Erythromycin (15 µg), Tetracycline (30 µg), Clindamycin (2 µg), Linezolid (30 µg), Ciprofloxacin (5µg), Quinupristin-dalfopristin (15µg), Cotrimoxazole (Trimethoprin sulfamethaxazole) (1.25/23.75µg).

RESULTS

Antimicrobial and antagonistic activity of *L. rhamnosus*

L. rhamnosus neat suspension had strong antimicrobial and antagonistic activity against 13.15% (n=10), intermediate and weak activities against 84.21% (n=64) and 2.63% (n=2) of *S. aureus* isolates respectively. Strong activity was found against 32.89% (n=25) isolates with the pH-adjusted suspension. Intermediate and weak activities turned out to be towards 65.78% (n=50) and 1.31% (n=1) isolates respectively. The mean value for neat suspensions of *L. rhamnosus* was 10.31 and that of pH-adjusted suspensions of *L. rhamnosus* was 10.92. The combined values of both neat and pH-adjusted suspensions showed an intermediate action against *S. aureus* with a mean value of 10.61. ANOVA Single Factor analysis (Table: 1) turned out to be significant for the antimicrobial and antagonistic activity of *L. rhamnosus* neat and pH-adjusted suspensions on *S. aureus*, as the *p-value* was <*0.05*. Therefore, pH-adjusted suspensions of *L. rhamnosus* have shown significantly higher probiotic activity than that of neat suspension.

Antimicrobial and antagonistic activity of *L. acidophilus*

The neat suspension of *L. acidophilus* showed strong activity against 3.94% (n=3) of *S. aureus* isolates(n=76) tested, intermediate activity towards 85.52% (n=65) and weak activity towards 10.52% (n=8) of the total isolates. pH-adjusted whole cell suspensions of *L. acidophilus* demonstrated strong activity against 7.89% (n=6), intermediate activity against 82.89% (n=65) and weak activity towards 13.15% (n=5) of the *S. aureus* isolates. The mean value for neat suspensions of *L. acidophilus* was 9.16 and that of pH-adjusted whole-cell suspensions of *L. acidophilus* was 9.52. The combined values of both neat pH-adjusted suspensions had a mean value of 9.34, with an intermediate action against *S. aureus.* Antimicrobial and antagonistic activity of *L. acidophilus* neat and pH-adjusted suspensions on *S. aureus* was analysed using the ANOVA single factor method (Table: 2) and was found to be not significant as the *p-value* was *>0.05*. Thus, neat and pH-adjusted suspensions of *L. acidophilus* did not show any difference in the probiotic activity.

Effect of neat suspensions of *L. rhamnosus* and *L. acidophilus*

The neat suspensions of both *L. rhamnosus* and *L. acidophilus* exhibited an overall intermediate activity against *S. aureus* isolates with a mean value of 10.31 by *L. rhamnosus* and 9.16 by *L. acidophilus*. *L. rhamnosus* neat suspension had strong activity against 13.15%, intermediate and weak activities against 84.21% and 2.63% of *S. aureus* isolates respectively. *L. acidophilus* has shown strong activity against 3.94% of *S. aureus* isolates tested, intermediate activity towards 85.52% and weak activity towards 10.52% of the total isolates tested. Strong inhibitions were found to be produced by *L. rhamnosus* neat suspensions (13.15%) than *L. acidophilus* (3.94%). The mean value of inhibition by *L. rhamnosus* neat suspensions (10.31) was also higher than *L. acidophilus* (9.16). The activity by neat suspensions of *L. rhamnosus* and *L. acidophilus* on *S. aureus* was analysed using ANOVA Single Factor method (Table: 3) and was found to be not significant as the *p-value* was *>0.05*. Thus, neat suspensions of *L. acidophilus and L. rhamnosus* didn't show any difference in their probiotic activity.

Effect of pH-adjusted suspensions of *L. rhamnosus* and *L. acidophilus*

L. rhamnosus and *L. acidophilus* pH-adjusted suspensions have shown an overall intermediate activity against *S. aureus* isolates with a mean value of 10.92 and 9.52 by *L. rhamnosus* and *L. acidophilus* respectively. Strong activity was found against 32.89% of isolates with the pH-adjusted suspension of *L. rhamnosus*, and intermediate and weak activities turned out to be towards 65.78% and 1.31% of isolates respectively. pH-adjusted cell suspensions of *L. acidophilus* demonstrated strong activity against 7.89%, intermediate activity against 85.52% and weak activity towards 6.57% of the *S. aureus* isolates. ANOVA Single Factor analysis (Table: 4) was found to be not significant for the antimicrobial and antagonistic activity of pH-adjusted suspensions of *L. rhamnosus* and *L. acidophilus* on *S. aureus*, as the *p-value* was *>0.05*. Thus, pH-adjusted suspensions of *L. rhamnosus* and *L. acidophilus* did not show any difference in their probiotic activity.

The total probiotic activity of *L. rhamnosus and L. acidophilus* on *S. aureus* was analysed using the ANOVA Single Factor method (Table: 5) and was found to be not significant as the *p-value* was *>0.05*. Therefore, *L. rhamnosus and L. acidophilus* did not show any difference in their probiotic activity despite having a difference in their mean values (Figure: 2).

Antimicrobial susceptibility testing of *S. aureus*

56.53% of *S. aureus* isolates were resistant to Cefoxitin and therefore considered as MRSA. All isolates were found to be sensitive to Gentamicin, Tetracycline, Linezolid and Cotrimoxazole. 45.6% of isolates have shown Inducible Clindamycin resistance by D zone formation and High-level Mupirocin resistance was found in 15.21% of isolates (Figure:3).

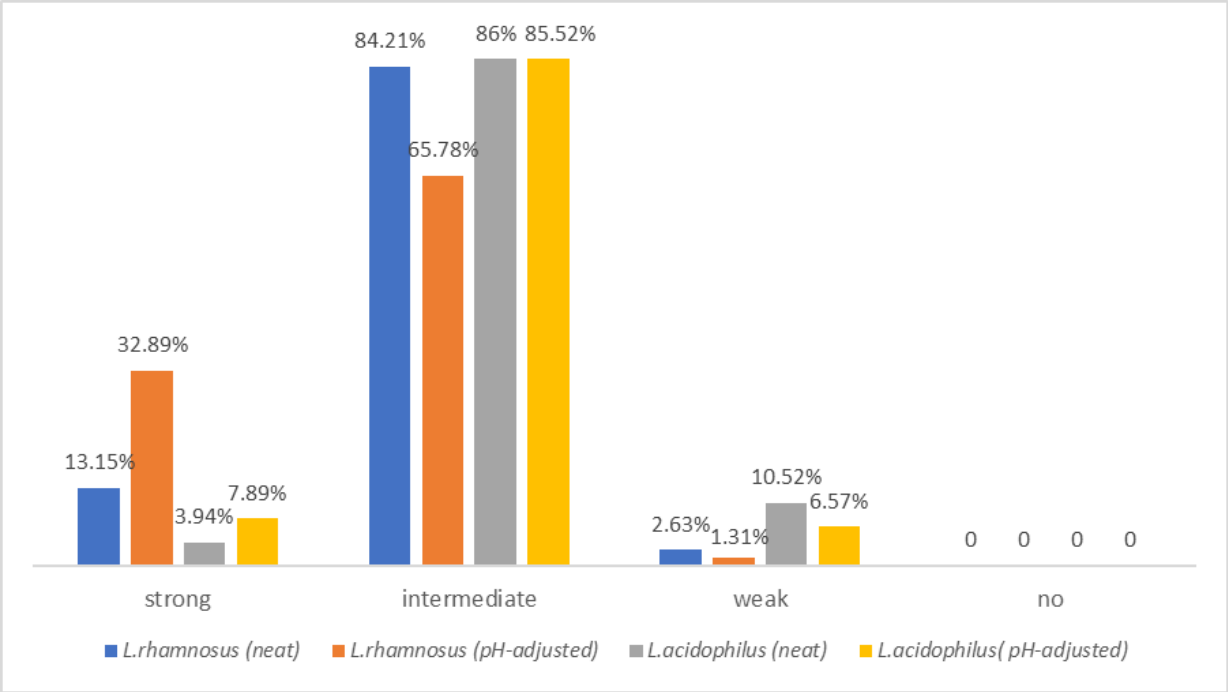

Figure 2: Graphical representation of Antimicrobial and antagonistic activity of *L. rhamnosus and L. acidophilus* on *S. aureus*.

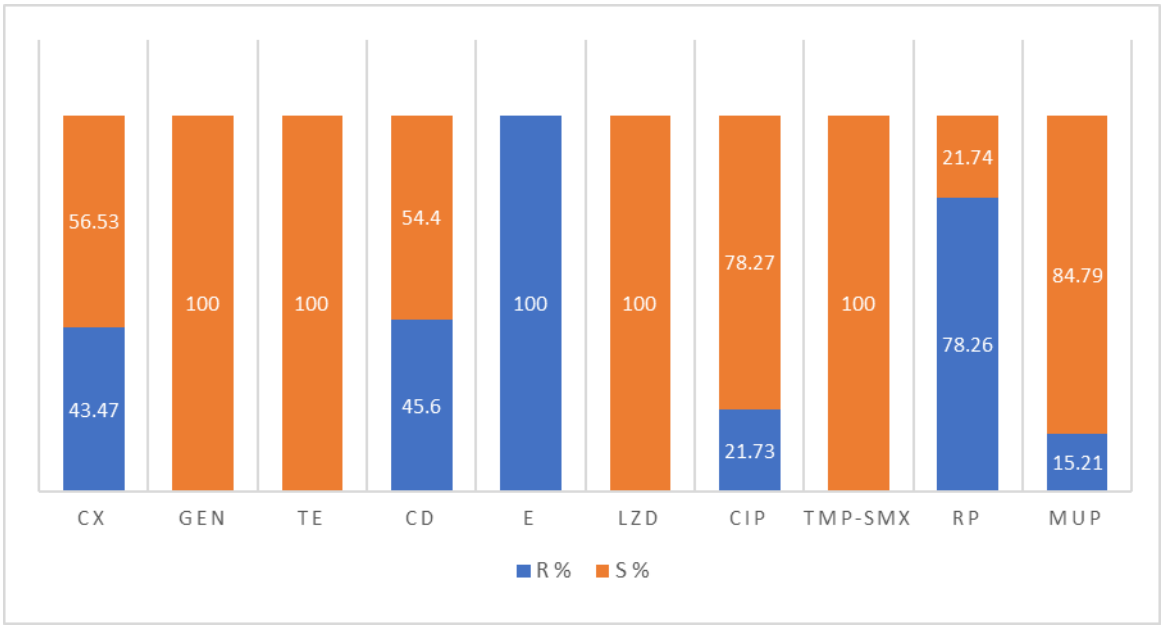

Figure 2: Graphical representation of antimicrobial susceptibility pattern of *S. aureus*

Table 1: ANOVA Single Factor analysis of probiotic activity by *L. rhamnosus* on *S. aureus*

ANOVA: Single Factor	***L. rhamnosus***					
Groups	*Count*	*Sum*	*Average*	*Variance*		STD ERROR
L. rhamnosus NEAT	76	784	10.31579	2.1972807		0.1700341
L. rhamnosus pH-ADJUSTED	76	830	10.92105	3.09368421		0.2017582
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	13.92105	1	13.92105	5.26219805	0.023182	3.9042019
Within Groups	396.8224	150	2.645482			
Total	410.7434	151				

Table 2: ANOVA Single Factor analysis of probiotic activity by *L. acidophilus* on *S. aureus*

ANOVA: Single Factor	***L. acidophilus***					
Groups	*Count*	*Sum*	*Average*	*Variance*		STD ERROR
L. acidophilus NEAT	76	696.25	9.161184	1.98783991		0.1617275
L. acidophilus pH-ADJUSTED	76	724	9.526316	2.28596491		0.1734315
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.066201	1	5.066201	2.37081517	0.125729	3.9042019
Within Groups	320.5354	150	2.136902			
Total	325.6016	151				

Table 3: ANOVA Single Factor analysis of probiotic activity by neat suspensions of *L. rhamnosus* and *L. acidophilus* on *S. aureus*

ANOVA: Single Factor	**NEAT**					
Groups	*Count*	*Sum*	*Average*	*Variance*		STD ERROR
L. acidophilus NEAT	76	696.25	9.161184	1.98783991		0.1617275
L. rhamnosus NEAT	76	784	10.31579	2.1972807		0.1700341

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	50.65831	1	50.65831	24.2087675	2.25E-06	3.9042019
Within Groups	313.884	150	2.09256			
Total	364.5424	151				

Table4: ANOVA Single Factor analysis of probiotic activity by pH-adjusted suspensions of *L. rhamnosus and L. acidophilus* on *S. aureus*

ANOVA: Single Factor	**pH-adjusted**					
Groups	*Count*	*Sum*	*Average*	*Variance*		STD ERROR
L. acidophilus pH-adjusted	76	724	9.526316	2.28596491		0.1734315
L. rhamnosus pH-adjusted	76	830	10.92105	3.09368421		0.2017582
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	73.92105	1	73.92105	27.4817375	5.3E-07	3.9042019
Within Groups	403.4737	150	2.689825			
Total	477.3947	151				

Table 5: ANOVA Single Factor analysis of total probiotic activity *L. rhamnosus and L. acidophilus* on *S. aureus*

ANOVA: Single Factor	**TOTAL MEAN**					
Groups	*Count*	*Sum*	*Average*	*Variance*		STD ERROR
L. acidophilus TOTAL	76	710.125	9.34375	1.48296875		0.139688
L. rhamnosus TOTAL	76	807	10.61842	2.03537281		0.1636497
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	61.74188	1	61.74188	35.0971491	2.06E-08	3.9042019
Within Groups	263.8756	150	1.759171			
Total	325.6175	151				

DISCUSSION

S. aureus is a major pathogen in nosocomial and community-acquired infections, and it poses a significant threat due to its high morbidity and mortality. *S. aureus* exhibits distinct antibiotic resistance mechanisms, including resistance to methicillin, a frequently prescribed antibiotic, which makes infection management difficult. *mecA* gene present in the mobile segments of the MRSA strains encodes for penicillin-binding protein 2a which has reduced affinity for β-lactam, enabling the survival of MRSA strains in the presence of β-lactam antibiotics. MRSA is endemic in India and the antimicrobial susceptibility patterns vary according to geographical region [24]. As strategies to control MRSA spread have already been proposed, natural compounds are also being investigated as an alternative treatment for these infections [25]. Lactobacilli strains may be considered as potential probiotic candidates to treat *S.aureus*, especially with antibiotic-resistant strains [26]. Many strains of lactobacilli isolated from a variety of sources have shown inhibition of the growth of *S. aureus* and clinical isolates of MRSA *in vitro*. *S. aureus* growth inhibition could be either or both by competition for adhesion/attachment sites, nutrients, and secretion of inhibitory substances [27].

There are many studies which evaluated the probiotic activity of *Lactobacillus spp.* against human pathogenic organisms. Most studies to our knowledge relied on natural or commercial sources for obtaining the test strains of *Lactobacillus spp.* This study evaluated the *in vitro* antimicrobial and antagonistic activity of both the neat and pH-adjusted whole-cell suspensions of *L. rhamnosus and L. acidophilus* towards *S. aureus*. From our comparative study, the mean value of inhibition zones produced by *L. rhamnosus* turned out to be higher than that of *L. acidophilus*, but the results were found to be statistically insignificant. Bhola and Bhadekar in their study, also reported a slightly higher growth inhibition zone of *S. aureus* by whole broth of *L. casei var rhamnosus* as compared to *L. acidophilus* [28]. Growth inhibition of *S. aureus* by agar spot test performed by Tejero-Sariñena S. et al. exhibited a zone of inhibition between 11 and 17 mm at 24 h for both *L. acidophilus* and *L. rhamnosus*, a slightly higher value than our research [29]. This may be attributed to a higher concentration of *S. aureus* isolates overlaid in our study. Study conducted by Kaur and Sharma using *L. acidophilus* showed strong inhibition zones in 16% of *S. aureus*, moderate zones in 33% and no inhibition zone in 50% of *S.aureus*, whereas the overall better activity in our study (Strong-3.94%, intermediate-85.52%, weak-10.52%, no inhibition-0) by *L. acidophilus* could be attributed to the use of standard strain instead of strains from fermented dairy products by Kaur and Sharma [30]. Barbara et al. in their study found a higher anti-MRSA activity by *L. acidophilus* with the extent of inhibition varying from 17-29 mm, analysed by themselves as improved activity of lactobacilli in milk-based medium [31].

Several researchers have studied the effect of pH on cell-free supernatants of *Lactobacillus spp.* in which mostly, pH-adjusted cell-free supernatants had lesser activity than neat suspensions attributing to the action of organic acids. It is known that lactobacilli survive a highly acidic gastric environment and exhibit probiotic activity. Our approach to this study was also to find out whether live lactobacilli possess probiotic activity in environments other than acidic conditions. Results of this study showed statistically significant data in which the pH-adjusted whole cell suspensions of *L. rhamnosus* exhibited larger inhibition zones than that of neat suspensions of the same. *Lactobacillus spp.* shows higher proteolytic activity at pH 7, by extracellular proteolytic enzyme production, also involving various types of exopeptidases with aminopeptidase, dipeptidase, tripeptidase, and carboxypeptidase activities, as well as a wide range of extracellular hydrolases [32,33]. The antimicrobial and antagonistic activity of pH-adjusted suspensions suggests the probiotic activity of *L. rhamnosus and L. acidophilus* in non-acidic environment, apart from its good activity in the acidic environment. In patients with Bacterial vaginosis, where vaginal pH rises due to dysbiosis in vaginal microflora, vaginal administration of *Lactobacillus spp.* is in practice, which according to our study is beneficial. Our finding also supports the use of *L. rhamnosus and L. acidophilus* as topical application on the skin, where they produce antimicrobial peptides that benefit cutaneous immune responses and eliminate pathogens, in particular for the treatment and decolonisation of *S. aureus* [34].

According to various research, the incidence of MRSA ranges from 21% to 45% among the Indian population and above 50% in the state of Kerala, which coincides with our results and findings of this study could possibly benefit the treatment and decolonisation of MRSA [35,36]. The antimicrobial and antagonistic activity of *lactobacillus spp.* can be due to many factors such as the production of organic acids, hydrogen peroxide, diacetyl, low molecular weight antimicrobial substances, bacteriocin etc. [29]. Our study could not attribute the activity between these modes of action, which turns out to be a possible limitation of our study. In conclusion, both *L. rhamnosus and L. acidophilus* possess anti-*Staphylococcus aureus* activity *in-vitro*, suggesting the use of the same in the probiotic treatment of staphylococcal infections and MRSA colonisation, avoiding the use of antimicrobials and thereby limiting the widespread of antibiotic resistance in *S. aureus*.

CONCLUSION

The current *in vitro* study shows anti-*Staphylococcus aureus* activity of *L. rhamnosus and L. acidophilus* suggesting their possible use as probiotic treatment options in the gastrointestinal tract as well as sites other than the GIT, with non-acidic environment. Our result supports the practice of local administration of *Lactobacillus spp.* in patients with Bacterial vaginosis, and the use as a topical application on the skin which will be impactful for the treatment and decolonisation of *S. aureus*.

Acknowledgments

Authors would like to thank Mrs. Rajumol B. Zacharia for her technical assistance.

Conflict of Interest

The authors declare no conflict of interest.

Funding

No external funding was received

ORCID ID

Harish Kumar K S: https://orcid.org/0000-0002-0504-7794

Ananthu Raj: https://orcid.org/0009-0006-8313-0768

Anju Anil: https://orcid.org/0009-0006-8634-2440

Nithyamol Kuriakose: https://orcid.org/0009-0005-6014-3980

Azhar M M: https://orcid.org/0009-0009-2332-3711

REFERENCES

1. Craft KM, Nguyen JM, Berg LJ, Townsend SD. Methicillin-resistant *Staphylococcus aureus* (MRSA): antibiotic-resistance and the biofilm phenotype. Medchemcomm. 2019;10(8):1231-1241.
2. Turner NA, Sharma-Kuinkel BK, Maskarinec SA, Eichenberger EM, Shah PP, Carugati M, Holland TL et al. Methicillin-resistant *Staphylococcus aureus*: an overview of basic and clinical research. Nat Rev Microbiol. 2019;17(4):203-218.
3. Cascioferro S, Carbone D, Parrino B, Pecoraro C, Giovannetti E, Cirrincione G et al. Therapeutic Strategies To Counteract Antibiotic Resistance in MRSA Biofilm-Associated Infections. Chem Med Chem. 2021;16(1):65-80.
4. Stapleton PD, Taylor PW. Methicillin resistance in *Staphylococcus aureus*: mechanisms and modulation. Sci Prog. 2002;85:57-72.
5. Katayama Y, Ito T, Hiramatsu K. A new class of genetic element, staphylococcus cassette chromosome mec, encodes methicillin resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother. 2000;44(6):1549-55.
6. Sharon J. Peacock and Gavin K. Paterson. Mechanisms of Methicillin Resistance in *Staphylococcus aureus*. Annual Review of Biochemistry 2015;84:1, 577-601
7. Saravolatz LD, Stein GE, Johnson LB. Ceftaroline: a novel cephalosporin with activity against methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis. 2011;52(9):1156-63.
8. Barzegari A, Kheyrolahzadeh K, Hosseiniyan Khatibi SM, Sharifi S, Memar MY, Zununi Vahed S. The Battle of Probiotics and Their Derivatives Against Biofilms. Infect Drug Resist. 2020;13:659-672.
9. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy D. Role of the normal gut microbiota. World J Gastroenterol. 2015;21(29):8787-803.
10. Food and Agriculture Organization and World Health Organization Expert Consultation. Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria. Córdoba, Argentina: Food and Agriculture Organization of the United Nations and World Health Organization; 2001.
11. A. S. Naidu, W. R. Bidlack & R. A. Clemens. Probiotic Spectra of Lactic Acid Bacteria (LAB), Critical Reviews in Food Science and Nutrition, 1999: 39:1, 13-126
12. Laleman I, Teughels W. Probiotics in the dental practice: a review. Quintessence Int. 2015;46(3):255-64.
13. Santos A, San Mauro M, Sanchez A, Torres JM, Marquina D. The antimicrobial properties of different strains of Lactobacillus spp. isolated from kefir. Syst Appl Microbiol. 2003;26(3):434-7.
14. Fijan S. Microorganisms with claimed probiotic properties: an overview of recent literature. Int J Environ Res Public Health. 2014;11(5):4745-67.
15. Zheng J, Wittouck S, Salvetti E, Franz CMAP, Harris HMB, Mattarelli P et al. A taxonomic note on the genus Lactobacillus: Description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. Int J Syst Evol Microbiol. 2020;70(4):2782-2858.
16. Kalliomäki M., Salminen S., Arvilommi H., Kero P., Koskinen P., Isolauri E. Probiotics in Primary Prevention of Atopic Disease: A Randomised Placebo-Controlled Trial. Lancet. 2001;357:1076–1079.
17. Mathipa-Mdakane MG, Thantsha MS. Lacticaseibacillus rhamnosus: A Suitable Candidate for the Construction of Novel Bioengineered Probiotic Strains for Targeted Pathogen Control. Foods. 2022;11(6):785.

18. Mital BK, Garg SK. Anticarcinogenic, hypocholesterolemic, and antagonistic activities of Lactobacillus acidophilus. Crit. Rev. Microbiol. 1995;21:175–214.
19. Saxelin M, Tynkkynen S, Mattila-Sandholm T, de Vos WM. Probiotic and other functional microbes: from markets to mechanisms. Curr. Opin. Biotechnol. 2005;16:204–211.
20. Fleming HP, Etchells JL, Costilow RN. Microbial inhibition by an isolate of pediococcus from cucumber brines. Appl Microbiol. 1975;30(6):1040-2.
21. Shokryazdan P, Sieo CC, Kalavathy R, Liang JB, Alitheen NB, Faseleh Jahromi M et al. Probiotic potential of Lactobacillus strains with antimicrobial activity against some human pathogenic strains. Biomed Res Int. 2014;2014:927268.
22. CLSI. Performance standards for antimicrobial disk susceptibility tests. 13th ed. CLSI standard M02.Wayne PA: Clinical and laboratory standards institute; 2018.
23. CLSI. Performance standards for antimicrobial disk susceptibility testing 33rd ed.CLSI supplement M100.Clinical and Laboratory Standards Institute; 2023
24. Patil SS, Suresh KP, Shinduja R, Amachawadi RG, Chandrashekar S, Pradeep S et al. Prevalence of Methicillin-resistant *Staphylococcus aureus* in India: A Systematic Review and Meta-analysis. Oman Med J. 2022;37(4):e440.
25. Muralinath E, Parasa L. *In vitro* investigation on antibacterial activity of selected probiotics against superbug of methicillin resistant *Staphylococcus aureus* (MRSA) isolated from hospital populations. World Journal of Pharmacy and Pharmaceutical Sciences 2015;4(6) 430-445.
26. Hafez MM, Maghrabi IA, Zaki NM. Toward an alternative therapeutic approach for skin infections: antagonistic activity of lactobacilli against antibiotic-resistant Staphylococcus aureus and Pseudomonas aeruginosa. Probiotics and antimicrobial proteins. 2013;5:216-26.
27. Sikorska H, Smoragiewicz W. Role of probiotics in the prevention and treatment of meticillin-resistant *Staphylococcus aureus* infections. Int J Antimicrob Agents. 2013;42(6):475-81.
28. Bhola J, Bhadekar R. *In vitro* synergistic activity of lactic acid bacteria against multi-drug resistant staphylococci. BMC Complement Altern Med. 2019;19(1):70.
29. Tejero-Sariñena S, Barlow J, Costabile A, Gibson GR, Rowland I. In vitro evaluation of the antimicrobial activity of a range of probiotics against pathogens: evidence for the effects of organic acids. Anaerobe. 2012;18(5):530-8.
30. Kaur S, Sharma M. Inhibitory potential of Lactobacillus species isolated from fermented dairy. J of Gastro Infs. 2013;3.1
31. Karska-Wysocki B, Bazo M, Smoragiewicz W. Antibacterial activity of Lactobacillus acidophilus and Lactobacillus casei against methicillin-resistant *Staphylococcus aureus* (MRSA). Microbiol Res. 2010;165(8):674-86
32. Novik GI, Samartsev AA, Astapovich NI, Kavrus MA, Mikhalyuk AN. Biological activity of probiotic microorganisms. Applied Biochemistry and Microbiology. 2006;42:166-72.
33. Minagawa E, Kaminogawa S, Tsukasaki F, Motoshima H, Yamauchi K. Exopeptidase profiles of bifidobacteria. Journal of Nutritional Science and Vitaminology. 1985;31(6):599-606.
34. Al-Ghazzewi FH, Tester RF. Impact of prebiotics and probiotics on skin health. Benef Microbes. 2014;5(2):99-107.
35. Rathinam KK, Immanuel JM. Prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in India: A Systematic Review and Meta-Analysis. https://ssrn.com/abstract=4100442
36. Kaniyarakkal V, Variamkandi SB, George K. Microbiological profile of significant multi-drug resistant organisms isolated in a tertiary care centre, Kerala, India. International Journal of Research and Review. 2021; 8(5):404-11.