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Antagonistic assessment of lactic acid bacteria against urinary tract pathogens

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Abstract

Urinary tract Infections (UTIs), is a common and significant global health issue which can lead to increased morbidity and healthcare cost. Lactic acid bacteria (LAB) have been known for their ability to inhibit microbial pathogens. However, there is a growing concern of antibiotic resistance in bacteria strains of food origin and its possible transmission through functional foods. In this study, antagonistic activities of LAB from *Ogi* (a fermented cereal gruel) and *Kunu* (a non-alcoholic fermented beverage) were demonstrated against clinical uropathogens. Potential probiotic LAB were also screened for their phenotypic antibiotic resistance profiles. LAB isolates were isolated and characterized. The antagonistic effect of the LAB isolates against the pathogens was determined by the agar well diffusion technique. The LAB isolates were examined for antimicrobial susceptibility with commercially available antibiotic discs, using the agar disc diffusion method. In total, 32 LAB isolates were isolated from the *ogi* and *kunu* samples. Using the agar well diffusion assay, a highest zone of inhibition of 12mm was exhibited by three respective LAB isolates from *ogi* samples against a *Klebsiella* spp. compared to a highest inhibitory zone of 9mm by a LAB isolate from *kunu* sample against an *E. coli* isolate. Twelve different antibiotic resistant phenotypes were expressed by the isolates of LAB examined. Our study shows LAB isolates to possess in vitro antimicrobial properties and the importance of identifying the presence of antibiotic resistant isolates in fermented foods to avoid the microbiological hazards link with horizontal transfer of antibiotic resistance genes.

Keywords: LAB; UTIS; Antibiotic Resistance; Antagonistic Activities

1. Introduction

Urinary tract infections (UTIs) can be regarded as a prevalent infection, affecting both men and women. Around 50–60% of women will experience a UTI in their lifetime (Al-Badr and Al-Shaikh, 2013). Furthermore, women are at increased risk of recurrent UTIs due to imbalanced microbiota in the reproductive system and anatomical differences (Uehara et al., 2006, Salvatore et al., 2011). A urinary tract infection (UTI) refers to infections that can develop in any area of the urinary system, including the kidneys, ureters, bladder, and urethra (Flores-Mireles et al., 2015). Clinical symptoms associated with urinary tract infections (UTIs) include dysuria, which is a burning sensation during urination, increased urinary frequency, occasional tenderness in the suprapubic area which can be indicative of cystitis or urethritis, flank pain, fever, and urgent urination which is associated with pyelonephritis (Bono and Leslie, 2025). Urinary tract infections (UTIs) are the second most prevalent infections caused by bacteria with *Escherichia coli* as the most frequent bacteria known to trigger UTIs and are responsible for about 90% of UTIs (Flores-Mireles et al., 2015). Other microbes, such as *Klebsiella species, Proteus mirabilis,* and *Staphylococcus saprophyticus* can also cause UTIs (Schaeffer, 2012).

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The rise of resistance, detrimental consequences of antibiotics, and other connected issues have led to the search for alternate techniques to managing urinary tract infections. Natural methods have been widely utilized for the therapy of many ailments to lower symptoms and enhance overall health (Fazly Bazzaz *et al*., 2021). One such approach is the implementation of probiotics through the use of lactic acid bacteria (LAB) found in varieties of fermented foods (Tamang and Kailasapathy, 2010). Lactic acid bacteria (LAB) are a type of microorganism which ferment carbohydrates to manufacture lactic acid (Wang *et al*., 2021). They are diverse collection of bacteria that contribute significantly to a range of fermentation procedures (Adesemoye et al., 2025). LAB are cocci shaped or rod shaped bacteria with high tolerance for a lower pH (Wang *et al*., 2021). These microorganisms can help to successfully compete with, and inhibit the growth of the uropathogens that cause urinary tract infections. Some lactic acid bacteria have been shown to possess antagonistic activity against many enteric and urinary tract bacterial pathogens (Ayeni et al., 2009, Mokoena et al., 2017). This activity is believed to be due to the production of various metabolites like bacteriocins, which are proteins or peptides with antimicrobial properties, hydrogen peroxide, organic acids etc (Mastromarino *et al*., 2002, Hao et al., 2004, Line et al 2008, Bamidele et al., 2013). These substances can hinder the proliferation of other bacterial species, thus protecting the host against infection (Line *et al*., 2008, Coda *et al*., 2011).

Although LAB are generally regarded as safe (GRAS) and can also be used as probiotics in food and pharma formulations, they should demonstrate absence of virulence factors such as transferable antibiotic resistance genes (Saarela et al., 2000, Ayeni et al., 2009, Sanders et al., 2010). Consuming probiotics helps balance the gut's commensal microorganisms by outcompeting pathogens for binding sites and nutrients, which can alleviate constipation and other gastrointestinal issues (Petrariu et al., 2024). However, there is a growing concern of antibiotic resistance in bacteria strains of food origin and its possible transmission through functional foods.

This study aimed to demonstrate the antagonistic activities of LAB from *Ogi* (fermented cereal gruel) and *Kunu* (a non-alcoholic fermented beverage) against clinical uropathogens. Potential probiotic LAB were also screened for their phenotypic antibiotic resistance profiles.

2. Materials and methods

In this study, Two (2) different varieties of the fermented cereal gruel "*Ogi*" were obtained from Okada market in Okada Edo State, Nigeria. "*Ogi*" made from white maize (*Zea mays*) and brown "*ogi*" made from Guinea corn (*Sorghum vulgare*) was obtained for this study. Yellow corn (Zea mays) was obtained from Okada market and brought to the laboratory. The method described by Afolayan et al., 2017 was used in preparing a different variety of the fermented cereal gruel, "*ogi*" using the yellow corn obtained. A bottle of *Kunu* sample was also obtained from a selected retail *Kunu* seller at the Hausa quarters from Okada market in Okada Edo State, Nigeria for this study. The non-alcoholic fermented beverage was also prepared using the method described by Sowemimo et al., 2021 with slight modifications. Both of the beverage samples were used in the study.

2.1. Lactic acid bacteria (LAB)

2.1.1. Isolation and Phenotypic Characterization of LAB

The LAB isolates were isolated from *Ogi* (a fermented cereal gruel) and Kunu (a non-alcoholic fermented beverage) and characterized using previously described standard microbiology procedures with slight modifications (Ayeni et al., 2009). One gram of the each of the three fermented cereal gruel varieties obtained were inoculated into 9ml of sterile MRS broth (ReadyMED) respectively and homogenized before incubating in a microaerophilic condition for 24hrs. For the *Kunu* (a non-alcoholic fermented beverage) sample, 1ml of the samples obtained was added to 9ml of sterile MRS broth (ReadyMED) respectively and homogenized before incubating in a microaerophilic condition for 24hrs. From appropriate tenfold serial dilution made for each sample, growth of LAB was aseptically determined by inoculating in MRS agar (ReadyMED) by spread plate method. The plates were incubated under microaerophilic conditions for 48hrs. Distinct colonies were picked aseptically from the plates based on their differences in morphology on MRS agar (ReadyMED) plates and isolated by streaking on MRS agar (ReadyMED) plates. Identified pure cultures were characterized based on colony and cell morphology, Gram-staining reaction, catalase, citrate, urease, indole, methyl red-voges proskauer and oxidase activity. Isolates identified as LAB were stored at -20°C in MRS broth (ReadyMED) with 50% (w/v) glycerol for further studies

2.1.2. UTI pathogens

UTI pathogens (*Escherichia coli* and *Klebsiella* spp.) were collected from the Medical Microbiology laboratory of the University of Benin teaching hospital to carry out the susceptibility test in the study. The isolates were pre-identified; hence, research ethics approval was not required. No contact was produced with patients and the original samples from

the hospital. Informed consent was not required by the institution. The isolate data were obtained from clinical records and anonymously handled. Isolates were sub-cultured on Mac conkey agar plates to obtain pure colonies. Previously described standard microbiological techniques (Cheesebrough 2006) were used for species identification. All the isolates were maintained on nutrient agar slants at 4°C.

2.2. Antagonistic activity

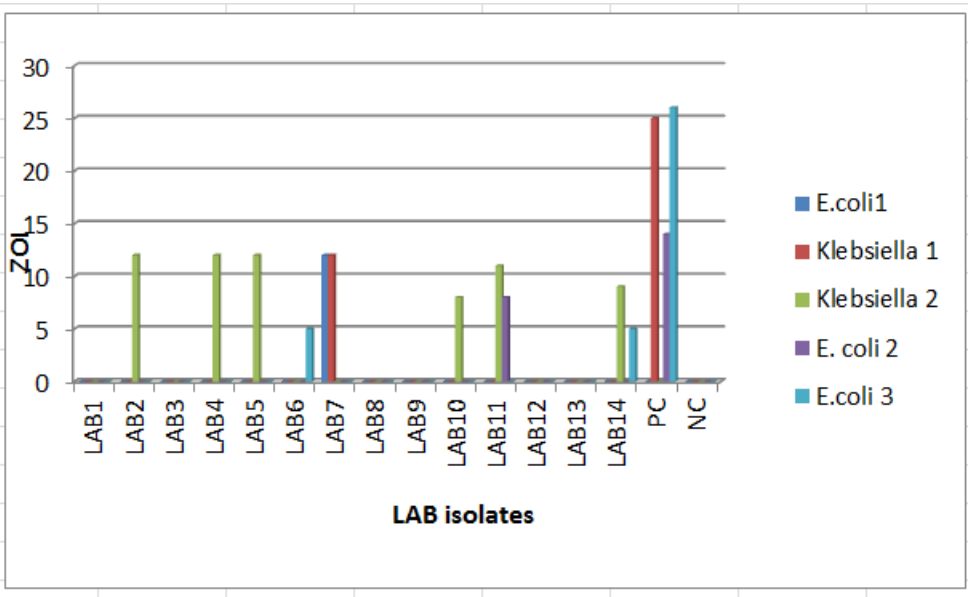
The antimicrobial effect of the LAB isolates against the pathogens was determined by the agar well diffusion technique (Bamidele et al., 2013). Cell free supernatants (CFS) of each isolates were prepared by centrifuging LAB culture obtained at 3000rpm for 15minutes and tested against clinical uropathogens (*Escherichia coli* and *Klebsiella* spp.). A volume of 100µl of the CFS of the LAB broth culture samples and control (10µg gentamicin) was introduced in Mueller Hinton agar plated seeded with UTI pathogens. The CFS was inoculated in holes made on the Mueller Hinton agar plates using a sterile cork borer, 6mm diameter. Plates were incubated at 37°C for 24hr under aerobic condition and zones of inhibition measured in millimeter.

2.3. Antibiotic susceptibility test

LAB were also screened for their phenotypic antibiotic resistance profiles using the disc diffusion method (Bauer et al., 1966). High-profile commercially available Gram-positive antibiotic discs (Celtech diagnostic) were used for the susceptibility testing. Nine antibiotics of varying concentrations in a multidisc which included, ciprofloxacin (5 µg), cefuroxime (30 µg), augmentin (30 µg), gentamycin (10 µg), erythromycin (5 µg), ofloxacin (30 µg), cefixime (5 µg), levofloxacin (5 µg), and azithromycin (15 µg) was employed. Results were interpreted based on standards previously described by Charteris et al., (1998).

3. Results

In total, Thirty (30) LAB isolates were isolated from the *ogi* and *kunu* samples based on their typical morphological appearance (small pinpointed and creamy white colonies), Gram-positive, catalase, citrate, urease, indole, methyl red-voges proskauer and oxidase test negative and non-motile, coccus and rod-shaped characteristics. Using the agar well diffusion assay, a highest zone of inhibition of 12mm was exhibited by three respective LAB isolates from *ogi* samples against a *Klebsiella* spp. compared to a highest inhibitory zone of 9mm by a LAB isolate from *kunu* sample against an *E. coli* isolate (Figure 1 and 2).

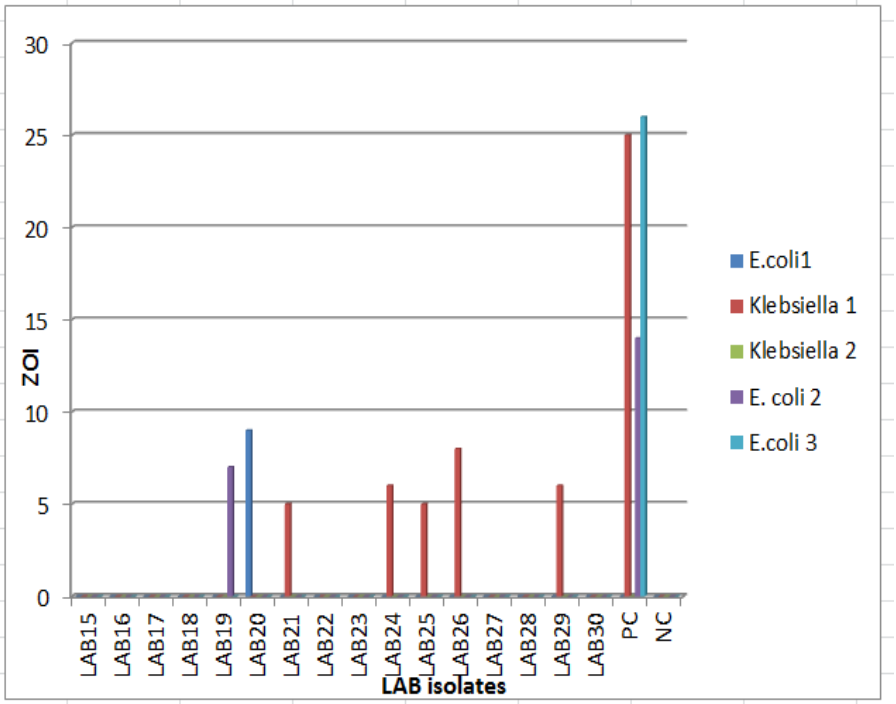


Key: ZOI- Zone of inhibition in mm, PC- Positive control, NC- Negative control

Figure 1 Antagonistic activity of LAB isolates from "*Ogi*" samples against UTI pathogens

The antimicrobial susceptibility of *Lactobacillus* species is presented in Table 1. A majority (8/15, 53%) of *Lactobacilli* studied were susceptible to cefuroxime while only two isolates (13%) were susceptible to the third-generation, broad-spectrum cephalosporin, cefixime. Only one isolate displayed moderate susceptibility to augmentin (amoxicillin/clavulanic acid). All *Lactobacilli* (100%) were resistant to the aminoglycoside, gentamicin studied. Most

Lactobacilli isolates (93%, 14/15) were resistant to ciprofloxacin and ofloxacin while 33% (5/15 isolates) were moderately susceptible to levofloxacin. A high number of isolates (73%, 11/15) were sensitive to erythromycin while 40% (6/15 isolates) were sensitive to azithromycin. Ten different antibiotic resistant phenotypes were expressed by the fifteen isolates that demonstrated antagonistic activity against UTI pathogens (Table 2). Generally, isolates from the "*kunu*" samples were resistant to more antibiotics than those from the "*ogi*" samples (Table 1 and 2).



Key: ZOI- Zone of inhibition in mm, PC- Positive control, NC- Negative control

Figure 2 Antagonistic activity of LAB isolates from "*Kunu*" samples against UTI pathogens

Table 1 Antibiogram of the LAB isolates

Isolates	Cxm 30µg	Gen 10µg	Zem 5µg	Lbc 5µg	Cip 5µg	Ofx 5µg	Azn 15µg	Aug 30µg	Ery 15µg
LAB2	25(S)	12(R)	12 (R)	14(MS)	R	26(S)	27(S)	R	22(S)
LAB4	23(S)	6(R)	R	14(MS)	R	R	R	R	24(S)
LAB5	23(S)	12(R)	R	17(MS)	R	R	R	R	R
LAB6	24(S)	R	15(MS)	14(MS)	17(MS)	R	20(S)	R	25(S)
LAB7	20*(S)	6(R)	14(R)	11(R)	3(R)	R	21(S)	R	15(S)
LAB10	R	R	R	R	R	R	R	R	R
LAB11	19(S)	12(R)	19(S)	R	R	R	19(S)	R	20(S)
LAB14	22(S)	R	11(R)	14(MS)	R	R	8(R)	R	10(R)
LAB 19	R	R	R	R	R	R	18(S)	R	14(MS)
LAB 20	R	R	R	R	R	R	R	19(MS)	18(S)
LAB 21	R	R	R	R	R	R	R	R	14(MS)
LAB 24	R	R	R	R	R	R	R	R	R
LAB 25	R	R	R	R	R	R	R	R	18(S)
LAB 26	R	R	R	R	R	R	R	R	14(MS)
LAB 29	16(MS)	R	R	R	R	R	14(MS)	R	18(S)

Key R- Resistant MS- Moderately susceptible S- Sensitive *zone of inhibition in mm

Table 2 Resistance phenotype of the LAB isolates

Isolates	Antibiotics	Number
LAB2	Cip, Aug , Gen, Zem	4
LAB4	Zem ,Cip, Ofx ,Azn ,Aug ,Gen	6
LAB5	Zem, Cip ,Ofx ,Azn, Aug, Ery, Gen	7
LAB6	Gen, Ofx,Aug	3
LAB7	Ofx ,Aug, Gen, Zem, Lbc, Cip	6
LAB10	Cxm, Gen, Zem, Lbc, Cip, Ofx, Azn, Aug ,Ery	9
LAB11	Lbc ,Cip ,Ofx ,Aug, Gen	5
LAB14	Gen ,Cip ,Ofx ,Aug, Zem, Azn ,Ery	7
LAB 19	Cxm, Gen, Zem, Lbc, Cip, Ofx, Aug	7
LAB20	Cxm, Gen, Zem, Lbc, Cip, Ofx, Azn, Aug	8
LAB21	Cxm, Gen, Zem, Lbc, Cip, Ofx, Azn, Aug	8
LAB 24	Cxm, Gen, Zem, Lbc, Cip, Ofx	6
LAB 25	Cxm, Gen, Zem, Lbc, Cip, Ofx, Azn, Aug ,Ery	9
LAB 26	Cxm, Gen, Zem, Lbc, Cip, Ofx, Aug	7
LAB 29	Gen, Zem, Lbc, Cip, Ofx, Aug	6

Key: Cxm – cefuroxime, Gen –gentamicin, Zem – cefixime, Lbc - levofloxacin Ofx - ofloxacin, Azn - azithromycin, Aug – Augmentin, Ery – erythromycin, Cip- Ciprofloxacin

4. Discussion

In Nigeria, where there are limited probiotic preparations on the market, the medicinal advantages of fermented foods can be explored in regards to the size and composition of LAB. Previous study confirms this discovery, stating that *Lactobacillus* species are more commonly found in fermented foods and have considerable health advantages (Holzapfel et al., 1998; Adesulu-Dahunsi et al., 2022). Among the characteristics of probiotics is the abundance of viable cells in sufficient amounts to confer health advantages (Afolayan et al., 2017).Our study shows LAB isolates isolated from the different food sources to possess in-vitro antimicrobial properties. The majority of isolates that were found belonged to the *Lactobacillus* species, which are well-known for their potent probiotic qualities and capacity to generate antimicrobial compounds. According to their morphological properties most of the isolates were gram positive bacilli or cocci which is a common characteristic of *lactobacillus* species. All isolates were indole, catalase, oxidase, methyl red, voges proskauer, citrate, urease negative which is also common to *lactobacillus* species and correlates with the result obtained by (Eji et al., 2023).

The current investigation assessed each isolate's cell-free supernatant's inhibitory potential. The isolated strains of lactic acid bacteria (LAB) were examined for their ability to inhibit UTI pathogens and their zones of inhibition which vary in diameter were noted. On the test organisms, a few of the isolated LAB displayed notable zones of inhibition (Figure 1 and 2). The zones of inhibition in the agar well diffusion studies demonstrated that LAB isolates produced possible pathogen-inhibiting chemicals such as organic acids, hydrogen peroxide, and bacteriocins. This is consistent with previous research, which found similar antibacterial activities in LAB isolated from diverse fermented foods (Ayeni et al., 2009, Afolayan et al., 2017). By producing lactic acid and other antimicrobial substances, LAB can lower pH levels and make environments less conducive to pathogen growth and survival. Reducing harmful bacteria is necessary for preserving urinary tract health, which makes this process important for preventing UTIs (Ouwehand et al., 2002, Kincses et al., 2021, Fayezi et al., 2025).

Fifteen *Lactobacillus* spp. isolates were assessed for their susceptibility to nine antibiotics. They exhibited varying levels of sensitivity to the antibiotics with all isolates showing 100% resistance to gentamicin. This agrees with previous studies where very little or no inhibition of *Lactobacillus* spp. by aminoglycosides was documented (Sharma et al., 2017, Duche et al., 2023). *Lactobacilli* have been mostly resistant to aminoglycosides (Zhou et al., 2005, Dec et al., 2018), while the aminoglycoside resistance phenotype has been reported as intrinsic; being mainly ascribed to two key factors: bacterial cell surface' low permeability to aminoglycosides, and the absence, in *lactobacilli*, of elements of cytochrome-mediated electron transport (Duskova et al., 2020). Resistances to flouroquinolones are also generally intrinsic to *lactobacilli* (Li et al., 2015, Sharma et al., 2016, Campedelli et al., 2018). In this study most *Lactobacillus* spp were

sensitive to erythromycin and resistant to ofloxacin and ciprofloxacin with 33% (5/15) of the isolates showing moderate susceptibility to levofloxacin (Table 1). This contrast with previous reports of Danielsen and Wind, 2003 and Duche et al., 2023 that reported that *lactobacilli* from food sources are more susceptible to ciprofloxacin and gatifloxacin than those from human sources. Isolates in this study were from food sources which showed high level of resistance to the flouroquinolones. Sensitivity of the isolates to erythromycin in this study compares with a previous study (Anisimova et al., 2022) but notably 60% (9/15) isolates were resistant to azithromycin. A previous report reported high-level resistance to macrolides among *lactobacilli* strains, with one-third of their isolates showing resistance to macrolides (Drago et al., 2011). Duche et al., 2023 reported resistance towards amoxiclav varied based on species with much higher resistance among *Lacticaseibacillus casei* (91.7%), than among *L. brevis* (45.5%) and *L. paracasei* (33.3%) strains. In this study, almost all isolates were resistant to Augmentin. The high level of resistance demonstrated by *Lactobacilli* to the third-generation, broad-spectrum cephalosporin, cefixime confirms previous findings (Charteris et al., 1998). Although the mechanisms of the *lactobacilli* resistance to cephalosporins remain limited, general processes, such as natural presence of broad-spectrum β-lactamases and/or non-specific multidrug transporters/cell wall impermeability, have been proposed as possible explanations (Delgado et al., 2005, Pfeifer et al., 2010). Antibiotic resistance to three or more antibiotics was demonstrated by all *Lactobacillus* species, indicating multi-drug resistance. It is important to consider this when selecting a probiotic since pathogenic bacteria can acquire antibiotic resistance genes from probiotic bacteria (Esezi-ovila et al., 2023). Recently, interest in LAB and their antibiotic resistances has increased in recent years, due to the observed horizontal transmission of antibiotic resistant determinants that can be transferred between bacterial species and also from beneficial bacteria to pathogens (Devirgiliis et al., 2013). The determination of antibiotic resistance of LAB as the main microbial community in fermented products is highly important and necessary in terms of identifying and understanding mechanisms of persistence and spread of resistance genes in the microbial world (Mathur and Singh, 2005). Resistance in LAB is enhanced by the large numbers of LAB in fermented products and in the gastrointestinal tract, but also from other bacteria in the environment. Once a LAB becomes resistant, amplified determinant can be transmitted to another host. Therefore, checking for signs of transferable antibiotic resistance in starter strains and bacteria used as feed and food additives is essential.

Antibiotic resistance can be "intrinsic" or "acquired". Intrinsic or natural resistance is inherent to a bacterial species and involves chromosomally coded different resistance mechanisms such as absence of the target, low cell permeability, antibiotic inactivation and the activity of an efflux system. The acquisition of antibiotic resistance is generated via the mutation of a pre-existing gene or by horizontal transmission. As intrinsic resistance and resistance by mutation are unlikely to be disseminated, so the risk is characterized by horizontally transferred genes, especially those carried on mobile genetic elements (Normark and Normark, 2002). Therefore, distinction between natural and acquired antibiotic resistance among the population of LAB is of a great importance, since only acquired resistance has the potential of being transferred (Galgano et al., 2025). The varying susceptibility result of the LAB isolates correlates with a previous report where Gfeller et al., (2003) reported the presence of potentially transferable genes conferring resistance to one or more of the antibiotics including penicillin, erythromycin, amoxicillin and tetracycline in several members of LAB. Thus, the presence of any specie resistant to many of these antibiotics among our isolates was an indication of the possibility that these populations possessed or acquired the resistance gene. Another previous report showed that lactic acid bacteria, the predominant microflora in fermented dairy products, may act as reservoirs of antibiotic resistant genes potentially transferable to human pathogens (Mathur and Singh, 2005).

Intrinsic resistance of lactic acid bacteria to many antibiotics may be considered as advantageous for those isolates with probiotic potential. Such resistance could be helpful for sustainable utilization of the strains in human intestine to maintain the natural balance of intestinal microflora during antibiotic therapy (Ketema et al., 2009). However, there is the danger of transferring multiple drug resistance to pathogens in the intestinal environment. Susceptibility of some of our LAB isolates to clinically important antimicrobials is beneficial as it minimizes the chances of disseminating resistance genes to pathogens both in the food matrix and/or in the gastrointestinal tract. Care should be taken in the use of LAB isolated from traditional fermented foods for the enhancement of consumer's health. The high rate of resistance calls for periodic monitoring of the level of drug resistance of LAB isolates.

5. Conclusion

This study shows that there are notable populations of lactic acid bacteria present in "*ogi*" and "*kunu*" with potent antagonistic activity against UTI pathogens which implies that they may be a good source of probiotic with potential use in the treatment of urinary tract infections. This study also highlights the importance to identify the presence of antibiotic resistant isolates in fermented foods to avoid the microbiological hazards link with horizontal transfer of antibiotic resistance genes. This study shows and validates other reports that documents the presence of antibiotic resistance genes in probiotic, as well as food-associated strains of lactobacilli (Duskova et al., 2020, Duche et al., 2023, Wang et al., 2019, Yang et al., 2022). After phenotypic resistance to antibiotics is observed, further research is necessary

to identify the molecular basis of such resistance; especially in food-associated microbial strains, to establish the possible transmissibility of such resistance.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper

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