Research Article

Antagonistic Activity of Lactobacillus Casei Against Bacterial Genera Isolated From Patients With Renal Failure and Acute Urinary Tract Infections

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Abstract

Background: Urinary tract infections are one of the most common bacterial infections globally. Given that the progression of UTIs may be directly linked to recurrent infections, the progression to acute kidney infection and chronic kidney disease is possible. Objective: Antagonistic activity of Lactobacillus casei against multidrug bacteria isolated from patients with renal failure. Methodology: A total of 208 urine samples were collected, identified, and tested for antimicrobial susceptibility using VITEK 2. DNA from isolates was extracted, employed to detect genes encoding beta-lactamase enzymes genes (bla SHV, bla TEM, bla CTX-M, and qnrA) in the studied bacterial isolates and antagonism activity of Lactobacillus casei against bacterial pathogens isolated from renal failure and acute UTIs. Results: cultivated samples showed that 124 bacterial isolates were identified and distributed into 83 (66.94%) from acute UTIs and 41(33.06%) from renal failure disease. Susceptibility testing results revealed that P. aeruginosa isolates were the most resistant to carbapenems, whereas E. coli isolates were completely susceptible. Using polymerase chain reaction, genes encoding resistance genes were found to be carried. On the other hand, L. casei isolates from cheese and Activia revealed high inhibitory activity and ability to suppress the growth of multidrug-resistant clinical pathogens. Conclusion: E. coli is the most common bacterial cause of UTIs in renal failure patients, these pathogens exhibited of multidrug resistance, molecularly supported by their carrying a number of beta-lactamase enzyme genes. However, L. casei, which is isolated from natural sources, is highly effective at inhibiting multidrug bacteria.

Keywords: Antagonism, L.casei, multidrug bacteria, UTIs, renal failure, β-lactamase.

INTRODUCTION

Urinary tract infections and interstitial nephritis are serious global health issues. Nephritis is characterized by both acute and chronic renal impairment; however, UTI is one of the most prevalent infections globally (Li et al., 2025). Urinary tract infections are microbial infections that impact the kidneys, bladder, ureters, and urethra, among other areas of the urinary system. Bacterial invasion and growth are the main causes of these illnesses (Putri et al., 2025). UTIs start when certain adhesions enable gut-resident uropathogens to colonize the urethra and then the bladder. Bacteria start to proliferate and produce toxins and enzymes that help them survive if the host's inflammatory response is unable to eradicate them altogether. If the pathogen penetrates the kidney epithelial barrier, further colonization of the kidneys may result in bacteremia (Zhou et al., 2023). Both Gram-positive and Gram-negative bacteria can cause UTIs, but the latter having the higher frequency rate of 74.4% of UTIs, Escherichia coli is the microbe that causes the majority of infections across all age groups and circumstances (Coutinho et al., 2021). They are deemed as causative agents of UTIs, with a consistent incidence of Gram-negative bacteria, in addition to E. coli, Streptococcus agalactiae, Klebsiella species, Pseudomonas aeruginosa, and Staphylococcus saprophyticus are all cause UTIs (Monroy-Higuera et al ., 2026). Antibiotic microbial resistance is a well-established and expanding issue in global healthcare systems. The predicted number of excess fatalities in the next 35 years is at least 300 million. Multi-drug resistant species, such E. coli that produce Extended Spectrum Beta-Lactamase, are challenging to treat, which leads to longer hospital stays and higher mortality rates(Ong et al., 2021). ESBLs are enzymes having significant hydrolyzing activity on a wide range of β-lactam antibiotics, including oxyminocephalosporins and aztreonam Other β-lactamases, including as TEM-type, SHV-type and CTX-M-type β-lactamases, have been found in this species in addition to class C cephalosporinase (Mirkalantari et al., 2020).

It is generally known that the use of excessive antibiotic contributes to the emergence of antibiotic resistance in microorganisms (Habboush et al., 2023). As a result, from ancient times, people have used dairy and fermented foods, which have been a known source of lactic acid bacteria (Ibrahim et al., 2024). Through the use of probiotics, and supplements, interventional studies have shown the potential of gut microbiota modification in enhancing kidney function. In patients with chronic kidney disease, formulations including, Lactobacillus casei, and Lactobacillus acidophilus have been demonstrated to decrease uremic toxins, lower serum levels of indoxyl sulfate, and lessen microinflammation (Liu et al., 2025). By shifting the composition of

the gut microbiota in favor of advantageous taxa like Subdoligranulum, Bifidobacteria, and Lactobacillus, these treatments improve estimated glomerular filtration rate and lower eprotein levels (Młynarska et al., 2024). The current aimed to test the antagonism activity of Lactobacillus casei against multidrug bacteria isolated from patients with renal failure.

METHODOLOG

Collection specimens:

Between September 2025 to March 2026. 208 samples of urine were collected from patients who suffer from renal failure and acute UTIs at Imam Hussein Teaching Hospital and Imam Hassan Al-Mujtaba Teaching Hospital in Karbala city. Urine samples were collected from the aforementioned groups using sterile cups and transferred to the laboratory in a short time.

Identification of bacterial isolates

Urine samples were cultured on the different media for isolating the UTI bacterial causative agents. Following culturing, all bacterial isolates were subjected to preliminary biochemical testing, which included growth. The VITEK 2 then used to accurately establish the identity of the bacteria isolates and the bacterial isolates were subjected to antibiotic susceptibility testing using the Vitek2 Compact System with the AST-GN222 card. All isolates were examined against 16 antibiotic agents related to 7 antibiotics classes.

DNA extraction and PCR technique:

A bacterial genomic DNA extraction kit manufactured by Geneaid was used to extract DNA from the bacterial isolates that were identified. PCR was utilized to detect the presence of four significant genes associated with antibiotic resistance: blaCTX-M, blaSHV, blaTEM, and the quinolone resistance gene qnrA. The reaction products were run over a 1.5% agarose gel using specific primers.

Table 1: The Reaction Conditions for each Tested Gene.

Gene.	PCR step	Temp.	Time	No. 0f cycles
	Initial denaturation	95	3 min	33 cycles
	Denaturation	94	30 sec	
bla SHV	Annealing	57	33 sec	
bla TEM		50		
CTX-M		58		
qnr A		58		
	Extension		55 sec	
	Final extension		5 min	

Isolated and Identification of Lactobacillus casei

L.casei were collected from a variety of food sources, including dairy products and aged cheese (ripened cheese). Then, samples were cultured on a selective medium (de Man Rogosa and Sharpe agar, MRS agar) and incubated anaerobically at 37°C for 48 h (Zhang et al., 2020). Following the incubation time, Microscopic examination was done Biochemical tests and Molecular detection.

Antagonism activity of L. casei against pathogenic bacteria

The inhibitory activity of Lactobacillus casei and its cell-free filtrate against antibiotic-resistant bacterial isolates was evaluated using the well diffusion method on Mueller-Hinton agar. Pathogens were diffused at a concentration of 0.5 McFarland, and 50 µL of the extract was added to 5 mm diameter wells, with two duplicates per sample. After the plates were incubated for 24 hours at 37°C, the inhibitory zone was clculated using a rule r(Divyashree et al., 2021).

Statistical analysis

Data were assessed for any significant differences using a one and two- way ANOVA, when possible. Results were presented as M ± S.D. The accepted level of significance was set at P ≤ 0.05, utilizing Mini Tab statistical software version 17 for the analysis, PA, USA, was used for conducting the statistical analysis.

RESULTS

Identification of bacterial isolates

Cultivated samples showed that 124 bacterial isolates were distributed into 83 (66.94%) from acute UTIs and 41(33.06%) from renal failure. Distribution of microbes showed that E. coli was 31 (37%), K. pneumoniae 28 (34%) and P. aeruginosa at (11%). The prevalence of Enterobacter cloacae and Morganella morganii accounted for 6% and 1%, respectively, whereas Gram-positive Staphylococcus spp. and Streptococcus spp. accounted for 6% and 5% of the cases, respectively. Identification of bacteria by culturing on Blood agar, MacConkey, Eosin Methylene Blue Agar and Cetrimide Agar. Microscopic examination was carried out using biochemical tests and API strip devices.

Antibiotic susceptibility testing (AST):

Antibiotics susceptibility of, The results of the susceptibility ESBL-producing strains of E. coli, K. pneumoniae, and P. aeruginosa testing showed a clear variation in resistance among the three bacterial species studied. For instance E. coli isolates exhibited high susceptibility to carbapenems (100%) for Ertapenem and Meropenem, with broad resistance to second-generation and third-generation cephalosporins ranging between 80% and 90%. Regarding the K. pneumoniae isolates demonstrated a more complicated resistance, with resistance to cephalosporins reaching 70% and ampicillin/clavulanic acid reaching 60%. Whereas, P. aeruginosa exhibited the highest levels of resistance, including total resistance to ampicillin/clavulanic acid, up to 40% resistance to carbapenems, and restricted sensitivity to current drugs such ceftazidime/avibactam.

Molecular Detection of genes encoded for Beta Lactamase Enzymes:

The data obtained from the PCR method for detecting beta-lactam resistance genes (bla SHV, bla TEM, bla CTX-M, and qnrA) in K. pneumoniae, E. coli and P. aeruginosa revealed significant differences among the species. K. pneumoniae isolates exhibited seven occurrences of the bla CTX-M gene, six carried the bla SHV gene, and only three contained the bla TEM gene Figure 1. In contrast, two isolates of P. aeruginosa were found to harbor both the bla TEM and qnrA genes, while bla CTX-M and bla SHV genes were identified in four isolates of P. aeruginosa Figure 2. The results of E. coli, showed that just four isolates were positive for the bla TEM gene despite thorough screening for all genes; the remaining genes were entirely absent from the remaining isolates of this species. As shown in Figure 3.

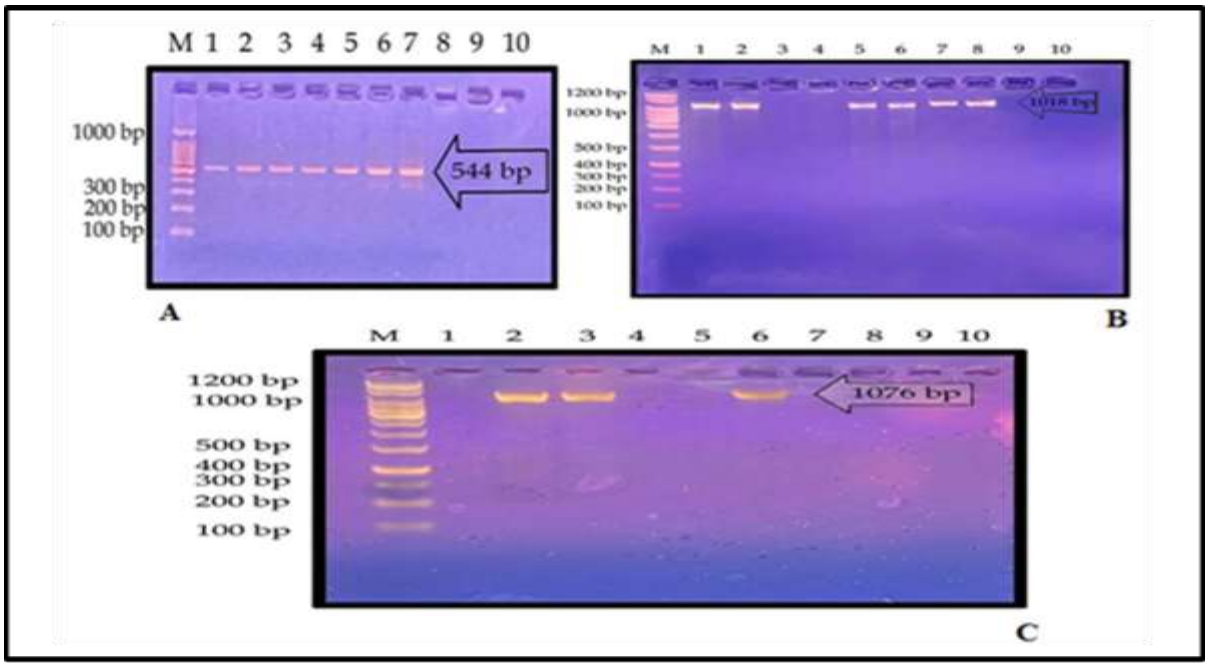

Figure1: Electrophoresis of PCR Amplicons of (A) blaCTX-M, (B) blaSHV and (C) bla-TEM from Klebsiella pneumoniae utilising a 1.5% Agarose Gel at 70 V. (Source: Authors)

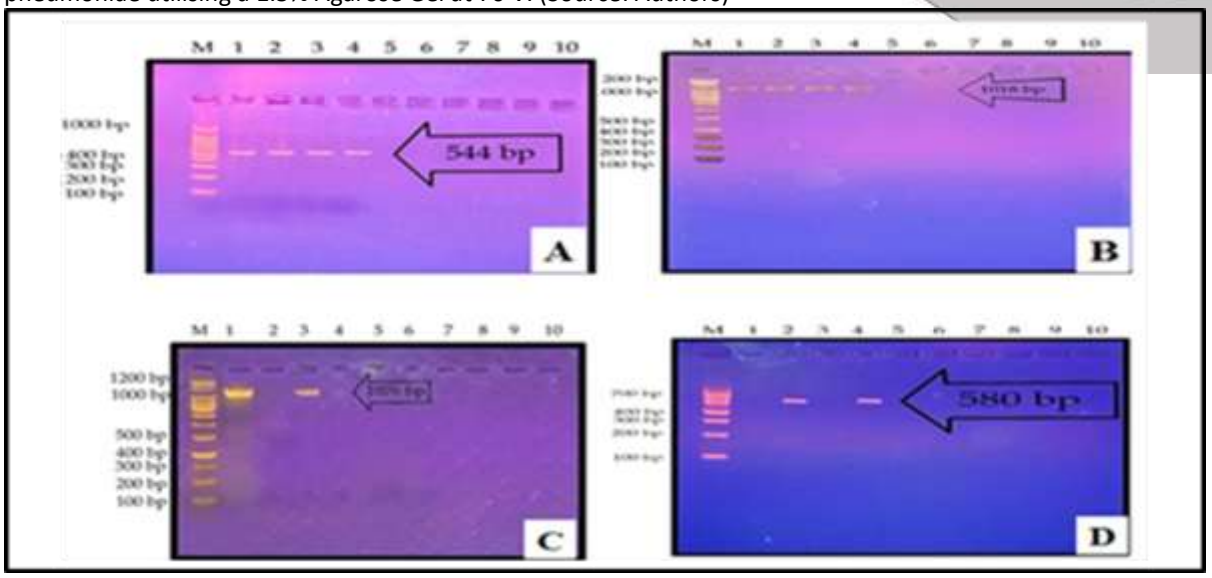

Figure 2: Electrophoresis of PCR Amplicons of (A) blaCTX-M, (B)bla SHV, (C)blaTEM and (D) qnrA genes from P. aeruginosa applying a 1.5% Agarose Gel at 70 V. (Source: Authors)

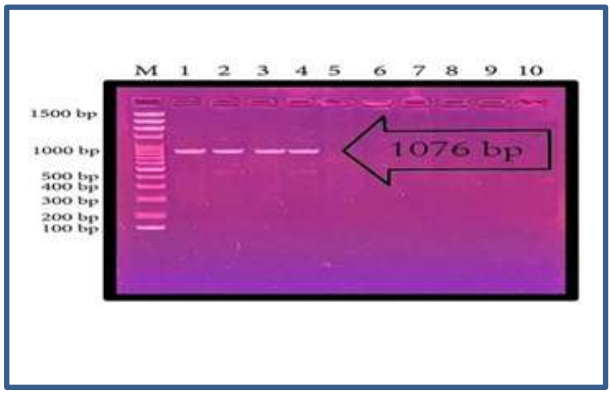

Figure3: Electrophoresis of PCR Amplicons of blaTEM from Escherichia coli utilizing a 1.5% Agarose Gel at 70 V. (Source: Authors)

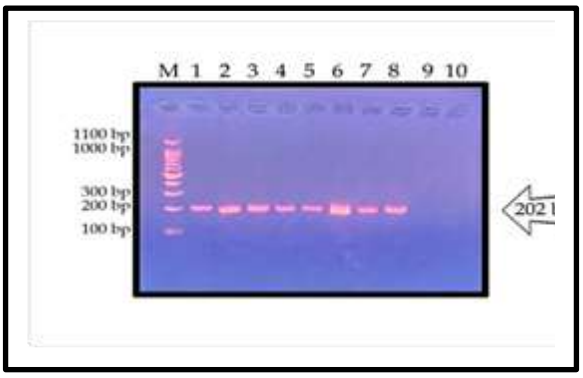

Figure4: Electrophoresis for PCR Amplicons of Diagnostic Gene from L.casei. (Source: Authors)

Diagnosis of Lactic Acid Bacteria (Lactobacillus casei)

Samples were cultured on a selective medium (de Man Rogosa and Sharpe agar, MRS agar) and incubated anaerobically at 37°C for 48 h. Following the incubation time, Microscopic examination was done Biochemical tests and Molecular detection as in Figure 4.

Antagonistic Activity of L. casei against Bacterial Pathogens

Antagonistic activity of Lactobacillus isolated from dairy products and aged cheese was evaluated using strains of L. casei against the isolated bacterial pathogen isolated from renal failure and acute UTIs. The findings demonstrated that L. casei isolates might inhibit the activity of multidrug-resistant clinical pathogens, such as Pseudomonas aeruginosa, Klebsiella pneumoniae, and Escherichia coli. On the other hand, statistical analysis did not show any significant differences ($P < 0.05$) attributable to the different sources of lactic acid bacteria isolation (cheese and Activia) or as a result of the different laboratory application methods used (cell-free supernatant method and direct method) against the bacteria isolates.

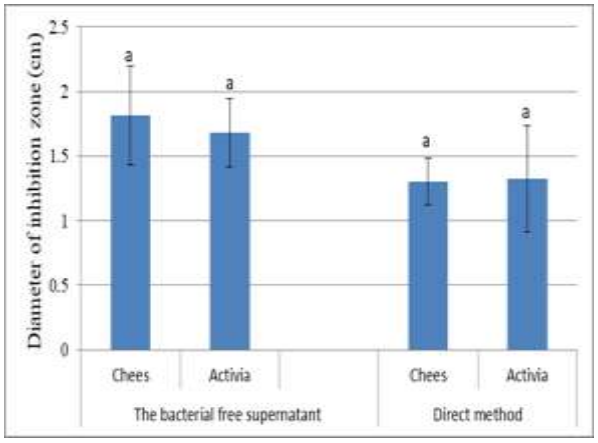

Figure5: The antagonism activity (the bacterial-free supernatantand direct method) of L. casei isolated from cheese and activia against P.aeruginosa identified from the urine samples. Columns having the same letters revealed no significant differences (P≥0.05). (Source: Authors)

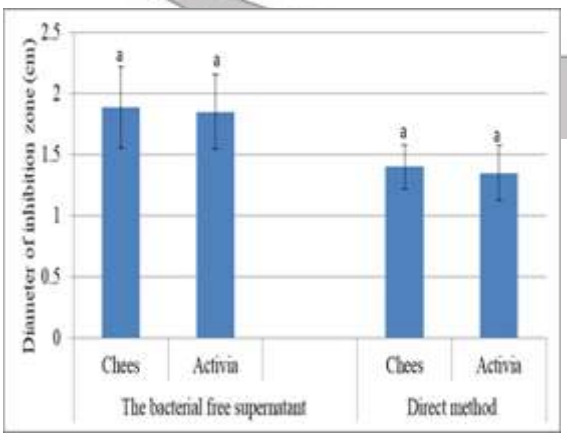

Figure 6 : The antagonistic activity of L. casei, isolated from cheese and Activia, against E. coli identified in urine samples was assessed using the cell- free supernatant and direct methods. Columns that share the same letters indicate no significant differences (P≥0.05). (Source: Authors)

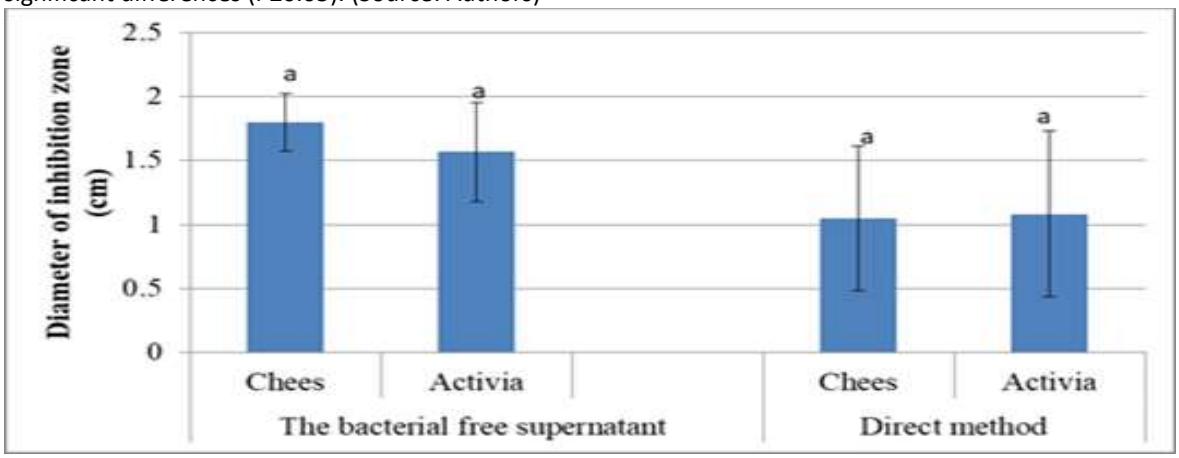

Figure 7 : The antagonistic activity of L. casei, isolated from cheese and Activia, against K. pneumoniae identified in urine samples, was assessed using both the cell- free supernatant and direct methods. Columns that share the same letters indicate no significant differences (P≥0.05). (Source: Authors)

DISCUSSION

Acute UTIs are a risk factor for infections in general due to the various immunological alterations described, and for UTIs specifically because of the comorbidities, advanced age and blockage of the urinary tract. However, E. coli is the most frequent cause of UTIs in this group of patients, and those with diabetic nephropathy, nephrotic syndrome, autosomal dominant polycystic disease, urolithiasis, and immunosuppressive medication appear to be at the most risk (Dicu-Andreescu et al., 2022). The findings of this study indicate that patients with chronic kidney disease (CKD) may be at a greater risk for infections, such as urinary tract infections, compared to individuals without the condition, which is consistent with the findings of Elsayed et al. (2025), demonstrated that E. coli was the most common pathogen in UTIs among our patients with CKD, followed by K. pneumoniae and other Gram-negative organisms. The most frequent cause of UTIs is uropathogenic E. coli. Changes in colonization, attachment, invasion, and intracellular replication to infiltrate the urothelium and survive intracellularly are characteristics of the adaptive evolution of UPEC (De Gaetano et al., 2023). The outer membrane of Gram-negative bacteria contains lipopolysaccharides, commonly known as endotoxins, particularly in pathogenic strains like K. pneumoniae. These components, along with a substantial extracellular polysaccharide layer called a capsule, play a crucial role in protecting the bacteria from the innate defenses of the host (Abbas et al., 2024) . Antibiotics susceptibility of ESBL-producing strains of E. coli isolates exhibited high susceptibility to carbapenems (100%) for Ertapenem and Meropenem, with broad resistance to second- and third-generation cephalosporins ranging between 80% and 90%.

Supporting this observation, ESBL-producing strains of E. coli exhibited very high resistance rates across most beta-lactams and cephalosporins; isolates were resistant to ceftriaxone and cefuroxime (Mir et al., 2025). While K. Pneumoniae isolates demonstrated a more complicated resistance, with resistance to cephalosporins reaching 70% and ampicillin/clavulanic acid reaching 60%. This finding is conceptually consistent with the local study conducted in Baghdad, Iraq Razooqi Al-Aajem et al., (2021) where K. pneumoniae exhibited the highest levels of resistance to Cefepime (86.11%), Cefazidime (80.56%), Piperacillin (75.0%), and Aztreonam (69.44%). In contrast to the previous findings, 90% of the isolates showed complete susceptibility to gentamicin, while 80% were susceptible to amikacin and trimethoprim/sulfamethoxazole. Additionally, 70% of the isolates were susceptible to ertapenem, meropenem, and fosfomycin, whereas 60% exhibited sensitivity to imipenem, nitrofurantoin, and ciprofloxacin. The present study demonstrated resistance to cephalosporins among all isolates, confirming the findings of Cristea (2017). This study reported high resistance levels in Klebsiella spp. strains tested against cephalosporins, with 71.43% resistance to cefazoline and 75%resistance to cefuroxime. Resistance levels staying below 20%, Imipenem 84.5% and Amikacin 79.6% had the highest susceptibility rates indicating their high efficacy against K. pneumoniae. Moreover, Piperacillin and Meropenem had moderate activity (around 70% susceptibility). However, P. aeruginosa demonstrated the highest levels of resistance, exhibiting total resistance to ampicillin/clavulanic acid, up to 40% resistance to carbapenems, and limited sensitivity to current drugs such as ceftazidime/avibactam. The present findings align with Dadjo et al. (2025), who demonstrated that P. aeruginosa isolated from patients with chronic kidney disease exhibited a significantly higher ciprofloxacin resistance rate. Efflux-mediated resistance to quinolones is well known. The most frequent mechanism of high-level resistance is caused by chromosomal mutations within a brief DNA sequence known as the quinolone resistance-determining regions in one or more of the genes encoding the type II topoisomerases for both Gram-positive and Gram-negative organisms (Miranda et al., 2022).

The results showed that the three isolates of K. pneumoniae were carried bla-TEM compared to 7 and 6 isolates were possess that bla-CTX-M and bla-SHV, respectively. β-lactamases, specifically the CTX-M-type extended-spectrum β-lactamases, represent a unique and rapidly expanding gene family. Mobile genetic elements, such as ISEcp1, facilitate the mobilization of the bla-CTX-M genes, transferring them to conjugative plasmids. This process contributes to the widespread distribution of these genes among various bacterial species. This transfer not only enhances the spread of antibiotic resistance among bacterial populations but also poses a significant challenge to public health. As these genes proliferate, the effectiveness of β-lactam antibiotics diminishes, leading to treatment failures and increased morbidity (Yu et al., 2024). However, two isolates of P. aeruginosa were identified to carry the bla-TEM and qnrA genes, while the blaCTX-M and blaSHV genes were detected in four isolates of P. aeruginosa. The primary cause of P. aeruginosa resistance to antibiotics, mainly cephalosporins and penicillin, is the ESBLs. The main ESBL genes responsible for this kind of resistance are blaCTX-M (Castanheira et al., 2021). Furthermore, bacterial microevolution may be caused by the accumulation of mutations in their DNA. Under these circumstances, strains are more likely to emerge as new bacterial variants that alter the expression of some virulence factors and increase the profile of antibiotic resistance. These bacterial variants may also exhibit novel cellular and metabolic morphologies that improve their chances of surviving in harsh environments (Flores-Vega et al., 2025). Conversely, four isolates of E. coli, showed were positive for the bla TEM gene despite thorough screening for all genes; the remaining genes were entirely absent. Notwithstanding their broad occurrence, the blaCTX-M and bla-SHV genes were completely absent from the remaining investigated isolates. These results clearly indicate that these specific resistance genes may not be universally prevalent among all strains, indicating potential variations in antibiotic resistance profiles. According to the study conducted by Ahmed and Hawezy (2023), some isolates may appear to be resistant to antibiotics without actually having these genes because they rely on complementary mechanisms such as active expulsion or altering cell membrane permeability, which supported the present results**.** Similarly, Goudarzi et al. (2015), found that none of the bacterial isolates had qnrA or qepA and qnrB was the most prevalent qnr gene**.**

These results highlight a concerning trend in antibiotic resistance, particularly in clinical settings where these pathogens are frequently encountered. Addressing this issue will require on going surveillance and the development of alternative treatment strategies to effectively manage infections caused by resistant strains.

The current findings closely agree with those of Fateh et al. (2024), which demonstrate that the average diameter of the inhibition zone produced by L. casei isolates against E. coli bacteria was 19.8 mm using the agar well diffusion method. As a researcher confirmed by Algroom (2023), L. casei successfully suppressed the E. coli pathotype. Probiotics have been shown in numerous studies to reduce the growth of a variety of illnesses. Due to live cells being able to stick to each other, they can block pathogenic microbes from colonizing them, thus preventing superiority (Alshatari and Ziarno, 2026). L. casei inhibits K. pneumoniae that is resistant to multiple drugs and was isolated from urinary catheters. L. casei also creates additional metabolites that

inhibit other bacteria in addition to compounds that resemble bacteriocin (Reza, 2019). The antimicrobial activity of the CSF from L. casei, which showed growth inhibition against strains E. coli and K. pneumoniae are in agreement with the present data (Rocha Ramírez et al., 2023). Biofilm formation and dispersion, particularly in the presence of oxidative elements like hydrogen peroxide, alongside the potential presence of proteins with bacteriocin, have been reported to contribute to the generation of organic acids. This may frequently explain the inhibitory activity of Lactobacillus strains against E. coli. Proteinaceous substances like bacteriocin and lactic, acetic, and propionic acids play a crucial role in inhibiting the growth of pathogenic bacteria (Divyashree et al., 2021). The growth of P. aeruginosa is significantly inhibited by CFS from L. casei strains. Hydrogen peroxide (H_2O_2) can act as a precursor to the production of bactericidal free radicals, such as superoxide (O_2^-) and hydroxyl (OH^-) radicals, which can damage DNA, and peroxidation of membrane lipids increases the permeability of the membrane. Lactobacillus casei is a probiotic bacterium that balances intestinal microbiota, controls intestinal diseases and affects the cellular immune response (Qin et al., 2022). Probiotics reduce pathogenic adherence to mucosal surfaces and colonization, hence competitively eliminating opportunistic pathogens. Probiotics' antibacterial action, which is provided by their metabolic byproducts (Aditya et al., 2020).

CONCLUSION

Different genera and species of the microbial community were identified, notably with a prevalence of E. coli. Moreover, most bacterial isolates were found to be resistant to various antibiotics, in particular P. aeruginosa, necessitating alternative treatments to manage their growth. Additionally, there is variation in the results obtained from both the phenotype and genotype methods. This suggests that there may be additional genes not explored in the current study that could be responsible for the antibiotic resistance observed in these bacteria. In this particular context, Lactobacillus casei revealed strong antagonism against most of the tested bacterial pathogens, highlighting its potential application in new functional probiotic products for treating these pathogens.

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Contribution of the author: Each author made an equal contribution to the research.
Conflict of interest: non

Ethical Approval

Before each patient was included in the study, informed consent was acquired from both the hospital administration and the patients themselves. To guarantee complete comprehension of the process, all procedures were thoroughly described to the patients or their guardians prior to sample collection. Patients had the option to decline participation in the study or refuse sample collection, and participation was completely optional.

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