Gut Microbiome derived Lactic Acid Bacteria (GM-d-LAB) from Dwarf Goats (*Capra aegagrus hircus*) inhibit Residence Multiple Antibiotics Resistance Bacterial pathogens.

Abstract

Background: The global health threat posed by antimicrobial resistance (AMR) has created an urgent need for developing alternative treatment methods. Probiotics, especially Lactic Acid Bacteria (LAB), are gaining interest in this context, as they demonstrate health-enhancing effects and potent antimicrobial activities. The intestines of goats could be a potential origin for developing new probiotics applications in animal feed and human health. Hence this study was carried out to determine the antibiotic resistance profiles of LAB and pathogens within the intestines of Nigerian dwarf goats (*Capra aegagrus hircus*), the antimicrobial activity of LAB against resident MDR pathogens, and subsequent identification of bioactive LAB isolated from goat faeces in Nigeria as potential probiotics in animal feed and human health.

Method: Selective isolation of the LAB was carried out using de Mann Rogosa Sharpe (MRS) agar while enteric pathogens were isolated on MacConkey agar. Preliminary identification was carried out based on Gram reaction, morphological, colonial, and biochemical characteristics of each isolate. Antibiotic susceptibility profiles of all isolates were determined using the Kirby-Bauer disc diffusion method. The most resistant LAB isolates were tested for antimicrobial activity against the enteric pathogens using the agar overlay method. Selected LAB isolates were identified by 16SrRNA sequencing.

Results: The antibiotics susceptibility profile showed that a majority (77%) of LAB isolates and minimal (≦10%) of enteric pathogens demonstrated resistance to at least three classes of antibiotics, indicating a pattern of multi-drug resistance. Over half (62%) of these LAB isolates displayed significant antimicrobial activity against at least five of the resident-resistant pathogens, illustrating their potential role in controlling these pathogens. The sequencing results identified the most active LAB isolates, revealing a mix of strains *including Pediococcus lolli* (46%), *Pediococcus pentosaceus* (23%), *Weissella confusa* (8%), *Enterococcus faecium* (8%), *Enterococcus hirae* (8%), and *Lactobacillus sanfranciscensis* (8%).

Conclusion: The discovery of a diverse range of LAB strains in goat intestines with significant antimicrobial activity against resident enteric pathogens is valuable. This finding suggests the potential use of these bacteria as natural alternatives to traditional antibiotics, especially in the context of growing AMR in animal husbandry.

Keywords: Lactic-acid bacterial, Probiotics, Antibiotics, Resistance, Nigerian-dwarf-goats.

Introduction

Antimicrobial resistance (AMR) is a critical global health issue that poses a significant threat to public health, modern medicine, and the treatment of bacterial infections[1-5]. AMR occurs when microorganisms, such as bacteria, viruses, fungi, and parasites, evolve to resist the effects of medications, making standard treatments ineffective[1-4]. Misuse and overuse of antimicrobial drugs in humans, animals, agriculture, and environment contribute to the development of resistance[6]. Resistant infections are more difficult to treat and often result in prolonged illness, higher healthcare costs, and increased mortality rates. Routine medical procedures, like surgeries, chemotherapy, and organ transplants, become riskier due to the potential for infections that are resistant to standard treatments[7; 8]. AMR knows no borders, and the movement of people, animals, and goods facilitates the spread of resistant microorganisms globally. Collaboration at the international level is crucial to address AMR comprehensively. In the face of this emerging health crisis, investment in the discovery of new antimicrobial agents is crucial. The development of novel drugs and alternative approach to health crisis is essential to stay ahead of evolving microbial resistance[9]. Lactic acid bacteria (LAB), commonly known as probiotics, have gained attention as potential alternatives to antibiotics in certain situations. Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits to the host[10-14]. While antibiotics are designed to kill or inhibit the growth of harmful bacteria, probiotics work by promoting the growth of beneficial bacteria and maintaining a balanced microbial environment in the body[14-16].

LAB are known for their extensive probiotic attributes such as improving intestinal flora balance, fostering nutrient digestion and absorption, enhancing immune response, growth performance, and disease resistance[16-18]. For example, antibiotics can disrupt the natural balance of gut bacteria, leading to antibiotic-associated diarrhea (AAD). Probiotics, particularly strains of Lactobacillus and Bifidobacterium, may help prevent or alleviate AAD by restoring a healthy gut

microbiota[19-21]. Some studies suggest that certain probiotic strains may help prevent recurrent UTIs by inhibiting the growth of harmful bacteria in the urinary tract[22-24]. Probiotics are also explored as alternatives to antibiotics in animal agriculture. They may be used to promote the health and growth of livestock while reducing the need for antibiotics in animal feed[25; 26]. With the quest for sustainable and biologically safe approaches in livestock production gaining momentum, probiotics have become increasingly significant. They provide an environmentally friendly avenue to boost animal health and productivity, thereby reducing reliance on antibiotics and curbing the spread of AMR[27-29]. The antimicrobial capacities of LAB hinge on their production of antimicrobial substances, including lactic acid, hydrogen peroxide, and bacteriocins, which impede the growth of pathogenic bacteria[30; 31]. For instance, LAB produce lactic acid as a byproduct of fermentation thereby creating an acidic environment which is inhospitable for many pathogenic bacteria. The low pH condition inhibits the growth of these harmful microorganisms[32; 33]. LAB also produced hydrogen peroxide; is a potent antimicrobial agent that can damage the DNA and proteins of bacteria, leading to their inhibition or destruction[34]. LAB are known to produce bacteriocins, which are proteinaceous substances with antimicrobial activity[35; 36]. Bacteriocins can selectively target and kill closely related bacteria, providing a competitive advantage to the producing strain[35; 36]. These antimicrobial peptides play a crucial role in the natural defense mechanisms of LAB against competing microorganisms[35; 36].

The search for antimicrobial compounds as alternative to antibiotics in uncommon environments is an area of ongoing research known as bioprospecting. Ruminants, like cows and goats, harbor a unique gastrointestinal microbiome crucial for sustaining their well-being and productivity. This has spurred increased endeavors to identify and delineate probiotic strains derived from the gastrointestinal tracts of these animals[37; 38]. LAB, when used as probiotics, can positively influence the microbial balance in the digestive tract of livestock. They contribute to a healthy gut microbiota, which is essential for proper digestion, nutrient absorption, modulate the immune

system of livestock, promoting a balanced immune response, and prevent or mitigate the effects of bacterial infections[27; 39; 40]. The Nigerian dwarf goat, *Capra aegagrus hircus*, well adapted to varied environments, contributes substantially to smallholder farmers' livelihoods through the provision of meat, milk, and fiber. Though probiotics have been identified in the stomach and gastrointestinal tracts of ruminant[41; 42], research on LAB in Nigerian Dwarf Goats, a crucial breed in the Nigerian livestock sector, is still limited. This study aims to generate data and information to fill the existing gap by isolating, characterizing and identifying the LAB strains through 16S rRNA molecular sequencing from the GIT of healthy Nigerian Dwarf Goats and assessing the antimicrobial efficacy of chosen LAB isolates against resident enteric pathogens.

Methodology

Sample collection

The intestinal contents of fifteen freshly slaughtered healthy goat were collected from abattoir market in Ibadan, Oyo State. Samples were collected into sterile glass flask, placed in ice-pack bag, transported to the Pharmaceutical Microbiology laboratory for microbiological analysis within a time frame of one hour from the moment of sample collection, thus ensuring the preservation of sample integrity.

Isolation and Characterization of Lactic Acid Bacteria

LAB were isolated from the intestinal samples of the goat using a selective medium known as de Man Rogosa Sharpe (MRS) agar. After medium inoculation, the MRS agar plates were subjected to an incubation period of 48 hours at a temperature of 37°C. These incubation conditions were established under microaerophilic conditions, which were achieved by placing the plates within an anaerobic jar supplemented with a CampyGenTM (Oxoid) gas pack to create the appropriate environment for LAB growth and isolation. After the incubation period, discrete colonies displaying distinctive morphological features were carefully chosen from each agar plate. These selected colonies were subsequently sub-cultured to procure pure cultures, ensuring the isolation

of individual strains of LAB for further analysis and characterization. The purified colonies underwent an initial identification process that assess their colonial and cell morphology followed by a catalase test for the isolates. For further molecular identification, only those isolates displaying the typical characteristics associated with LAB were chosen. These characteristics included spherical cell shapes, a cream or off-white color, a Gram-positive staining pattern, and a negative result in the catalase test. These selected isolates were then preserved for future use by storing them in 50% glycerol stock at a temperature of -80°C.

Isolation procedures for the Enterobacteriaceae (Enteric pathogens)

Enteric pathogens present in the dwaft goat intestinal samples were isolated using MacConkey agar. The process involved inoculating the agar plates and incubating them at a temperature of 37°C for 24 hours. After the incubation period, distinct colonies with unique morphological characteristics were carefully chosen from each plate. These selected colonies were then sub-cultured to obtain pure cultures for further analysis. The purified colonies underwent an initial identification process, which encompassed evaluating colonial morphology, cellular morphology involving Gram staining, and performing a biochemical test using the oxidase test. For subsequent molecular identification, only isolates displaying the combined characteristics of being Gram-negative and oxidase-negative were retained. These specific isolates were preserved for future use by storing them in 50% glycerol stock at a temperature of -80°C.

Antibiotic susceptibility testing of the LAB isolates

In accordance with the stipulations outlined by the European Food Safety Authority (EFSA) for the safety evaluation of bacteria intended for use as probiotics, it is imperative that these microorganisms do not demonstrate acquired resistance to antibiotics that hold significant medical importance. This criterion underscores the importance of ensuring that probiotic strains used in food and health supplements do not contribute to the ongoing issue of antibiotic resistance, thereby safeguarding public health. The antibiotic susceptibility of all isolated LAB

strains was assessed using the disk diffusion method. The following antibiotics from Oxoid, UK, each with their respective concentrations were employed in the analysis. Ceftazidime (30 µg), Cefuroxime (30 µg), Gentamicin (10 µg), Ceftriaxone (30 µg), Erythromycin (5 µg), Cloxacillin (5 µg), Ofloxacin (5 µg), and Augmentin (30 µg). A lawn of the LAB was made with approximately 5 x 10^7 CFU/mL (equivalent to 0.5 McFarland standard) on 18 mL semi solid MRS agar. These plates were subsequently incubated at 37°C for 24 hours under microaerophilic conditions. Clear zones of inhibition surrounding the antibiotic disks served as visual indicators of the LAB strains' sensitivity to the respective antibiotics. Interpretation of the antimicrobial susceptibility results was compared with the standards set by the Clinical and Laboratory Standards Institute[43].

Antibiotic susceptibility testing of the Enterobacteriaceae (Enteric pathogens)

The antibiotic susceptibility profile of the pathogen isolates was determined using antibiotics disc diffusion methods each with corresponding concentration. Ceftazidime (30 µg), Cefuroxime (30 µg), Gentamicin (10 µg), Cefixime (5 µg), Ofloxacin (5 µg), Augmentin (30 µg), Nitrofurantoin (300 µg), and Ciprofloxacin (5 µg). Enterobacteriaceae culture at approximately 5 x 10^7 CFU/mL (equivalent to 0.5 McFarland standard) was seeded on Muller Hilton agar and incubated at 37°C for 24 hours in aerobic conditions. Clear zones of inhibition surrounding the antibiotic disks signified the susceptibility of the test organisms to the corresponding antibiotics. Interpretation of the susceptibility results was determined as compared to the standards set by the Clinical and Laboratory Standards Institute[43].

Determination of the antimicrobial activity of isolated Lactic Acid Bacteria isolates

The antimicrobial activity of viable LAB cells was determined using the Agar overlay method described by Ayeni *et al*[41] with slight modification. An inoculating loop was used to transfer LAB grown in MRS broth onto MRS agar plates, streaking a line approximately 2 mm wide and

30 mm in length. These plates were incubated under microaerophilic conditions at 37°C for 24 hours. Subsequently, the MRS agar plates were overlaid with 10 mL of Mueller Hinton (MH) soft agar (0.7% agar-agar) that had been inoculated with about 10^5 CFU/mL of an overnight broth culture of each test pathogen. Once the overlay was set, the plates were incubated at 37°C in aerobic conditions for another 24 hours. Post-incubation, the plates were examined for clear zones of inhibition around the line of LAB, and the clear zones were measured in millimeters (mm).

Molecular identification of isolates

The genomic DNA of the lactic acid bacteria strains was extracted using the Jena Bioscience DNA extraction kit, following the manufacturer's prescribed protocols. This extracted DNA was subsequently employed as a template in Polymerase Chain Reaction (PCR) amplification, with the target region being the 16S rRNA gene. Specific primers designed for this gene were used in the PCR amplification process: LACIF 5'- AGCAGTAGGGAATCTTCCA- '3 and LAB0677R 5'-CACCGCTACACATGGAG- '3 for Lab isolated and 16S 10F 5'-AGTTTGATCATGGCTCAGATTG- '3 and 16S 1507R 5'-TACCTTGTTACGACTTCACCCCAG- '3 for the enteric bacteria. The PCR products were visualized on an agarose gel to confirm successful amplification. These amplified PCR products were then purified and sequenced using the Sanger sequencing method. The sequences obtained were compared with those in the National Center for Biotechnology Information (NCBI) database via the Basic Local Alignment Search Tool (BLAST) program, facilitating the identification of the isolates.

Results.

Microbial load and colonial morphological characteristics

The analysis of the microbial loads in the intestinal samples collected from fifteen (15) healthy goat revealed distinct populations of LAB and enteric pathogens. The average colony count for the LAB isolates was determined to be 9.84 x 10^8 CFU/mL, with a range spanning from 2.2 x 10^8 CFU/mL to 19.6 x 10^8 CFU/mL (**Figure 1**). The preliminary identification of these isolates gave a total of 80 LAB isolates based on the results of Gram staining and catalase test reactions, which confirmed their Gram-positive and catalase-negative characteristics. These isolates represent a diverse group of LAB strains that were further examined and characterized for their potential applications in antimicrobial studies. The analysis of the goat intestinal samples unveiled an average colony count of enteric pathogens at 3.36 x 10^8 colony-forming units per milliliter (CFU/mL). The range of pathogen colony counts spanned from 1.0 x 10^6 CFU/mL to 9.6 x 10^8 CFU/mL (**Figure 1**). Following Gram staining and oxidase test reactions, which demonstrated Gram-negative and oxidase-negative attributes, a total of 70 enteric pathogen isolates were successfully obtained. These isolates represent a subset of Gram-negative bacteria with oxidase-negative properties.

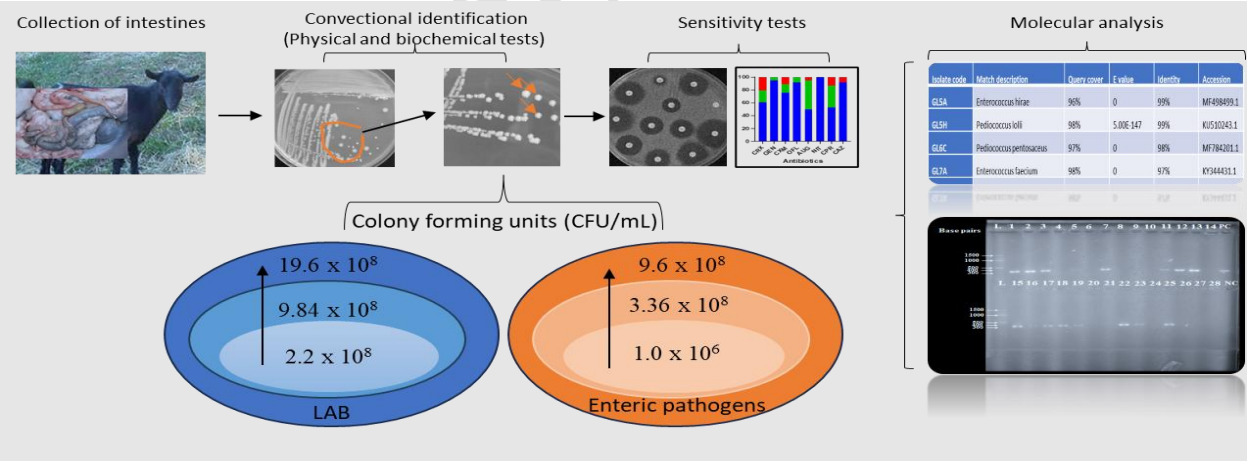

Figure 1. Schematic overview of the study design. Goat intestine were collected and plate on selective media and colonies were conuted, physical and biochemical parameters were also determined. Isolated organims were subjected to susecptibility testing, 16SrRNA sequencing, agorose gel electrophoresis and BLAST for identification

Antibiotic Susceptibility Profile of LAB and enteric pathogens

The susceptibility profiles of Gram-positive and catalase-negative LAB isolates to eight (8) antibiotic panel as shown in (**Figure 2a**) was utilized using the Kirby Bauer disc diffusion method. Augmentin demonstrated the highest effectiveness against LAB with susceptibility rates of 92%. Next was Cefuroxime (84%), Ceftriaxone (42%), Cloxacillin at 38%, and Erythromycin at 31%. Gentamicin and Ceftazidime reported minimal susceptibility rates at 2% and 5%, respectively. Remarkably, Ofloxacin exhibited no effect on the LAB isolates, registering a 0% susceptibility rate. These results provide valuable insights into the antibiotic susceptibility profiles of the LAB isolates, which can inform future research and applications in areas such as probiotics and antimicrobial interventions. For Gram-negative and oxidase-negative enteric pathogens, an alternative antibiotic panel was selected, as illustrated in Figure 2b. Nitrofurantoin exhibited exceptional efficacy, displaying a 100% susceptibility rate. Next were Gentamicin, Ofloxacin, and Ceftazidime, each demonstrating a susceptibility rate of 92.1%. Subsequently, Cefixime and Cefuroxime showed susceptibility rates of 76.3% and 60.5%, respectively. Augmentin and Ciprofloxacin exhibited the lowest activity, with susceptibility rates of 50% and 52.6%, respectively.

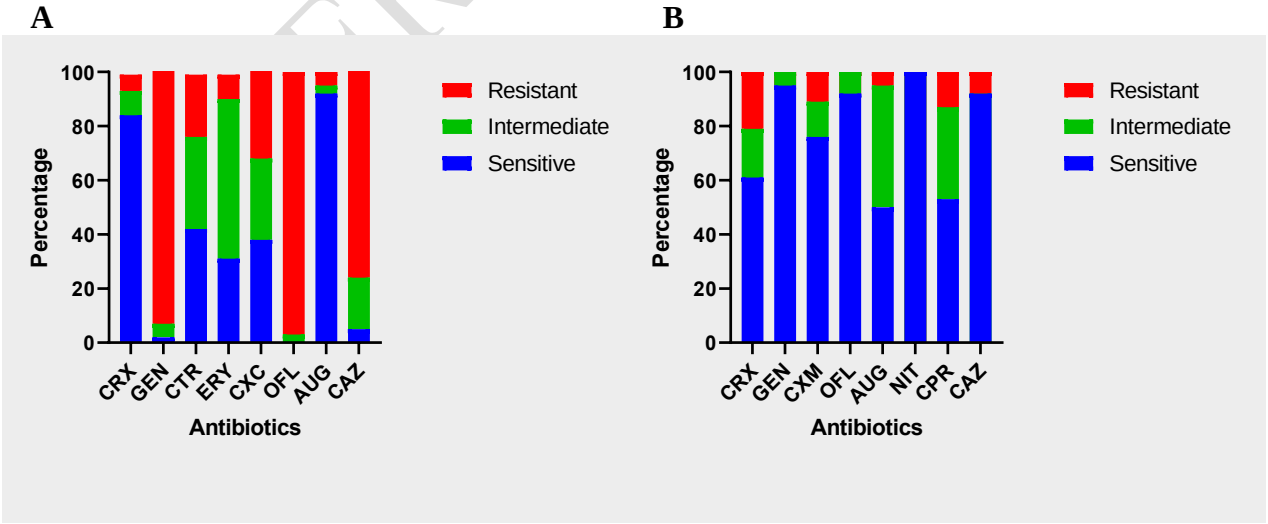

Figure 2: Antibiotics susceptibility patterns isolates from goat intestine (**A**) LAB isolates (**B**) enteric pathogen.

Antimicrobial activity of LAB against enteric pathogens isolated from the intestine of goat.

The most antibiotic-resistant LAB isolates were selected to undergo challenge tests against the most resistant enteric pathogens. A total of twenty-nine (29) LAB isolates were challenged against twelve (12) enteric pathogens obtained from the intestine of goats using the agar overlay method to test their antimicrobial activity. The results of the challenge tests revealed that 18 of the 29 LAB isolates tested (approximately 62%) exhibited significant antimicrobial activity. This was defined by a clearance zone measuring ≥ 20 mm against at least five of the pathogens they were tested against (**Table 1**). The isolates demonstrating the most potent antimicrobial activity were selected for further analysis. The characterization of these selected isolates was conducted through molecular identification, utilizing 16S rRNA gene sequencing. Furthermore, the identified LAB isolates with the potential of being good probiotic candidates were streamlined based on their antagonistic profile to four pathogens isolated from the same environment (**Fig.3**).

Table 1: Antimicrobial Activity of LAB isolates against Enteric pathogen isolated from goat intestine.

S/N	CODE	Zones of inhibition (< 4 mm +, 4 – 10 mm ++, 10 – 20 mm +++)											
		GP 1B	GP 4C	GP 5A	GP 7C	GP 10D	GP 11A	GP 12B	GP 12D	GP 13A	GP 13D	GP 13F	GP 14A
1	GL2E	+	+++	-	-	+++	+++	+++	-	+++	+++	+++	+++
2	GL3A	-	-	-	-	-	-	-	-	-	-	-	-
3	GL3G	++	+++	++	+++	+++	+++	+++	-	+++	+++	++	+++
4	GL5A	++	+++	++	+++	+++	+++	+++	-	+++	+++	+++	+++
5	GL5C	+	+++	-	-	-	+++	+++	-	-	+++	-	+++
6	GL5H	++	+++	++	++	-	++	+++	-	-	+++	-	+++
7	GL6A	++	+++	++	++	-	+++	+++	-	-	+++	++	+++
8	GL6C	-	-	-	-	-	-	-	-	-	-	-	+++
9	GL6D	++	+++	+++	-	-	+++	+++	-	-	+++	+++	+++
10	GL6E	++	+++	++	+++	++	-	+	++	+++	++	+++	+++
11	GL6G	++	+++	++	+++	-	+++	+++	+++	++	+++	-	-
12	GL7A	++	+++	++	++	-	+++	+++	-	++	+++	-	-
13	GL7B	++	+++	++	+++	+++	+++	++	-	++	+++	-	-
14	GL7C	++	+++	++	+++	+++	++	++	-	+++	++	-	-
15	GL8D	-	++	+	++	-	-	-	-	-	-	-	-
16	GL8H	-	+++	-	++	-	-	-	-	+++	++	-	-
17	GL1B	-	-	-	-	-	-	-	-	-	-	-	-
18	GL1C	-	-	-	-	-	-	-	-	-	-	-	-
19	GB11A	++	+++	++	++	-	+++	+++	+++	++	-	+++	-
20	GB11C	+	+++	++	++	-	+++	+++		-	-	-	-
21	GB11D	++	+++	++	-	-	+++	+++	+++	+++	-	+++	-
22	GB12A	++	+++	++	-	-	+++	+++	++	++	-	+++	-
23	GB12B	++	+++	++	-	+++	++	++	-	-	-	+++	+++
24	GB12E	++	+++	++	+++	+++	+++	+++	-	+++	-	+++	+++
25	GB13B	++	+++	++	+++	++	+++	+++	-	+++	-	+++	++
26	GB14D	++	+++	++	-	++	+++	+++	-	++	-	++	+++
27	GB15A	++	+++	+++	+++	-	+++	+++	+++	-	-	+++	-
28	GB15E	++	++	++	+++	-	+++	++	+++	-	-	-	-
29	GB15F	+	+++	++	+++	-	++	+++	+++	-	-	+++	-

Molecular identification of LAB and Enteric pathogen isolates from the intestine of Goat.

The phenotypically identified LAB with good antimicrobial activity were further identified genotypically. A total of 17 LAB and 5 enteric pathogens were analyzed molecularly targeting the 16SrRNA gene. The gel image showing the expected band size of the different isolates is presented in Figures 2a & b. The amplicons were sent for sequencing at Inqaba Biotechnological in South Africa to identify them to the species level. Thirteen (13) out of the 17 LAB isolates and 4 of the enteric pathogen DNA were successfully identified when compared to the GenBank data in NCBI (**Table 2 and 3**).

A **B**

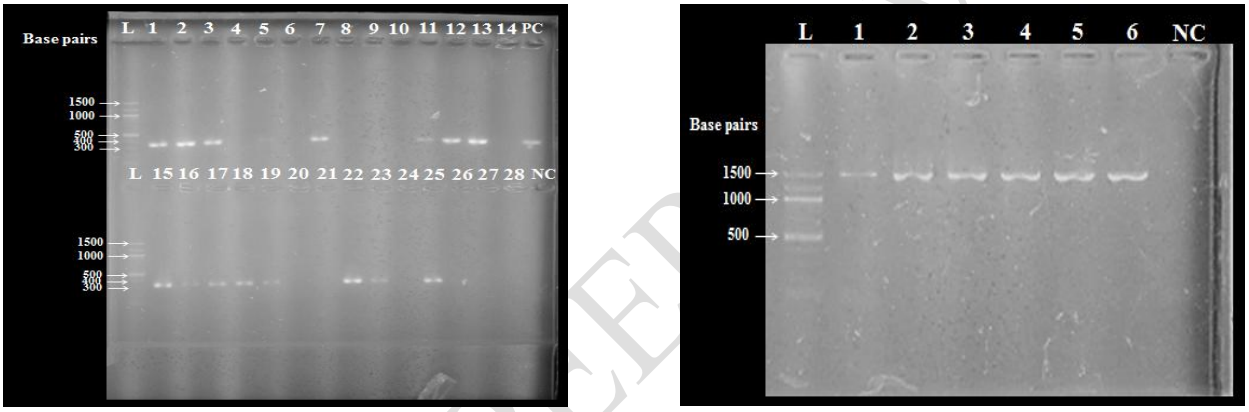

Figure 3: Agarose gel electrophoresis image showing a portion of 16S rRNA genes amplification. (**A**) LACIF and LAB0677R (LAB strains), Lane L: 100-bp Leader, Lane 1, 2, 3, 5, 7, 11, 12, 13, 15, 16, 17, 18, 19, 22, 23, 25: Test samples, Lane 4, 6, 8, 9, 10, 14, 20. 21, 24. 26: No band, Lane NC: Negative control, Lane PC: Positive. (**B**) 10F and 1507R primers (enteric pathogen strains), Lane L: 100-bp Leader, Lane 1-6: Test samples, Lane NC: Negative control.

Table 2: Molecular identification (16srRNA) of the LAB strains isolated from the intestine of Goat.

Isolate code	Match description	Query cover	E value	Identity	Accession
GL5A	*Enterococcus hirae*	96%	0	99%	MF498499.1
GL5H	*Pediococcus lolli*	98%	5.00E-147	99%	KU510243.1

GL6C	*Pediococcus pentosaceus*	97%	0	98%	MF784201.1
GL7A	*Enterococcus faecium*	98%	0	97%	KY344431.1
GL7B	*Pediococcus lolli*	93%	6.00E-153	98%	KU510243.1
GL7C	*Pediococcus lolli*	94%	3.00E-01	98%	KU510243.1
GB11A	*Pediococcus pentosaceus*	98%	8.00E-151	99%	MF967224.1
GB11C	*Pediococcus lolli*	97%	6.00E-132	94%	KU510243.1
GB11D	*Lactobacillus sanfranciscensis*	98%	7.00E-13	91%	MF967224.1
GB13B	*Weissella confuse*	94%	2.00E-159	99%	KT62405.1
GB14D	*Pediococcus pentosaceus*	98%	2.00E-146	99%	MF967224.1
GB15A	*Pediococcus lolli*	97%	5.00E-147	99%	KU510243.1
GB15E	*Pediococcus lolli*	94%	3.00E-156	98%	KU510243.1

Table 3: Molecular identification (16srRNA) of the Enteric Pathogen isolated from the intestine of Goat.

Isolate code	Match description	Query cover	E value	Identity	Accession
GP4C	*Escherichia coli*	98%	0	97%	MF429390.1
GP5A	*Escherichia coli*	97%	0	98%	KJ477008.1
GP12B	*Escherichia coli*	99%	0	97%	KX162656.1
GP14A	*Escherichia coli*	98%	0	98%	LS992192.1

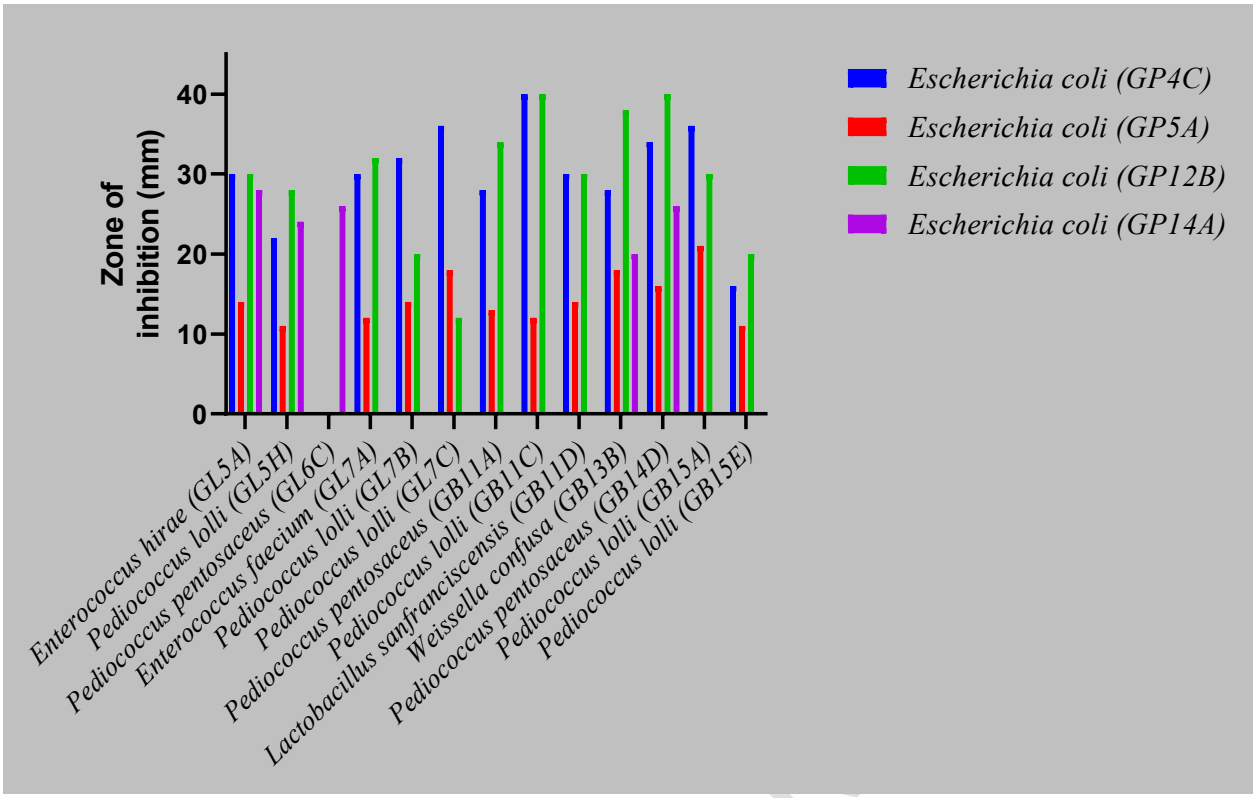

Figure 4: The Antagonistic effect of LAB isolates against some enteric pathogens isolated from goat intestine. Diameter (mm) of the zone of inhibition

Discussion

Lactic acid bacteria (LAB) play a pivotal role in the gastrointestinal microbiota of animals and humans, bestowing health advantages to the host. Their presence is known to offer multifaceted health advantages, including bolstering the host immune system, assisting with lactose intolerance, and contesting pathogens for nutrients due to their broad nutrient utilization spectrum. While studies have been conducted on LAB from the gastrointestinal tracts of few ruminants[41; 42], there is a notable scarcity of literature specifically focusing on LAB derived from goats. Our results uncovered a substantial presence of lactic acid bacteria in the gut microbiota of goats, with average LAB colony counts reaching approximately 9.84 x 10^8 CFU/mL **(Figure 1)**. When compared to the counts of enteric pathogens (3.36 x 10^8 CFU/mL), these results align well with previous findings from cattle[44]. The available data indicates an apparent inverse relationship between LAB and Enterobacteriaceae populations. This suggests that as concentrations of lactic acid bacteria increase, the load of Enterobacteriaceae tends to decrease. This observation is consistent with literature that underscores the health advantages associated with a higher LAB concentration, including stabilization of intestinal flora, acidification of the gut environment, and bacteriocin production that inhibits specific pathogens[10-16; 45; 46].

In accordance with the stipulations outlined for the safety evaluation of probiotics, the LAB isolates in our samples showed significant resistance to Ofloxacin, Gentamicin, and Ceftazidime. This resistance is crucial for probiotics, allowing them to persist in the gastrointestinal tract during antibiotic treatments, ensuring their efficacy without unintended elimination. In sharp juxtaposition, most of the Enterobacteriaceae isolates in our goat samples exhibited a high susceptibility to the antibiotics under examination. This distinct susceptibility pattern, in contrast to the results reported by Adeniyi *et al.*,[44] indicates a minimal likelihood of transmitting multidrug-resistant bacteria through the consumption of goat meat sourced from the Bodija abattoir in Ibadan. In delving into the antagonistic capacities of the LAB strains, we specifically

chose those with the utmost antibiotic resistance and assessed their efficacy against the most resilient pathogens within the same environment. The encouraging findings revealed that approximately 62% of the LAB isolates exhibited robust antagonistic activity against a minimum of five enteric pathogens (refer to Table 1). This underscores the potential of LAB as a health-promoting factor in goats and potentially positions it as a viable substitute for antibiotics in animal husbandry.

Additionally, through 16S rRNA sequencing, we identified an array of LAB strains, some of which include, *Pediococcus pentosaceus, Pediococcus lolli, Lactobacillus sanfranciscensis, Weissella confusa, Enterococcus hirae, and Enterococcus faecium*. While *Lactobacillus* sp. continues to be a prevalent probiotic, our research validates prior studies that underscore the probiotic potential of other LAB strains. This includes various strains of *Enterococcus*, *Pediococcus*, and *Weissella*, highlighting the diverse range of LAB with promising probiotic attributes beyond the commonly recognized *Lactobacillus* species[47-49]. *Pediococcus spp*., typically isolated from plants such as cereals and fruits, were identified in the gut of goats in our study. *P. pentosaceus* is renowned for its multifaceted roles as an antibacterial, anti-inflammatory, antioxidant, and many more[47; 48]. It's worth noting that bacteriocins or bacteriocin-like inhibitory substances (BLISs) were identified as the pivotal agents behind *P. pentosaceus's* antibacterial efficacy. Other studies on *P. pentosaceus* isolated from different sources also emphasized its broad antagonistic activity against various pathogens[50]. Additionally, we identified *L. sanfranciscensis*, a strain predominantly utilized in the baking industry, renowned for its notable probiotic capabilities. Multiple studies, including the work of Silva and colleagues[51], have highlighted its resilience in gastric conditions and its effectiveness against pathogenic bacteria. Moreover, Torres-Maravilla et al[52] have provided insights into its anti-inflammatory properties, further emphasizing the diverse and beneficial characteristics of *L. sanfranciscensis*[51; 52]. *Weissella confusa*, frequently isolated from fruits and vegetables, is

recognized for its robust probiotic properties, including tolerance to various stresses and its antimicrobial prowess due to ethanol and bacteriocin-like substances production[53; 54]. Another well-studied LAB strain with good probiotics activity is *Enterococcus sp*, it is ubiquitous as various studies have isolated it from the gut of the animals, various plants and vegetables, diaries, and even from aquatic organisms. In this research, we identified two different strains of Enterococcus which are *E. hirae* and *E. faecium* from the gut of goat. Past research has lauded *E. hirae* for its potential probiotic properties and diverse antimicrobial activities[55; 56]. Likewise, the probiotic capabilities of *E. faecium* have been thoroughly documented, from its bacteriocin production to its positive effects on epithelial integrity[57; 58]. The collective findings from these highlighted studies affirm the alignment of our research with a substantial potential for the identified LAB strains from the goat intestine.

Conclusion

This study unveils a promising landscape of potential probiotics originating from goat intestines. These strains not only emerge as potential health enhancers for goats but also open avenues for future research into their application in human health and nutrition. Particularly, *Pediococcus lolli* and *Pediococcus pentosaceus* show promise for the development of novel probiotic applications in both animal feeds and human health. Further research is imperative to comprehensively characterize these LAB isolates, assess their safety, evaluate their efficacy in vivo, and elucidate the molecular mechanisms underlying their antimicrobial activity. This comprehensive understanding will ultimately facilitate the development of effective strategies to address the global challenge of antimicrobial resistance and enhance the overall health of both animals and humans.

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