Infektiologie und Mikrobiologie (Schwerpunkt Bakteriologie)/ Infectiology and Microbiology (Focus Bacteriology)

Redaktion: P. Ahmad-Nejad/ B. Ghebremedhin

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Normal and abnormal vaginal microbiota

Normale und abnormale vaginale Mikrobiota

DOI 10.1515/labmed-2016-0011
Received January 31, 2016; accepted July 11, 2016; previously published online August 5, 2016

Abstract: The normal and abnormal vaginal microbiome are an ecosystem of up to 200 species influenced by genetic, ethnic, environmental and behavioral factors. Cultural methods release only a small clinically unimportant spectrum. Lactobacilli are the most dominant and maintain a pH value between 3.8 and 4.5. They support a defense system against dysbiosis and infections to care for a healthy outer and inner genital tract, a balanced restitution after intercourse and normal pregnancy and childbirth. Bacterial vaginosis (BV) is the most frequent dysbiosis with a lack of lactobacilli and an overgrowth of anaerobic bacteria. Special *Gardnerella vaginalis* strains work together with *Atopobium vaginae*, *Clostridiales* and others, but also *Lactobacillus iners* in a vaginal polymicrobial biofilm, which is sexually transmitted and cannot be destroyed by the recommended antibiotics.

Keywords: bacterial vaginosis; lactobacilli; microbiota; probiotics; vagina.

Zusammenfassung: Das normale und das abnormale vaginale Mikrobiom sind ein Ökosystem, das von Genen, Ethnie, Umwelt- und Verhaltensfaktoren bestimmt wird. Es wurden über 200 Bakterienarten in der gesunden Scheide identifiziert. Kulturelle Bestimmungsmethoden bilden nur ein klinisch unbedeutendes Spektrum ab. Laktobazillen dominieren meist bei der geschlechtsreifen Frau und sorgen für einen normalen pH – Wert von 3,8 bis 4,5. Sie unterstützen ein Abwehrsystem gegen Dysbiosen und Infektionen, um für einen gesunden äusseren und inneren Genitaltrakt zu sorgen, für eine balancierte Restitution nach Geschlechtsverkehr und für eine gesunde Schwangerschaft und zeitgerechte Geburt. Die bakterielle Vaginose ist die häufigste Dysbiose mit einem Verlust von Laktobazillen und einem starken Anstieg der Zahl anaerober Gram-negativer und Gram-positiver Bakterien. Spezielle Stämme von *Gardnerella vaginalis*, *Atopobium vaginae*, *Clostridiales* und andere, aber auch *Lactobacillus iners* arbeiten bei der Entstehung vaginaler, typischer polymikrobieller Biofilme mit, die sexuell übertragbar sind und nicht von den leitliniengerechten Antibiotika aufgelöst werden.

Schlüsselwörter: bakterielle Vaginose; Laktobazillen; Mikrobiota; Probiotika; Vagina.

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History

Albert Döderlein (1860–1941) (Leipzig, Germany) (for all historical references under "History" refer to [1]) was the first to describe the importance of bacteria in the vagina of women, which produce lactic acid and are responsible for the inhibition of other, facultatively anaerobic, pathogenic bacteria (Döderlein 1892). Krönig (1895), a co-worker of Döderlein's, was the first to differentiate lactobacilli from anaerobic bacteria and to illustrate the curved rods. The lactobacilli were cultured by Curtis (1913) and were later named *Mobiluncus curtisii* (Spiegel and Roberts 1984). The name *Lactobacillus acidophilus* was subsequently proposed by Stanley Thomas (1928) (Bethlehem, PA, USA). Lauer, Helming and Kandler (1980) (Germany) characterized different *Lactobacillus* species by DNA-hybridization, which were previously identified by culture or biochemically as *Lactobacillus acidophilus*. Gynecologists still use the term "Döderlein-bacteria" today.

Manu af Heurlin (1914) [Helsingfors (today called Helsinki), Finland] was the first European to characterize the vaginal flora of pregnant and not pregnant women, from girls to elderly women and to attempt to classify a grading system ranging from healthy to disturbed flora. Robert Schröder (1921) (Rostock, Germany) distinguished three bacteriologically different vaginal flora types which are still used today by some German gynecologists to determine the difference between normal flora, bacterial

vaginosis (BV) and other flora. Later, Ludwig Nürnberger (1930) (Halle, Germany) summerized the infectiological knowledge for the gynecologists of his time in 463 pages and discussed Schröder's "Reinheitsgrade" (grades of vaginal cleanliness). He concurred with Döderlein's opinion to differ only two flora types: normal and abnormal flora – from the modern point of view very visionistic!

Otto Jirovec (Jirovec, Peter and Malek 1948) (Prague, Czechoslovakia) classified six different vaginal flora types (normal, abnormal, abnormal with many leucocytes, gonorrhea, trichomoniasis and candidosis).

Novel research was triggered by the description of "*Haemophilus vaginalis*" by Herman Gardner and Charles Dukes (Houston, TX, USA) (Gardner and Dukes, 1955), today known as *Gardnerella* (*G.*) *vaginalis* (Greenwood and Pickett, 1980). Gardner and Dukes pointed to the importance of the microscopy of vaginal fluids, defined as diagnostic criteria "clue cells" and were sure, that *G. vaginalis* and this disease, today known as bacterial vaginosis (BV), is a sexually transmitted infection.

Per-Anders Mardh (Lund, Sweden) and his colleagues, nearly 30 years later in a symposium in Stockholm, summarized: "BV is a replacement of lactobacilli by characteristic groups of bacteria, accompanied by changed properties of the vaginal fluid" (Mardh et al. 1984). This definition is still used today.

In recent year an explosion of new knowledge has arisen from using new molecular and genetic technologies to determine bacteria, which has led to the characterization of new, formerly unknown bacteria, including lactobacilli, the interactions of bacteria and of bacterial biofilms, and genetically determined differences of flora types in the vagina.

The normal vaginal microbiota

Vaginal lactobacilli

Both the exterior skin and some interior surfaces of a vaginally-born newborn are primarily colonized by the vaginal flora of the mother. After birth, exposed surfaces are also colonized by the skin and mouth flora of the mother and by lactobacilli in the mother's breast milk, especially *Lactobacillus* (L.) *gasseri* [2]. In young, premenarchal girls and postmenopausal women without hormone replacement therapy, a lack of estrogen (except in the first 2–4 weeks of life due to placental hormones) causes the vaginal flora to mainly consist of skin and gut flora, although the vagina can also harbor some lactobacilli [3].

During the reproductive phase of a women's life, estrogen and progesterone change the environmental conditions to promote the growth of lactobacilli. Estrogens promote the proliferation of the vaginal epithelium and the production of intraepithelial glycogen. Progesterone promotes the cytolysis of vaginal epithelial cells, releasing glycogen. The glycogen is metabolized by lactobacilli and other bacteria to form glucose and maltose and then metabolized further to lactic acid. It is currently thought that the production of lactic acid, together with other factors, contributes to maintaining a normal vaginal pH of 3.8–4.4. At least 120 different *L. actobacillus* species have been identified [4]. Commonly, more than 10 bacterial species can be found in a woman's vagina, although one or two species is usually dominant, frequently *L. crispatus, L. gasseri, L. jensenii* or *L. iners*. Many lactobacilli also produce hydrogen peroxide (H_2O_2), and it is thought that a low number of H_2O_2-producing-lactobacilli in the vagina promote the development of BV. Vaginal lactobacilli with physiological functions can also produce bacteriocins, which synergize with lactic acid and H_2O_2 to inhibit the growth of facultatively anaerobic, pathogenic bacteria. Bacteriocins, biosurfactants and coaggregating molecules inhibit the adhesion of such bacteria to the vaginal wall.

Lactobacilli are sensitive to β-lactam antibiotics, but less sensitive to doxycycline or metronidazole. Clindamycin, however, inhibits lactobacilli.

The normal vaginal flora – a delicate balance of a mixture of different bacteria

The bacterial diversity

Today, over 250 species of bacteria have been identified in the vagina using genomic sequencing. Examples include (in alphabetical order) *Actinomyces, Aerococcus, Allisonella, Alloscardovia, Anaerococcus, Arcanobacterium, Atopobium, Bacteroides, Balneimonas, Bifidobacterium, Blastococcus, Blautia, Bulleidia, Campylobacter, Citrobacter, Coriobacteriacea, Corynebacterium, Enterobacter, Escherichia, Facklamia, Faecalibacterium, Finegoldia, Gardnerella, Gemella, Haemophilus, Lachnospiracea, Massilia, Megasphera, Mobiluncus, Mollicutes, Moryella, Olsinella, Parvimonas, Peptinophilus, Peptostreptococcus, Prevotella, Porphyromonas, Proteobacteria, Providencia, Rhizobialis, Ruminococcaceae, Salmonella, Shigella, Shuttleworthia, Sneathia, Solobacterium, Staphylococcus, Streptococcus, Veillonella, Ureaplasma*, and many lactobacilli species [5]. A healthy vaginal flora is usually

dominated by lactobacilli, which maintains a pH of <4.5. However, this composition can change.

The composition of vaginal flora, and therefore increased susceptibility to BV, is independently influenced by sexual activity, receptive anal before vaginal intercourse, cigarette smoking, sex with an uncircumcised male partner, lack of vaginal H_2O_2-producing lactobacilli, the detection of herpes simplex virus (HSV) type 2 serum antibodies and Black ethnicity [6]. Culture-independent methods, for instance, 16S rRNA gene identification and polymerase chain reaction (PCR) amplification methods have revealed very different results compared to culture methods [7]. It was once thought that lactobacilli were necessary for a healthy vagina and hence the development of diagnostic tools, such as scoring according to Nugent criteria (zero points for the presence of many lactobacilli). However, this is no longer the prevailing view, instead being replaced with the notion of several types of a vaginal microbial community [8].

The Human Microbiome Project, operating since 2008, was a 5-year effort to determine the microorganisms in the human body using next-generation sequencing. Within that project, the Vaginal Microbiome Project, performed by the Virginia Commonwealth University examined thousands of women to determine the relationship between the vaginal microbiome and various physiological and infectious conditions [3]. Different "vagitypes" have been identified many of which are dominated by a single bacterial taxon and others by a broad spectrum of different bacteria.

"Vagitypes", ethnic influences

Previous research has suggested a link between ethnic background and the vaginal flora. Ravel et al. [7] revealed that there was a significantly different vaginal *Lactobacillus* flora, and subsequently different pH-values, in 396 North American women between 12 and 45 years old with different ethnic backgrounds.

White/Caucasian women's flora were dominated by *L. iners* (45% relative quantity) with a mean pH-value of 4.2±0.3. Forty-two percent of Asian women's floras were dominated by *L. crispatus* (42.7%) with a mean pH-value of 4.4±0.59. The vaginal flora of Black women were dominated by *L. jensenii* (40.4%) with a mean pH-value of 4.7±1.04. The most common bacteria in the flora of Hispanic women were *L. jensenii* (38.1%) with a mean pH-value of 5.0±0.59. Caucasian and Asian women's flora were dominated by one *Lactobacillus* species in 80.2% and 89.7% of cases, respectively. Whereas, in Black and Hispanic women's flora, only 61.9% and 59.6% of cases, respectively, were dominated by one species. Interestingly, a significant group of 108 women harbored no lactobacilli at all in the vagina (named the "diversity group") and such was the case for 38.9% of Black and 34.3% of Hispanic women. The Nugent scores were between 7 and 10 (indicating BV) mostly in the diversity- and the *L. iners*-groups, but not in the group dominated by *L. crispatus*. All women reported that they felt healthy. This study raises the question as to what is the normal composition of vaginal flora.

In addition to Ravel's findings, Jespers et al. [9] (Antwerp, Belgium) identified three types of vaginal flora in healthy, premenopausal women and in women in an STD clinic at risk of acquiring BV.

One group of women's flora was dominated by *L. crispatus, L. iners, L. jensenii* and *L. vaginalis*.

A second group's flora preferentially harbored *L. gasseri* and *L. vaginalis*. The third group's flora was dominated by *L. gasseri, A. vaginae* and *L. iners* and most women had African or Asian ethnicity.

The most frequently occurring lactobacilli of healthy women in Vienna in the late first trimester of pregnancy were *L. crispatus, L. gasseri, L. jensenii* and *L. rhamnosus* [10].

Vaginal samples from 494 asymptomatic, reproductive-age, Estonian women indicated five major bacterial community groups, each with a distinct diversity and species composition (Figure 1) [11]. It was found that the species diversity increased with higher vaginal pH-values and with BV (symptomatic or asymptomatic) This study also showed a much higher diversity and number of 196 fungal operational taxonomic units (OTUs), including the identification of 16 OTUs of *Candida* species (in 50% of all women; mean 7–8 per sample; mean relative abundance of *Candida albicans* 36.9% (Figure 2).

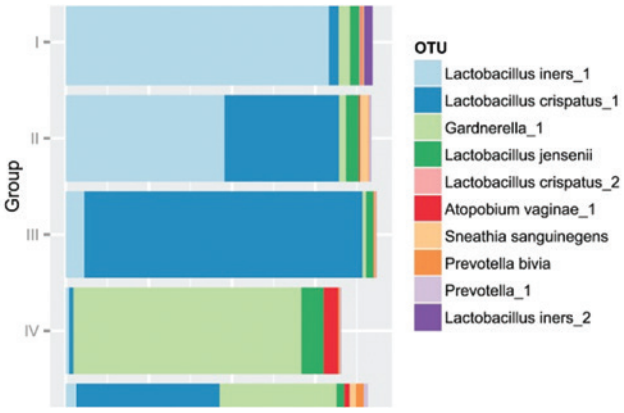

Figure 1: Distribution of 10 most relatively abundant operational taxonomic units (OTU) in determined bacterial community groups [11].

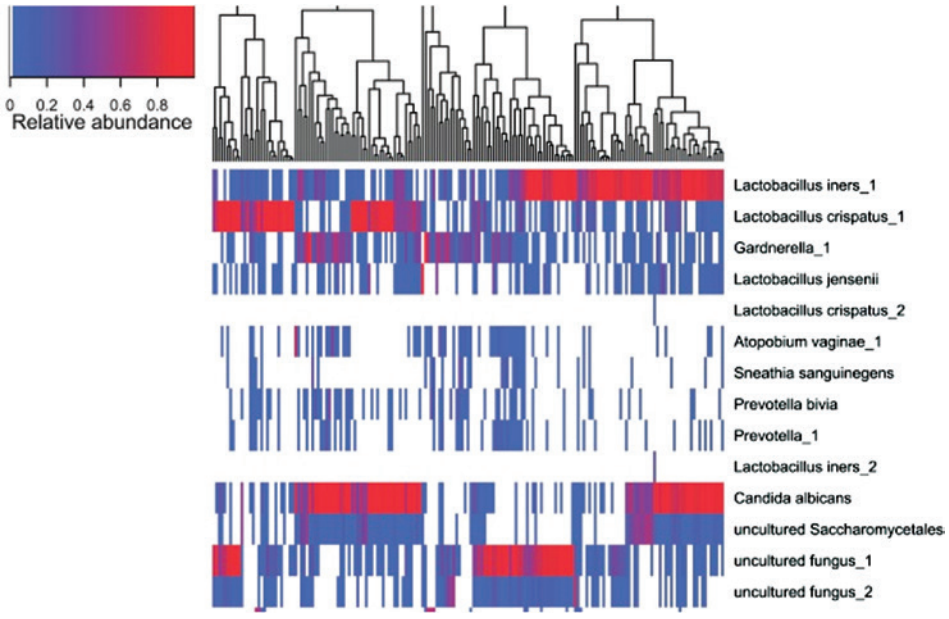

Figure 2: Relative abundance of the most abundant bacterial and fungal operational taxonomic units (OTUs) found in the vaginal communities of 181 women [11].

Individual temporal dynamics

The community types underlie the dynamic variations during the menstrual cycle and are influenced by external circumstances, for instance, sexual behavior. However, the flora compositions are thought to remain in a rather stable balance and it is clear that a healthy vaginal system can correct disturbances caused by external factors. This was demonstrated by Gajer et al. [12] in a longitudinal study of individual women, who were monitored for 16 weeks with weekly vaginal swabs for bacterial identification by 16S rRNA determination and PCR. According to Ravel's study, the women had markedly different vaginal "community state types". During the study, the women documented daily their menstruation, tampon use, vaginal, anal or oral sex, sex toys, digital penetration and lubricants. The vaginal flora of some women was heavily disturbed by some of these interactions, but others were not disturbed in spite of very frequent vaginal manipulations. Black women were once again significantly different in their "community state types".

Several recent studies using molecular methods have identified vaginal flora of mixed species, which were previously unidentified using cultural methods, for example, BV-associated bacteria (BVAB) 1, 2 and 3. Nearly all of these bacteria are unknown in clinical practice, because culture techniques are biased towards bacteria with undemanding nutritional needs, like *Escherichia coli*.

Fredricks et al. [13] observed similar results in women with normal and with abnormal flora. It has become clear that *L. iners,* which belongs to the normal vaginal flora acts as a "poisoned apple in the basket" because the presence of *L. iners* is strongly connected to a shift from normal to abnormal flora. All studies to date have demonstrated that *L. crispatus* stabilizes the normal flora and that *L. iners*, and also *L. gasseri*, predispose to flora instability and susceptibility to BV [14–17].

Vaginal-oral-anal similarities

The vaginal flora is also influenced by the anal and the oral flora. Petricevic et al. [17] have found that in around 80% of 30 pregnant women and in 40% of 30 postmenopausal women, there were one or more *Lactobacillus* ssp. in the vagina and in the rectum, and they were in 80% resp. 40% of the same identity. In these groups of women, 50% of pregnant and 30% of postmenopausal women contained one or more *Lactobacillus* ssp. in their mouth. Women who harbored *L. crispatus* in at least two of the three locations were significantly less likely to develop BV than others (p=0.02). Women who harbored BV-associated bacteria, especially *G. vaginalis* and *Leptotrichia/Snethia* or *Megasphera,* in higher concentrations in the mouth or anus, were significantly more likely to develop BV (p=0.001) [16]. On the other hand, although there are similarities between the bacterial species in the vaginal

and rectal flora in women with normal and with abnormal flora, the polymicrobial biofilms associated with BV (see Section "Polymicrobial bacterial biofilms in BV and sexual transmission") can only be found in the vagina and are not present in the anal region [18].

The morphology of *Atopobium vaginae* can vary greatly, from elliptic cocci to curved rods, which can make clinical diagnoses of normal flora or BV, using smell or microscopy, problematic [19]. Once again, the underlying question remains "what is normal vaginal flora?".

Morbidity by too less or too many lactobacilli

A healthy, balanced vaginal microbiota protects not only against ascending infections or HIV acquisition, but also against premature birth [20–23]. The most important factor for morbidity is the lack of lactobacilli.

On the other hand, an overgrowth of vaginal lactobacilli or abnormally long lactobacilli can cause vestibular pruritus, itching or dysuria, which are more pronounced in the luteal phase. Known as "cytolytic vaginosis" or "lactobacillosis", this condition is sometimes misdiagnosed clinically as candidosis. The diagnostic criteria for microscopic examination are an increased number of lactobacilli, very long lactobacilli (60 µm long compared to the normal 10 µm), cytolysis, normal numbers of leucocytes, normal pH, the absence of *Candida*, *Trichomonas* or BV in discharge and the presence of associated symptoms. Cytolytic vaginosis has been diagnosed in 1.83% of 2947 Papanicolaou-stained vaginal smears [24].

Gene polymorphisms and vaginal immunity

The vaginal flora is not only influenced by ethnic background, but also by gene polymorphisms. The capacity of the individual to produce differing levels of anti- or promicrobial factors influences the composition of the vaginal flora. Polymorphisms in the interleukin-1 receptor antagonist gene or the Toll-like receptor4 (*TLR4*) gene, both of which are involved in the innate recognition of Gram-negative bacteria, influence the quantities of vaginal bacteria and can influence the individual's susceptibility to pregnancy complications [25]. Polymorphisms such as these vary between different racial groups and may be linked to environmental differences between populations [19]. BV, and also periodontal disease, are influenced by gene polymorphisms and both conditions are associated with preterm birth [26].

The innate immune system which acts to protect the vagina consists of soluble factors such as mannose-binding lectin (MBL), defensins, secretory leucocyte protease inhibitor, nitric oxide, in addition to membrane-associated factors, TLRs (11 TLRs have been identified) and phagocytes. Different TLRs recognize specific lipoproteins and peptidoglycans present on the surface of Gram-positive bacteria, the lipopolysaccharide of Gram-negative bacteria, flagellins, and other bacterial components [19, 27]. Vaginal production of specialized defensins is stimulated by estrogens and inhibited by progesterone. Bacterial vaginosis in pregnant women has been associated with lower vaginal concentrations of defensin 3 [27].

TLR ligands and fatty acids, which are produced by a large proportion of vaginal bacteria, have dramatic effects on the vaginal immune function. The anaerobic bacteria associated with BV produce bad smelling amines (putrescine, cadaverine and others), succinate, sialidases, and immunomodulatory substances such as lipopolysaccharides, lipoteichoic acids and peptidoglycans. These substances influence cytokines and other immune responses [26].

Bacterial vaginosis (BV)

Gardner and Dukes [1], in 1955, termed the vaginal disorder "*Haemophilus vaginalis* vaginitis", which was later known as "bacterial vaginosis". BV is characterized by "the replacement of lactobacilli by characteristic groups of bacteria accompanied by changed properties of the vaginal fluid" [1]. The change in definition occurred because it became clear that the cause of the dysbiosis was not only *G. vaginalis*, but other bacteria too. The diagnostic criteria for BV are summarized by Amsel et al. [28]: gray-white, milky discharge, pH >4.5, a bad "fishy" smell, especially if 10% KOH solution is added, and at least 20% of the sample contains "clue cells". Around 20% of pregnant women in Germany have BV; however, not all are symptomatic [22]. In order to increase the reproducibility of diagnoses using wet mounts, Nugent et al. [29] proposed a scoring system using Gram-staining criteria. The Nugent score involves counting the numbers of lactobacilli, Gram-negative rods and *Mobiluncus* species and a Nugent score of 0–3 indicates normal health, 4–6 indicates intermediate health and 7–10 indicates BV. The factors considered essential for the development of BV were a lack of H_2O_2-producing lactobacilli, an overgrowth of *G. vaginalis*, anaerobic Gram-negative rods and anaerobic Gram-positive cocci. Due to the nature of culture-based methods, the predominating bacterial genera grown are *Gardnerella*, *Prevotella*, *Porphyromonas*, *Bacteroides*,

Mobiluncus, Mycoplasma, Ureaplasma and *Peptostreptococcus*. These bacteria often exist in low numbers (about 10^2–10^5/mL) in the healthy vagina, but grow in much larger numbers (10^6–10^8/mL) when grown in culture.

Culture-independent methods reveal a much more diverse flora in BV [3, 5, 9–11, 23, 30]. *Clostridiales*, such as bacterial vaginosis-associated bacteria (BVAB) 1, –2 and –3, *A. vaginae, Megasphera, Leptotrichia, Dialister, Eggerthella, Peptinophilus lacrimalis* and other anaerobic, Gram-negative bacteria, and also *L. iners* appear to play a much greater role in the etiology of BV than *Gardnerella, Mobiluncus* or *Mycoplasma* [16]. There are four different *G. vaginalis* strains, although only two strains produce the BV marker sialidase and only one strain is found in large numbers in women with BV [31]. Therefore, the existence of *G. vaginalis* strains in the vagina does not equal a predisposition to BV.

BV is influenced by environmental and genetic factors. The effects of the flora in the vagina, the mouth and the rectum have already been discussed. Sexual practices, especially receptive oral sex and digital vaginal penetration are significant risk factors for BV (which is perhaps an explanation for a higher risk of acquiring BV in lesbian women) [16]. Other risk factors include cigarette smoking, Black race and receptive anal sex before vaginal intercourse [12]. It should be noted that when Gardner and Dukes [1] transferred cultivated *G. vaginalis* from a woman with BV to a healthy woman, this did not result in the spread of BV. However, if they transferred the discharge from a woman with BV to a healthy woman's vagina, then this resulted in the healthy woman acquiring BV. Therefore, it is unlikely to be a high abundance of a single species of bacterium that causes BV, but a critical mixture of BVAB and specialized lactobacilli combined with a decrease in other lactobacilli that together cause BV. It is thought that *L. iners* and *A. vaginae* also play an underestimated role in the development of BV [21].

Decreased estrogen levels affect the number and diversity of vaginal lactobacilli and are in some women a risk factor for urogenital infections.

Gene polymorphisms influence the levels of *G. vaginalis* and *A. vaginae* in the vagina [13].

There are at least six types of BV, each possessing different bacterial communities. Two types of BV have been investigated which do not contain *G. vaginalis*.

Polymicrobial bacterial biofilms in BV and sexual transmission

For many years, the nature and properties of bacterial biofilms have been well studied and understood by biologists, however these were unfamiliar in medicine until around the year 2000. In gynecology, the first bacterial biofilm to be described was in women with BV [32, 33]. The epithelial cells of the vagina in healthy premenarchal or postmenopausal women or girls are free from bacteria. However, BV is characterized by a structured polymicrobial biofilm which adheres to the epithelial cells of the vagina. "Clue cells", which Gardner and Dukes had seen microscopically when examining vaginal epithelial cells in discharge, originate from the biofilm coating the vaginal wall. The biofilm mainly consists of *G. vaginalis* (>50% up to 90%) and *A. vaginae* (10%–40%), but also contains lactobacilli and other bacteria [32, 34, 35]. It is not clear whether the lactobacilli found in the biofilm are *L. iners* or another species. If the patient is treated with metronidazole according to the guidelines, the bacterial biofilm is not disrupted. This is thought to be the reason for the high rates of recurrence of BV, approximately 30% recurrence 3 months' post treatment or 60% 6 months' post treatment [36]. A typical BV biofilm can also be found in the epithelial cells in the urine of females with BV and additionally, in the urine of their partners. It can sometimes be found on cryopreserved (washed) donor semen and is commonly found in the endometrium of non-pregnant women, in the tissue of a miscarriage or abortion, and even in the fallopian tubes of women with BV [36]. Post-void residual urine was collected from men after having pulled back the preputium and no biofilm-coated cells were found. This corroborated with other investigators who proposed that male circumcision reduces the risk of acquisition of ulcerations, trichomoniasis and BV [37]. Circumcision is associated with a significant change in the microbiota and with a significant decrease in putative anaerobic bacteria, especially *Clostridiales* and Prevotellaceae [38].

Women who have been treated for BV have a higher risk for recurrence if they have intercourse with the same partner without using condoms [16, 39]. The formation of a biofilm in BV is clearly dependent upon the combination of a mixture of bacteria. In vitro, *G. vaginalis* was found to form significantly stronger biofilms with the addition of *Fusobacterium nucleatum* or *Prevotella bivia* [40].

In summary, *Gardnerella* is not the only contributor to the bacterial biofilm, but is a component of every biofilm formed in BV patients. No single bacterial species, nor a surplus of different bacteria, are the cause of the BV biofilm, but instead a delicate mixture of co-operating bacteria determines the health of the vagina [16, 39]. The recurrence of BV after treatment can be reduced by vaginal or oral application of lactobacilli or probiotics and is especially effective in Caucasian women [41].

Diagnostic considerations

The wet mount preparation of vaginal discharge (examined using 400-fold phase contrast microscopy) and measurement of the pH-value are the recommended methods in gynecological practice to distinguish between normal and abnormal flora [1, 23, 42, 43].

It is not critical to know if *E. coli, G. vaginalis, Mycoplasma* or *Ureaplasma* species (except *M. genitalium*) are present in flora, because, unassisted, these species are not known to cause a genital infection. Using Gram-staining and the Nugent score is the standard for most scientific studies (although this technique is becoming more and more inadequate).

Nenadic et al. [44] recently proposed that the analysis of Gram-stained vaginal samples using a 200-fold magnification was a faster and simpler method, and that it better reflected the complexity of the vaginal flora compared to Nugent's 1000-fold method. Bacteriological cultures or the PAP test lack clinical value (except for *Streptococcus agalactiae* infections during pregnancy/delivery, infections from *Streptococcus pyogenes, Neisseria gonorrhoeae or Chlamydia trachomatis,* or in other special cases).

A DNA hybridization test can be used to detect the presence of *Gardnerella vaginalis* (Affirm VP III®, Becton Dickinson) and OSOM BV Blue can be used to detect the sialidase activity of vaginal fluid (OSOM BV Blue Test, Sekisui Diagnostics, Lexington, USA). Both methods have accepted value compared to the Gram stain. DNA-based methods for the detection of BV-associated bacteria are not yet recommended by the Centers for Disease Control (CDC) [43]. However, it has been found that a PCR-based assay used for nine bacteria commonly associated with a healthy or diseased vagina (*L. crispatus, L. gasseri, L. iners, L. jensenii, G. vaginalis, A. vaginae*, BVAB 2 and *Megaspheraphylotype* 1 or 2) is highly accurate and useful in assisting the diagnosis of BV [45]. Moreover, increased inflammatory cervicovaginal cytokine markers can predict asymptomatic genital inflammation due to BV and therefore, increased risk of sexually transmitted infections HIV infection in women [46].

Acknowledgments: The author thanks Mrs. Georia Goodhew (University of Bath) for excellent support in translating the English.
Author contributions: The author has accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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