Enferm Infecc Microbiol Clin. 2019;37(9):592–601

Enfermedades Infecciosas y Microbiología Clínica

www.elsevier.es/eimc

CONTINUING MEDICAL EDUCATION: SEXUALLY TRANSMITTED INFECTIONS

Vaginosis. Vaginal microbiota☆

Fernando Vazquez[a,b,c,d,e,*], Ana Fernández-Blázquez[a], Beatriz García[b,c,d]

[a] Servicio de Microbiología, Hospital Universitario Central de Asturias, Oviedo, Spain
[b] Departamento de Biología Funcional, Universidad de Oviedo, Oviedo, Spain
[c] Fundación de Investigación Oftalmológica, Instituto Oftalmológico Fernández-Vega, Oviedo, Spain
[d] Fundación para la Investigación y la Innovación Biosanitaria del Principado de Asturias (FINBA), Oviedo, Spain
[e] Grupo GEITS de la SEIMC

ARTICLE INFO

Article history:
Received 1 November 2018
Accepted 18 November 2018
Available online 15 June 2019

Keywords:
Aerobic vaginitis
Bacterial vaginosis
Vaginal microbiota

ABSTRACT

The latest advances in the vaginal microbiome and molecular diagnosis of bacterial vaginosis have allowed for a better knowledge of this entity, characterising aspects of its pathogenesis and the establishment of the vaginal biolayer, the models and new theories of its aetiology, how it is transmitted, with it being considered nowadays as a probable sexually transmitted infection, the separation of other entities such as aerobic vaginosis, its molecular diagnosis and treatment with new molecules to prevent frequent relapses. This entity and the study of the vaginal microbiome have made it possible to consider these infections as a polymicrobial syndrome, putting an end to the dogma: one microorganism, one disease. In addition, a lesser-known entity such as aerobic vaginosis and the methods for its detection are updated.

Vaginosis. Microbiota vaginal

Palabras clave:
Vaginitis aerobia
Vaginosis bacteriana
Microbiota vaginal

RESUMEN

Los últimos avances en el microbioma vaginal y el diagnóstico molecular de la vaginosis bacteriana han permitido un mayor conocimiento de esta entidad caracterizando aspectos de su patogenia y el establecimiento de la biocapa vaginal, los modelos y nuevas teorías de la etiología de la misma, cómo se transmite al considerarse hoy como una probable infección de transmisión sexual, la separación de otras entidades como la vaginitis aerobia, el diagnóstico molecular de la misma y el tratamiento y nuevas moléculas que eviten las recaídas frecuentes. Esta entidad y el estudio del microbioma vaginal han permitido considerar estas infecciones como un síndrome polimicrobiano acabando con el dogma: un microorganismo, una enfermedad. Además, se actualiza una entidad menos conocida como es la vaginitis aerobia y los métodos para su detección.

Introduction

The human vagina is a balanced and dynamic ecosystem, with a complex population of aerobic and anaerobic bacteria, which reach up to 10^9 CFU/ml of vaginal fluid.[1–3] Typically, the presence of *Lactobacillus* spp. is considered to have a protective effect in this ecosystem due to three complementary mechanisms: (a) exclusion by means of the formation of a biofilm, which covers the epithelial cell receptors and blocks the binding of pathogenic microorganisms; (b) growth inhibition due to generation of different antimicrobial compounds: (b1) lactic acid from the fermentative catabolism of sugars, especially glucose, which excretes glycogen and hydrolysis that makes the pH of the vagina 3.5–4.5, (b2) production of hydrogen peroxide (H_2O_2), fundamentally from *Lactobacillus crispatus* and *Lactobacillus jensenii*, (b3) bacteriocins whose effect has only been demonstrated in vitro; and (c)

DOI of original article: https://doi.org/10.1016/j.eimc.2018.11.009
☆ Please cite this article as: Vazquez F, Fernández-Blázquez A, García B. Vaginosis. Microbiota vaginal. Enferm Infecc Microbiol Clin. 2019;37:592–601.
* Corresponding author.
E-mail address: opsklins@gmail.com (F. Vazquez).

Table 1
Differential characteristics between aerobic vaginitis and bacterial vaginosis.

Characteristic	Aerobic vaginitis	Bacterial vaginosis
Microorganisms involved	*Escherichia coli*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Enterococcus* (increased 3–5-fold). It is not known if they are the cause or association due to the fact that they alter the immune status which influences the microbiota	GV and anaerobes (see in BV)
Lactobacillus	Reduced or absent	Reduced or absent
Production of		
High pH	Increased (more than in BV)	Increased
Lactate	Decreased	Decreased
Succinate	Normal	Increased
Prevalence	Not clear: 5–10% and 4–8% in pregnant women (between 7 and 26%)	Clear: 12% to >50% (7–77%)
Clinical characteristics		
Inflammation	Yes; red vagina, oedematous with small erosions or ulcerations	No
Discharge	Increased with unpleasant odour Colour: yellowish-greenish, thick and mucoid	Increased with fishy odour Colour: whitish or grey, thin and homogeneous
Dyspareunia	Yes	No
Associated with other clinical symptoms	BV, candidiasis, other STIs (HPV, HIV, CT)	With AV and the rest the same as AV
Diagnosis	Wet mount examination with phase contrast (×400) (see Table 2)	Amsel criteria, Nugent criteria and Hay-Ison criteria (see Table 4)
Treatment	Unclear recommendations	Clear recommendations (see Table 7)

co-aggregation with pathogens. These effects increase the barrier function in the epithelial cells and stimulate innate immunity.

The onset of genital discomfort (exudate, pruritus, dyspareunia and unpleasant odour) is common in women and is of varied aetiology: vulvodynia, contact dermatitis, atrophic vaginitis or lichen sclerosus. Within the infections, there are those caused by yeasts, trichomonas and bacterial vaginosis (BV), which represent 19% of diagnoses in these women.[4] These infections produce dysbiosis, in which another more recently recognised clinical entity also intervenes: aerobic vaginitis (AV), which is sometimes confused with BV.[5]

The focus of this review was to update aspects of the clinical entity known as BV, and the characteristics which enable the diagnosis and treatment of AV in relation to the role of vaginal microbiota are included.

Aerobic vaginitis

Definition

This entity was named in 2002, and it can be defined as an alteration of the microbiota (less lactobacilli and greater quantities of enteric aerobic bacteria) with variable levels of inflammation, deficiency of epithelial maturation and of immune response with local elevation of IL-1, IL-6 and IL-8. In pregnant women, it is associated with a risk of preterm birth, chorioamnionitis and funisitis of the foetus, and also cervical dysplasia in general.[5,6] It is important to differentiate AV from BV (Table 1), although sometimes it is not easy. The majority of clinicians agree with Nugent's score of 7 or more for BV and 3 or less for normal microbiota, but the meaning of intermediate microbiota with a score of 4–6 is not clear.[5] In this intermediate group, AV may explain unclear aspects of BV: (a) the very concept of this intermediate microbiota; (b) the variability of the symptoms with Nugent's score; (c) so-called inflammatory BV, and (d) the failure of treatment with metronidazole to prevent preterm birth in many women with BV.[5] Therefore, it is believed that this intermediate group may be a mixed group that may include women with AV associated with BV.

Prevalence

There are no very reliable data due to the lack of studies performed, but it is estimated in between 5 and 10% of non-pregnant women and 4–8% of pregnant women,[7] although it may vary between 7 and 26%.[6] The risk factors are similar to those of BV.

Signs and symptoms

A purulent yellowish-greenish discharge with inflammation and altered epithelial cells is produced. The signs are a reddened, inflamed vagina, with foul-smelling discharge, burning with ecchymotic haemorrhages, erosions and dyspareunia. The symptoms may be prolonged for up to 12 months or more, and it is sometimes indistinguishable from desquamative inflammatory vaginitis.[5]

Diagnosis

The most accurate and preferred method is the wet mount examination with phase-contrast microscopy (×400) (Table 2),[6] applying a score like in Nugent's criteria: from 0 to 2 means absence of AV; between 3 and 4, mild AV; between 5 and 6, moderate AV; and from 7 to 10, severe. Some studies consider a score of between 5 and 10 to be pathological.

This method is not very widespread and alternative methods are usually used, such as quantitative PCR (its sensitivity [SEN] and specificity [SPE]) are unknown, cultures to detect *Streptococcus pyogenes* and/or *Trichomonas vaginalis* (TV) or histological analysis. The observation of the coccoid microbiota under the microscope is a rapid technique, but only reflects a subgroup of patients with AV.[6]

There is also another test for measuring five enzymatic indicators[6]: (a) activity of hydrogen peroxidase (indicator of H_2O_2-producing lactobacilli); (b) activity of leukocyte esterase (indicates inflammation); (c) activity of sialidase (due to high production in AV by *Streptococcus agalactiae* and in BV by *Atopobium vaginae* [AV], *Gardnerella vaginalis* [GV] and *Prevotella bivia*); (d) activity of beta-glucuronidase (specific to *Escherichia coli*), and (e) coagulase activity (of *Staphylococcus aureus* and *Enterococcus faecalis*); by means of these five indicators obtains a SEN of 90%, but its SPE has not been studied.

Other less common methods are: measuring the oestrogen content in blood (a low oestrogen content in the vagina suggests the presence of immature epithelial or parabasal cells, but it is not relevant when measuring in serum); the pH test, by self-testing, for screening (SEN of 90% but low SPE because it also increases the

Table 2
Diagnosis of aerobic vaginitis via a wet mount examination with phase-contrast microscopy (×400).

Score	Microbiota/*Lactobacillus*	Background microbiota	White blood cell count	Proportion of toxic leukocytes	Proportion of parabasal cells
0	I (normal microbiota with predominance of *Lactobacillus* and few or absence of cocci) IIa (slight mixture of *Lactobacillus* with other microorganisms)	No characteristic or cytolysis	≤10	None or sporadic	None or <1%
1	IIb (moderate alteration of the microbiota)	Small coliform bacilli	>10 (and ≤10 epithelial cells)	≤50%	≤10%
2	III (no *Lactobacillus* and numerous bacteria)	Cocci or chains	>10	>50%	>10%

Parabasal epithelial cells: immature, round, small epithelial cells with a high nucleus-to-cytoplasm ratio.
Toxic leukocytes: leukocytes with abundant secretory granules.

Table 3
Historical milestones of bacterial vaginosis.

Year	Milestone
1950s	Description of *Gardnerella vaginalis*
1955	Discovery as causative agent of nonspecific vaginosis
1964	Introduction of the term vaginosis
1981	Introduction of the term bacterial vaginosis
2016	The term polymicrobial vaginosis is suggested

pH in TV, BV, yeasts, due to sperm or due to menstrual blood) and next-generation sequencing (NGS).

Treatment

It is not clear and there are no conclusive data with the use of antibiotics. The following can be used: (a) antiseptics such as dequalinium chloride or nifuratel 500 mg intravaginal for 10 days; and (b) antibiotics such as clindamycin, kanamycin in 100-mg ovules for six days, rifaximin vaginal for six days or oral moxifloxacin 400 mg for six days in one dose. Metronidazole is not appropriate as it does not cover the microorganisms involved in AV. The treatment guidelines advise: when there is atrophy, prescribe estradiol with or without probiotics; if there is inflammation, prescribe local corticosteroids; and when there is clear infection (grade IIb or III [Table 2] and/or abundant growth of *E. coli*, *S. pyogenes*, *S. agalactiae* or staphylococci in culture) include antibiotics. During pregnancy it seems better to use clindamycin, which is active for AV and BV, in addition to reducing preterm birth, but again this regimen is controversial.[6]

Bacterial vaginosis

Introduction

Until the emergence of molecular techniques, the concept of "a microorganism, a disease" was a dogma, as well as that microorganisms were in planktonic form and as a single species, when in reality they are complex polymicrobial communities forming a biofilm.[8] Since 1892, the year in which Albert Döderlein discovered the bacilli that bear his name or *Lactobacillus*, these have been considered as normal vaginal microbiota.[1] This normal microbiota consists of aerobic and anaerobic bacteria, with *Lactobacillus* representing >95% of all of them. They therefore maintain an acidic pH, ensuring that the H_2O_2 is present in the environment.[9]

The term vaginosis emerged in the literature in 1964, but it was not until 1981 that the term BV was used, which is now questioned by that of polymicrobial vaginosis. Table 3 shows the historical milestones of BV.[10,11]

Definition

For some time, BV has been considered as a syndrome with different aetiologies. With current methods such as fluorescence in situ hybridisation (FISH), the characteristics of these biofilms in the case of BV have been checked.[12] BV represents dysbiosis of the vaginal microbiota[13] and the concentration of *Lactobacillus* is reduced while that of bacterial pathogens is increased in this polymicrobial syndrome.[9] Despite the fact that there is not only one microorganism whose presence potentially confirms the diagnosis, GV and AV are strong indicators of BV.[14]

Although it is accepted that BV is caused by a conglomerate of microorganisms, there is a debate regarding the trigger which would start the alterations: if the founding microorganism of this altered microbiota is GV, if it is a consortium of microorganisms, or if it is due to other factors such as the use of a vaginal douche.

Prevalence

It affects 12% of women in Australia, 29% in the US and >50% of women in the Sub-Saharan area,[15] although there is great variation between 7 and 70% of women.[16,17]

Epidemiology

BV is the most common vaginal dysbiosis in women of childbearing age,[1] and the most common cause of vaginitis with abundant vaginal discharge and odour in pregnant and non-pregnant women.[9] Furthermore, it is more common in women who have sex with women (WSW) or a mixture of women and men, than in women who have sex with men (WSM). The mechanism could be due to sharing sexual objects, but it is not known if the vaginal fluid shared between women is more efficient than masculine penetration. In addition, the transmission of anaerobes is less efficient, meaning that it would be in favour of the hypothesis of primary transmission of GV.[18]

There is a debate surrounding whether it can be defined as a true sexually transmitted infection (STI). It was suggested historically in the 1950s when Gardner and Dukes saw the transmission from infected women to non-infected women, but this theory was abandoned as (a) there was no counterpart theory in men; (b) the treatment of men did not reduce BV in couples (although these studies were of low methodological quality), and (c) the presence of various pathogens went against this possibility.

Currently, the theory in favour of an STI has been taken up again due to various noteworthy and highly significant aspects[15]:

(a) There are many cross-sectional and longitudinal studies linked to sexual activity.

(b) It is associated with low condom use.
(c) Women with BV have a greater number of sexual partners than women without BV.
(d) It appears earlier in sexually active women.
(e) There is a reservoir in men, which hosts the microorganisms involved in the subpreputial space and distal urethra.
(f) Only tobacco (and not vaginal douches, dietary factors and stress) has been consistently associated.
(g) It has been linked to sexual transmission between women, to a female partner with symptoms or a history of BV and to receptive oral sex.
(h) There is concordance of biotypes and oligotypes between men and women who are a couple.
(i) Circumcision reduces the probability in women who are the partner of these men.
(j) Non-gonococcal urethritis (NGU) is more common in men who have female partners with BV, but only *Sneathia*, BV-associated bacteria (BVAB), such as BVAB2 and BVAB3, has been found in a non-statistically significant manner.
(k) It is linked to balanoposthitis caused by GV in men.

Diagnosis

The concept of BV is influenced by the diagnostic method which is carried out:

(a) Clinical, using the Amsel criteria.[17]
(b) Research, using the Nugent criteria[16] or their modification of the Hay-Ison criteria.[19]

The Amsel criteria have been used in clinical studies, while the Nugent criteria based on the Gram stain have been used in research studies (Table 4): 0–3 normal, 4–6 intermediate with GV/Bacteroides morphotypes and from 7 to 10 with numerous GV/Bacteroides and appearance of curved anaerobic bacilli (Mobiluncus and possibly BVAB1) in 9–10.

The Amsel criteria generate problems of overdiagnosis, as there would be many asymptomatic women diagnosed with BV. In a study in which 29% of women presented with BV according to the Amsel criteria, only 15.7% were symptomatic.[20] However, this group of women had more complications associated with BV, regardless of whether or not they had symptoms.

The Nugent criteria have high diagnostic accuracy and reliability, with high inter- and intra-centre and interobserver reproducibility. However, the identification of microorganisms in the clue cells in the Gram stain is not always accurate. It has been demonstrated using quantitative PCR and FISH that the curved gram-negative bacilli that were believed to represent *Mobiluncus* spp. are more likely to be (in a factor of 100 to 1) BVAB1.[21] In addition, a correlation between a greater score in accordance with the Nugent criteria and the presence of more symptoms in women has not been observed, although there are few studies conducted in this regard.[18]

For this reason, the National Institutes of Health (NIH) working group recommends using a combination of the Nugent criteria with at least a score of 7 and one positive Amsel criteria for the definition of BV, regardless of the symptoms.[22]

(c) Another alternative is the visualisation of the biofilm in desquamated cells in the urinary sediment with FISH with a diagnostic accuracy of 0.94, a SEN of 83% and a SPE of 97%, a positive predictive value of 83% and a negative predictive value of 97%.[21] This method is appropriate for clinical and epidemiological studies, as the sample is easy to obtain.
(d) Currently, the culture of GV is not recommended nor is the use of specific probes for GV. The Gram stain has shown greater SPE than the culture in recent trials based on the study of the microbiome. The GV culture does not suggest nor test for BV or the need for its treatment, as 50% of asymptomatic women present with a positive culture. The FDA recently approved the Max Vaginal Panel (Becton-Dickinson, New Jersey, USA) for the study of the microbiome, which makes it possible to carry out two to 24 tests simultaneously. It enables the detection of *L. crispatus*, *L. jensenii*, GV, AV, BVAB2, *Megasphaera* type 1, *Candida* group (*C. albicans*, *C. parapsilosis*, *C. dubliniensis*, *C. glabrata* and *C. krusei*) and TV.[23]

Table 4
Amsel criteria, Nugent criteria and Hay-Ison criteria.

Amsel criteria (clinical)[a]	Characteristics (%)
Vaginal discharge	Stringy, homogeneous, white, stuck to the walls and even
Vaginal pH	>4.5 (90%)
10% KOH	Fishy smell
Clue cells (×40)	>20% (>90%)
Nugent criteria (Gram stain)[b]	**Count per oil immersion field (value)**
Lactobacillus type gram-positive bacteria	>30 (0) 5–30 (1) 1–4 (2) <1 (3) 0 (4)
GV and *Bacteroides* type gram-negative bacilli	>30 (4) 5–30 (3) 1–4 (2) <1 (1) 0 (0)
Curved gram-variable bacilli	>5 (2) <1–4 (1) 0 (0)
Hay-Ison criteria (Gram stain)	**Grade**
Predominance of *Lactobacillus* type gram-positive bacteria	1. Normal
Mixed microbiota of a *Lactobacillus* and GV and *Mobiluncus* type gram-negative bacilli	2. Intermediate
Predominance of GV and/or *Mobiluncus* with few/no *Lactobacillus*	3. BV

National Institutes of Health (NIH): Diagnosis of BV with the Nugent criteria (at least a score of 7) and one positive Amsel criteria, regardless of the symptoms.
The presence of polymorphonuclear leukocytes suggests the coexistence of another process such as cervicitis.
[a] Interpretation: at least 3 of the signs or symptoms.
[b] Interpretation: 0–3, normal vagina; 4–6, intermediate pattern; 7–10, BV.

Microorganisms in bacterial vaginosis

The concept of BV as a syndrome was established many years ago, but FISH techniques have enabled biofilms where GV is the predominant microorganism to be seen, followed by AV and, to a lesser extent, by *Bacteroides*, *Veillonella*, *Ruminococcus* and the genus *Streptococcus*.[12]

Although traditionally it has been associated with the presence of GV, nowadays BV is associated with bacterial conglomerates, as shown in Table 5.[1] Using molecular methods in the vaginal microbiota, vulva/labia, cervix and uterus, more than 250 bacterial species, yeasts, *Chlamydia*, *Archaea*, viruses and protozoa have been detected.[24] In one study, up to six clusters or types of community statuses of the vaginal microbiota were observed using genome

Table 5
Microorganisms associated and/or found in bacterial vaginosis.

Microorganism	Characteristic and species	Comments
Gardnerella vaginalis	Small, pleomorphic gram-positive bacillus. Observed as a gram-negative or gram-variable bacillus in Gram stain	Tolerates pH 4–5
	Facultative anaerobe	Has virulence factors
	Non-motile	Cultivable
Atopobium vaginae	Small gram-positive cocci in pairs or short chains	It is the best marker of BV (>80% of cases)
	Facultative anaerobe	2.6 times better than the Amsel criteria
	Immobile	19 times better than the Nugent criteria
		It rarely appears without GV
		It may be normal microbiota
		It may be responsible for resistance to metronidazole and explains why it reappears in recurrences
BV-associated bacteria (BVAB)	3 species have been observed using FISH:	BVAB1 predominates more than *Mobiluncus* spp. in clue cells (it tends to be that which is seen in the Gram stain)
Related to the phylum *Clostridium*	-BVAB1: curved, thin bacteria	Gives off unpleasant, fishy smell due to polyamines (putrescine, cadaverine and trimethylamine)
	-BVAB2: straight bacilli	BVAB3 the only one which is characterised and cultivated
	-BVAB3 (*Mageeibacillus indolicus*): straight, broad and long bacilli	The role of these bacteria in the aetiology of BV has not been well studied
Other		
Actinomyces, *Eggerthella*, *Mobiluncus*, *Prevotella*	They produce non-volatile fatty acids (NVFA)	Succinic acid produces acid fermentation. *Mobiluncus*, malic acid which causes irritation of the membranes and vaginal mucosa
	And volatile fatty acids (VFA)	Formic acid and acetic acids
Megasphaera, *Propionibacterium*		Propionic acids
Mobiluncus, *Prevotella*	*Prevotella* (*P. amnii*, *P. bivia*, *P. buccalis*, *P. corporis*, *P. disiens*)	Isobutyl, valeric and isovaleric acids
Anaerococcus spp., *Bacteroides* spp. (*B. levii*, *B. ureolyticus*), *Dialister* spp., *Finegoldia* spp., *Fusobacterium nucleatum*, *Leptotrichia* spp., *Peptococcus*, *Peptostreptococcus*, *Sneathia* spp., *viridans* group streptococci *Mycoplasma hominis* *Ureaplasma urealyticum*	*Peptostreptococcus* (*P. anaerobius*, *P. prevotii*, *P. tetradius*) *Viridans* group streptococci (*S. acidominimus*, *S. constellatus*, *S. morbillorum*, *S. mutans*, *S. sanguis II*)	

sequencing by Illumina (NGS): two associated with normal vaginal microbiota (one dominated by *Lactobacillus iners* and another by *L. crispatus*) and four associated with BV (dominated by *P. bivia*, GV, *Lachnospiraceae* or a mixture of different species).[24] Similarly, other authors have described a cluster with the presence of *Lactobacillus* associated with normal microbiota, and another three groups with dysbiosis with the order *Lachnospiraceae* and genera *Sneathia* and *Prevotella* as dominant microorganisms.[25] Finally, studies have been performed in pregnant and non-pregnant HIV-negative women, with five groups being described: (1) *L. crispatus*; (2) *Lactobacillus gasseri*; (3) *L. iners*; (4) *Peptoniphilus*, *Prevotella* and *Anaerococcus* spp. and a high quantity of GV or *Ureaplasma*; and (5) *L. jensenii*. Using transcriptomics, it has been observed that *L. iners* adapts to very different environments of BV.[24,26]

There is high variability among these studies with regard to sampling, the storage of samples, the processing of samples, the DNA extraction kit, technical variations in PCR amplification and the use of different *primers*, as well as with regard to the statistical methods used. In addition, changes due to menstruation, sexual activity, spermicides, douches and antibiotics were not taken into account.

In summary, molecular studies have demonstrated, as classic studies did previously using cultures, that there is a high presence of *Lactobacillus* in a healthy vagina and that it is not possible to associate it with one single microbiota composition.[24] Therefore, different clusters of vaginal microbiota can be defined, each one correlated with the predominance of one or more microorganisms.[9] If the Nugent criteria are compared with the vaginal microbiota defined molecularly, a good, although not a total, correlation is observed.[27]

The substances released by this conglomerate of bacteria which make up the biofilms and which may be involved in BV are varied:

(a) Immunomodulatory such as haemolysins, volatile fatty acids (VFA) and non-volatile fatty acids (NVFA), proteases and sialidases. Sialidases inhibit serum IgA, increasing the risk of preterm birth due to cytokines, and also increase IL-1b, IL-8 and TNF-alpha, which, along with prolidase, confer greater susceptibility to HIV infections and herpes simplex virus type 2 (HSV-2).
(b) Proinflammatory substances, such as lipopolysaccharide (LPS), which acts on cytokines, IL-1b, IL-6 and TNF-alpha, favouring preterm birth.

Some of the mechanisms of production of BV are shown in Fig. 1.

An alteration of the protein content of the cervicovaginal fluid has been detected in BV by proteome analysis: neutrophil elastase, kaliocin-1, defensin-1 of neutrophils, lambda-2 chain C regions of Ig and protein S100-A7. This seems to indicate that the alteration of the microbiota is capable of interfering with the immune response mechanisms.[28]

Regarding the role of GV, a phenotypic and genotypic heterogeneity has been observed, in addition to a variability in the virulence potential. Four GV clades have been described. It has been attempted to correlate them with the vaginal microbiota clusters. The hypothesis is that there is colonisation by different GV clades in the vagina which express different determinants of virulence.[27] The role of some virulence factors such as haemolysin or vaginolysin (which is a cholesterol-dependent cytolysin), sialidase or prolidase

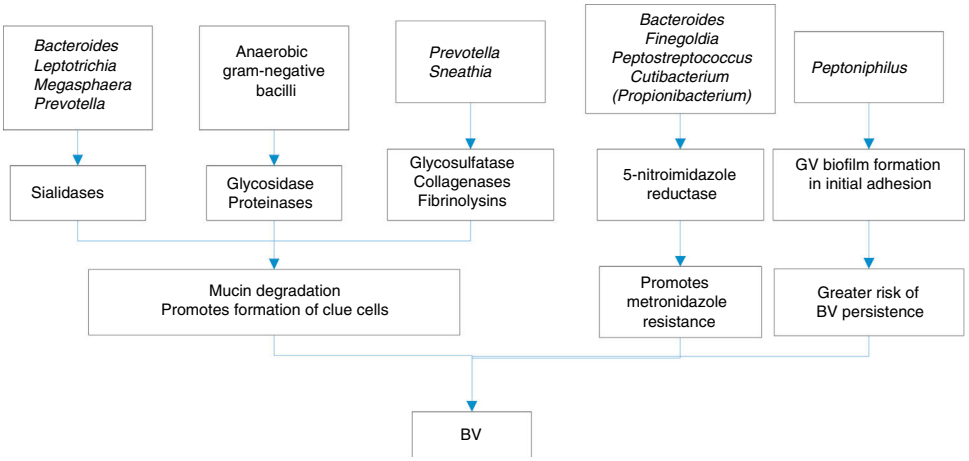

Fig. 1. Some of the mechanisms of production of bacterial vaginosis.

is not clear. Furthermore, GV clade A is lipase-positive and clade B is sialidase-positive, which could indicate that different clades are involved either in the resolution of BV re-establishing the *Lactobacillus*, or, on the other hand, in the transition to infection by yeasts or AV.[1]

GV appears in the vagina in two forms: one in a scattered form ($10^{6–8}$ cells per gram in the vaginal fluid) and another in the infectious or transmissible form in the cohesive form a biofilm ($10^{10,11}$ cells per gram).[29] This pattern is also seen in men and in semen. It is not clear if it is different strains of GV, although all those which form part of the biofilms present genes involved in the synthesis of exopolysaccharide.[14]

Nor are there specific data on the cell surface receptors for GV, although various studies have demonstrated significant differences in the binding capacity and cytotoxicity between the pathogenic and non-pathogenic strains of this microorganism.[30,31] Various GV ecotypes are capable of altering the adhesion of *Lactobacillus*, measured by cell-surface glycosaminoglycans, thanks to the production of glycosidases which degrade these receptors.[31,32] In addition, the production of sialidases and vaginolysin is capable of altering the epithelial cells, therefore affecting the bound microbiota.[33] Furthermore, lactobacilli are capable of affecting both the binding and the internalisation of GV, interfering with its pili and with fibronectin-binding proteins.[30]

How does the protection and alteration of the vaginal ecosystem occur?

Lactobacilli play a key role in the protection of the vaginal mucosa and in the inhibition of colonisation by pathogenic bacteria through a series of mechanisms[1]:

(a) Adhesion to glycolipid receptors on the surface of vaginal epithelial cells. The presence of high volumes of *Lactobacillus* inoculum in the vaginal discharge of healthy premenopausal women (10^{7}–10^{8} CFU/g) prevents the binding of genitourinary pathogens to these receptors by means of a competitive exclusion mechanism.
(b) Coaggregation of *Lactobacillus* with pathogens and autoaggregation or quorum sensing.

Both mechanisms contribute to the formation of the biofilm in a healthy vagina and to the inhibition of the growth of pathogens. In a study of three vaginal populations of lactobacilli (*Lactobacillus acidophilus*, *L. gasseri* and *L. jensenii*), the autoaggregation ability measured by surface proteins or lipoproteins was demonstrated. In addition, the three strains adhered strongly to vaginal epithelial cells by glycoproteins (*L. acidophilus* and *L. gasseri*) and carbon hydrates (*L. jensenii)*, while the lactobacilli recovered from other sources, such as dairy products, adhere in significantly lower numbers, which indicates that adherence is an idiosyncratic property of vaginal lactobacilli.[34]

(c) Vaginal acidification with the production of lactic acid. Metabolomic studies[24] have observed the production of lactic acid, acetic acid, glycerol and others in a healthy vagina, while in BV there is 2-hydroxyvalerate, gamma-hydroxybutyrate or succinate. A diagnostic test could be developed to detect gamma-hydroxybutyrate in vaginal swabs with an elevated pH.
(d) The production of H_2O_2, bacteriocins and biosurfactants contributes to the inhibition of the growth of some pathogens.

On the other hand, the factors which alter the ecosystem would be:

(a) Excessive vaginal douches. Vaginal douches[35] are defined as the use of a liquid solution in the vagina. It is estimated that they are used by 27–59% of women depending on ethnic, cultural and educational differences. Most studies advise against their use, although some see them as beneficial with the use of acetic acid to eliminate semen and the possible transmission of pathogens, unpleasant vaginal odour and to relive vaginal irritation. However, it is known that it increases the risk of developing BV an average of 2.1-fold (use of vaginal douches ≥once per month increases the risk 1.6-fold; ≥twice per month increases the risk 2.5-fold; and douches in the last two months increases the risk 2.9-fold).
(b) Use of spermicides (nonoxynol-9).
(c) Antibiotics.
(d) Phages (not tested). It is postulated that there is sexual transmission of phages with the ability to destroy the *Lactobacillus* population. Tobacco behaves as a facilitating factor, through the production of benzo[a]pyrene diol epoxide type promoters.

Models and new theories of the aetiology of bacterial vaginosis

Three explanatory models of BV have been proposed,[1] and all of them are in agreement in that there is: reduction of *Lactobacillus* spp., exposure and growth of BVAB and other BV-associated bacteria, and an increase in vaginal pH.

Table 6
Process and chronological stages of the formation of the biofilm in bacterial vaginosis.

Phase of formation	Outline of the processes
1st. Adhesion	GV expresses the domains "*Rib*" by the gene *bap*L, coded by the protein BapL and forms the biofilm as a precursor species *Lactobacillus iners* and *Peptoniphilus* spp. probably also intervene
2nd. Formation of microcolonies and coaggregation	A. Microcolonies are formed before producing the non-extracellular polymeric substances (EPS) B. Then, coaggregation occurs with the second colonising species, in two ways: (a) Due to a cascade in suspension before binding to the surface (probable mechanism of *Mobiluncus* spp. and perhaps of *Atopobium vaginae*, *Fusobacterium nucleatum*, *Escherichia coli* or *Enterococcus faecalis*) (b) After binding to the primary species C. Subsequently, they bind to other species
3rd. Maturation of the biofilm	GV releases extracellular DNA which stimulates the production of EPS controlled by molecules perhaps of quorum sensing and the biofilm forms in a brick-like shape GV may also encode glycosyltransferases from families I, II and IV involved in the production of EPS
4th. Dispersion	The hypothesis is that it occurs in the following ways: A. Active, essential process in BV during menstruation or when BV-associated bacteria grow due to the increase in vaginal pH with menstrual blood and polyamines B. Passive, due to erosion of the epithelium and detachment to sialidase, glycosulfatase, glycosidase, proteinase, collagenase and fibrinolysin

The three proposed models are: (a) depletion of *Lactobacillus* spp. model, (b) primary pathogen model and (c) polymicrobial pathogen model:

(a) In the first model, it is established that there is a reduction of H_2O_2-producing *Lactobacillus* spp., prior to the increase in vaginal pH which triggers the overgrowth of anaerobes producing BV. However, in opposition to this theory, there is the theory that there are healthy women without *Lactobacillus* and, furthermore, there are women in whom AV is isolated who produce lactic acid. According to this model, a treatment strategy would be the use of probiotics and acidifying agents.
(b) In the primary pathogen model, a pathogen such as GV would be introduced due to sexual activity which creates the conditions, due to its virulence factors and the formation of the biofilm, for the growth of an intermediate microbiota and, finally, the establishment of the BV-associated microbiota. Contrary to this model is the fact that GV is also detected in healthy women who are not sexually active, although it could be different genotypes and biotypes (e.g. biotype 5 is observed in healthy women). According to this model, agents which alter the GV biofilm could be used as treatment.
(c) In the polymicrobial pathogen model, it would be a set of BV bacteria which trigger colonisation after sexual activity, without the presence of an intermediate microbiota, with synergisms among them which would reduce the *Lactobacillus* population. In this case, and in opposition to this model, is the lower virulence of these other BV bacteria compared to that presented by GV. Treatment in this model should include agents which alter the polymicrobial biofilm.
(d) There are other proposed models or theories apart from the above-mentioned three:

(d1) BV-associated bacteria are internalised in the epithelial cells, meaning that microorganisms such as GV, AV and *P. bivia* would escape the defences and the action of antibiotics such as clindamycin and metronidazole. There is no evidence of this mechanism.
(d2) Alkaline semen would reduce the acidity of the vagina by increasing the pH after sexual intercourse, promoting the growth of GV. In this case, it could be defined as a sexually associated infection more than as an STI in the strict sense of the term.
(d3) Genetic polymorphisms would promote BV. There are no data.

Formation of the biofilm in bacterial vaginosis

As in other locations, for example in the oral biofilm, the formation of the biofilm in BV has advantages for GV: it increases its tolerance to H_2O_2 five-fold and to lactic acid four to eight-fold.[1] The stages of biofilm formation are detailed in Table 6.

Table 7
Established treatments of bacterial vaginosis.

Antimicrobial	Dose	Duration in days	Cure rate (%)	Grade of recommendation
Metronidazole	500 mg/oral/twice daily	7	75–85	A
Metronidazole	0.75% gel (1 application of 5 g intravaginal once daily)	5	75–85	A
Alternatives:				
Tinidazole	1 g/day	5–7	75–85 (reduces gastrointestinal effects and number of doses)	B
	2 g	Single dose		B
Metronidazole	2 g/oral	Single dose		A
Clindamycin	300 mg/oral/twice daily	7		A
Recurrences:				
Metronidazole	500 mg/twice daily	10–14	Using a condom can reduce recurrences in sexually active women	A
	0.75% gel (1 application of 5 g intravaginal once daily)	10, after 3–6 months twice a week		A
Symptomatic pregnant women:				
Metronidazole	500 mg/oral/twice daily	7	Topical treatment is not recommended and there is a high recurrence	A
Clindamycin	300 mg/oral/twice daily	7		A

Signs and symptoms

The symptoms which appear in BV are: homogeneous discharge (90%) and fishy vaginal odour during menstruation, after sexual intercourse and with minimal itching or irritation. There is perivaginal irritation and rarely dysuria or dyspareunia, occasional abdominal pain, discharge at the introitus, there is no erythema or oedema of the labia and vulva.

The following are described as complications: chorioamnionitis, endometritis, salpingitis and pelvic inflammatory disease [PID] (BV treatment reduces PID after induced abortion). In pregnant women it can trigger: spontaneous abortion, premature rupture of membranes, preterm birth, premature baby, low birthweight, postpartum endometritis and infections of the postoperative wound.[9] It also increases the risk of acquiring other STIs (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, HSV-2 and HIV) and transmission of HIV to male partners. There is also a negative impact on self-esteem, sexual relationships and quality of life.

Treatment

Standard treatment is carried out with metronidazole or clindamycin (Table 7). In the short-term, a cure rate of 80–90% is obtained, although 50–70% of women have a recurrence after three to six months. In the long-term, a cure rate of up to 80% is achieved. It is therefore worth confirming the diagnosis in these cases. It is not clear if recurrence is due to (a) resistance (it does not seem probable as metronidazole is active against anaerobic gram-negative bacteria, *Mobiluncus mulieris* and less active against GV, anaerobic cocci and *Mobiluncus curtisii*) and inactive against *Mycoplasma hominis* and AV; (b) recurrence or reinfection, which seems more

Table 8
Alternative treatments tested in bacterial vaginosis.

Compound	Dose	Duration in days	Grade of recommendation	Comments
Antimicrobials				
Metronidazole	Ovules 500 mg/1 dose	5	A	
Metronidazole	Ovules 2 g	Single dose	B	
Tinidazole	Vaginal tablet 500 mg	14	B	
Ornidazole	Vaginal tablet 500 mg	7	C	
	Oral 500 mg/twice daily + vaginal tablet 500 mg/once daily	5	B	
Secnidazole	Oral 1–2 g/1 dose		B	
	Oral 2 g + 500-mg tablets/1 dose	Single dose + 5 days	B	
Clindamycin	2% vaginal cream		A	
Rifaximin (rifamycin derivative)	25-mg vaginal tablets/1 dose	5	B	
Antiseptics				
Benzydamine				Little data
Chlorhexidine	Vaginal wash 1 dose	Single dose	C	
Dequalinium chloride	Vaginal tablets 1 day	10	A	
Polyhexamethylene biguanide				Little data
Povidone-iodine	Vaginal washes once daily	7	D	Little data
Hydrogen peroxide	Vaginal washes 3% once daily		B	More failures than with metronidazole Caustic effect
Nifuratel	25-mg vaginal tablets	10	B	
	Vaginal tablet 500 mg	8		
	20-mg oral tablet/3 times daily	5	B	
Vaginal acidifying agents				
Acetic acid	0.92% vaginal gel twice daily	7	B	Not superior to placebo
Lactic acid	Gel, suppositories and impregnated tampons		–	Less effective than metronidazole No appropriate studies
Polycarbophil-carbopol	Gel 1 dose daily	5 weeks	B	Seems effective, but there are little data
Ascorbic acid	250-mg silicone-coated vitamin C vaginal tablets	6 days and then 3 times a week	A	Does not irritate and has been used in pregnant women
Prebiotics and probiotics				
Lactate			B	Little data
Lactobacillus: L. crispatus (*LACTIN-V*) or *L. reuteri* and *L. rhamnosus*	Vaginal tablets various studies 1 dose per day (they could be improved probiotics or vaginal microbiota transplantation with use of a sponge which collects the microbiota from the donor or a synthetic preparation of it)	5 days to 3 months	B	No benefit. No appropriate studies and possible need to repeat applications of the same so that they are effective or perhaps as adjuvant treatment associated with metronidazole
Saccharomyces cerevisiae			–	Little data
Compounds against biofilms				
Octenidine	Not defined		–	There are recurrences and resistances appear
Tobramycin	Not defined		–	Does not act on established biofilm
Sodium cocoamphoacetate amphoteric surfactant	Not defined		–	Reduces the biofilm by 50%, potential use with metronidazole
DNase	Not defined		–	In vitro acts together with metronidazole
Retrocydin (vaginolysin inhibitor)	Not defined		–	Inhibits the formation of GV biofilm in vitro (hence its potential) or inhibits quorum sensing
Synthetic retrocyclin and thymol	Not defined		–	In vitro studies
Boric acid + EDTA (TOL-463)	Aqueous gel with polyethylene glycol 250 mg or 500 mg + EDTA	Vaginal/once at night/7 doses	–	50% cure rate

Table 9
Evidence in the diagnosis and treatment of bacterial vaginosis.

Process	Evidence
Diagnosis *It will be performed with clinical (Amsel) or laboratory criteria (Gram stain with an objective scoring system) (Nugent and Hay-Ison)*	II-2A
Symptomatic BV treatment *It will be treated with oral metronidazole 500 mg twice daily for 7 days* *Alternatives* Vaginal metronidazole gel Oral clindamycin or in vaginal cream	IA
BV treatment with multiple recurrences *Lengthen the course of treatment*	IA
Alternative treatments *Limited efficacy of treatment with probiotics and vitamin C*	I Based on Amsel criteria and few days of follow-up

likely, due to various possible factors although none demonstrated reliably: (b1) an exogenous source (male or female sexual partner), (b2) endogenous source (rectal reservoir), (b3) formation of biofilms, (b4) risk factors such as vaginal douches and smoking, (b5) recolonisation failure or (b6) activation of phages.[23] There is no evidence of complications associated with BV, meaning that standard treatment is only recommended in symptomatic women.

In general, there is a 58–92% cure rate after one month. No nitroimidazole has demonstrated superiority over others from the same family. The combination of oral use plus vaginal use seems most efficient (80–86% cure rate versus 75–86% without the combination). Oral or local use of clindamycin or metronidazole presents the same cure rates.[36]

Due to these cure rates, different treatment regimens or new molecules which would act in light of this new knowledge on how pathogenesis occurs in BV have been sought, such as the case of biofilm formation (Table 8).[23,35–38]

In the case of the use of lactobacilli, the ideal lactobacilli strains with probiotic potential would be those which produce lactic acid, H_2O_2, which form biofilms and whose minimum inhibitory concentration (MIC) for metronidazole, clindamycin and nonoxynol-9 is high. Endogenous lactobacilli from the vagina have not presented carcinogenetic potential and do not code for antibiotic resistance determinants transmissible to the present microbiota, despite containing numerous plasmids. Furthermore, lactobacilli which produce genital conditions have not been reported, and very rarely are they the cause of infections in other anatomical regions.[39–41]

Table 9 shows current evidence of diagnosis and BV treatment.[36]

In conclusion, an important breakthrough in the knowledge of these two clinical entities has occurred, which enables better management and diagnosis in light of new studies on the vaginal microbiome.

Conflicts of interest

None declared.

References

1. Jung H, Ehlers M, Lombaard H, Redelinghuys M, Kock M. Etiology of bacterial vaginosis and polymicrobial biofilm formation. Crit Rev Microbiol. 2017;43:651–67, http://dx.doi.org/10.1080/1040841x.2017.1291579.
2. Bartlett JG, Onderdonk AB, Drude E, Goldstein C, Anderka M, Alpert S, et al. Quantitative bacteriology of the vaginal flora. J Infect Dis. 1977;136:271–7, http://dx.doi.org/10.1093/infdis/136.2.271.
3. Bartlett JG, Polk BF. Bacterial flora of the vagina: quantitative study. Rev Infect Dis. 1984;6 Suppl. 1:S67–72, http://dx.doi.org/10.1093/clinids/6.supplement_1.s67.
4. Reichman O, Luwisch H, Sela HY, Samueloff A. Genital discomfort: yeast, trichomonas and bacterial vaginosis are only the tip of the iceberg. Eur J Obstet Gynecol Reprod Biol. 2017;214:200–1, http://dx.doi.org/10.1016/j.ejogrb.2017.04.049.
5. Donders G, Bellen G, Rezeberga D. Aerobic vaginitis in pregnancy. BJOG. 2011;118:1163–70, http://dx.doi.org/10.1111/j.1471-0528.2011.03020.x.
6. Donders G, Bellen G, Grinceviciene S, Ruban K, Vieira-Baptista P. Aerobic vaginitis: no longer a stranger. Res Microbiol. 2017;168:845–58, http://dx.doi.org/10.1016/j.resmic.2017.04.004.
7. Tansarli GS, Kostaras EK, Athanasiou S, Falagas ME. Prevalence and treatment of aerobic vaginitis among non-pregnant women: evaluation of the evidence for an underestimated clinical entity. Eur J Clin Microbiol Infect Dis. 2013;32:977–84, http://dx.doi.org/10.1007/s10096-013-1846-4.
8. Peters BM, Jabra-Rizk MA, O'May GA, Costerton JW, Shirtliff ME. Polymicrobial interactions: impact on pathogenesis and human disease. Clin Microbiol Rev. 2012;25:193–213, http://dx.doi.org/10.1128/cmr.00013-11.
9. Verstraelen H, Swidsinski A. The biofilm in bacterial vaginosis: implications for epidemiology, diagnosis and treatment. Curr Opin Infect Dis. 2013;26:86–9, http://dx.doi.org/10.1097/QCO.0b013e32835c20cd.
10. Gardner HL, Dukes CD. Haemophilus vaginalis vaginitis: a newly defined specific infection previously classified non-specific vaginitis. Am J Obstet Gynecol. 1955;69:962–76, http://dx.doi.org/10.1016/0002-9378(55)90095-8.
11. Onderdonk AB, Delaney ML, Fichorova RN. The human microbiome during bacterial vaginosis. Clin Microbiol Rev. 2016;29:223–38, http://dx.doi.org/10.1128/cmr.00075-15.
12. Swidsinski A, Mendling W, Loening-Baucke V, Ladhoff A, Swidsinski S, Hale L, et al. Adherent biofilms in bacterial vaginosis. Obstet Gynecol. 2005;106:1013–23, http://dx.doi.org/10.1097/01.aog.0000183594.45524.d2.
13. Kenyon CR, Osbak K. Recent progress in understanding the epidemiology of bacterial vaginosis. Curr Opin Obstet Gynecol. 2014;26:448–54, http://dx.doi.org/10.1097/gco.0000000000000112.
14. Yeoman CJ, Yildirim S, Thomas SM, Durkin AS, Torralba M, Sutton G, et al. Comparative genomics of *Gardnerella vaginalis* strains reveals substantial differences in metabolic and virulence potential. PLoS One. 2010;5:e12411, http://dx.doi.org/10.1371/journal.pone.0012411.
15. Unemo M, Bradshaw CS, Hocking JS, de Vries HJC, Francis SC, Mabey D, et al. Sexually transmitted infections: challenges ahead. Lancet Infect Dis. 2017;17:e235–79, http://dx.doi.org/10.1016/S1473-3099(17)30310-9.
16. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of Gram stain interpretation. J Clin Microbiol. 1991;29:297–301.
17. Amsel R, Totten PA, Spiegel CA, Chen KCS, Eschenbach D, Holmes KK. Nonspecific vaginitis: diagnostic criteria microbial epidemiologic associations. Am J Med. 1983;74:14–22, http://dx.doi.org/10.1016/0002-9343(83)91112-9.
18. Olson KM, Boohaker LJ, Schwebke JR, Aslibekyan S, Muzny CA. Comparisons of vaginal flora patterns among sexual behaviour groups of women: implications for the pathogenesis of bacterial vaginosis. Sex Health. 2018;15:61–7, http://dx.doi.org/10.1071/sh17087.
19. Ison CA, Hay PE. Validation of a simplified grading of Gram stained vaginal smears for use in genitourinary medicine clinics. Sex Transm Infect. 2002;78:413–5, http://dx.doi.org/10.1136/sti.78.6.413.
20. Koumans EH, Sternberg M, Bruce C, McQuillan G, Kendrick J, Sutton M, et al. The prevalence of bacterial vaginosis in the United States 2004; associations with symptoms, sexual behaviors, and reproductive health. Sex Transm Dis. 2007;34:864–9, http://dx.doi.org/10.1097/olq.0b013e318074e565.
21. Srinivasan S, Morgan MT, Liu C, Matsen FA, Hoffman NG, Fiedler TL, et al. More than meets the eye: associations of vaginal bacteria with gram stain morphotypes using molecular phylogenetic analysis. PLoS One. 2013;8:e78633, http://dx.doi.org/10.1371/journal.pone.0078633.
22. Marrazzo JM, Martin DH, Watts DH, Schulte J, Sobel JD, Hillier SL, et al. Bacterial vaginosis: identifying research gaps proceedings of a workshop sponsored by DHHS/NIH/NIAID. Sex Transm Dis. 2010;37:732–44, http://dx.doi.org/10.1097/olq.0b013e3181fbbc95.
23. Miller JM, Binnicker MJ, Campbell S, Carroll KC, Chapin KC, Gilligan PH, et al. A guide to utilization of the Microbiology Laboratory for diagnosis of infectious diseases: 2018 Update by the Infectious Diseases Society of America and the American Society for Microbiology. Clin Infect Dis. 2018;67:e1–94, http://dx.doi.org/10.1093/cid/ciy381.

24. Reid G. Therapeutic opportunities in the vaginal microbiome. Microbiol Spectrum. 2017;5, http://dx.doi.org/10.1128/microbiolspec.bad-0001-2016.
25. Braga PC, Dal Sasso M, Culici M, Spallino A. Inhibitory activity of thymol on native and mature *Gardnerella vaginalis* biofilms: in vitro study. Arzneimittelforschung. 2010;60:675–81, http://dx.doi.org/10.1055/s-0031-1296346.
26. Macklaim JM, Fernandes AD, Di Bella JM, Hammond JA, Reid G, Gloor GB. Comparative meta-RNA-seq of the vaginal microbiota and differential expression by *Lactobacillus iners* in health and dysbiosis. Microbiome. 2013;1:12, http://dx.doi.org/10.1186/2049-2618-1-12.
27. Schellenberg JJ, Patterson MH, Hill JE. *Gardnerella vaginalis* diversity and ecology in relation to vaginal symptoms. Res Microbiol. 2017;168:837–44, http://dx.doi.org/10.1016/j.resmic.2017.02.011.
28. Ferreira CST, da Silva MG, de Pontes LG, dos Santos LD, Marconi C. Protein content of cervicovaginal fluid is altered during bacterial vaginosis. J Low Genit Tract Dis. 2018;22:147–51, http://dx.doi.org/10.1097/lgt.0000000000000367.
29. Danielsson D, Teigen PK, Moi H. The genital econiche: focus on microbiota bacterial vaginosis. Ann N Y Acad Sci. 2011;1230:48–58, http://dx.doi.org/10.1111/j.1749-6632.2011.06041.x.
30. Ojala T, Kankainen M, Castro J, Cerca N, Edelman S, Westerlund-Wikström B, et al. Comparative genomics of *Lactobacillus crispatus* suggests novel mechanisms for the competitive exclusion of *Gardnerella vaginalis*. BMC Genomics. 2014;15:1070, http://dx.doi.org/10.1186/1471-2164-15-1070.
31. Cornejo O, Hickey RJ, Suzuki H, Forney LJ. Focusing the diversity of *Gardnerella vaginalis* through the lens of ecotypes. Evol Appl. 2017;11:312–24, http://dx.doi.org/10.1111/eva.12555.
32. Martín R, Martín C, Escobedo S, Suárez JE, Quirós LM. Surface glycosaminoglycans mediate adherence between HeLa cells and *Lactobacillus salivarius* Lv72. BMC Microbiol. 2013;13:210, http://dx.doi.org/10.1186/1471-2180-13-210.
33. Castro J, Alves P, Sousa C, Cereija T, França Â, Jefferson KK, et al. Using an in-vitro biofilm model to assess the virulence potential of bacterial vaginosis or non-bacterial vaginosis *Gardnerella vaginalis* isolates. Sci Rep. 2015;5:11640, http://dx.doi.org/10.1038/srep11640.
34. Boris S, Suárez JE, Vázquez F, Barbés C. Adherence of human vaginal lactobacilli to vaginal epithelial cells and interaction with uropathogens. Infect Immun. 1998;66:1985–9.
35. Aslan E, Bechelaghem N. To 'douche' or not to 'douche': hygiene habits may have detrimental effects on vaginal microbiota. J Obstet Gynaecol. 2018;38:678–81, http://dx.doi.org/10.1080/01443615.2017.1395398.
36. Donders G, Zodzika J, Rezeberga D. Treatment of bacterial vaginosis: what we have and what we miss. Expert Opin Pharmacother. 2014;15:645–57, http://dx.doi.org/10.1517/14656566.2014.881800.
37. Sabbatini S, Monari C, Ballet N, Mosci P, Decherfe AC, Pélerin F, et al. *Saccharomyces cerevisiae*-based probiotic as novel anti-microbial agent for therapy of bacterial vaginosis. Virulence. 2018;9:954–66, http://dx.doi.org/10.1080/21505594.2018.1464362.
38. Schalkwyk J, Yudin MH, Infectious Disease Committee. Vulvovaginitis screening for and management of trichomoniasis, vulvovaginal candidiasis, and bacterial vaginosis. J Obstet Gynaecol Can. 2015;37:266–74, http://dx.doi.org/10.1016/s1701-2163(15)30316-9.
39. Marrazzo JM, Dombrowski JC, Wierzbicki MR, Perlowski C, Pontius A, Dithmer D, et al. Safety and efficacy of a novel vaginal anti-infective TOL-463, in the treatment of bacterial vaginosis and vulvovaginal candidiasis: a randomized, single-blind, phase 2, controlled trial. Clin Infect Dis. 2018, http://dx.doi.org/10.1093/cid/ciy554 [in press].
40. Martín R, Soberón N, Vaneechoutte M, Flórez AB, Vazquez F, Suárez JE. Characterization of indigenous vaginal lactobacilli from healthy women as probiotic candidates. Intern Microbiol. 2008;11:261–6, http://dx.doi.org/10.2436/20.1501.01.70.
41. Martín R, Soberón N, Vazquez F, Suárez JE. Vaginal microbiota: composition, protective role, associated pathologies, and therapeutic perspectives. Enferm Infecc Microbiol Clin. 2008;26:160–7, http://dx.doi.org/10.1157/13116753.