Association between hydrogen peroxide Lactobacillus species and bacterial vaginosis-related bacteria in vaginal fluid of pregnant women

MALGORZATA WASIELA1, GRAZYNA MISIAK1, ANNA PIECZARA1, JAROSŁAW KALINKA2

Abstract

Aim: Bacterial vaginosis (BV) is well-known risk factor of preterm delivery. Lactobacilli, principally the strains that are hydrogen peroxide (H₂O₂) producing, may have a protective effect against vaginal colonization by pathogenic species such as those that cause BV, but it is still unclear which species of H₂O₂ producing strains are inhibitory to other bacterial species. The main aim of this paper was to evaluate the quantitative and qualitative characteristics of BV-related flora in correlation to hydrogen peroxide-producing Lactobacillus species among pregnant women. Material and methods: 196 pregnant women randomly selected from the patients of 10 districts maternity units in the Łódź region, Central Poland. Only singleton pregnancies between 8 and 16 weeks of gestation were qualified for inclusion in the survey. Vaginal fluids were collected from all the women. BV was diagnosed by Gram stain method according to Spiegel’s criteria while BV-related bacteria and lactobacilli were identified by cultures on the basis of biochemical methods. Results: BV was diagnosed in 55 (28.1%) pregnant women, grade I (normal) flora were found in 70% (53.7%) and grade II (intermediate) flora in 71 (36.2%) of women. Among women with BV the following bacteria: Bacteroides sp., Prevotella sp., Mobiluncus mulieris, G. vaginalis, and genital mycoplasmas – M. hominis and U. urealyticum were isolated more frequently and in the higher concentration (≥10⁵ CFU/ml, CFU – colony forming units) compared to other groups. Any Lactobacillus sp. was detected in 139 women while among 136 women H₂O₂ – producing strains were detected. 5 species of Lactobacillus sp. were identified: L. jenseni, L. fermentum, L. acidophilus, L. catenaformis, and L. minutus. The majority of isolates of H₂O₂ producing strains were detected significantly more frequently among women with grade I and II microflora (94.2% and 90.1% respectively) compared to only 9.1% among women with BV. Quantitative analysis showed that all of them were also isolated in higher concentrations in groups I and II (≥10⁵ CFU/ml) as compared to women with BV(< 10⁵ CFU/ml). Conclusions: Our results showed that pregnant women with BV lack lactobacilli, especially hydrogen peroxide producing ones. The presence of H₂O₂ producing Lactobacillus as L. acidophilus, L. fermentum, L. minutus, L. jenseni, and L. fermentum seems to protect against the development of BV during pregnancy. The exact role of various lactobacilli species in pathogenesis of preterm delivery should be evaluated in further studies.

Introduction

The human vagina is a complex ecosystem containing an abundance of microorganisms. The healthy vaginal flora in reproduction age is usually predominated by Lactobacillus species, a genus of Gram-positive, non-motile rod-like bacteria. Vaginal microbiota protect the mucosa against the establishment of pathogenic microorganisms through complementary mechanisms: specific adherence to the epithelium which blocks colonization of pathogens, production of antimicrobial compounds and coaggregation with pathogens. Their metabolic products, such as hydrogen peroxide (H₂O₂), lactic acid, and bacteriocin play an important role in maintenance of the normal vaginal flora also during pregnancy [1]. Principally, the strains that are H₂O₂ producing, may have a protective effect against vaginal colonization by pathogenic species such as those that cause bacterial vaginosis (BV) [1-4]. Microbiological analyses have shown that the predominant Lactobacillus species in the normal lactobacillary flora are as following: L. crispatus, L. gasseri, L. iner, L. acidophilus and L. jensenii [1, 3, 5] but only 9% of some strain of this species produce H₂O₂, whereas almost all strains of L. crispatus and L. jensenii produce H₂O₂ [4].

Bacterial vaginosis is the disturbed vaginal flora, in which normal lactobacilli are replaced by an overgrowth of various anaerobic bacteria [6]. This condition is common in women of reproductive age [6, 7] and may cause vaginal discharge with fishy odour, although many women may stay asymptomatic [8]. It was well documented that bacterial vaginosis is a strong risk factor of various perinatal complications including mainly preterm birth and preterm rupture of membrane (pPROM) [9, 10]. Some reports have also suggested that H₂O₂ producing lactobacilli in the vagina may protect pregnant women against ascending infection to the chorioamniotic membranes and uterine cavity [11, 12].

Clinical study concerning the role of hydrogen peroxide producing lactobacilli in BV are controversial. There have been few studies regarding the frequencies of the BV-related bacteria and Lactobacillus strains in healthy and abnormal vaginal flora in pregnant women, but this is still unclear which species of H₂O₂ producing Lactobacillus strains are inhibitory to other pathologic bacterial species. Bacteria detected in BV mainly included Gardnerella vaginalis, Mobiluncus sp., Mycoplasma hominis and other anaerobic bacteria such as Prevotella sp., and Bacteroides sp. [6, 7, 14, 15].

As BV is a strong risk factor of preterm delivery the main aim of this study was to evaluate the prevalence, quantitative and qualitative characteristics of BV-related bacteria with correlation to the hydrogen peroxide-producing Lactobacillus species in lower genital tract among randomly selected pregnant women in Łódź, Poland.

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Table 1. Distribution of bacterial vaginosis-related bacteria in women with BV, intermediate (II) and normal (I) flora

<table>
<thead>
<tr>
<th>BV-related bacteria</th>
<th>I (n = 70)</th>
<th>II (n = 71)</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; $10^5$</td>
<td>$\geq 10^5$</td>
<td>n (%)</td>
</tr>
<tr>
<td>Bacteroides spp.</td>
<td>0</td>
<td>1.8</td>
<td>4%</td>
</tr>
<tr>
<td>Prevotella spp.</td>
<td>14</td>
<td>144</td>
<td>25.5</td>
</tr>
<tr>
<td>Mobiluncus mulleris</td>
<td>6</td>
<td>109</td>
<td>9.1</td>
</tr>
<tr>
<td>Gardnerella vaginalis</td>
<td>8</td>
<td>145</td>
<td>29</td>
</tr>
<tr>
<td>Mycoplasma hominis</td>
<td>&lt; $10^5$</td>
<td>$\geq 10^5$</td>
<td>n (%)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>14.5</td>
<td>15</td>
</tr>
<tr>
<td>Ureaplasma urealyticum</td>
<td>9</td>
<td>16.4</td>
<td>16</td>
</tr>
</tbody>
</table>

BV group: a B. fragilis, b B. capillosus, c P. melaninogenica, d P. disiens (6), P. oralis (4), P. intermedia (3), P. ruminicola (1)  
II group: a B. capillosus, b B. capillosus, B. urealyticum, c P. oralis, P. disiens, d P. oralis (2), P. disiens

Table 2. Presence of Lactobacillus in study population

<table>
<thead>
<tr>
<th>Lactobacillus</th>
<th>H$_2$O$_2$ +</th>
<th>H$_2$O$_2$ -</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>any Lactobacillus</td>
<td>136</td>
<td>23</td>
<td>159</td>
</tr>
<tr>
<td>L. jensenii</td>
<td>79</td>
<td>17</td>
<td>96</td>
</tr>
<tr>
<td>L. fermentum</td>
<td>41</td>
<td>5</td>
<td>46</td>
</tr>
<tr>
<td>L. acidophilus</td>
<td>1</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>L. catenaformis</td>
<td>8</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>L. minutus</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

Material

A group of 196 pregnant women was selected randomly from 10 districts maternity units in the Łódź region, Central Poland. Only singleton pregnancies between 8 and 16 weeks of gestation were qualified for inclusion in the survey. Women with chronic diseases diagnosed during the first prenatal visit were not considered in the study. A standard questionnaire covering medical, demographic, constitutional and environmental aspects was administered to every subject and verified with medical records. This study was approved by the Ethical Committee of Medical University in Łódź, Poland, No RNN/536/97. Each participant provided written consent for participation in the study.

BV-detection

Vaginal smear were also tested for bacterial vaginosis by Gram stain according to Spiegel’s criteria [17] and vaginal fluid were diagnosed as: grade I – normal flora, predominant Lactobacillus morphotypes, grade II – intermediate, mixed flora, and grade III – BV, predominate Gram-negative small rods.

Bacterial isolates

For the quantitative and qualitative assessment of vaginal flora, cervical and vaginal swabs were collected from the pregnant women under study. At first, cervical swabs were made to check for Mycoplasma hominis and Ureaplasma urealyticum. For isolation, identification and differential titration of mycoplasmas, the commercially available Mycoplasma DUO kits (Sanofi Diagnostics Pasteur) were used. Identification of mycoplasmas, the commercially available Mycoplasma DUO kits (Sanofi Diagnostics Pasteur) were used. Identification of mycoplasmas, the commercially available Mycoplasma DUO kits (Sanofi Diagnostics Pasteur) were used. Identification of mycoplasmas, the commercially available Mycoplasma DUO kits (Sanofi Diagnostics Pasteur) were used. Identification of mycoplasmas, the commercially available Mycoplasma DUO kits (Sanofi Diagnostics Pasteur) were used. Identification of mycoplasmas, the commercially available Mycoplasma DUO kits (Sanofi Diagnostics Pasteur) were used. Identification of mycoplasmas, the commercially available Mycoplasma DUO kits (Sanofi Diagnostics Pasteur) were used. Identification of mycoplasmas, the commercially available Mycoplasma DUO kits (Sanofi Diagnostics Pasteur) were used. Identification of mycoplasmas, the commercially available Mycoplasma DUO kits (Sanofi Diagnostics Pasteur) were used. Identification of mycoplasmas, the commercially available Mycoplasma DUO kits (Sanofi Diagnostics Pasteur) were used. Identification of mycoplasmas, the commercially available Mycoplasma DUO kits (Sanofi Diagnostics Pasteur) were used. Identification of mycoplasmas, the commercially available Mycoplasma DUO kits (Sanofi Diagnostics Pasteur) were used. Identification of mycoplasmas, the commercially available Mycoplasma DUO kits (Sanofi Diagnostics Pasteur) were used. Identification of...
Hydrogen peroxide Lactobacillus species and bacterial vaginosis-related bacteria in vaginal fluid

**Table 3. Distribution of lactobacilli in pregnant women with normal (I), intermediate (II) microflora and with bacterial vaginosis (BV)**

<table>
<thead>
<tr>
<th>Grade</th>
<th>L. jenseni</th>
<th>L. fermentum</th>
<th>L. acidophilus</th>
<th>L. catenaformis</th>
<th>L. minutus</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (No. 70)</td>
<td>49 (70)</td>
<td>14 (20)</td>
<td>7 (10)</td>
<td>6 (8.6)</td>
<td>2 (2.9)</td>
</tr>
<tr>
<td>CFU/ml</td>
<td>≥10^5</td>
<td>≥10^5</td>
<td>≥10^5</td>
<td>≥10^5</td>
<td>≥10^5</td>
</tr>
<tr>
<td>II (No.71)</td>
<td>28 (39.4)</td>
<td>27 (38)</td>
<td>6 (8.5)</td>
<td>2 (2.8)</td>
<td>2 (2.8)</td>
</tr>
<tr>
<td>CFU/ml</td>
<td>≥10^5</td>
<td>≥10^5</td>
<td>≥10^5</td>
<td>≥10^5</td>
<td>≥10^5</td>
</tr>
<tr>
<td>BV (No.55)</td>
<td>2(3.6)</td>
<td>2 (3.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>CFU/ml</td>
<td>≤10^3</td>
<td>≤10^3</td>
<td>≤10^3</td>
<td>≤10^3</td>
<td>≤10^3</td>
</tr>
</tbody>
</table>

*Lactobacilli* were isolated from 68 (97.1%) of women with normal flora (grade I), 67 (94.4%) of women with intermediate flora (grade II) and only among 22 (40%) of women with BV. All of *Lactobacilli* species were isolated from vaginal fluid of pregnant women with normal and intermediate flora while in pregnant women with BV only *L. jenseni*, *L. fermentum* and *L. minutus* were isolated.

**H₂O₂ production and Gram-stain graded**

The majority of H₂O₂ producing *Lactobacilli* strains were isolated among pregnant women with grade I and II microflora (94.2% and 90.1% respectively) as compared to only 9.1% detected among women with bacterial vaginosis. Quantitative analysis showed that in groups I and II all of them were also isolated in higher concentrations as compared to women with BV (Table 3).

**Association between BV-isolated bacteria and H₂O₂ producing Lactobacillus isolates**

The relationships between H₂O₂ producing lactobacilli species and BV-associated flora is shown in figure 1. *L. jenseni* and *L. fermentum* species were isolated with all BV-related flora.

![Fig. 1. Association between BV-isolated bacteria and H₂O₂ producing Lactobacillus](image)

*L. jenseni* was isolated frequently together with *Bacteroides* sp. and *G. vaginalis* (60% and 51.1% co-isolations), whereas *L. fermentum* with *Bacteroides* (40%). Both species were isolated in the same frequency together with *M. mulieris* (15.4%), in 21.0% and 18.2% with *M. hominis* and in 20% and 28.6% with *U. urealyticum*. We detected very low co-isolations of *L. fermentum* with *Prevotella* sp. and *G. vaginalis* (5.0% and 4.3% respectively). The other species of Lactobacillus: *L. acidophilus*, *L. catenaformis* and *L. minutus* were isolated in very low percentage (from 1 to 2%) only with some BV-related bacteria, as *G. vaginalis*, *M. hominis* and *U. urealyticum*. The all species of Lactobacillus were isolated in low concentrations (<10^5 CFU/ml), while BV-related bacteria in high concentrations (>10^5 CFU/ml).

**Discussion**

There are only few reports describing *Lactobacillus* species found in the vagina of pregnant women and the relationship between those species and BV [1, 13, 18-21]. The range of species isolated from human vagina is relatively small and different from those isolated from the mouth and intestine [22, 23]. *L. crispatus*, *L. gasseri*, *L. vaginalis*, *L. jenseni*, *L. acidophilus* are common *Lactobacillus* species found in the vaginal fluid, with *L. iners* described recently as a new species of the vaginal microbiota [4, 24, 25, 26]. The relative proportion of these species varies between studies and differences in the prevalence of species in various studies could reflect geographical variations [26-28].

In the present study performed among pregnant Polish women at early pregnancy (8-16 weeks) we identified only 5 Lactobacilli species: 2 previously described species *L. jenseni*, and *L. acidophilus*, and 3 another species like: *L. fermentum*, *L. catenaformis*, and *L. minutus*. Two recently described vaginal species such as *L. fornicalis* and *L. iners* were not isolated in our study. It could be the result of different methods of isolations used in our study (cultures) compared for example to 16S rRNA sequencing used by others. Vaginal *Lactobacilli* were described as *L. acidophilus* mainly on the basis of the site of isolation rather than on any specific biochemical or genetic property. In this study we isolated *L. acidophilus sensu stricto* among 13 cases, only from pregnant women with grade I (10.0%) 8.5% and grade II (8.5%) microflora, but non in BV group.
Most isolates of vaginal *Lactobacilli* produced some detectable H$_2$O$_2$. As the presence of H$_2$O$_2$ producing *Lactobacilli* in the vaginal fluid is associated with a reduced risk of BV and because the concentration of these bacteria is low in women with BV, the H$_2$O$_2$ producing ability of species is thought to play a significant role in protecting vaginal ecosystem from BV [2, 18, 20, 29, 30]. The mechanisms by which *Lactobacilli* resist antimicrobial activity of H$_2$O$_2$ is still unknown.

The results of present study confirm that H$_2$O$_2$ producing *Lactobacilli* were common strains in pregnant women with normal and intermediate vaginal microflora. All of the *Lactobacillus* isolated species were less prevalent in pregnant women with BV. In nearly all of isolated strains of *Lactobacillus* the ability to produce H$_2$O$_2$ was observed: only 17 isolates vs 79 did not produce H$_2$O$_2$. The results of our study also shown that the concentrations of *Lactobacillus* species were significantly higher among women with normal flora as compared to women with BV. In contrast, 6 main BV-related bacteria, *i.e.*, *Bacteroides sp.*, *Prevotella sp.*, *M. mulieris*, *G. vaginalis*, *M. hominis* and *U. urealyticum* were detected at higher prevalence in pregnant women with BV.

It has been demonstrated in *in vitro* studies that H$_2$O$_2$ producing vaginal lactobacilli have an inhibitory effect on colonization by the bacteria that predominate in BV [31]. Our observation suggest that the highest ability to inhibit the BV-related bacteria had *L. acidophilus, L. fermentum* and *L. minutus*. This observation is consistent with the notion that H$_2$O$_2$ producing ability of *Lactobacilli* is important in protecting the healthy vaginal ecosystem during pregnancy.

Bacterial vaginosis is a well known cause of various perinatal complications including preterm delivery and PROM [6, 7, 9, 32]. The relationship between BV and pregnancy outcome has been studied extensively, whereas the relationship between *Lactobacilli* colonizing the vagina and pregnancy outcome has received less attention [11-13]. Kim et al. [13] observed that distribution of hydrogen peroxide-producing *Lactobacilli* in vaginal flora as defense factors for infection may have an important role in the pathophysiology of preterm labor. Usui et al. [11] suggest that tests determining the presence of vaginal *Lactobacilli* may be clinically useful tools for identifying women at an increased risk of preterm delivery below 33 weeks of gestation. As our results show that pregnant women with BV lack *Lactobacilli*, especially hydrogen peroxide producing ones, it seems that the role of various *Lactobacilli* species in pathogenesis of preterm delivery should be evaluated in further studies.

**Conclusion**

Our results showed that pregnant women with BV lack lactobacilli, especially hydrogen peroxide producing ones. The presence of H$_2$O$_2$ producing lactobacilli as *L. acidophilus, L. fermentum, L. minutus* and *L. jensenii*, and *L. fermentum* seems to protect against the development of BV during early pregnancy. The role of various lactobacilli species in pathogenesis of preterm delivery should be evaluated in further studies.

**References**


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