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Prevalence and Antimicrobial Properties of Lactic Acid Bacteria in Nigerian Women During the Menstrual Cycle

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Abstract

The composition of vagina lactic acid bacteria (LAB) differs within the different ethnic group. This study is aimed at determining the prevalence of LAB with their antimicrobial properties in Nigerian women's vagina during different stages of the menstrual cycle. Microorganisms were isolated from vaginal swabs of ten Nigerian women during different stages of the menstrual cycle and identified by partial sequencing of the 16S rRNA gene. The antimicrobial properties of the LAB were tested against the multidrug-resistant uropathogens. The prevalence of LAB was higher during ovulation period while during menstruation period, it declined. Twenty-five LAB isolates were identified as three species, namely: *Lactobacillus plantarum* (15), *Lactobacillus fermentum* (9), *Lactobacillus brevis* (1) and one acetic acid bacteria – *Acetobacter pasteurianus*. The LAB had antimicrobial activities against the three uropathogens with zones of inhibition from 8 to 22 mm. The presence of LAB inhibits the growth of *Staphylococcus* sp. GF01 also in the co-culture. High LAB counts were found during ovulation period with *L. plantarum* as a dominant species while during menstruation, there was a decrease in the LAB counts. The isolated LAB has antimicrobial properties against the urogenital pathogens tested thus exhibiting their potential protective role against uropathogens.

Key words: menstrual cycle, Nigerian women, lactobacilli, uropathogens

Introduction

A healthy human vagina is primarily colonized by the genus *Lactobacillus* (Shiraishi et al. 2011) and it builds a barrier separating the pathogens from the epithelium, thereby, protecting the vagina. The pH ~4.5 also maintains the balance of the vaginal ecosystem as well as antimicrobial substances e.g. hydrogen peroxide against pathogens (Ayeni and Adeniyi 2013; Ghartey 2014). Occasional and recurrent vaginal yeast and bacterial imbalances are common among premenopausal women, which can be due to hormonal changes during menstrual cycle, antibiotic treatment, pregnancy, sexual intercourse, excessive intimate hygiene and use of tampons, which may predispose a woman to infections.

The hormonal changes occur during the reproductive stages with the resulting fluctuating levels of hormones that regulate the menstrual cycle. This is an important influence on the vaginal microbiota during human reproductive years (Farage et al. 2010). Women of different racial groups may exhibit different composition of microbial communities and, correspondingly, different susceptibility to vaginal infections. Women are more prone to urinary tract infections (UTI) than men due to the position of the urethra. The reduction in protective vaginal flora may increase the risk of these infections (Gupta et al. 2017).

Lactic acid bacteria (LAB) have been shown to inhibit the *in vitro* growth of pathogenic microorganisms, e.g. *Klebsiella* spp. *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli*, etc. (Ayeni and Adeniyi 2012; Adeoshun and Ayeni 2016). This can be achieved mainly through the action of lactic acid (Graver and Wade 2011). During menstruation, the diminished population of lactobacilli and the presence of menstrual fluid make the vaginal less acidic, therefore, more prone to colonization by pathogenic microorganisms.

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Changes between different bacterial species in the vagina are associated with menses (Gajer et al. 2012).

The antimicrobial activity of the vaginal fluids correlates with an increased lactic acid content, low pH and competitive exclusion. The increased susceptibility to disease may be also related to vaginal microbiota fluctuations (Gajer et al. 2012). Ayeni and Adeniyi (2013) and Agboola et al. (2014) had reported the presence of organisms in healthy and menstruating women with their antimicrobial properties in Nigeria. However, there is no information to establish changes in vaginal microbiota at different stages of the menstrual cycle and the antimicrobial effects of the isolated LAB. Therefore, this study aimed at determining the prevalence of LAB at different stages of the menstrual cycle in Nigerian women with their potential antimicrobial properties.

Experimental

Materials and Methods

Pathogens. Ten clinical strains of uropathogens: *Klebsiella* spp., *Staphylococcus* spp. and *Pseudomonas* spp. (isolated from urine) were collected from the culture collection of the Medical Microbiological Laboratory of the University College of Medicine (UCH), Ibadan.

Sample collections, isolation, and lactic acid bacteria viability counts. Ethical approval (UI/EC/13/0258) was obtained from the Institutional Ethical Committee (IEC), Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Ibadan, Nigeria. Ten Nigerian women volunteers aged between 20–40 years were recruited into this study. They were premenopausal, self-declared healthy, not on antibiotics or hormonal therapy for at least four months, not on contraceptives, having regular menstrual cycle, i.e. a complete menstrual cycle between 25–30 days every month, and exhibiting the three different stages within the cycle. For each volunteer, Day 1 of menstrual flow is noted as day one of the menstrual cycle. For a five-days flow, samples were taken on day 3 and for a 4-days flow, samples were taken on day 2. Safe period is between 6^{th}–12^{th} day of the menstrual cycle while the ovulation period is between 13^{th}–15^{th} day of the menstrual cycle. The safe period samples were taken on day 6 after menstrual flow stopped. The ovulation period samples were obtained midway of the menstrual cycle. The signed informed consent was obtained from each volunteer. The samples were collected from the volunteers during different stages of their menstrual cycle between August and September 2014. The volunteers used sterile swab sticks to swab the vagina according to the standard protocols. The samples were collected during the three different stages of the menstrual cycle and immediately inoculated in 10 ml MRS broth, (Oxoid, UK) adjusted to pH 5, shaken vigorously for 10 s and incubated under the microaerophilic condition using CampyGen™ at 37°C for 24 h. Serial dilutions were done using sterile normal saline and the suspension of the suitable dilution factor was plated out on MRS agar by pour plate method, then incubated under microaerophilic condition at 37°C for 48 h. The initial colony counts were noted and colonies were picked according to the differences in their colony morphology on MRS agar plates and isolated by streaking onto another MRS agar to obtain a distinct colony. Gram staining and catalase test were carried out and only the colonies that were Gram-positive and catalase-negative were picked and stored in MRS broth containing 20% w/v glycerol at –20°C for further characterization and identification.

Identification of Lactic Acid Bacteria. The DNA of the LAB isolated were extracted by QuickExtract™ DNA extraction solution (Epicentre, Wisconsin) according to the manufacturer's instructions. The PCR mixture consisted of a total volume of 20 µl (1 µl of DNA extract, 10 pmol of each primer, and 25 µl of 2-fold concentrated RedTaq Ready Mix (SigmaAldrich, Germany)). The primers used for amplification were (5'-TGTAA AACGGCCAGTAGAGTTTGATC(AC)TGGCTCAG) and (5'-CAGGAAACAGCTATGACCG(AT)ATTAC CGCGGC(GT)GCTG), containing an M13 primer sequence (Montanaro et al. 2016). PCR conditions were 95°C for 5 min; 35 cycles each of 95°C for 15 s, 58°C for 30 s, and 72°C for 45 s; and a final step at 72°C for 10 min. Ten microliters of the amplified products were analyzed on 1.5% agarose gels and subsequently sequenced using the BigDye Terminator v3.1 sequencing kit (Applied Biosystems, California). The sequence was blasted against the NCBI database for species identification. The nucleotide sequences for the 16S rRNA genes have been deposited in the GenBank database under accession numbers KX261342 to KX261366.

Antibiotic susceptibility of uropathogens. Ten uropathogenic strains were collected and screened against seven antibiotics by disk diffusion methods. A bacterial lawn was accomplished by spreading inoculum from 10^8 dilution factor of the pathogen culture which is approximately equivalent to 0.5 McFarland standards by a sterile swab stick. The antibiotic disks containing ceftazidime (30 µg), cefotaxime (30 µg), cefuroxime (30 µg), Augmentin (amoxicillin clavulanate) (10 µg), ciprofloxacin (30 µg), ofloxacin (30 µg), and gentamycin (30 µg) were placed on the surface of the solidified agar with the aid of sterile forceps and incubated aerobically at 37°C for 24 h. The susceptibility of the test organisms to the antibiotics used was documented by measuring the diameter of the clear zones of inhibition in millimeter (mm) around the antibiotics disks, and the results were interpreted according to the guidelines of European Committee on Antimicrobial Susceptibility Testing (2015). The resistant strains were selected for LAB antimicrobial study.

Determination of LAB antimicrobial activities against bacterial uropathogens. To study the antimicrobial potential of the LAB against clinical isolates of uropathogens, three different methods were employed, which are: using the cell free supernatant *via* agar well diffusion method, using the viable LAB cells *via* agar overlay method, and co-culture of the LAB and uropathogens.

Determination of the antimicrobial activity of the cell-free supernatant. The LAB isolates were grown in MRS broth overnight under the microaerophilic condition at 37°C, centrifuged at 10 000 rpm for 10 min and the supernatant decanted. The antimicrobial activities of the cell-free supernatant were determined twice, i.e. before and after neutralization to pH of 6.5 with 1 M NaOH, using the agar well diffusion assay against *Staphylococcus* sp. GF01, *Pseudomonas aeruginosa* GF01, and *Klebsiella* sp. GF01.

Determination of the antimicrobial activity of viable lactic acid bacterial cells. The modified agar overlaid method as described by Ayeni et al. (2011) was used in this study. In summary, the LAB cells in broth were inoculated in two 2-cm-long lines on an MRS agar surface and then incubated at 37°C for 24–48 h in microaerophilic conditions. The plates were overlaid with 0.2 ml of an overnight broth culture of the test pathogen vehiculated in 10 ml soft nutrient agar and incubated at 37°C under aerobic condition. The plates were then examined for a clear zone of inhibition around the line of the LAB and the clear zones were measured in millimeters.

Coculture of lactic acid bacteria and uropathogens. The interference of the LAB strains with the growth of uropathogenic strains was evaluated by coincubating *Staphylococcus* sp. GF01 with four representative strains of LAB (*Lactobacillus brevis* GF021, obtained from the menstruation period, *Lactobacilus fermentum* GF002, *Lactobacillus plantarum* GF011, obtained from the safe period and *Lactobacillus fermentum* GF019, obtained from the ovulation period). This was done in two series of experiments.

In the first experiment, an overnight culture of *Staphylococcus* sp. GF01 was inoculated into 5 ml double strength nutrient broth and then added to 5 ml of overnight culture of the LAB and the mixture was incubated for 24 h. The monoculture of the mixture, the LAB and *Staphylococcus* sp. GF01 (control) was evaluated at time zero (t_0) and after incubation. For the second experiment, 5 ml of *Staphylococcus* sp. GF01 was incubated for 8 h, after which it was centrifuged and the supernatant discarded, 5 ml of double strength nutrient broth was added to resuspend the pellets, vortexed and added to a 5 ml of overnight culture of the LAB. *Staphylococcus* sp. GF01 monoculture and the mixture was plated out at 8 h and 24 h to evaluate the growth of *Staphylococcus* sp. GF01.

Results

The three organisms used exhibited high resistance (i.e. 0 mm zones of inhibition) towards most of the antibiotics used. The *Pseudomonas* and *Staphylococcus* strains tested were resistant to ceftazidime, cefotaxime, cefuroxime, Augmentin (amoxicillin clavulanate) but sensitive to ciprofloxacin, ofloxacin, and gentamycin, while the *Klebsiella* sp. GF01 strain was completely resistant to all the antibiotics.

Ten volunteers were assessed for a level of LAB in their vagina at different stages of the menstrual cycle. It was observed that in seven (70%) out of the ten volunteers, there was a significant shift of the LAB level from low to high (8×10^5 to 7.6×10^9) CFU/ml over the course of the menstrual cycle. In the remaining three (30%) volunteers, the presence of LAB was not observed throughout the menstrual cycle (Table I).

A total of twenty-seven (27) bacterial species were identified from the three different stages of menstrual cycle as five species (*L. plantarum* (15), *L. fermentum* (9), *Lactobacillus brevis* (1), *Bacillus safensis* (1) and *Acetobacter pasteurianus* (1)) and their percentage occurrence at different stages of the menstrual cycle was shown in Table I. Twenty-five (25) isolates (92.59%) belonging to the genus *Lactobacillus* occurred in the three stages, while one (1) isolate (3.70%) each belonged to *Bacillus* and *Acetobacter*, both noted during safe (follicular) period, only. The organism with the highest frequency of occurrence (60%) among the *Lactobacillus* species was *L. plantarum* and it constituted 55.55% among all the isolates studied, and its highest occurrence was during the ovulation period. *L. brevis* has the lowest frequency of occurrence of 4% among the *Lactobacillus* spp. and 3.7% among the total isolates, and it occurred during the menstruation period.

The cell-free supernatants and viable cells showed a clear inhibitory antimicrobial activity against *Pseudomonas aeruginosa* GF01, *Klebsiella* sp. GF01 and *Staphylococcus* sp. GF01. Out of 27 LAB isolates used against *P. aeruginosa* GF01, 20 (74.07%) of the isolates had zones of inhibition ranging from 8 to 22 mm against *Klebsiella* sp. GF01, 24 (88.89%) had zones of inhibition ranging from 10 to 20 mm, while 20 (74.07%) had inhibition zones ranging from 10 to 20 mm against *Staphylococcus* sp. GF01. *Staphylococcus* sp. GF01 was the least susceptible to the LAB isolated while *Klebsiella* sp. GF01 was the most susceptible (Table II).

Table I
Evaluation of the LAB counts at different stages of the menstrual cycle.

Week	Menstruation Period		Safe Period		Ovulation Period	
	Total CFU/ml	LAB CFU/ml	Total CFU/ml	LAB CFU/ml	Total CFU/ml	LAB CFU/ml
1	1.02×10^8	4.2×10^7	1.81×10^{10}	9.3×10^9	2.22×10^{10}	1.51×10^{10}
2	5.6×10^7	1.0×10^6	1.91×10^{10}	5.2×10^9	1.90×10^{10}	1.14×10^{10}
3	7.8×10^7	1.2×10^6	1.89×10^{10}	7.4×10^9	2.53×10^{10}	1.51×10^{10}
4	7.0×10^7	2.2×10^6	1.87×10^{10}	1.89×10^{10}	9.8×10^9	–
5	9.2×10^7	1.4×10^6	1.52×10^{10}	4.2×10^9	1.87×10^{10}	1.02×10^{10}
6	6.1×10^7	8×10^5	8.3×10^9	3.0×10^9	1.12×10^{10}	7.6×10^9
7	1.13×10^8	2.5×10^6	2.11×10^{10}	5.4×10^9	3.26×10^{10}	1.02×10^{10}
8	2.0×10^3	Nil	1.05×10^4	–	1.05×10^4	Nil
9	3.8×10^3	Nil	8.5×10^3	–	5.8×10^3	Nil
10	4.2×10^3	Nil	1.12×10^4	–	8.5×10^3	Nil

Note – Nil means no count of bacteria

Table II
Determination of the antimicrobial activity of the cell-free supernatant and viable cells.

	Cell-free supernatant			Viable Cell*		
	P. aeruginosa GF01	*Klebsiella* sp. GF01	*Staphylococcus* sp. GF01	*P. aeruginosa* GR01	*Klebsiella* sp. GR01	*Staphylococcus* sp. GF01
L. fermentum GF002	16	12	20	20	15	20
A. pasteurianus GF004	20	13	0	20	18	12
L. plantarum GF005	15	14	0	18	20	0
L. fermentum GF006	15	14	12	18	20	15
L. plantarum GF007	20	10	15	20	15	18
L. fermentum GF008	15	15	15	20	18	18
L. plantarum GF009	10	12	13	19	20	15
L. plantarum GF010	20	12	10	15	20	20
L. plantarum GF011	22	13	17	20	16	20
L. fermentum GF012	19	14	0	20	20	18
L. fermentum GF013	22	12	0	13	18	20
L. plantarum GF015	18	10	0	20	15	20
L. plantarum GF016	19	12	0	20	20	20
L. fermentum GF018	19	20	20	20	20	15
L. fermentum GF019	18	20	16	20	15	20
L. brevis GF021	19	20	15	20	20	20
L. plantarum GF022	8	10	0	0	20	15
L. plantarum GF023	10	0	0	12	15	0
L. fermentum GF024	14	13	15	12	15	15
L. plantarum GF025	10	15	0	10	18	0
L. fermentum GF026	0	18	0	0	18	0
L. plantarum GF029	0	0	0	0	0	0
L. plantarum GF030	0	0	0	10	0	0
B. safensis GF031	0	13	0	10	15	0
L. plantarum GF032	0	10	0	0	10	0
L. plantarum GF033	0	18	18	10	18	18
L. plantarum GF036	0	10	0	0	12	0

* Antimicrobial activity is expressed as diameters of inhibition zones in mm

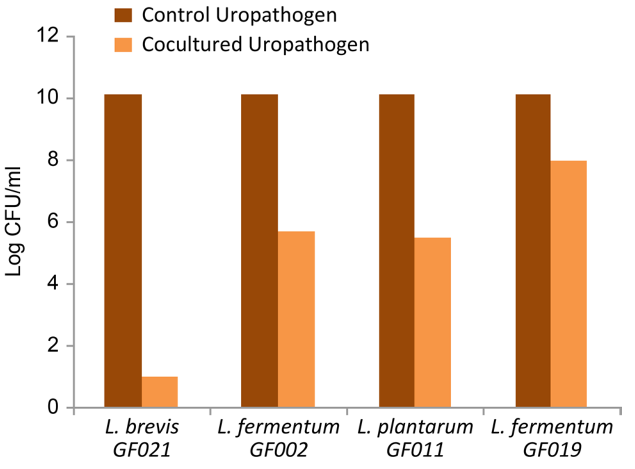

Fig 1. Inhibition of *in vitro* growth of *Staphylococcus* sp. GF01 by *L. brevis* GF021, *L. fermentum* GF002, *L. plantarum* GF011, and *L. fermentum* GF019 after 24 h – coincubation.

After the neutralization of the cell-free supernatant, no obvious antimicrobial activity was observed against the uropathogens.

The capability of the LAB strains to inhibit the *in vitro* growth of *Staphylococcus* sp. GF01 was evaluated in coculture experiment which was carried out in two parts. In the first experiment, *L. brevis* GF021 was active against *Staphylococcus* sp. GF01 with 6 $\log_{10}$ reduction after 24 h, had and 5 $\log_{10}$ reduction of *Staphylococcus* sp. GF01 after incubation with *L. fermentum* GF002 or *L. plantarum* GF011. *L. fermentum* GF019 had a low activity against *Staphylococcus* sp. GF01, which demonstrated only 3 $\log_{10}$ reduction in numbers of CFU. It was observed that *Staphylococcus* sp. GF01 did not have an effect on any of the LAB strains (Fig. 1). In the second experiment, *Lactobacillus brevis* GF021 was active against *Staphylococcus* sp. GF01 with a 6 $\log_{10}$ reduction. *L. fermentum* GF002 and *L. plantarum* GF011 were not active against *Staphylococcus* sp. GF01 while *L. fermentum* GF019 showed a low activity against *Staphylococcus* sp. GF01 with just 1 $\log_{10}$ reduction. *L. fermentum* GF019 had the least activity and *L. brevis* GF021 had the highest activity (6 $\log_{10}$ reduction) on *Staphylococcus* sp. GF01 (Fig. 2).

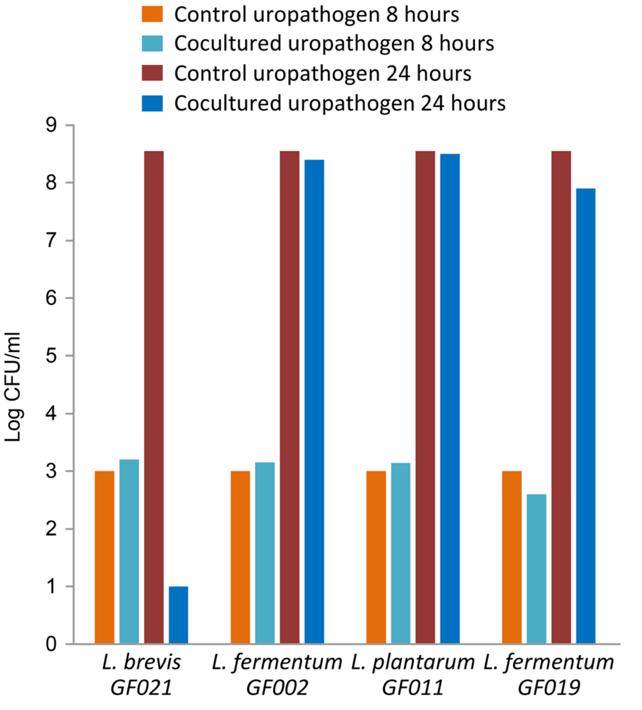

Fig 2. Inhibition of *in vitro* growth of *Staphylococcus* sp. GF01 by *L. brevis* GF021, *L. fermentum* GF002, *L. plantarum* GF011, and *L. fermentum* GF019 at 8 and 24 h of coincubation.

Discussion

The resistance to broad-spectrum antibiotics is a persistent challenge in the management of infections (Ayeni et al. 2011). In this study, *Klebsiella* sp. GF01, *P. aeruginosa* GF01 and *Staphylococcus* sp. GF01 were isolated from urogenital infections. These uropathogens were found to be multidrug resistant, especially *Klebsiella* sp. GF01, which was completely resistant to all the antibiotics tested in this study. This high phenotypic resistance is making the present antibiotic therapy for bacterial infections ineffective thereby resulting in more search for naturally occurring remedies, e.g. LAB (Ayeni et al. 2011).

The species of beneficial bacteria identified from the vaginal samples in this study were *L. fermentum, L. brevis*, *L. plantarum*, *B. safensis*, and *A. pasteurianus.* Out of 27 isolates, 25 isolates were lactobacilli where *L. plantarum* and *L. fermentum* were the most predominant species, with *L. plantarum* having the highest occurrence during the ovulation period. Dareng et al. (2016) also reported *Lactobacillus iners* and *Lactobacillus crispatus* in the vagina of Nigerian women, while a study of vaginal *Lactobacillus* strain in the pregnant Korean women reported prevalence of *L. crispatus* and *L. iners*, followed by *L. gasseri* and *L. jensenii* (Kim et al. 2017). There are versatility and species diversity in the prevalent lactobacilli present in the vagina. This can stem from different lifestyles, geographical and environmental conditions. There is usually a predominance of *Lactobacillus* in healthy women, including *L. iners* and *L. crispatus* in women in the reproductive age (Xu et al. 2013; Ghartey et al. 2014). However, there may be also a complete absence of lactobacilli in the other apparently healthy women. This is in accordance with this study, in which twenty-seven LAB were isolated from the vagina of seven healthy Nigerian women out of the ten volunteers, while no the LAB was detected in three of the women. This result of the LAB absence in women could be due to immunosuppression. Other factors could include infections, stress, nutrition intake, etc.

Many researchers have reported the prevalence of different LAB species isolated from the vagina of women from different geographical area (Gajer et al. 2012; Chaban et al. 2014; Shiraishi et al. 2011), but few have been able to report the type of LAB present or absent during the different stages of a woman's menstrual cycle in different countries and specific ethnic aspects; this could influence the structure of the microbiota in specific niches. It was observed that during the menstruating period, the LAB count was low while at the safe/follicular period, the presence of the LAB was greater, but a large amount of LAB was found at the latter part of the cycle. Thereby, there was a significant shift in the LAB level from low to high over the course of the menstrual cycle. Menstruation may enhance a distortion of the bacterial microbiota around the vulva (Shiraishi et al. 2011) and influence *Lactobacillus* spp. which were the dominant organism in most girls before the onset of menses from the early to middle stages of puberty (Hickey et al. 2015).

The absence or low the LAB count during menses may suggest the growth of yeast which can outgrow the bacteria in immunocompromised patients causing yeast and other urogenital infections. However, during safe and ovulation periods when the LAB count is increasing there is a decrease in the yeast count probably due to the antagonistic effect of these LAB on yeast or the hormonal changes taking place during these periods (Relloso et al. 2012). Women may be more susceptible to urogenital tract infections during the menstruation period compared to the ovulation period due to the high prevalence of the LAB during the ovulation period. The dynamic nature of the vaginal environment leads to changes in the microbiota of the vagina as a result of exposure to pathogens and physiologic fluctuations of the menstrual cycle (Farage et al. 2010). In the course of this study, *L. plantarum* dominated during the ovulation period, *L. brevis* was found during menstruation and *B. safensis* during the safe period. The presence of these organisms at different periods can be attributed to change in vagina pH, hormonal change and even the blood flow during menses.

Lactobacilli isolated from the vagina have a prominent role as a prophylactic aimed at improving the vaginal microbiota defense against bacterial infections. The cell-free supernatant and the viable LAB cells exhibited capabilities to inhibit the growth of the uropathogens, albeit to a different extent. The vaginal strains of *L. acidophilus* had been reported to inhibit the growth of *Klebsiella* sp. and some other uropathogenic strains (Ayeni and Adeniyi 2012; Adeoshun and Ayeni 2016). Most of the *L. plantarum* strains showed the most impressive effect. The antagonistic activity of *L. brevis* GF021 was also appreciable. It was suggested that the organic acid produced by these LAB play a major role in the antagonistic activity because after neutralization, there was no obvious effect. This result agrees with Ayeni et al. (2011) who reported that the antimicrobial properties of LAB are related to their metabolic products such as organic acids and hydrogen peroxide. Non-lactic acid bacterial strains i.e. *B. safensis* and *A. pasteurianus* also could inhibit the uropathogens growth. To the best of our knowledge, this is the first study that will report the presence of these two species in the vagina of a woman. *B. safensis* has biotechnological and industrial potentials (Larboda et al. 2014) while *A. pasteurianus* is important in vinegar production (Viana et al. 2017). The mechanism by which this organism inhibits the three uropathogens used in this study is probably due to the production of acetic acid.

The resistance of *S. aureus* strain to the cell-free supernatant from most of the LAB strains in this study prompted another mechanism of antagonism through co-culture experiment. Bamidele et al. (2013) also reported that methicillin-resistant *S. aureus* (MRSA) strains were resistant to the cell-free supernatants of the LAB but higher activities were shown when the LAB was in contact with the pathogens. Different LAB strains have different rates of the killing of *Staphylococcus* sp. GF01. In the first experiment, the growth of *Staphylococcus* sp. GF01 was not influenced by the presence of the lactobacilli while for the second experiment, the 8 h already grown *Staphylococcus* sp. GF01 overpowered the LAB activity, except for *L. brevis.* Very good activity demonstrated the strain *L. brevis* GR01. There was an inhibition (5 $\log_{10}$ reduction) observed only when the pathogen was freshly introduced but no effect was noted towards the 8 h already grown pathogen. The study of Adetoye et al. (2018) reveals a similar process where it was reported that an effective inhibition was observed when the LAB was co-cultured with the pathogens. The presence of the LAB inhibited the growth of *Staphylococcus* sp. GF01 freshly introduced, while for the already 8 h grown *Staphylococcus* sp. GF01, the effect of LAB was not obvious, except for *L. brevis.* It was suggested that *Staphylococcus* sp. GF01 was able to overpower or suppress the activity of the LAB. The decrease in the number of *Staphylococcus* sp. GF01 reveals the antimicrobial activity of the LAB cells against *Staphylococcus* sp. GF01. These data support the result obtained previously using the cell-free supernatant and the viable LAB cells confirming the high resistance of *Staphylococcus* sp. GF01 to the LAB strains.

Conclusion

The high LAB counts were found during the ovulation period while during menstruation, there was a decrease in the LAB counts. The highest occurrence in the vagina of Nigerian women was shown for *L. plantarum* that mostly was found during the ovulation period. The LAB isolated has the antimicrobial properties against multidrug-resistant urogenital pathogens what may be applicable *in vivo*. The fermented foods such as Ogi, yogurt, etc. can be consumed during menstruation in order to replenish the beneficial bacteria. To the best of our knowledge, this is the first study in Nigeria to report a prevalence of the LAB with their protective role at different stages of a woman's menstrual cycle and also the first study to show the presence of *B. safensis* and *A. pasteurianus* in the vagina of a woman.

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Limitation of the study

The only lactic acid bacteria mechanism of antibacterial activity investigated in this study is an organic acid. Other mechanisms might be responsible. Also, the volunteers were self-declared healthy.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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