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Antibacterial Potential of Lactobacillus-Derived Bacteriocin-Like Inhibitory Substances (BLIS) Against Group B Streptococcus (GBS): A Biotherapeutic Approach

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Abstract

Background: Lactic acid bacteria (LAB) are a diverse group of Gram-positive, non-spore-forming bacteria known for their ability to produce antimicrobial substances such as bacteriocins and bacteriocin-like inhibitory substances (BLIS). **Aim:** This study aimed to isolate, characterize, and evaluate the antimicrobial activity of BLIS produced by LAB against Group B Streptococcus (GBS) from vaginal samples of pregnant women attending Federal University Teaching Hospital Wukari, Nigeria. **Methods:** Vaginal swabs were collected from 50 pregnant women and processed for the isolation of LAB and GBS using selective media and biochemical characterization. Bacteriocin-like inhibitory substances were extracted from LAB isolates and tested against GBS using the disk diffusion method. The effects of temperature and pH on BLIS activity were also evaluated. **Results:** The results revealed that LAB isolates exhibited varying degrees of antimicrobial activity against GBS. The BLIS produced were found to be moderately heat-stable, retaining activity up to 90°C but showing loss at 100°C. BLIS produced by **selected**

isolates (S8, S4, and S2) maintained antimicrobial activity across a broad temperature range (30°C –100°C) and different pH conditions (5.5–8.0). The inhibitory effects of BLIS remained stable across different pH levels, reinforcing their potential as alternative antimicrobial agents. There was no significant difference in activity between crude and partially purified BLIS, suggesting they may be low-molecular-weight peptides. Antibiotic susceptibility testing showed that GBS isolates were resistant to levofloxacin, amoxicillin, and ampiclox, while ciprofloxacin, erythromycin, chloramphenicol, and streptomycin remained effective. **Conclusion:** This study underscores the potential of LAB-derived BLIS as natural antimicrobial agents for managing GBS infections. The effectiveness and stability of Lactobacillus-derived BLIS in various environmental conditions suggest its suitability for pharmaceutical and food industry applications. Further research on purification and molecular characterization could enhance its use in antimicrobial therapy.

Keywords: Lactic acid bacteria, Bacteriocin-like inhibitory substances, Group B Streptococcus, Antimicrobial activity, Pregnant women, Wukari

INTRODUCTION

Lactic acid bacteria (LAB) are known to be Gram-positive, non-spore-forming rods, cocci and cocco-bacilli non-aerobic but aerotolerant, able to ferment carbohydrates into energy and lactic acid [1]. They are the dominant bacteria of a healthy human vagina, and their presence and number are influenced estrogen production, which under goes age and menstrual cycle dependant changes. A health vaginal ecosystem is dominated by certain species of lactobacillus which exert a significant influence on the microbiology of the vagina [2]. The lactic acid bacteria (LAB) group includes more than 25 *Lactobacillus* genera, including *Lactobacillus*, *Acetilactibacillus*, *Agrilactobacillus*, *Amylolactibacillus*, *Furfurilactobacillus*, *Fructilactobacillus*, *Holzapfelia*, *Latilactobacillus*, *Lactiplantibacillus*, *Loigolactibacillus*, *Paralactobacillus*, *Schleifer ilactobacillus*, etc [3].

According to Yusuf and Hamid [1], LAB belong to the phylum Firmicutes. The different major genera of LAB include: *Lactobacillus*, *Weissella Lactococcus*, *Melissococcus*, *Enterococcus*, *Lactosphaera*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Vagococcus*, *Carnobacterium* and *Tetragenococcus*. Other genera include: *Aerococcus*, *Propionibacterium*, *Microbacterium*, and *Bifidobacterium*. In addition *Lactobacillus*, *Streptococcus*, *Carnobacterium*, *Enterococcus*, *Lactococcus*, *Pediococcus* and *Weissella* are also under in the LAB group [3]. According to Karaoglu *et al.*

[4] among LAB members, the *lactobacilli* are composed of a diverse group of homofermentative and heterofermentative species. The production of bacteriocins by LAB has been known for many years [4].

Bacteriocins are heterogeneous group of peptides or proteins with antimicrobial activity synthesized ribosomally and released extracellularly by bacteria belonging to nearly all taxonomic groups; these are classified according to their genetic, structural and biochemical characteristics [5]. According to the original definition, the term bacteriocin refers to proteins of the colicin type, characterized by lethal biosynthesis, intraspecific activity, and adsorption to specific receptors. Those produced by gram-positive bacteria fit closely to the classical colicin model [4]. Bacteriocins are active metabolic peptides that are ribosomally synthesized by certain lactic acid bacteria (LABs). These are characterized by being non-toxic, and are either electrically neutral or positively charged [3].

Bacteriocins produced by different LABs differ from each other by their unique biochemical, structural, genetic, ecological and metabolic activity [3, 6]. Bacteriocins are found within each of the four major classes of antimicrobial proteins produced by lactic acid bacteria. Class I (lantibiotics) was only recently discovered in the Lactobacillaceae. Lantibiotics are small membrane active peptides (<5 kDa) containing the unusual aminoacid lanthionine. Class II; small heat-stable, non-lanthionine containing membrane-active peptides (<10kDa) characterized. The class III bacteriocins, which have to date only been found in *Lactobacillus*, include heat-labile proteins of large molecular mass. Class IV is a complex bacteriocin group. These proteins are associated with other lipid or carbohydrate moieties, which appear to be required for activity. The bacteriocin was relatively hydrophobic and heat stable [4].

The vaginal microbiome of healthy women is a dynamic ecosystem, and it is colonized by a variety of microorganisms. The composition of the vaginal microbiome is influenced by a number of factors such as age, hormonal levels, sexual activity, hygiene, phase of menstrual cycle, or diet [7, 8]. In healthy premenopausal women, bacteria of the genus Lactobacillus are dominant in the vaginal microbiome at 10^7–10^8 CFU/g of vaginal fluid [8]. The most frequently found are species of *L. crispatus*, *L. gasseri*, *L. iners*, and *L. jensenii*, but this depends on ethnic group or geographic location. There are also differences between pregnant and non-pregnant women; lower strain diversity was found in pregnant women. On the other hand, lactobacilli in pregnant women are more stable than in non-pregnant

women [7-9]. The healthy urogenital tract plays a significant role in protecting against vaginal infections, and vaginal lactobacilli are important because of their protective functions (adhesion to the vaginal tissue and production of antimicrobial substances) [8].

Group B Streptococcus (GBS), also known as *Streptococcus agalactiae*, is a facultative, gram-positive diplococcus [10]. According to Patras and Nizet, [11], *Streptococcus agalactiae* [Group B Streptococcus (GBS)] is an encapsulated Gram-positive bacterium that colonizes the lower gastrointestinal tract, and in females, the urogenital tract, of 20–30% of healthy human adults. *Streptococcus agalactiae* is a leading source of perinatal and neonatal infections globally, accounting for 1.8 new born infections per 1000 live births each year [12]. The organism can be acquired after delivery or in utero via transfer from maternal vaginal or anorectal mucosa [13]. It is estimated that 10 to 30% of all pregnant women are colonized with GBS in their gastrointestinal and genital tracts [10]. Group B Streptococcus utilizes multiple adhesins and stress response mechanisms, defences against other microbes, and immune evasion strategies to achieve persistent or intermittent vaginal colonization. During the peripartum period, GBS gains access to a new host, the immune-deficient neonate, where GBS can again serve as a commensal organism or transition to an invasive pathogen resulting in sepsis or meningitis. GBS displays an arsenal of virulence factors, including a potent hemolytic toxin and multiple surface proteins to invade host tissues, as well as molecular mimicry and proteases to impede host immune recognition and responses [11].

MATERIALS AND METHODS

Description of the Study Area

Wukari Metropolis, the headquarters of Wukari Local Government Area in Taraba State, is crossed by the River Donga and River Benue [14, 15]. It is one of the 16 local government areas in the state, located at latitude 7.53'43'N and longitude 9.47'59'E, with a population ranging from 5,000 to 10,000. Covering 4,308 km² [16, 17]. Wukari is historically significant as the administrative center of the Kwararafa Kingdom and is predominantly inhabited by the Jukun people. The region features Savannah vegetation and an economy driven by farming, fishing, livestock rearing, commerce, and civil service [14, 17, 18]. The main languages spoken include Jukun, Hausa, Fulani, and Tiv [14].

Ethical Considerations

Before collecting the samples, the Head of Department Microbiology provided a letter of introduction that was submitted to the Education Secretary Wukari Local Government through the administration of Federal University Teaching Hospital Wukari for clearance. Samples were gathered, as well as collection schedules at the Federal University Teaching Hospital Wukari.

Collection of Specimen

Vaginal fluids were taken aseptically from the lateral vaginal wall of 50 pregnant women [2] attending Federal University Teaching Hospital Wukari with a sterile swab stick and promptly delivered to the Microbiology Laboratory Federal University Wukari.

Isolation Characterization and Identification of *Lactobacillus Agalactiae*

Samples were delivered for the isolation of *Lactobacillus Agalactiae*. The medium were created in accordance with the manufacturer's instructions [19]. The vaginal swab samples transported in Thioglycollate Broth Medium were inoculated onto Rogosa SL agar and incubated at 37 ºC in a candle jar for 48 hours before the colonies were subculture to obtain pure culture for further analysis [4]. The lactobacilli were identified on the basis of growth on selective Rogosa SL agar (pH 5.2), cell morphology, gram staining, and catalase activity. Further identification of the species of these lactobacilli was performed according to carbohydrate fermentation patterns, esculin hydrolysis and growth at 15 ºC and 45 ºC in the Lactobacilli DeMan Rogosa Sharpe (MRS) broth (Difco), as described in Bergey's Manual of Systematic Bacteriology. Purified cultures were maintained at –80 ºC in MRS broth with 10% glycerol [4].

Isolation, Characterization and Identification of Group B *Streptococcus*

Streptococcus Vaginal Swabs for Group B Streptococcus isolate were immersed in peptone broth for 1 minute before being gently pressed against the edge of the tube. The tube was incubated at 37°C for 24 hours under anaerobic conditions. Growth in the culture broth was streaked onto 5% sheep blood agar plates and incubated at 37°C for 24 hours. Colonies with clear zones were selected and sub-cultured. Gram staining was performed on a freshly produced culture, and the catalase test was seen.

Bacterial Identification

The isolates were identified based on their morphological, cultural, and biochemical characteristics. *Lactobacillus spp*. were identified by comparing observed isolate features to those of lactic acid bacteria as given in Bergey's Manual of Determinative Bacteriology [20]. The catalase test, Christie, Atkins, and Munch-Peterson (CAMP) test and Gram staining were used to identify Group B Streptococcus [21].

Crude Bacteriocin-like Inhibitory Substance (BLIS)

Bacteriocin-like Inhibitory Substance production involves the extracellular release of proteins into the culture broth. Three hundred (300) mL of MRS broth were inoculated with 1% v/v of the overnight cultures of the identified Lactobacillus isolates and anaerobically incubated at 37°C for 48 hours in triplicate. Following incubation, cells were sorted by centrifugation at 5000 g for 30 minutes. The supernatants were adjusted to pH 6.5 with Sodium Hydroxide (NaOH) to eliminate the influence of organic acid, and 5 mg/ml catalase was added to remove the inhibiting effect of hydrogen peroxide. The supernatants were filtered through a 0.45 μm membrane filter to extract crude BLIS from the cell-free supernatant [2, 21]. To produce partially pure BLIS, ammonium sulphate precipitation of bacteriocin (90% saturation) was performed by gradually dissolving 90.45 g of the salt in the cell-free supernatant and stirring until completely dissolved. The precipitate were re-dissolved in 6 ml of 0.05 M Sodium Phosphate buffer (pH 7.0) after centrifugation and then kept under cold condition for further use [21].

Media Preparation

Any media used for microbe isolation must be prepared so that it does not produce erroneous results. The work bench was properly sterilized with 70% alcohol. Material for media preparation was assembled on the workbench. Then, the manufacturer's directions were properly followed. Autoclaving sterilized the media, which was then prepared according to the manufacturer's instructions [18].

Production of Crude Bacteriocin-like Inhibitory Substance (BLIS)

BLIS proteins are secreted into the culture broth. To extract them, 300 ml of MRS broth was inoculated with 1% (v/v) overnight cultures of the identified isolates and incubated anaerobically at 37°C for 48 hours. Following incubation, the cells were removed by centrifugation at 5000 g for 30 minutes. The obtained supernatants were split into two

portions for crude and refined extract preparation. One portion was adjusted to pH 6.5 using NaOH to neutralize organic acids, and catalase (5 mg/ml) was added to eliminate the effects of hydrogen peroxide. Finally, the supernatants were filtered through a 0.45 μm membrane to obtain the crude BLIS extract [2].

Preparation of Group B Streptococcus

The Group B Streptococcus overnight culture was inoculated into the broth and incubated at 37°C for 24 hours. The microbial count was determined using a routine plate count method. A tenfold serial dilution was carried out by transferring 1 mL of the stock solution into 9 mL of sterile normal saline, continuing up to the tenth dilution. From each dilution, 0.1 mL was plated on nutrient agar using the spread plate technique. After overnight incubation, colony counts were recorded. The sensitivity test was conducted using a culture containing approximately 5×10^8 cells/mL [21].

Determination of Inhibitory Spectrum of Bacteriocin

The bacteriocin assay was performed using the disk diffusion method as described by Bhunia [22]. Whatman No. 1 filter paper was punched into 8.0 mm discs, which were then soaked with 100 μl of the extract. These discs were sterilized in a dry heat oven at 140°C for one hour and cooled before use. A sterile glass rod was used to spread 100 μl of an overnight broth culture (containing approximately 5×10^8 cells/ml of the test organism) onto nutrient agar, which was left to stand briefly. The sterilized discs, pre-soaked in 100 μl of the extract, were placed on the inoculated agar using sterile forceps. Sterile phosphate buffer (pH 7.0) served as a control. The plates were incubated aerobically at 37°C for 24 hours, and inhibition zones were observed around the discs, indicating antimicrobial activity. The diameters of these zones were measured in millimeters, with antagonistic activity expressed as the average inhibition zone size.

Physicochemical Characterization of BLIS

Effect of Temperature on BLIS

The impact of crude and partially purified extracts on the target organism was evaluated at different temperatures over a 10-minute period. Each sample was placed in a 3 mL test tube, sealed with cotton, and heated using a water bath to temperatures of 30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C, and 100°C. After heating, the samples were immediately

cooled in a refrigerator before being tested against the target organism using the previously described method [21, 23].

Effect of hydrogen ion concentration (pH) on BLIS

The effects of crude and purified extracts on the target organism were analyzed at different pH levels (5.5, 6.0, 7.5, and 8.0). The pH was adjusted using 1 N NaOH and 1 N HCl with the help of a Hanna pH meter [21, 23]. After adjustment, the samples were filtered through 0.45 μm membrane filters and tested against GBS following the previously described method. The sample's activity at its original pH (7.0) was used as a control [21].

Isolation and Identification of *Lactobacillus* and Group B *Streptococcus*

Samples were cultured on selective media, and isolates were identified through biochemical assays. The presence of *Lactobacillus* and Group B Streptococcus (GBS) was confirmed using Gram staining, catalase testing, and carbohydrate fermentation analysis.

Antimicrobial Activity of *Lactobacillus* Isolates

The antibacterial activity of *Lactobacillus* isolates against Group B Streptococcus (GBS) was evaluated using agar well diffusion and microdilution techniques. The effectiveness of crude and partially purified bacteriocin-like inhibitory substances (BLIS) was determined by measuring inhibition zones and assessing their potency through minimum inhibitory concentration (MIC) analysis.

Statistical Analysis

Descriptive data analysis was used to assess the prevalence and antibacterial activity, with statistical significance set at $p < 0.05$.

RESULTS AND DISCUSSIONS

Among the 50 samples collected from the vaginas of pregnant women in different laboratories and clinics of Wukari Taraba State, some samples showed the microscopic characteristics of *Lactobacillus and streptococcus algalactiae.* Table 1 showed the microscopically characteristics of the 14 isolates used in the study. Some of the isolates did not comply with the biochemical tests while also some do not produce any inhibitory effect. Microscopic examination revealed that the tested isolates were Gram positive, rods appearing in pairs or

in chains. The test organism used (GBS) was Gram positive, spherical in shape and they occur in chains. It had a wide clear zone on nutrient agar.

Table 1: Microscopic characteristics of isolates from sample collected at Federal University Teaching Hospital Wukari

Isolate symbol	Gram reaction	Microscopic appearance	Cellular arrangement
S1	+	Bacilli	Chains
S14	+	Bacilli	Chains
S3	+	Cocci	Chains
S12	+	Cocci	Chains
S4	-	Cocci	Pairs
SB	+	Cocci	Chains
S7	+	Short-Bacilli	Chains
S8	+	Cocci	Pairs
S2	-	Cocci	Pairs
S10	+	Cocci	Pairs
S13	+	Bacilli	Chains
S9	-	Cocci	Pairs
S6	+	Cocci	Chains
S5	+	Bacilli	Chains

The microscopic characteristics (Table 1) confirmed the presence of both Gram-positive and Gram-negative bacterial isolates, with varied cellular arrangements such as chains and pairs. The majority of isolates were Gram-positive bacilli and cocci, which are typical characteristics of ***Lactobacillus* spp.** and **Group B Streptococcus (GBS)**. Similar studies by **Liasi *et al.* [24]** and **Williams *et al.* [25]** have also identified *Lactobacillus* isolates as Gram-positive, rod-shaped, and occurring in chains.

Table 2: Biochemical characteristics of isolates from sample collected at Federal University Teaching Hospital Wukari

Isolate symbol	Colony Morphology	Biochemical test				Sugar fermentation test			
		Catalase Test	Oxidase Test	Coagulase Test	Camp Test	Glucose	Fructose	Galactose	Lactose	Maltose
S1	Smooth, circular non pigmented colonies	-	-	-	+	+	-	+	-	w
S14	Rough, irregular, pigmented colonies	+	-	+	-	+	+	-	+	+
S3	Smooth, flat, non pigmented colonies	-	-	-	+	+	+	w	+	-
S12	Smooth, irregular, pigmented colonies	-	-	+	+	-	+	+	+	+
S4	Raised pigmented, concave colonies	+	+	-	-	+	+	+	+	+
SB	Smooth, non pigmented colonies	-	-	-	+	w	+	+	+	+
S7	Rough ,non pigmented colonies	+	-	-	+	+	+	w	-	+
S8	Smooth, regular, flat colonies	-	-	-	+	+	-	+	-	-
S2	Moderate, non pigmented colonies	-	+	-	-	+	w	+	+	-
S10	Small, smooth, nonpigmented colonies	-	-	-	+	+	+	+	+	+
S13	Rhizoid, pigmented colonies	-	+	-	-	-	+	+	w	+
S9	Pigmented, translucent rhizoid colonies	-	+	-	-	+	+	+	+	-
S6	Non pigmented, smooth colonies	-	+	-	-	+	w	+	+	+
S5	Rough non pigmented colonies	-	+	+	-	-	+	+	`-	-

Keys + = positive; - = negative; w = weak reaction. All the isolates fermented glucose, fructose and galactose.

The biochemical tests (Table 2) further classified the isolates based on their enzymatic activity and carbohydrate fermentation patterns. The catalase-negative isolates, such as S1, S3, S12, and SB, align with the properties of **Lactobacillus spp.**, which are known for their catalase-negative nature due to their fermentative metabolism. Studies by **Hemme & Foucaud-Scheunemann [26]** confirmed that catalase-negative Lactobacillus species contribute to antimicrobial activity through acid and bacteriocin production.

Moreover, the **Camp test** positivity in S1, S3, S12, SB, S7, S8, and S10 supports the identification of **Group B Streptococcus (GBS)** strains, as **GBS is Camp test-positive**, a distinguishing characteristic that differentiates it from other streptococci [27].

Table 3: Positive Samples after Anaerobic Incubation for 48hrs using Peptone Broth

Isolates	Test
S14	Turbid
S1	Turbid
S2	Turbid
S6	Turbid
S4	Turbid
S9	Turbid
S5	Turbid
S12	Turbid
S3	Turbid
S10	Turbid
S7	Turbid

Table 3 presents the turbidity observed after 48-hour incubation, which confirms bacterial viability and growth under anaerobic conditions. The presence of turbidity in isolates such as S14, S1, S6, S4, and S9 suggests that these isolates thrive in low-oxygen environments. This observation aligns with previous findings by Centeno ***et al.*** **[28]**, who reported the facultative anaerobic nature of **Lactobacillus spp.** and **GBS**, allowing them to survive in the gastrointestinal and urogenital tracts.

Figure 1 is the inhibitory spectrum of crude and partially purified extract of *Lactobacillus* species against the test organism. The BLIS from all isolates had their strongest activity at 30°C (Fig 2 - 4). The **Bacteriocin-Like Inhibitory Substances (BLIS)** extracted from ***Lactobacillus*** **spp.** demonstrated significant inhibitory activity against the test organism **GBS** (Figure 1). The **agar well diffusion assay** revealed **clear inhibition zones**, confirming the presence of antimicrobial peptides.

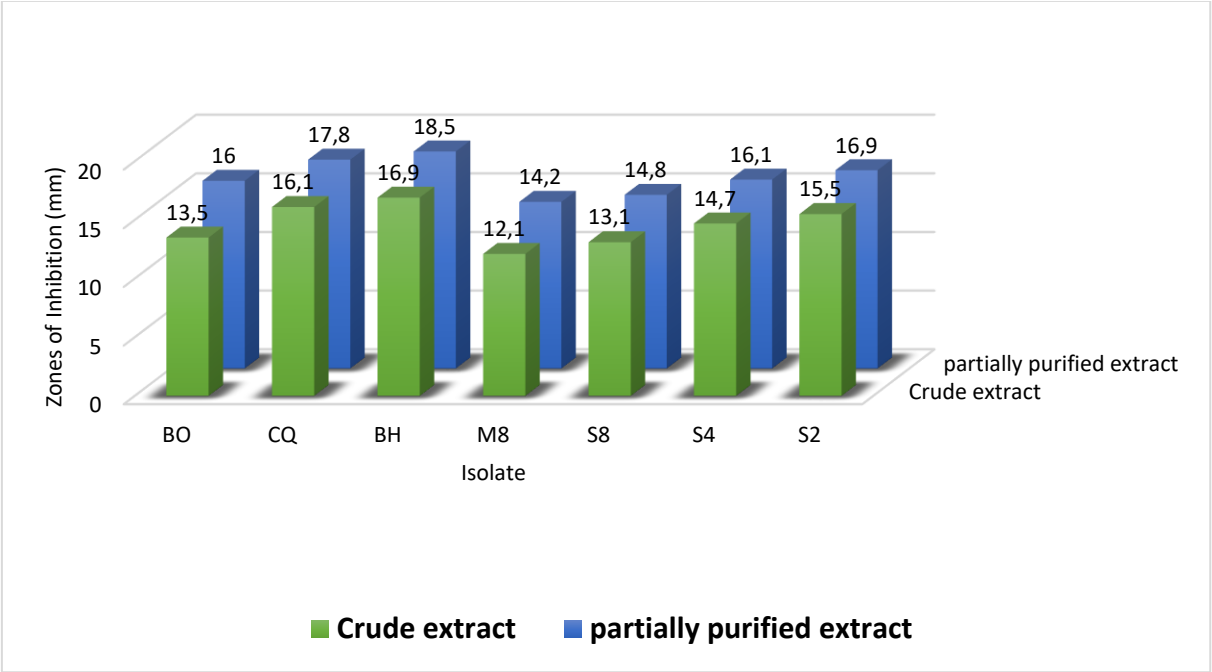

Figure 1: Inhibitory spectrum of crude and partially purified extract of *Lactobacillus* species against the test organism

Figure 2 shows the activity of BLIS from isolate S8, its activity was maintained from 30°C - 50°C in the crude extract, while the activity of partially purified BLIS was stable at 80^0C - 100^0C with an average of 12.5mm zone of inhibition. Figure 3 shows the activity of BLIS in the crude extract to be steady at high temperature while the activity of partially purified BLIS was not steady at all temperature ranges. Partial loss of activity was seen for BLIS from isolate S2 as the temperature increased. The partially purified BLIS lost its activity at 100°C (Figure 4).

The results in Figures 2, 3, and 4 highlight the **temperature stability of BLIS**. The inhibitory activity was retained at temperatures up to **70°C**, but a decline was observed at higher temperatures (90°C and 100°C). Similar findings were reported by **Todorov & Dicks [29]**, where Lactobacillus bacteriocins retained activity at moderate heat but lost potency at extreme temperatures.

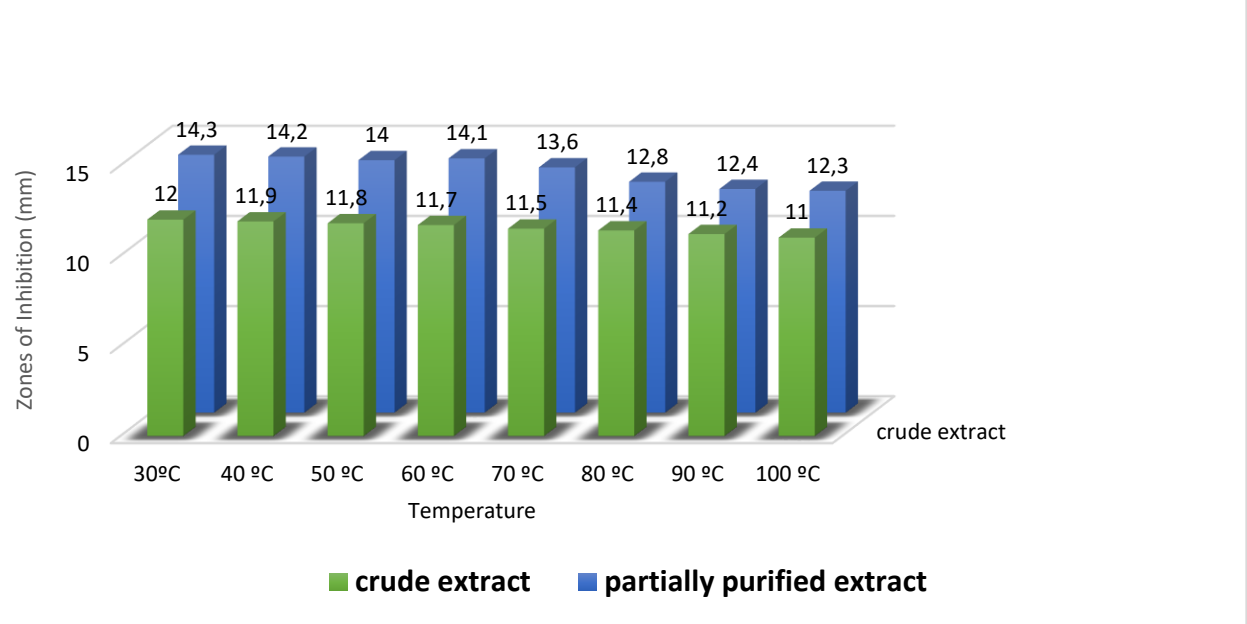

Figure 2: Effect of BLIS Produced by Isolate S8 at Different Temperature Ranges

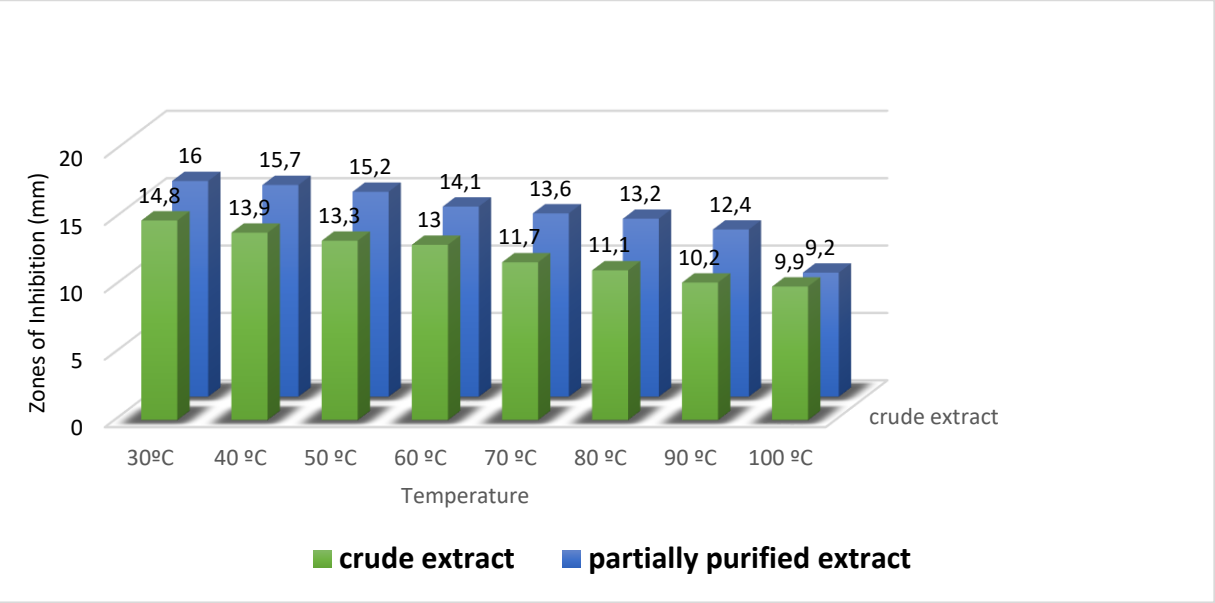

Figure 3: Effect of BLIS Produced by Isolate S4 at Different Temperature Ranges

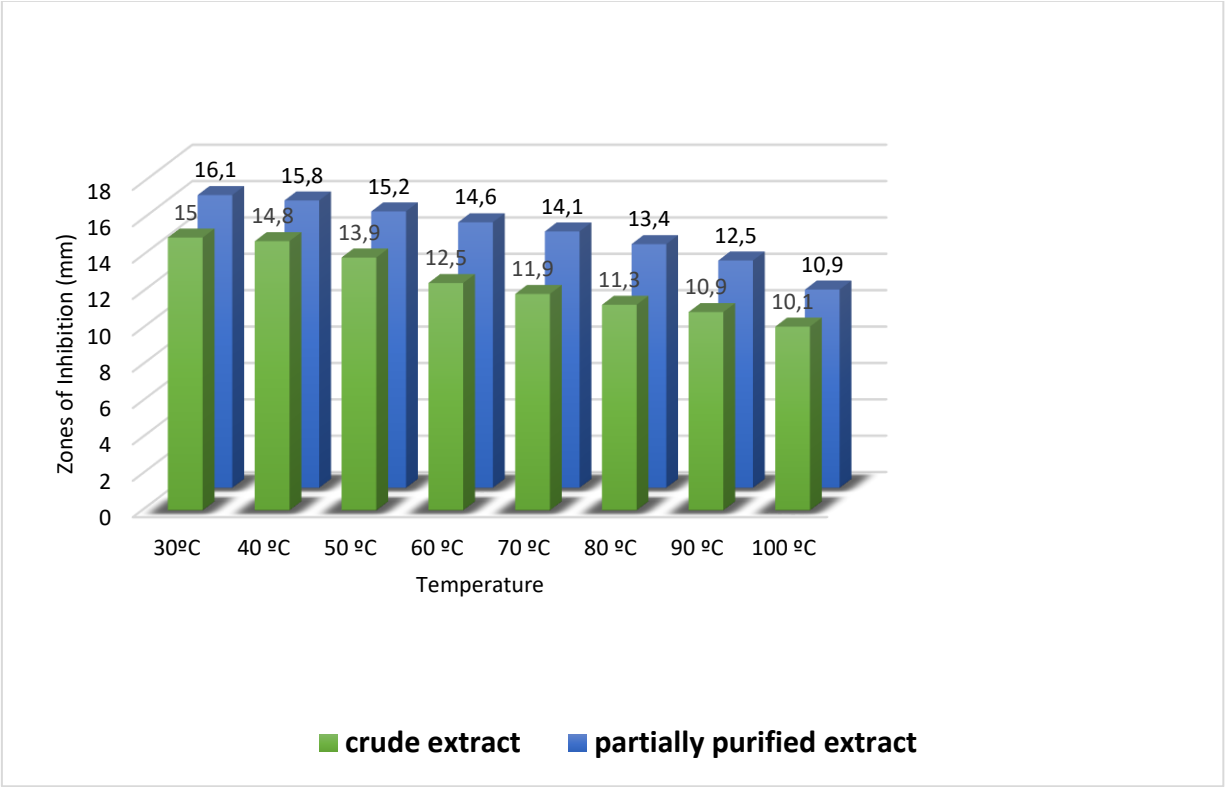

Figure 4. Effect of BLIS Produced by Isolate S2 at Different Temperature Ranges

Each inhibitory compound was assayed for its stability after 1 hour incubation at different pH levels (pH 5.5 – pH 8.0). The partially purified inhibitory compounds were active at a wide range of pH. Isolates S4 showed highest activity at pH 5.5. S8 and S2 were more sensitive against the target organism at pH 6.0. BLIS from isolate S2 had the strongest activity with 17.8 mm zone of inhibition. Generally the activities of the isolates were more active at acidic pH than at alkaline pH. The **pH stability** study (not shown in tables) also indicated that BLIS remained active at a **wide pH range (5.5–8.0)**, consistent with bacteriocins reported by **Mokoena [30]**.

The results of the inhibitory capacity assessment revealed that S2 and S8 exhibited the strongest activity against the target organism. When these two inhibitory compounds were combined, their effectiveness increased, as indicated by larger zones of inhibition. The crude extract of the combined compounds produced a 19.06 mm inhibition zone, while the partially purified extract showed an even greater zone of 21.07 mm. Further analysis of the partially purified extract across different pH levels demonstrated activity over a broad pH

range (5.5 - 8.0). Additionally, the extract maintained its inhibitory effect when exposed to temperatures between 30°C and 100°C for 10 minutes, showing stability particularly within the 30°C to 60°C range.

Table 4: Antibiotics Sensitivity Test for Positive Isolates

Test Isolate	Antimicrobial Agent	Zone of Inhibition (mm)	Disk Content (µg)	Zone Diameter (Interpretative Criteria)		
				S	I	R
S1	Amoxicillin	26	10			✓
	Streptomycin	20	10			✓
	Chloramphenical	22	30	✓		
	Ciprofloxacin	17	5		✓	
	Norflaxacin	18	10	✓		
	Erythromycin	28	10	✓		
	Colistin	26	10	✓		
	Ampiclox	20	20	✓		
	Rifampin	28	5	✓		
	Levofloxacin	26	5	✓		
S14	Ciprofloxacin		5	✓		
S3	Amoxicillin	13	10			✓
	Streptomycin	14	10	✓		
	Chloramphenical	28	30	✓		
	Ciprofloxacin	28	5	✓		
	Norflaxacin	23	10	✓		
	Erythromycin	25	10	✓		
	Colistin	22	10		✓	
	Ampiclox	10	20			✓
	Rifampin	16	5	✓		
	Levofloxacin	22	5	✓		
S12	Amoxicillin		10	✓		
	Streptomycin	24	10	✓		
	Chloramphenical	24	30	✓		
	Ciprofloxacin		5	✓		
	Norflaxacin	18	10			✓
	Erythromycin		10	✓		
	Colistin		10	✓		
	Ampiclox		20	✓		
	Rifampin		5			✓
	Levofloxacin		5	✓		

S4	Amoxicillin		10			✓
	Streptomycin	19	10			✓
	Chloramphenical	20	30			✓
	Ciprofloxacin		5			✓
	Norflaxacin	18	10	✓		
	Erythromycin		10	✓		
	Colistin		10			✓
	Ampiclox		20			✓
	Rifampin		5	✓		
	Levofloxacin		5	✓		
SB	Amoxicillin		10	✓		
	Streptomycin	21	10	✓		
	Chloramphenical		30		✓	
	Ciprofloxacin	16	5	✓		
	Norflaxacin		10			✓
	Erythromycin	18	10	✓		
	Colistin	17	10		✓	
	Ampiclox	15	20	✓		
	Rifampin	14	5			✓
	Levofloxacin		5			✓
S7	Ciprofloxacin	24	5	✓		
S8	Ciprofloxacin	27	5	✓		
S2	Amoxicillin		10			✓
	Streptomycin	21	10	✓		
	Chloramphenical		30			✓
	Ciprofloxacin		5	✓		
	Norflaxacin		10			✓
	Erythromycin		10	✓		
	Colistin		10		✓	
	Ampiclox		20			✓
	Rifampin		5	✓		
	Levofloxacin	18	5	✓		

S10	Ciprofloxacin	24	5	✓	
S13	Ciprofloxacin	20	5	✓	
S9	Amoxicillin		10	✓	
	Streptomycin		10		✓
	Chloramphenical	15	30	✓	
	Ciprofloxacin	17	5		✓
	Norflaxacin		10	✓	
	Erythromycin		10		✓
	Colistin		10		✓
	Ampiclox		20	✓	
	Rifampin		5		✓
	Levofloxacin		5	✓	
S6	Ciprofloxacin	23	5	✓	
S5	Ciprofloxacin	19	5	✓	

Note: Multiple spectrum and single spectrum antibiotic disk was used for this assay, the single spectrum used was Ciprofloxacin.

The **antibiotic sensitivity test** revealed that many isolates were **resistant to Amoxicillin, Ampiclox, and Colistin**, which is concerning due to the increasing **antimicrobial resistance (AMR)**. However, most isolates were sensitive to **Streptomycin, Erythromycin, and Ciprofloxacin**, showing promising treatment options. Resistance to **β-lactam antibiotics (Amoxicillin, Ampiclox)** aligns with reports by **Simeoni *et al.* [31]**, who observed that **GBS strains** possess β-lactamase enzymes that degrade penicillins. Sensitivity to **Ciprofloxacin and Erythromycin** supports previous findings by **Ruh *et al.* [32]**, where similar strains of **GBS** were susceptible to **fluoroquinolones and macrolides**.

***Lactobacillus* strains isolated from dairy and fermented foods** have been reported to produce heat-stable bacteriocins with a broad inhibitory spectrum [33]. **GBS resistance to penicillin and ampicillin** has increased over time, as noted in studies on neonatal

infections [32]. **Bacteriocin-producing *Lactobacillus*** species from probiotic sources have been explored as alternatives to antibiotics [30], which supports the potential application of BLIS in combating drug-resistant pathogens.

During the course of this study, a total number of 50 samples were obtained from the vaginas of pregnant women and identified based on cultural and biochemical characteristics. The biochemical characteristics of this research isolates are comparable with vaginal *Lactobacillus* isolated by some researchers. *Lactobacillus plantrum* is found to be a normal inhabitant of healthy women [2]. Isolate S4 and S2 which were provisionally identified as *L. acidophilus* in this study, has been isolated from other studies [4]. Aslim *et al.* [2] found 16% *L. acidophilus* in healthy women. According to Li *et al.* [34], the rate of vaginal *L. acidophilus* in pregnant women was 76.9%. According to Pascaul *et al.* [35] *L fermentum* is also a normal inhabitant of healthy women. Lactobacillus species are commonly isolated from the vaginas of healthy women. The presumptive Lactobacilli identified in this study closely resemble those reported by previous researchers.

Bacteriocins or bacteriocin-like inhibitory substances (BLIS) produced by *Lactobacillus* serve as a protective mechanism against pathogenic and opportunistic microorganisms in the vagina. Research has highlighted the antimicrobial properties and beneficial effects of bacteriocins/BLIS from vaginal *Lactobacillus* on Gram-positive and closely related organisms [36]. However, their impact on Group B Streptococci (GBS) remains largely unexplored.

The BLIS produced in this study successfully inhibited the growth of the test organism (GBS), aligning with the findings of Strus *et al.* [37], who reported that *Lactobacillus* species could suppress GBS growth. Similarly, Ruiz *et al.* [38] demonstrated that BLIS from *L. fermentum* and *L. rhamnosus* exhibited inhibitory effects on GBS. Furthermore, the combination of BLIS from both *Lactobacillus* species showed stronger inhibitory activity than individual BLIS, a trend also observed in this study.

The antimicrobial activity observed in this study was attributed to bacteriocins or bacteriocin-like inhibitory substances (BLIS), as their inhibitory effects remained unchanged after treatment with catalase or pH adjustment to 6.5. The BLIS produced by the isolates were partially characterized and evaluated for their in vitro antimicrobial activity against the test organism under varying temperature and pH conditions. The BLIS

exhibited moderate heat stability, as their effects were tested for only 10 minutes. However, isolate S4 lost its activity at 100°C within this timeframe, suggesting it is heat-labile.

The results showed no discernible difference between crude and partially purified BLIS activity, indicating that low molecular weight molecules may make up the BLIS. Certain antibiotics, such as ciprofloxacin, erythromycin, chloramphenicol, and streptomycin, demonstrated resistance in the antibiotic assay used to treat infections, while others, such as levofloxacin, amoxicillin, and ampiclox, demonstrated effectiveness. Some antibiotics showed sensitivity that was at the intermediate stage.

CONCLUSION

This study demonstrated the antimicrobial potential of lactic acid bacteria (LAB) isolated from the vaginal microbiota of pregnant women in Wukari, Nigeria, against Group B Streptococcus (GBS). The bacteriocin-like inhibitory substances (BLIS) produced by these LAB isolates exhibited significant inhibitory activity against GBS, suggesting their potential as natural antimicrobial agents. The BLIS were found to be moderately heat-stable and effective across a range of pH levels, further supporting their viability for therapeutic applications. Additionally, the antibiotic susceptibility testing revealed that some GBS isolates were resistant to commonly used antibiotics such as levofloxacin, amoxicillin, and ampiclox, while ciprofloxacin, erythromycin, chloramphenicol, and streptomycin remained effective. These findings highlight the need for alternative antimicrobial strategies, such as LAB-derived BLIS, in managing GBS infections, particularly in pregnant women where GBS colonization poses risks of neonatal infections. Further research is needed to fully characterize the molecular properties of these BLIS, optimize their production, and explore their potential applications in probiotic formulations or alternative antimicrobial therapies. This study contributes to the growing body of evidence supporting LAB as beneficial microorganisms in the human microbiome with promising clinical applications.

Conflict of Interest

There were not any conflicts of interest between the authors from beginning of the study to the end. Everything went well as design and agrees on the proposal.

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