Inhibitory Potential of Lactobacillus Species Against Bacterial Vaginosis Associated Bacteria

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Abstract: This study aimed at testing the inhibitory potential of Lactobacillus species isolates against causal agents of Bacterial vaginosis (BV). A cross-sectional study was done by taking samples from female patients who had been treated for BV in a randomized controlled trial. A presumptive identification for Lactobacillus spp and BV associated bacteria was done on isolates from a pelvic inflammatory disease laboratory. Fifty organisms identified presumptively as Lactobacillus spp were removed from stock by culturing them in fresh Brucella agar (oxoid) media for characterization. The BV associated organisms were also removed from stock and cultured in fresh media and identified through various biochemical tests. The biochemical data, H2O2 production data and data on inhibitory potential of Lactobacillus was then carried out. Lactobacillus jensenii, L. salivarius, L. fermentum, L. paraplantanarum, L. crispatus, L. iners, L. casei, L. plantanarum, L. vaginals, and L. acidophilus were isolated. L. jensenii and L. salivarius were the most predominant Lactobacillus species isolated. L. jensenii was significantly (p<0.5) more abundant than other lactobacillus species. 54% of lactobacilli inhibited G. vaginalis, 56% inhibited Mobiluncus spp and 26% inhibited P. bivia. None of the selected species of lactobacilli were found to be inhibitory to Bacteroides fragilis. It was concluded that H2O2 contributes to the inhibition of BV associated organisms and should be a factor to consider when characterizing Lactobacilli for probiotic use. Administration of preparations containing a well characterized probiotic strain to human could be used to prevent acquisition or cure both BV and gastrointestinal disorders.

Key words: Inhibitory potential, Lactobacillus species, Bacterial vaginosis, Probiotic strain

INTRODUCTION

Lactobacilli genera ecologically occur as a predominant group of bacteria in the normal vaginal microflora found in women of reproductive age (Hillier, 2005; Redondo-Lopez, 1990). Lactobacilli species which dominate the normal vaginal flora are gram positive, facultative anaerobe or strict anaerobes that are non-motile, non-spore-forming, and fermentative organotrophs (Donders, 2000; Altermann, 2005).

Species of the genera lactobacilli include; L. fermentum, L. acidophilus, L. plantanum, L. brevis, L. jensenii, L. vaginalis, L. dubrekii, L. salivarius (Redondo-Lopez, 1990). These species play a role in the stabilization of the vaginal microflora by producing bacteriocins and other antimicrobial compounds like hydrogen peroxide and lactic acid which lower the pH, which in turn inhibit the growth of other bacteria including Gardnerella vaginalis, Bacterioides spp, Prevotella and Mobiluncus spp (Song, 1999).

The present lifestyle of women where certain practices have evolved like vaginal douching, changing sexual partners, using contraceptives and taking antibiotics like ampicillin and amoxillin has resulted in the loss or reduction in population of Lactobacillus species (Wilson, 2002). This encourages the growth of commensal pathogenic bacteria in the vaginal tract a condition known as bacterial vaginosis (BV) (Wilson, 2002).

Bacterial vaginosis is a polymicrobial syndrome whose species could be aerobic, anaerobic and micro-aerobic. The normal vaginal Lactobacilli are replaced by strict anaerobes and facultative bacteria (Hillier, 2005). BV is now associated with obstetrical complications including amnionitis, premature rupture of membranes, post delivery endometritis and pre-term birth, pelvic inflammatory disease (PID) and increased acquisition of HIV-1 (Calzolari, 2000).
At present in Kenya, antibiotic therapy is the recommended treatment of BV associated bacteria. Oral administration of metronidazole or clindamycin is thus a common treatment. However, there is evidence of development of resistance and chronic toxicity of BV associated bacteria of up to 50% of treated patients (Hillier, 2005). Some alternative treatments have been tried elsewhere like the use of in vitro inhibition potential of Lactobacillus species. Such natural antagonism by Lactobacillus species isolated from Kenyan women has not been tried in Kenya.

This study aimed at testing the inhibitory potential of Lactobacillus species isolated from Kenyan women against causal agents of BV.

**MATERIALS AND METHODS**

**Study Site and Design:**
A cross-sectional study was done by taking samples from female patients who had been treated for BV in a randomized controlled trial. A presumptive identification for Lactobacillus spp and BV associated bacteria was done on isolates in the pelvic inflammatory disease (PID) laboratory at the Centre for Microbiology Research Institute, Kenya Medical Research Institute (KEMRI).

**Sampling:**
Fifty organisms identified presumptively as Lactobacillus spp were removed from stock by culturing them in fresh Brucella agar (oxoid) media for characterization. The BV associated organisms were also removed from stock and cultured in fresh media and identified through various biochemical tests.

**Laboratory Analysis:**

**Characterization of Lactobacillus Species:**
A minimum of 50 organisms from a pool of 300 presumptive Lactobacillus isolates were cultured in fresh Brucella agar (oxoid) media for characterization. They were stained by Gram's Method to describe the cell appearance. To identify the Lactobacillus to species level, biochemical tests were carried out. They included tests for indole production, catalase, nitrate reduction and preformed enzyme tests. Identification was confirmed by the ability to ferment lactose, cellobiose, rhamnose, mannitol, sucrose, melezitose, xylose, raffinose and arabinose. To characterize the BV associated bacteria (Gardnerella vaginalis, Bacteroides spp, Prevotella spp and Mobiluncus spp.), the organisms to be tested for inhibition were isolated from another study and stored at -700C in the freezer. They were sub-cultured in fresh media and identified through biochemical tests as follows;

Gardnerella vaginalis were confirmed by showing pleomorphic gram variable rods on gram stain, catalase and oxidase negative, positive hippurate hydrolysis test and sensitive to Sodium Polyanethol Sulfonate (SPS) potency disks.

Bacteroides species were confirmed as gram-negative rod, growth in Bacteroides Bile Esculin (BBE) agar and resistant to Kanamycin (1mg), vancomycin (5ug), and colistin (10ug) impregnated disks.

Prevotella species were confirmed by appearance as gram-negative rods, negative growth on BBE, and resistance to Kanamycin (1mg), vancomycin (5ug), and colistin (10ug) impregnated disks.

Mobiluncus species were confirmed by appearance as curved gram variable rods and identity confirmed by Rapid test (Rapid ANA, biomereiux).

**Identification of Hydrogen Peroxide Producing Lactobacillus Species:**
Confirmed Lactobacillus colonies were cultured in freshly prepared Tetramethylbenzide (TMB) media which contained 12.9g of Brucella agar; 3ml Horseradish peroxide; 3ml hemin: 0.06 ml Vitamin K. 3g of starch for each one litre of batch of the medium. The plates were incubated anaerobically for three days at 37oC after wish they were exposed to ambient air for up to 30 minutes. H2O2 production was noted by appearance of blue colonies due to the oxidation of TMB by Horseradish peroxidase in presence of hydrogen peroxide. Hemin was added to TMB medium to stimulate the growth of heme requiring anaerobic bacteria. Vitamin K is required for the growth of some vaginal microorganisms and starch was added as a preferred carbon source for these microorganisms. This was a qualitative method.

**Assay for the Inhibitory Potential of Lactobacillus Species:**
The inhibitory potential of the Lactobacillus species to the growth of BV associated bacteria was assessed by a deferred antagonism well assay as described by McLean and Rosenstein (McLean, 2000). The test bacteria were seeded in Brucella agar.
100μl of Lactobacillus culture suspension was added to 10mm diameter wells cut in freshly prepared agar containing the test organism. Plates were incubated anaerobically at 37°C for 48 hrs and the zone of inhibition measured and recorded. The results were interpreted as sensitive, if there was any zone of inhibition and the zone was related to inhibitory capacity, the larger the zone size, the stronger the inhibitory potential.

**Statistical Analysis:**

The biochemical data, H₂O₂ production data and data on inhibitory potential of Lactobacillus was analyzed using SPSS software for the prevalence of H2O2 production with 95% confidence interval. Difference between species and H2O2 production was analyzed using Chi Square test while difference between species, H2O2 and inhibitory activity was tested by analysis of variance (ANOVA).

**RESULTS AND DISCUSSION**

**Strain Identification Using Sugars:**

In a pool of 300, a total of 50 Lactobacillus isolates were selected by random sampling and identified to species level as shown in Table 1.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Total Isolates n (%)</th>
<th>H₂O₂ Positive n (%)</th>
<th>H₂O₂ negative n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.jensenii</td>
<td>10 (22)</td>
<td>5 (22)</td>
<td>6 (22)</td>
</tr>
<tr>
<td>L. salvarius</td>
<td>7 (14)</td>
<td>6 (26)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>L. fermentum</td>
<td>6 (12)</td>
<td>1 (4)</td>
<td>5 (19)</td>
</tr>
<tr>
<td>L. paraplantarum</td>
<td>4 (8)</td>
<td>1 (4)</td>
<td>3 (11)</td>
</tr>
<tr>
<td>L. crispatus</td>
<td>4 (8)</td>
<td>2 (9)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>L. iners</td>
<td>2 (4)</td>
<td>1 (4)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>L. casei</td>
<td>4 (8)</td>
<td>2 (9)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>L. plantarum</td>
<td>2 (4)</td>
<td>1 (4)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>L. vaginalis</td>
<td>2 (4)</td>
<td>0 (0)</td>
<td>3 (11)</td>
</tr>
<tr>
<td>Not identified</td>
<td>3 (6)</td>
<td>0 (0)</td>
<td>3 (11)</td>
</tr>
<tr>
<td>L. acidophilus</td>
<td>5 (10)</td>
<td>4 (17)</td>
<td>1 (4)</td>
</tr>
</tbody>
</table>

Out of 50 strains tested Lactobacillus jenseni were the majority and the difference was significant (p < 0.0001) (Table 1). Among the hydrogen peroxide producing strains Lactobacillus strains, L. salivarius and L.jensenii strains were more abundant, 26% and 22% respectively although it was not significantly different (p=0.1696).

**Inhibition of the Indicator Strains by H₂O₂ Positive and Negative Lactobacilli:**

The inhibition of the indicator bacteria by the H₂O₂ positive and H₂O₂ negative Lactobacillus species was assessed. Out of the 50 isolates 46% were positive for H₂O₂ while the 54% were negative.

![Fig. 1: The percentage of Hydrogen peroxide production.](image)

Overall, 54% Lactobacilli inhibited G.vaginalis, 56% inhibited Mobiluncus spp, and 26% inhibited P.bivia. None of the selected strains of Lactobacilli were found to be inhibitory to Bacteroides fragilis (Figure 2). There was no significant (p = 0.083) variation between the numbers of Lactobacillus that inhibited each of the four indicator strains.
Fig. 2: Overall inhibition of the different indicator strains i.e. G.vaginalis, Mobiluncus spp, P. bivia and B. fragilis.

**Inhibitory Zones:**

The inhibitory zones were measured and the radii of inhibition ranged between 1mm to 7mm. The zones were grouped into several categories as shown in figure 3, 4 and 5. Each of the individual strains was assessed for inhibition comparing the H$_2$O$_2$ positive and negative.

**Inhibition of G.vaginalis:**

Among the H$_2$O$_2$ positive lactobacilli, 91% were inhibitory to G.vaginalis while 9% were not inhibitory to the indicator bacteria. In the H$_2$O$_2$ negative Lactobacilli group, only 19% were inhibitory while 81% were not inhibitory. There was significant (p< 0.0001) variation in inhibitory potential among the Lactobacilli that produced H$_2$O$_2$ and those that did not produce H$_2$O$_2$; chi square test with Yates correction. Those that were producing hydrogen peroxide were significantly inhibitory.

Among the H$_2$O$_2$ positive, those that inhibited the G.vaginalis, 4%, 22%, 52%, and 13% produced zones of between 1-2.9 mm, 3-4.9 mm, 5-6.9 mm and >7 mm respectively. Among the H2O2 negative strains, 4% and 7% produced zones of >7 mm and 1-2.9 mm, 3-4.9 mm each respectively. None among these produced zones of 5-6.9 mm (figure 3).

**Inhibition of Mobiluncus Spp:**

Twenty eight strains were inhibitory to Mobiluncus spp, among these, 75% were H$_2$O$_2$ producing while 25% were H$_2$O$_2$ negative. Twenty two strains did not inhibit this indicator bacteria, 9% produced H$_2$O$_2$ while 91% were negative. The more H$_2$O$_2$ Lactobacilli were significantly inhibitory to Mobiluncus spp (p< 0.0001).
Among the H2O2 producing strains, 26%, 43%, 4% and 17% produced zones of inhibition of 1-2.9 mm, 3-4.9 mm, 5-6.9 mm and >7 mm respectively. Among the H2O2 negative strains, 11% and 7% produced zones of 3-4.9 mm and 1-2.9 mm each respectively. None of the H2O2 negative produced zones of >7 mm (figure 4).

Only 13 strains were inhibitory to Prevotella bivia, among these 85% were H2O2 producing while 15% were H2O2 negative.

Thirty seven strains did not inhibit this indicator, 32% produced H2O2 while 68% were negative. Those Lactobacilli that produced hydrogen peroxide were more inhibitory than the rest, this variation was significant (p =0.0035).

When the zones were measured and compared to H2O2 production, 35% and 13% of the H2O2 positive strains produced zones of 1-2.9 mm and 5.0-6.9 mm respectively. None among these produced zones of 3.0-4.9 mm and >7 mm. Among the H2O2 negative strains 4% each produced zones of 1-2.9 mm and 5.0-6.9 mm. Similar to H2O2 positive none among these produced zones of 3.0-4.9 mm and >7 mm (figure 5).

**Inhibition of Three Indicator Strains Combined:**

Lactobacilli that inhibited the three susceptible indicator strains were evaluated for hydrogen peroxide production. Overall, 22% were inhibitory to G. vaginalis, Mobiluncus and Prevotella bivia. Among these, 36%, 18% and 9% each were L. jensenii, L. salivarius and L. fermentum, L. crispatus, L. casei, L. acidophilus and L. plantarum respectively.
Among the hydrogen peroxide producing Lactobacilli that inhibited all three strains, 33%, 22% and 11% each were L.jensenii, L.salivarius and L. fermentum, L. crispatus, L. acidophilus and L. plantanarum respectively. None of the hydrogen peroxide producing L.casei was inhibitory. This distribution was not significant (p=0.4279). Only one strain each of L. jensenii and L. casei which did not produce hydrogen peroxide were inhibitory. The vaginal ecosystem harbours a micro biota that is increasingly recognized as protecting it from invading pathogens including those that cause urinary tract infections and sexually transmitted diseases (Hillier, 2005). Lactobacilli are dominant in this habitat at 107 to 108 Colony Forming Units/g of vaginal fluid in healthy premenopausal women (Hillier, 2005). The species L. gasseri, L. vaginalis, L. acidophilus, L. delbrueckii subspecies lactis, L. crispatus, L. plantanarum, L. cellobiosus, L. jensenii, L. salivarius, L. curatius, L. brevis and L. oris have been reported by other workers (Song, 1999). In this study L. acidophilus and L. vaginalis were the predominant Lactobacilli species isolated (Song, 1999). In a Japanese study L. iners, L. gasseri, L. plantanarum, L. crispatus, L. vaginalis, and L. rhamnosus were also isolated with L. crispatus and L. gasseri reported as the predominant lactobacillus species (Song, 1999). In general L.crspiratus, L.jensenii, L.acidophilus and L.fermentum have been reported to be the predominant Lactobacillus species in vaginal tracts of majority white women (Ouwehand, 2002; Benno, 1989).

<table>
<thead>
<tr>
<th>Strains</th>
<th>H₂O₂ Positive n (%)</th>
<th>H₂O₂ negative n (%)</th>
<th>Total strains n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. acidophilus</td>
<td>1 (11)</td>
<td>0 (0)</td>
<td>1 (9)</td>
</tr>
<tr>
<td>L. casei</td>
<td>0 (0)</td>
<td>1 (50)</td>
<td>1 (9)</td>
</tr>
<tr>
<td>L. crispatus</td>
<td>1 (11)</td>
<td>0 (0)</td>
<td>1 (9)</td>
</tr>
<tr>
<td>L. fermentum</td>
<td>1 (11)</td>
<td>0 (0)</td>
<td>1 (9)</td>
</tr>
<tr>
<td>L. jensenii</td>
<td>3 (33)</td>
<td>1 (50)</td>
<td>4 (36)</td>
</tr>
<tr>
<td>L. plantanarum</td>
<td>1 (11)</td>
<td>0 (0)</td>
<td>1 (9)</td>
</tr>
<tr>
<td>L. salivarius</td>
<td>2 (22)</td>
<td>0 (0)</td>
<td>2 (18)</td>
</tr>
</tbody>
</table>

There have not been reports before on the predominant Lactobacillus species in vagina of women in sub-Saharan Africa where BV is highly prevalent. To our knowledge this is the first report which presents the results of the predominant Lactobacillus species in the vaginal swabs obtained from Kenyan women. In our study we isolated L. jensenii, L. salivarius, L. fermentum, L. paraplantanarum, L. crispatus, L. iners, L. casei, L. plantanarum, L. vaginalis, and L. acidophilus. L. jensenii and L. salivarius were the most predominant Lactobacillus species isolated. Although other researchers reported the isolation of L. gasseri as being one of the predominant species, we never isolated L. gasseri. This could be due to the limitation of the sugars we used. In most cases L. gasseri is isolated when the molecular method of identification is used other than the metabolic method that we used.

Lactobacillus can act competitively to exclude pathogens inhibiting their colonization and subsequently preventing infections. The inhibition of growth has been attributed to production of organic acids such as lactic acid, H₂O₂ production, bacteriocins and competitive inhibition of the pathogens to adhesion and competition for nutrients by lactobacillus (Oyetayo, 2004; McAuliffe, 2001; Vesterlund, 2006). Although BV is a polymicrobial disease it is acknowledged that the species associated with BV have a symbiotic relationship (McAuliffe, 2001). The inhibitory activity of different lactobacillus species maybe attributed to H₂O₂ after a loss of activity was maintained after proteinase K treatment of the lactobacillus suspension (Kaewsrichan, 2006).

In a related study 60 vaginal isolates of lactobacilli were tested on their ability to inhibit the growth of G.vaginalis, Bacteroides fragilis, and P.bivia and all the tested lactobacilli were found to be inhibitory to each of the indicator bacteria (McLean, 2000). The lactobacilli produced zones of inhibition of growth in the range 4.0-13.5mm for G. vaginalis, 2.0-7.0 mm for Bacteriodes fragilis and 1.5-8.0mm for P. bivia.

In our study we found that 54% of lactobacilli inhibited G. vaginalis, 56% inhibited Mobiluncus spp and 26% inhibited P.bivia. None of the selected species of lactobacilli were found to be inhibitory to Bacteroides fragilis (see fig 2). We also found that the lactobacilli produced zones of inhibition of growth in the range of 1-7mm for G.vaginalis and Mobiluncus spp, 1-6.9 mm for P.bivia. Among the H₂O₂ positive lactobacilli, 91% were inhibitory to G.vaginalis, 75% were inhibitory to Mobiluncus while 85% were inhibitory to P.bivia. We found Mobiluncus to be the most susceptible BV indicator organism. To our knowledge this may be the first report on the inhibition of lactobacilli to Mobiluncus spp. It however shows that the susceptibility of Mobiluncus follows the same line with the other indicator organism other than B. fragilis. The resistance of B. fragilis could be attributed to the fact that B. fragilis is catalase positive, therefore it produces the enzyme catalase which breaks down the H₂O₂ produced by the lactobacilli to water and oxygen gas hence diminishing its antimicrobial effects.
Also the growth rate of B. fragilis is faster than that of lactobacilli species hence B. fragilis become well established before the lactobacillus start producing H2O2 to levels toxic to B.fragilis. Although the present study assessed the inhibitory activity of lactobacilli to G. vaginalis, Mobiluncus spp, P.bivia and B. fragilis only, it is likely that the inhibitory environment produced by the lactobacilli would prevent the colonization by other species also associated with BV e.g. M. hominis, which appear in large numbers only in the late and most severe stage of BV.

It has been suggested that H2O2 producing strains of L. crispatus or L. jensenii should be considered for vaginal probiotic as these strains appear not only to produce H2O2 but also to persist in the vagina during pregnancy (Vallor, 2001). The findings of our study also support this suggestion. In addition L. salivarius should also be considered as it's among the prevalent strains and also produces.

Lactobacilli have previously been used to inhibit the growth of gram positive and gram negative bacteria and other micro organisms. Lactobacilli species has been used to inhibit the growth in vitro of Methicillin resistance S. aureus 02 and S. aureus (Supayang, 2006). The growth of both strains was effectively inhibited by the presence of lactobacillus. A number of metabolic by products such as H2O2 and bacteriocins are believed to contribute to their antimicrobial activities (Supayang, 2006).

Due to the inhibitory activity of most of the lactobacilli species, lactobacilli have been used as probiotics in both humans and animals to treat a broad range of gastrointestinal and/or vaginal disorders (Hallen, 1992). L. reuteri is among the common available probiotic products fulfilling the FAO/WHO guidelines. Lactobacillus species and the H2O2 they produce are increasingly recognized as essential component of a healthy vaginal environment. H2O2 is toxic to many micro organisms including those associated with BV at concentrations that are typical in the vaginal fluid and, thus, provides an intrinsic protective mechanism in the vaginal compartment (Hillers, 2005). Most isolates of vaginal lactobacillus produce some detectable H2O2. H2O2 production by lactobacillus may contribute to the control of vaginal flora, particularly in the presence of peroxidase and a halide ion both of which are found in the vagina (Hillers, 2005). H2O2 positive lactobacillus have been shown to be an important factor in the prevention of preterm labor and it has been demonstrated that levels of H2O2 producing lactobacilli are significantly reduced in women with preterm labour (Yoon, 2001).

Published studies have assigned a protective role of H2O2 producing lactobacilli, because women colonized by H2O2 producing strains have decreased acquisition of HIV, Neisseria gonorrhoea infection and BV (Martin, 1999; Hawes, 1996). It has been been demonstrated that women with normal vaginal flora are mostly colonized with H2O2 positive lactobacillus as compared to those with a disturbed flora (Hillers, 2005; Vallor, 2001).

In a study of 947 women with BV reported the isolation of 191 (20.2%) H2O2 producing lactobacilli whereas 222 women (23.4%) had non-H2O2 producing Lactobacillus species present and the remaining 534 (56.4%) had no lactobacilli present (Hillers, 2005). The last two categories were latter combined which resulted in a group of 756 (79.8%) who lacked H2O2 producing lactobacilli.

In one study nearly all L. crispatus and L. jensenii isolates produced H2O2 (97% and 99% respectively), whereas the production of H2O2 was detected in only 6% of L.iners isolates (Vallor, 2001). The group 246 of H2O2 producing lactobacillus was composed predominantly of L.crispatus (47%), L. jensenii (35%), and L. gasseri (13%). Colonization by H2O2 producing L. crispatus or L. jensenii isolate was associated with a decreased prevalence of BV (p < 0.001) only 13 (9%) of 147 females colonized by H2O2 producing L. crispatus and/or L. jensenii were positive for BV.

In this study we found that 27 (54%) isolates were negative for H2O2 production while 23 (46%) were positive for H2O2 production. We also found that among the H2O2 producing species of lactobacilli, L. salivarius and L. jensenii were more abundant 26% and 22% respectively although the distribution was however not significantly different (p= 0.17). These results are however consistent with other previous findings (Antonio, 2003). It is evident from the above findings that H2O2 producing lactobacillus species are also isolated from women with BV therefore BV may develop in some women despite the presence of H2O2 producing species of lactobacilli. Overgrowth of the BV indicator organisms may therefore be important than the reduction of H2O2 producing lactobacilli.

Conclusion and Recommendation:

Conclusion:

This study indicates that in vitro methods can be used to determine the Lactobacilli showing the greatest inhibitory potential and their antimicrobial property. Mobiluncus spp was the most susceptible followed by G. vaginalis then lastly P. bivia. B. fragilis was resistant to all the Lactobacilli species tested. H2O2 have been shown to significantly contribute to the inhibition of BV associated organisms and should be a factor to consider when characterizing Lactobacilli for probiotic use.
Administration of preparations containing a well characterized probiotic strain to human could be used to prevent acquisition or cure both BV and gastrointestinal disorders. This approach may overcome problems relating to drug- resistant strains, chronic toxicity, as well as the loss of normal micro-biota.

**Recommendations:**

Further studies should be done to determine the adherence of the Lactobacilli that have shown to be inhibitory and producing H2O2 to the vaginal epithelial cells to prevent them from being flushed out during voiding of urine so that they can produce their protective effects. Phenotypic/molecular methods should be used to identify the lactobacilli to species level as these methods are both sensitive and specific as compared to metabolism methods. Other antimicrobial properties produced by lactobacillus species should also be studied to determine the significance of their contribution to the inhibitory potential of lactobacilli to BV associated organisms to enhance the choice of the appropriate lactobacilli for probiotic use.

**REFERENCES**


