International Journal of Research in Medical Sciences
Anil A et al. Int J Res Med Sci. 2024 Apr;12(4):1140-1146
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20240834

Original Research Article

Evaluation of antimicrobial activity of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* against clinically isolated *Klebsiella pneumoniae*: an *in vitro* study

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Received: 27 January 2024
Revised: 01 March 2024
Accepted: 06 March 2024

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ABSTRACT

Background: *Klebsiella pneumoniae* are Gram-negative opportunistic pathogen, belonging to the family *Enterobacteriaceae* that can cause severe nosocomial infections particularly in immuno-compromised individuals. They exhibit co-resistance to multiple antibiotics which emphasize the need for non-antibiotic therapies. The goal of the presented study was to investigate the antimicrobial ability of probiotic Lactobacilli on clinical isolates of *K. pneumoniae*.

Methods: In this cross-sectional study, antimicrobial activities of probiotic *L. acidophilus* and *L. rhamnosus* on *K. pneumoniae* were evaluated by Agar overlay interference technique. Clear zone around Lactobacilli were taken as positive inhibition. Antibiotic susceptibility profiles of *K. pneumoniae* were determined by Kirby-Bauer disk diffusion method, analyzed using interpretive standards of CLSI M100-S33 and categorized into MDR, XDR and Non MDR groups. Statistical analysis was done using descriptive statistics such as mean and standard error and inferential statistics such as ANOVA single factor.

Results: *K. pneumoniae* exhibited positive inhibition with both the probiotic strain. On comparing the zone of inhibition of *L. acidophilus* and *L. rhamnosus* (both treated-pH adjusted and untreated), *L. acidophilus* had greater zone of inhibition against *K. pneumoniae* but concluded that statistically the values are insignificant ($p>0.05$). Based on antibiotic susceptibility pattern of *K. pneumoniae*, 63% of isolates were XDR, 3% were MDR and 34% were Non MDR

Conclusions: It can be concluded that *L. acidophilus* and *L. rhamnosus* had significant inhibitory effect against *K. pneumoniae in vitro* and should be further studied for their human health benefit.

Keywords: Probiotic, Lactobacilli, *Klebsiella pneumoniae*, Agar overlay interference technique

INTRODUCTION

Recently recognized as a significant threat to global public health, *Klebsiella pneumoniae* is an opportunistic gram-negative bacterial pathogen.[1] Colonizing various body sites, including the human gut, *Klebsiella pneumoniae* remains embedded, forming biofilm that shields it from antibiotic action, thereby causing infections that are more challenging to treat.[2] Well-known for their ability to develop and transfer antibiotic resistance determinants, such as the production of extended-spectrum β-lactamase (ESBL), these pathogens confer resistance to β-lactam antibiotics.[3] Worldwide, the critical increase in the spread of ESBL-producing gram-negative bacilli poses one of the most growing problems, limiting treatment options significantly for clinicians.[4]

The major mechanisms conferring antibiotic resistance to *K. pneumoniae* include enzymatic inactivation of the drug, alteration of drug targets, drug efflux, biofilm formation, and reduced permeability due to porin loss or modification.[5] For combating antibiotic resistance in *K. pneumoniae* infections, several therapies are currently being developed, among them probiotic therapy is gaining importance.

The World Health Organization defined probiotics as "*Live microorganisms which, when administered in adequate amounts, confer a health benefit on the host*".[6] Commonly used microorganisms as probiotics are Lactic acid bacteria especially *Lactobacillus* and *Bifidobacterium*. The lactobacilli, which are generally recognize as safe (GRAS), are the prime members of the intestinal microbiota of humans, and had already been reported as alternative to antibiotics as a promising candidate to compete with the harmful bacteria.[7] They produce antimicrobial metabolites like organic acids that prevent the rise of pathogenic bacteria. Clinical trials have demonstrated the effectiveness of probiotics against a wide range of pathological conditions, including diarrhoea, constipation, ulcerative colitis, polycystic ovary syndrome, stress and anxiety, inflammatory bowel disease, breast cancer, and diabetes.[8]

The use of probiotic therapy to eliminate pathogenic organisms are increasing globally. The need of such new therapies is important in the treatment of health care-associated infections caused by antibiotic resistant (MDR: multidrug resistant; XDR: extensively drug-resistant; PDR: pan-drug resistant) bacteria.[9] The lactobacilli have extensively been studied for their remarkable capacity to inhibit the growth of other microorganisms through various mechanisms.[10,11] As drug resistant strains of *K. pneumoniae* are increasing at an alarming rate, antimicrobial susceptibility pattern was also determined to identify the resistant strains and to investigate whether probiotics have any action on the same. The present study investigates the antimicrobial activity of *L. acidophilus* and *L. rhamnosus* (now renamed as *Lacticaseibacillus rhamnosus)* on *Klebsiella pneumoniae* by Agar overlay interference technique.

METHODS

The present cross-sectional study was conducted at school of medical education (SME), Centre for professional and advanced studies, Kottayam, Kerala between January 2023 and September 2023. 100 clinical isolates of *K. pneumoniae* were collected from various diagnostic microbiology laboratories in Gandhinagar, Kottayam.

Inclusion and exclusion criteria

K. pneumoniae isolates showing pure growth in clinical samples were only included. Patients who have undergone any antimicrobial therapy in the past three months was excluded from the study.

Bacterial stains for the study

Lactobacillus acidophilus MTCC 10307 and *Lactobacillus rhamnosus* MTCC 1408 were the bacterial strains used in the study that were procured from institute of microbial technology (IMTECH), Chandigarh.

Microbial growth media

De Man, Rogosa and Sharpe Agar (MRS), Brain heart infusion broth (BHIB) and Mueller Hinton agar (MHA) was purchased from HiMedia Laboratories.

Antibiotic disc used

Gentamicin (10µg), Tetracycline (30µg), Ciprofloxacin (5µg), Imipenem (10µg), Cefuroxime (30µg), Cefoxitin (30µg), Aztreonam (30µg), Cefixime (5µg), Ceftazidime (30µg), Cefotaxime (30µg), Amikacin (30µg).

Microbiological methods

Isolates of *K. pneumoniae* were collected from various diagnostic laboratories in Gandhinagar, Kottayam, Kerala. These isolates were reconfirmed by subculturing on to MacConkey agar, followed by Gram staining and routine biochemical tests.

Antibiotic susceptibility test

Antibiotic susceptibility profiles of the *K. pneumoniae* were determined by the Kirby-Bauer disk diffusion as prescribed by CLSI M02-A13[12] conditions and analyzed using interpretive standards of CLSI M100-S33 and are categorized into MDR, XDR and Non MDR groups based on CDC/ECDC guidelines.[13,14]

Agar-overlay interference method

Probiotic activity was detected using Agar overlay method described by Fleming et al with minor modifications. Briefly, a layer of MRS agar was prepared and allowed to solidify.[15] Both *L. acidophilus* and *L. rhamnosus* were inoculated to BHI broth and incubated at 37°C overnight. Similarly, *K. pneumoniae* isolates were incubated in BHI broth at 37°C for 24 hours. Then, the surface of MRS agar was spot inoculated with 5µl of an overnight culture of *L. acidophilus* and *L. rhamnosus* (untreate), each was done in duplicate and mean was calculated. Also, the overnight culture of *Lactobacillus spp.* was adjusted to 6.5-7.0 pH using 1N NaOH (treated) and were spot inoculated on MRS agar. Then the plates were incubated for 24 hr at 37˚C in 5-10% CO_2. After incubation visible spot appears on the surface of the MRS medium. Then the MRS agar plates with *Lactobacillus* spots were thereafter overlaid with 7ml of molten BHI soft agar (0.75%) cooled to 40-45˚C, which was seeded with 100µL of *K. pneumoniae* to be tested. After 24 hours

of incubation at 37˚C in 5-10% CO_2, the inhibition zones around *Lactobacillus* spots were diametrically measured and expressed in millimeters and interpreted following Shokryazdan et al with modifications.[16] The zone of inhibition diameter ≥12 mm, 8-11 mm, 4-7 mm and <4 mm were considered as strong, intermediate, weak and no inhibition respectively.

Data analysis

The data was analyzed using Microsoft Excel 2019. Descriptive statistics such as mean and standard error and inferential statistics such as ANOVA single-factor were employed in the present study, p value <0.05 was considered statistically significant.

RESULTS

During the study, 100 *K. pneumoniae* were obtained from various samples-urine (N=66), sputum (N=39), and blood (N=5). Of the 100 samples 56% (N=56) were obtained from females and 44% (N=44) from males as depicted in (Figure 1).

Antibiotic susceptibility

Antibiotic Susceptibility pattern of *K. pneumoniae* were calculated by measuring zone of inhibition around the respective antibiotic disc (Figure 2). Based on the susceptibility pattern, 63% of isolates were XDR, 3% were MDR and 34% were Non MDR.

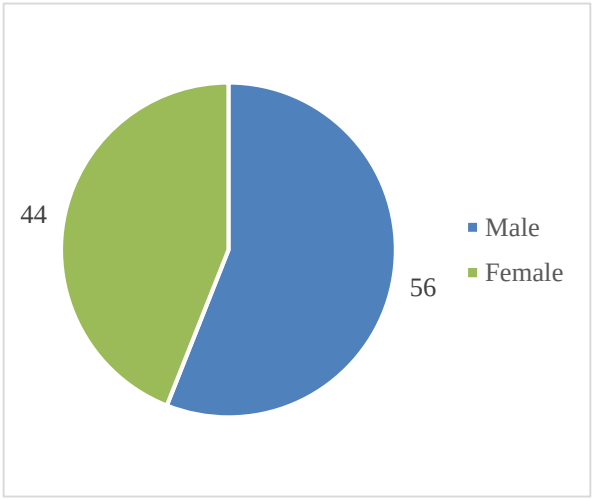

Figure 1: Distribution of clinical isolates of *K. pneumoniae.*

Growth inhibition of K. pneumoniae

K. pneumoniae was inhibited by *L. acidophilus* and *L. rhamnosus* (both treated and untreated) as visible zone of inhibition using agar overlay interference technique (Figure 3-4).

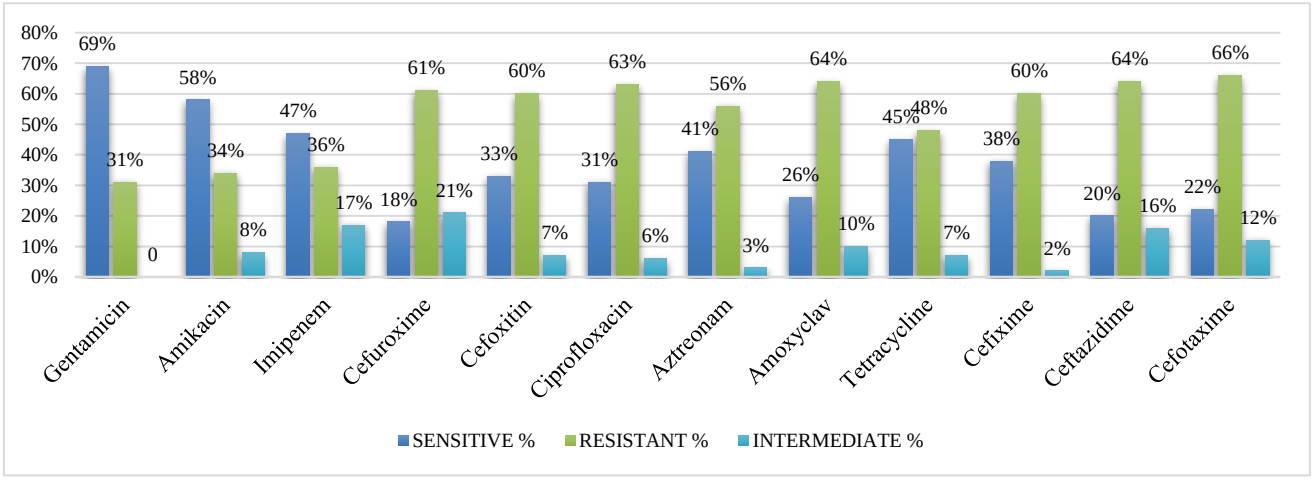

Figure 2: Antibiotic susceptibility pattern of *K. pneumoniae.*

The mean zone of inhibition (mm) exhibited by *L. acidophilus* treated and untreated against *K. pneumoniae* were 9.27 and 11.32 respectively. The mean zone of inhibition of *K. pneumoniae* by *L. rhamnosus* treated and untreated were 8.12 and 9.80 respectively (Figure 5). *L. acidophilus* (both treated and untreated) exhibited greater zone of inhibition against *K. pneumoniae* than *L. rhamnosus*. On comparing the zone of inhibition of treated and untreated suspension of *L. acidophilus* and *L. rhamnosus*, untreated exhibited greater zone of inhibition against *K. pneumoniae* in both cases.

Comparative activity of L. acidophilus and L. rhamnosus against K. pneumoniae by using ANOVA single factor

The difference in antimicrobial activity between treated suspension of *L. acidophilus and L. rhamnosus* and between untreated suspension of *L. acidophilus* and *L. rhamnosus* was analyzed using ANOVA one way method (Table 1-2). ANOVA one way method was also used to analyse the difference in antimicrobial activity between *L. acidophilus*- treated and untreated and *L. rhamnosus* treated and untreated.

Table 1: comparative activity of treated suspension of *L. acidophilus* and *L rhamnosus* against *K. pneumoniae*.

Groups	Count	Sum	Average	Variance	Standard Error	
***L. acidophilus* (Treated)**	100	927	9.27	2.52	0.16	
***L. rhamnosus* (Treated)**	100	812.5	8.125	4.62	0.21	
ANOVA						
Source of variation	**SS**	**df**	**MS**	**F value**	**P value**	**F crit**
Between groups	65.55	1	65.55	18.35	2.8688E-05	3.89
Within groups	707.39	198	3.58			
Total	772.95	199	-			

Significant if p<0.05

Table 2: Comparative activity of untreated suspension of *L. acidophilus* and *L. rhamnosus* against *K. pneumoniae*.

Groups	Count	Sum	Average	Variance	Standard Error	
***L. acidophilus* (Untreated)**	100	1131.5	11.315	3.20	0.18	
***L. rhamnosus* (Untreated)**	100	980.5	9.805	2.47	0.16	
ANOVA						
Source of variation	**SS**	**df**	**MS**	**F value**	**P value**	**F crit**
Between groups	114.1	1	114.1	40.22	1.50911E-09	3.89
Within groups	561.27	198	2.83			
Total	675.28	199	-			

Significant if p<0.05

Table 3: Grading of antimicrobial activity of *L. acidophilus* (both treated and untreated) on XDR, MDR and Non MDR *K. pneumoniae* isolates.

Non MDR/MDR/ XDR	Interpretation of *L. acidophilus* action on *K. pneumoniae*							
	***L. acidophilus* treated (%)**				***L. acidophilus* untreated (%)**			
	Strong	**Intermediate**	**Weak**	**No action**	**Strong**	**Intermediate**	**Weak**	**No action**
Non MDR	2.96	88.23	8.82	0	58.82	38.24	2.94	0
MDR	0	100	0	0	66.67	33.33	0	0
XDR	4.76	77.78	17.46	0	53.97	44.44	1.59	0

Table 4: Grading of antimicrobial activity of *L. rhamnosus* (both treated and untreated) on XDR, MDR and Non MDR *K. pneumoniae* isolates.

Non MDR/MDR/ XDR	Interpretation of *L. rhamnosus* action on *K. pneumoniae*							
	***L. rhamnosus* treated (%)**				***L. rhamnosus* untreated (%)**			
	Strong	**Intermediate**	**Weak**	**No action**	**Strong**	**Intermediate**	**Weak**	**No action**
Non MDR	0	73.59	26.47	0	23.53	70.59	5.88	0
MDR	0	33.33	66.67	0	33.33	33.33	33.33	0
XDR	1.59	63.49	34.92	0	14.29	79.36	6.35	0

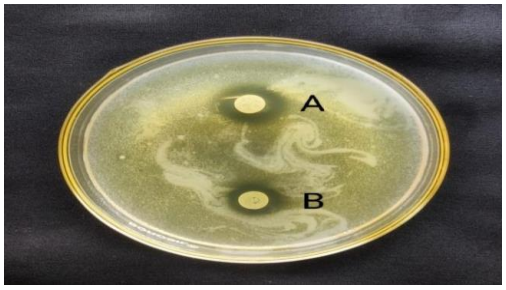

Figure 3: Inhibition of *K. pneumoniae* by treated suspension of *L. acidophilus* and *L. rhamnosus* as visible zone of inhibition using Agar overlay interference technique (A-*L. acidophilus* treated, B-*L. rhamnosus* treated).

Grading of antimicrobial activity of L. acidophilus and L. rhamnosus (both treated and untreated) on XDR, MDR and non MDR K. pneumoniae isolates

L. acidophilus treated had intermediate action on MDR, XDR and Non MDR strains of *K. pneumoniae* whereas *L. acidophilus* untreated has strong action against the same (Table 3).

L. rhamnosus treated had intermediate action against XDR and Non MDR and weak action against MDR *K. pneumoniae*.

L. rhamnosus untreated had intermediate action against MDR, XDR and Non MDR (Table 4).

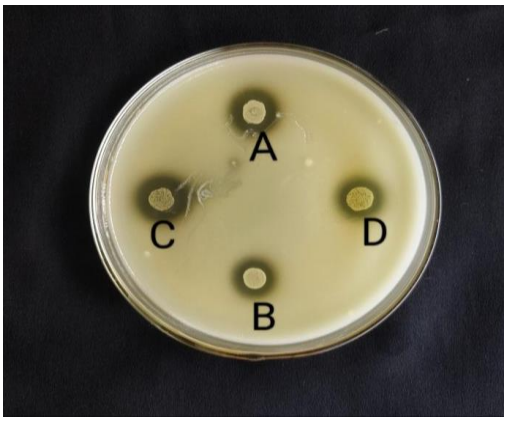

Figure 4: Inhibition of *K. pneumoniae* by untreated suspension of *L. acidophilus* and *L. rhamnosus* as visible zone of inhibition using Agar overlay interference technique (A-*L. acidophilus* untreated 1, B-*L. acidophilus* untreated 2, C-*L. rhamnosus* untreated 1, D-*L. rhamnosus* untreated 2).

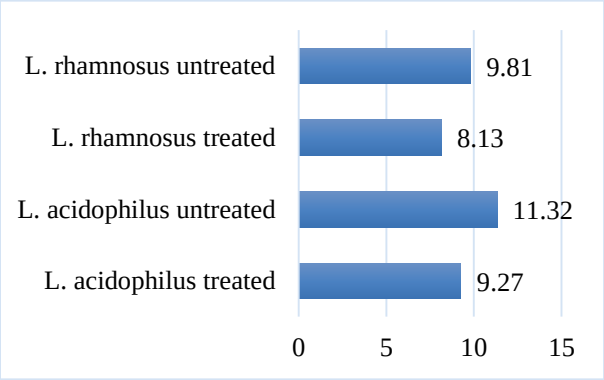

Figure 5: Antimicrobial activity of *L. acidophilus* and *L. rhamnosus* (both treated and untreated) against *K. pneumoniae* based on zone of inhibition.

DISCUSSION

Lactobacilli are non-pathogenic, associated with the human microbiota generally regarded as safe bacteria and commonly used as probiotics. Their probiotic effect relies on their ability to modulate the host immune system, fight pathogen colonization via competitive exclusion, enhance the epithelial barrier function, or production of antimicrobial molecule.[17] The gram-negative enteric bacteria, *K. pneumoniae* possesses the capacity to cause community-acquired as well as healthcare associated infections, such as bloodstream infection, urinary tract infection, pneumonia, surgical site infections, intra-abdominal infection, skin and soft tissue infection, liver abscess and meningitis.[18,19] In addition, the antibiotic resistance, among *K. pneumoniae*, is an emerging global healthcare crisis, and of particular the emergence of MDR and XDR *K. pneumoniae* incites impediment in the antibiotic therapy.[20,21] Health promoting bacteria exerts inhibitory activities even against MDR pathogens which supports the employment of these antimicrobial probiotics to fight infectious diseases, furthermore, it is assumed that the loss of their activity against the targeted pathogens is an unlikely or rare event, in contrast to the frequent development of resistance by human pathogens towards antibiotics that are commonly used.[22,23] Since the last two decades, because of its worldwide spread as a multidrug-resistant (MDR) pathogen, *K. pneumoniae* has gained importance.[24] In our study, 63% of the clinical isolates of *K. pneumoniae* tested were XDR and 3% were MDR. In recent years the drug resistant strains are increasing. According to study conducted by Sharma et al there was a significant increase in resistant *K. pneumoniae* from 7.5% in 2018 to 21.4% in 2022, while XDR *K. pneumoniae* among mechanically ventilated ICU patients significantly increased from 62.5% in 2018 to 71% in 2022.[25] Multi-drug-resistant *K. pneumoniae* has become an urgent risk to public health[9]. Thus, in the era of antimicrobial resistance, the possible use of probiotics as antibacterial agents is an emerging issue with a rapidly expanding field of applications.[26]

To date, most of the studies on the antibacterial activity of probiotics have focused on bacteria-free supernatants of Lactobacilli.[27-29] Only limited works have investigated the antibacterial activity of live Lactobacilli. Using Cell Free Supernatant and live cells in a study by Fernández et al.[30] showed that when the live cells of *L. rhamnosus* were used strongest antimicrobial activity was observed. Herein, we tested the ability of live *L. acidophilus* and *L. rhamnosus* treated (pH adjusted) and untreated, to exert antimicrobial activity against *K. pneumoniae* by Agar overlay method. According to Cadirci and Citak, for the evaluation of inhibitory activity of Lactobacilli against Gram negative bacteria, the agar overlay method is most effective when compared to agar well diffusion method.[31] Although *in vitro* assessments may not be able to totally mimic the actual in situ conditions, they remain powerful tools for rapid screening of potential strains. They permit an enormous level of simplification of the system under study, allowing a large number of strains to be investigated for a specific probiotic property. The use of *in vivo* studies for initial investigation of probiotic properties of new potential probiotic strains is not only time-consuming but also expensive. Thus, the use of *in vitro* assays to assess and select the most effective strain for *in vivo* investigations is a more logical option.[32,33]

According to our study, both *L. acidophilus* and *L. rhamnosus* has inhibitory effect on *K. pneumoniae*. This has been supported by the investigation carried out by Davoodabadi et al.[34] who reported the antimicrobial effect of *Lactobacillus* strain of human origin and suggested their usefulness as probiotics in controlling *E. coli* infections. Another study by Monteagudo-Mera et al showed that probiotic properties of *L. rhamnosus* from pharmaceutical sachets showed growth inhibitory properties against gram-positive as well as gram-negative

bacterial strain.[35] Chen et al showed that some *Lactobacillus* strains are able to inhibit Carbapenem-resistant *Enterobacteriaceae*. In this study we compared the mean zone of inhibition of *L. acidophilus* and *L. rhamnosus* (both treated and untreated) for which *L. acidophilus* exhibited more inhibitory effect against *K. pneumoniae* but concluded that statistically the differences are insignificant ($p>0.05$), therefore both have independent action against *K. pneumoniae*.[36] This finding is in contrast to the result obtained by Halder and Mandal who got greater zone of inhibition values for *L. rhamnosus* than *L. acidophilus* against *K. pneumoniae* isolates.[9] Such differences in the antibacterial activity of the Lactobacilli could be due to the strain and their source variation, in the later study the strains were obtained from curd (*L. acidophilus LMEM8* and *L. rhamnosus LMEM9*) while in our study we used standard strains of *L. acidophilus* and *L. rhamnosus* (*L. acidophilus* MTCC 10307 and *L. rhamnosus* MTCC 1408). In this study, we also compared the inhibitory action of treated and untreated suspension of *L. acidophilus* and *L. rhamnosus* and found out that untreated suspension possess greater inhibitory effect in both cases. This finding is in accordance with the work carried out by Tejero-Sariñena et al who observed that lower the pH, the higher the diameter of zone of inhibition against pathogenic organisms.[37] Treating the suspension and raising the pH to 7 reduced the antimicrobial action but still could observe some effects. Therefore, we cannot rule out that other mechanisms could inhibit the growth of the pathogens in some way.

CONCLUSION

In conclusion, within the limitations of the present study, we can conclude that probiotics especially *L. acidophilus* and *L. rhamnosus* are effective in inhibiting the growth of *K. pneumoniae* (MDR, XDR and Non-MDR strains) and had an intermediate action against them in general. The extent to which these *In vitro* results corresponds to *in vivo* conditions remains to be determined. Further studies are warranted to extend and authenticate the current indications.

ACKNOWLEDGEMENTS

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

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Paczosa MK, Mecsas J. Klebsiella pneumoniae: going on the offense with a strong defense. Microbiol Mol Biol Rev. 2016;80(3):629-61.
2. Lebeaux D, Ghigo JM, Beloin C. Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. Microbiol Mol Biol Rev. 2014; 78(3):510-43.
3. Ahmed SH, Daef EA, Badary MS, Mahmoud MA, Abd-Elsayed AA. Nosocomial blood stream infection in intensive care units at Assiut University Hospitals (Upper Egypt) with special reference to extended spectrum beta-lactamase producing organisms. BMC Res Notes. 2009;2:76.
4. Sheu CC, Lin SY, Chang YT, Lee CY, Chen YH, Hsueh PR. Management of infections caused by extended-spectrum β-lactamase-producing Enterobac-teriaceae: current evidence and future prospects. Expert Rev Anti Infect Ther. 2018;16(3): 205-18.
5. Mulani MS, Kamble EE, Kumkar SN, Tawre MS, Pardesi KR. Emerging Strategies to Combat ESKAPE pathogens in the era of antimicrobial resistance: a review. Front Microbiol. 2019;10:539.
6. FAO/WHO Report of a Joint FAO/WHO expert consultation on guidelines for the evaluation of probiotics in food. Available at: https://www.who.int. Accessed on 20 November 2023.
7. Halder D, Mandal M, Chatterjee SS, Pal NK, Mandal S. Indigenous probiotic lactobacillus isolates presenting antibiotic like activity against human pathogenic bacteria. Biomedicines. 2017;5(2):31.
8. Kechagia M, Basoulis D, Konstantopoulou S, Dimitriadi D, Gyftopoulou K, Skarmoutsou N, et al. Health benefits of probiotics: a review. ISRN Nutr. 2013;481651.
9. Shyamapada M, Debashis H. Exploring Anti-Klebsiella pneumoniae Activity of Probiotic Lactobacilli of Curd Origin. Acta Sci Microbiol. 2018;1(4) 49-53.
10. Halder D, Mandal S. Antibacterial potentiality of commercially Available Probiotic Lactobacilli and Curd Lactobacilli strains, Alone and in combination, against Human Pathogenic Bacteria. Transl Biomed. 2016;7(2):61.
11. Halder D, Mandal S. Curd Lactobacilli with Probiotic Potentiality. Transl Biomed. 2015;6(2):1.
12. Performance standards for antimicrobial disk susceptibility tests. Available at: https://clsi.org/standards/products/microbiology/documents/m100/. Accessed on 20 November 2023.
13. Performance Standards for antimicrobial susceptibility Testing. Available at: https://clsi.org/standards/products/microbiology/documents/m100/. Accessed on 20 November 2023.
14. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-81.

15. Fleming HP, Etchells JL, Costilow RN. Microbial inhibition by an isolate of Pediococcus from cucumber brines. Appl microbiol. 1975;30(6):1040-2.
16. Shokryazdan P, Sieo CC, Kalavathy R, Liang JB, Alitheen NB, Faseleh JM, et al. Probiotic potential of Lactobacillus strains with antimicrobial activity against some human pathogenic strains. Biomed Res Int. 2014;2014:927268.
17. Raheem A, Liang L, Zhang G, Cui S. Modulatory effects of probiotics during pathogenic infections with emphasis on immune regulation. Front Immunol. 2021;12:616713.
18. Yu WL, Chuang YC. Clinical features, diagnosis, and treatment of Klebsiella pneumoniae infection. Biomed Res Int. 2018.
19. Lu CH, Chang WN, Wu HS. Klebsiella pneumoniae meningitis: analysis on clinical features of thirty-two adult patients. Zhonghua Yi Xue Za Zhi. 1997;60(6): 296-302.
20. Lin WP, Wang JT, Chang SC, Chang FY, Fung CP, Chuang YC, et al. The Antimicrobial Susceptibility of Klebsiella pneumoniae from Community Settings in Taiwan, a Trend Analysis. Sci Rep. 2016;6:36280.
21. Gupta N, Limbago BM, Patel JB, Kallen AJ. Carbapenem-resistant Enterobacteriaceae: epidemiology and prevention. Clin Infect Dis. 2011;53(1):60-7.
22. Simons A, Alhanout K, Duval RE. Bacteriocins, antimicrobial peptides from bacterial origin: overview of their biology and their impact against multidrug-resistant bacteria. Microorganisms. 2020;8(5):639.
23. Todorov SD, de Melo Franco BDG, Tagg JR. Bacteriocins of Gram-positive bacteria having activity spectra extending beyond closely-related species. Benef Microbes. 2019;10(3):315-28.
24. Navon-Venezia S, Kondratyeva K, Carattoli A. Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol Rev. 2017;41(3):252-75.
25. Sharma A, Thakur A, Thakur N, Kumar V, Chauhan A, Bhardwaj N. Changing trend in the antibiotic resistance pattern of klebsiella pneumonia isolated from endotracheal aspirate samples of icu patients of a tertiary care hospital in north India. Cureus. 2023; 15(3):e36317.
26. Silva DR, Sardi JO, Pitangui NS, Roque SM. Probiotics as an Alternative Antimicrobial Therapy: Current Reality and Future Directions. J Funct Foods. 2020;731:4080.
27. Jeyanathan A, Ramalhete R, Blunn G, Gibbs H, Pumilia CA, Meckmongkol T, et al. Lactobacillus cell-free supernatant as a novel bioagent and biosurfactant against Pseudomonas aeruginosa in the prevention and treatment of orthopedic implant infection. J Biomed Mater Res Appl Biomater. 2021; 109(10):1634-43.
28. 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;13:543-52.
29. Chappell TC, Nair NU. Engineered lactobacilli display anti-biofilm and growth suppressing activities against Pseudomonas aeruginosa. NPJ Biofil Microb. 2020;6(1):48.
30. Fernández MF, Boris S, Barbés C. Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract. J Appl Microbiol. 2003; 94(3):449-55.
31. Cadirci BH, Citak S. A comparison of two methods used for measuring antagonistic activity of lactic acid bacteria. Pak J Nutr. 2005;4:237-41.
32. Ehrmann MA, Kurzak P, Bauer J, Vogel RF. Characterization of lactobacilli towards their use as probiotic adjuncts in poultry. J Appl Microbiol. 2002; 92(5):966-75.
33. Nemcová R. Criteria for selection of lactobacilli for probiotic use. Vet Med. 1997;42(1):19-27.
34. Davoodabadi A, Soltan DMM, Lashani E, Tajabadi Ebrahimi M. Antimicrobial Activity of Lactobacillus spp. Isolated From Fecal Flora of Healthy Breast-Fed Infants Against Diarrheagenic Escherichia coli. Jundishapur J Microbiol. 2015;8(12):e27852.
35. Monteagudo MA, Rodríguez AL, Rúa J, Martínez BH, Navasa N, García AMR, et al. In vitro evaluation of physiological probiotic properties of diferent lactic acid bacteria strains of dairy and human origin. J Funct Foods. 2012;4(2):531-41.
36. Chen CC, Lai CC, Huang HL, Huang WY, Toh HS, Weng TC, et al. Antimicrobial Activity of Lactobacillus Species Against Carbapenem-Resistant Enterobacteriaceae. Front Microbiol. 2019; 10:789.
37. 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.

Cite this article as: Anil A, Raj A, Azhar MM, Kuriakose N, Murali R, Kumar HKS. Evaluation of antimicrobial activity of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* against clinically isolated *Klebsiella pneumoniae*: an *in vitro* study. Int J Res Med Sci 2024;12:1140-6.