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## PROBIOTIC POTENTIALS OF LACTIC ACID BACTERIA ISOLATED FROM VAGINAL SWABS ON SELECTED GENITAL PATHOGENS

F.M. Agboola<sup>1</sup>, B.A. Adeniyi Agboola\*<sup>1</sup>, B.A. Adeniyi<sup>1</sup>, C.A. Okolo<sup>3</sup>, T.O. Ogunbanwo<sup>2</sup>, F.A. Ayeni<sup>1</sup>, C.A. Okolo<sup>3</sup>, T.O. Ogunbanwo<sup>2</sup> and F.A. Ayeni<sup>1</sup>

<sup>1</sup>Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Nigeria

<sup>2</sup>Department of Microbiology, Faculty of Science, University of Ibadan, Ibadan, Nigeria

<sup>3</sup>Department of Pathology, Faculty of Basic Medical Science, University of Ibadan, Nigeria

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### Correspondence to Author:

**Dr. Bola A. Adeniyi**

Professor of Pharmaceutical  
Microbiology, Department of  
Pharmaceutical Microbiology,  
Faculty of Pharmacy, University of  
Ibadan, Ibadan, Nigeria

**E-mail:** baadeniyi@yahoo.co.uk

**ABSTRACT:** Oral probiotics has been found useful in treatment or prevention of urogenital infections. The ability of probiotic organisms to serve as biotherapeutic agents can be attributed to various properties they possess. A total of thirty-two lactic acid bacteria (LAB) strains isolated from the vagina swab samples of healthy women in selected areas in southwestern Nigeria were characterised as *Lactobacillus fermentum*, *Lactobacillus jensenii*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Lactobacillus lactis*. *L. fermentum* exhibited a high antagonistic effect with zones of inhibition of 24mm and 25mm against *Klebsiella sp.* and *E. coli* respectively. *L. jensenii* also had a high activity with inhibition zone of 24mm against *Klebsiella sp.* Percentage antibiotic resistance of the isolates reflects that 86% of the isolates exhibited resistance to both amoxicillin and gentamicin; 73% to ampiclox and 64% to rocephine while low percentage resistance from 0% to 5% was observed for the fluoroquinolones and augumentin. The knowledge of the antimicrobial resistance of exogenously applied probiotic is of interest to predict their behaviour in patients subject to any type of chemotherapy, as well as to consider the concomitant use of the probiotic and antibiotics for the restoration of the normal urogenital flora. Production of lactic acid and hydrogen peroxide was detected in all the LAB isolates and they were observed for up to 96hours. *L. fermentum* produces more hydrogen peroxide when compared with the other isolates, it had the highest zones of inhibition against genital pathogens, hence, may be considered as an isolate with outstanding probiotic potential.

**INTRODUCTION:** Genital infections in women may result in vaginal discharge, mucosal ulceration producing local discomfort and pain on intercourse.

Continuous infection of the upper genital tract leads to infertility, ectopic pregnancies and chronic pelvic pain. In men, genital infection may cause urethral discharge, pain on voiding, and painful scrotal swellings<sup>1</sup>.

Some of pathogens isolated from the seminal fluid include *Enterococci*, *S. aureus*, *Klebsiella* species, *Escherichia coli* and other gram negative bacilli, *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.

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Unhealthy vagina cultures can also yield *Staphylococcus aureus* and beta-hemolytic streptococci. Urogenital infections are a risk factor for susceptibility to sexually transmitted diseases<sup>2</sup>. It is estimated that annually, several hundred million women suffer from urinary tract infections (UTI), with costs to health care providers amounting to over \$6 billion annually worldwide<sup>3</sup>. This figure could even be an under estimation given that there are three billion females in the world and the incidence of uncomplicated UTI in women as 0.5 episodes/person/year, with a recurrence rate of between 27% and 48%<sup>4</sup>.

UTI is also a problem in pregnancies affecting around 5% of women, and of those 20% may acquire pyelonephritis. The infection has a prevalence of 4.1 to 7.1% during infancy<sup>5</sup>. Elderly patients are more vulnerable to the infections because of their underlying diseases<sup>6</sup>.

The management of these infections usually involves the prescription of antibiotics such as tetracyclines, metronidazoles, Nystatin, terconazole, Benzathine, Penicillin G, Cephalosporins and Macrolides. Other typical treatment for these involves ampicillin or the mixture of trimethoprim and sulfamethoxazole amongst others. However, strictly chemotherapeutic treatment has its' popular high concerns which includes a high incidence of microbial resistance<sup>7</sup>, side effects, re-occurrences of infection and the high cost of maintenance. Meanwhile, diagnosis of some genital infection are extremely difficult because of the mixed infectious agents involved<sup>8</sup>.

Oral probiotics have been found useful in treatment or prevention of urogenital infections. Clinical evaluations have been conducted on the influence on *Lactobacilli* on treatment of bacterial vaginosis using intravaginal suppositories and for prevention of recurrent candidal and bacterial vaginal infections<sup>9</sup>. Several of these studies do suggest that administration of *Lactobacilli*, either orally or intravaginally, can play a prophylactic role in the etiology of this disease, presumably through the recolonization of the vaginal tract with *Lactobacilli*<sup>10</sup>. Probiotic organisms, as defined by the Food and Agricultural Organization of the United Nations, are "live microorganisms administered in adequate

amounts which confer a beneficial health effect on the host<sup>11</sup>. Lactic acid bacteria (LAB) are natural habitants of the vaginal microflora and they represent a group of probiotic bacteria<sup>12</sup>.

The ability of probiotic organisms to serve as biotherapeutic agents can be attributed to various properties which they possess. One of these is their ability to adhere to and colonize tissues; another is the capacity to inhibit the pathogenesis of disease causing organisms. They also produce biosurfactants and several anti-adhesion molecules which inhibit the attachment and colonization of a broad range of pathogens<sup>13</sup>. Other properties include the ability to produce inhibitory substances such as hydrogen peroxide, lactic acids and bacteriocins which are believed to be important in vaginal colonization. Some of these agents have the ability to resist spermicides which could normally destroy the normal flora<sup>14</sup>.

For a population such as those of developing world that is inadequate in primary health care and considering the side effects and high costs of some chemotherapeutics, the use of probiotic LAB strains may offer an alternative choice and therapeutic value in the treatment of genital infections and effectiveness against drug resistant strains. This study therefore employed human vaginal strains of lactic acid bacteria; reports their antagonistic activities against selected genital pathogens; and studied their antimicrobials production. The synergism of the concomitant use of the probiotics and antibiotics for the restoration of the normal genital flora was also considered by assessing the vaginal LAB strains' susceptibility to various antibiotics.

## MATERIAL AND METHODS:

**Lactic Acid Bacteria Isolation and Genital Pathogen Strains:** Fifty-five vaginal swab samples of healthy female volunteers were self-collected between September 2008 and April 2009 in Nigeria. Informed consent was obtained from the volunteers before sample collection. One milliliter of sterile water was added to each swab stick after sample has been collected. The samples were carefully packaged and taken down to the laboratory within 30mins of collection. The sticks were removed from their tubes after they had been

vigorously shaken and the wool end rubbed and pressed to the sides of their tubes and 9 mls of sterile distilled water was then added to each sample. From appropriate tenfold dilutions for each sample, growth of LAB was determined by inoculating in MRS agar (Oxoid, Basingstoke, UK), adjusted to pH 5 with 1 M HCL, by pour plate method and incubating for 48 hours in a microaerophilic environment.

Colonies were picked according to differences in their morphology on MRS agar plates and isolated by streaking onto fresh MRS agar. The pure cultures of lactic acid bacteria isolates were sub cultured into maintenance medium consisting of MRS broth supplemented with glycerol 12% (v/v) and incubated at 30°C until growth were visible. The stock cultures were then stored at 4°C for subsequent use and sub cultured at 4 weeks intervals.

Ten strains of vagina and semen pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella spp.*, and *Candida albicans*) were used. These were clinical strains identified in the microbiological laboratory of State Specialist Hospital, Akure. Re-validation of the isolates was done by subjecting them to specific biochemical confirmation tests.

**Characterization and Identification:** The isolates were characterized using cultural, cellular and biochemical properties. The cultural properties involved observing the isolates based on shape, colour, elevation, size, edge and surface of the colonies on agar plates. Physiological and biochemical tests such as Gram staining, catalase test, motility test, growth at different temperatures and in 4% NaCl, Oxidase test, starch hydrolysis, hydrogen sulphide production, Casein hydrolysis, Methyl-red and Voges-Proskauer tests, Homo-fermentative and heterofermentative tests, gas production from glucose, and various specific sugar fermentation tests. Bergey's Manual of Systematic Bacteriology<sup>15</sup>, "An Approach to the Classification of Lactobacilli"<sup>16</sup> and "Species differentiation of human vaginal Lactobacilli"<sup>17</sup> were used as references for identification based on the results of the various biochemical tests and characteristics observed.

**Antibiotics Sensitivity Tests for Vagina LAB Isolates:** These were performed using the disc diffusion (Kirby-Bauer) method to antimicrobial agents according to the National Committee for Clinical Laboratory Standards<sup>18</sup>. Antibiotic multi-discs which include Septrin 30µg, Chloramphenicol 30µg, Sparfloxacin 10µg, Ciprofloxacin 10µg, Amoxicillin 30µg, Augmentin 30µg, Gentamycin 10µg, Perfloxacin 30µg, Tanvid 10µg, Streptomycin 30µg, Ampiclox 30µg, Zinnacef 20µg, Rocephin 25µg, and Erythromycin 10µg were placed on MRS agar medium seeded with the LAB isolates that had been adjusted to 0.5 Mcfarland standard. The plates were incubated aerobically at 37°C for 24hours. The relative susceptibility of each isolates to each antibiotic was determined by clear zone of inhibition<sup>19</sup>.

**In-vitro Antimicrobial Activity of Lactic Acid Bacteria against Vagina and Semen Pathogens:** Mueller Hinton agar was prepared in sterile McCartney bottles and inoculated with 0.5ml of each of the pathogens; the mixture was poured into sterile Petri dishes. They were allowed to set after which wells of 8mm in diameter were cut on the agar using a sterile cork borer. 50µl of lactic acid bacteria supernatant which were obtained by centrifugation of MRS broth of LAB cultures at 10,000g for 10 mins at 4°C was added to each well. These were allowed to diffuse into the agar during a five hour pre-incubation period at room temperature, followed by aerobic incubation at 37°C for 24 hours and 25°C for 24-28 hours for *Candida*. The antimicrobial effects were recorded by measuring the zone of inhibition around the well<sup>20, 21</sup>.

**Quantitative Determination of Antimicrobial Compounds Produced by LAB:** Lactic acid and hydrogen peroxide productions were carried out by growing the test organisms in MRS broth for 96 hours and samples collected at 24-hourly intervals and centrifuged at 3000g for 15 mins.

**Lactic Acid Production:** This was determined by titrating 25ml of the supernatant fluid of the test organism and adding three drops of phenolphthalein as indicator, 0.1M NaOH was slowly added from a burette into the samples until a pink colouration appeared. Each ml of 0.1M NaOH is equivalent to 90.08mg of lactic acid<sup>22</sup>.

**Hydrogen Peroxide Production:** Twenty milliliters of 0.1M diluted H<sub>2</sub>SO<sub>4</sub> was added to 25ml of the supernatant fluid of the test organisms. Titration was carried out with potassium permanganate (KMnO<sub>4</sub>). Each ml of 0.1M potassium permanganate is equivalent to 1.079mg of hydrogen peroxide solution. Decolorization of the sample was regarded as the end point. The volume of hydrogen peroxide produced was then calculated according to the A.O.A.C<sup>22</sup>.

**RESULTS:** The selected lactic acid bacteria were identified as *Lactobacillus fermentum*, *Lactobacillus jensenii*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Lactobacillus lactis*. The percentage occurrence of each species is presented in **Table 1**.

**TABLE 1: NUMBER AND PERCENTAGE OCCURRENCE OF LACTIC ACID BACTERIA SPECIES ISOLATED FROM HUMAN VAGINA**

Lactic acid bacteria spp.	Number	Percentages (%)
<i>Lactobacillus fermentum</i>	8	25.0
<i>Lactobacillus jensenii</i>	9	28.1
<i>Lactobacillus delbrueckii</i>	5	15.6
<i>Lactobacillus plantarum</i>	4	12.5
<i>Lactobacillus acidophilus</i>	3	9.4
<i>Lactobacillus lactis</i>	3	9.4
<b>Total</b>	<b>32</b>	<b>100</b>

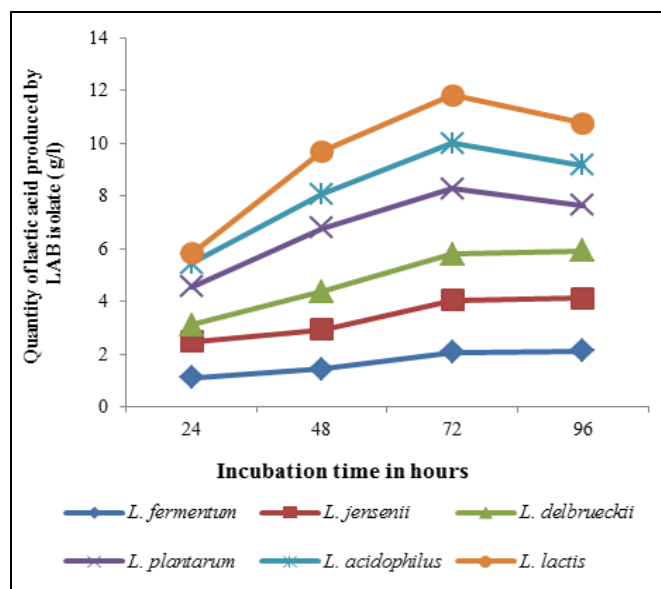
Drug resistance and sensitivity pattern of the LAB isolates is presented in Table 2. Percentage antibiotic resistance of the isolates reflects that 86% of the isolates exhibited resistance to both amoxicillin and gentamicin; 73% to ampiclox and 64% to Zinnacef while low percentage resistance from 0% to 5% was observed for the fluoroquinolones and augumentin. Rocephin, Streptomycin and Chloramphenicol had relatively low percentage resistance values of 18%, 18% and 36% respectively. Ampiclox and Zinnacef were on the high side exhibiting percentage resistance of 73% and 64%.

The antagonistic activity of metabolites of the vagina lactobacilli isolates against selected genital pathogens is presented in Table 3. There was antagonistic activity on all the genital pathogens except in some tests on *Candida albicans*. This includes the activities of *L. jensenii* Amh1A and *L. jensenii* Iba28B against both *Candida albicans*

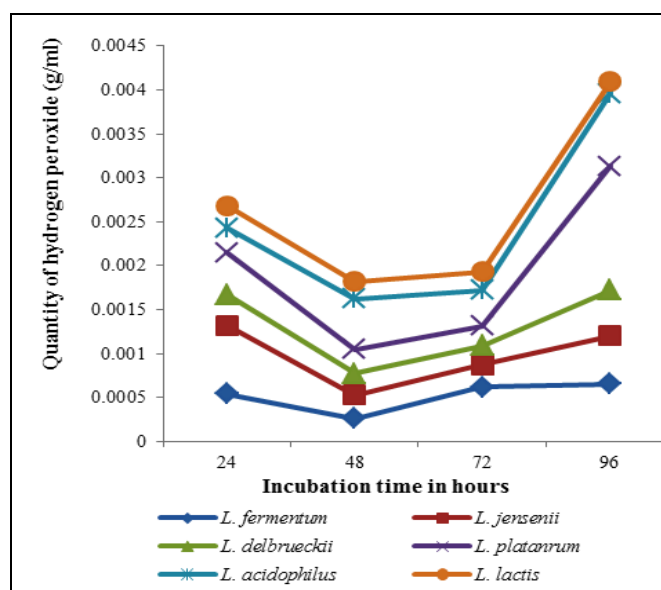
strains and that of *L. fermentum* Iba13 against *Candida albicans* SSH1505 where there was no zone of inhibition.

62.5% of the bacterial genital pathogens were resistant to up to six different antibiotics used. *Candida albicans* showed resistance to all antifungals used except metronidazole (**Table 4**).

The quantities of lactic acid and hydrogen peroxide produced by the LAB species are presented in **figures 1 & 2**.



**FIGURE 1: PRODUCTION OF LACTIC ACID BY VAGINA LAB ISOLATE WITH INCREASING INCUBATION TIME**



**FIGURE 2: QUANTITY OF HYDROGEN PEROXIDE PRODUCED BY VAGINA LAB ISOLATE WITH INCREASING INCUBATION TIME**



**TABLE 2: ANTIBIOTICS SENSITIVITY PATTERN OF THE VAGINA LACTIC ACID BACTERIA ISOLATES (mm)**

Antibiotics (µg)→ Isolates↓	SXT (30)	CH (30)	SP (10)	CPX (10)	AM (30)	AU (30)	CN (10)	PEF (30)	OFX (10)	S (30)	PEF (10)	CN (10)	APX (30)	Z (20)	AM (30)	R (25)	CPX (10)	S (30)	SXT (30)	E (10)
<i>L. fermentum</i> (Iba17A)	0.0	0.0	8.0	17.0	0.0	12.0	0.0	20.0	20.0	20.0	18.0	0.0	0.0	18.0	0.0	10.0	21.0	0.0	0.0	0.0
<i>L. fermentum</i> (Akr6B)	8.0	5.0	10.0	15.0	0.0	10.0	0.0	12.0	13.0	15.0	12.0	0.0	10.0	0.0	0.0	0.0	10.0	12.0	3.0	0.0
<i>L. fermentum</i> (Iba13B)	4.0	0.0	15.0	10.0	0.0	8.0	0.0	15.0	10.0	0.0	10.0	0.0	0.0	12.0	0.0	8.0	12.0	0.0	0.0	0.0
<i>L. fermentum</i> (Iba28A)	5.0	0.0	10.0	5.0	0.0	0.0	0.0	10.0	15.0	10.0	6.0	0.0	12.0	0.0	0.0	0.0	0.0	10.0	5.0	0.0
<i>L. jensenii</i> (Amh1A)	10.0	19.0	22.0	25.0	0.0	0.0	0.0	20.0	17.0	21.0	12.0	0.0	0.0	0.0	0.0	10.0	18.0	15.0	0.0	0.0
<i>L. jensenii</i> (Iba28B)	9.0	12.0	16.0	20.0	0.0	0.0	0.0	16.0	14.0	8.0	15.0	0.0	0.0	0.0	0.0	5.0	15.0	10.0	6.0	0.0
<i>L. delbrückii</i> (Amh1B)	8.0	16.0	18.0	22.0	0.0	5.0	0.0	17.0	20.0	10.0	20.0	0.0	0.0	15.0	0.0	9.0	25.0	12.0	0.0	10.0
<i>L. delbrückii</i> (Oau2A)	0.0	13.0	15.0	20.0	12.0	0.0	0.0	10.0	15.0	10.0	20.0	0.0	18.0	15.0	15.0	9.0	23.0	20.0	0.0	10.0
<i>L. plantarum</i> (Iba25B)	0.0	20.0	18.0	24.0	0.0	4.5	0.0	22.0	22.0	15.0	22.0	10.0	0.0	0.0	12.0	25.0	25.0	20.0	14.0	6.0
<i>L. acidophilus</i> (Iba17c)	0.0	19.0	15.0	18.0	0.0	0.0	0.0	16.0	15.0	9.0	15.0	8.0	0.0	0.0	0.0	16.0	20.0	15.0	0.0	5.0
<i>L. lactis</i> (Iba18A)	8.0	0.0	23.0	25.0	0.0	0.0	0.0	20.0	24.0	0.0	15.0	13.0	0.0	0.0	0.0	10.0	25.0	4.0	0.0	0.0

**Key:** SXT= Septrin CH= Chloramphenicol; SP= Sparfloxacin; CPX= Ciprofloxacin; AM= Amoxicillin; AU= Augumentin; CN=Gentamycin; PEF= Perfloxacin; OFX= Tanvid; S= Streptomycin; APX= Ampiclox; Z= Zinnacef; R= Rocephin; E= Erythromycin

**TABLE 3: ANTAGONISTIC ACTIVITIES OF SELECTED VAGINA LAB ISOLATES METABOLITE AGAINST PATHOGENS ASSOCIATED WITH GENITAL INFECTIONS (mm)**

Lactic acid bacteria→ Target organisms↓	<i>L. fermentum</i> (Iba17A)	<i>L. fermentum</i> (Akr6B)	<i>L. fermentum</i> (Iba13B)	<i>L. fermentum</i> (Iba28A)	<i>L. jensenii</i> (Amh1A)	<i>L. jensenii</i> (Iba28B)	<i>L. delbrückii</i> (Amh1B)	<i>L. delbrückii</i> (Oau2A)	<i>L. plantarum</i> (Iba25B)	<i>L. acidophilus</i> (Iba17C)	<i>L. lactis</i> (Iba18A)
<i>Candida albicans</i> SSH1504	12.0	13.0	8.0	10.0	0.0	0.0	10.0	14.0	10.0	13.0	10.0
<i>Candida albicans</i> SSH1505	10.0	13.0	0.0	8.0	0.0	0.0	12.0	8.0	13.0	9.0	8.0
<i>Klebsiella spp</i> SSH1506	20.0	22.0	12.0	18.0	22.0	24.0	10.0	10.0	15.0	22.0	15.0
<i>Klebsiella spp</i> SSH1507	14.0	10.0	12.0	15.0	14.0	14.0	10.0	12.0	10.0	9.0	9.0
<i>Klebsiella spp</i> SSH1508	20.0	24.0	15.0	18.0	20.0	22.0	18.0	10.0	15.0	16.0	14.0
<i>Escherichia coli</i> SSH1509	25.0	8.0	20.0	24.0	20.0	18.0	8.0	13.0	20.0	20.0	10.0
<i>Escherichia coli</i> SSH1510	20.0	12.0	20.0	18.0	13.0	8.0	15.0	18.0	20.0	10.0	13.0
<i>Escherichia coli</i> SSH1511	15.0	18.0	10.0	20.0	15.0	13.0	20.0	9.0	18.0	10.0	12.0
<i>Staphylococcus aureus</i> SSH1512	17.0	13.0	15.0	20.0	15.0	20.0	12.0	14.0	16.0	15.0	13.0
<i>Staphylococcus aureus</i> SSH1513	14.0	15.0	9.0	15.0	10.0	18.0	12.0	13.0	14.0	15.0	15.0

**TABLE 4: ANTIBIOGRAM SHOWING DRUG RESISTANCE AND SENSITIVITY PATTERNS OF THE GENITAL PATHOGENS**

Pathogens	Antibiogram									
<i>Staphylococcus aureus</i> SSH1513	CO <sup>R</sup>	FX <sup>S</sup>	AP <sup>R</sup>	CD <sup>S</sup>	AU <sup>R</sup>	OF <sup>S</sup>	E <sup>S</sup>	CIP <sup>S</sup>	GN <sup>S</sup>	CX <sup>R</sup>
<i>Staphylococcus aureus</i> SSH1512	CO <sup>R</sup>	FX <sup>S</sup>	AP <sup>R</sup>	CD <sup>S</sup>	AU <sup>R</sup>	OF <sup>S</sup>	E <sup>S</sup>	CIP <sup>S</sup>	GN <sup>S</sup>	CX <sup>R</sup>
<i>Escherichia Coli</i> SSH1511	AM <sup>R</sup>	*GN <sup>R</sup>	N <sup>R</sup>	CIP <sup>S</sup>	TE <sup>R</sup>	NB <sup>S</sup>	AX <sup>R</sup>	OF <sup>S</sup>	C <sup>R</sup>	CF <sup>S</sup>
<i>Escherichia Coli</i> SSH1510	AM <sup>R</sup>	*GN <sup>R</sup>	N <sup>R</sup>	CIP <sup>S</sup>	TE <sup>R</sup>	NB <sup>S</sup>	AX <sup>R</sup>	OF <sup>S</sup>	C <sup>R</sup>	CF <sup>S</sup>
<i>Escherichia Coli</i> SSH1509	AM <sup>S</sup>	*GN <sup>R</sup>	N <sup>R</sup>	CIP <sup>S</sup>	TE <sup>R</sup>	NB <sup>R</sup>	AX <sup>R</sup>	OF <sup>S</sup>	C <sup>R</sup>	CF <sup>S</sup>
<i>Klebsiellaspp</i> SSH1508	AM <sup>R</sup>	*GN <sup>R</sup>	N <sup>S</sup>	CIP <sup>S</sup>	TE <sup>R</sup>	NB <sup>S</sup>	AX <sup>S</sup>	OF <sup>S</sup>	C <sup>R</sup>	CF <sup>S</sup>
<i>Klebsiellaspp</i> SSH1507	AM <sup>R</sup>	*GN <sup>R</sup>	N <sup>R</sup>	CIP <sup>S</sup>	TE <sup>R</sup>	NB <sup>S</sup>	AX <sup>R</sup>	OF <sup>S</sup>	C <sup>R</sup>	CF <sup>S</sup>
<i>Klebsiellaspp</i> SSH1506	AM <sup>R</sup>	*GN <sup>S</sup>	N <sup>R</sup>	CIP <sup>S</sup>	TE <sup>R</sup>	NB <sup>R</sup>	AX <sup>R</sup>	OF <sup>S</sup>	C <sup>R</sup>	CF <sup>S</sup>
<i>Candida albicans</i> SSH1505	TND <sup>R</sup>	MTD <sup>R</sup>	NST <sup>R</sup>	KTC <sup>R</sup>						
<i>Candida albicans</i> SSH1504	TND <sup>R</sup>	MTD <sup>S</sup>	NST <sup>R</sup>	KTC <sup>R</sup>						

Key: CO=Cotrimoxazole 50µg FX=Ceptriaxone 30µg AP= Ampicillin/Cloxacillin 30µg;CD= Clindamycin 10µg OF= Ofloxacin 5µg AU= Augumentin GN= Gentamycin 50µg; E= Erythromycin 10µg CIP= Ciprofloxacin 5µg CX= Cephalexin 30µg AM= Ampicillin 10µg; \*GN= Gentamycin 10µg N= Nitrofuratoin 100µg TE= Tetracycline 50µg NB= Norfloxacin 10µg; AM= Amoxycillin 20µg C= Chloramphenicol 10µg CF= Cefuroxime 30µg; TND=Tindazole 5µg/ml MTD=Metronidazole 4µg/ml NST= Nystatin 500IU/ml; KTC= Ketoconazole 2µg/ml.<sup>R</sup>=Resistant <sup>S</sup>=Sensitive

**DISCUSSION:** The species of LAB isolates identified from the vaginal samples worked with in this study are *Lactobacillus fermentum*, *Lactobacillus jensenii*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Lactobacillus lactis*. *L. plantarum* and *L. fermentum* among others were isolated from women in Benin city, Nigeria <sup>23</sup>; *L. fermentum*, *L. jensenii* and *L. plantarum* isolated from Korean and Ugandan women <sup>24</sup>; *L. acidophilus*, *L. delbrueckii* subsp. *delbrueckii*, *L. delbrueckii* subsp. *lactis* isolated from Brazilian women <sup>25</sup>, and *L. jensenii* amongst others isolated from Swedish women <sup>26</sup>. The cultural and biochemical characteristics of the LAB isolated in this study were as stated in Bergey’s Manual of Systematic Bacteriology <sup>15</sup>.

In this study, *Lactobacillus jensenii* and *Lactobacillus fermentum* were the most predominant species reported from the isolation and identification processes of LAB isolates from the vagina swab samples. Petrova, et al. <sup>27</sup> also reported that the most active strains amongst a pre-selected twenty vagina LAB isolates from

Bulgarian women belonged to the species *L. fermentum*, *L. gasserriand* *L. salivarius*. A study of vaginal lactobacilli strain prevalence of Korean women reported *Lactobacilli crispatus* as the most prevalent strain <sup>28</sup>. Some researchers worked with 125 vaginal strains of lactobacillus and reported that the majority of strains belonged to *L. acidophilus*: *L. acidophilus sensu stricto*, *L. crispatus*, *L. gasserriand* *L. johnsonii* as well as *L. fermentum* and *L. plantarum* species <sup>29</sup>. There is versatility and species diversity in the prevalent species type of lactobacilli present in the vagina. This may be as a result of different lifestyles and other geographical and environmental conditions.

The population of the beneficial bacteria is usually depleted and not always restored in majority of patients following antibiotic therapy <sup>30</sup>. In this work, the sensitivity patterns of the isolated LAB strains to some antibiotics were studied. Gentamicin and amoxacillin appear friendly with all the vagina lactobacilli isolates, having no zone of inhibition except in only one isolate *L. delbrueckii* Oau2A.

Lactobacillus strains isolated from the blood, cerebrospinal fluid, respiratory tract, hospitalized immunocompromised patients however were inhibited by both gentamicin and amoxicillin<sup>31</sup>. This study also observed high susceptibility of the isolated vagina lactobacilli to sparfloxacin, streptomycin, augumentin and perfloxacin. Vagina lactobacilli had been reported to be susceptibility to norfloxacin, ampicillin, cefaclor, trimethoprim - sulfamethoxazole, nitrofurantoin and nalidixic acid<sup>32</sup>. The knowledge of the antimicrobial susceptibility or resistance is therefore of interest to predict the behavior of an exogenously applied probiotic formula in patients subject to any type of chemotherapy, as well as to consider the concomitant use of the probiotic and antibiotics for the restoration of the normal urogenital flora. Ultimately, antimicrobial susceptibility of exogenously applied microorganisms needs to be known for treating eventual collateral effects<sup>32</sup>.

Lactobacilli administered to the genital tract have a prominent role as a prophylactic aimed at improving the genital microfloral defence against bacterial infections<sup>33, 34</sup>. The antagonistic activity of the isolated vagina LAB metabolites on ten pathogens from hospital patient's high vagina swabs and semen samples was carried out in this study. The tested LAB exhibited various degrees of growth inhibition against all the tested pathogens except in a few cases of *Candida albicans*. It has been suggested that the lactic acid produced by these lactobacilli play a major role in the antagonistic activity. Vagina strains of *L. acidophilus* had been reported to inhibit the growth of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella sp.* and some other uropathogenic strains<sup>35</sup>. Majority of the detected LAB antagonistic activities on *Candida* under study were bacteriostatic (the inhibition zones were visible but not clear). *Lactobacillus delbrueckii* Oau2A showed the most impressive effect. The antagonistic activities of some *L. fermentum*, *L. acidophilus* and *L. plantarum* were also appreciable. This agrees with another study where vagina isolates of *Candida* were subjected to the antagonistic activities of over one hundred and twenty lactobacilli species where only eighteen showed detectable inhibitory activity<sup>12</sup>. During a secondary screening, a strain of *L. fermentum* was found to give an exceptional desired activity.

Also a study of twenty five vaginal *Lactobacillus* isolates of different species, those identified as *Lactobacillus delbrueckii* exhibited the strongest inhibitory activity against *C. albicans*<sup>29</sup>. The in vitro inhibitory capabilities of these vaginal lactic acid bacteria indicate a good probiotic potential.

The antimicrobial properties of LAB have been related to their metabolic products such as organic acids, bacteriocins and hydrogen peroxide<sup>36</sup>. Lactic acid quantification assay was carried out on all the vagina lactobacilli isolates that were studied. There was appreciable production of the acid varying degrees by the isolates, and *L. plantarum* had the highest production. The organism produced the highest yield throughout the test period. *L. plantarum* had been noted for this outstanding property in previous works<sup>37, 38, 39</sup>. The *L. lactis* isolated in this study produced the lowest lactic acid quantity in contrast to the strain isolated from 'massa', fermented maize dough where the highest quantity of lactic acid was reported<sup>41</sup>. The qualities of a particular strain of LAB may be peculiar to its site of isolation.

Hydrogen peroxide producing lactobacilli is a very important factor of a healthy vagina. This is supported by a study that proved that pregnant women colonized by hydrogen peroxide lactobacilli were less likely to have bacterial vaginoses, vulvo-vaginal candidiasis, vaginal *trichomoniasis* or vaginal colonization by other pathogens than women with hydrogen peroxide negative lactobacilli<sup>42</sup>. Though not all strains of lactobacilli produces hydrogen peroxide<sup>43</sup>, its production was detected in all the vaginal lactobacilli isolates in this study. *L. fermentum* had the highest production of all the strains that was studied. A strain of *L. fermentum* designated as *L. fermentum* Ess-1 has been reported as the first *Lactobacillus* strain described with significant growthinhibition activity against both *C. albicans* and *C. glabrata*. The metabolites produced by *L. fermentum* Ess-1 is believed to show this exceptional fungistatic property against these vulvo-vaginal pathogens<sup>12</sup>.

There is a trend towards natural remedies and the search for alternatives to develop and achieve a sustainable balanced level of development in all aspects of life.

The human genital health is of great importance and its knowledge is therefore entitled to growth in alternative therapy. This study provides additional facts to the amount of that knowledge in existence and hopes to encourage the acceptance, pursuit and development of a novel probiotic for therapeutic and maintenance of the genital system. In summary, it was observed that vaginal strains of LAB resists the antibiotic actions of gentamicin and amoxicillin, but are highly susceptible to sparfloxacin, streptomycin, augumentin and perfloxacin. This fact is very important when prescribing antibiotics or considering the concomitant use of probiotic and antibiotics for the restoration of the normal urogenital flora.

The vagina lactic acid bacteria strains also produces metabolites which has great antagonistic effects on pathogens isolated from high vaginal swabs and semen samples, hence, will serve at improving the genital microfloral defence against genital infections. *L. fermentum* produces more hydrogen peroxide when compared with other vagina LAB isolates and also produces the highest zones of inhibition against genital pathogens, hence, can be considered has an isolate with outstanding probiotic potential. We plan and recommend follow up research work on genetic characterization/identification, studying bacteriocin and biosurfactant productions, bile salt hydrolase activity and the *in-vitro* adhesion properties of these vaginal LAB strains. Further research should also be done on using urogenital cell lines for *in-vivo* tests and the immunomodulatory and immunostimulatory effects of the strains

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