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Combined Effects of Bacteriocin-Like Inhibitory Substances from Vaginal *Lactobacillus* Isolated from Clinical Sample on Group B *Streptococcus*

Samuel Tamunoiyowuna Cockeye Brown[1], Usman Ikrimah Mohammed[2*], Eze Emmanuel Onyemaechi[3], Benjamin Nanisi Daniel[4]

[1,2,4]Federal University Wukari, Taraba State, Nigeria

[3]I. M. Sechenov Medical University Moscow, Russia

ikrimah25@gmail.com

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Abstract

This study investigates the prevalence of Group B Streptococcus (GBS) and the potential inhibitory effects of bacteriocin-like substances produced by *Lactobacillus* species in pregnant women in Wukari, Taraba State, Nigeria. *Lactobacillus* species, known as non-pathogenic Gram-positive rods, are commonly found in the vaginal microbiota of healthy premenopausal women, where they play a crucial role in maintaining vaginal health through their antimicrobial properties. The study aimed to determine the incidence of GBS colonization and explore the antimicrobial activity of crude and partially purified bacteriocin-like inhibitory substances from *Lactobacillus* isolates against both Gram-positive and Gram-negative bacteria. A total of 50 vaginal swab samples were collected from pregnant women. The results revealed that 11 women (22%) were colonized by Group B Streptococcus, while 14 women (28%) tested positive for *Lactobacillus* presence. The bacteriocin-like inhibitory substances produced by the *Lactobacillus* isolates demonstrated significant antimicrobial activity against GBS, with optimal inhibitory effects observed at 30°C and pH 5.5. Furthermore, antibiotic susceptibility testing showed that

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ciprofloxacin, erythromycin, chloramphenicol, and streptomycin were effective against GBS isolates. The findings underscore the potential role of *Lactobacillus*-derived bacteriocins as natural antimicrobial agents in the prevention and control of GBS infections, particularly in pregnant women. This study also reinforces the importance of monitoring GBS colonization due to its implications for maternal and neonatal health.

Keywords: *Lactobacillus*; Group B Streptococcus; Bacteriocin-Like Inhibitory Substances; Antimicrobial Activity; Vaginal Microbiota; Antibiotic Susceptibility

INTRODUCTION

Lactobacillus species are nonpathogenic Gram-positive rods that are predominantly isolated from the vagina of healthy premenopausal women [1] The vaginal flora in healthy premenopausal women contains *Lactobacillus* spp*,* which occur in the range of 10^7 – 10^8 cfu/g fluid [2]. Some Lactobacilli produce bacteriocins and other compounds like hydrogen peroxide and lactic acid as a means of protecting the vagina against pathogenic/opportunistic microorganism [3]. Some strains such as *L. gasseri* have been shown to specifically inhibit the growth of pathogens associated with bacterial vaginosis [4]. The protective role of Lactobacilli in the vagina becomes evident when their concentrations drop as a result of the use of antibiotics or in an immune-compromised host. This drop in concentration favors colonization by intestinal bacteria and the overgrowth of opportunistic organisms [5]. Bacteriocin-like substances (BLIS) are ribosomal-synthesized antimicrobial peptides that are not completely defined and do not fit the typical criteria of bacteriocins. Unlike bacteriocins with activities only on species closely related to the producing organism, BLIS have been shown to have broader activities and are reported to inhibit a wide range of both Gram-positive and Gram-negative bacteria as well as fungi [6]. Most bacteriocins from lactic acid bacteria have been isolated from species of the genus *Lactobacillus,* because of the diversity of its species and habitats [7]. Group B *Streptococci* (GBS) are among the opportunistic organisms that colonize the lower gastrointestinal and genitourinary tracts of 30 to 50% of healthy adults [8]. An estimated 10 to 30% of all pregnant women are carriers of GBS [9].

Streptococcus agalactiae (GBS) is a significant cause of perinatal and neonatal infections worldwide and is responsible for 1.8 neonatal infections per 1000 live birth per year [10].

The organism can be acquired during delivery or in utero by transmission from maternal vaginal or anorectal-colonized mucosa [11]. Colonization in late pregnancy can result in poor pregnancy outcome because GBS has the ability to penetrate intact amniotic membrane causing amnionitis which could lead to miscarriage [12].

MATERIALS AND METHODS

Study Area

The study was conducted in Wukari, Taraba State, Nigeria. The town of Wukari is home to its headquarters. As of the 2006 census, its area was 4,308 km^2, and its population was 241,546. The area's postal code is 670. Jukun is the language used locally (Wapan, Jibu, Nyifon). Wukari is located between longitude 9047'59" East and latitude 7055'42" North. Jukun, Kutep, Tiv, Hausa, and Fulani are the principal languages spoken there [13]. The research took place during the rainy season. Majority of the patients are women from low background and have little or no idea about personal hygiene and the prevalence of this organisms in their vagina tract leading to high morbidity and mortality in newborns.

Ethical considerations

A letter of introduction was obtained from the Head of department Microbiology and submitted to the Education secretary Wukari Local Government for approval before the samples were collected. From the office of the Education Secretary another letter of introduction to the laboratories, clinic and hospitals where samples were collected and schedules for sample collection at the Federal University Teaching Hospital Wukari.

Specimen Collection and Isolation of *Lactobacillus Agalactiae*

A total number of 50 samples were collected from pregnant women at the Wukari Federal University Teaching Hospital and possibly other hospitals in different regions of Wukari Local Government Area Taraba state Nigeria. Vaginal fluids were collected from the lateral wall of the vagina using a sterile cotton swab aseptically and were transported immediately to the Microbiology Laboratory, Federal University Wukari. Vaginal swabs were inoculated on Chocolate, MacConkey and Blood agar plates by streaking method and incubated at 37°C for 48hrs were suspected colonies were sub-cultured to obtain pure culture for further analysis.

Isolation and Identification of Group B *Streptococcus*

Streptococcus Vaginal swabs for Group B *Streptococcus* isolation was immersed in peptone broth for 1 minute and pressed lightly against the side of the tube. The tube was incubated at 37°C for 24hrs under anaerobic condition. Growth in the culture broth was streaked on 5% sheep blood agar plates and incubated at 37°C for 24hrs. Colonies showing clear zones was picked and sub-cultured. Gram staining was carried out with a freshly prepared culture and catalase test was observed.

Bacterial Identification

The isolates were identified according to their morphological, cultural and biochemical characterization. Identification of *Lactobacillus* spp. was based on comparison of observed characteristics of isolates with those of lactic acid bacteria described in the Bergey's Manual of Determinative Bacteriology [14]. Group B *Streptococcus* was identified using Gram staining and catalase test.

Production of Crude Bacteriocin-like inhibitory substance (BLIS) are proteins produced extracellularly 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 incubated at 37°C for 48hrs anaerobically in triplicates. After incubation, cells were separated by centrifugation at 5000g for 30min. The supernatants were adjusted to pH 6.5 using Sodium Hydroxide (NaOH) to remove the effect of organic acid and 5 mg/ml catalase was also added to remove the inhibitory effect of hydrogen peroxide. The supernatants were filtered using 0.45 μm pore size membrane filter to obtain crude BLIS present in the cell-free supernatant [15]. Production of partially purified BLIS, Ammonium Sulphate precipitation of bacteriocin (90% saturation) was carried out by gently dissolving 90.45 g of the salt in the cell-free supernatant and stirring gently till the salt was 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.

Media Preparation

For any media to be used for the isolation of microorganisms it must be prepared in such a way that it will not give false result. The working bench was sterilized properly with 70% alcohol. Material for the media preparation will be assembled at the working bench. Then the manufacturer's instructions were followed carefully. Sterilization of

media was achieved by autoclaving after which the media was prepared according to the manufacturer's instruction [13].

Production of Crude Bacteriocin-like Inhibitory Substance (BLIS)

BLISes are proteins produced extracellularlly into the culture broth. Three hundred (300) ml of MRS broth were inoculated with 1% v/v of the overnight cultures of the identified isolates and incubated at 37°C for 48 h anaerobically in triplicates. After incubation, cells were separated by centrifugation at 5000 g for 30 mins (using Galenkamp centrifuge, England). The supernatants were divided into two for the production of the crude extracts and purified extracts. The one half of the supernatants were adjusted to pH 6.5 using Sodium Hydroxide (NaOH) to remove the effect of organic acid and 5 mg/ml catalase (Sigma, England) was also added to remove the inhibitory effect of hydrogen peroxide. The supernatants were filtered using 0.45 μm pore size membrane filter to obtain crude BLIS present in the cell-free supernatant [15].

Preparation of the Test Organism

Overnight culture of GBS was inoculated in broth. It was incubated at 37°C for 24 hrs. The number of organism present in the culture was estimated using standard plate count. Ten-fold serial dilution was carried out by adding 1ml of the stock solution to 9 ml of sterile normal saline. Dilutions were made until the 10^{th} test tube. A 0.1 ml of the solution from each test tube was plated on nutrient agar using spread plate method. The plates were incubated overnight and the colonies were counted. Culture containing about 5 x 10^8 cells/ml was used for the sensitivity test.

Determination of Inhibitory Spectrum of Bacteriocin

Bacteriocin assay was carried out by disk diffusion method as described by Bhunia [16]. Whatman No 1 filter paper was punched using puncher to obtain disc of 8.0 mm in diameter. Discs were arranged to absorb 100 μl of the extract. These were placed in a sterile screw-caped Bijuo bottles and sterilized in dry heat oven at 140°C for 1 hrs. The discs were allowed to cool before use. One hundred (100) μl of the overnight broth culture containing about 5 x 10^8 cells/ml of the test organism were spread on nutrient agar using sterile glass rod and allowed to stand for some time. The sterile discs were immersed in 100 μl of the extracts and were asceptically placed on the seeded nutrient agar using a sterile forceps. Sterile phosphate buffer (pH 7.0) was used as controls. Plates were incubated at 37°C for 24 h aerobically and examined for the presence of inhibition zones around the wells as

indicator of inhibiting activity. The diameter of the inhibition zones were measured in milimetre. Antagonistic activity were expressed and recorded as the mean of inhibition zone values.

Effect of Temperature and Hydrogen ion Concentration on BLIS

The effects of the crude and partially purified extracts on the target organism were examined at different temperatures for 10 mins. The samples were dispensed in 3 ml test tubes and covered with cotton wool. The samples were heated at 30°C, 40°C, 50°C; 60°C, 70°C, 80"C, 90°C and 100°C using water bath. The samples were cooled immediately in the fridge before testing against the target organism as described earlier. The effects of the crude and purified extracts on the target organism were examined at different ranges of pH level (pH 5.5, 6.0, 7.5, 8.0). The samples were adjusted to different pH using 1 N NaOH and 1 N Hydrochloric acid (HCI) using pH meter (Hanna pH meter model). The samples were filtered with 0.45 μm membrane filters after the adjustment. The treated samples were tested against GBS as described earlier. The activity of sample at initial pH (7.0) was used as a control.

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

Samples were cultured on selective media, and isolates were identified using biochemical tests. The identification process included Gram staining, catalase test, and carbohydrate fermentation tests to confirm the presence of *Lactobacillus* and Group B *Streptococcus* (GBS).

Antimicrobial Activity of *Lactobacillus* Isolates

The antimicrobial activity of Lactobacillus isolates against Group B *Streptococcus* (GBS) was assessed using agar well diffusion and microdilution methods. The inhibition zones were measured to determine the effectiveness of crude and partially purified bacteriocin-like inhibitory substances (BLIS). Minimum inhibitory concentrations (MICs) were determined to establish the potency of the BLIS.

Statistical Analysis

Data were analyzed using descriptive statistics to determine the prevalence and antimicrobial activity. Statistical significance was set at $p < 0.05$.

RESULTS

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 and 2 showed the microscopically characteristics of the 24 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: Microscopically Characteristics Of Isolates Collected From Kwararafa Hospital, Grace Lab And Beco Lab In Wukari.

Isolate symbol	Gram reaction	Microscopic appearance	Cellular arrangement
SBO	+	Cocci	Pairs
SB10	+	Bacilli	Chains
SB9	-	Cocci	Pairs
SB14	-	Cocci	Pairs
SB8	+	Cocci	Pairs
SBH	+	Cocci	Pairs
SCH	+	Bacilli	Chains
SM8	+	Bacilli	Chains
SB12	-	Cocci	Chains
SM1	+	Cocci	Pairs
SMB	+	Cocci	Pairs
SC8	+	Cocci	Pairs
SB9	-	Bacilli	Chains
SB10	+	Cocci	Chains
SC3	+	Bacilli	Chains
SM9	+	Cocci	Pairs
SM8	+	Bacilli	Chains
SCQ	-	Cocci	Pairs
SCS	-	Cocci	Pairs
SBA	+	Cocci	Pairs
SB12	+	Cocci	Chains
SBJ	+	Cocci	Pairs
SD	+	Bacilli	Chains
SB1	+	Cocci	Pairs
SCP	+	Cocci	Chains
SBC	+	Cocci	Clusters
SCE	+	Bacilli	Chains
SBN	+	Cocci	Chains
SMA	-	Bacilli	Chains
SBK	-	Cocci	Pairs
SMH	+	Cocci	Pairs

Table 2: Microscopically Characteristics Of Isolates Collected From 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

Table 3 and 4 outline the cultural and biochemical characteristics of isolates after incubation at 37^0C. BC, MH, MS, BT, BJ, BO, CE, BL,MP, BD, CG, MB, BK, BN, CQ,BT, BN, BF and MA were able to grow at 15°C while at 45°C is BC, MH, BJ, BD, MB, CQ, BT, BN, BF, MA were able to show turbidity. Also MB, CQ was able to produce gas from glucose. The isolates were catalase negative as bubbles was not produced during the reaction. Some of the isolates were capable of fermenting glucose, fructose, maltose and galactose, except BO, SB10, SCH, SM8 and some isolates that do not ferment galactose and gave weak reaction for maltose.

Table 3: Biochemical Characteristics Of Isolates Collected From Kwararafa Hospital, Grace Lab And Beco Lab In Wukari.

Isolate symbol	Colony Morphology	Biochemical test				Sugar fermentation Test				
		Catalase Test	Oxidase Test	Coagulase Test	Camp Test	Glucose	Fructose	Galactose	Lactose	Maltose
SBO	Flat, smooth and non pigmented colonies.	-	-	-	+	+	-	-	-	W
SB10	Rough, pigmented, raised colonies	+	-	+	+	+	+	-	+	+
SB9	Creamy, milky ,raised colonies	+	-	-	-	+	+	+	+	+
SB14	Smooth, non pigmented colonies	-	-	+	-	+	+	+	+	+
SB8	Raised, large and pigmented colonies.	+	-	+	-	+	+	+	+	+
SBH	Smooth ,flat, convex colonies.	+	-	-	+	W	+	+	+	+

SCH	Smooth, flat, non pigmented colonies	+	-	-	+	+	+	+	-	+
SM8	Raised,irregular pigmented colonies	W	+	+	+	+	-	+	-	-
SB12	Smooth, flat and non pigmented colonies	+	-	-	-	+	-	+	+	-
SM1	Regular, smooth, non pigmented colonies.	-	-	-	+	+	+	+	+	+
SMB	Punctiform, irregular colonies	+	+	-	-	-	+	+	+	+
SC8	Moderate, irregular ,rhizoid colonies	-	-	-	+	+	+	+	+	+
SB9	Smooth, flat, glistering non pigmented colonies	-	-	-	-	+	w	+	+	+
SB10	Circular ,rhizoid and tiny colonies	-	+	+	-	+	+	+	+	+
SC3	Smooth, regular, non pigmented colonies	-	-	-	+	W	+	+	+	W
SM9	Smooth, flat, mucoid olonies.	-	-	-	-	+	+	+	+	+
SM8	Irregular, non pigmented colonies	W	-	-	+	+	+	+	+	+
SCQ	Rough, spindle colonies	-	+	+	-	-	+	+	+	+
SCS	Irregular, smooth , non pigmented colonies	-	-	w	+	+	+	+	+	+
SBA	Smooth raise, regular colonies	-	-	+	+	+	-	+	W	+
SB12	Smooth, flat, regular, non pigmented colonies	-	-	-	+	+	+	-	+	+
SBJ	Irregular, rhizoid colonies	-	+	+	-	+	+	+	+	+
SBD	Pigmented , circular colonies	+	+	-	-	+	+	+	+	W
SB1	Glistering, mucoid colonies	+	+	+	+	+	+	-	+	+
SCP	Smooth, non pigmented, convex colonies	-	-	-	+	+	-	+	-	+
SBC	Rough, mucoid colonies	W	+	-	-	+	+	+	+	+
SCE	Irregular, mucoid colonies	+	+	-	+	+	-	+	+	-
SBN	Convex, regular, circular colonies	-	-	-	+	+	+	+	+	+
SMA	Concave, smooth and irregular colonies	-	+	+	+	+	+	-	-	+
SBK	Smooth, irregular colonies	+	+	+	+	+	+	+	W	+
SMH	Non pigmented, regular colonies	-	-	-	+	+	-	+	+	+

Table 4: Biochemical Characteristics Of Isolates Collected From 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.

Table 5: Positive Samples after Anaerobic Incubation for 48hrs Using Peptone Broth

BC	Turbid
MH	Turbid
BD	Turbid
MB	Turbid
CQ	Turbid
BT	Turbid
BN	Turbid
BF	Turbid
MA	Turbid
S14	Turbid
S1	Turbid
S2	Turbid
S6	Turbid
S4	Turbid
S9	Turbid
S5	Turbid
S12	Turbid
S3	Turbid
S10	Turbid
S7	Turbid

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 - 8).

Figure 2 shows the activity of BLIS from isolate BO at different temperatures. There was a partial loss of activity as the temperature increased. At the temperature of 30°C - 70°C and between 70°C - 90°C, the activity of its crude BLIS were relatively steady. For the partially purified BLIS, activity was steady between 50°C - 70°C and 80°C - 100°C.

Figure 3 shows the activity of BLIS from isolate CQ. Its activity decreased slightly as the temperature increased. The activity of the partially purified BLIS was steady between 40 °C – 60°C with an average of 15mm zone of inhibition. It was also stable between 80°C - 100°C with an average of 9.1 mm.

Figure 4 shows the activity of BLIS from isolate BH. The activity of the partially purified BLIS was stable between 30°C - 50°C with an average of 15.2 mm zone of inhibition but with continuous decrease from 60°C - 100°C.

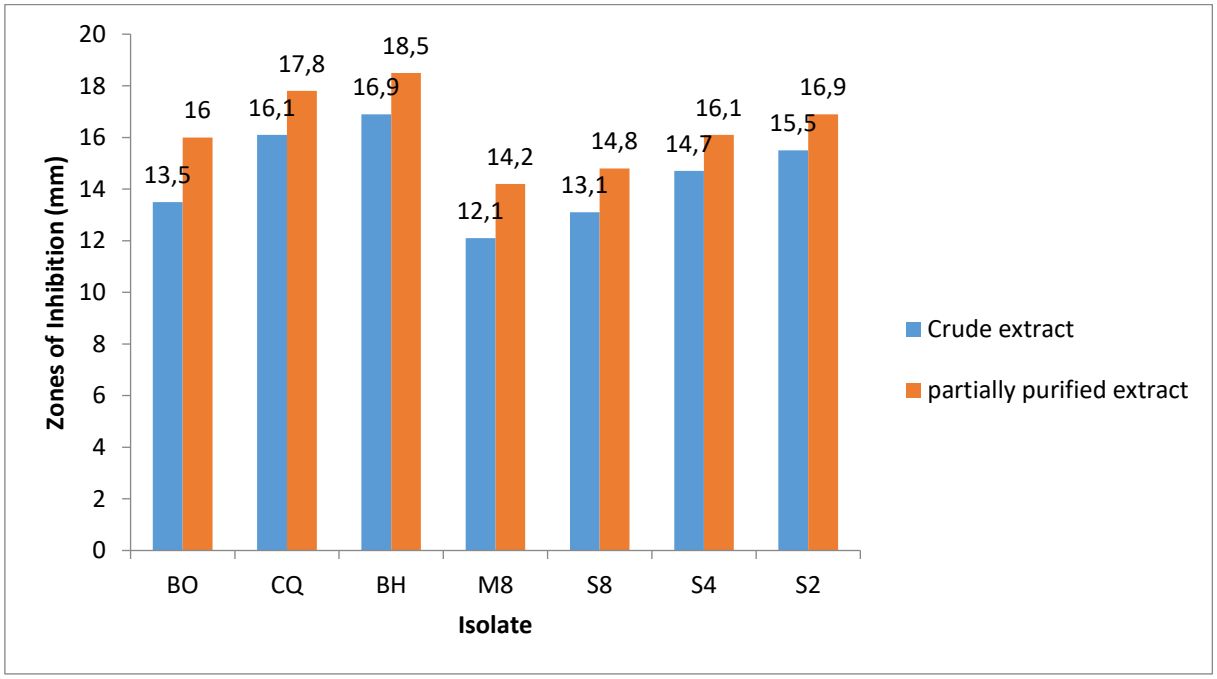


Figure 1: Inhibitory spectrum of crude and partially purified extract of *Lactobacillus* species against the test organism

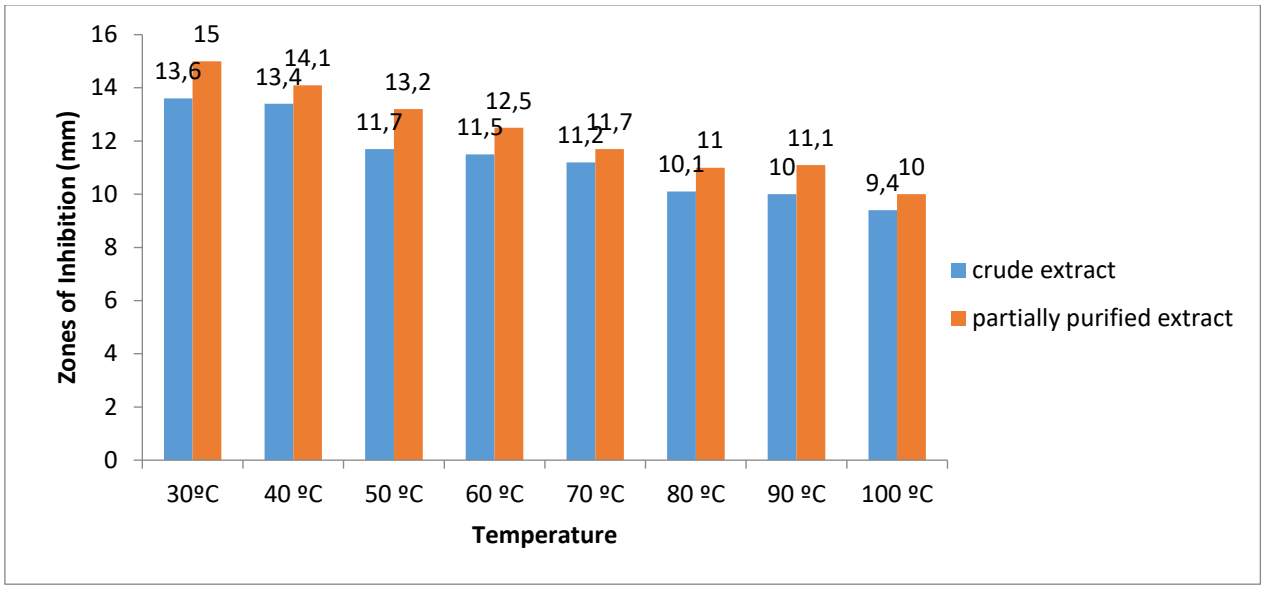


Figure 2: Effect of Blis Produced by Isolate BO at Different Temperature Ranges

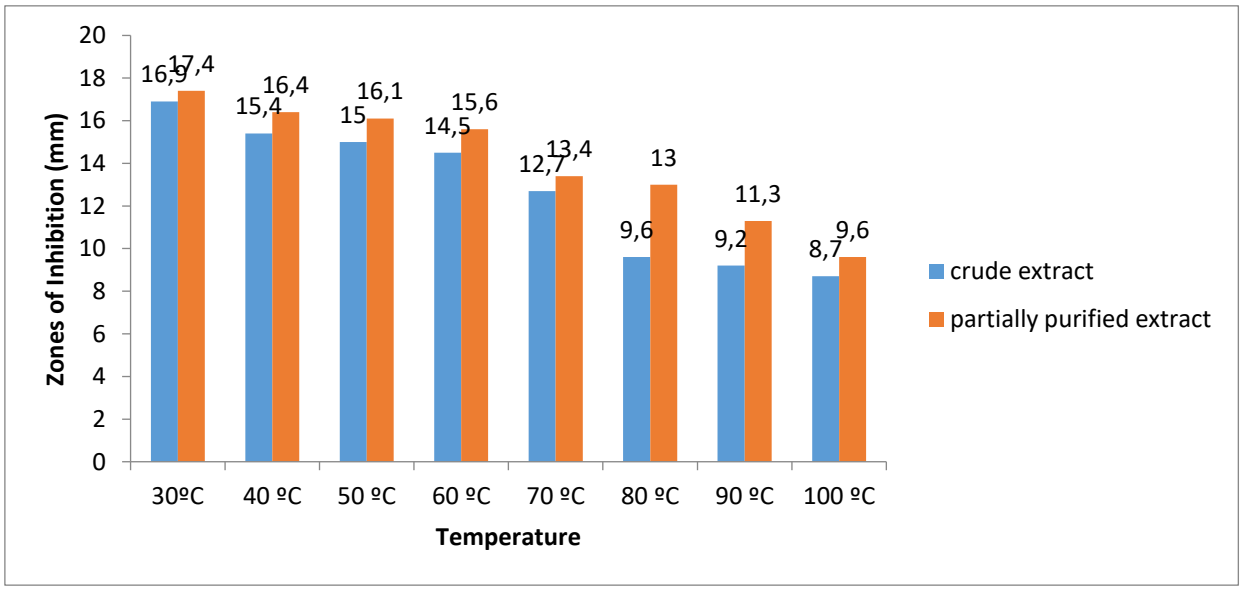


Figure 3: Effect of BLIS Produced by Isolate CQ at Different Temperature Ranges

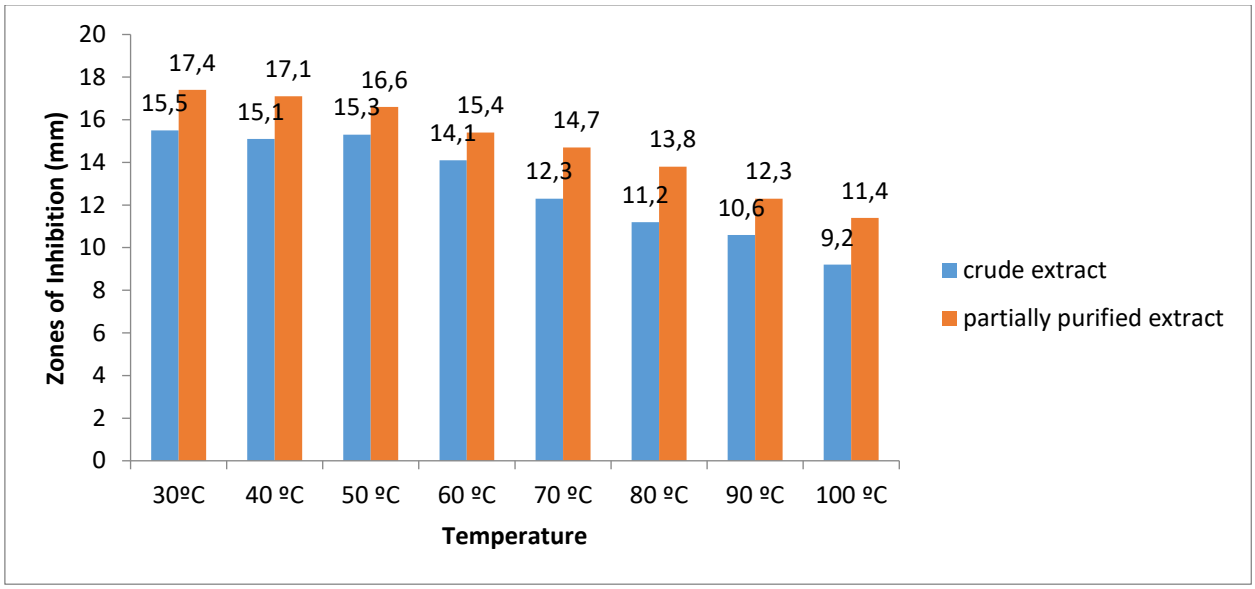


Figure 4: Effect of BLIS Produced by Isolate BH at Different Temperature Ranges

Figure 5 shows the activity of BLIS from isolate M8. Its activity was maintained throughout the temperature range but with slight decrease as the temperature increased. The activity of the partially purified BLIS was stable between 30°C - 50°C with an average of 13.3 mm zone of inhibition.

Figure 6 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 7 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 8).

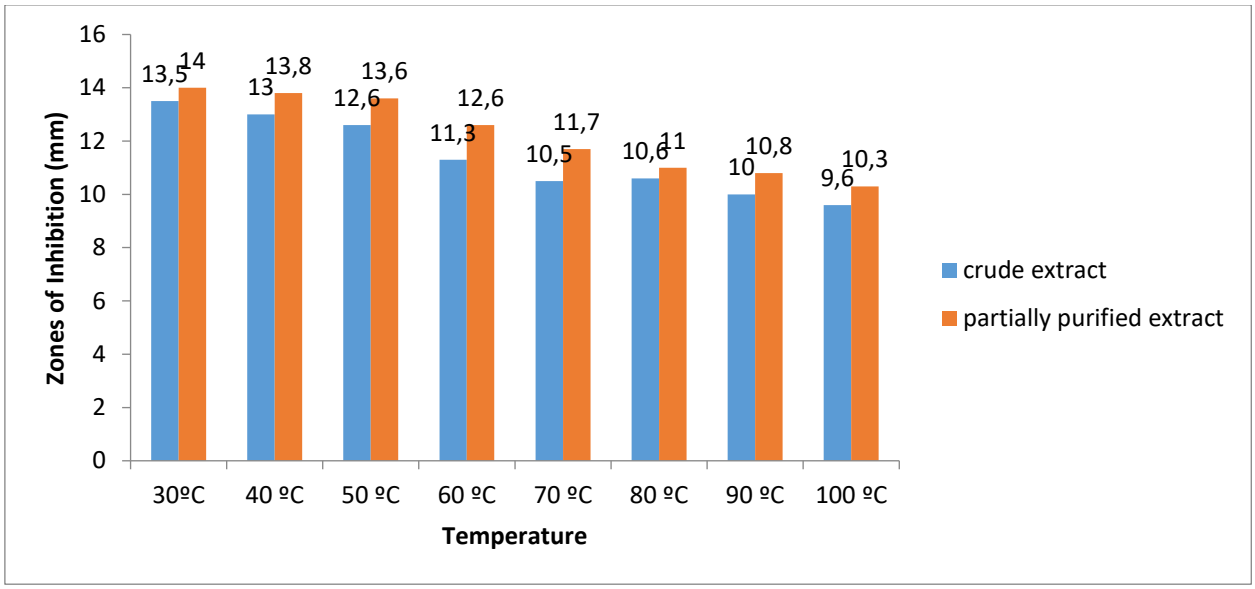


Figure 5: Effect of BLIS Produced by Isolate M8 at Different Temperature Ranges

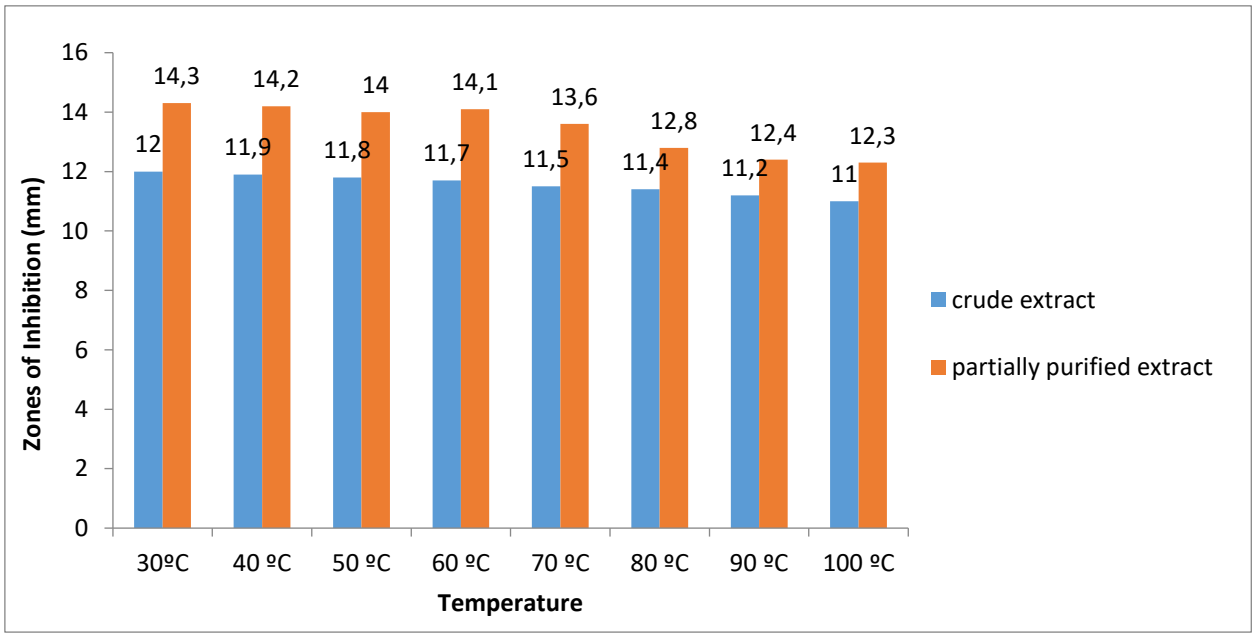


Figure 6: Effect of Blis Produced by Isolate S8 at Different Temperature Ranges

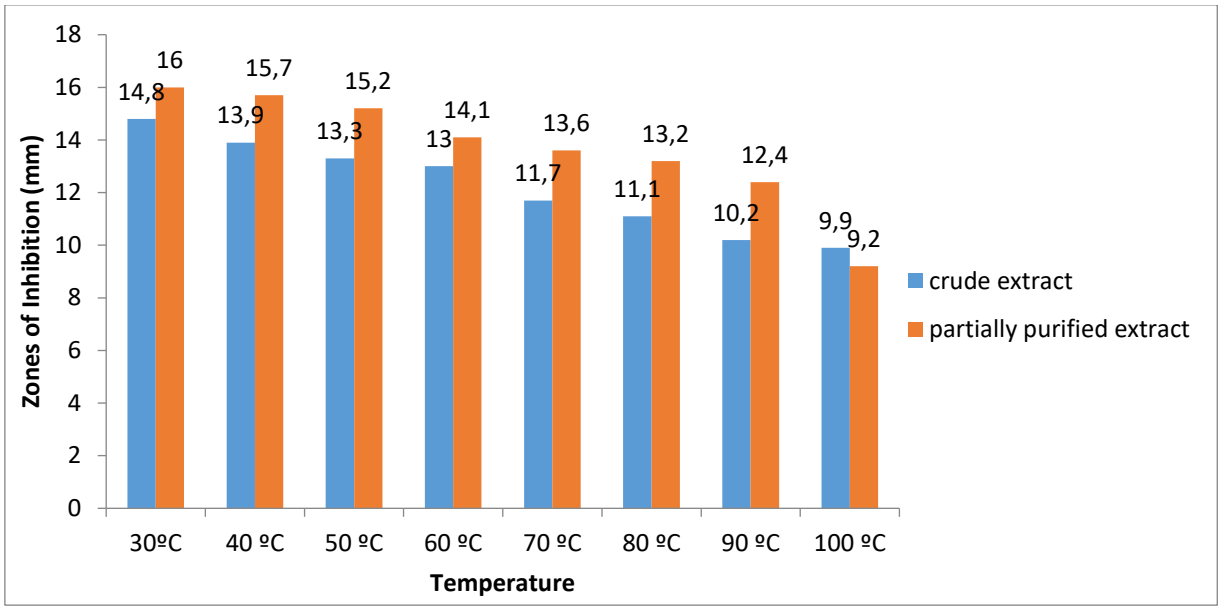


Figure 7: Effect of Blis Produced by Isolate S4 at Different Temperature Ranges

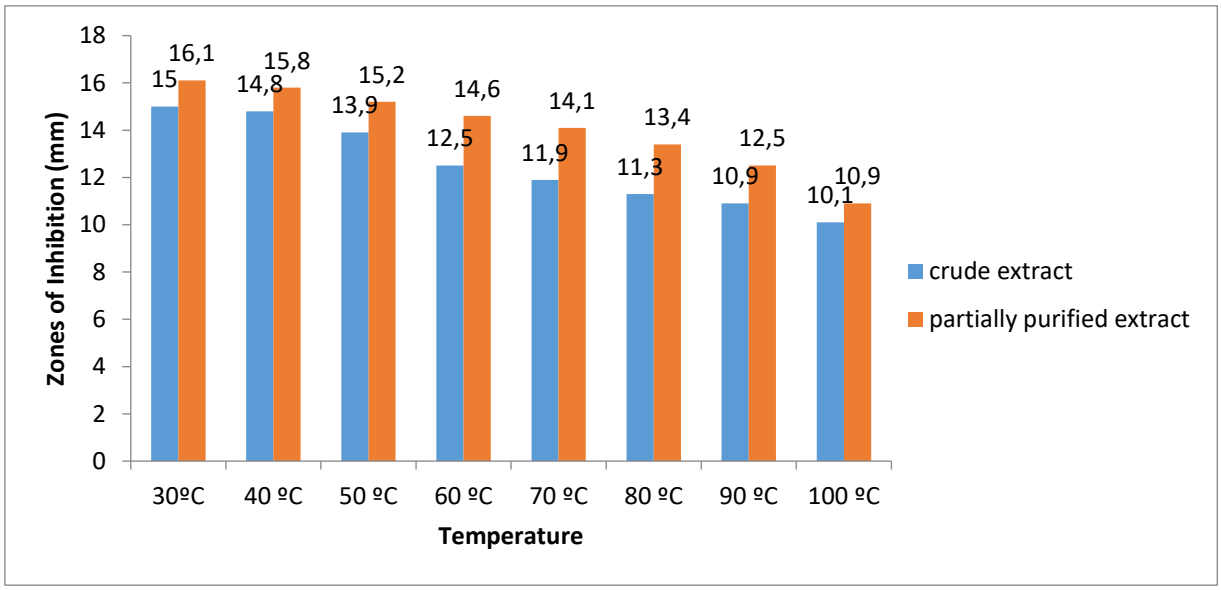


Figure 8. Effect of Blis Produced by Isolate S2 at Different Temperature Ranges

Each inhibitory compound was assayed for its stability after 1h 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 BO, CQ, BH, M8, and 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, followed by CQ at 17.4 mm. Generally the activities of the isolates were more active at acidic pH than at alkaline pH.

The result of the individual inhibitory capacity shows that S2 and S8 had the highest activity against the target organism. The combination of the two inhibitory compounds resulted in increased activity against the target organism which was shown by increase in the zones of inhibition. The crude extract of the combined inhibitory compounds had 19.06 mm zone of inhibition, while the partially purified extracts had 21.07 mm zone of inhibition. The inhibitory activity of the partially purified extract was further evaluated at various pH levels. The combined inhibitory compound was active at a wide range of pH (5.5 - 8.0). They were also evaluated at different temperature range (30°C - 100°C) for 10 mins. The data showed that the combined inhibitory compound had inhibitory capacity at the temperature ranges tested. They were stable between temperatures 30°C to 60°C.

Table 6: Antibiotics Sensitivity Test for Positive Isolates

Test Isolate	Antimicrobial Agent	Zone of Inhibition (mm)	Disk Content (µg)	Zone Diameter (Interpretative Criteria)		
				S	I	R
SMH	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			
SBF	Ciprofloxacin		5			
SMA	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			
SBL	Amoxicillin		10			
	Streptomycin	24	10			
	Chloramphenical	24	30			
	Ciprofloxacin		5			
	Norflaxacin	18	10			
	Erythromycin		10			
	Colistin		10			
	Ampiclox		20			
	Rifampin		5			
	Levofloxacin		5			
SBO	Amoxicillin		10			
	Streptomycin	19	10			
	Chloramphenical	20	30			
	Ciprofloxacin		5			
	Norflaxacin	18	10			
	Erythromycin		10			
	Colistin		10			
	Ampiclox		20			
	Rifampin		5			
	Levofloxacin		5			
SBN	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			
SCQ	Ciprofloxacin	24	5			
SBJ	Ciprofloxacin	27	5			
SBC	Amoxicillin		10			

	Streptomycin	21	10			
	Chloramphenical		30			
	Ciprofloxacin		5			
	Norflaxacin		10			
	Erythromycin		10			
	Colistin		10			
	Ampiclox		20			
	Rifampin		5			
	Levofloxacin	18	5			
SBF	Ciprofloxacin	24	5			
SBN	Ciprofloxacin	20	5			
SMB	Amoxicillin		10			
	Streptomycin		10			
	Chloramphenical	15	30			
	Ciprofloxacin	17	5			
	Norflaxacin		10			
	Erythromycin		10			
	Colistin		10			
	Ampiclox		20			
	Rifampin		5			
	Levofloxacin		5			

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

DISCUSSION

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 Deman Rogosa [17]. *Lactobacillus plantrum* is found to be a normal inhabitant of healthy women [15]. Isolate M8 in this study is probably *L. plantarum* based on biochemical findings. Isolate S4 and S2 which were provisionally identified as *L. acidophilus* in this study, has been isolated from other studies [18]. Aslim *et al.* [15] found 16% *L. acidophilus* in healthy women. According to Li *et al.* [19], the rate of vaginal *L. acidophilus* in pregnant women was 76.9%. *L fermentum* is also a normal inhabitant of healthy women [20]. *Lactobacillus* species are generally isolated from the vaginas of healthy women. The presumptive *Lactobacilli* species found in this study were relatively same to the species described by the authors above. This indicated that the vary finding correspond with the research of Aslim *et al.* [15], Deman Rogosa [17], Alpay *et al.* [18] and Li *et al.* [19].

Bacteriocins/Bacteriocin-like inhibitory substances (BLIS) produced by *Lactobacillus* is one of its protective mechanism against pathogenic and opportunities microorganisms in the vagina. There have been researches on the antimicrobial activity and positive effects of bacteriocin/BLIS produced by vaginal *Lactobacillus* on Gram positive and closely related organism [21]. However, the effect of vaginal *Lactobacillus* on the test organism, Group B *Streptococci* (GBS), has been scarcely examined.

BLIS produced by these research were able to inhibit the growth of the test organism (GBS). This study is in agreement with the study of Strus *et al.* [22]. They reported that species of *Lactobacillus* were able to inhibit the growth of GBS. This findings are also consistent with the findings of Ruiz *et al.* [23] where BLIS produced by *L. fermentum* and *L. rhamnosus* were found to inhibit the growth of GBS. The combination of the BLIS from the two *Lactobacillus* species showed higher inhibitory activity than the individual BLIS which was also seen in our present study.

The antimicrobial activity produced by the isolates of this study were identified to be as a result of bacteriocin/bacteriocin-like inhibitory substances, as their inhibitory activities were not lost after treatment with catalase or adjustment of pH to 6.5. Activity of BLIS produced by this isolates were partially characterized and tested for their *in vitro* antimicrobial activity against the test organism at different temperature and pH

levels. Activities of BLIS from the isolates were moderately heat stable as the effect of temperature on them was effect tested for only 10 mins. Isolate S4 lost its activity at 100°C in 10 mins and might be said to be heat-labile.

Many of the bacteriocins/bacteriocin-like inhibitory substances produced by lactic acid bacteria are only stable at acid and neutral pH and are inactivated at a pH above 8.0. These findings are consistent with this results; BLIS from this isolates were active over a wide pH range (pH 5.5-8.0) with highest activities at the acidic pH. The activity of the BLIS decreased at the alkaline pH. BLIS from isolates BO, BH, CQ, S2, and S4 had highest activity at pH 5.5.

The findings showed that there is no significant difference between the activity of partially purified BLIS and the crude BLIS. This suggests that the BLIS may be lower molecular weight molecules. From the antibiotic assay carried out for the treatment of these infections, some antibiotic were resistant, (levofloxacin, amoxicillin and ampiclox), some were sensitive (ciprofloxacin, erythromycin, chloramphenicol and streptomycin) and very few were at intermediate. Dreadlock

CONCLUSION

The BLIS produced by *Lactobacilli* isolated from vagina of pregnant women in Wukari Taraba state inhibited the growth of GBS at different pH and temperature ranges, which suggests its usefulness as a probiotics and an alternative to antibiotics. The antagonistic effect of the combined BLIS was stronger with higher heat stability than individual BLIS, suggesting that combination of bacteriocins may be more effective. This study revealed a notable prevalence of GBS and *Lactobacillus* among pregnant women in Wukari. The antimicrobial properties of *Lactobacillus*-derived BLIS against GBS highlight their potential as therapeutic agents. Further research is recommended to explore the clinical applications of these findings and to develop alternative treatments for vaginal infections.

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