The Vaginal Microbiota: What Have We Learned after a Decade of Molecular Characterization?

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Abstract

We conducted a systematic review of the Medline database (U.S. National Library of Medicine, National Institutes of Health, Bethesda, MD, U.S.A) to determine if consistent molecular vaginal microbiota (VMB) composition patterns can be discerned after a decade of molecular testing, and to evaluate demographic, behavioral and clinical determinants of VMB compositions. Studies were eligible when published between 1 January 2008 and 15 November 2013, and if at least one molecular technique (sequencing, PCR, DNA fingerprinting, or DNA hybridization) was used to characterize the VMB. Sixty three eligible studies were identified. These studies have now conclusively shown that lactobacilli-dominated VMB are associated with a healthy vaginal micro-environment and that bacterial vaginosis (BV) is best described as a polybacterial dysbiosis. The extent of dysbiosis correlates well with Nugent score and vaginal pH but not with the other Amsel criteria. Lactobacillus crispatus is more beneficial than L. iners. Longitudinal studies have shown that a L. crispatus-dominated VMB is more likely to increase the incidence of BV. In Research settings, BV is also defined by molecular techniques (sequencing, PCR, DNA fingerprinting, or DNA hybridization) to determine if consistent molecular VMB can be discerned. Data on VMB determinants are scarce and inconsistent, but a robust consensus has been reached that BV is characterized by a decrease in Lactobacillus spp., an increase in Gardnerella vaginalis, and a decrease in the diversity of the VMB. This article is a review of the published literature and does not report on any original data.

Introduction

It has been known for some time that the most vaginal microflora (VMB) consist predominantly of lactobacilli and that VMB alterations can cause symptoms. The most familiar condition is bacterial vaginosis (BV), which has traditionally been characterized as a reduction of vaginal lactobacilli and an overgrowth of other (facultative) anaerobic bacteria. In clinical settings, BV is typically diagnosed using Amsel criteria (three of the following four criteria should be present: 1) clue cells on wet mount microscopy; 2) a ‘fishy’ odor after adding 10% KOH to vaginal secretions; 3) vaginal pH>4.5; and 4) thin, homogenous vaginal discharge) [2]. In research settings, BV is also often defined by Gram stain Nugent scoring, which is based on microscopic visualization of three bacterial morphotypes (a Nugent score of 0–3 is considered normal, 4–6 intermediate microflora, and 7–10 BV) [3]. BV is not highly inflammatory and is therefore often asymptomatic; this is why it is referred to as a vaginosis and not a vaginitis [1]. Two common types of microbiological vaginitis are vaginal candidiasis and trichomoniasis [1]. Vaginal candidiasis is often highly inflammatory and is typically diagnosed by wet mount microscopy and/or culture of Candida species. Trichomoniasis is caused by the sexually transmitted single-celled parasite Trichomonas vaginalis, which can be detected by microscopy, culture or PCR.

Other types of vaginitis have been described (such as aerobic vaginitis, desquamative inflammatory vaginitis, and atrophic vaginitis) but occur less frequently [4–6]. The term ‘aerobic vaginitis’ is used by some clinicians to refer to vaginal inflammation and/or culture of Group B streptococcus) and Escherichia coli [4]. While the roles of vaginal Streptococcus agalactiae (also known as Group B streptococcus) and E. coli in invasive maternal and neonatal infections have been well-
documented [7], their potential roles in causing a vaginosis syndrome distinct from BV is not universally accepted.

Altered communities of micro-organisms in the vagina are not only implicated in septic postpartum and neonatal infections but also in pelvic inflammatory disease [8], miscarriage and pre-term birth [9], and increased HIV acquisition and onward transmission [10–12]. VMB alterations may therefore be of much greater public health importance than was previously assumed.

In the last decade, phylogenetic analyses of vaginal samples (mostly bacterial 16S ribosomal RNA gene sequencing) have shown that bacterial communities in the vagina are more complex than previously thought. The first study using molecular methods to characterize the VMB was published in 2002 [13]. In a review of studies conducted between 2002 and 2008, Srinivasan and colleagues concluded that BV is indeed associated with a loss of lactobacilli and the introduction and/or overgrowth of other (facultative) anaerobic bacteria, and identified important VMB bacteria that had previously been missed by culture-based methods [14]. These bacteria included species in the Lactobacillus genus (e.g. L. mers) and bacteria associated with BV (e.g. Atopobium vaginae and three bacteria in the Lachnospiraceae family temporarily named BVAB1, 2 and 3) [13,15,16].

Since 2008, high throughput molecular techniques have become more affordable and accessible, and many more VMB characterization studies have been performed. We conducted a systematic review of the published literature from 2008 to date, to synthesize current knowledge about the VMB and its determinants, and to identify research gaps.

**Methods**

We conducted a systematic review according to the PRISMA 2009 guidelines [17].

Our first objective was to determine if any consistent VMB composition patterns can be discerned after a decade of molecular testing, despite the fact that different groups have used different molecular techniques and/or operating procedures. Our second objective was to review correlations between molecular compositions, Amsel criteria, and Nugent scoring. Our third objective was to assess which determinants (sociodemographic, physiological, and behavioral risk factors, and the presence of pathogens in the genital tract) have been consistently associated with certain VMB composition patterns in different studies.

**Search strategy and selection criteria**

Eligible studies included studies that used at least one molecular technique (sequencing, PCR, DNA fingerprinting, or DNA hybridization). We only included PCR and DNA hybridization studies if multiple bacterial species or genera were assessed (either by multiple individual assays or by multiplex assays). We excluded studies that focused on viral, archaeal, fungal, or protozoal diversity, or on development of diagnostic assays. We only considered randomized controlled intervention trials if the baseline data, prior to the intervention, could be used to address one of our objectives. Article selection was based on the first objective; not all articles also addressed the second and third objectives.

We searched the Medline database (U.S. National Library of Medicine, National Institutes of Health, Bethesda, MD, U.S.A.) for articles between 1 January 2008 and 15 November 2013, limiting our search to articles written in English. We started our review in 2008 as opposed to 2002 (when the first molecular VMB data were published) because Srinivasan and Fredericks published a review of the early studies in 2008 [14]. We searched titles and abstracts using the search term “vaginal microbiome”. Two authors (JvdW and HB) assessed the articles for eligibility, and hand-searched the reference lists of eligible articles to identify additional articles. Five authors (JvdW, HB, RV, VJ, and TC) extracted data from all eligible articles using predefined data extraction tables, which included the data categories presented in tables 1 and 2, a description of the VMB compositions and correlates that the study had identified, and study strengths and weaknesses. Each article was reviewed independently by two authors.

**Results**

**Study selection**

Our Medline database search yielded 475 results, of which 50 were eligible. We identified 20 additional eligible articles from the reference lists of the initial 50 articles. After data extraction, a further seven articles were rejected because they did not address our objectives appropriately (mostly because they focused on technical laboratory issues or diagnostic assay development). A total of 63 articles are therefore included in this review [18–80].

**Study characteristics**

Table 1 shows characteristics of the 63 articles (references are included in the table and are not repeated here). It should be noted that one article could include data from more than one data extraction category, which is why some column totals exceed 63. Most of the articles reported data from North America (31 articles), followed by Europe (13), Africa (10), Asia (9), and Central America (3). Most sample sizes were small, with only 19 articles reporting a sample size larger than 100. Most of the study populations were non-pregnant adult women of reproductive age, with or without BV as the only diagnosed condition (28 articles). Other study populations included adolescents/virgins (3 articles), pregnant women (7), postmenopausal women (5), women attending a sexual health clinic (7) or with confirmed HIV, HPV or other infections (16), female sex workers (2), women who have sex with women (MSW; 3), and women undergoing in-vitro fertilization (IVF) (2). Sequencing was the most commonly employed molecular technique; earlier studies tended to sequence DNA isolated from individual colonies obtained by bacterial culture (15 articles), whereas later studies extracted DNA directly from genital samples followed by next generation sequencing (18 studies used 454 sequencing (454 Life Sciences Corporation), Branford, CT, US) and four used other platforms. PCR was also commonly used with 19 studies using quantitative PCR (qPCR) for individual species/genera, and four studies using qualitative multiplex PCR. Other molecular techniques included DNA fingerprinting techniques (13), phylogenetic DNA microarrays (4), and hybridization to oligonucleotide probes coupled to beads (1). One of the main aims in all articles was to describe the VMB in a particular study population, and 20 articles included longitudinal data (indicated in Table 1 as longitudinal VMB changes); three articles also described cervical and/or endometrial microbiota and three rectal and/or oral reservoirs of vaginal bacteria; and 17 employed a clustering technique to characterize bacterial communities.

**Definitions used for a healthy VMB and for BV**

Most of the 63 articles that we reviewed used a Nugent score of 0–3 to define a healthy VMB (26 articles), with an additional 12 using a Nugent score of 0–3 plus the presence of fewer than three Amsel criteria, and nine using a Nugent score of 0–6. Fourteen articles did not provide a definition, and the remaining 27 articles used a variety of definitions based on microscopy, vaginal pH, and clinical symptoms. In the 37 articles that described a comparison between a healthy VMB and BV, BV was mostly defined as a...
Table 1. Characteristics of molecular vaginal microbiota articles published between 1 January 2008 and 15 November 2013.

| Year of publication | Number of articles | References |
|---------------------|-------------------|------------|
| 2008                | 6                 | 18–23      |
| 2009                | 11                | 24–34      |
| 2010                | 7                 | 35–41      |
| 2011                | 13                | 42–54      |
| 2012                | 14                | 55–68      |
| 2013<sup>1</sup>    | 12                | 69–80      |
| **TOTAL**           | **63**            |            |

| Country<sup>2</sup> | Number of articles | References |
|---------------------|--------------------|------------|
| USA & Canada        | 31                 | 19–21, 23–25, 27, 28, 30–32, 35–39, 42, 44, 49, 59–61, 64–70, 77, 78 |
| Belgium             | 6                  | 26, 33, 34, 54, 62, 63 |
| Europe – other<sup>3</sup> | 7 | 18, 22, 43, 55, 72, 76, 79 |
| China               | 6                  | 29, 41, 47, 57, 74, 80 |
| Asia – other<sup>4</sup> | 3 | 35, 50, 71 |
| South Africa        | 4                  | 45, 46, 53, 73 |
| East Africa<sup>5</sup> | 4 | 25, 40, 48, 77 |
| West Africa<sup>6</sup> | 2 | 51, 58 |
| Central America<sup>7</sup> | 3 | 52, 56, 75 |
| **TOTAL**           | **66**             |            |

| Sample size (women) | Number of articles | References |
|---------------------|--------------------|------------|
| ≤10                 | 4                  | 28, 29, 56, 70 |
| 11–50               | 22                 | 18–20, 22, 23, 25–27, 31, 36–38, 43, 44, 48–50, 54, 59, 61, 69, 73 |
| 51–100              | 18                 | 24, 30, 33, 41, 45–47, 52, 55, 57, 58, 62, 63, 65, 66, 74, 78, 80 |
| 101–200             | 14                 | 21, 32, 34, 35, 39, 40, 53, 63, 67, 68, 71, 75–77, 79 |
| >200<sup>8</sup>    | 5                  | 42, 51, 60, 64, 72 |
| **TOTAL**           | **63**             |            |

| Study population    | Number of articles | References |
|---------------------|--------------------|------------|
| Adults, healthy or with BV | 28 | 19, 22, 23, 26, 28, 29, 35, 36, 38, 41–43, 50, 54, 57, 59, 60, 62–64, 67, 68, 70, 72, 73, 75, 79, 80 |
| Adolescents/virgins | 3                  | 24, 25, 66 |
| Pregnant women      | 7                  | 33, 34, 52, 55, 58, 68, 78 |
| Postmenopausal women| 5                  | 43, 49, 55, 57, 71 |
| STD clinic attendees | 7                | 19, 37, 38, 60, 62, 65, 76 |
| Female sex workers  | 2                  | 25, 48 |
| WSW                 | 3                  | 21, 39, 61 |
| By HIV status       | 11                 | 20, 30, 40, 44–46, 48, 51, 53, 58, 77 |
| By HPV/cytology status | 5             | 46, 56, 66, 71, 74 |
| Women undergoing IVF| 2                  | 18, 69 |
| **TOTAL**           | **78**             |            |

| Molecular technique<sup>2</sup> | Number of articles | References |
|----------------------------------|--------------------|------------|
| NGS sequencing 454<sup>9</sup>   | 18                 | 20, 36, 41, 42, 44, 48, 56, 58–60, 64, 65, 67, 68, 70–72, 76 |
| NGS sequencing other<sup>9</sup> | 4                  | 40, 49, 56, 67 |
| Culture & sequencing             | 15                 | 18, 19, 23, 28, 29, 35, 50, 53, 54, 56, 57, 69, 73, 75, 78 |
| qPCR                             | 19                 | 21, 26, 30, 37–39, 41, 42, 47, 51, 55, 57, 61, 62, 63, 66, 77, 79, 80 |
| Multiplex PCR                    | 4                  | 27, 43, 51, 55 |
| Fingerprinting<sup>10</sup>      | 13                 | 20, 24, 31, 33–36, 41, 43, 47, 52, 55, 74 |
| Hybridisation<sup>11</sup>       | 5                  | 22, 25, 32, 45, 46 |
| **TOTAL**                        | **78**             |            |

| Main topic of research<sup>2</sup> | Number of articles | References |
|------------------------------------|--------------------|------------|
| Clustering of VMB                 | 17                 | 24, 28, 31, 35, 36, 40, 42, 48, 50, 56, 58, 60, 62, 65, 71, 72, 79 |
| Improving BV diagnosis             | 5                  | 30, 47, 76, 79, 80 |
| Descriptive – other<sup>12</sup>  | 27                 | 18, 21, 24, 28, 33, 35, 38, 39, 42, 48, 51, 54–56, 59, 60, 62, 63, 66, 68–70, 72, 73, 77–79 |
| Longitudinal VMB changes           | 20                 | 18, 21, 23, 25, 26, 33, 38, 39, 42, 49, 54, 56, 59, 62, 63, 66, 67, 69, 77, 78 |

**TOTAL** | 78 |
Nugent score of 7–10 (20 articles), with an additional nine using a Nugent score of 7–10 plus the presence of three or more Amsel criteria, and the remaining eight using a variety of other definitions based on microscopy and clinical symptoms.

### Vaginal bacterial communities by clustering

The 17 studies that used a clustering technique to characterize the composition of VMB bacterial communities can be subdivided into those that used comprehensive data based on next generation sequencing of DNA extracted from vaginal samples (10 articles) [36,40,42,48,56,58,60,65,71,72] and those that used less comprehensive data by sequencing DNA of bacterial culture colonies (2) [36,40,42,48,56,58,60,65,71,72] and those that used less comprehensive data based on next generation sequencing of DNA extracted from vaginal samples, the 25 most abundant taxa consistently (in at least 50% of studies) included the following additional taxa: *A. vaginae, Eggerthella* spp., *Mobилиncus* spp., *Lachnoclostridia* (including the species BVAB1-3), *Dialister* spp., *Megaglossa* spp., *Parvimonas* (formerly *Peptostreptococcus*) spp., *Veillonella* spp., *Streptococcus* spp., *Staphylococcus* spp., *Gemella* spp., *Prevotella* spp., *Parvimonas* spp., *Bacteroides* spp., *Sneathia* spp., *Lepotrichia* spp., *Mycoplasma* spp., *Ureaplasma* spp., and *Escherichia/Shigella* spp. Other bacterial taxa were often found but either in low abundance or not consistently. Most articles identified more than one cluster with mixed taxa; these clusters typically did not differ significantly in the total number or types of bacterial taxa present but they did differ in their relative proportions. We were not able to discern consistent patterns across studies. Only three of the 17 articles reported clusters dominated by streptococci, staphylococci, *Proteus* spp., or *Escherichia/Shigella* spp. (Table 2).

### Longitudinal VMB patterns

One of the conclusions of the Human Microbiome Project was that within-subject microbiota variation over time was lower than between-subject variation for all habitats, including the vagina [67]. Similarly, several longitudinal VMB studies showed that a majority of women have a stable VMB microbiome [23,26,54,59,62]. A study in post-menopausal women showed that the VMB is usually stable in that group as well [49]. However, while one study suggests that an increased VMB diversity is

| Number of articles | References |
|--------------------|------------|
| Extravaginal reservoirs | 6 | 34, 43, 47, 55, 56, 61 |
| VMB associations with HIV | 11 | 20, 30, 40, 44, 45, 46, 48, 51, 53, 58, 77 |
| VMB associations with HPV | 5 | 46, 56, 66, 71, 74 |
| VMB associations with other STIs | 4 | 51, 64, 72, 79 |
| VMB associations with other infections | 5 | 27, 32, 47, 65 |
| VMB associations with non-infectious reproductive health outcomes | 4 | 32, 49, 69, 78 |
| VMB associations with immune activation | 1 | 22 |
| **TOTAL** | **105** |
associated with a decreased stability [23], others suggest that this is not necessarily the case: for example, a BV-associated VMB can be stable and persist for a long time [59]. The few articles that not necessarily the case: for example, a BV-associated VMB can be stable and persist for a long time [59]. The few articles that

Extravaginal reservoirs of VMB bacteria

The Human Microbiome Project also concluded that vaginal microbial communities are relatively ‘simple’ at genus-level compared to oral and gut communities, but have a higher diversity of Lactobacillus spp. [67]. Solt et al. identified 673 genera in the rectum, 275 in the mouth, and 112 in the vagina [81]. Three studies assessed the presence of lactobacilli and other VMB taxa in rectal and oral specimens, as well as vaginal specimens, to test the hypothesis that the gut and mouth act as extravaginal reservoirs of VMB bacteria [34,55,61]. Lactobacilli and various BV-associated bacteria were indeed often found in the rectum, while lactobacilli were sometimes, and G. vaginalis consistently, found in the mouth. In one study, women who had high quantities of G. vaginalis in the mouth or rectum, or Megasphaera, Leptotrichia, or Sneathia spp. in the rectum, were more likely to develop clinical BV during follow-up; in contrast, women who had L. crispatus in the rectum were less likely to develop clinical BV [61].

VMB associations with traditional BV diagnostics

More than half of the articles (37) described molecular VMB data in relation to BV diagnosis by Amsel and/or Nugent criteria [19–23,25–27,30,32,33,37,38,40–42,45,47,48,50–54,56,59,60,62,63,65,67,72,73,75,76,79,80]. Two important areas of consensus emerged. First, almost all women carry vaginal lactobacilli regardless of their BV status by Nugent or Amsel criteria [19,20,22,23,25,26,33,37–42,45,47,48,50–54,56,59,60,62,63,65,67,72,73,75,76,79,80]. A second area of consensus is that BV diagnosis is predominately found in BV-negative women [20,22,23,25,32,33,37,38,40,42,45,48,50,54,59,60,62,63,65,73,75,76,80] whereas L. iners (and to a lesser extent L. gasseri) is also found in women with intermediate microbiota or BV [21,23,25,33,37,38,40–42,47,50,54,59,60,62,63,65,73,76,79,80].

Table 2. Vaginal microbiota communities identified by clustering techniques in 17 articles.

| Cluster | Molecular techniques | References | Total |
|---------|----------------------|------------|-------|
| Led by Lactobacillus crispatus | All except qPCR | 24, 31, 35, 36, 40, 42, 50, 56, 65, 71, 72 | 11 |
| Led by L. iners | All | 24, 31, 35, 36, 40, 42, 48, 50, 56, 58, 60, 65, 71, 72, 79 | 15 |
| Led by L. jensenii | Direct sequencing and fingerprinting | 35, 42 | 2 |
| Led by L. gasseri | Direct sequencing and fingerprinting | 31, 35, 36, 42, 56 | 5 |
| Led by lactobacilli but unspecified | Sequencing of culture colonies | 28 | 1 |
| Led by Lactobacillus iners | All | 24, 35, 60, 62, 65 | 5 |
| Led by Gardnerella vaginalis | Direct sequencing and qPCR | 40, 48, 56, 79 | 4 |
| Mixture of lactobacilli and G. vaginalis | Direct sequencing and sequencing of culture colonies | 28, 40, 56 | 3 |
| Mixture of lactobacilli, G. vaginalis and other anaerobes | All except qPCR | 28, 31, 42, 48, 56, 58, 60, 65 | 8 |
| Mixed anaerobes with few/no lactobacilli | All | 24, 28, 35, 36, 40, 50, 56, 58, 60, 62, 71, 72, 79 | 13 |
| Led by aerobes | Direct sequencing and fingerprinting | 35, 58, 65 | 3 |

qPCR = quantitative polymerase chain reaction.

1 Includes direct sequencing (next generation sequencing) of DNA extracted from vaginal samples (10), sequencing of culture colonies (2), fingerprinting (3), and qPCR (2); the 5 studies using DNA hybridisation techniques did not employ data clustering and this technique is therefore not represented in this table.

2 One qPCR study only assessed L. iners and the other qPCR study did not find clusters dominated by just one Lactobacillus species.

3 Other than Lactobacillus spp., the 25 most abundant taxa in the 10 direct sequencing studies consistently (in at least 50% of studies) include: Phylum Actinobacteria: G. vaginalis, A. vaginae, Eggerthella spp., Mobiluncus spp.; Phylum Firmicutes: Lachnospiraceae (including BVAB1-3), Dialister spp., Megasphaera spp., Parvimonas (formerly Peptostreptococcus) spp., Veillonella spp., Streptococcus spp., Staphylococcus spp., Gemella spp.; Phylum Sphingobacteria: Prevotella spp., Paraphyromonas spp., Bacteroides spp.; Phylum Fusobacteria: Sneathia spp., Leptotrichia spp.; Phylum Tenericutes: Mycoplasma spp., Ureaplasma spp.; Phylum Proteobacteria: Escherichia/Shigella spp.

4 Includes Streptococcus spp., Staphylococcus spp., Escherichia/Shigella spp., Proteus spp.

Molecular Characterization of Vaginal Microbiome
bacteria [26,41,47,62,76,80]. It is not yet clear from the studies we reviewed whether BV is associated with a higher overall bacterial load than healthy lactobacilli-dominated microbiota. Importantly, several studies showed that *G. vaginalis* and *Prevotella* spp. are often found regardless of BV status by Nugent or Amsel criteria, but their abundances increase in BV; furthermore, a synergistic effect between them was noted [26,37,41,42,62,79]. The role of streptococci, staphylococci, and enterococci is generally not well described; when present, they are usually present in low abundance. One study (using DNA hybridization) reported that their presence in the VMB did not differ by BV status [32], whereas another study (also using DNA hybridization) reported higher levels in women with intermediate microbiota by Nugent score [22].

Higher bacterial diversity and/or higher levels of individual BV-associated bacteria are consistently associated with a higher vaginal pH [30,42,60,67,72,80] and Nugent score [42,47,51,80]. Vice versa, increasing abundance of lactobacilli is consistently associated with a lower vaginal pH [30,40,67,80] and Nugent score [80]. Associations with the other Amsel criteria have been less well studied, but one study found that only the *Leptotrichia* and *Eggerthella* genera were associated with all four Amsel criteria [60].

**VMB associations with other clinical outcomes**

Vaginal colonization with *Candida* spp. seems more common in women with a lactobacilli-dominated VMB than in women with BV [26,31,72], as had been noted previously in epidemiological studies using traditional diagnostic methods [11]. In contrast, *T. vaginalis* has often been strongly associated with BV in the past, and this was confirmed in a study using 454 sequencing [64]. Convincing patterns of associations between bacterial sexually transmitted infections (STIs) and the molecular composition of the VMB did not emerge, most likely due to the fact that women with bacterial STIs were often excluded or the prevalence rates were low.

Eleven studies assessed the VMB by HIV status [20,30,40,44–46,48,51,53,58,77] and five studies by HPV status [46,56,66,71,74]. One study found no relationship between HIV and the VMB [53], but most found trends towards decreased lactobacilli (and particularly *L. crispatus*) [44,46,48] and increased bacterial diversity [45,51], particularly in women who had both HIV as well as BV by Nugent score [20]. Similar trends were found related to HIV-1 RNA load in the genital tract [30,77]. One study found an increased prevalence of *E. coli*-dominated VMB in HIV-positive women [48], and another one found increased HIV transmission from mother to child with increasing VMB diversity in the mother (although this did not reach statistical significance) [58]. HPV also seems to be associated with a reduction in lactobacilli [71] and increased VMB diversity [46,71,74]. In one study, *Sneathia* spp. were strongly associated with the presence of high risk HPV [71].

The cervical microbiota are similar to the VMB, except that bacterial loads are lower [47]. A comprehensive qPCR study showed that cervical bacterial diversity is highest in women with BV, followed by women with cervicitis and healthy women, with only small differences between the latter two; BV was associated with a dramatic reduction in lactobacilli in the vagina and cervix, whereas cervicitis with a reduction in the cervix only [47]. The authors conclude that the VMB does not play a large role in cervicitis. Another study found good agreement between PCR results of five BV-associated species in cervical and endometrial samples of women with pelvic inflammatory disease, although this did not reach statistical significance [27].

BV and gingivitis were also reported to be associated, with counts of *P. bivia*, *P. disiens*, *M. curtisi*, and *M. mulieris* being particularly high in women with both BV and gingivitis [32]. Finally, a lactobacilli-dominated VMB was associated with a reduced risk of pre-term birth, a higher likelihood of IVF resulting in a live birth, and a reduced risk of vaginal dryness in postmenopausal women in one study each [49,69,78].

We found only one study that correlated molecular VMB composition (using both culture and a DNA-DNA checkerboard including *L. iners* and 12 BV-associated species) with vaginal immune responses [22]. Total viable bacterial counts and the presence of BV-associated bacteria were positively associated with cervicovaginal IL-1α and IL-1β (and BV-associated bacteria also with IL-6 and IL-8), whereas *L. iners* was negatively associated with IL-1β. The relationships with secretory leukocyte protease inhibitor (SLPI) were the other way around.

**VMB associations with demographic and behavioral characteristics**

Data on the association between the molecular VMB and age were inconsistent. Six studies did not find an association but four of these only included a narrow age range (exclusively reproductive age or post-menopausal women) [42,49,62,72]; one study did not find a difference between reproductive age and post-menopausal women [76] and another one did not find a difference between adolescents and women of reproductive age [24]. However, three studies that quantified multiple *Lactobacillus* spp. found lower overall levels, as well as reduced *L. crispatus* levels and *Lactobacillus* diversity, in post-menopausal women compared to women of reproductive age [43,55,57]. Several articles report that Black African and African-American women compared to Caucasian or Asian women are less likely to carry *L. crispatus*, *L. jensenii*, *L. gasseri* and/or *L. vaginalis* and more likely to carry *L. iners*, and are more likely to have a higher bacterial diversity [35,42,60,62,77,78]. One study found the same for U.S. Hispanic women [42].

Few studies found significant associations between the VMB at the molecular microbial level and sexual behavior. However, detailed sexual behavior data were mostly not collected, sample sizes were small, or analyses focused on risk factors for BV by Amsel or Nugent criteria even though bacterial molecular data were also available. One study found that the detection of prostate-specific antigen (as a marker of sexual activity within the last 48 hours) was negatively associated with *L. crispatus* and positively with *L. iners* and *L. gasseri* [62]. In another study, the prevalence of various BV-associated bacterial genera was increased with an increasing number of sexual partners [51]. Finally, a comprehensive study found a slight gain of *G. vaginalis* after sexual debut, but no significant gain of other BV-associated bacteria or loss of lactobacilli [66].

Even though most studies that evaluated the influence of the menstrual cycle were small, they consistently suggest that high levels of estradiol (assessed by phase in the menstrual cycle or in serum of IVF patients) promote lactobacilli, and particularly *L. crispatus* [18,59,69,70]. Studies also consistently suggest that menses is the largest disturbing factor during the menstrual cycle, with sometimes large reductions in lactobacilli [38,59,62,63], shifts from *L. crispatus* to *L. iners* [38,70], or the appearance of BV-associated bacteria, streptococci or other Gram-positive cocci [54,70]. Pregnancy, which is also accompanied by high estradiol levels, is associated with high levels of lactobacilli, particularly *L. crispatus*, and low bacterial diversity [53,68]. However, one study found an increasing bacterial diversity in late term pregnancies [68]. In another study, a VMB dominated by *L. iners* or *L. gasseri*
in the first trimester was more likely to evolve to BV later on during pregnancy; L. crispatus had the opposite effect [33].

**Discussion**

Despite the fact that many different molecular techniques and operating procedures with specific advantages and disadvantages have been used (reviewed in [14]) and despite the fact that these technical differences can result in under- or overrepresentation of bacterial species [82], we found several areas of consensus about the VMB composition. Studies have now conclusively shown that lactobacilli-dominated VMB are associated with a healthy vaginal micro-environment, and that BV is best described as a polymicrobial dysbiosis: the Lactobacillus load decreases, and both the diversity and bacterial load of other facultative anaerobic bacteria increase [24,28,31,35,36,40,42,48,50,56,58,60,62,65,71,72,79]. Furthermore, the bacteria associated with this dysbiosis are now well described [24,28,31,35,36,40,42,48,50,56,58,60,62,65,71,72,79]. Some are consistently found (G. vaginalis, A. vaginae, bacteria in the Lachnospiraceae family (including BVAB1-3), and species in the following genera: Prevotella, Eggerthella, Dialister, Megaplasma, Streptococcus, Lactobacillus, Parvimonas (formerly Peptostreptococcus), Veillonella, Bacteroides, Mobiluncus, Porphyromonas, Mycoplasma, Ureaplasma, Staphylococcus, Gemella, and Escherichia/Shigella) whereas others are not consistently found but can be part of a long tail of minority species. G. vaginalis and Prevotella spp. are also often present in healthy women, but their bacterial loads increase significantly in dysbiosis. Consensus is also emerging about the relative importance of different Lactobacillus species: L. iners is present in almost all women worldwide including those with dysbiosis; L. crispatus is mostly present in healthy women and might be less common in women of African or Hispanic descent; and L. jensenii, L. crispatus, and L. gasseri, and L. vaginalis are much less common [24,28,31,35,36,40,42,48,50,56,58,60,62,65,71,72,79]. Furthermore, longitudinal studies have shown that a L. crispatus-dominated VMB might transition to a L. iners-dominated VMB but is less likely to transition directly to a dysbiotic state (and vice versa) [33,59]. The gut, and to a lesser extent the mouth, serve as extravaginal reservoirs of common VMB bacteria [34,55,61].

An increase of bacterial diversity and BV-associated bacteria is consistently associated with an increase in Nugent score and/or vaginal pH, but not with the other three Amsel criteria [30,42,47,51,60,67,72,80]. This is reassuring because our current knowledge about the epidemiology of vaginal dysbiosis is mostly based on Nugent scoring. A recent study by Srinivasan and colleagues, however, questioned the microbial interpretation of Nugent scoring [83]. This study showed that the Mobiluncus morphotype more likely represents BVAB-1 than Mobiluncus spp., and the Bacteroides morphotype more likely represents Porphyromonas and Prevotella spp than Bacteroides spp. While these are important observations, the clinical relevance is unclear because all of these bacterial species are associated with vaginal dysbiosis. The composition and significance of the intermediate Nugent category remains unclear. One molecular study suggested that this category is a transition state from a lactobacilli-dominated VMB to dysbiosis or vice versa [40], but another study found an association with VMB clusters dominated by the facultative anaerobic bacteria that have been implicated in aerobic vaginitis [4,22]. While it is important to investigate this further to allow for the proper interpretation of epidemiological studies that have used/are using Nugent scoring to characterize the VMB, it is likely that future studies will replace Nugent scoring by molecular VMB characterization and quantification.

We found much less consensus on VMB associations with sociodemographic, behavioral, and clinical characteristics, mostly because few studies were designed to evaluate these. Three areas of consensus stood out: Vaginal colonization with Candida spp. was consistently more common in women with a lactobacilli-dominated VMB than women with bacterial dysbiosis [26,31,72], infection with Trichomonas vaginalis is associated with vaginal dysbiosis [64], and a high level of estradiol is consistently associated with lactobacilli [18,59,69,70]. The latter is also supported by many studies that evaluated the VMB by microscopy (reviewed in [84]). The data on the associations between the VMB and HIV and HPV infection are not entirely consistent but also point in the direction of decreased lactobacilli and increased bacterial diversity when a STI is present. We recently confirmed this in a study in women at high risk of HIV and other STIs in Rwanda [85]. This study showed that women with L. crispatus-dominated VMB had the lowest prevalence of HIV, HPV, and herpes simplex type 2 (and had no bacterial STIs), with a slight increase in women with a L. iners-dominated VMB, and a significant increase in women with vaginal dysbiosis. A similar trend was found for HIV-1 RNA shedding in the genital tract of HIV-positive women. Since the study was cross-sectional, the temporality of these relationships remains to be elucidated.

It is worth emphasizing that the molecular studies did not identify large VMB differences between adolescent, reproductive age, and post-menopausal women [24,79], except in post-menopausal women with vaginal atrophy and dryness [49]. Post-menopausal women have lower estrogen levels, which might lead to less protection from dysbiosis. However, they no longer menstruate, and are therefore protected from the potentially negative effects of menstrual blood and increased vaginal pH on the VMB.

Our review also highlighted many research gaps. Most importantly, we still do not sufficiently understand how the VMB is established and maintained, and how bacterial dysbiosis develops and resolves. In particular, the roles of L. crispatus (which seems to inhibit dysbiosis), L. iners (which does not seem to inhibit dysbiosis), and G. vaginalis and Prevotella spp. (which are often present in healthy women in low abundance but greatly increase in abundance in the dysbiotic state) are not well understood. The role of L. iners is particularly controversial [9,86,87]. L. iners is well adapted to the vaginal niche, is present in many different types of VMBs, and often persists after antibiotic treatment. This could mean that L. iners easily tolerates the presence of other bacteria (which in turn could lead to dysbiosis), or that it helps to restore a lactobacilli-dominated VMB during and after dysbiosis and/or antibiotic treatment. One appealing hypothesis regarding the development of dysbiosis is the formation of a vaginal biofilm [88]. Current evidence suggests that G. vaginalis can be present in the vagina as dispersed bacteria or as biofilm-associated (cohesive) bacteria, with the former associated with a lower total bacterial load than the latter [89]. In-vitro studies suggest that when the concentration of G. vaginalis increases, it starts to adhere to the vaginal epithelium, providing a scaffolding to which other species adhere [90]. Initial human biopsy studies focused on A. vaginae as another potentially important biofilm member [91], and one study found that L. iners increases G. vaginalis adherence in-vitro (although this did not reach statistical significance) [92]. However, more research is needed to properly evaluate the potential role of all relevant lactobacilli and dysbiosis-associated bacteria in biofilm formation. Furthermore, it is not yet entirely clear whether the dispersed and cohesive forms of G. vaginalis represent different G.
Other short-chain fatty acids as the end product of metabolism [87]. These changes likely result in the production of succinate and butyrate, which differentially expresses over 10% of its genome in dysbiotic conditions. Focused on L. iners, synergies and dependencies of the various bacterial communities have been well studied in that regard, and studies have indeed shown that the greater the density of colonization, the greater the probability of invasive disease in postpartum women and their neonates [98].

Whether bacterial dysbiosis is symptomatic or not most likely depends on the degree and nature of the dysbiosis, bacterial loads, and type and quantity of virulence factors expressed by bacteria, and the intensity and nature of the host’s immune responses [97]. ‘Thresholds’ (in terms of bacterial loads and diversity) might exist. While most of the above-mentioned dysbiosis-associated bacteria are never pathogenic in immune-competent hosts, streptococci, staphylococci, and E. coli can cause invasive disease when present in sufficiently high abundance. S. agalactiae has been particularly well studied in that regard, and studies have indeed shown that the greater the density of colonization, the greater the probability of invasive disease in postpartum women and their neonates [98].

Only three molecular studies included in our review reported VMB clusters dominated by streptococci, staphylococci, and/or E. coli [35,58,65] but many additional studies showed presence of these bacteria in low abundance; this is in agreement with studies using selective culture media [7]. In-vitro studies confirm that S. agalactiae only inhibits growth of other bacteria at concentrations higher than 10^5 colony forming units per ml (but does not inhibit S. aureus and E. coli), and such high concentrations are rarely seen in-vivo [98]. If aerobic vaginitis is defined as a VMB composition dominated by these bacteria, we conclude that it does exist but is not common. Future studies of invasive infections by streptococci, staphylococci, and E. coli should determine vaginal concentrations and not just vaginal presence.

We also do not yet sufficiently understand the metabolic synergies and dependencies of the various bacterial communities that are commonly found in the vagina. Recent studies have focused on L. iners, which is present in almost all women worldwide, in healthy and dysbiotic states. These studies suggest that L. iners is highly adapted to the vaginal compartment [9,86], but it differentially expresses over 10% of its genome in dysbiotic compared to healthy states, with increased expression of a cytolysin, mucin, glycerol transport and related metabolic enzymes [97]. These changes likely result in the production of succinate and other short-chain fatty acids as the end product of metabolism as opposed to lactic acid, leading to an increased vaginal pH. L. iners might also be the first Lactobacillus species to recover after dysbiosis [59], which suggests a bidirectional relationship between L. iners and vaginal pathogens or dysbiosis. Other studies have noted synergistic effects between G. vaginalis and Prevotella spp., perhaps due to metabolic dependencies [91,98]. At the moment, metagenomic studies of vaginal bacteria are ongoing but difficult to conduct because the public sequence databases do not yet contain all relevant bacterial genomes.

While molecular techniques have significantly improved our understanding of the VMB, some limitations should be noted. Molecular techniques detect viable as well as non-viable organisms, some cannot reliably differentiate species within a genus, some cannot adequately detect minority species, and most are not fully quantitative. We have taken this into account in our data interpretations as much as possible. Furthermore, even when the same molecular techniques were used, different laboratories used different operating procedures. Not all of these are important, but some (such as DNA extraction methods, amplification platform, choice of amplification target or of variable 16S region, choice or design of primers, and the presence or absence of proper negative controls to detect contamination) might result in significant inter-laboratory variation [14]. We were fully aware of these limitations and therefore focused this review on areas of consensus.

Now that the VMB of women with and without dysbiosis in different parts of the world have been well described, and molecular techniques have become more accessible and affordable, we believe that the time has come to incorporate these techniques into larger epidemiological studies with clinical outcomes. These studies should investigate the etiology and pathogenesis research gaps that were outlined above, but also provide transmission patterns of VMB bacteria, and the temporal relationships between the VMB and adverse reproductive health outcomes, such as HIV/STIs, pelvic inflammatory disease, adverse pregnancy outcomes, and invasive infections in pregnant/postpartum women and their neonates. At the moment, most treatment guidelines only advise clinicians to treat symptomatic vaginal dysbiosis, but this might have to be re-evaluated in specific at risk population groups (such as pregnant women or women highly exposed to HIV) if dysbiosis is identified as a strong risk factor for adverse outcomes in sufficiently powered longitudinal studies. In parallel, interventions that prevent dysbiosis, disrupt biofilms, and restore and maintain lactobacilli-dominated microbiota, should continue to be optimized and tested. The VMB studies discussed in this review have provided us with the tools to properly evaluate the safety and efficacy of such interventions. If safe, efficacious and affordable interventions are identified, they could potentially have a significant public health impact.

**Supporting Information**

**Checklist S1** PRISMA checklist.

**Diagram S1** PRISMA Flow-Diagram.

**Author Contributions**

Contributed to the writing of the manuscript: JvdW. Conceived the idea for this systematic review: JvdW. Selected the articles: JvdW HB. Extracted the data: JvdW HB VJ RV TC. Reviewed and commented on the manuscript: JvdW HB VJ RV TC SF HV.

**References**

1. Holmes KK, Sparling PF, Stamm WE, Piot P, Wasserheit JN, et al. (2008) Sexually Transmitted Diseases. 4th ed New York: McGraw-Hill Medical.
2. Ansel R, Totten PA, Spiegel CA, Chen KCS, Eschenbach D, et al. (1983) Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations. Am J Med 74: 14–22.
3. Nugent RP, Krohn MA, Hillier SL. (1991) Reliability of diagnosing bacterial vaginosis is improved by a standardized method of Gram stain interpretation. J Clin Microbiol 29: 297–301.
4. Donders GGG, Vereecken A, Boomsma E, Dekeresmaeker A, Salesbier G, et al. (2002) Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis: aerobic vaginitis. BJOG 109: 34–35.
30. Mitchell C, Moreira C, Fredricks D, Paul K, Caliendo AM, et al. (2009) Does bacterial vaginosis cause pelvic inflammatory disease? Sex Transm Dis 36: 117–122.

29. Shi Y, Chen L, Tong J, Xu C (2009) Preliminary characterization of vaginal bacteria. Sex Transm Infect 85: 242–248.

28. Biagi E, Vitali B, Pugliese C, Candela M, Donders GG, et al. (2009) Multiplex detection of bacteria associated with normal microbiota and with bacterial vaginosis. J Clin Microbiol 47: 4067–4077.

27. Haggerty CL, Totten PA, Ferris M, Martin DH, Hoffer M, et al. (2009) Comparison of the diversity of the vaginal microbiota in HIV-infected and HIV-uninfected women with or without bacterial vaginosis. J Infect Dis 199: 1110–1116.

26. Marrazzo JM, Thomas KK, Fiedler TL, Ringwood K, Fredricks DN (2008) Comparison of the diversity of the vaginal microbiota in HIV-infected and HIV-uninfected women with or without bacterial vaginosis. J Infect Dis 198: 1113–1140.

25. Marrazzo JM, Thomas KK, Fiedler TL, Ringwood K, Fredricks DN (2008) Identification of clinically relevant vaginal bacteria in relation to bacterial vaginosis. J Infect Dis 198: 1110–1116.

24. Yamamoto T, Zhou X, Williams CJ, Hochwalch A, Forney LJ (2009) Bacterial vaginosis and Mycoplasma hominis. J Infect Dis 191: 958–966.

23. Persson R, Holt J, Schellenberg J, Golecki J, Clemente C, et al. (2011) Vaginal microbiota of women with frequent vulvovaginal candidiasis. Infect Dis Obstet Gynecol 17: 4130–4135.

22. Verhelst R, Verstraeten H, Vaneechoutte M, Temmernag M (2011) Group A streptococcal vaginitis: an unrecognized cause of vaginal symptoms in adult women. Arch Gynecol Obstet 284: 93–96.

21. Marrazzo JM, Thomas KK, Fiedler TL, Ringwood K, Fredricks DN (2008) Comparison of the diversity of the vaginal microbiota in HIV-infected and HIV-uninfected women with or without bacterial vaginosis. J Infect Dis 198: 1110–1116.

20. Venkatesh PR, McGee L, Schrag SJ (2010) Prevention of perinatal group B streptococcal disease-revised guidelines from CDC. 2010. MMWR Recomm Rep 59: 1–36.

19. Taylor BD, Darville T, Haggerty CL (2013) Does bacterial vaginosis cause pelvic inflammatory disease? Sex Transm Dis 40: 203–210.

18. Jakobsson T, Forsum U (2008) Changes in the predominant human lactobacillus species in healthy postmenopausal women. J Med Microbiol 57: 79–86.

17. Moher D, Liberati A, Tetzlaff J, Altman DG, and the PRISMA Group (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 6: e1000097. doi:10.1371/journal.pmed.1000097.

16. Fredricks DN, Fiedler TL, Marrazzo JM (2005) Molecular identification of vaginal microbiota in East African commercial sex workers. Appl Environ Microbiol 71: 428–431.

15. Jakobsson T, Forsum U (2008) Changes in the predominant human lactobacillus species in healthy postmenopausal women. J Med Microbiol 57: 79–86.

14. Srinivasan S, Fredricks DN (2008) The human vaginal bacterial biota and bacterial vaginosis. Interdiscip Perspect Infect Dis 2008: 750479. doi:10.1155/2008/750479.

13. Burton JP, Reid G (2002) Evaluation of the bacterial vaginal flora of 20 pregnant women by direct (Nugent score) and molecular (polymerase chain reaction and denaturing gradient gel electrophoresis) techniques. J Infect Dis 186: 1770–1780.

12. Hayes R, Watson-Jones D, Celum C, van de Wijgert J, Wasserheit J (2010) Temporal variability of human vaginal bacteria and relationship with bacterial vaginosis. PLoS One 5: e10197. doi:10.1371/journal.pone.0010197.

11. Zozaya-Hinchliffe M, Lillis R, Martin DH, Ferris MJ (2010) Quantitative PCR assessment of bacterial species in women with and without bacterial vaginosis. J Clin Microbiol 48: 1012–1019.

10. Srinivasan S, Liu C, Mitchell CM, Fiedler TL, Thomas KK, et al. (2010) Temporal variability of human vaginal bacteria and relationship with bacterial vaginosis. PLoS One 5: e10197. doi:10.1371/journal.pone.0010197.

9. Shi Y, Chen L, Tong J, Xu C (2009) Preliminary characterization of vaginal bacteria. Sex Transm Infect 85: 242–248.

8. Biagi E, Vitali B, Pugliese C, Candela M, Donders GG, et al. (2009) Multiplex detection of bacteria associated with normal microbiota and with bacterial vaginosis. J Clin Microbiol 47: 4067–4077.

7. Verani JR, McGee L, Schrag SJ (2010) Prevention of perinatal group B streptococcal disease-revised guidelines from CDC. 2010. MMWR Recomm Rep 59: 1–36.

6. Yamamoto T, Zhou X, Williams CJ, Hochwalch A, Forney LJ (2009) Bacterial vaginosis and Mycoplasma hominis. J Infect Dis 191: 958–966.

5. Sobel JD, Reichman O, Misra D, Yoo W (2011) Prognosis and treatment of bacterial vaginosis. Interdiscip Perspect Infect Dis 2008: 750479. doi:10.1155/2008/750479.

4. Persson R, Holt J, Schellenberg J, Golecki J, Clemente C, et al. (2011) Vaginal microbiota of women with frequent vulvovaginal candidiasis. Infect Dis Obstet Gynecol 17: 4130–4135.

3. Persson R, Holt J, Schellenberg J, Golecki J, Clemente C, et al. (2011) Vaginal microbiota of women with frequent vulvovaginal candidiasis. Infect Dis Obstet Gynecol 17: 4130–4135.

2. Verhelst R, Verstraeten H, Vaneechoutte M, Temmernag M (2011) Group A streptococcal vaginitis: an unrecognized cause of vaginal symptoms in adult women. Arch Gynecol Obstet 284: 93–96.
62. Jespers V, Menten J, Smet H, Poradosu S, Abdellati S, et al. (2012) Characterisation of the oral, vaginal and rectal Lactobacillus flora in healthy, pregnant and postmenopausal women. Eur J Obstet Gynecol Reprod Health 160: 93–99.

63. Smith BC, McAndrew T, Chen Z, Harari A, Barris DM, et al. (2012) The cervical microbiome over 7 years and a comparison of methodologies for its characterisation. PLoS One 7: e40425. doi:10.1371/journal.pone.0040425.

64. Zhang R, Daroczy K, Xiao B, Yu L, Chen R, et al. (2012) Extravaginal reservoirs of vaginal bacteria as risk factors for incident bacterial vaginosis. J Infect Dis 205: 1580–1588.

65. Aagaard K, Riehle K, Ma J, Segata N, Mistretta TA, et al. (2012) A temporal dynamics of the vaginal microbiota. Sci Transl Med 4: 132ra52. doi:10.1126/scitranslmed.3003605.

66. Mitchell CM, Fredricks DN, Winer RL, Koutsky L (2012) Effect of sexual debut on vaginal microbiota with human papillomavirus infection in a Korean twin cohort. PLoS One 7: e37818. doi:10.1371/journal.pone.0037818.

67. Brotman RM, Bradford LL, Conrad M, Gajer P, Ault K, et al. (2012) Bacterial communities over time. BJOG 120: 695–704.

68. Martin DH, Zozaya M, Lillis R, Miller J, Ferris MJ (2012) The microbiota of the cervical microbiome during infertility therapy with in vitro fertilization-embryo transfer. J Assist Reprod Genet 29: 105–115.

69. Yeoman CJ, Yildirim S, Thomas SM, Durkin AS, Torralba M, et al. (2010) Reciprocal interference between Lactobacillus spp. and Gardnerella vaginalis on initial adherence to epithelial cells. Int J Med Sci 10: 1193–1198.

70. Macklaim JM, Gloor GB, Anukam KC, Cribby S, Reid G (2011). At the molecular level: characterisation of vaginal lactobacilli species isolated from vaginal secretions of healthy and bacterial vaginosis-associated women. BMC Genomics 11: 375. doi:10.1186/1471-2164-11-375.

71. Verstraelen H, Swidsinski A (2013) The biofilm in bacterial vaginosis: implications for epidemiology, diagnosis and treatment. Curr Op Infect Dis 26: 86–89.

72. Verstraelen H, Swidsinski A, Doerrfël Y, Loening-Baucke V, Swidsinski S, Verstraelen H, et al. (2010) Gardnerella biofilm involves females and males and is transmitted sexually. Gynecol Obset Invest 70: 256–263.

73. Patterson JL, Snell-Lane A, Gireid PH, Jefferson KK (2010) Analysis of adherence, biofilm formation and cytotoxicity suggests a greater virulence potential of Gardnerella vaginalis relative to other bacterial vaginosis-associated anaerobes. Microbiology 156 (Pt 2): 392–399.

74. Verstraelen H, Mendingh W, Loening-Baucke V, Swidsinski S, Verstraelen H, et al. (2000) Adherent biofilms in bacterial vaginosis. Obstet Gynecol 106(1 Pt 1): 1013–1023.

75. Castro J, Henriquez A, Machado A, Henriquez M, Jefferson KK, et al. (2013) Experimental evidence for a interaction between Gardnerella vaginosis and the gut microbiota in the development of glandular breast carcinomas. Cancer Epidemiol 37: 948–953.

76. Verstraelen H, Swidsinski A (2009) Gardnerella biofilm formation in the mammalian host. Microbiology 155 (Pt 1): 189–197.