Journal of Advanced Biomedical Sciences

https://jabs.fums.ac.ir/

Online ISSN: 2783-1523

Fasa University of Medical Sciences

The Antimicrobial Effect of *Lactobacillus casei* against *Klebsiella pneumoniae* and *Escherichia coli* Isolated from Urinary Samples

Mozhgan Fateh, Fatemeh Foroohi, Fatemeh Rafiee

Department of Microbiology, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran

Article Info

Article Type:

Research Article

Article history:

Received

11 Mar 2024

Received in revised form

29 Mar 2024

Accepted

20 Apr 2024

Published online

05 May 2024

Publisher

Fasa University of Medical Sciences

Abstract

Background & Objectives: Urinary tract infection (UTI) is a serious issue affecting both men and women resulting from the invasion of microbial agents into the urinary system. This study aimed to investigate the antimicrobial activity of *Lactobacillus casei* (*L. casei*) against *Klebsiella pneumoniae* (*K. pneumoniae*) and *Escherichia coli* (*E. coli*) isolated from UTI.

Materials & Methods: In this study, 100 urine specimens were obtained from medical laboratories in western Tehran. *E. coli* and *K. pneumoniae* isolates were identified and subsequently confirmed using polymerase chain reaction (PCR). Antibiotic susceptibility patterns were determined using the disk diffusion method. The antimicrobial activity of *L. casei* against these strains (four multidrug-resistant isolates from each species) was then evaluated using the agar well diffusion method.

Results: From 100 urine specimens, 76 *E. coli* and 14 *K. pneumoniae* isolates were identified. Antimicrobial susceptibility testing revealed that imipenem and nitrofurantoin were the most effective antibiotics against *E. coli*, while amikacin demonstrated the highest efficacy against *K. pneumoniae*. In the agar well diffusion assay, *L. casei* generated growth inhibition zones measuring 19.8 mm ± 3 for *E. coli* and 20.3 mm ± 4 for *K. pneumoniae*.

Conclusions: *Lactobacillus casei* demonstrates notable antimicrobial efficacy against both *E. coli* and *K. pneumoniae*, suggesting its potential as an alternative therapeutic option for UTIs.

Keywords: *Lactobacillus casei*, *Escherichia coli*, *Klebsiella pneumoniae*, Urinary Tract Infection

Cite this article: Fateh M, Foroohi F, Rafiee F. The Antimicrobial Effect of *Lactobacillus casei* against *Klebsiella pneumoniae* and *Escherichia coli* Isolated from Urinary Samples. J Adv Biomed Sci.2024; 14(2): 94-103.
DOI: 10.18502/jabs.v14i2.15755

[DOI: 10.18502/jabs.v14i2.15755]

Introduction

Urinary tract infection (UTI) is a prevalent issue affecting many individuals globally, ranking second after respiratory infections with millions affected annually (1, 2). Typically, urine passes through the urinary system without contamination. However, bacteria can enter from outside the body, leading to infection and inflammation, most commonly in the lower urinary tract (3). Symptoms vary based on the affected area, age, sex, and catheter use but generally include flank or pelvic pain (4), lower abdominal pressure (5), persistent urge to urinate, burning sensation during urination, frequent urination, cloudy or dark urine, hematuria, foul-smelling urine, dyspareunia, and genital pain (6). UTIs are the most

Corresponding Author: Fatemeh Foroohi, Department of Microbiology, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran
Email: f_foroohi@ymail.com

common hospital-acquired infections, especially in individuals with diabetes mellitus or compromised immune systems (7, 8). Primarily caused by *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) (3, 9, 10), UTIs range from mild discomfort to severe complications, highlighting the need for timely treatment. Untreated or asymptomatic UTIs can lead to kidney damage, bloodstream infections, and recurrent infections, significantly impacting quality of life (3).

The excessive and inappropriate use of antibiotics has led to the emergence of resistant strains, complicating treatment efforts. Consequently, alternative antimicrobial approaches, such as probiotics, are being explored (10-13). Probiotics, beneficial microorganisms, offer a versatile approach to combat infections, including UTIs (14). Typically administered orally or topically, they restore and maintain a healthy microbial balance in the gut and vagina, inhibiting the growth of pathogens like *E. coli* and *K. pneumoniae* (10, 15-18). They also enhance immune function, supporting natural defenses against infections (19).

Probiotics, particularly *Lactobacillus* spp., are known for their antimicrobial activity against pathogens (20-23). *Lactobacillus casei (L. casei)*, a Gram-positive bacterium found in the digestive and urinary tracts, produces antimicrobial substances that enhance immune response and compete against urinary tract pathogens (24, 25). Recent research has highlighted *L. casei* potential to inhibit pathogenic bacteria, suggesting it as a promising antimicrobial agent for UTI treatment (9, 11, 26-28). Therefore, in this study, we aimed to investigate the antimicrobial effect of *L. casei* on *K. pneumoniae* and *E. coli* isolated from urinary specimens, with the goal of developing a strategy to combat antibiotic-resistant UTIs.

Materials and Methods

Bacterial Sampling and Isolation

To conduct this study, 100 urine specimens were obtained from individuals diagnosed with UTIs at medical diagnostic laboratories in western Tehran. To adhere to ethical principles and maintain patient confidentiality, access to patient records was not granted. The specimens were processed and prepared according to standard laboratory protocols to ensure accurate bacterial identification and isolation. None of the patients had indwelling urinary catheters. One microliter of uncentrifuged urine was inoculated onto MacConkey agar, blood agar, and eosin methylene blue agar for observation of the characteristic green metallic sheen of *E. coli* colonies. Cultures were incubated at 37°C for 24 hours. Standard biochemical tests (sulfide indole motility [SIM], triple sugar iron [TSI], indole, methyl red, Voges-Proskauer, and citrate [IMViC], and urease) were performed and incubated at 37°C for 24 hours (Table 1). Confirmed strains of *E. coli* and *K. pneumoniae* were isolated, cultured on MacConkey agar and agarose gel, and incubated at 37°C for 48 hours for subsequent DNA extraction and antimicrobial susceptibility testing.

Table 1. Biochemical Test Diagnosis Table

Tests/Isolates	Urea Hydrolysis	Citrate (Simmons)	Voges-Proskauer	Methyl Red	Motility	Indole Production	TSI	H_2S (TSI)
K. pneumoniae	+	+	+	-	-	-	A/A	+
E. coli	-	-	-	+	+	+	K/A A/A	-

[DOI: 10.18502/jabs.v14i2.15755]

Antibiotic Sensitivity Testing

Antibiotic susceptibility testing was conducted using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MHA) medium (Merk, Germany) following the Clinical and Laboratory Standards Institute (CLSI) version 2022 guidelines. The antibiotic discs included amikacin (30 µg), cefalotin (30 µg), ciprofloxacin (5 µg), ceftriaxone (30 µg), gentamicin (10 µg), imipenem (10 µg), nitrofurantoin (300 µg), co-trimoxazole (23.75 µg sulfamethoxazole + 1.75 µg trimethoprim), and cefepime (30 µg). *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 13883 were used as quality control strains. The results were interpreted according to CLSI 2022 guidelines.

Extraction of *E. coli* and *K. pneumoniae* acterial DNA

Bacterial DNA was extracted using the Sinagen Company extraction kit (Iran). Five to ten colonies of the target bacteria were picked and suspended in 100 µL of water. Then, 400 µL of lysis solution were added and vortexed for 15-20 seconds to lyse the cells and extract DNA. Subsequently, 300 µL of precipitation solution were added, vortexed for 5 seconds, and centrifuged for 10 minutes at 12,000 × g. The supernatant was discarded and the tubes were dried on a paper towel. Next, 1 mL of wash buffer was added and centrifuged for 5 minutes at 12,000 × g. The supernatant was again discarded and the tube was dried. For long-term preservation, 20 µL of TE buffer at 60°C added to dissolve the DNA completely and stored at -70°C. However, since long-term preservation was not intended, 20 µL of nuclease-free water was added to the dried tube containing DNA for immediate use.

Polymerase chain reaction

For the PCR procedure, distinct reaction mixtures were prepared for each bacterial species (*E. coli* and *K. pneumoniae*) as outlined in Table 2. The reactions were carried out using the specified primers from Table 3, following the designated thermocycler programs: For *E. coli*: initial denaturation at 95°C for 5 minutes, followed by 30 thermal cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 60 seconds, with a final extension at 72°C for 5 minutes (29). For *K. pneumoniae*: initial denaturation at 94°C for 4 minutes, followed by 30 thermal cycles of denaturation at 94°C for 45 seconds, annealing at 59°C for 45 seconds, and extension at 72°C for 45 seconds, with a final extension at 72°C for 10 minutes (30). A 1.5% agarose gel was used for electrophoresis of both PCR products.

Assessing *Lactobacillus casei* antibacterial activity via agar-well diffusion

Initially, *L. casei* ATCC 39392 was cultured in de Man, Rogosa and Sharpe (MRS) broth (Merck, Germany) and incubated for 24 hours at 37°C under anaerobic conditions using an anaerobic glove box (Anoximat incubator, Germany) with a gas mixture of 95% N_2, 5% H_2, 6% O_2, and 5% CO_2 (31). The cell-free supernatant (CFS) obtained from *L. casei* was prepared following the methodology outlined in a previous study (32). The selected isolates of *K. pneumoniae* and *E. coli* (four multidrug-resistant (MDR) isolates from each species) were adjusted to a 0.5 McFarland standard suspension in sterile tubes. Subsequently, individual isolates were swabbed onto the MHA media using a sterile cotton swab. Agar wells were then created using a sterilized cork borer with a diameter of 10 mm. Next, 100 µL of CFS derived from *L. casei* was added to each agar well on the plate. The media were then incubated at 37°C for 24 hours. The presence of an inhibition zone around the wells indicated antimicrobial activity, with the diameter of the zone measured in millimeters (32).

[DOI: 10.18502/jabs.v14i2.15755]

Table 2. PCR reaction components

Components	Volume	Final Con
Distilled H2O	14 µL	-
10 X PCR buffer	2.5 µL	0.2 mM each
10 mM dNTP Mix	0.5 µL	0.2 mM each
50 mM $MgCl_2$	0.75 µL	1.5 mM
Forward Primer	1 µL	0.5 mM
Reverse Primer	1 µL	0.5 mM
Control DNA	5 µL	20 pg
Taq DNA Polymerase	0.2 µL	1 unit
Mineral Oil	25 µL	-

Table 3. Primer sequences used in the study

Bacteria	Target Gene	Primer Sequence	Size (bp)	Reference
K. pneumoniae	*16srRNA*	F:5′ ATT TGA AGA GGT TGC AA CGA T 3′	1300	(30)
		R:5′ TTC ACT CTG AAG TTT TCT TGT GTTC 3′		
E. coli	*16srRNA*	F: 5′ AGA GTT TGA TCM TGG CTC AG 3′	919	(29)
		R: 5′ CCG TCA ATT CAT TTG AGT TT 3′		

Statistical Analysis

In the present study, the chi-square (χ^2) test was used to assess independence. The correlations between gender and UTI, and between gender and antibiotic resistance of bacteria, were calculated using the chi-square test via SPSS version 20 (SPSS, USA). A p-value < 0.05 was considered statistically significant.

Results

Bacterial isolates

Out of 100 urine specimens examined, 76 *E. coli* isolates were obtained, with 65 isolates derived from female subjects and 11 from male subjects. Additionally, 14 isolates of *K. pneumoniae* were identified, comprising 11 isolates from female subjects and three from male subjects (Figure 1). Statistical analysis revealed a significant association between UTI and gender ($p < 0.05$), indicating a predominance of UTIs among females compared to males. Subsequently, four MDR *E. coli* and four MDR *K. pneumoniae* isolates were selected for further investigation.

[DOI: 10.18502/jabs.v14i2.15755]

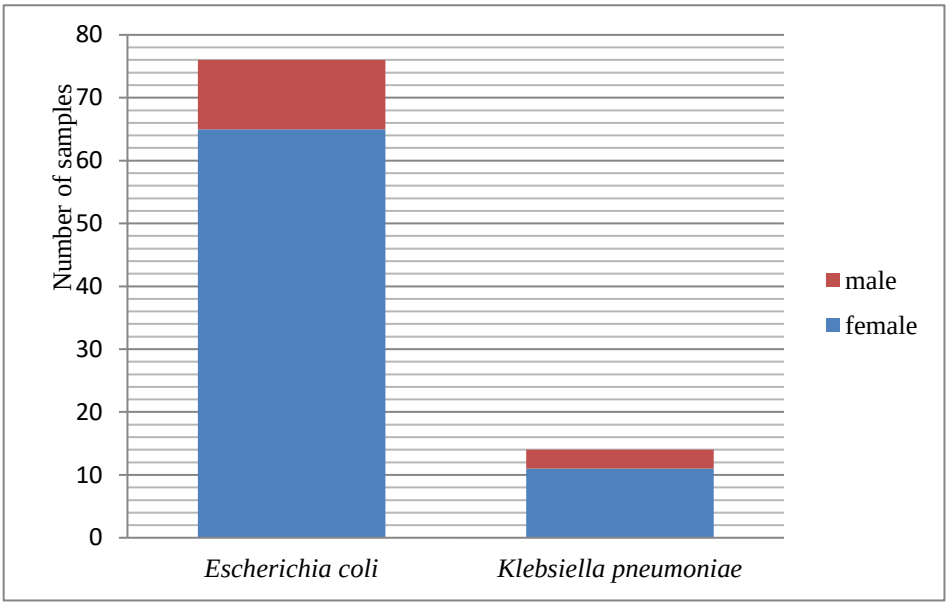

Chart 1. Distribution of isolates based on gender, distinguishing between *E. coli* and *K. pneumoniae*

Polymerase Chain Reaction

Based on the banding patterns, the isolated samples were identified as *E. coli* and *K. pneumoniae*. As observed in Figure 1, the five samples on the right side correspond to *K. pneumoniae*, while the five samples on the left side correspond to *E. coli*. The fifth sample is the control (Ladder). According to the control sample, the sequence size for *E. coli* is 919 bp, and for *K. pneumoniae* is 1300 bp.

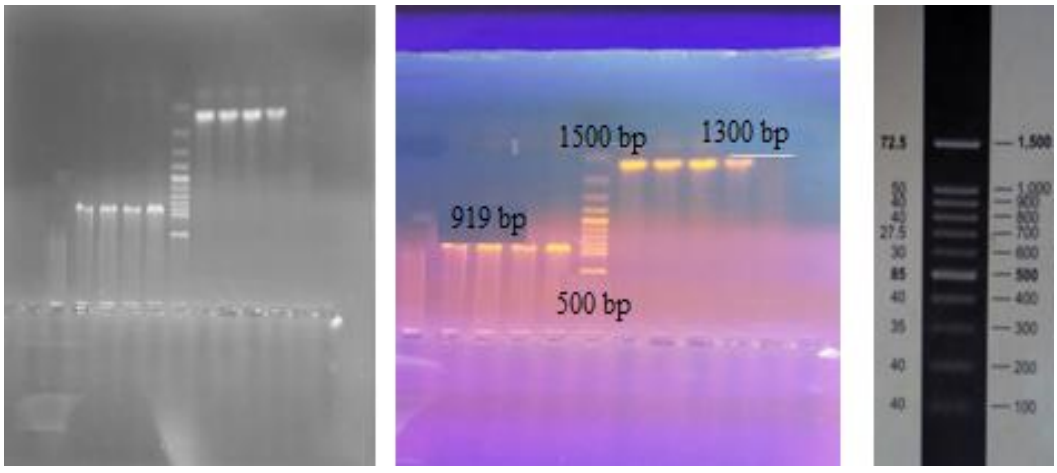

Figure 1. Imaging gel electrophoresis and control sample (Ladder)

[DOI: 10.18502/jabs.v14i2.15755]

Table 4. Antibiotic resistance patterns of isolated *E. coli* and *K. pneumoniae* from urine samples

Antibiotics *E. coli*	R (Resistant)	I (Intermediate)	S (Sensitive)
FM (Nitrofurantoin)	0 (0%)	3 (3.94%)	73 (96.05%)
SXT(Trimethoprim Sulfamethoxazole)	40 (52.63%)	0 (0%)	36 (36.47%)
CZ (Cefazolin)	42 (55.26%)	0 (0%)	34 (44.73%)
GM (Gentamicin)	13 (17.10%)	0 (0%)	63 (82.89%)
CP (Ciprofloxacin)	45 (59.21 %)	3 (3.94%)	28 (36.84%)
AN (Amikacin)	2 (2.63%)	1 (1.31%)	73 (96.05%)
CRO (Ceftriaxone)	38 (50%)	0 (0%)	38 (50%)
IMP (Imipenem)	0 (0%)	0 (0%)	76 (100%)
FEP (Cefepime)	30 (39.47%)	0 (0%)	47 (61.84%)
Antibiotics *K. pnumoniae*	R (Resistant)	I (Intermediate)	S (Sensitive)
CZ (Cefazolin)	7 (50%)	3 (21.42%)	4 (28.57%)
CP (Ciprofloxacin)	3 (21.42%)	4 (28.57%)	7 (50%)
IMP (Imipenem)	2 (14.28%)	5 (35.71%)	7 (50%)
CRO (Ceftriaxone)	3 (21.42%)	4 (28.57%)	7 (50%)
FEP (Cefepime)	2 (14.28%)	5 (35.71%)	7 (50%)
SXT (Trimethoprim Sulfa methoxazole)	2 (14.28%)	6 (42.85%)	7 (50%)
FM (Nitrofurantoin)	1 (7.14%)	4 (28.57%)	9 (64.28%)
GM (Gentamicin)	1 (7.14%)	5 (35.71%)	8 (57.14%)
AN (Amikacin)	0 (0%)	5 (35.71%)	9 (64.28%)

Antibiotic Sensitivity Testing

The findings from the antibiotic sensitivity test, displayed in Table 4, indicate that 73 (96.05%) of the *E. coli* isolates demonstrated sensitivity to imipenem and nitrofurantoin. Additionally, nine (64.28%) of the *K. pneumoniae* isolates exhibited sensitivity to nitrofurantoin and amikacin. No significant correlation was observed between gender and antibiotic resistance ($p>0.05$). The antibiotic sensitivity test plates for *E. coli* and *K. pneumoniae* are shown in Figures 2 and 3, respectively.

[DOI: 10.18502/jabs.v14i2.15755]

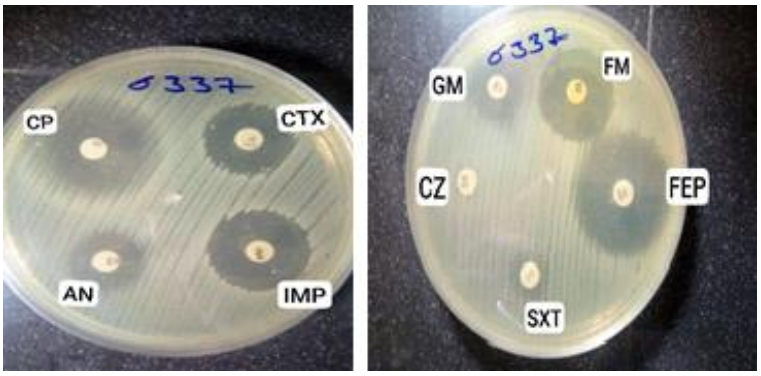

Figure 2. The antibiotic Sensitivity Test of *E. coli*

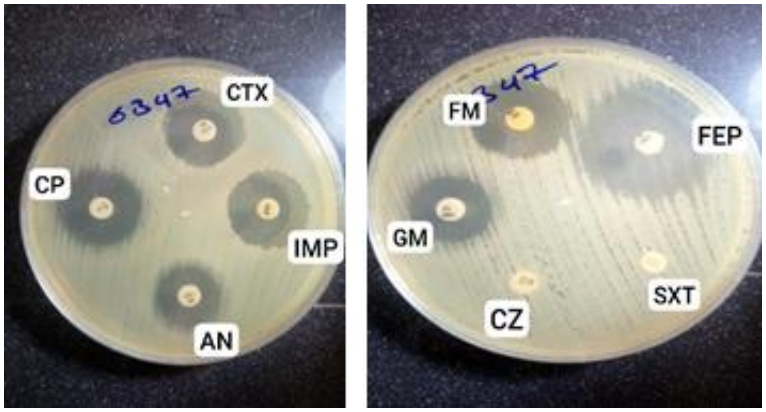

Figure 3. The antibiotic Sensitivity Test of *K. pneumonia*

Assessing *Lactobacillus casei* antibacterial activity via agar-well diffusion

In the agar well diffusion assay, the mean diameter of growth inhibition zones around *L. casei* well was 19.8 mm ± 3 for *E. coli* and 20.3 mm ± 4 for *K. pneumoniae* (Figure 4).

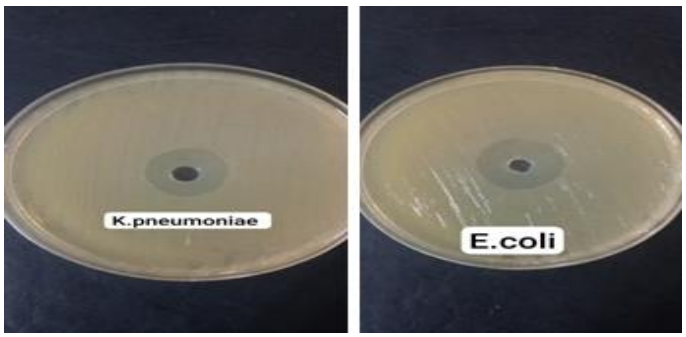

Figure 4. Antibacterial activity of *L. casei* against *K. pneumoniae* (right) and *E. coli* (left)

[DOI: 10.18502/jabs.v14i2.15755]

Discussion

UTIs are highly prevalent, second only to respiratory infections, and are common hospital-acquired infections (2, 33). The ability of an organism to cause infection depends on both host susceptibility and the pathogen's properties. Specific factors enable pathogens to infect hosts and cause disease. Common UTI pathogens include *Enterobacteriaceae* Gram-negative bacilli, especially *E. coli* and *K. pneumoniae* (34, 35). Recent studies highlight increasing drug resistance in *E. coli* and *K. pneumoniae*, particularly to beta-lactams (36, 37). The extensive use of beta-lactams has led to the continuous production and mutation of beta-lactamases, expanding their activity spectrum against new antibiotics (17, 38, 39). Despite easy diagnosis, treating these infections poses challenges, emphasizing the need to recognize antibiotic resistance in infectious agents. The rising drug resistance has increased UTI-related mortality in hospitals globally, affecting various communities and regions. Consequently, researchers are exploring alternative treatments, including effective probiotics (40).

Probiotics, beneficial microorganisms that improve human health, are found in yogurt, dairy products, fermented foods, and naturally in the human digestive system. *L. casei*, a probiotic, has garnered interest for its antimicrobial properties against *E. coli* and *K. pneumoniae* (13, 41, 42). It inhibits *K. pneumoniae* growth by acidifying the environment and producing organic acids, hydrogen peroxide, and bacteriocins, which attack the bacterial cell walls (43). *L. casei* also competes for nutrients, alters pH, and enhances the host immune response, aiding in defense against these infections (12).

A study by Soudeh Bandari et al. investigated the effect of probiotics *Lactobacilli* on the binding ability and biofilm formation of *E. coli* strains isolated from UTIs. The results inferred that *L. casei*'s anti-adhesive effect against pathogenic bacteria was 58%, and *L. plantarum*'s effect was 62%, indicating that *Lactobacilli* prevent the binding of pathogenic bacteria (44).

In our study, the most effective antibiotics against *E. coli* were imipenem, amikacin, and nitrofurantoin, whereas ciprofloxacin, cefazolin, and cotrimoxazole were the least effective. For *K. pneumoniae*, the most effective antibiotics were amikacin and nitrofurantoin, with cefazolin being the least effective. The agar well diffusion method showed that *L. casei* had a greater growth inhibition zone compared to the antibiotics, particularly against *K. pneumoniae*, indicating a better preference for *L. casei*. A study by Naeimeh Soltani et al. reported that *L. casei* formed a 15 mm inhibition zone against *E. coli*, while *L. acidophilus* formed a 16 mm zone (9). Another study by Amir Emami et al. showed *L. casei* and *L. acidophilus* had significant antimicrobial activity against common hospital-acquired infection strains, including *E. coli* and *K. pneumoniae* (7). Our findings demonstrate a significant increase in the growth inhibition zones of *L. casei* compared to previous research. This expansion indicates an enhanced capacity of *L. casei* to inhibit the growth of pathogenic bacteria, potentially offering promising prospects for its utilization as an effective antimicrobial agent in UTI treatment.

Conclusion

The antimicrobial activity demonstrated by *L. casei* against *E. coli* and *K. pneumoniae* isolated from UTIs supports its potential as a promising therapeutic agent for UTI management. The observed growth inhibition zones in the agar well diffusion method signify the effectiveness of *L. casei* against these pathogens. These findings suggest a promising avenue for the development of alternative treatments for UTIs, especially in the context of increasing antibiotic resistance. Further research is essential to elucidate the clinical applicability and optimize the use of *L. casei* in UTI management protocols.

Acknowledgements

The authors thank the staff of the Department of Microbiology at Shahr-e-Qods Branch, Islamic Azad

[DOI: 10.18502/jabs.v14i2.15755]

University, Tehran, Iran, for providing the essential equipment.

Conflict of Interests

The authors declare no conflicts of interest relevant to this article.

Funding

No financial support was provided relevant to this article.

Code of Ethics

This study is derived from the master's thesis of Mozhgan Fateh, identified by code 162771048

Consent for publication

All authors have read and agreed to the published version of the manuscript.

Data availability statement

All data are mentioned in the article.

References

1.Reid G. Perspective: microbial interventions in the urinary tract. Microbiome Res Rep. 2023;2(1): 3-11.
2.Shahkolahi S, Shakibnia P, Shahbazi S, Sabzi S, Badmasti F, Asadi Karam MR, et al. Detection of ESBL and AmpC producing *Klebsiella pneumoniae* ST11 and ST147 from urinary tract infections in Iran. Acta Microbiol Immunol Hung. 2022;69(4):303-13.
3.Kim DS, Lee JW. Urinary Tract Infection and Microbiome. Diagnostics. 2023;13(11):1921.
4.Kaur R, Kaur R. Symptoms, risk factors, diagnosis and treatment of urinary tract infections. Postgrad Med J. 2021;97(1154):803-12.
5.Desie N, Willems A, De Laet I, Dits H, Van Regenmortel N, Schoonheydt K, et al. Intra-abdominal pressure measurement using the FoleyManometer does not increase the risk for urinary tract infection in critically ill patients. Ann Intensive Care. 2012;2:1-9.
6.Singh B, Tilak R, Srivastava R, Katiyar D. Urinary tract infection and its risk factors in women: an appraisal. J Pure Appl Microbiol. 2014;8(5):1-8.
7.Shahbazi R, Alebouyeh M, Shahkolahi S, Shahbazi S, Hossainpour H, Salmanzadeh-Ahrabi S. Molecular study on virulence and resistance genes of ST131 clone (uropathogenic/enteropathogenic *Escherichia coli*) hybrids in children. Future Microbiol. 2023;18(18):1353-61.
8.Rahimzadeh M, Shahbazi S, Sabzi S, Habibi M, Asadi Karam MR. Antibiotic resistance and genetic diversity among *Pseudomonas aeruginosa* isolated from urinary tract infections in Iran. Future Microbiol. 2023;18(16):1171-83.
9.Soltani N, Abbasi S, Baghaeifar S, Taheri E, Jadid MFS, Emami P, et al. Antibacterial and antibiofilm activity of *Lactobacillus* strains secretome and extraction against *Escherichia coli* isolated from urinary tract infection. Biotechnol Rep. 2022;36:e00760.
10.Naderi A, Kasra-Kermanshahi R, Gharavi S, Fooladi AAI, Alitappeh MA, Saffarian P. Study of antagonistic effects of *Lactobacillus* strains as probiotics on multi drug resistant (MDR) bacteria isolated from urinary tract infections (UTIs). Iran J Basic Med Sci. 2014;17(3):201.
11.Qasemi A, Lagzian M, Rahimi F, Majd FK, Bayat Z. The Power of Probiotics to Combat Urinary Tract Infections: A Comprehensive Review. Res Biotechnol Environ Sci. 2023;2(1):1-11.
12.Sharma P, Das S, Sadhu P, Pal S, Mitra S, Ghoshal A, et al. Therapeutic Role of Probiotics In Managing Various Diseases. J Surv Fish Sci. 2023;10(1S):6378-80.
13.Chen Y-C, Lee W-C, Chuang Y-C. Emerging non-antibiotic options targeting uropathogenic mechanisms for recurrent uncomplicated urinary tract infection. Int J Mol Sci. 2023;24(8):7055.
14.Bustamante M, Oomah BD, Oliveira WP, Burgos-Díaz C, Rubilar M, Shene C. Probiotics and prebiotics potential for the care of skin, female urogenital tract, and respiratory tract. Folia Microbiol. 2020;65:245-64.
15.Akgül T, Karakan T. The role of probiotics in women with recurrent urinary tract infections. Turk J Urol. 2018;44(5):377.
16.Bustamante M, Oomah BD, Oliveira WP, Burgos-Díaz C, Rubilar M, Shene C. Probiotics and prebiotics potential for the care of skin, female urogenital tract, and respiratory tract. Folia Microbiologica. 2020;65:245-64.
17.Shahbazi S, Habibi M, Badmasti F, Sabzi S, Farokhi M, Karam MRA. Design and fabrication of a vaccine candidate based on rOmpA from *Klebsiella pneumoniae* encapsulated in silk fibroin-sodium alginate nanoparticles against pneumonia infection. Int Immunopharmacol. 2023;125:111171.
18.Shahbazi S, Karam MRA, Habibi M, Talebi A, Bouzari S. Distribution of extended-spectrum β-lactam, quinolone and carbapenem resistance genes, and genetic diversity among uropathogenic *Escherichia coli* isolates in Tehran, Iran. J Glob Antimicrob Resist. 2018;14:118-25.
19.Gupta V, Nag D, Garg P. Recurrent urinary tract infections in women: how promising is the use of probiotics? Iran J Basic Med Sci. 2017;35(3):347-54.

[DOI: 10.18502/jabs.v14i2.15755]

20.Zommiti M, Chikindas ML, Ferchichi M. Probiotics—live biotherapeutics: a story of success, limitations, and future prospects—not only for humans. Probiotics Antimicrob. 2020;12:1266-89.
21.Jeong J-J, Park HJ, Cha MG, Park E, Won S-M, Ganesan R, et al. The Lactobacillus as a probiotic: focusing on liver diseases. Microorganisms. 2022;10(2):288.
22.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(1):927268.
23.Choi A-R, Patra JK, Kim WJ, Kang S-S. Antagonistic activities and probiotic potential of lactic acid bacteria derived from a plant-based fermented food. Front Microbiol. 2018;9:365192.
24.Vagios S, Hesham H, Mitchell C. Understanding the potential of *lactobacilli* in recurrent UTI prevention. Microb Pathog. 2020;148:104544.
25.Miglani R, Parveen N, Kumar A, Dewali S, Rawat G, Mishra R, et al. Bacteriocin and its biomedical application with special reference to Lactobacillus. Recent Advances and Future Perspectives of Microbial Metabolites: Elsevier; 2023. p. 123-46.
26.Petricevic L, Rosicky I, Kiss H, Janjic N, Kaufmann U, Holzer I, et al. Effect of vaginal probiotics containing *Lactobacillus casei rhamnosus* (Lcr regenerans) on vaginal dysbiotic microbiota and pregnancy outcome, prospective, randomized study. Sci Rep. 2023;13(1):7129.
27.Tommasi C, Equitani F, Masala M, Ballardini M, Favaro M, Meledandri M, et al. Diagnostic difficulties of *Lactobacillus casei* bacteraemia in immunocompetent patients: A case report. J Med Case Rep. 2008;2:1-4.
28.Ng QX, Peters C, Venkatanarayanan N, Goh YY, Ho CYX, Yeo W-S. Use of *Lactobacillus* spp. to prevent recurrent urinary tract infections in females. Med Hypotheses. 2018;114:49-54.
29.Webster G, Blazejak A, Cragg BA, Schippers A, Sass H, Rinna J, et al. Subsurface microbiology and biogeochemistry of a deep, cold-water carbonate mound from the Porcupine Seabight (IODP Expedition 307). Environ. Microbiol. 2009;11(1):239-57.
30.Asadpour L, Nahavandinejhad M. Frequency of Extended Spectrum Beta Lactamase Producing Multidrug Resistant *Klebsiells Pnemoniae* in Urinary Tract Infections in Rasht. J Ilam Univ Med Sci. 2017;25(2):82-90.
31.Saadatzadeh A FM, Jamalifar H, Dinarvand R. Probiotic Properties of Lyophilized Cell Free Extract of *Lactobacillus casei*. Jundishapur J Nat Pharm Prod. 2013;8(3):131-7.
32.Abdelhalim MM, Saafan GS, El-Sayed HS, Ghaith DM. In vitro antibacterial effect of probiotics against Carbapenamase-producing multidrug-resistant *Klebsiella pneumoniae* clinical isolates, Cairo, Egypt. J Egypt Public Health Assoc 2022;97(1):19.
33.Shivaee A, Shahbazi S, Soltani A, Ahadi E. Evaluation of the prevalence of broad-spectrum beta-lactamases (ESBLs) and carbapenemase genes in *Klebsiella pneumoniae* strains isolated from burn wounds in patients referred to Shahid Motahari Hospital in Tehran. Med Sci. 2019;29(3):232-9.
34.Goodarzi NN, Fereshteh S, Sabzi S, Shahbazi S, Badmasti F. Construction of a chimeric FliC including epitopes of OmpA and OmpK36 as a multi-epitope vaccine against *Klebsiella pneumonia*. Health Biotechnol Biopharma. 2021;5:44-60.
35.Shivaee A, Mirshekar M, Mohammadzadeh R, Shahbazi S. Association between ESBLs genes and quinolone resistance in uropathogenic *Escherichia coli* isolated from patients with urinary tract infection. Infect Epidemiol Microbiol. 2019;5(1):15-23.
36.Shahbazi S, Badmasti F, Habibi M, Sabzi S, Noori Goodarzi N, Farokhi M et al . In silico and in vivo Investigations of the Immunoreactivity of *Klebsiella pneumoniae* OmpA Protein as a Vaccine Candidate. IBJ. 2024; 28 (4) :2-2
37.Mirshekar M, Zadeh RG, Moghadam MT, Shahbazi S, Jazi FM. Upregulation of *pmr*A, *pmr*B, *pmr*C, *pho*Q, *pho*P, and *arn*T genes contributing to resistance to colistin in superbug *Klebsiella pneumoniae* isolates from human clinical samples in Tehran, Iran. New Microbes New Infect. 2024;59:101275.
38.Taati Moghadam M, Mirzaei M, Fazel Tehrani Moghaddam M, Babakhani S, Yeganeh O, Asgharzadeh S, et al. The challenge of global emergence of novel colistin-resistant *Escherichia coli* ST131. Microb Drug Resist. 2021;27(11):1513-24.
39.Shivaee A, Meskini M, Shahbazi S, Zargar M. Prevalence of *flm*A, *flm*H, *mrk*A, *ecp*A, and *mrk*D virulence genes affecting biofilm formation in clinical isolates of *K. pneumonia*. Feyz Med Sci J. 2019;23(2):168-76.
40.Rastogi S, Singh A, Nandi A, Gupta A, Agarwal J, Kostova I. Can the Therapeutic Spectrum of Probiotics be Extended: Exploring Potential of Gut Microbiome. Recent Adv Antiinfect Drug Discov. 2023;18(2):120-147.
41.Ali MS, Lee E-B, Hsu WH, Suk K, Sayem SAJ, Ullah HA, et al. Probiotics and postbiotics as an alternative to antibiotics: An emphasis on pigs. Pathogens. 2023;12(7):874.
42.Chen C-C, Lai C-C, Huang H-L, Huang W-Y, Toh H-S, Weng T-C, et al. Antimicrobial activity of *Lactobacillus* species against carbapenem-resistant *Enterobacteriaceae*.
Front Microbiol. 2019;10:789.
43.Moglad EH. Antibiotics profile, prevalence of extended-spectrum beta-lactamase (ESBL), and multidrug-resistant Enterobacteriaceae from different clinical samples in Khartoum State, Sudan. Int J Microbiol. 2020;2020(1):8898430.
44.Bandari S, Arbab Soleimani N, Tajbakhsh E. The effect of probiotic lactobacilli on the attachment power and biofilm formation of *Escherichia coli* isolated from urinary tract infections. J Microbiol. 2018;11(3):278-87.

[DOI: 10.18502/jabs.v14i2.15755]