International Society for the Study of Vulvovaginal Disease Recommendations for the Diagnosis and Treatment of Vaginitis

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- to promote international communication among gynecologists, pathologists, dermatologists, and other healthcare providers;
- to establish international agreement on terminology and definitions of vulvovaginal diseases;
- to promote clinical investigation, basic research and dissemination of knowledge in this field.

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INDEX

INDEX OF FIGURES

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PREFACE

Vulvovaginitis is among the most common gynecologic diagnoses in both primary care and lower genital tract specialist care worldwide, with most women experiencing at least one lifetime episode. Accordingly, the need for uniform, simplified and standardized management directives to both diagnose and treat vulvovaginal infection is great globally and therefore on a national basis, medical professional societies have already undertaken to publish guidelines to optimize therapy, but often with considerable differences given variation in availability of diagnostic tests, clinical expertise, drug availability and access. Moreover, the rapid progress in development and availability of new diagnostic tests and therapeutic agents dictates that guidelines be updated frequently. Unfortunately, timely updates are frequently not forthcoming. So do practitioners need yet another version of instruction and guidelines? The International Society for the Study of Vulvovaginal Disease (ISSVD) is unique, with a membership that is worldwide reflecting the variable needs and standards of different communities. So the international design of the "writing" teams afforded an opportunity to standardize guidelines to reflect the variable needs of women in societies with differences in patient needs and practitioner availability. The new ISSVD recommendations are designed to overcome cultural, social and financial differences in global societies utilizing our team approach. Also unique to ISSVD recommendations was the inclusion of a strong educational background for each clinical entity together with treatment rationale. Authors recognized that there have been major advances in diagnostic tests reflecting the application of advances in molecular technology in new superior diagnostic tests. Author opinion emphasized that the "syndromic" approach is no longer acceptable and that empiricism in treatment selection has to be avoided at all costs. Final guidelines followed extensive review and discussion. The new ISSVD recommendations will be updated on a regular and frequent basis and represent the views and experience of the society membership including highly respected experts with global reputations.

Producing the 2023 recommendations not only represents a major contribution to women's health but serves as an act of altruism by all contributors.

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NOTE

Members and non-members of the International Society for the Study of Vulvovaginal Disease (ISSVD), acknowledged as experts in the field of vulvovaginitis, from different countries and backgrounds, were invited to participate in this mission.

Participants were involved in one or more working groups, according to their expertise and interest.

Each group performed a systematic review and produced a draft based on that. The next step of the process consisted of discussion of the drafts, open to all participants involved in the development of this document. Finally, all drafts were reviewed by the editors and sent back for discussion in case of need.

The levels of evidence and grades of recommendation in the final tables of each chapter were based on the "Oxford Centre for Evidence-Based Medicine: Levels of Evidence".[1]
The final version of the document was accepted by all authors.

[1]Oxford Centre for Evidence-Based Medicine: Levels of Evidence.
https://www.cebm.ox.ac.uk/resources/levels-of-evidence/oxford-centre-for-evidence-based-medicine-levels-of-evidence-march-2009

THE NORMAL DISCHARGE

1

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1.1
The vaginal microbiome and other components of the normal discharge

Vaginal discharge is described as the fluid excreted from the vagina. It may be pathological or physiological.[1] Normal discharge is usually clear or white, and without an offensive odor. The consistency varies from thick and sticky to stretchy.[2] The normal amount of vaginal discharge is about 1-3 mL daily.[3] Women may have different concepts concerning what is a normal discharge.[4] Sometimes, women may note an increased discharge (as a symptom) and have a "normal" discharge. Nevertheless, more knowledge and markers of normality are needed.[5,6]

Fluids present in the vagina include those that originate in the vagina itself, but also from the cervix and the upper genital tract and some not produced by the woman.[7] (Figure 1.1)

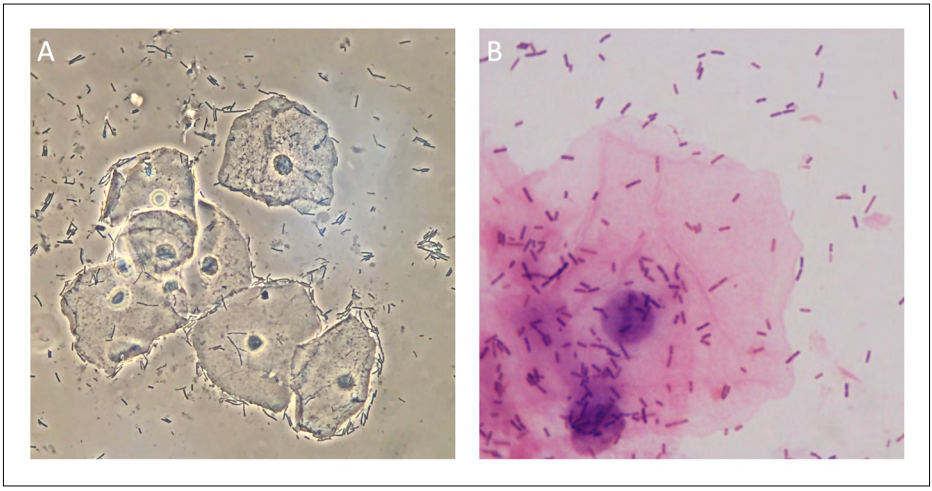

Figure 1.1 Normal vaginal discharge.

A– Wet mount microscopy (400x, phase contrast) B– Gram stain (1000x, oil immersion).

Consequently, the pH value of the vaginal fluid results from the mixture of those from the cervix, vagina, and semen if the woman recently had unprotected intercourse.
Vaginal lubrication depends on the amount and quality of the transudate from the arterial circulation. Its amount represents the predominant force of pressure from the vessels and its counterpart, the epithelial pressure. The interstitial fluid passes to the cavity and, accordingly to its rheological properties, spreads and covers the entire vagina.[8]

Among the substances contained in the vaginal fluid, there are some volatile ones that cause its particular odor, like acetic acid or cresol.[9] As lactic acid is the predominant product of lactobacilli metabolism and one of the main acidifying agents of the vaginal fluid, it is expected that lactic odor will be representative of normality. Nevertheless, the absence of perception of odor is also considered normal.

The future will show whether among the more than a thousand proteins present in the vaginal fluid there are some that will be useful in differentiating normal from non-normal fluid.[10]
The components of the vaginal discharge can be categorized into: host cells, microorganisms, and soluble components. All three create the color, odor, viscosity, and amount of the fluid.

Host cell components

Host cell components include epithelial cells and leukocytes. Multiple layers of stratified squamous epithelium line the vagina. Epithelial cells are continually shedding into the vaginal lumen.[11] Healthy vaginal fluid predominantly contains cells of the vaginal superficial layer and ectocervical epithelium, because they are not held together by tight junctions.[11, 12] It takes approximately 96 hours for epithelial cells to transit from the basal layer to the apical one. One cell layer is lost every 4 hours; however the rate of desquamation varies with intercourse, vaginal product use, and hormonal status. Disintegration of the epithelial cells is a major source of glycogen – the main substrate for lactobacilli. The junctions between epithelial cells are weaker compared with skin ones and do not keratinize or form a lipid envelope. The permeability is increased for all components, including for leucocytes.[11]

Leucocytes are also part of the cells present in a healthy vagina, with T-lymphocytes comprising the dominant type.[13] Granulocytes, B-lymphocytes and macrophages are also detectable, but are minor components.[13, 14] The composition of leucocytes differs from blood indicating that they are not a result of "passive" infiltration through the tissue.[13] However, natural killers in the vagina resemble those in the blood stream, contrary to those identified in the upper genital tract, and play an important role in limiting viral infections.[14] Cervical ectropion (a normal developmental finding in which the squamocolumnar junction is located in the ectocervix), when prominent, can cause discharge with leucocytes.[15] Transient presence of leucocytes from a partner's sperm can also occur and be a source of disease transmission. This must be considered when interpreting the presence of inflammatory cells in wet mount microscopy, therefore making it important to know the time elapsed since last intercourse.

Soluble components and mucus

Soluble components include secretions of glandular cells from the cervix and upper reproductive tract, the remains of desquamated vaginal epithelial cells, microorganisms´ metabolites, as well as multiple products transduced to the vagina from the systemic circulation. Cervical mucus coats the vaginal surface and forms a protective barrier. The composition of vaginal mucus includes 2 to 5% mucin glycoproteins and 1% of other secreted agents such as antibodies, antibacterial proteins, and peptides. Secreted mucins form a viscoelastic gel. Carbohydrates in the fluid are responsible for more than 80% of the mucinal weight and consist of N-acetyl-glucosamine, N-acetyl-galactosamine, galactose, fucose, and sialic acid.[16] Estrogens and progesterone influence the vaginal pH, viscosity and protein content.[16, 17] The subsequent release of glycogen from the shedded cells and its breakdown by vaginal amylase provide a major source of nutrients that are utilized by lactobacilli.[18] Data show that vaginal amylase is produced by both the host and various bacteria (i.e. *Lactobacillus crispatus, L. iners, Bifidobacterium lacrimalis,* and *B. vaginale*).[19] It degrades glycogen to monosaccharides, disaccharides, and trisaccharides, making it available for the lactobacilli's metabolism.[19, 20]

Vaginal concentrations of neutrophil gelatinase-associated lipocalin, matrix metalloproteinase 8, and D- and L-lactic acid levels have been reported.[18] Vaginal epithelial cells are a component of the innate immune system and release antimicrobial compounds, as well as cytokines that activate antigen-specific immunity, which are part of the soluble media of vaginal discharge.[21] The concentration of immune-active cells and compounds in the vagina varies with the composition of the vaginal microbiota. Levels are typically lower when *L. crispatus* is the dominant bacterium.[22]

Microorganisms

Bacteria, fungi, viruses, archaea, and protozoans are present in the vaginal fluids.[23] The diverse saccharolytic population, mainly composed of lactobacilli, are often referred to as Döderlein bacilli and are the most common acidifying organisms of the vaginal milieu.
The following is a general description of the vaginal microbiota and other components of the vagina that are typically present in healthy reproductive age women. It must be acknowledged, however, that due to variations in genetics, physiological factors and environmental exposures it is difficult to define the "normal" vaginal environment that encompasses all healthy women.[23]

Bacteria

Microbiota release metabolites and degrade macronutrients. The lexicon describing different aspects of the microbiome has been clarified by Verstraelen *et al.*.[23] In the majority of women, one of four species of the genus *Lactobacillus* is numerically dominant in the vagina: *L. crispatus, L. iners, L. jensenii* or *L. gasseri*.[24, 25] Lactobacilli produce lactic acid and regulate pH, modulate local immunity and release bacteriocins.[26] The reason why usually only one of these species of lactobacilli becomes predominant in a specific woman remains undetermined. The most often cited classification of vaginal microbiomes is the one established by Ravel *et al.* in 2011, which divides it into five community state types (CSTs).[24] Four of the CSTs are dominated by lactobacilli: CST-I, CST-II, CST-III, and CST-V, in which the predominant species are *L.*

crispatus, L. gasseri, L. iners, and *L. jensenii*, respectively.[23, 24] CST-IV is characterized by high bacterial diversity and is usually considered a "risky" community state in the scientific literature.[23, 27]

When lactobacilli are not numerically abundant, the most frequent alternatives are dominance by *Gardnerella* spp. or a situation in which no bacterium constitutes over 50% of the total bacterial species identified and, instead, there is a mixture of variable composition of multiple species of anaerobic and facultative bacteria. Dominance by *L. crispatus, L. jensenii* and *L. gasseri* has historically been associated with vaginal health, while the predominance of *L. iners* or diversity of bacteria are associated with vaginal dysbiosis.[28, 29] It must be noted that the majority of women, in whom the latter microbes predominate, are asymptomatic. *L. iners* is described both as having superior adaptation capacities due to resistance to hydrogen peroxide and tolerance to environmental fluctuation (pH, menstrual bleeding, mucus concentration, infection, hormones) as well as contributing to bacterial vaginosis (BV) through secretion of inerolysin.[29, 30] Long term health of women and their offspring, rather than only absence of symptoms, should be considered when evaluating the "normality" of the vaginal microbiome.[23]

Non-lactobacilli species are also present in the vagina and sometimes prevalent. This prevalence may vary according to the different stages of life and ethnical/racial factors.[23] For instance, *Prevotella* spp. or *Sneathia* spp. may dominate in neonates while *Gardnerella* spp. and *Bifidobacterium* spp. may be encountered more often in post-menopausal women.[31, 32] *Leptotrichia amnionii* and *Fannyhessea (Atopobium) vaginae* are more common among African Americans.[33] Molecular methods allow the detection of a huge variety of bacteria, but the pathogenicity of the majority remains unknown. *Mycoplasma* spp. and *Ureaplasma* spp. are examples of such.[34] Detection of bacteria (with the exclusion of cases such as *Chlamydia trachomatis* or *Neisseria gonorrhoeae*) does not define normality or abnormality of vaginal discharge. For instance, *L. iners* is present in both women with and without vaginal dysbiosis.[34] Moreover, detection of *Gardnerella* spp. is not evidence of dysbiosis.[23] Abundance and diversity of bacteria, fluctuation of CST during menstrual cycle and life span has been described in the scientific literature, showing both instability of the microbiome and, in particular cases, stability of fluctuation patterns.[3, 32, 35, 36]

Viruses

Recent studies have added to the list of identified viruses in the vagina of healthy women. Two broad group of viruses have been described: bacteriophages (viruses that infect bacteria) and other eucaryotic viruses.[37, 38]

The dominant bacteriophages belong to the Caudovirales order, especially members of the Myoviridae, Siphoviridae and Podoviridae families.[37, 39] However, this dominance may be due to reporting bias. Other bacteriophage families are Herelleviridae and Ackermannviridae, Inoviridae, Microviridae, Lipothrixviridae, Tectiviridae and Plasmaviridae. Bacteriophages play an important role in vaginal mucosa inflammation by inducing an inflammatory type-1 interferon response.[37, 40] Da Costa *et al.* in 2021, evaluating samples from 107 pregnant women, described the prevalence of phage species as: *Bacillus* spp. phages in 43.6% of women, *Escherichia* spp. phages in 40.9%, *Staphylococcus* spp. phages in 36.4%, Gokushovirus in 30.0% and *Lactobacillus* spp. phages in 26.4%.[41]

Among eucaryotic viruses, the Papillomaviridae predominate, followed by other double stranded deoxyribonucleic acid (DNA) viruses including Polyomaviridae, Herpesviridae, Genomoviridae, Adenoviridae, and Poxviridae and single-stranded DNA viruses such as those of the Anelloviridae family.[37, 42-44] DNA viruses such as Herpesviridae, Papillomaviridae, Polyomaviridae, Poxviridae and Adenoviridae families are considered pathogenic as well as ribonucleic acid (RNA) viruses such as human immunodeficiency virus (HIV) and Zika.[37] While the presence of an apparently non-pathogenic herpesvirus has been shown to increase immune sensitivity to endogenous vaginal bacteria in mice, the influence of DNA viruses in the vagina on bacterial composition has not been reported.[45]

Yeasts

The colonization of the vagina of healthy women by *Candida* spp., especially *C. albicans*, occurs frequently.[46, 47] Immune system components present in the vagina of healthy women are usually able to prevent the conversion of *C. albicans* from a benign colonization yeast morphology to an invasive hyphal form and also to limit its capacity of replication.[48, 49] In most healthy women, the presence of a low level of *C. albicans* has no apparent influence in the composition of the vaginal bacteria.[50, 51] Even more, some researchers hypothesized potential benefits, such as inhibition of *E. coli* in colonization cases.[52] *C. glabrata*, a non-hyphae forming yeast, is the second most common fungus that can be isolated from the vagina.[53, 54] In some women, especially if diabetic type I, this yeast may be responsible for symptoms.[53, 55] It seems that not the specific species, but rather the interaction of the pathogen (i.e. candidalysin secretion), host (i.e. inflammatory cytokines) and environment (i.e. microbiome, hormones, sexual activity) may lead to symptoms.[56]

1.2
Normal vaginal discharge variations during the menstrual cycle

The amount of cervical secretion decreases over the menstrual cycle, while the vaginal transudate increases. Approximately 1-3 mL of discharge are produced daily close to the menstruation. Its consistency and distribution remain stable during the whole cycle.[3]

Cyclic fluctuations of estrogen and progesterone have impact in the genital mucosal immune milieu.[57, 58] Abundance of proteins differ in follicular, ovulatory and luteal phases. The luteal and follicular phases are associated with higher activation of neutrophils/leukocytes and cell migration pathways. During the ovulatory phase the antimicrobial and wound-healing pathways are increased, while that of inflammatory cytokines is reduced. The microbiome modulates luteal phase-dependent alterations to the vaginal mucosal proteome, leading to a mucosal barrier function decrease in that phase.[57]

The proportion of leucocytes observed in wet mount microscopy slides tends to be stable during the menstrual cycle, and not correlated with white blood cell count.[3] The neutrophil, antimicrobial, and tissue homeostasis pathways may be significantly changed during the menstrual phase.[57]

Vaginal microbiome of healthy woman can be stable in each menstrual cycle or fluctuate.[36, 59, 60] Menstruation dramatically changes the microbiome composition. Two thirds of women

have high amounts of lactobacilli at the beginning of menses.[3] However, the abundance of *L. crispatus* decreases more than 100 fold, while the proportion of *L. iners* increases.[61] Vaginal microbiome diversity is much higher compared to that seen during the follicular or luteal phase.[59] Heavy growth of non-lactobacilli species is observed in the last days of the menstrual cycle, namely *Gardnerella* spp., *P. bivia,* and *F. vaginae*.[3, 61] The process is associated with vaginal pH increase. After the menses, the abundance of group B streptococci, *E. coli*, *Gardnerella* spp. and *Prevotela* spp. slightly decreases, while the amount of *C. albicans*, *Bacteroides fragilis* and *Ureaplasma urealiticum* increases.[3]

Menstruation patterns also influence the microbiome, with heavier flow associated with higher abundance of *Propionibacterium acnes* in cervical samples. Regular periods are negatively correlated with *L. vaginalis, L. johnsonii*, and *Weissella* spp., and lower levels of plasma metabolites (androstenedione, testosterone, and serum low density lipoprotein).[62]

Contraception use may lead to microbiome changes in some women, while in others the impact is minimal.[63, 64] The use of combined oral contraceptives or levonorgestrel intrauterine systems (LVN-IUS) does not seem to have a deleterious effect in the vaginal microbiome composition or diversity.[59, 65, 66] In fact, some data suggest that the use of sex hormones for contraception promotes eubiosis; this effect is not clear for progestin-only contraceptives.[64, 65] Shifts in the vaginal microbiome are mostly observed in women not using hormonal contraception and is less noticed for LVN-IUS, even after excluding women without menstrual bleeding.[59]

In conclusion, the vaginal microbiome is very sensitive to menstrual cycle and to circulating hormones. However, it is still unknown why some women have a stable microbiome and in others microbial diversity and abundance changes very rapidly.

1.3
Normal vaginal discharge in physiological estrogen deficiency (pre-menstrual girls, postpartum, and post-menopausal women)

Normal vaginal discharge varies during the different stages of life, as the vaginal microbiome is a dynamic system, depending on the host (inflammatory factors), the environment and on the adaptation of vaginal bacteria (domination of species in an ecological niche and their metabolites) to the environment (hormonal factors, sexual activity).[23, 56]

During a woman´s life cycle there are three physiologic hypotrophic vaginal periods: 1) during infancy, 2) during the post-partum and early lactation period and 3) after established menopause.[67]

After birth, the newborn's vagina is colonized by maternal lactobacilli.[31] During the first month of life, the vaginal mucosa is under the influence of maternal estrogens.[68] Due to the consequent high levels of vaginal glycogen, the amount of lactobacilli is high, the vaginal pH is low and discharge can be perceived.[69] *Lactobacillus* spp., *Prevotella* spp., or *Sneathia* spp. can be detected amongst the vaginal microbiota of newborns and tend to be similar to the mother's vaginal or skin microbiome, in case of vaginal birth or cesarian section, respectively.[23, 31] After this short period, due to vaginal mucosa de-estrogenization, the vaginal pH increases, as well as the diversity of the vaginal microbiota.

This hypoestrogenic environment, and increased pH, is maintained until puberty.[23] The vaginal microbiota in prepubertal girls is abundant in non-lactobacilli species.[68, 70] The discharge in girls is usually scarce. As the circulating levels of sex hormones gradually increase, the lactobacilli-deficient microbiota gradually shifts toward lactobacilli dominance.[23, 71, 72] Still, it remains unclear which CST and how stable the microbiome will be for the adolescent girl. The correlation of the microbiome composition between mothers and their daughters remains unestablished.[71, 73-75]

The second period of hypoestrogenic state is the post-partum period. The number of lactobacilli decreases dramatically.[73] The process can be associated with a decreased estrogen level after delivery and during breastfeeding. (Figure 1.2) Another theory claims that alkaline lochial discharge impedes *Lactobacillus* spp. growth.[76, 77] A reduced amount of lactobacilli is followed by an increase in the proportion of *Clostridia* spp., *Bacteroidia* spp., *Prevotella* spp., *Finegodia magna*, *Streptococcus anginosus* and other rare species.[77] These communities are similar to gut microbiome post-partum.[73]

Some authors state that an increased vaginal pH without symptoms of BV or other form of dysbiosis is an indicator of menopause.[78] The correlation between estradiol level and pH is well established.[79] Nevertheless, the process is more complex: during perimenopause, the level of circulating hormones decrease, reducing lactobacilli dominance and increasing the diversity of other species.[23] After the menopause, the vaginal mucosa again turns into a de-estrogenized state that leads to thinning of the epithelium. Due to the decrease in glycogen, and consequent lactobacilli reduction and elevated pH, the diversity of species present increases.[68] Gliniewizc *et al*. described six ecological clusters for postmenopausal women in accordance to dominant species: *L. crispatus, L. iners, L. gasseri, Gardnerella* spp., *Bifidobacterium* spp. and co-dominance by several taxa.[32] Previous studies that have characterized the vaginal microbiota of postmenopausal women have reported associations between different taxonomic compositions and vaginal symptoms. For example, Brotman *et al*. found that a vaginal microbiome dominated by *Fannyhessea (Atopobium*) spp. (CST IVB) was associated with mild or moderate atrophy, while dominance by *Streptococcus* spp. and *Prevotella* spp. (CST IVA) was associated with severe symptoms,[80] and Shen *et al*. reported that postmenopausal women with *L. gasseri/L. jensenii* dominated communities had less vaginal dryness compared to other women.[81]

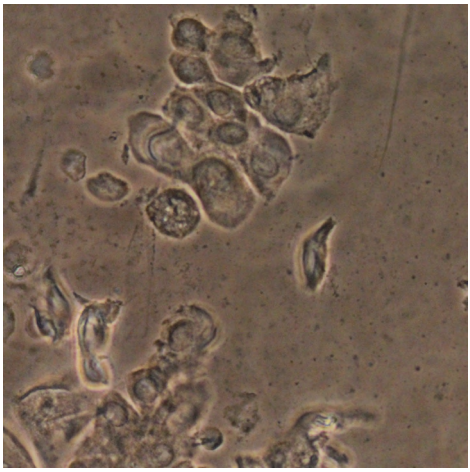

Figure 1.2 Wet mount microscopy of a vaginal sample collected from a breastfeeding woman (400x, phase contrast). Note the absence of lactobacilli and of intermediate and superficial epithelial cells.

The importance of the microbiome in relation to urinary incontinence and other urinary symptoms remains unclear.[23, 81]

1.4
Normal vaginal discharge during pregnancy

The physiological state of pregnancy is led by hormonal changes associated with immune modulation, behavioral changes, physio-chemical changes in the mucosa, and changes in the genital tract. These factors modulate the vaginal microbiome, that is different from that of non-pregnant women.[73] The amount of vaginal discharge during pregnancy increases, presumably due to increased transudation associated with vaginal congestion. The fluid is usually white or yellowish and creamy.

Lactobacilli usually dominate during pregnancy.[61, 77] The community becomes more stable and less diverse with the progression of pregnancy, an effect probably mediated by the increase in estrogen levels.[23, 73, 82] Physiological changes increase deposition of glycogen that will be broken down into lactic acid and consequently lead to a decrease of pH.[35, 61, 73, 83, 84] Upregulation of pro-inflammatory processes and D-lactic acid induce autophagy of bacteria.[61, 85] Increased ratio of D- to L-lactic acid promotes the expression of vaginal extracellular matrix metalloproteinase inducer, which in turn can activate matrix metalloproteinase-8 and subsequently alter the cervical integrity.[77, 86] Lactic acid enhances the release of IL-1β and IL-8 from vaginal epithelial cells, suggesting a synergistic relationship between inflammatory activation in the host and microbial composition.[77] These mechanisms decrease the probability of aerobic vaginitis during pregnancy, inhibit *E. coli* and stimulate *L. crispatus* dominance.[61, 87, 88] Studies show that, besides *L. crispatus* dominance among Caucasian and Asian pregnant women, *L. jensenii* and *L. gasseri* dominated communities are also common. African American women vaginal microbiota is more likely to be dominated by *L. iners* during pregnancy.[77]

1.5
Factors contributing to variations in the composition of the vaginal discharge

Stress

Stress is associated with higher risk of BV.[89, 90] Stress initiates the release of cortisol and norepinephrine from the adrenal cortex. Cortisol affects estrogen level and has an inhibitory effect on the maturation of the vaginal epithelial cells. As a consequence, due to the decrease in the amount of glycogen, the proportion of lactobacilli is reduced, and thus less lactic acid is produced.[91-93] Several studies have shown that even when co-administered with estrogens, cortisol inhibits glycogen deposition.[89, 93] This change decreases the anti-inflammatory properties of lactobacilli products and potentiates proinflammatory response, leading to abundance of facultative anaerobes or worsening symptoms of vulvovaginitis.[91] Increase of norepinephrine potentiates pro-inflammatory response and affects stability of the microbiota.[94]

Sexual activity

A study on sexual workers has shown that recent sexual activity is related with increased microbiome diversity.[95] Certain sexual behaviors increase the probability of BV.[60, 96-99] Identified risk factors include: frequency, increased number of sex partners, unprotected penile-vaginal sex or women with a female partner. On the other hand, condom use is a protective factor.[93, 100, 101]

Douching

Douching has been associated with changes in vaginal discharge, namely BV, in some women. The washing of the vagina mechanically reduces the abundance of bacteria, including lactobacilli.[93] However, inhibition of bacteria is not always the main reason for disturbance due to douching; according to Hesham *et al*., some products (i.e. vinegar) do not inhibit bacterial growth.[102] Nevertheless, there was an increased induction of vaginal epithelial cell death, triggered by a proinflammatory response with elevation of IL-6, IL-1β when vinegar or iodine were used.[93, 102] Douching inhibited *E. coli* growth. If more lactobacilli are present, less epithelial cell death is observed.[102] Some researchers observed that products, even without effect on the vaginal pH, increase the diversity of anaerobic bacteria and promote occurrence of symptomatic candidiasis.[103, 104] However, some studies have shown that stopping vaginal douching in women with BV is not enough to restore a lactobacilli dominated microbiota.[105] After careful evaluation of cultural background, douching should be discouraged.

Smoking

Cigarette smoking is associated with BV and higher prevalence of CST-IV.[106] This may be due to promotion of the growth of *Gardnerella* spp. and *Mobiluncus* spp., rather than lactobacilli depletion.[107] Biogenic amines, such as agmatine, cadaverine, putrescine, tryptamine and tyramine, affecting the virulence of infective pathogens and contributing to vaginal malodor, were higher among cigarette users.[108]

Diet

A number of studies have investigated the relationship between diet and vaginal microbiota composition. The association between the vaginal microbiota and sugar, fiber, protein, or fat intake remains unclear, despite some studies hypothesizing that a starch rich diet increases vaginal glycogen and thus may have a positive effect on lactobacilli.[109, 110] However, a high glycemic load has been related with progression of BV.[111] Also, Saraf *et al*. noted that a high fat diet contributes to estrogen increase and consequently to vaginal glycogen level.[112] Alternatively, Naggers *et al.* found that high fat intake was associated with severe BV.[112] Vegetarian diets can be related to higher vaginal microbial diversity.[109]

1.6
Racial differences in the composition of the vaginal discharge and vaginal microbiota

It is well established that the numerically dominant bacterium in the vagina varies with race. *L. crispatus, L. jensenii* and *L. gasseri* are typically more prevalent in White and Asian women than in Black and Hispanic women.[113] In African American women, the most common profile is dominance by *L. iners*, followed by *Gardnerella* spp., BV-associated bacteria (BVAB) 1, and *L. crispatus*.[114] A similar pattern has been observed among Hispanic women as well.[113] Vaginal community composition in young Black women was related to glycogen levels, not estradiol and psychosocial stress.[113] Accordingly, the mean vaginal pH is also higher in these

groups.[24, 115] Reasons for these variations in vaginal microbiota composition remain speculative. While differences in vaginal microbiota composition across different ethnic or racial groups have been reported, the association between ethnicity and vaginal microbiota composition is likely to be confounded by other factors known to influence it, including douching, sexual networks, cultural practices and other factors discussed above.

1.7 Summary and conclusions

Vaginal discharge can be both a sign and a symptom. The characteristics of the discharge that were discussed earlier included amount, color, consistency, odor, and pH value. There is no uniform pattern of normality for the vaginal discharge, as its characteristics change during menstruation, in accordance with lifestyle, over time and have racial differences. In fact and taking particularly into account the relevant role of sexual hormones in the physiology of the vagina, different normal states are defined for the newborn, the prepubertal girl, the non-pregnant woman during the reproductive age and the post menopause involutional stage. In between, transitional stages must be considered: puberty, pregnancy, puerperium and perimenopause. For decades, it was assumed almost dogmatically that the "healthy vagina" was necessarily dominated by lactobacilli. However, more recently it has been shown that a significant number of asymptomatic women harbor a non-lactobacilli dominated microbiota.[24]

Molecular methods that enable comprehensive characterization of the vaginal microbiome, metabolome and proteome, will provide further insight into the composition and function of the vaginal microbiome in health and disease.

Recommendations

Recommendation	Quality of evidence	Strength of recommendation
A perceived increased discharge is not necessarily pathological and should not automatically prompt treatment.	4	C
There is no recommendation to treat a non-ideal vaginal microbiota in the absence of symptoms.	5	D
The use of hormonal contraceptives promotes eubiosis.	4	C

References

1. Mitchell, H., Vaginal discharge--causes, diagnosis, and treatment. Bmj 2004, 328, (7451), 1306-8.
2. Rao, V. L.; Mahmood, T., Vaginal discharge. *Obstetrics, Gynaecology & Reproductive Medicine* 2020, 30, (1), 11-18.
3. Eschenbach, D. A.; Thwin, S. S.; Patton, D. L.; Hooton, T. M.; Stapleton, A. E.; Agnew, K.; Winter, C.; Meier, A.; Stamm, W. E., Influence of the normal menstrual cycle on vaginal tissue, discharge, and microflora. *Clin Infect Dis* 2000, 30, (6), 901-7.
4. Karasz, A.; Anderson, M., The vaginitis monologues: women's experiences of vaginal complaints in a primary care setting. *Soc Sci Med* 2003, 56, (5), 1013-21.

5. Anderson, M.; Karasz, A.; Friedland, S., Are vaginal symptoms ever normal? a review of the literature. *MedGenMed* 2004, 6, (4), 49.
6. Chaturvedi, S. K.; Chandra, P. S.; Issac, M. K.; Sudarshan, C. Y., Somatization misattributed to non-pathological vaginal discharge. *J Psychosom Res* 1993, 37, (6), 575-9.
7. Owen, D. H.; Katz, D. F., A vaginal fluid simulant. *Contraception* 1999, 59, (2), 91-5.
8. Dubinskaya, A.; Guthrie, T.; Anger, J. T.; Eilber, K. S.; Berman, J. R., Local Genital Arousal: Mechanisms for Vaginal Lubrication. *Current Sexual Health Reports* 2021, 13, (2), 45-53.
9. Huggins, G. R.; Preti, G., Vaginal odors and secretions. *Clin Obstet Gynecol* 1981, 24, (2), 355-77.
10. Kim, Y. E.; Kim, K.; Oh, H. B.; Lee, S. K.; Kang, D., Quantitative proteomic profiling of Cervicovaginal fluid from pregnant women with term and preterm birth. *Proteome Sci* 2021, 19, (1), 3.
11. Anderson, D. J.; Marathe, J.; Pudney, J., The structure of the human vaginal stratum corneum and its role in immune defense. *Am J Reprod Immunol* 2014, 71, (6), 618-23.
12. Donders, G. G., Definition and classification of abnormal vaginal flora. *Best Pract Res Clin Obstet Gynaecol* 2007, 21, (3), 355-73.
13. Givan, A. L.; White, H. D.; Stern, J. E.; Colby, E.; Gosselin, E. J.; Guyre, P. M.; Wira, C. R., Flow cytometric analysis of leukocytes in the human female reproductive tract: comparison of fallopian tube, uterus, cervix, and vagina. *Am J Reprod Immunol* 1997, 38, (5), 350-9.
14. Monin, L.; Whettlock, E. M.; Male, V., Immune responses in the human female reproductive tract. *Immunology* 2020, 160, (2), 106-115.
15. Mancuso, A. C.; Ryan, G. L., Normal Vulvovaginal Health in Adolescents. *J Pediatr Adolesc Gynecol* 2015, 28, (3), 132-5.
16. Moncla, B. J.; Chappell, C. A.; Debo, B. M.; Meyn, L. A., The Effects of Hormones and Vaginal Microflora on the Glycome of the Female Genital Tract: Cervical-Vaginal Fluid. *PLoS One* 2016, 11, (7), e0158687.
17. Chappell, C. A.; Rohan, L. C.; Moncla, B. J.; Wang, L.; Meyn, L. A.; Bunge, K.; Hillier, S. L., The effects of reproductive hormones on the physical properties of cervicovaginal fluid. *Am J Obstet Gynecol* 2014, 211, (3), 226.e1-7.
18. Nasioudis, D.; Beghini, J.; Bongiovanni, A. M.; Giraldo, P. C.; Linhares, I. M.; Witkin, S. S., α-Amylase in Vaginal Fluid: Association With Conditions Favorable to Dominance of Lactobacillus. *Reprod Sci* 2015, 22, (11), 1393-8.
19. Nunn, K. L.; Clair, G. C.; Adkins, J. N.; Engbrecht, K.; Fillmore, T.; Forney, L. J., Amylases in the Human Vagina. *mSphere* 2020, 5, (6).
20. Spear, G. T.; French, A. L.; Gilbert, D.; Zariffard, M. R.; Mirmonsef, P.; Sullivan, T. H.; Spear, W. W.; Landay, A.; Micci, S.; Lee, B. H.; Hamaker, B. R., Human α-amylase present in lower-genital-tract mucosal fluid processes glycogen to support vaginal colonization by Lactobacillus. *J Infect Dis* 2014, 210, (7), 1019-28.
21. Linhares, I. M.; Sisti, G.; Minis, E.; de Freitas, G. B.; Moron, A. F.; Witkin, S. S., Contribution of Epithelial Cells to Defense Mechanisms in the Human Vagina. *Curr Infect Dis Rep* 2019, 21, (9), 30.
22. Dabee, S.; Barnabas, S. L.; Lennard, K. S.; Jaumdally, S. Z.; Gamieldien, H.; Balle, C.; Happel, A. U.; Murugan, B. D.; Williamson, A. L.; Mkhize, N.; Dietrich, J.; Lewis, D. A.; Chiodi, F.; Hope, T. J.; Shattock, R.; Gray, G.; Bekker, L. G.; Jaspan, H. B.; Passmore, J. S., Defining characteristics of genital health in South African adolescent girls and young women at high risk for HIV infection. *PLoS One* 2019, 14, (4), e0213975.
23. Verstraelen, H.; Vieira-Baptista, P.; De Seta, F.; Ventolini, G.; Lonnee-Hoffmann, R.; Lev-Sagie, A., The Vaginal Microbiome: I. Research Development, Lexicon, Defining "Normal" and the Dynamics Throughout Women's Lives. *J Low Genit Tract Dis* 2022, 26, (1), 73-78.
24. Ravel, J.; Gajer, P.; Abdo, Z.; Schneider, G. M.; Koenig, S. S.; McCulle, S. L.; Karlebach, S.; Gorle, R.; Russell, J.; Tacket, C. O.; Brotman, R. M.; Davis, C. C.; Ault, K.; Peralta, L.; Forney, L. J., Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci U S A* 2011, 108 Suppl 1, (Suppl 1), 4680-7.
25. Lamont, R. F.; Sobel, J. D.; Akins, R. A.; Hassan, S. S.; Chaiworapongsa, T.; Kusanovic, J. P.; Romero, R., The vaginal microbiome: new information about genital tract flora using molecular based techniques. *Bjog* 2011, 118, (5), 533-49.
26. Kovachev, S., Defence factors of vaginal lactobacilli. *Crit Rev Microbiol* 2018, 44, (1), 31-39.
27. McKinnon, L. R.; Achilles, S. L.; Bradshaw, C. S.; Burgener, A.; Crucitti, T.; Fredricks, D. N.; Jaspan, H. B.; Kaul, R.; Kaushic, C.; Klatt, N.; Kwon, D. S.; Marrazzo, J. M.; Masson, L.; McClelland, R. S.; Ravel, J.; van de Wijgert, J.; Vodstrcil, L. A.; Tachedjian, G., The Evolving Facets of Bacterial Vaginosis: Implications for HIV Transmission. *AIDS Res Hum Retroviruses* 2019, 35, (3), 219-228.
28. Han, Y.; Liu, Z.; Chen, T., Role of Vaginal Microbiota Dysbiosis in Gynecological Diseases and the Potential Interventions. *Front Microbiol* 2021, 12, 643422.
29. Petrova, M. I.; Reid, G.; Vaneechoutte, M.; Lebeer, S., Lactobacillus iners: Friend or Foe? *Trends Microbiol* 2017, 25, (3), 182-191.

30. Macklaim, J. M.; Gloor, G. B.; Anukam, K. C.; Cribby, S.; Reid, G., At the crossroads of vaginal health and disease, the genome sequence of Lactobacillus iners AB-1. *Proc Natl Acad Sci U S A* 2011, 108 Suppl 1, (Suppl 1), 4688-95.
31. Dominguez-Bello, M. G.; Costello, E. K.; Contreras, M.; Magris, M.; Hidalgo, G.; Fierer, N.; Knight, R., Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A* 2010, 107, (26), 11971-5.
32. Gliniewicz, K.; Schneider, G. M.; Ridenhour, B. J.; Williams, C. J.; Song, Y.; Farage, M. A.; Miller, K.; Forney, L. J., Comparison of the Vaginal Microbiomes of Premenopausal and Postmenopausal Women. *Front Microbiol* 2019, 10, 193.
33. Srinivasan, S.; Hoffman, N. G.; Morgan, M. T.; Matsen, F. A.; Fiedler, T. L.; Hall, R. W.; Ross, F. J.; McCoy, C. O.; Bumgarner, R.; Marrazzo, J. M.; Fredricks, D. N., Bacterial communities in women with bacterial vaginosis: high resolution phylogenetic analyses reveal relationships of microbiota to clinical criteria. *PLoS One* 2012, 7, (6), e37818.
34. Lev-Sagie, A.; De Seta, F.; Verstraelen, H.; Ventolini, G.; Lonnee-Hoffmann, R.; Vieira-Baptista, P., The Vaginal Microbiome: II. Vaginal Dysbiotic Conditions. *J Low Genit Tract Dis* 2022, 26, (1), 79-84.
35. Romero, R.; Hassan, S. S.; Gajer, P.; Tarca, A. L.; Fadrosh, D. W.; Nikita, L.; Galuppi, M.; Lamont, R. F.; Chaemsaithong, P.; Miranda, J.; Chaiworapongsa, T.; Ravel, J., The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. *Microbiome* 2014, 2, (1), 4.
36. Chaban, B.; Links, M. G.; Jayaprakash, T. P.; Wagner, E. C.; Bourque, D. K.; Lohn, Z.; Albert, A. Y.; van Schalkwyk, J.; Reid, G.; Hemmingsen, S. M.; Hill, J. E.; Money, D. M., Characterization of the vaginal microbiota of healthy Canadian women through the menstrual cycle. *Microbiome* 2014, 2, 23.
37. Madere, F. S.; Monaco, C. L., The female reproductive tract virome: understanding the dynamic role of viruses in gynecological health and disease. *Curr Opin Virol* 2022, 52, 15-23.
38. Happel, A. U.; Balle, C.; Maust, B. S.; Konstantinus, I. N.; Gill, K.; Bekker, L. G.; Froissart, R.; Passmore, J. A.; Karaoz, U.; Varsani, A.; Jaspan, H., Presence and Persistence of Putative Lytic and Temperate Bacteriophages in Vaginal Metagenomes from South African Adolescents. *Viruses* 2021, 13, (12).
39. Jakobsen, R. R.; Haahr, T.; Humaidan, P.; Jensen, J. S.; Kot, W. P.; Castro-Mejia, J. L.; Deng, L.; Leser, T. D.; Nielsen, D. S., Characterization of the Vaginal DNA Virome in Health and Dysbiosis. *Viruses* 2020, 12, (10).
40. Van Belleghem, J. D.; Dąbrowska, K.; Vaneechoutte, M.; Barr, J. J.; Bollyky, P. L., Interactions between Bacteriophage, Bacteria, and the Mammalian Immune System. *Viruses* 2018, 11, (1).
41. Da Costa, A. C.; Moron, A. F.; Forney, L. J.; Linhares, I. M.; Sabino, E.; Costa, S. F.; Mendes-Correa, M. C.; Witkin, S. S., Identification of bacteriophages in the vagina of pregnant women: a descriptive study. *Bjog* 2021, 128, (6), 976-982.
42. Wylie, K. M.; Wylie, T. N.; Cahill, A. G.; Macones, G. A.; Tuuli, M. G.; Stout, M. J., The vaginal eukaryotic DNA virome and preterm birth. *Am J Obstet Gynecol* 2018, 219, (2), 189.e1-189.e12.
43. Wylie, K. M.; Mihindukulasuriya, K. A.; Zhou, Y.; Sodergren, E.; Storch, G. A.; Weinstock, G. M., Metagenomic analysis of double-stranded DNA viruses in healthy adults. *BMC Biol* 2014, 12, 71.
44. Eskew, A. M.; Stout, M. J.; Bedrick, B. S.; Riley, J. K.; Omurtag, K. R.; Jimenez, P. T.; Odem, R. R.; Ratts, V. S.; Keller, S. L.; Jungheim, E. S.; Wylie, K. M., Association of the eukaryotic vaginal virome with prophylactic antibiotic exposure and reproductive outcomes in a subfertile population undergoing in vitro fertilisation: a prospective exploratory study. *Bjog* 2020, 127, (2), 208-216.
45. Cardenas, I.; Mor, G.; Aldo, P.; Lang, S. M.; Stabach, P.; Sharp, A.; Romero, R.; Mazaki-Tovi, S.; Gervasi, M.; Means, R. E., Placental viral infection sensitizes to endotoxin-induced pre-term labor: a double hit hypothesis. *Am J Reprod Immunol* 2011, 65, (2), 110-7.
46. Sobel, J. D., Recurrent vulvovaginal candidiasis. *Am J Obstet Gynecol* 2016, 214, (1), 15-21.
47. Sobel, J. D., Vulvovaginal candidosis. *Lancet* 2007, 369, (9577), 1961-71.
48. Verma, A.; Gaffen, S. L.; Swidergall, M., Innate Immunity to Mucosal Candida Infections. *J Fungi (Basel)* 2017, 3, (4).
49. Rosati, D.; Bruno, M.; Jaeger, M.; Ten Oever, J.; Netea, M. G., Recurrent Vulvovaginal Candidiasis: An Immunological Perspective. *Microorganisms* 2020, 8, (2).
50. Brown, S. E.; Schwartz, J. A.; Robinson, C. K.; O Hanlon, D. E.; Bradford, L. L.; He, X.; Mark, K. S.; Bruno, V. M.; Ravel, J.; Brotman, R. M., The Vaginal Microbiota and Behavioral Factors Associated With Genital Candida albicans Detection in Reproductive-Age Women. *Sex Transm Dis* 2019, 46, (11), 753-758.
51. D'Enfert, C.; Kaune, A. K.; Alaban, L. R.; Chakraborty, S.; Cole, N.; Delavy, M.; Kosmala, D.; Marsaux, B.; Fróis-Martins, R.; Morelli, M.; Rosati, D.; Valentine, M.; Xie, Z.; Emritloll, Y.; Warn, P. A.; Bequet, F.; Bougnoux, M. E.; Bornes, S.; Gresnigt, M. S.; Hube, B.; Jacobsen, I. D.; Legrand, M.; Leibundgut-Landmann, S.; Manichanh, C.; Munro, C. A.; Netea, M. G.; Queiroz, K.; Roget, K.; Thomas, V.; Thoral, C.; Van den Abbeele, P.; Walker, A. W.; Brown, A. J. P., The impact of the Fungus-Host-Microbiota interplay upon Candida albicans infections: current knowledge and new perspectives. *FEMS Microbiol Rev* 2021, 45, (3).

52. De Seta, F.; Lonnee-Hoffmann, R.; Campisciano, G.; Comar, M.; Verstraelen, H.; Vieira-Baptista, P.; Ventolini, G.; Lev-Sagie, A., The Vaginal Microbiome: III. The Vaginal Microbiome in Various Urogenital Disorders. *J Low Genit Tract Dis* 2022, 26, (1), 85-92.
53. Kennedy, M. A.; Sobel, J. D., Vulvovaginal Candidiasis Caused by Non-albicans Candida Species: New Insights. *Curr Infect Dis Rep* 2010, 12, (6), 465-70.
54. Powell, A. M.; Gracely, E.; Nyirjesy, P., Non-albicans Candida Vulvovaginitis: Treatment Experience at a Tertiary Care Vaginitis Center. *J Low Genit Tract Dis* 2016, 20, (1), 85-9.
55. Ray, D.; Goswami, R.; Banerjee, U.; Dadhwal, V.; Goswami, D.; Mandal, P.; Sreenivas, V.; Kochupillai, N., Prevalence of Candida glabrata and its response to boric acid vaginal suppositories in comparison with oral fluconazole in patients with diabetes and vulvovaginal candidiasis. *Diabetes Care* 2007, 30, (2), 312-7.
56. Willems, H. M. E.; Ahmed, S. S.; Liu, J.; Xu, Z.; Peters, B. M., Vulvovaginal Candidiasis: A Current Understanding and Burning Questions. *J Fungi (Basel)* 2020, 6, (1).
57. Bradley, F.; Birse, K.; Hasselrot, K.; Noël-Romas, L.; Introini, A.; Wefer, H.; Seifert, M.; Engstrand, L.; Tjernlund, A.; Broliden, K.; Burgener, A. D., The vaginal microbiome amplifies sex hormone-associated cyclic changes in cervicovaginal inflammation and epithelial barrier disruption. *Am J Reprod Immunol* 2018, 80, (1), e12863.
58. Wira, C. R.; Rodriguez-Garcia, M.; Patel, M. V., The role of sex hormones in immune protection of the female reproductive tract. *Nat Rev Immunol* 2015, 15, (4), 217-30.
59. Krog, M. C.; Hugerth, L. W.; Fransson, E.; Bashir, Z.; Nyboe Andersen, A.; Edfeldt, G.; Engstrand, L.; Schuppe-Koistinen, I.; Nielsen, H. S., The healthy female microbiome across body sites: effect of hormonal contraceptives and the menstrual cycle. *Hum Reprod* 2022, 37, (7), 1525-1543.
60. Gajer, P.; Brotman, R. M.; Bai, G.; Sakamoto, J.; Schütte, U. M.; Zhong, X.; Koenig, S. S.; Fu, L.; Ma, Z. S.; Zhou, X.; Abdo, Z.; Forney, L. J.; Ravel, J., Temporal dynamics of the human vaginal microbiota. *Sci Transl Med* 2012, 4, (132), 132ra52.
61. Amabebe, E.; Anumba, D. O. C., The Vaginal Microenvironment: The Physiologic Role of Lactobacilli. *Front Med* (Lausanne) 2018, 5, 181.
62. Jie, Z.; Chen, C.; Hao, L.; Li, F.; Song, L.; Zhang, X.; Zhu, J.; Tian, L.; Tong, X.; Cai, K.; Zhang, Z.; Ju, Y.; Yu, X.; Li, Y.; Zhou, H.; Lu, H.; Qiu, X.; Li, Q.; Liao, Y.; Zhou, D.; Lian, H.; Zuo, Y.; Chen, X.; Rao, W.; Ren, Y.; Wang, Y.; Zi, J.; Wang, R.; Liu, N.; Wu, J.; Zhang, W.; Liu, X.; Zong, Y.; Liu, W.; Xiao, L.; Hou, Y.; Xu, X.; Yang, H.; Wang, J.; Kristiansen, K.; Jia, H., Life History Recorded in the Vagino-cervical Microbiome Along with Multi-omics. *Genomics Proteomics Bioinformatics* 2021.
63. Balle, C.; Konstantinus, I. N.; Jaumdally, S. Z.; Havyarimana, E.; Lennard, K.; Esra, R.; Barnabas, S. L.; Happel, A. U.; Moodie, Z.; Gill, K.; Pidwell, T.; Karaoz, U.; Brodie, E.; Maseko, V.; Gamieldien, H.; Bosinger, S. E.; Myer, L.; Bekker, L. G.; Passmore, J. S.; Jaspan, H. B., Hormonal contraception alters vaginal microbiota and cytokines in South African adolescents in a randomized trial. *Nat Commun* 2020, 11, (1), 5578.
64. Bastianelli, C.; Farris, M.; Bianchi, P.; Benagiano, G., The effect of different contraceptive methods on the vaginal microbiome. *Expert Rev Clin Pharmacol* 2021, 14, (7), 821-836.
65. Ratten, L. K.; Plummer, E. L.; Bradshaw, C. S.; Fairley, C. K.; Murray, G. L.; Garland, S. M.; Bateson, D.; Tachedjian, G.; Masson, L.; Vodstrcil, L. A., The Effect of Exogenous Sex Steroids on the Vaginal Microbiota: A Systematic Review. *Front Cell Infect Microbiol* 2021, 11, 732423.
66. Bassis, C. M.; Allsworth, J. E.; Wahl, H. N.; Sack, D. E.; Young, V. B.; Bell, J. D., Effects of intrauterine contraception on the vaginal microbiota. *Contraception* 2017, 96, (3), 189-195.
67. Pérez-López, F. R.; Vieira-Baptista, P.; Phillips, N.; Cohen-Sacher, B.; Fialho, S.; Stockdale, C. K., Clinical manifestations and evaluation of postmenopausal vulvovaginal atrophy. *Gynecol Endocrinol* 2021, 37, (8), 740-745.
68. Godha, K.; Tucker, K. M.; Biehl, C.; Archer, D. F.; Mirkin, S., Human vaginal pH and microbiota: an update. *Gynecol Endocrinol* 2018, 34, (6), 451-455.
69. Farage, M.; Maibach, H., Lifetime changes in the vulva and vagina. Arch Gynecol Obstet 2006, 273, (4), 195-202.
70. Zuckerman, A.; Romano, M., Clinical Recommendation: Vulvovaginitis. *J Pediatr Adolesc Gynecol* 2016, 29, (6), 673-679.
71. Hickey, R. J.; Zhou, X.; Settles, M. L.; Erb, J.; Malone, K.; Hansmann, M. A.; Shew, M. L.; Van Der Pol, B.; Fortenberry, J. D.; Forney, L. J., Vaginal microbiota of adolescent girls prior to the onset of menarche resemble those of reproductive-age women. *mBio* 2015, 6, (2).
72. Biro, F. M.; Pinney, S. M.; Huang, B.; Baker, E. R.; Walt Chandler, D.; Dorn, L. D., Hormone changes in peripubertal girls. *J Clin Endocrinol Metab* 2014, 99, (10), 3829-35.
73. Gupta, P.; Singh, M. P.; Goyal, K., Diversity of Vaginal Microbiome in Pregnancy: Deciphering the Obscurity. *Front Public Health* 2020, 8, 326.
74. Rasmussen, M. A.; Thorsen, J.; Dominguez-Bello, M. G.; Blaser, M. J.; Mortensen, M. S.; Brejnrod, A. D.; Shah, S. A.; Hjelmsø, M. H.; Lehtimäki, J.; Trivedi, U.; Bisgaard, H.; Sørensen, S. J.; Stokholm, J., Ecological succession in the vaginal microbiota during pregnancy and birth. *The ISME Journal* 2020, 14, (9), 2325-2335.

75. France, M. T.; Brown, S. E.; Rompalo, A. M.; Brotman, R. M.; Ravel, J., Identification of shared bacterial strains in the vaginal microbiota of related and unrelated reproductive-age mothers and daughters using genome-resolved metagenomics. *PLOS ONE* 2022, 17, (10), e0275908.
76. Odogwu, N. M.; Onebunne, C. A.; Chen, J.; Ayeni, F. A.; Walther-Antonio, M. R. S.; Olayemi, O. O.; Chia, N.; Omigbodun, A. O., Lactobacillus crispatus thrives in pregnancy hormonal milieu in a Nigerian patient cohort. *Sci Rep* 2021, 11, (1), 18152.
77. MacIntyre, D. A.; Chandiramani, M.; Lee, Y. S.; Kindinger, L.; Smith, A.; Angelopoulos, N.; Lehne, B.; Arulkumaran, S.; Brown, R.; Teoh, T. G.; Holmes, E.; Nicoholson, J. K.; Marchesi, J. R.; Bennett, P. R., The vaginal microbiome during pregnancy and the postpartum period in a European population. *Sci Rep* 2015, 5, 8988.
78. Lehtoranta, L.; Ala-Jaakkola, R.; Laitila, A.; Maukonen, J., Healthy Vaginal Microbiota and Influence of Probiotics Across the Female Life Span. *Front Microbiol* 2022, 13, 819958.
79. Caillouette, J. C.; Sharp, C. F., Jr.; Zimmerman, G. J.; Roy, S., Vaginal pH as a marker for bacterial pathogens and menopausal status. *Am J Obstet Gynecol* 1997, 176, (6), 1270-5; discussion 1275-7.
80. Brotman, R. M.; Shardell, M. D.; Gajer, P.; Fadrosh, D.; Chang, K.; Silver, M. I.; Viscidi, R. P.; Burke, A. E.; Ravel, J.; Gravitt, P. E., Association between the vaginal microbiota, menopause status, and signs of vulvovaginal atrophy. *Menopause* 2014, 21, (5), 450-8.
81. Shen, J.; Song, N.; Williams, C. J.; Brown, C. J.; Yan, Z.; Xu, C.; Forney, L. J., Effects of low dose estrogen therapy on the vaginal microbiomes of women with atrophic vaginitis. *Sci Rep* 2016, 6, 24380.
82. Walther-António, M. R.; Jeraldo, P.; Berg Miller, M. E.; Yeoman, C. J.; Nelson, K. E.; Wilson, B. A.; White, B. A.; Chia, N.; Creedon, D. J., Pregnancy's stronghold on the vaginal microbiome. *PLoS One* 2014, 9, (6), e98514.
83. Smith, S. B.; Ravel, J., The vaginal microbiota, host defence and reproductive physiology. *J Physiol* 2017, 595, (2), 451-463.
84. Hay, P., Vaginal discharge. *Medicine* 2018, 46, (6), 319-324.
85. Ramos Bde, A.; Kanninen, T. T.; Sisti, G.; Witkin, S. S., Microorganisms in the female genital tract during pregnancy: tolerance versus pathogenesis. *Am J Reprod Immunol* 2015, 73, (5), 383-9.
86. Witkin, S. S.; Mendes-Soares, H.; Linhares, I. M.; Jayaram, A.; Ledger, W. J.; Forney, L. J., Influence of vaginal bacteria and D- and L-lactic acid isomers on vaginal extracellular matrix metalloproteinase inducer: implications for protection against upper genital tract infections. *mBio* 2013, 4, (4).
87. Donders, G.; Bellen, G.; Rezeberga, D., Aerobic vaginitis in pregnancy. *Bjog* 2011, 118, (10), 1163-70.
88. Vieira-Baptista, P.; Lima-Silva, J.; Pinto, C.; Saldanha, C.; Beires, J.; Martinez-de-Oliveira, J.; Donders, G., Bacterial vaginosis, aerobic vaginitis, vaginal inflammation and major Pap smear abnormalities. *Eur J Clin Microbiol Infect Dis* 2016, 35, (4), 657-64.
89. Nansel, T. R.; Riggs, M. A.; Yu, K. F.; Andrews, W. W.; Schwebke, J. R.; Klebanoff, M. A., The association of psychosocial stress and bacterial vaginosis in a longitudinal cohort. *Am J Obstet Gynecol* 2006, 194, (2), 381-6.
90. Turpin, R.; Slopen, N.; Borgogna, J. C.; Yeoman, C. J.; He, X.; Miller, R. S.; Klebanoff, M. A.; Ravel, J.; Brotman, R. M., Perceived Stress and Molecular Bacterial Vaginosis in the National Institutes of Health Longitudinal Study of Vaginal Flora. *Am J Epidemiol* 2021, 190, (11), 2374-2383.
91. Amabebe, E.; Anumba, D. O. C., Psychosocial Stress, Cortisol Levels, and Maintenance of Vaginal Health. *Front Endocrinol (Lausanne)* 2018, 9, 568.
92. Padgett, D. A.; Glaser, R., How stress influences the immune response. Trends Immunol 2003, 24, (8), 444-8.
93. Kwon, M. S.; Lee, H. K., Host and Microbiome Interplay Shapes the Vaginal Microenvironment. *Front Immunol* 2022, 13, 919728.
94. Witkin, S. S.; Linhares, I. M., Why do lactobacilli dominate the human vaginal microbiota? *Bjog* 2017, 124, (4), 606-611.
95. Sivro, A.; Mwatelah, R.; Kambaran, C.; Gebrebrhan, H.; Becker, M. G.; Ma, H.; Klatt, N. R.; Zevin, A. S.; King'ola, N.; Wambua, S.; Gichangi, P.; Cheuk, E.; McLaren, P. J.; Mishra, S.; Becker, M.; McKinnon, L. R., Sex Work Is Associated With Increased Vaginal Microbiome Diversity in Young Women From Mombasa, Kenya. *J Acquir Immune Defic Syndr* 2020, 85, (1), 79-87.
96. Ratten, L. K.; Plummer, E. L.; Murray, G. L.; Danielewski, J.; Fairley, C. K.; Garland, S. M.; Hocking, J. S.; Tachedjian, G.; Chow, E.; Bradshaw, C. S.; Vodstrcil, L. A., Sex is associated with the persistence of non-optimal vaginal microbiota following treatment for bacterial vaginosis: a prospective cohort study. *Bjog* 2021, 128, (4), 756-767.
97. Plummer, E. L.; Vodstrcil, L. A.; Fairley, C. K.; Tabrizi, S. N.; Garland, S. M.; Law, M. G.; Hocking, J. S.; Fethers, K. A.; Bulach, D. M.; Murray, G. L.; Bradshaw, C. S., Sexual practices have a significant impact on the vaginal microbiota of women who have sex with women. *Sci Rep* 2019, 9, (1), 19749.
98. Wessels, J. M.; Lajoie, J.; Vitali, D.; Omollo, K.; Kimani, J.; Oyugi, J.; Cheruiyot, J.; Kimani, M.; Mungai, J. N.; Akolo, M.; Stearns, J. C.; Surette, M. G.; Fowke, K. R.; Kaushic, C., Association of high-risk sexual behaviour with diversity of the vaginal microbiota and abundance of Lactobacillus. *PLoS One* 2017, 12, (11), e0187612.
99. Schwebke, J. R.; Richey, C. M.; Weiss, H. L., Correlation of behaviors with microbiological changes in vaginal flora. *J Infect Dis* 1999, 180, (5), 1632-6.

100. Fethers, K. A.; Fairley, C. K.; Hocking, J. S.; Gurrin, L. C.; Bradshaw, C. S., Sexual risk factors and bacterial vaginosis: a systematic review and meta-analysis. *Clin Infect Dis* 2008, 47, (11), 1426-35.
101. Vodstrcil, L. A.; Muzny, C. A.; Plummer, E. L.; Sobel, J. D.; Bradshaw, C. S., Bacterial vaginosis: drivers of recurrence and challenges and opportunities in partner treatment. *BMC Med* 2021, 19, (1), 194.
102. Hesham, H.; Mitchell, A. J.; Bergerat, A.; Hung, K.; Mitchell, C. M., Impact of vaginal douching products on vaginal Lactobacillus, Escherichia coli and epithelial immune responses. *Sci Rep* 2021, 11, (1), 23069.
103. Van der Veer, C.; Bruisten, S. M.; van Houdt, R.; Matser, A. A.; Tachedjian, G.; van de Wijgert, J.; de Vries, H. J. C.; van der Helm, J. J., Effects of an over-the-counter lactic-acid containing intra-vaginal douching product on the vaginal microbiota. *BMC Microbiol* 2019, 19, (1), 168.
104. Gondwe, T.; Ness, R.; Totten, P. A.; Astete, S.; Tang, G.; Gold, M. A.; Martin, D.; Haggerty, C. L., Novel bacterial vaginosis-associated organisms mediate the relationship between vaginal douching and pelvic inflammatory disease. *Sex Transm Infect* 2020, 96, (6), 439-444.
105. Brown, S. E.; He, X.; Shardell, M. D.; Ravel, J.; Ghanem, K. G.; Zenilman, J. M.; Brotman, R. M., Douching cessation and molecular bacterial vaginosis: a reanalysis of archived specimens. *Sex Transm Infect* 2022.
106. Brotman, R. M.; He, X.; Gajer, P.; Fadrosh, D.; Sharma, E.; Mongodin, E. F.; Ravel, J.; Glover, E. D.; Rath, J. M., Association between cigarette smoking and the vaginal microbiota: a pilot study. *BMC Infect Dis* 2014, 14, 471.
107. Tužil, J.; Filková, B.; Malina, J.; Kerestes, J.; Doležal, T., Smoking in women with chronic vaginal discomfort is not associated with decreased abundance of Lactobacillus spp. but promotes Mobiluncus and Gardnerella spp. overgrowth - secondary analysis of trial data including microbio-me analysis. *Ceska Gynekol* 2021, 86, (1), 22-29.
108. Nelson, T. M.; Borgogna, J. C.; Michalek, R. D.; Roberts, D. W.; Rath, J. M.; Glover, E. D.; Ravel, J.; Shardell, M. D.; Yeoman, C. J.; Brotman, R. M., Cigarette smoking is associated with an altered vaginal tract metabolomic profile. *Sci Rep* 2018, 8, (1), 852.
109. Song, S. D.; Acharya, K. D.; Zhu, J. E.; Deveney, C. M.; Walther-Antonio, M. R. S.; Tetel, M. J.; Chia, N., Daily Vaginal Microbiota Fluctuations Associated with Natural Hormonal Cycle, Contraceptives, Diet, and Exercise. mSphere 2020, 5, (4).
110. Miller, E. A.; Beasley, D. E.; Dunn, R. R.; Archie, E. A., Lactobacilli Dominance and Vaginal pH: Why Is the Human Vaginal Microbiome Unique? *Front Microbiol* 2016, 7, 1936.
111. Thoma, M. E.; Klebanoff, M. A.; Rovner, A. J.; Nansel, T. R.; Neggers, Y.; Andrews, W. W.; Schwebke, J. R., Bacterial vaginosis is associated with variation in dietary indices. *J Nutr* 2011, 141, (9), 1698-704.
112. Saraf, V. S.; Sheikh, S. A.; Ahmad, A.; Gillevet, P. M.; Bokhari, H.; Javed, S., Vaginal microbiome: normalcy vs dysbiosis. *Arch Microbiol* 2021, 203, (7), 3793-3802.
113. Nunn, K. L.; Ridenhour, B. J.; Chester, E. M.; Vitzthum, V. J.; Fortenberry, J. D.; Forney, L. J., Vaginal Glycogen, Not Estradiol, Is Associated With Vaginal Bacterial Community Composition in Black Adolescent Women. *J Adolesc Health* 2019, 65, (1), 130-138.
114. Fettweis, J. M.; Brooks, J. P.; Serrano, M. G.; Sheth, N. U.; Girerd, P. H.; Edwards, D. J.; Strauss, J. F.; The Vaginal Microbiome, C.; Jefferson, K. K.; Buck, G. A., Differences in vaginal microbiome in African American women versus women of European ancestry. *Microbiology (Reading)* 2014, 160, (Pt 10), 2272-2282.
115. Sun, S.; Serrano, M. G.; Fettweis, J. M.; Basta, P.; Rosen, E.; Ludwig, K.; Sorgen, A. A.; Blakley, I. C.; Wu, M. C.; Dole, N.; Thorp, J. M.; Siega-Riz, A. M.; Buck, G. A.; Fodor, A. A.; Engel, S. M., Race, the Vaginal Microbiome, and Spontaneous Preterm Birth. *mSystems* 2022, 7, (3), e0001722.

DIAGNOSTIC TOOLS

2

(alphabetical order)
Ana Rita Silva
Carlos Sousa
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2.1 Introduction

The diagnosis of vaginitis is often done empirically, based on symptoms and mere gynecological observation, leading frequently to misdiagnosis and mistreatment. Before initiating any therapy, confirmation of the diagnosis must be obtained, thus minimizing those risks.[1, 2] Nevertheless, diagnostic tests often are not used. In a study performed in 2021 in the US, in women with symptoms of vaginitis, it was shown that pH measurement, whiff test and wet mount microscopy (WMM) were performed in only 15%, 21% and 17% of cases, respectively.[3]

2.2 pH

Despite significant ethnic and geographical variation, as well as across the woman's life cycle, the mean pH ranges between 3.8-5.0.[4, 5] Lactobacilli, under the influence of estrogens, are responsible for maintaining the low pH, thus, dysbiotic conditions associated with decreased lactobacilli are typically associated with an increased pH.[6] Other genera, such as *Fannyhessea (Atopobium)*[7], *Megasphaera*, and *Leptotrichia* are also lactic acid–producing bacteria and can contribute to reduce the vaginal pH. The relation is bidirectional, as not only the bacteria modulate the pH, but the opposite is also true.[8]

Nevertheless, a "normal" pH cannot be assumed as equivalent to absence of "vaginitis". For instance, *Candida* spp. which itself does not affect the vaginal pH, can be found across the whole spectrum of the vaginal pH and for other conditions, such as bacterial vaginosis (BV), there is an overlap between normal and abnormal pH.[9]

Evaluation of the vaginal pH is not useful in the presence of blood, recent exposure to semen or to vaginal medication. In postmenopausal women not using menopause hormone treatment, the pH is typically increased – making it a good predictor of hypoestrogenism, but of limited value for the diagnosis of vaginitis.

In the presence of an elevated pH, the diagnosis of BV, aerobic vaginitis (AV)/desquamative inflammatory vaginitis (DIV), trichomoniasis, atrophic vaginitis or of cervicitis (*Chlamydia trachomatis* or *Neisseria gonorrhoeae*) must be considered.[10] While candidiasis can occur at any pH, it is more common and more symptomatic at pH ≤4.5.[8] Within the range of normal to low pH, the diagnosis of cytolytic vaginosis (CV) must also be considered.[11]

The pH is one of the four Amsel criteria, which are used for the diagnosis of BV (see section 2.7). While the sensitivity of the isolated pH measurement (using a cut-off of 4.5) is relatively good for the diagnosis of BV (79.0% [95% CI 72.08–84.95%]), the specificity and positive predictive values are low (56.4% [95% CI 52.22–60.43] and 34.2% [31.53–36.97], respectively).[12] This comes as no surprise, given the overlap of situations that course with a high pH. Better sensitivities can be achieved by reducing the cut-off, but that leads to an unacceptable drop in the specificity and positive predictive value.[12] Of note, the 4.5 cut-off value is still within the normal range of vaginal pH, especially when considering Black and Hispanic populations.[5]

For measurement, a pH strip with an adequate range and intervals can be placed directly, for a few seconds, in contact with the vaginal wall or with the discharge collected in the blade of the (unmoistened) speculum, avoiding cervical secretions. (Figure 2.1)

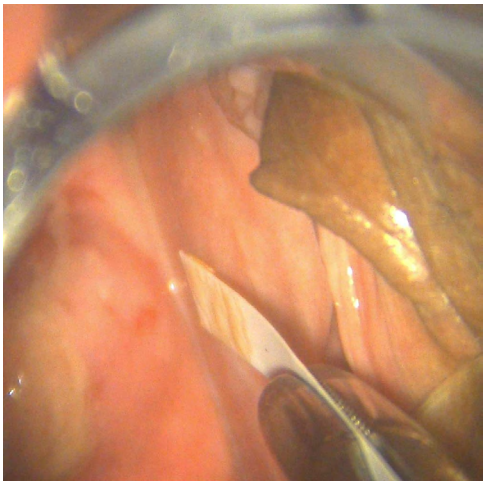

Figure 2.1 Vaginal pH measurement.

Alternatively, the discharge can be collected with an unmoistened spatula or swab and put in contact with the pH strip. Another possible strategy may be applying the sample into the microscopy slide and checking the pH on the slide, before the application of saline.[13] The reading should be made within 5 minutes after the collection. One study suggests that if the pH is measured after the adding of saline to the sample, it may be reasonable to subtract 0.5 from the pH reading, but we do not recommend this approach.[14]

In research, a broad range pH scale may be desirable (i.e. Macherey-Nagel 3.6–6.1 [Macherey–Nagel GmbH & Co. KG, Duren, Germany]).[15, 16] For clinical practice, a narrower interval may be more practical (i.e. 4.0-5.5).

One study showed that self-evaluation of the pH, by collecting a sample with a gloved finger and spreading it into a slide to which a pH strip was attached, allowed effective identification of abnormal vaginal microbiota and was considered "easy" by more than 90% of the participating women.[17, 18] Other studies showed that self-sampling, using a swab, was also feasible in both adolescents and adult women and had a moderate (Cohen's κ 0.53) and almost perfect agreement (Cohen's κ 0.90), respectively, with the clinicians interpretation.[19, 20]

Several tests are commercially available, under the recommendation to be used in case of symptoms of vaginitis.[21] Their usefulness is relative and based on a dichotomic and insufficient concept (candidiasis *vs*. BV). Some studies show a very good performance for these tests – surprisingly superior to that of pH evaluation by clinicians in the setting of clinical studies.[22]

There are commercial tests, such as the FemExam® (Cooper Surgical, CT, USA) and the QuickVue Advance pH and Amines® (Quidel Corporation, CA, USA) that evaluate the pH along with the presence of specific enzymes (see section 2.8).[23, 24]

The role of vaginal pH is limited in settings in which a microscope, cultures and molecular tests are available. Nevertheless, in settings in which these are not available and when identifying dysbiosis is relevant (i.e. during pregnancy), it can be useful.[8] Also, it has been suggested as possibly being useful for "screening" of BV and/or trichomoniasis during pregnancy.[25, 26]

2.3 Whiff test

The whiff test is another of the four components of the Amsel criteria. A whiff-amine test is considered positive when a fishy odor occurs following the addition of a drop of 10% potassium hydroxide (KOH) to a sample of vaginal discharge. In cases in which the fishy odor is readily noticed, there is no need to add the KOH. The alkalinization of the discharge leads to the release of volatile amines, which are perceived as a rotten or fishy odor. A positive whiff test is highly suggestive of BV and/or trichomoniasis.

It must be kept in mind that KOH is caustic and thus must be handled carefully. For that reason, the slide should not be placed too close to the face or directly under the nose. Also, it can damage the microscope objectives.[27]

Different studies showed the whiff test to have an excellent specificity (>90%), but a very low sensitivity (around 40%).[12, 28]

The Cohen's kappa agreement rate between users is substantial (0.68). Possible causes for disagreement include different KOH concentrations, delay in the performance of the test, insufficient discharge sample, interference of the collecting device (i.e. cotton swab), and different abilities to smell.[29] There are evidence showing promising results with the use of "electronic noses".[30, 31]

2.4 Wet mount microscopy

WMM is currently the most important tool in the diagnosis of women with symptoms of vulvovaginitis, despite being performed only by a limited number of practitioners.[3] While it is not a perfect test, it is the gold standard for the diagnosis of AV/DIV and CV and performs very well in the diagnosis of BV (sensitivity 82-100% and specificity 93-97%). The performance of WMM for candidiasis seems to be more discrepant across studies (sensitivity 44-78% and specificity 75-89%). In general, it is considered insufficient for the diagnosis of trichomoniasis (sensitivity 25-82% and specificity 98-100%). WMM can be a very useful resource to evaluate "mixed infections", the presence of inflammation and the maturation status of the vaginal mucosa.[27]

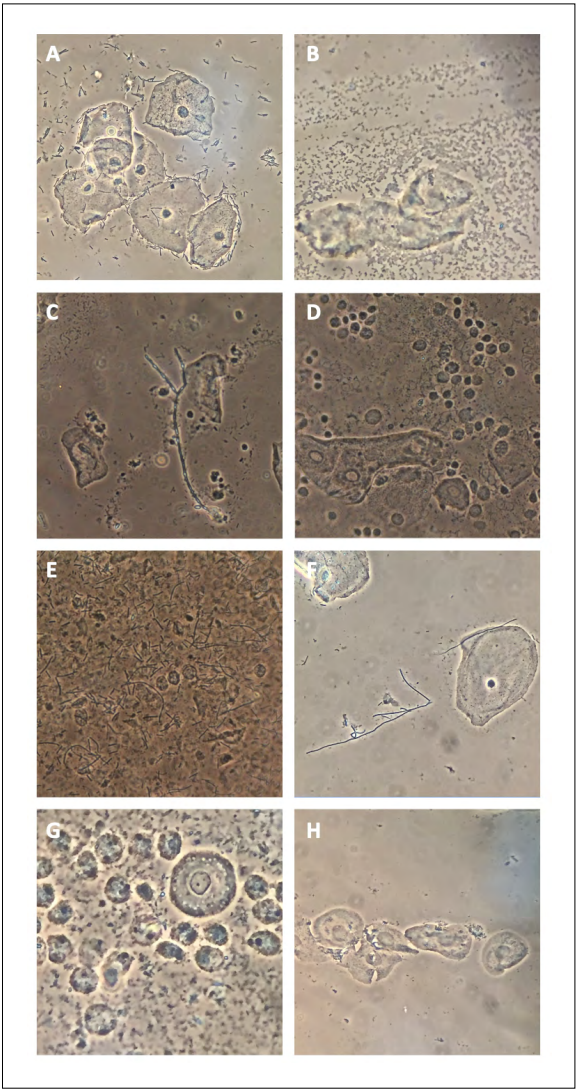

Figure 2.2 Wet mount microscopy (400x, phase constrast).

A– Normal
B– Bacterial vaginosis
C– Candidiasis
D– Trichomoniasis
E– Cytolytic vaginosis
F– Leptothrix
G– Desquamative inflammatory vaginitis (severe aerobic vaginitis)
H– Vaginal atrophy

Apart from the investment in a microscope, this an inexpensive technique, that often allows an immediate diagnosis and appropriate treatment. It has been shown that a training of merely 10 hours is enough to acquire the skills to perform and accurately interpret WMM.[32] The ISSVD recommends that all providers diagnosing and treating women with vulvovaginal symptoms should have training on this technique.[27]

Specific details on the WMM diagnosis of vaginitis will be provided in the diagnosis section of each of the addressed entities and are summarized in table 2.1; examples can be seen in figure 2.2.

Further details on the performance and interpretation of WMM can be found in "The International Society for the Study of Vulvovaginal Disease Vaginal Wet Mount Microscopy Guidelines: How to Perform, Applications, and Interpretation", published in 2021.[27]

We recommend the use of phase contrast, despite the initial higher investment it represents. Since the slides are usually evaluated under a 400x magnification, only the 40x objective lens needs to be phase contrast, in order to reduce the investment.[27] The use of phase contrast allows better identification of fungal structures, as well as of cells and background microbiota. (Figure 2.3)

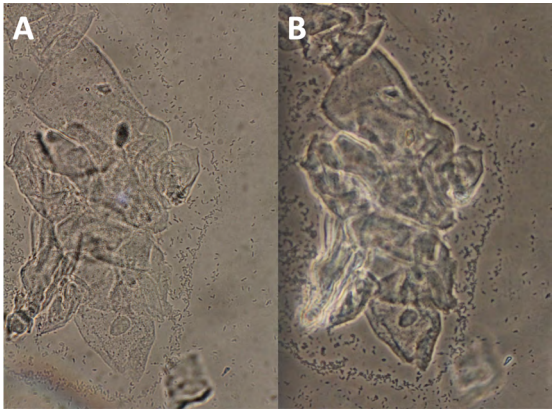

Figure 2.3 Wet mount microscopy (400x); granular microbiota suggestive of bacterial vaginosis.

A– Without phase contrast B– With phase contrast

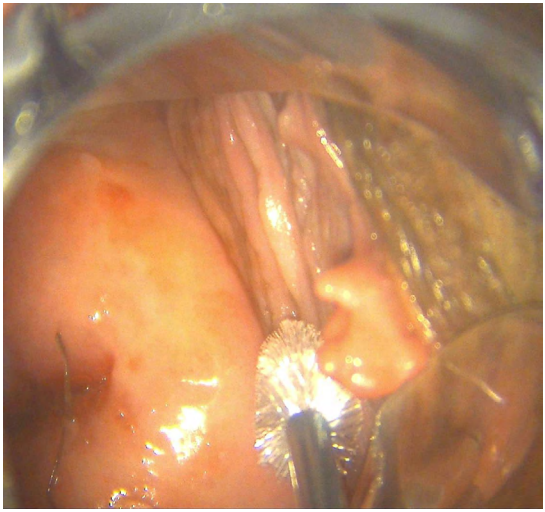

Figure 2.4 Sampling of vaginal discharge for wet mount microscopy

Nevertheless, it does not solve the lack of agreement between observers, concerning the evaluation of inflammation.[33] The use of KOH is not necessary if phase contrast is used.[27]

The sample should ideally be collected when the woman is symptomatic, and without having used any vaginal products, nor sexual intercourse or bleeding in the previous 48-72 hours.[34] For women with suspected BV, the follicular phase may be the best moment for evaluation, while the luteal phase may be better if candidiasis or CV are suspected.[35]

There is no consensus on the best place from where to collect the sample. However, the posterior fornix should be avoided, as it tends to be more exposed to the cervical secretions and thus, have a higher pH and more inflammatory cells. For the same reason, touching the cervix should be avoided. (Figure 2.4)

One study showed a higher sensitivity for *Candida* spp. and BV if the sampling is performed from the lower third of the vagina; for CV, the best results were achieved when sampling from the anterior fornix. However, the study was performed on mostly asymptomatic women and these differences may be less relevant in symptomatic women.[36]

There are no studies validating the use of self-sampling. Except for the diagnosis of trichomoniasis, there seems to be no advantage in the use of a speculum for sampling.

TABLE 2.1 Wet mount microscopy characteristics of different conditions.
AV – aerobic vaginitis, BV – bacterial vaginosis, DIV – desquamative inflammatory vaginitis, NAAT – nucleic acid amplification tests

	Lactobacilli	Background microbiota	Inflammation	Epithelial cells	Other/ comments
Candidiasis	Usually normal, but may coexist with any type of background microbiota	Usually lactobacilli dominance	Absent or mild/ moderate	According to expected for hormonal status	Presence of blastospores Sometimes mycelium present (suggestive of *C. albicans*)
Bacterial vaginosis	Absent or scarce	Granular microbiota Curved motile rods may be present (*Mobiluncus* spp.)	Absent	A significant portion covered by bacteria (clue cells)	"Partial BV" is possible
Trichomoniasis	Usually absent or scarce	Granular microbiota (BV) often present	Usually present (moderate/ severe) "Toxic" (swollen) leukocytes often present	Parabasal and basal cells often present	Presence of motile protozoans (absence does not exclude the hypothesis) Erythrocytes sometimes present
Cytolytic vaginosis	Abundant	Only lactobacilli	Absent	Variable degrees of cellular lysis (bare nuclei and cytoplasm debris)	Evaluate preferably in the 2nd phase of the cycle
Lactobacillosis / leptothtrix	Presence of elongated lactobacilli (8-15x the normal size) May coexist with normal lactobacilli	Variable	Variable	Variable	Evaluation preferably in the 2nd phase of the cycle Can coexist with any other type of microbiota Lactobacillosis used sometimes to describe increased lactobacilli, without cytolysis
Aerobic vaginitis/ desquamative inflammatory vaginitis	Absent or scarce	Dominance of cocci (sometimes in chains) or small rods	Moderate/severe inflammation "Toxic" leukocytes often present	Parabasal and basal cells often present	Trichomoniasis should be excluded in severe forms

Vaginal atrophy	Absent or scarce	Absent or presence of cocci or small rods	Absent	Mostly parabasal and basal cells (usually low quantity)	Erythrocytes often present
Atrophic vaginitis	Absent or scarce	Absent or presence of cocci or small rods	Moderate/severe inflammation "Toxic" leukocytes often present	Parabasal and basal cells	Differential diagnosis with AV/DIV, trichomoniasis and lichen planus
Cervicitis	Normal or decreased	Normal/mixed	Moderate/severe Sometimes with mucous filaments	According to expected hormonal status	NAAT test (*C. trachomatis* and *N. gonorrhoea*) needed if cervicitis is suspected
Lichen planus	Often decreased	Normal/mixed/ absent	Moderate/severe	Parabasal cells, sometimes with cellular wall defects	Vulvar involvement common

Thus, not using a speculum may be a good option in women with vulvodynia or other causes of vulvar pain (i.e. herpes), for those who refuse speculum examination, or girls. If a speculum is used, it should be unmoistened.[37, 38]

The sample should be collected avoiding touching the cervix or scraping the vaginal epithelium. Several devices can be used, including an endocervical brush, a plastic spatula, a Dacron swab or even a talc-free gloved finger.[39] Cotton swabs and wooden spatulas are not ideal, as they can leave fibers or absorb water, respectively.[27]

The preparation of the slide can be done either by applying a tiny drop of saline and adding a small amount of discharge to it or by spreading the sample on the slide and then adding the drop of saline (the first method may be preferable if the discharge is profuse, as it will help to dilute it and allow a better visualization of its components, with less overlap). Afterwards, a coverslip is placed and pressed to avoid the formation of air bubbles, and the excessive amount of fluid is removed using absorbent paper. Some authors opt to prepare another slide, using KOH. As mentioned before, it seems less relevant if using phase contrast. If used, extra care should be taken to clean the excess amount of fluid, as it can damage the objective lens.

The sample should be read immediately, in order to increase the sensitivity for *T. vaginalis* (warming the slide can increase its motility and allow a better identification).[40] Nevertheless, deferred reading is also an option. In that case, the sample is spread onto the slide, allowed to air dry, and later rehydrated.

2.5
Gram and other staining techniques

Gram stain has been used for the diagnosis of BV for almost 60 years.[41] The Nugent score, applied on Gram-stained slides, is considered the gold standard for the diagnosis of BV.[42] It allows an accurate evaluation of the background bacterial morphotypes, despite some debate on whether or not it is superior to WMM, as the former is associated with some degree of wash out of the background bacteria during the fixation and staining process.[43] Self-sampling can be used for Nugent score, with an excellent agreement with physician collected samples.[44, 45] For the best results, the smear should be spread evenly and be very thin, to reduce the overlap of cells.

For the calculation of the Nugent score, the amount of the different bacterial morphotypes is semi-quantitively evaluated directly in the Gram-stained smears of vaginal discharge and the presence of epithelial cells covered with bacteria (clue cells) is also evaluated (Table 2.2). That quantification is subjective: the scoring intervals are relatively narrow, only a limited number BV associated bacteria are evaluated and it relies on the identification of bacterial morphotypes rather than on that of species. The evaluated bacterial genera are 1) *Lactobacillus* spp., 2) *Gardnerella* spp./*Bacteroides* spp., and 3) *Mobiluncus* spp.[46] This may be improved in the future by the use of multiplex peptide nucleic acid fluorescence *in situ* hybridization, targeting the different evaluated species of bacteria, rather than relying on morphological identification.[47]

The bacterial morphotypes are counted using an oil immersion objective (1000x magnification). The score achieved for each of the evaluated bacterial morphotypes is added together, with a total score of 0-3 considered normal (healthy microbiota) and a score of 7 or worse is consistent with BV. A score of 4-6 is classified as intermediate[48], which constitutes another disadvantage of the Nugent score because it does not represent a "partial" or "light" form of BV and there is no specific management for it. (Table 2.2)

TABLE 2.2 Calculation of the Nugent score.
The reading is performed under a high-power magnification.
Score 0-3 normal, 4-6 intermediate, >6 bacterial vaginosis

Score	**Lactobacilli** (Gram positive rods)	***Gardnerella* spp./*Bacteroides* spp.** (tiny, Gram-variable coccobacilli and rounded, pleomorphic Gram-negative rods)	***Mobiluncus* spp.** (curved, Gram-negative rods)
0	>30	0	0
1	5-30	<1	1-5
2	1-4	1-4	>5
3	<1	5-30	--
4	0	>30	--

The Nugent score is typically used in research, but as the result is not readily available, it is time consuming, more expensive than other alternatives (i.e. WMM, Amsel criteria), and requires experienced technicians and facilities, it is often not the first choice in clinical practice.

In the future, clinical criteria for the diagnosis of AV/DIV and CV may be further developed and allow its accurate diagnosis using Gram stain samples. Also, artificial intelligence may be applied to Gram stain and increase the role of this technique in the evaluation of women with vulvovaginal symptoms.[49]

An interesting alternative to the Nugent score is the Ison and Hay criteria, in which a quantitative estimation of the bacterial morphotypes is not performed. Instead, an evaluation of the relationship between the amounts of bacteria (lactobacilli, mixed bacteria, and cocci morphotypes) is performed. As the evaluation is relative, nor the field size of the microscope nor the "concentration" of the samples influence the impression.[46, 50] Additionally, it provides a more comprehensive evaluation of the vaginal microbiome, as it includes a pattern with absent bacteria (grade 0) and one dominated by cocci (grade IV), probably corresponding partially to AV/DIV. (Table 2.3)

TABLE 2.3 Ison and Hay criteria (adapted from Ison *et al.*)[46]

Grade 0	No lactobacilli or other microbiota present
Grade I	*Lactobacillus* spp. morphotypes only
Grade II	Reduced *Lactobacillus* spp. morphotype with mixed bacterial morphotypes
Grade III	Mixed bacterial morphotype with few or absent *Lactobacillus* spp. morphotypes
Grade IV	Gram-positive cocci only

This classification system can be used on slides with different staining methods and also on non-stained smears, but further validation is needed in this field. This simplified assessment of Gram stained smears can be used as an alternative to Nugent score; a good agreement between both has been shown.[51]

While Gram stain is currently not the first recommendation for the diagnosis of cervicitis, if an endocervical sample is collected and significant inflammation is present (>30 polymorphonuclear leukocytes/high power field), and specially in a suggestive clinical context, it must be considered. In cases of gonococcal cervicitis, the presence of Gram-negative diplococci can be noted. Nevertheless, a negative microscopy does not rule out the infection and, if indicated, a molecular test should be performed.

While the Pap test can provide useful information regarding the presence of microorganisms and/or inflammation, it is not a recommended test for the study of a woman with suspected vaginitis, due to low sensitivity.[52] The specificity for the diagnosis of candidiasis, trichomoniasis and BV is usually high, but the sensitivities may be as low as 25%, 61.4% and 55%, respectively. One possible explanation for this performance may be the fact that the collected sample is cervical rather than vaginal. The performances may be different for conventional and liquid based cytology (increased in the latter for the presence of *Candida* spp. and *T. vaginalis* and decreased for BV).[53, 54] Nevertheless, it can point to less common diagnostics, such as presence of *C. trachomatis*, herpes simplex virus, cytomegalovirus, *Enterobius vermicularis or Schistosoma* spp.[53]

2.6
Cultures

Cultures have a role in the diagnosis of vaginitis, especially in the case of candidiasis, for which it is considered the gold standard. Even though, in most cases of acute candidiasis, there may be no advantage in performing routine cultures, as long as WMM is performed and confirms the diagnosis. Cultures for *Candida* are mandatory in cases of recurrent candidiasis, suspicion of non-*albicans* candidiasis, negative microscopy and symptoms suggestive of candidiasis and in cases of therapeutic failure.[54, 55]

Antifungal sensitivity tests are not routinely used, in part because they are not easily available, but should be considered when a resistant strain is suspected. It must be taken into consideration that the pH at which these are routinely performed (usually 7.0) is higher than that of the vagina and does not reflect the real sensitivities in clinical practice. It has been shown that the minimal inhibitory concentration (MIC) is increased at lower pH for miconazole, clotrimazole, fluconazole, and nystatin.[56, 57]

The samples for mycological cultures should preferably be transported using a transport means (i.e. Amies or Stuart) and should be cultivated in no more than six hours.[58]

There seems to be no disparities between the different culture means (Sabouraud agar, Nickerson medium, or Microstix-Candida medium).[54]

We do not recommend routine cultures for bacteria for the study of vaginitis, group A streptococci infection suspicion being the exception.[59] In general, a positive result does not distinguish colonization from infection. Nevertheless, the presence of *N. gonorrhea* should always prompt treatment, as it is always pathogenic. On the other hand, a positive culture for *Gardnerella* spp., sometimes wrongly considered a surrogate for BV, is not useful, due to its very low specificity: it can be cultivated from more than half of healthy, asymptomatic women.[60]

The usefulness of cultures in women with suspected AV/DIV is also of limited interest, as the diagnosis is established by microscopy.[61]

Cultures are an option for the diagnosis of trichomoniasis. The media used can be the Modified Diamond medium or the InPouch® TV (BioMed Diagnostics Inc., USA), which have a similar performance.[62] The reported sensitivities and specificities range between 44-96% and up to 100%, respectively. [63-65] Cultures are especially useful in cases of suspected resistance but are not readily available commercially. Cultures using Modified Diamond medium can take up to seven days; for the InPouch® TV, the majority of positive results can be identified in the first three days.[63]

While the molecular approach is the recommended one for the diagnosis of gonorrhea, a sample of endocervical exudate should be cultured to evaluate the antibiotic susceptibility profiles, in case of treatment failure. A selective medium for *N. gonorrhoeae* (Thayer-Martin or Martin-Lewis) should be used, along with a general medium, such as blood agar and chocolate agar, as some strains of *N. gonorrhoeae* can be inhibited in selective media.[66]

2.7
Amsel criteria

In 1983, Amsel R *et al*. evaluated 397 consecutive women presenting to a gynecology office and investigated different criteria for the diagnosis of "non-specific vaginitis".[67] Later, these would be known as the "Amsel criteria" and the "non-specific vaginitis" as BV.

These include:
1. A thin grey or white, homogeneous vaginal discharge coating the vaginal walls;
2. Vaginal pH>4.5;
3. A fishy/rotten smell (before or after the addition of KOH [whiff test]);
4. Presence of clue cells on wet mount microscopy.

To be considered positive, a minimum of three out of the four criteria must be present.
The reported ranges of sensitivity across studies are very broad (37-70%), while the specificity is systematically very high.[42]

These criteria have the advantage of allowing an immediate diagnosis, being of low complexity and cheap. While it may be useful in clinical practice, in the absence of expertise or availability of a microscope or other tests (i.e. enzymatic or molecular tests), there are significant limitations to its use. For instance, the evaluation of the characteristics of the vaginal discharge are subjective and may be affected by the use of previous vaginal medication or douches. Traditionally, it has been considered that the "normal" pH is lower than 4.5, a concept that is now under challenge; a study published in 2021 showed that using this cut-off the sensitivity of the pH for the diagnosis of BV is 79% but the specificity is only 56%.[5, 12] Also, this criterion is of very limited value in postmenopausal women not using hormone treatments for menopause/atrophy. The reading of the pH in paper strips is also subjective, as previously mentioned. The same is also true for the evaluation of odor. Also, given the microbiological heterogeneity of BV, there is a chance that bacteria producing volatile amines may not be significantly present.[68] The evaluation of clue cells implies the existence of a microscope and expertise in its use – limiting the Amsel use in most settings to the other three criteria. Under these circumstances, the sensitivity of the Amsel criteria may drop to as low as 22.8%.[12] On the other hand, if there is expertise in the use of WMM, it is an excellent tool for the diagnosis of BV, by evaluating not only the presence of clue cells, but also the background microbiota.[27]

The Amsel criteria are still recommended by some scientific societies, but its role is currently questioned and considered unacceptable by some for a definite diagnosis.[42, 69-71] If used, it must be kept in mind that there is a risk of overlooking "mixed infections", specially of BV and trichomoniasis or candidiasis (coexisting with BV in 60-80% and 20-30% of cases, respectively). (Figure 2.5)

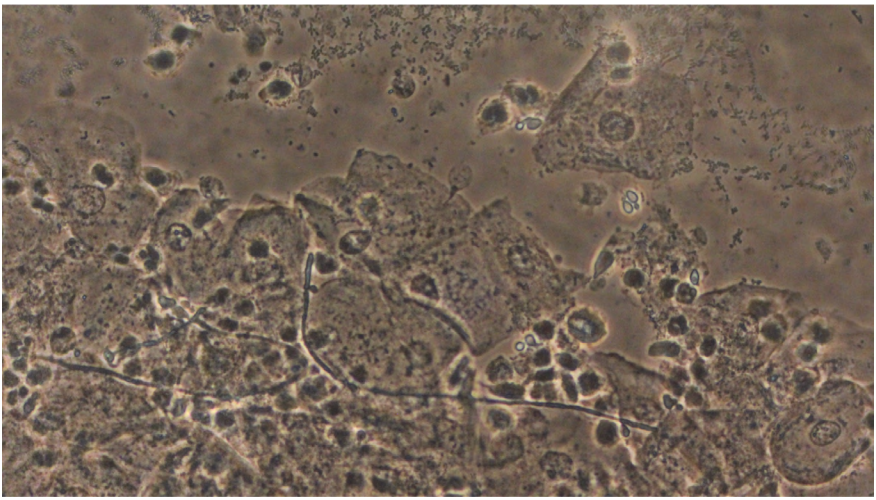

Figure 2.5 [72] Wet mount microscopy with phase contrast (400x) showing the presence of a "mixed infection" (*Candida* spp. and bacterial vaginosis)

In a possible (future) context of screening (i.e. pregnancy), the use of these criteria may prove to be more challenging, as its performance may be lower in these circumstances (lack of significant discharge, no odor). These limitations, in part, translate the complexity of BV, in which, up to now no specific isolated bacterial marker has been proved to be universally present – and different bacteria and its interaction may lead to different symptoms/signs.[68]

2.8 Enzymatic tests

The OSOM® BVBlue® (Sekisui Diagnostics, MA, USA) is a commercially available chromogenic enzymatic test for the diagnosis of BV, with a reported sensitivity of 88-94% and a specificity of around 96%.[73, 74] It evaluates the levels of the enzyme sialidase in a sample of vaginal discharge. The results are available in 10 minutes. Self-sampling for sialidase tests has been shown to be specific (90%) but to have a much lower sensitivity (40%).[19]

The FemExam® (Cooper Surgical, CT, USA) is a diagnostic kit, composed of two plastic cards, with the approximate size of a credit card, designed for a fast and inexpensive diagnosis of BV. The first card is used to evaluate the presence of an elevated pH and of trimethylamine; the second card measures the proline iminopeptidase (PIP) activity of *Gardnerella* spp. (discussed ahead). The vaginal discharge sample is rubbed in the indicated place in the cards and will show a positive sign for pH if it is >4.7 and for trimethylamine if it is detected (acting as a surrogate for a positive whiff test). One study showed, that a positive result for pH and trimethylamine had a sensitivity of 71.4% (61.7–79.8) and a specificity of 72.8% (63.7–80.7).[23] Card 2 of the FemExam® evaluates the PIP activity of *Gardnerella* spp.. Its sensitivity and specificity for the

diagnosis of BV are 70.0% (55.4–82.1) and 80.9% (69.1–71.6), respectively. If both cards are used and the test is considered positive when at least two out of the three evaluated variables are present, the sensitivity and specificity are 91.0% (83.1–96.0) and 61.5% (50.7–71.6), respectively.[23] Samples can be self-collected and results are available in five minutes; the use of this test is not recommended when blood is present.[23, 75]

QuickVue Advance pH and Amines® (Quidel Corp, San Diego, CA), has a reported sensitivity of 53% and a specificity of 97%.[24] In this test, the pH cut-off is 4.5 and the amine evaluated is trimethylamine.

These tests can be an option in settings where microscopy is not available and perform well for the diagnosis of BV. It must be kept in mind that they do not provide information concerning other conditions, such as candidiasis or trichomoniasis, which frequently coexist with BV.

2.9 Molecular tests

Given the limitation of microscopy and point-of-care tests, and in line with what is occurring in other fields of medicine, there is a trend towards the use of molecular tests for diagnostic purposes. These allow the diagnosis in a reasonable time (hours), are precise and reproducible, capable of high throughput, and allow the identification of fastidious microorganisms. Also, the demand for self-sampling is growing in women's health and molecular tests will likely fulfill this requirement; there are already data suggesting that this approach is not inferior to the use of clinician collected samples.[76] Currently, in most settings, price is still a limitation to its widespread use.

The "first generation" of molecular tests for the diagnosis of vaginitis were direct probe assays. These are nucleic acid probes that bind to sequences (usually ribosomal RNA) specific to the targeted microorganisms in a vaginal discharge sample.

The BD Affirm™ VPIII (BD Diagnostic Systems, Sparks, Maryland) is a test based on DNA probes, that detects the presence of *Candida* spp. (including *C. albicans, C. glabrata, C. kefyr, C. krusei, C. parapsilosis,* and *C. tropicalis*), *T. vaginalis* and *Gardnerella* spp., in vaginal samples, providing results in 45 minutes.[77] The sampling is usually from the vaginal walls, but it has been suggested that if done from the speculum blades leftover the performance is similar.[78] The reported sensitivity and specificity for *Candida* spp., are 69.4 to 82.76% and 98.80 to 100%, respectively.[77, 79-81] The sensitivity for BV has been reported to range between 75.9 and 96.2% and the specificity from 60.6 to >95%.[27, 77, 81-83] While the specificity for trichomoniasis is also high, the sensitivity is lower (46.3-100%).[77, 79, 84] One study comparing the Affirm™ VPIII with the BD Max™ vaginal panel (Table 2.4) showed the latter to be more accurate: higher specificity for BV and more sensitive to *Candida* spp., without a decrease in specificity.[77] It can be used in symptomatic women, along with pH and whiff test, for a better performance.[42]

In the Affirm™ VPIII, the diagnosis of BV is based on the detection of *Gardnerella* spp. (being positive if the load is higher than 5×10^5 colony forming units [CFUs] per milliliter). While targeting a single agent for the diagnosis of BV seems a strategy of limited interest, the results are reasonable.[41, 85]

While one study showed that the performance of the Affirm™ VPIII for the diagnosis of BV is

unchanged in pregnant women[86], another one, using self-collected vaginal swabs concluded that it had insufficient sensitivity for BV, candidiasis and trichomoniasis during pregnancy.[87]

Besides the provided transport means, the use of the ESwab™ (Copan Diagnostics, Murrieta, CA) has also been validated as an option.[88]

Among a survey of members of the American College of Obstetricians and Gynecologists, performed in 2018, 25% noted using this test in their clinical practice.[89]

More recently, nucleic acid amplification tests (NAATs), including polymerase chain reaction (PCR) entered the market and are very promising approaches for the diagnosis and management of vaginitis. (Table 2.4) These have been acknowledged as the gold standard for the diagnosis of trichomoniasis for some years.[42] Adding the possibility of using a molecular approach to test at once not only trichomoniasis, but also candidiasis and BV is of great interest. For *T. vaginalis* and *Candida* spp. the challenge was not that high (it was requested to identify one species or genus), in striking contrast with BV (a complex multibacterial condition, of which there is no specific marker).[6] While correctly identifying bacteria or other microorganisms is an uncomplicated task for NAATs, defining BV through this technology is much more complex: it requires identifying and quantifying diverse BV associated bacteria, as well as lactobacilli, to evaluate their relative proportions.[90]

There are several validated and commercially available tests for the isolated diagnosis of trichomoniasis (i.e. Xpert® TV, Aptima TV[91, 92]) and BV (i.e. Allplex™ Bacterial Vaginosis assay, MDL OneSwab® BV Panel AmpliSens®).[93-95]

More relevant for the clinical practice are the tests that allow the concomitant diagnosis of BV, candidiasis and trichomoniasis. We found clinical validation data for:

- Allplex™ Vaginitis (Seegene, Seoul, Korea);
- BD Max™ Vaginal Panel (Becton Dickinson, USA);
- Hologic Aptima® BV (Hologic, USA);
- NuSwab® VG (LabCorp, USA).

Another available test, to which we could not identify published validation data is the Quest Diagnostics SureSwab® BV (Quest Diagnostics, USA).[96]

In general, the available tests have shown great performance, for all three conditions. One study showed a lower sensitivity for the BD Max™ vaginal panel, but it must be taken into account that the comparison was made against amplicon sequencing of the 16S ribosomal RNA, which is currently not standardized.[97]

Van der Pol *et al*. showed a very high agreement in the detection of *T. vaginalis* between the BD Max™ Vaginal Panel and the BD Max™ CT/GC/TV assay.[98]

These tests have the advantage of being unaffected by the presence of multiple infections or by prevalence variation of the conditions.[16, 76, 79] Real world data shows that the use of NAATs can improve value-based care and even lead to a 12% risk reduction of preterm delivery in symptomatic pregnant women.[99] A molecular approach leads to more accurate diagnosis, decreasing both nondiagnosis and overdiagnosis and, consequently, allowing timely and correct treatment.[100]

TABLE 2.4 Nucleic acid amplification tests for the diagnosis of vaginitis.
*Calculated from the data in the paper.; § clinician collected sample; + patient collected sample.
BV – bacterial vaginosis, BVAB – bacterial vaginosis associated bacteria, NA not applicable; NAC – non-*albicans* candidiasis

Test	Study	Bacterial vaginosis		Candida		Trichomoniasis		Notes
		Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	
Allplex™ Vaginitis (Seegene®, Seoul, Korea)	Vieira-Baptista *et al*[16]	91.7% (86.49-95.40%)	86.6% (83.57-89.24%)	All species 91.1% (82.23–96.08%) *C. albicans* 88.1% (77.82–94.70%) NAC 100% (85.18–100.00%)	All species 95.6% (93.65–97.12%) *C. albicans* 98.2% (96.83–99.11%) NAC 97.5% (95.73–98.38%)	94.4% (72.71–99.86%)	99.9% (99.25–100%)	- One swab from the upper third of the vagina - NAC includes: *C. glabrata, C. tropicalis, C. parapsilosis, C. krusei, C. lusitaniae* and *C. dubliniensis* - BV diagnosis based on the evaluation of: *Fannyhessea (Atopobium) vaginae, Gardnerella* spp., *Lactobacillus* spp., *Mobiluncus* spp
Aptima® CV/TV + Aptima® BV (Hologic, San Diego, USA)	Richter S *et al*[27]	90.0% (76.4–96.6%)	92.3% (81.3–97.5%)	NA	NA	NA	NA	- Candida group includes: *C. albicans, C. parapsilosis, C. tropicalis, C. dubliniensis* - Separably identifies: *C. glabrata* - BV diagnosis based on evaluatiom of *Lactobacillus* spp. *Gardnerella*. spp and *F. vaginae*
	Schwebke JR *et al*[76]	95.0% (93.1–96.4%)§ 97.3% (95.8–98.2%)+	89.6% (87.1–91.6%)§ 85.8% (83.1–88.2%)+	Candida group: 91.7% (88.7–94.0%)§ Candida group: 92.9 (905.0–95.0%)+ *C. glabrata:* 84.7% (73.5–91.8%)§ *C. glabrata:* 86.2% (75.1–92.8%)+	Candida group: 99.1% (98.4–99.5%)§ Candida group: 98.7% (98.0–99.2%)+ *C. glabrata:* 95.1% (93.8–96.2%)§ *C. glabrata:* 98.9% (98.2–99.4%)+	96.5% (92.0–98.5%)§ 97.1% (92.9–98.9%)+	95.1% (93.8–96.2%)§ 98.9% (98.2–99.4%)+	

BD Max™ Vaginal Panel (Becton Dickinson, USA)	Sherrard J[103]	94.4% (86.2-98.4%)	79.0% (70.8-85.8%)	All species 86.4% (75.0-94.0%)	All species: 86% (79.1- 91.4%)	77.8% (40.0-97.2%)	98.9% (96.2-99.9%)	- One vaginal swab - Candida group includes: *C. albicans, C. tropicalis, C. parapsilosis*, and *C. dubliniensis*) - Separably identifies: *C. glabrata,* and *C. krusei* - BV diagnosis based on evaluatiom of *L. crispatus* and *L. jensenii* and BV associated bacteria (*Gardnerella* spp., *F. vaginae*, *Megasphaera-1*, and BVAB-2);
	Aguirre -Quiñonero *et al.*[104]	89.8% (85.0–93.1%)	96.5% (95.1–97.6%)	All species: 97.2% (94.8–98.5%) *C. glabrata:* 100% (85.7–100%) *C. krusei:* 83.3% (43.6–97.0%)	All species: 96.8% (95.3–97.9%) *C. glabrata*: 100% (99.6–100%) *C. krusei*: 100% (99.6–100%)	100% (79.6–100%)	100% (99.6–100%)	
	Van den Munckhof *et al*[97]	63.9% (47.58-77.52%)	98.7% (93.17-99.78%)	Not evaluated	Not evaluated	Not evaluated	Not evaluated	
	Thompson A *et al*[77]	96.2% (89.3–99.2%)	96.1% (89.8–98.7%)	All species: 98.4% (91.3–99.6%)	All species: 95.4% (90.3–98.3%)	87.5% (47.4-99.7%)*	100% (98-100%)*	
NuSwab® (LabCorp, Burling-ton,NC)	Danby C *et al*[105]	78.7% (67.7–87.3%)	97.6% (91.6–99.7%)	92.4% (83.2–97.5%)	100% (96.1–100%)	100% (79.4–100%)	100% (97.4–100%)	- Identifies *C. albicans* and *C. glabrata* - BV diagnosis based on evaluation of A. vaginae, BVAB 2, and *Megasphaera* spp.
	Cartwright C *et al*[79]	96.9% (94.5-99.3%)	92.6% (87.7-97.5%)	97.7% (93.3-100%)	93.2% (86.8-99.6%)	98.1% (94.5-100%)	Not calculated	

The molecular approach is not free of pitfalls, including the need for clinical validation of the results. For instance, similar to what happens with cultures, it does allow distinguishing colonization *vs.* infection in cases in which *Candida* spp. is identified. While the identification of *T. vaginalis* will of necessity lead to treatment, the same may not be true in cases of BV. The symptoms of different vulvovaginal conditions tend to be similar and overlap and that may be problematic, especially with the trend to move to self-collection of samples: significant pathology may be missed, and the symptoms wrongly attributed to whatever returns positive in the molecular test.

Also, the landscape of vaginitis is wider than BV, trichomoniasis and candidiasis – these platforms will have to adapt in the future, as knowledge increases in the field. Currently, there are no validated molecular approaches for the diagnosis of CV. The molecular diagnosis of AV/DIV seems feasible but is not yet validated nor commercially available.[95]

NAATs should be used for the diagnosis of the agents involved in cervicitis. There are different platforms on the market for the joint detection of *C. trachomatis* and *N. gonorrhoeae* in the same sample

Recommendations

Recommendation	Quality of evidence	Strength of recommendation
Empirical diagnosis of vaginitis is not recommended.	1b	A
A normal pH is insufficient to exclude the presence of vaginitis.	2b	B
Evaluation of the vaginal pH is not useful in the presence of blood, recent exposure to semen or to vaginal medication, or in postmenopausal women not using hormone therapy.	4	C
In clinical practice, a narrow pH interval scale may be used (i.e. 4.0-5.5).	5	D
Self-sampling for vaginal pH measurement can be used.	3b	C
In cases in which the fishy odor is readily noticed, there is no need to add KOH.	5	D
A positive whiff test is highly suggestive (specific) of bacterial vaginosis and/or trichomoniasis, but the sensitivity is low.	2a	B
The use of wet mount microscopy is recommended as the initial step in the diagnosis of vaginitis.	2a	B
All providers diagnosing and treating women with vulvovaginal symptoms should have training on wet mount microscopy.	5	D
The use of phase contrast is considered preferrable.	3b	C
Wet mount microscopy is the gold standard for the diagnosis of cytolytic vaginosis and aerobic vaginitis/desquamative inflammatory vaginitis.	5	D
The sample for microscopy should be collected when the woman is symptomatic, and without having used any vaginal products, nor sexual intercourse or bleeding in the previous 48-72 hours.	5	D
The posterior fornix and touching the cervix should be avoided when sampling the vagina for microscopy.	4	C
The use of a speculum is not mandatory for sampling for microscopy.	4	C

The use of cotton swabs and wooden spatulas should be avoided for sampling for microscopy.	5	D
The sample for microscopy should be read immediately, but deferred reading is possible.	5	D
The Nugent score, applied on Gram-stained slides, is considered the gold standard for the diagnosis of bacterial vaginosis.	1a	A
The Ison-Hay criteria are an alternative for the diagnosis of bacterial vaginosis.	3b	C
Self-sampling can be used for Nugent score.	4	C
Wet mount microscopy is a reliable tool for the diagnosis of bacterial vaginosis in clinical practice.	1b	A
The Pap test should not be used for the diagnosis of vaginitis.	4	C
Cultures are the gold standard for the diagnosis of candidiasis.	1a	A
Cultures may be omitted in acute candidiasis, but should be performed in chronic/recurrent cases, treatment failure, if non-*albicans* species is suspected or if symptoms present and microscopy negative.	5	D
Sensitivity tests are only recommended is case of suspected resistance (Candida).	5	D
The samples for mycological cultures should preferably be transported using a transport means (i.e. Amies or Stuart) and should be cultivated in no more than six hours.	5	D
There seem to be no disparities between the different culture means (Sabouraud agar, Nickerson medium, or Microstix-Candida medium) for Candida.	2b	B
Routine cultures for bacteria for the study of vaginitis are not recommended.	5	D
Cultures are not recommended for the diagnosis of aerobic vaginitis/ desquamative inflammatory vaginitis.	4	C
Cultures of trichomonas can be considered when resistance is suspected.	5	D
The Amsel criteria may be used in clinical practice, in the absence of expertise or availability of a microscope or other tests.	1b	A
Enzymatic tests for the diagnosis of bacterial vaginosis can be used in settings in which there is no expertise in microscopy.	4	C
Validated nucleic acid amplification tests can be used for the diagnosis of candidiasis, trichomoniasis and bacterial vaginosis.	1b	A
Validated nucleic acid amplification tests are recommended for the diagnosis of cervicitis.	1a	A
Next generation sequencing is currently not recommended for the diagnosis of vaginitis.	5	D

References

1. Landers, D. V.; Wiesenfeld, H. C.; Heine, R. P.; Krohn, M. A.; Hillier, S. L., Predictive value of the clinical diagnosis of lower genital tract infection in women. *Am J Obstet Gynecol* 2004, 190, (4), 1004-10.
2. Cerca, N., Addressing the challenges with bacterial vaginosis pharmacotherapy. *Expert Opin Pharmacother* 2022, 1-3.
3. Hillier, S. L.; Austin, M.; Macio, I.; Meyn, L. A.; Badway, D.; Beigi, R., Diagnosis and Treatment of Vaginal Discharge Syndromes in Community Practice Settings. *Clin Infect Dis* 2021, 72, (9), 1538-1543.
4. Carr, P. L.; Felsenstein, D.; Friedman, R. H., Evaluation and management of vaginitis. *J Gen Intern Med* 1998, 13, (5), 335-46.
5. Ravel, J.; Gajer, P.; Abdo, Z.; Schneider, G. M.; Koenig, S. S.; McCulle, S. L.; Karlebach, S.; Gorle, R.; Russell, J.; Tacket, C. O.; Brotman, R. M.; Davis, C. C.; Ault, K.; Peralta, L.; Forney, L. J., Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci U S A* 2011, 108 Suppl 1, (Suppl 1), 4680-7.
6. Lev-Sagie, A.; De Seta, F.; Verstraelen, H.; Ventolini, G.; Lonnee-Hoffmann, R.; Vieira-Baptista, P., The Vaginal Microbiome: II. Vaginal Dysbiotic Conditions. *J Low Genit Tract Dis* 2022, 26, (1), 79-84.
7. Konschuh, S.; Jayaprakash, T.; Dolatabadi, A.; Dayo, E.; Ramay, H.; Sycuro, L., O02.3 Reclassification of Atopobium vaginae as three novel Fannyhessea species: implications for understanding their role in bacterial vaginosis. *Sexually Transmitted Infections* 2021, 97, (Suppl 1), A18-A18.
8. Linhares, I. M.; Summers, P. R.; Larsen, B.; Giraldo, P. C.; Witkin, S. S., Contemporary perspectives on vaginal pH and lactobacilli. *Am J Obstet Gynecol* 2011, 204, (2), 120.e1-5.
9. Benyas, D.; Sobel, J. D., Mixed Vaginitis Due to Bacterial Vaginosis and Candidiasis. *J Low Genit Tract Dis* 2022, 26, (1), 68-70.
10. Pavletic, A. J.; Hawes, S. E.; Geske, J. A.; Bringe, K.; Polack, S. H., Experience with routine vaginal pH testing in a family practice setting. *Infect Dis Obstet Gynecol* 2004, 12, (2), 63-8.
11. Cibley, L. J.; Cibley, L. J., Cytolytic vaginosis. *Am J Obstet Gynecol* 1991, 165, (4 Pt 2), 1245-9.
12. Vieira-Baptista, P.; Silva, A. R.; Costa, M.; Figueiredo, R.; Saldanha, C.; Sousa, C., Diagnosis of bacterial vaginosis: Clinical or microscopic? A cross-sectional study. *Int J Gynaecol Obstet* 2022, 156, (3), 552-559.
13. Donders, G.; Slabbaert, K.; Vancalsteren, K.; Pelckmans, S.; Bellen, G., Can vaginal pH be measured from the wet mount slide? *Eur J Obstet Gynecol Reprod Biol* 2009, 146, (1), 100-3.
14. Bakir, S.; Elas, D.; Stockdale, C. K.; Zimmerman, M. B.; Hardy-Fairbanks, A., Accuracy of Vaginal pH Testing Before and After Addition of Sterile Saline. *J Low Genit Tract Dis* 2021, 25, (2), 181-185.
15. Donders, G. G.; Caeyers, T.; Tydhof, P.; Riphagen, I.; van den Bosch, T.; Bellen, G., Comparison of two types of dipsticks to measure vaginal pH in clinical practice. *Eur J Obstet Gynecol Reprod Biol* 2007, 134, (2), 220-4.
16. Vieira-Baptista, P.; Silva, A. R.; Costa, M.; Aguiar, T.; Saldanha, C.; Sousa, C., Clinical validation of a new molecular test (Seegene Allplex™ Vaginitis) for the diagnosis of vaginitis: a cross-sectional study. *Bjog* 2021, 128, (8), 1344-1352.
17. Donders, G. G.; Gonzaga, A.; Marconi, C.; Donders, F.; Michiels, T.; Eggermont, N.; Bellen, G.; Lule, J.; Byamughisa, J., Increased vaginal pH in Ugandan women: what does it indicate? *Eur J Clin Microbiol Infect Di*s 2016, 35, (8), 1297-303.
18. Donders, G. G.; Andabati, G.; Donders, F.; Michiels, T.; Eggermont, N.; Bellen, G.; Lulé, J., Acceptance of self-testing for increased vaginal pH in different subsets of Ugandan women. *Int J STD AIDS* 2012, 23, (1), 30-5.
19. Huppert, J. S.; Hesse, E. A.; Bernard, M. C.; Bates, J. R.; Gaydos, C. A.; Kahn, J. A., Accuracy and trust of self-testing for bacterial vaginosis. *J Adolesc Health* 2012, 51, (4), 400-5.
20. Roy, S.; Caillouette, J. C.; Faden, J. S.; Roy, T.; Ramos, D. E., Improving appropriate use of antifungal medications: the role of an over-the-counter vaginal pH self-test device. *Infect Dis Obstet Gynecol* 2003, 11, (4), 209-16.
21. Lin, Y. P.; Chen, W. C.; Cheng, C. M.; Shen, C. J., Vaginal pH Value for Clinical Diagnosis and Treatment of Common Vaginitis. *Diagnostics (Basel)* 2021, 11, (11).
22. Shen, C. J.; Yang, C. Y.; Chen, H. Y.; Chen, W. C.; Chang, T. C.; Cheng, C. M., Clinical Evaluation of a Self-Testing Kit for Vaginal Infection Diagnosis. *J Healthc Eng* 2021, 2021, 4948954.
23. West, B.; Morison, L.; Schim van der Loeff, M.; Gooding, E.; Awasana, A. A.; Demba, E.; Mayaud, P., Evaluation of a new rapid diagnostic kit (FemExam) for bacterial vaginosis in patients with vaginal discharge syndrome in The Gambia. *Sex Transm Dis* 2003, 30, (6), 483-9.
24. Charonis, G.; Larsson, P. G., Use of pH/whiff test or QuickVue Advanced pH and Amines test for the diagnosis of bacterial vaginosis and prevention of postabortion pelvic inflammatory disease. *Acta Obstet Gynecol Scand* 2006, 85, (7), 837-43.
25. Gjerdingen, D.; Fontaine, P.; Bixby, M.; Santilli, J.; Welsh, J., The impact of regular vaginal pH screening on the diagnosis of bacterial vaginosis in pregnancy. *J Fam Pract* 2000, 49, (1), 39-43.
26. Hosny, A.; El-Khayat, W.; Kashef, M. T.; Fakhry, M. N., Association between preterm labor and genitourinary tract infections caused by Trichomonas vaginalis, Mycoplasma hominis, Gram-negative bacilli, and coryneforms. *J Chin Med Assoc* 2017, 80, (9), 575-581.

27. Vieira-Baptista, P.; Grincevičienė, Š.; Oliveira, C.; Fonseca-Moutinho, J.; Cherey, F.; Stockdale, C. K., The International Society for the Study of Vulvovaginal Disease Vaginal Wet Mount Microscopy Guidelines: How to Perform, Applications, and Interpretation. *J Low Genit Tract Dis* 2021, 25, (2), 172-180.
28. Modak, T.; Arora, P.; Agnes, C.; Ray, R.; Goswami, S.; Ghosh, P.; Das, N. K., Diagnosis of bacterial vaginosis in cases of abnormal vaginal discharge: comparison of clinical and microbiological criteria. J Infect Dev Ctries 2011, 5, (5), 353-60.
29. Cohrssen, A.; Anderson, M.; Merrill, A.; McKee, D., Reliability of the whiff test in clinical practice. *J Am Board Fam Pract* 2005, 18, (6), 561-2.
30. Hay, P.; Tummon, A.; Ogunfile, M.; Adebiyi, A.; Adefowora, A., Evaluation of a novel diagnostic test for bacterial vaginosis: 'the electronic nose'. *Int J STD AIDS* 2003, 14, (2), 114-8.
31. Chandiok, S.; Crawley, B. A.; Oppenheim, B. A.; Chadwick, P. R.; Higgins, S.; Persaud, K. C., Screening for bacterial vaginosis: a novel application of artificial nose technology. *J Clin Pathol* 1997, 50, (9), 790-1.
32. Donders, G. G.; Marconi, C.; Bellen, G.; Donders, F.; Michiels, T., Effect of short training on vaginal fluid microscopy (wet mount) learning. *J Low Genit Tract Dis* 2015, 19, (2), 165-9.
33. Donders, G. G.; Larsson, P. G.; Platz-Christensen, J. J.; Hallén, A.; van der Meijden, W.; Wölner-Hanssen, P., Variability in diagnosis of clue cells, lactobacillary grading and white blood cells in vaginal wet smears with conventional bright light and phase contrast microscopy. *Eur J Obstet Gynecol Reprod Biol* 2009, 145, (1), 109-12.
34. Santiago, G. L.; Cools, P.; Verstraelen, H.; Trog, M.; Missine, G.; El Aila, N.; Verhelst, R.; Tency, I.; Claeys, G.; Temmerman, M.; Vaneechoutte, M., Longitudinal study of the dynamics of vaginal microflora during two consecutive menstrual cycles. *PLoS One* 2011, 6, (11), e28180.
35. Morison, L.; Ekpo, G.; West, B.; Demba, E.; Mayaud, P.; Coleman, R.; Bailey, R.; Walraven, G., Bacterial vaginosis in relation to menstrual cycle, menstrual protection method, and sexual intercourse in rural Gambian women. *Sex Transm Infect* 2005, 81, (3), 242-7.
36. Azevedo, S.; Lima-Silva, J.; Vieira-Baptista, P., Impact of the Sampling Site in the Result of Wet Mount Microscopy. *J Low Genit Tract Dis* 2019, 23, (2), 176-181.
37. Frobenius, W.; Bogdan, C., Diagnostic Value of Vaginal Discharge, Wet Mount and Vaginal pH - An Update on the Basics of Gynecologic Infectiology. *Geburtshilfe Frauenheilkd* 2015, 75, (4), 355-366.
38. Audisio, T.; Penacino, M.; Cannistraci, R.; Bertolotto, P., Detection of bacterial vaginosis, Trichomonas vaginalis infection, and vaginal Candida infection: a comparative study of methods of extracting exudates, with and without a speculum, during pregnancy. *J Low Genit Tract Dis* 2005, 9, (4), 213-5.
39. Hemalatha, R.; Ramalaxmi, B. A.; Swetha, E.; Balakrishna, N.; Mastromarino, P., Evaluation of vaginal pH for detection of bacterial vaginosis. *Indian J Med Res* 2013, 138, (3), 354-9.
40. Kissinger, P. J.; Dumestre, J.; Clark, R. A.; Wenthold, L.; Mohammed, H.; Hagensee, M. E.; Martin, D. H., Vaginal swabs versus lavage for detection of Trichomonas vaginalis and bacterial vaginosis among HIV-positive women. *Sex Transm Dis* 2005, 32, (4), 227-30.
41. Coleman, J. S.; Gaydos, C. A., Molecular Diagnosis of Bacterial Vaginosis: an Update. *J Clin Microbiol* 2018, 56, (9).
42. Workowski, K. A.; Bachmann, L. H.; Chan, P. A.; Johnston, C. M.; Muzny, C. A.; Park, I.; Reno, H.; Zenilman, J. M.; Bolan, G. A., Sexually Transmitted Infections Treatment Guidelines, 2021. *MMWR Recomm Rep* 2021, 70, (4), 1-187.
43. Donders, G. G.; Vereecken, A.; Dekeersmaecker, A.; Van Bulck, B.; Spitz, B., Wet mount microscopy reflects functional vaginal lactobacillary flora better than Gram stain. *J Clin Pathol* 2000, 53, (4), 308-13.
44. Camus, C.; Penaranda, G.; Khiri, H.; Camiade, S.; Molet, L.; Lebsir, M.; Plauzolles, A.; Chiche, L.; Blanc, B.; Quarello, E.; Halfon, P., Acceptability and efficacy of vaginal self-sampling for genital infection and bacterial vaginosis: A cross-sectional study. *PLoS One* 2021, 16, (11), e0260021.
45. Kashyap, B.; Singh, R.; Bhalla, P.; Arora, R.; Aggarwal, A., Reliability of self-collected versus provider-collected vaginal swabs for the diagnosis of bacterial vaginosis. *Int J STD AIDS* 2008, 19, (8), 510-3.
46. Ison, C. A.; Hay, P. E., Validation of a simplified grading of Gram stained vaginal smears for use in genitourinary medicine clinics. *Sex Transm Infect* 2002, 78, (6), 413-5.
47. Machado, A.; Cerca, N., Multiplex Peptide Nucleic Acid Fluorescence In Situ Hybridization (PNA-FISH) for Diagnosis of Bacterial Vaginosis. *Methods Mol Biol* 2017, 1616, 209-219.
48. Joesoef, M. R.; Hillier, S. L.; Josodiwondo, S.; Linnan, M., Reproducibility of a scoring system for gram stain diagnosis of bacterial vaginosis. *J Clin Microbiol* 1991, 29, (8), 1730-1.
49. Dong, M.; Wang, C.; Li, H.; Yan, Y.; Ma, X.; Li, H.; Li, X.; Wang, H.; Zhang, Y.; Qi, W.; Meng, K.; Tian, W.; Wang, Y.; Fan, A.; Han, C.; Donders, G. G. G.; Xue, F., Aerobic Vaginitis Diagnosis Criteria Combining Gram Stain with Clinical Features: An Establishment and Prospective Validation Study. *Diagnostics (Basel)* 2022, 12, (1).

50. Larsson, P. G.; Carlsson, B.; Fåhraeus, L.; Jakobsson, T.; Forsum, U., Diagnosis of bacterial vaginosis: need for validation of microscopic image area used for scoring bacterial morphotypes. *Sex Transm Infect* 2004, 80, (1), 63-7.
51. Forsum, U.; Jakobsson, T.; Larsson, P. G.; Schmidt, H.; Beverly, A.; Bjørnerem, A.; Carlsson, B.; Csango, P.; Donders, G.; Hay, P.; Ison, C.; Keane, F.; McDonald, H.; Moi, H.; Platz-Christensen, J. J.; Schwebke, J., An international study of the interobserver variation between interpretations of vaginal smear criteria of bacterial vaginosis. *Apmis* 2002, 110, (11), 811-8.
52. Levi, A. W.; Harigopal, M.; Hui, P.; Schofield, K.; Chhieng, D. C., Comparison of Affirm VPIII and Papanicolaou tests in the detection of infectious vaginitis. *Am J Clin Pathol* 2011, 135, (3), 442-7.
53. Fitzhugh, V. A.; Heller, D. S., Significance of a diagnosis of microorganisms on pap smear. *J Low Genit Tract Dis* 2008, 12, (1), 40-51.
54. Sobel, J. D., Vulvovaginal candidosis. *Lancet* 2007, 369, (9577), 1961-71.
55. Farr, A.; Effendy, I.; Tirri, B. F.; Hof, H.; Mayser, P.; Petricevic, L.; Ruhnke, M.; Schaller, M.; Schäfer, A. P. A.; Willinger, B.; Mendling, W., Vulvovaginal Candidosis (Excluding Mucocutaneous Candidosis): Guideline of the German (DGGG), Austrian (OEGGG) and Swiss (SGGG) Society of Gynecology and Obstetrics (S2k-Level, AWMF Registry Number 015/072, September 2020). *Geburtshilfe Frauenheilkd* 2021, 81, (4), 398-421.
56. Liu, W.; Zhang, X.; Liu, Z.; Luo, X., Impact of pH on the antifungal susceptibility of vaginal Candida albicans. *Int J Gynaecol Obstet* 2011, 114, (3), 278-80.
57. Danby, C. S.; Boikov, D.; Rautemaa-Richardson, R.; Sobel, J. D., Effect of pH on in vitro susceptibility of Candida glabrata and Candida albicans to 11 antifungal agents and implications for clinical use. *Antimicrob Agents Chemother* 2012, 56, (3), 1403-6.
58. Donders, G. G.; Bellen, G.; Mendling, W., Management of recurrent vulvo-vaginal candidosis as a chronic illness. *Gynecol Obstet Invest* 2010, 70, (4), 306-21.
59. Donders, G.; Greenhouse, P.; Donders, F.; Engel, U.; Paavonen, J.; Mendling, W., Genital Tract GAS Infection ISIDOG Guidelines. *J Clin Med* 2021, 10, (9).
60. Stockdale, C. K., A Positive Culture Result for Gardnerella Is Not Diagnostic of Bacterial Vaginosis. *J Low Genit Tract Dis* 2016, 20, (4), 281-2.
61. Donders, G. G. G.; Bellen, G.; Grinceviciene, S.; Ruban, K.; Vieira-Baptista, P., Aerobic vaginitis: no longer a stranger. *Res Microbiol* 2017, 168, (9-10), 845-858.
62. Levi, M. H.; Torres, J.; Piña, C.; Klein, R. S., Comparison of the InPouch TV culture system and Diamond's modified medium for detection of Trichomonas vaginalis. *J Clin Microbiol* 1997, 35, (12), 3308-10.
63. Vieira-Baptista, P.; Bornstein, J., Candidiasis, Bacterial Vaginosis, Trichomoniasis and Other Vaginal Conditions Affecting the Vulva. In Vulvar Disease: Breaking the Myths, Bornstein, J., Ed. *Springer International Publishing: Cham,* 2019; pp 167-205.
64. Ohlemeyer, C. L.; Hornberger, L. L.; Lynch, D. A.; Swierkosz, E. M., Diagnosis of Trichomonas vaginalis in adolescent females: InPouch TV culture versus wet-mount microscopy. *The Journal of adolescent health: official publication of the Society for Adolescent Medicine* 1998, 22, (3), 205-8.
65. Hobbs, M. M.; Sena, A. C., Modern diagnosis of Trichomonas vaginalis infection. *Sex Transm Infect* 2013, 89, (6), 434-8.
66. Ng, L. K.; Martin, I. E., The laboratory diagnosis of Neisseria gonorrhoeae. *Can J Infect Dis Med Microbiol* 2005, 16, (1), 15-25.
67. Amsel, R.; Totten, P. A.; Spiegel, C. A.; Chen, K. C.; Eschenbach, D.; Holmes, K. K., Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. *Am J Med* 1983, 74, (1), 14-22.
68. Srinivasan, S.; Hoffman, N. G.; Morgan, M. T.; Matsen, F. A.; Fiedler, T. L.; Hall, R. W.; Ross, F. J.; McCoy, C. O.; Bumgarner, R.; Marrazzo, J. M.; Fredricks, D. N., Bacterial communities in women with bacterial vaginosis: high resolution phylogenetic analyses reveal relationships of microbiota to clinical criteria. *PLoS One* 2012, 7, (6), e37818.
69. Van Schalkwyk, J.; Yudin, M. H., Vulvovaginitis: screening for and management of trichomoniasis, vulvovaginal candidiasis, and bacterial vaginosis. *J Obstet Gynaecol Can* 2015, 37, (3), 266-274.
70. Keane, F. E.; Maw, R.; Pritchard, C.; Ison, C. A., Methods employed by genitourinary medicine clinics in the United Kingdom to diagnose bacterial vaginosis. *Sex Transm Infect* 2005, 81, (2), 155-7.
71. Brusselaers, N.; Shrestha, S.; van de Wijgert, J.; Verstraelen, H., Vaginal dysbiosis and the risk of human papillomavirus and cervical cancer: systematic review and meta-analysis. *Am J Obstet Gynecol* 2019, 221, (1), 9-18.e8.
72. Sobel, J. D.; Subramanian, C.; Foxman, B.; Fairfax, M.; Gygax, S. E., Mixed vaginitis-more than coinfection and with therapeutic implications. *Curr Infect Dis Rep* 2013, 15, (2), 104-8.
73. Bradshaw, C. S.; Morton, A. N.; Garland, S. M.; Horvath, L. B.; Kuzevska, I.; Fairley, C. K., Evaluation of a point-of-care test, BVBlue, and clinical and laboratory criteria for diagnosis of bacterial vaginosis. *J Clin Microbiol* 2005, 43, (3), 1304-8.
74. Sumeksri, P.; Koprasert, C.; Panichkul, S., BVBLUE test for diagnosis of bacterial vaginosis in pregnant women attending antenatal care at Phramongkutklao Hospital. *J Med Assoc Thai* 2005, 88 Suppl 3, S7-13.

75. Miller, L., Can Fem Exam Card Use Facilitate Bacterial Vaginosis Diagnosis on Day of Abortion to Prevent Postabortion Endometritis? *Obstetrics & Gynecology* 2001, 97, (4), 58S-59S.
76. Schwebke, J. R.; Taylor, S. N.; Ackerman, R.; Schlaberg, R.; Quigley, N. B.; Gaydos, C. A.; Chavoustie, S. E.; Nyirjesy, P.; Remillard, C. V.; Estes, P.; McKinney, B.; Getman, D. K.; Clark, C., Clinical Validation of the Aptima Bacterial Vaginosis and Aptima Candida/Trichomonas Vaginitis Assays: Results from a Prospective Multicenter Clinical Study. *J Clin Microbiol* 2020, 58, (2).
77. Thompson, A.; Timm, K.; Borders, N.; Montoya, L.; Culbreath, K., Diagnostic performance of two molecular assays for the detection of vaginitis in symptomatic women. *Eur J Clin Microbiol Infect Dis* 2020, 39, (1), 39-44.
78. Mulhem, E.; Boyanton, B. L., Jr.; Robinson-Dunn, B.; Ebert, C.; Dzebo, R., Performance of the Affirm VP-III using residual vaginal discharge collected from the speculum to characterize vaginitis in symptomatic women. *J Low Genit Tract Dis* 2014, 18, (4), 344-6.
79. Cartwright, C. P.; Lembke, B. D.; Ramachandran, K.; Body, B. A.; Nye, M. B.; Rivers, C. A.; Schwebke, J. R., Comparison of nucleic acid amplification assays with BD affirm VPIII for diagnosis of vaginitis in symptomatic women. *J Clin Microbiol* 2013, 51, (11), 3694-9.
80. Brown, H. L.; Fuller, D. D.; Jasper, L. T.; Davis, T. E.; Wright, J. D., Clinical evaluation of affirm VPIII in the detection and identification of Trichomonas vaginalis, Gardnerella vaginalis, and Candida species in vaginitis/vaginosis. *Infect Dis Obstet Gynecol* 2004, 12, (1), 17-21.
81. Byun, S. W.; Park, Y. J.; Hur, S. Y., Affirm VPIII microbial identification test can be used to detect gardnerella vaginalis, Candida albicans and trichomonas vaginalis microbial infections in Korean women. *J Obstet Gynaecol Res* 2016, 42, (4), 422-6.
82. Sheiness, D.; Dix, K.; Watanabe, S.; Hillier, S. L., High levels of Gardnerella vaginalis detected with an oligonucleotide probe combined with elevated pH as a diagnostic indicator of bacterial vaginosis. *J Clin Microbiol* 1992, 30, (3), 642-8.
83. Gazi, H.; Degerli, K.; Kurt, O.; Teker, A.; Uyar, Y.; Caglar, H.; Kurutepe, S.; Surucuoglu, S., Use of DNA hybridization test for diagnosing bacterial vaginosis in women with symptoms suggestive of infection. *Apmis* 2006, 114, (11), 784-7.
84. Andrea, S. B.; Chapin, K. C., Comparison of Aptima Trichomonas vaginalis transcription-mediated amplification assay and BD affirm VPIII for detection of T. vaginalis in symptomatic women: performance parameters and epidemiological implications. *J Clin Microbiol* 2011, 49, (3), 866-9.
85. Richter, S. S.; Otiso, J.; Goje, O. J.; Vogel, S.; Aebly, J.; Keller, G.; Van Heule, H.; Wehn, D.; Stephens, A. L.; Zanotti, S.; Johnson, T.; Leal, S. M.; Procop, G. W., Prospective Evaluation of Molecular Assays for Diagnosis of Vaginitis. *J Clin Microbiol* 2019, 58, (1).
86. Witt, A.; Petricevic, L.; Kaufmann, U.; Gregor, H.; Kiss, H., DNA hybridization test: rapid diagnostic tool for excluding bacterial vaginosis in pregnant women with symptoms suggestive of infection. *J Clin Microbiol* 2002, 40, (8), 3057-9.
87. Dessai, F.; Nyirenda, M.; Sebitloane, M.; Abbai, N., Diagnostic evaluation of the BD Affirm VPIII assay as a point-of-care test for the diagnosis of bacterial vaginosis, trichomoniasis and candidiasis. *Int J STD AIDS* 2020, 31, (4), 303-311.
88. Rivers, C. A.; Lee, J. Y.; Sharples, N.; Ledeboer, N. A.; Schwebke, J. R., ESwab as an optional collection device for use with the Affirm VPIII microbial test system. *J Clin Microbiol* 2014, 52, (5), 1698-700.
89. Rompalo, A. M.; Castleberry, N.; Widdice, L.; Schulkin, J.; Gaydos, C. A., Patterns of point-of-care test use among obstetricians and gynaecologists in the US. *Sex Health* 2018, 15, (4), 318-324.
90. Brotman, R. M.; Ravel, J., Ready or not: the molecular diagnosis of bacterial vaginosis. *Clin Infect Dis* 2008, 47, (1), 44-6.
91. Schwebke, J. R.; Gaydos, C. A.; Davis, T.; Marrazzo, J.; Furgerson, D.; Taylor, S. N.; Smith, B.; Bachmann, L. H.; Ackerman, R.; Spurrell, T.; Ferris, D.; Burnham, C. A.; Reno, H.; Lebed, J.; Eisenberg, D.; Kerndt, P.; Philip, S.; Jordan, J.; Quigley, N., Clinical Evaluation of the Cepheid Xpert TV Assay for Detection of Trichomonas vaginalis with Prospectively Collected Specimens from Men and Women. *J Clin Microbiol* 2018, 56, (2).
92. De Salazar, A.; Espadafor, B.; Fuentes-López, A.; Barrientos-Durán, A.; Salvador, L.; Álvarez, M.; García, F., Comparison between Aptima Assays (Hologic) and the Allplex STI Essential Assay (Seegene) for the diagnosis of Sexually transmitted infections. *PLoS One* 2019, 14, (9), e0222439.
93. Drew, R. J.; Murphy, T.; Broderick, D.; O'Gorman, J.; Eogan, M., An interpretation algorithm for molecular diagnosis of bacterial vaginosis in a maternity hospital using machine learning: proof-of-concept study. *Diagn Microbiol Infect Dis* 2020, 96, (2), 114950.
94. Hilbert, D. W.; Smith, W. L.; Chadwick, S. G.; Toner, G.; Mordechai, E.; Adelson, M. E.; Aguin, T. J.; Sobel, J. D.; Gygax, S. E., Development and Validation of a Highly Accurate Quantitative Real-Time PCR Assay for Diagnosis of Bacterial Vaginosis. *J Clin Microbiol* 2016, 54, (4), 1017-24.
95. Rumyantseva, T. A.; Bellen, G.; Savochkina, Y. A.; Guschin, A. E.; Donders, G. G., Diagnosis of aerobic vaginitis by quantitative real-time PCR. *Arch Gynecol Obstet* 2016, 294, (1), 109-14.
96. Muzny, C. A.; Balkus, J.; Mitchell, C.; Sobel, J. D.; Workowski, K.; Marrazzo, J.; Schwebke, J. R., Diagnosis and Management of Bacterial Vaginosis: Summary of Evidence Reviewed for the 2021 Centers for Disease Control and Prevention Sexually Transmitted Infections Treatment Guidelines. *Clin Infect Dis* 2022, 74, (Suppl_2), S144-s151.

97. Van den Munckhof, E. H. A.; van Sitter, R. L.; Boers, K. E.; Lamont, R. F.; Te Witt, R.; le Cessie, S.; Knetsch, C. W.; van Doorn, L. J.; Quint, W. G. V.; Molijn, A.; Leverstein-van Hall, M. A., Comparison of Amsel criteria, Nugent score, culture and two CE-IVD marked quantitative real-time PCRs with microbiota analysis for the diagnosis of bacterial vaginosis. *Eur J Clin Microbiol Infect Dis* 2019, 38, (5), 959-966.
98. Van Der Pol, B.; Daniel, G.; Kodsi, S.; Paradis, S.; Cooper, C. K., Molecular-based Testing for Sexually Transmitted Infections Using Samples Previously Collected for Vaginitis Diagnosis. *Clin Infect Dis* 2019, 68, (3), 375-381.
99. Ackerman, S. J.; Knight, T.; Wahl, P. M.; Cartwright, C. P., Health care utilization and costs following amplified versus non-amplified molecular probe testing for symptomatic patients with suspected vulvovaginitis: a US commercial payer population. *Clinicoecon Outcomes Res* 2019, 11, 179-189.
100. Broache, M.; Cammarata, C. L.; Stonebraker, E.; Eckert, K.; Van Der Pol, B.; Taylor, S. N., Performance of a Vaginal Panel Assay Compared With the Clinical Diagnosis of Vaginitis. *Obstet Gynecol* 2021, 138, (6), 853-859.
101. Lynch, T.; Peirano, G.; Lloyd, T.; Read, R.; Carter, J.; Chu, A.; Shaman, J. A.; Jarvis, J. P.; Diamond, E.; Ijaz, U. Z.; Church, D., Molecular Diagnosis of Vaginitis: Comparing Quantitative PCR and Microbiome Profiling Approaches to Current Microscopy Scoring. *J Clin Microbiol* 2019, 57, (9).
102. Vieira-Baptista, P.; Eleutério Jr., J., Diagnosis of vaginitis: time to improve and move on. DST - *J bras Doenças Sex Transm* 2020, 32, (e203214), 1-3.
103. Sherrard, J., Evaluation of the BD MAX™ Vaginal Panel for the detection of vaginal infections in a sexual health service in the UK. *Int J STD AIDS* 2019, 30, (4), 411-414.
104. Aguirre-Quiñonero, A.; Castillo-Sedano, I. S.; Calvo-Muro, F.; Canut-Blasco, A., Accuracy of the BD MAX™ vaginal panel in the diagnosis of infectious vaginitis. *Eur J Clin Microbiol Infect Dis* 2019, 38, (5), 877-882.
105. Danby, C. S.; Althouse, A. D.; Hillier, S. L.; Wiesenfeld, H. C., Nucleic Acid Amplification Testing Compared With Cultures, Gram Stain, and Microscopy in the Diagnosis of Vaginitis. *J Low Genit Tract Dis* 2021, 25, (1), 76-80.

BACTERIAL VAGINOSIS

3

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3.1 Introduction

The female genital tract consists of a hormonally driven tissue structure with estrogenic hormones driving trophic and maturing effects on the epithelium. Even before the vagina was thought of as an ecosystem with its resident microbial communities, microscopic and culture-based observations indicated lactobacilli colonization appeared abundant after an infant became colonized, declined until puberty, became prominent through the reproductive years, and declined in menopause. Modern molecular methods have opened a range of new possibilities for the characterization of the vaginal microbiota (VMB), allowing us to not only establish which microorganisms are present, but also to begin to understand their functional properties.[1, 2]

Growing evidence suggests that low diversity, *Lactobacillus* spp. dominated VMB is associated with lower inflammation, and that this is protective. On the other hand, non-*Lactobacillus* spp.-dominated, higher diversity VMB (sometimes termed "dysbiotic") is associated with risk of infections (including human immunodeficiency virus [HIV]) and, possibly, obstetric complications.[3-5] Vaginal dysbiosis may be physiological for some women, or pathological, depending on the interplay of metabolic and microbial factors.[6]

The clinical syndrome that is currently known as bacterial vaginosis (BV), involving multiple bacterial species which vary from woman to woman, has been extensively studied for the last six decades. BV is a polymicrobial disorder of the vaginal microbiome that is characterized by the absence of protective lactobacilli. The most frequently detected bacterial taxa include: *Gardnerella* spp., *Mycoplasma hominis, Fannyhessea (Atopobium) vaginae, Bacteroides, Clostridiale, Fusobacterium* spp., *Mobiluncus* spp., *Peptostreptococcus* spp., *Porphyromonas* spp., *Prevotella* spp., and others that have been described and may cause dysbiosis. Dysbiosis may be generated when conditions disrupt, modify, reduce, block, fluctuate or deplete the dominant lactobacilli.[7]

The prevalence of these communities, with a paucity of *Lactobacillus* spp. varies among women, and epidemiological studies have associated them with an increased risk of adverse health outcomes: preterm labor and birth (PTB), premature rupture of membranes (PROM), chorio-amnionitis, funisitis, post abortion infections, and increased risk of acquiring sexually transmitted infections (STIs).[4, 5] The mechanisms that drive these associations are yet to be described in detail, with few studies establishing causative relationships. Despite advances in our understanding of BV, it remains an enigmatic condition. While the associated clinical symptoms are relatively uncomplicated and easily measured, the fact that not all symptoms occur in all women diagnosed with BV remains problematic. This is not surprising given the complexity of the vaginal microbiome, host immunity, and the variability in individual responses to potential inflammatory mediators produced by an array of microorganisms.[8]

The link between non-*Lactobacillus* spp.-dominated high diversity VMB/BV and a variety of adverse outcomes for women's sexual and reproductive health has been well-established. This suggests that treatment or prevention of vaginal dysbiosis/BV may improve women's health outcomes.

3.2 Etiology and physiopathology

BV is considered a polymicrobial dysbiosis of the VMB. The optimal VMB of reproductive aged women is typically dominated by lactic acid producing lactobacilli which maintain a vaginal pH<4.7. In contrast, BV is characterized by an increase in the load of facultative and strict anaerobic bacteria, a reduction in beneficial lactobacilli, and a corresponding increase in vaginal pH.[9-14] Although the exact etiological agent(s) responsible for BV is(are) not known, cultivation studies and more recently, molecular studies, have identified a large number of bacteria that are associated with BV, collectively referred to as BV-associated bacteria (BVAB). These organisms include *Gardnerella* spp., *Prevotella* spp., *F. vaginae, Mobiluncus* spp., *Megasphaera* spp., *Sneathia* spp., among others.[11, 12, 15-17] Of note, specific *Gardnerella* spp. are thought to play a key role in BV pathogenesis, potentially as a founder or initiating organism.[18-21] Until recently, *Gardnerella vaginalis* was the only species present in the *Gardnerella* genus. In 2019, thirteen *Gardnerella* species were proposed;[22] three new species (*G. swidsinskii, G. piotii and G. leopoldii*) were given official taxonomic standing, and the description of *G. vaginalis* was narrowed and amended. To reflect the current taxonomy, *Gardnerella* spp. will be used below in place of *G. vaginalis*. Also, recently, *Atopobium vaginae* was reclassified into the new genus *Fannyhessea*.[23]

Gardnerella spp. have been shown to adhere to vaginal epithelial cells and initiate biofilm formation, which is thought to be a key factor in BV pathogenesis.[24-26] *Gardnerella* spp. are often the predominant species present in BV-biofilms, and have demonstrated ability to form a biofilm in acidic environments,[27] which further supports its role as an integral organism involved in the initiation of BV. However, synergistic relationships between *Gardnerella* spp. and other BVAB exist.[18] It is hypothesized that *Gardnerella* spp. act to lower the oxidation-reduction potential of the vaginal environment which promotes growth of strict anaerobic bacteria including *Prevotella* spp. and *F. vaginae*.[18, 20] Production of amino acids by *Gardnerella* spp. fur-

ther enhances the growth of *Prevotella* spp., which in turn produces ammonia, which enhances growth of *Gardnerella* spp..[28] Additionally, *F. vaginae* and *Prevotella* spp. are also present in BV-biofilms alongside *Gardnerella* spp..[29] Production of virulence factors (e.g. sialidase) by *Gardnerella* spp. and *Prevotella* spp. degrade the protective cervicovaginal mucosa, further enhancing biofilm formation and facilitate attachment of other BVAB,[29-31] which contribute to BV symptoms and sequelae.[32] Exfoliation of vaginal epithelial cells produces the clue cells that are characteristic of BV,[33] and the increased load of anaerobic bacteria is associated with production of amines, which contributes to the malodorous discharge observed in BV.[34-36]

Importantly, the event that triggers the vaginal dysbiosis observed in BV is not well understood. It is not known if BV results from acquisition of a single organism (e.g. specific *Gardnerella* spp.) or a polymicrobial consortium, or is a consequence of overgrowth of BVAB in response to specific host or behavioral factors.[37] Importantly, both epidemiological and molecular data indicate that sexual transmission is involved in both the acquisition and recurrence of BV.[38-45] The epidemiological profile of BV is similar to that of STIs, with a meta-analysis of 43 studies finding that BV was associated with inconsistent condom use, as well as new and increased number of sexual partners.[46] BV is associated with early age of sexual debut,[47, 48] and is rare among women without a history of coital or noncoital sexual contact.[48] Furthermore, risk factors for BV acquisition among women with female partners include having a sexual partner with a history of BV, BV symptoms or microbiologically confirmed BV.[39, 45] BV has also been associated with other behavioral practices including smoking[49-52] and intravaginal douching.[53-56]

3.3 Prevalence and epidemiology

Global and regional estimates of bacterial vaginosis prevalence

The prevalence of BV varies widely across countries and between different population groups and is influenced by differences in diagnostic and sampling methodology. In a recent systematic review and meta-analysis (122 publications, up to 2017), the global prevalence of BV among reproductive-aged women in the general population was high, ranging from 23 to 29%, with marked racial disparities.[57] Pooled estimates by geographical region included: Europe and Central Asia, 23%; East Asia and Pacific, 24%; Latin America and Caribbean, 24%; Middle East and North Africa, 25%; sub-Saharan Africa, 25%; North America, 27%; South Asia, 29%).[57] Within sub-Saharan Africa, BV prevalence was lower in Western and Central Africa (20.6%; 95% confidence interval [CI], 6.1–40.6%) than in Southern and Eastern Africa (33.3%; 95% CI 17.4–51.5%), although this was not statistically significant. The review found BV prevalence varied with ethnicity within specific geographical regions. For example, within North America overall BV prevalence was 27% (95% CI 24- 31%), but prevalence estimates were higher in Black and Hispanic women (33% and 31%, respectively) compared to White and Asian women (23% and 11%, respectively).[57] Overall, there was an approximately 2-fold higher BV prevalence among majority Black populations when compared to majority non-Black populations in this meta-analysis (46.5%; 95% CI 37.5–55.6% *vs.* 21.3%; 95% CI 16.7–26.3%; $p<0.001$).[57]

A meta-analysis among women participating in HIV prevention studies (n=18) across three primary region and population groups in sub-Saharan Africa (South Africa community-based, Southern/Eastern Africa community-based, and Eastern Africa higher-risk populations), reported summary estimates for BV prevalence in excess of 30%.[58] Among 15 to 24-year-olds in South African community-based populations, the summary estimate for BV prevalence was 42.1% (95% CI 35.6-49.0%), in Southern and Eastern African clinic and community based populations it was 35.2% (95% CI 27.7-43.6%), and in the higher-risk populations in Eastern Africa, BV prevalence was 49.5% (95% CI 42.2-56.8%). Prevalence was similar among women aged 25 to 49 years, with high heterogeneity across studies.[58]

Bacterial vaginosis prevalence among pregnant women

There have been three large meta-analyses examining BV prevalence in pregnant women. In a systematic review and meta-analysis of malaria, STIs and BV prevalence among pregnant women attending antenatal care facilities in sub-Saharan Africa from 1990-2011 (340,904 women), the burden of BV was higher than that of any STI.[59] The pooled BV prevalence estimate in East and Southern Africa was 50.8% (43.3-58.4%; n=4280) and in West and Central Africa was 37.6% (18.0-57.2%; n=1208).[59] A more recent meta-analysis of BV prevalence during pregnancy in sub-Saharan Africa, which included publications dating from 2015 to 2020 (48 studies, n=5042 women), yielded a pooled estimate of BV prevalence of 36.6% (27.1–46.6%).[60] Pooled BV prevalence estimates ranged from 28.5% (24.5–32.8%, n=1030) in Eastern Africa to 52.4% (33.5–70.9%, n=2305) in Southern Africa.[60] A subgroup analysis of pregnant women in the large global meta-analysis by Peebles *et al*. also reported data outside Africa, with pooled BV prevalence estimates in pregnant women ranging from 11.7% in South Asia (95% CI 9.0–14.7%) to 33.2% in Latin America and the Caribbean (95% CI 14.8–54.7%). Within the United States, they confirmed the racial and ethnic disparities observed in non-pregnant women. Prevalence of BV was highest among Black (49.0%; 95% CI 40.2–57.8%) and Hispanic pregnant women (42.7%; 95% CI 36.4–49.1%) and lowest among Asian (20.3%; 95% CI 5.4–41.2%) and White pregnant women (19.9%; 95% CI 8.0–35.5%).[61] Overall, there are limited pooled BV prevalence estimates from the Middle East for pregnant women, but a systematic review and meta-analysis of BV prevalence in Iran, that included studies up to 2017, reported the prevalence of BV in pregnant women to be 16.5% (95% CI 12.5–21.6%), compared to 28% (95% CI 15.1–45.9%) in non-pregnant women.[62]

Bacterial vaginosis prevalence among other populations/sub-groups of women

BV prevalence estimates are generally reported to be high in women with female partners. In a systematic review of BV prevalence among lesbian women, BV was the most frequent genital infection reported, and prevalence ranged from 25.7 to 42.8%.[63]

Peebles *et al*. undertook a sub-group analysis in their global meta-analysis, and found that BV prevalence was approximately 20% higher (33.5%; 95% CI 30.5-40.7%) in women with female partners than in women of the general population (p=0.007).[61] Peebles *et al*. also un-

dertook a sub-group analysis examining BV prevalence among women living with HIV (two studies from Southeast Asia and four from sub-Saharan Africa). Relative to women of the general population, BV prevalence was also higher among women living with HIV (35.6%; 95% CI 25.7–46.2% *vs.* 25.6; 95% CI 22.6–28.7%; p= 0.054).[61]

A recent systematic review and meta-analysis of women undergoing *in vitro* fertilization (IVF) found the overall prevalence of vaginal dysbiosis (BV by microscopy or dysbiosis by molecular methods including qPCR, 16S rRNA gene sequencing and interspace profiling) to be 18% (95% CI 17–19%) (644/3543) with considerable heterogeneity across studies (prevalence varying from 4 to 44%).[64] Importantly, there was no significant difference in the BV prevalence ratio between microscopy and molecular methods (0.87; 95% CI 0.74–1.02). Studies based on microscopy (n=13) found an overall prevalence of 17% (517/3091), and studies using molecular methods found a prevalence of 19% (171/889).[64]

While current diagnostic methods for BV are impacted by the menopausal status, a meta-analysis of all published studies reporting BV prevalence in post-menopausal women up to 2020, found that BV prevalence ranged from 2.0 to 57.1%, with a summary estimate of 16.93% (95% CI 8.5–27.4%). There was significant heterogeneity between studies and quality varied considerably.[65]

3.4 Risk factors

Table 3.1 lists common risk factors for BV. BV is more common among African American women.[66] It is sexually associated, including sexual activity with male and female partners. Fethers *et al*. identified sexual contact with new and multiple male and female partners to be associated with an increased risk of BV in a systematic review and meta-analysis.[46] Further studies have noted BV is highly prevalent (25 to 50 percent) in females who have sex with females and is associated with having a female partner with symptomatic BV, shared used of sex toys, and increasing numbers of female sexual partners.[39, 45, 67-70]

Presence of STIs appears to be associated with an increased prevalence of BV and presence of BV may also be a risk factor for HIV and other STIs.[71-78]

Other risk factors identified include inconsistent condom use, cigarette smoking, douching, and obesity.[50, 53, 55, 79-83] Past or current tobacco use has been reported to modify the vaginal milieu increasing bacterial virulence, as well as promoting an antiestrogen environment with additional vaginal amines.[84] Vaginal douching has been connected with modifications of the vaginal milieu and optimal VMB therefore, favoring an increased risk of BV.[85-87] However, cessation of douching does not seem to promote the return to a lactobacilli dominated VMB.[88]

The use of copper intrauterine devices has been associated with an elevated risk of BV.[85]

TABLE 3.1 Risk factors for bacterial vaginosis. HIV – human immunodeficiency virus; BV – bacterial vaginosis; STI – sexually transmitted infection
African American race
Sexual activity with male and female partners
Multiple sexual partners
New sexual partner
Female sexual partner with BV symptoms
Shared use of sex toys
Copper intrauterine devices
Douching
Cigarette smoking
Obesity
Inconsistent condom use
Previous or concurrent STI

3.5 Complications

Major sequelae of BV include an increased risk of PTB, postpartum endometritis, post hysterectomy vaginal cuff cellulitis, post abortal infection, pelvic inflammatory disease (PID), and STIs (including increased risk of HIV acquisition and transmission).

Preterm birth

While BV has been associated with PTB and greater risk of early pregnancy loss, early identification and treatment of asymptomatic women has failed to impact those rates.

A 2020 meta-analysis of studies concerning pregnancy outcomes according to vaginal microbiota, in sub-Saharan Africa, showed that an association between BV and PTB was not systematically reported (positive in seven out of nine studies).[86] Another recent meta-analysis including 44 studies found no difference in the incidence of PTB and related outcomes from treatment of asymptomatic women with BV in a general obstetric population.[87] Thus, the United States Preventative Services Task Force recommends against screening for BV in pregnant women not at increased risk for preterm delivery and concludes there is insufficient evidence to assess the benefits and harms of screening for BV in pregnant persons at increased risk for preterm delivery.[88]

Endometritis/ postpartum fever

As with PTB, BV has been associated with adverse postpartum complications including endometritis and postpartum fever. A Cochrane Systematic Review found that antibiotic prophylaxis given during the second or third trimester reduced the risk of postpartum endometritis when routinely administered to all pregnant women.[89] However, the authors noted

substantial bias due to the small numbers of studies available for analysis and high rate of loss to follow-up and concluded there was insufficient evidence to support routine use of antibiotics during pregnancy to prevent infectious adverse effects.

Post hysterectomy vaginal cuff cellulitis

Vaginal bacterial contamination is a major cause of febrile morbidity including post vaginal cuff cellulitis and pelvic abscess following total hysterectomy.[90] Thus, current standard of therapy includes vaginal cleaning with hexachlorophene or povidone-iodine in addition to the standard surgical preparation and prophylactic antibiotics.[90] Despite these measures, infections related to vaginal contamination persist and there remains a need to improve vaginal antisepsis for hysterectomy.

Post abortal infection

Risk factors for post abortal infection include history of PID, lower genital tract infection, BV, and age less than 20.[91] As with hysterectomy, vaginal bacterial contamination is a major cause of febrile morbidity with operative vaginal procedures despite current standard of therapy to reduce bacterial load (including prophylactic antibiotics and standard procedural preparation).

Pelvic inflammatory disease

PID has a multimicrobial etiology. BV (by Nugent score) has been associated with clinical as well as subclinical PID infections.[92, 93]

Other sexually transmitted infections

BV is associated with increased risk of acquisition of HIV and other STIs.[76-78, 94] Alteration in the vaginal microenvironment, by the lack of hydrogen peroxide producing lactobacilli in women with BV, has been postulated to increase the risk for STI acquisition. BV is frequently seen as a co-infection with cervical and vaginal STIs.[95, 96] Schwebke *et al*. conducted a randomized trial of metronidazole gel *vs.* observation in women with asymptomatic BV and found significantly fewer cases of *Chlamydia* in the treated group (1.58 *vs.* 2.29 per person-year).[95]

3.6
Signs and symptoms

BV is confined to an asymptomatic state in at least half of the cases, though it still is a leading cause of vulvovaginal symptoms worldwide.[97] Unlike single pathogen vaginal infections, BV is in fact thought of as a set of common clinical signs and symptoms that can be provoked by a plethora of bacterial species and communities, with different bacterial species showing different associations with presenting signs and symptoms, hence explaining considerable variability in clinical presentation.[8, 16] Key symptoms in BV, if present, do not seem to result primarily from inflammation, but rather from bacterial strategies deployed by the BV microbiota to colonize the vaginal niche. Breakdown of the protein backbone as well as of the sugar coating of

cervical mucins through bacterial mucinase action is thought to underlie at least in part vaginal discharge in this setting.[98] Symptomatic patients with BV may typically complain of copious vaginal discharge, that is thin, off-white to greyish, and sometimes described as foamy.[99] (Figure 3.1)

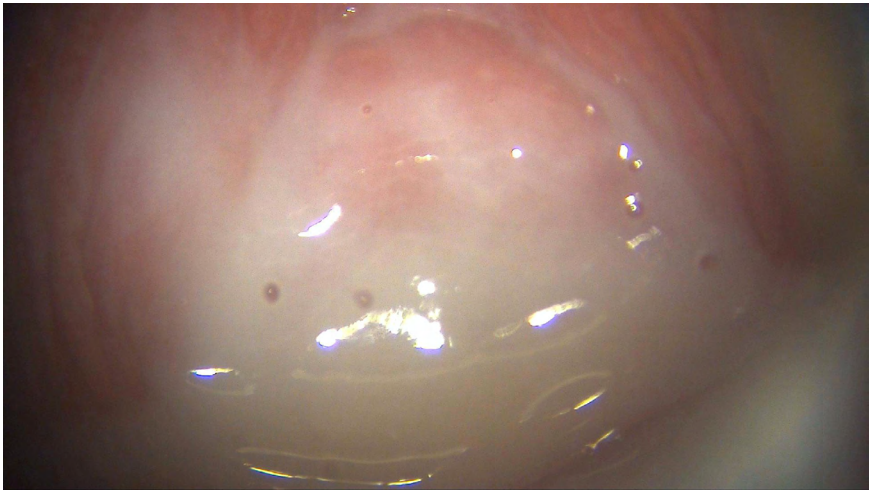

Figure 3.1 Typical discharge associated with bacterial vaginosis

The production of biogenic polyamines by a few bacterial species associated with BV, relates to the "fishy odor" or "fishy smelling" vaginal discharge, which is as a rather specific symptom of BV.[100, 101] Symptomatic patients may describe an even stronger smell after sexual intercourse and some perceive the odor also to be more noticeable during and following their period. Notably, a lack of perceived odor in patients with vulvovaginal symptoms makes the diagnosis of BV rather unlikely.[101] Conversely, symptoms other than fishy-smelling discharge, such as itch, dyspareunia, and dysuria, are not typically expected in BV, though such complaints may be present with mixed vaginitis, i.e. combined BV and vulvovaginal candidiasis.[102] To the attending health care provider, there are usually no overt or even appreciable signs of disease in patients with BV, though the typical "discharge" and "odor" may also be apparent as signs on clinical exam.

3.7 Diagnosis

While patient history and symptoms of "fishy odor" or "fishy smelling" vaginal discharge in particular, may be highly suggestive of BV, ultimate diagnosis will rely on microbiological confirmation, in addition to clinical presentation. Several point-of-care tests (POCTs) as well laboratory tests for diagnosis of BV are available to that purpose.

Clinical diagnosis

In clinical practice, the diagnostic criteria originally described by Amsel *et al*. have proven a useful diagnostic tool.[33] Clinical diagnosis of BV according to Amsel's criteria is conventionally made if three of the four following signs are present: (1) adherent and homogenous grayish-white vaginal discharge; (2) a vaginal pH exceeding a value of 4.5; (3) the presence of so-called clue cells (vaginal epithelial cells with such a heavy coating of BVAB that the peripheral borders are obscured) on saline wet mount (Figure 3.2); and (4) a fishy or amine odor after the addition of a 10% potassium hydroxide solution (positive whiff or sniff test).[33]

Despite being widely used, Amsel's criteria have been criticized, particularly because the appearance of the discharge and, to some extent, the appraisal of the odor, tend to be subjective, difficult to standardize, and hence prone to misdiagnosis. It has been suggested however, that Amsel's approach can be simplified to a modified combination of merely two criteria, without significant loss of overall sensitivity and specificity.[103-105] Elevated vaginal pH (>4.5) in particular, is consistently found as the most sensitive of all Amsel's criteria. It is important to acknowledge that many other factors may alter vaginal pH and/or interfere with vaginal pH assessment, notably menses/blood (even when not obvious on clinical exam), but also semen, as well as any inserted product (lubricants, creams, suppositories, etc.). Such artifacts generally involve a pH elevation and hence threaten specificity, rather than diagnostic sensitivity. Positioning of the pH strip close the external *os* of the cervix and close to the cervical mucus flow may also distort the picture. The presence of clue cells, in turn, is considered the single most specific predictor of BV.[97] The clue cell criterion is often cited as the presence of clue cells representing ≥20% of epithelial cells on microscopic examination, although the 20% cut-off was not originally mentioned by Amsel *et al*., but added later on as to increase specificity and overall accuracy.[33, 104, 106]

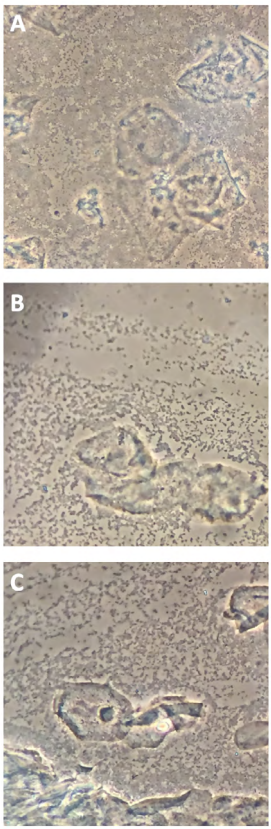

Figure 3.2 Wet mount microscopy (400x, phase contrast).

A-C– Bacterial vaginosis: absence of lactobacilli, granular microbiota and presence of clue cells (seen in A)

Amsel's approach has also been critiqued for its low sensitivity when compared to Gram-stain based or molecular diagnosis of BV. Across comparative studies, a wide range of sensitivities and specificities has been reported, with a sensitivity as low as 37% in one study and as high as 98.2% for the presence of clue cells on wet mount examination alone.[103, 107] Clearly, the performance of Amsel's criteria, whether modified or not, is highly dependent on the assessor's experience, time, and equipment. Furthermore, it should be acknowledged that Amsel's criteria have not

been developed as a screening tool, but rather as a diagnostic aid in case of vulvovaginal symptoms suggestive for BV. Overall, in the absence of molecular or biochemic POCTs, Amsel's clinical criteria remain the best option for in-office testing for BV. Clinical diagnosis of BV can be quickly obtained at very low cost, but does require the presence of a microscope and microscopy skills. In the presence of the latter, the diagnosis can be established using wet mount microscopy, with sensitivity and specificity ranging between 82-100% and 93-97%, respectively.[108]

Gram-stain diagnosis

Gram-stain based diagnosis has been the mainstay of BV diagnosis, especially in research settings, and is broadly accepted as the gold standard in that respect. (Figure 3.3)

This approach has several advantages, including a high frequency of interpretable results, a permanent record, and low cost. Gram-stained vaginal smears can also be interpreted repeatedly or independently by more than one assessor, thereby increasing diagnostic reliability.[97] Gram-stain diagnosis of BV does, however, require a specific laboratory setting and considerable skill, experience, and time. The most widely performed Gram-stain based method is the scoring system developed by Nugent *et al*..[14] Briefly, the Nugent scoring system accounts for three bacterial cell morphotypes – that is, *Lactobacillus* spp. morphotypes (large Gram-positive rods), *Gardnerella* spp. and *Bacteroides* spp. morphotypes (small Gram-variable or Gram-negative rods), and curved Gram-variable rods (such as *Mobiluncus* spp.). Although the taxonomic assignment of the morphotypes has been revised since then, the overall approach remains valid.[109] In particular, Srinivasan *et al*. found that the "Bacteroides morphotype," was primarily represented by *Prevotella* spp. and *Porphyromonas* spp., whereas *Mobiluncus* spp. morphotypes are more likely BVAB1 (*Candidatus* Lachnocurva *vaginae*). [109, 110] Based on the abundance of each of the aforementioned morphotypes per oil immersion field, quantitated from 0 to 4+, a summary score is obtained which equates the overall Nugent score (See section 2.5 and table 2.2).

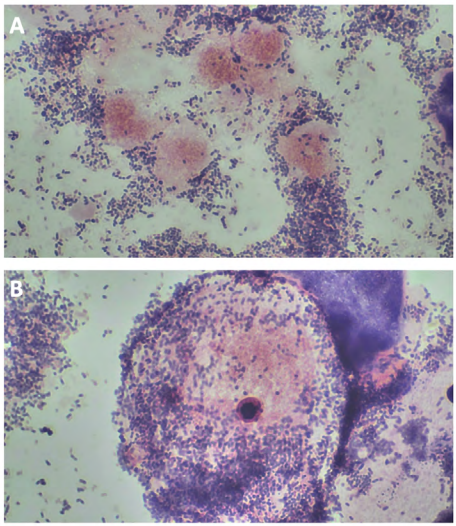

Figure 3.3 Gram stain (1000x, oil immersion).
A and B– Bacterial vaginosis (clue cell seen in B)

Diagnosis of BV is accepted when a score of 7 or higher is obtained. A score of 4–6 corresponds to intermediate vaginal microbiota, and a score of 0–3 is considered to represent non-BV microbiota. Hay and Ison developed a similar, simplified scoring system, which is known as the Ison-Hay criteria, in which smears are graded qualitatively as normal (grade

I), intermediate (grade II), or consistent with BV (grade III), further complemented with two additional grades, i.e. grade 0 for epithelial cells only with no bacteria, and grade IV for Gram positive cocci only. The Ison-Hay criteria tends to perform equally well, but is less widely used than the Nugent system.[111]

Overall, the Nugent scoring system for Gram-stained vaginal smears shows a high degree of accuracy and high reliability, as well as high intraobserver and interobserver reproducibility. This does not imply however that the Nugent scoring system is without shortcomings. Firstly, of concern is the defined lack of standardized pre-analytical and analytical conditions. Forsum *et al*. have reported on that account: different sampling devices and procedures, different ways of spreading the vaginal specimen on the glass slide, hence with variable homogeneity of the sample and thickness of the smear, different fixation methods and time, and differences in the area of the high-power oil immersion field at magnification ×1000, all of which may affect Gram stain interpretation.[112-114] Secondly, no definite criteria have been proposed to distinguish between the three basic morphotypes handled in the Nugent scoring system.[114] It may be added here, that the significance of the intermediate Nugent (scores 4 to 6) or grade II Ison-Hay category remains undetermined, although studies have suggested that about one third to one half of women with this category actually do have BV.[21, 115]

Cultures

Culture of *Gardnerella* spp. has no role in BV diagnostics. Most laboratories will report to the clinician who obtained a vaginal swab the results of Gram stain analysis as well as of culture on general and more specific media. A positive culture for *Gardnerella* spp. *per se* does not provide any information on the community state of the vaginal microbiota, as *Gardnerella* spp. is commonly part of the latter, also in women who do not have BV or intermediate microbiota.

Point-of-care tests (non-molecular)

As the most commonly used diagnostic approaches, Amsel's and Nugent's method, require time, skill, equipment, and experience, a defined need for rapid POCTs is perceived by many healthcare providers workers. Several such tests have been developed and commercialized, primarily in the US, though none are widely used. POCTs would ideally also allow for diagnosis of self-collected vaginal swabs or even self-diagnosis. Commercial POCTs typically rely on the detection of metabolites, specifically biogenic polyamines, such as trimethylamine, or short-chain fatty acids (SCFAs), either on the detection of the enzymes proline aminopeptidase and sialidase produced by several bacteria in women with BV.[21, 97] Among the better documented POCTs in this respect are the OSOM BV Blue test (Genzyme Diagnostics, Cambridge, MA, USA) and the FemExam card (Cooper Surgical, Shelton, CT, USA).

The OSOM BV Blue assay is a chromogenic dipstick test, based on the measurement of sialidase levels in vaginal fluid. The test is particularly fast, with results available within 10 minutes, and performs rather accurate, with reported sensitivities of 88 to 94% and specificities of 91 to 98%, compared to Nugent and Amsel's criteria, respectively.[21, 97]

The FemExam card, in turn, is a POCT that consists of two plastic cards, one for pH assessment and detection of trimethylamine, and a second one for proline aminopeptidase measurement.

The FemExam card is even faster than the OSOM BV Blue test, with results within two minutes, comparable sensitivity (91%), however with significantly lower specificity (estimated 61%).[21, 97]

Molecular diagnostics

Over the past two decades vaginal microbiome and BV research, has witnessed a marked shift towards molecular characterization techniques, primarily 16S rRNA gene, and to a smaller extent cpn60 gene amplification-based methods, such as next-generation sequencing. Molecular techniques will likely also replace existing in-office and laboratory BV diagnostics in the future. Current molecular diagnostic assays can be broadly divided in direct probe assays and nucleic acid amplification techniques (NAATs).[21] Their use is recommended only in symptomatic women.[116]

Direct probe assays make use of DNA probes that directly bind bacterial sequences, i.e. without an intermediate amplification step. The best-known example of a direct probe assay for BV is the so-called Affirm VPIII assay (Becton Dickinson, Sparks, MD, USA), which can provide results in less than one hour. This assay specifically targets *Gardnerella* spp. with a detection limit of $5x10^5$ colony forming units/mL of vaginal fluid. The Affirm VPIII assay performs well in comparison to the detection of clue cells with a sensitivity of 90% and a specificity of 97%, respectively, and in comparison with Nugent score-based BV diagnosis with a sensitivity of 94% and a specificity of 81%, respectively. A related test by the same company, Affirm VPIII microbial identification test (Becton Dickinson, Sparks, MD, USA) allows for simultaneous diagnosis of other common causes of vaginitis, such as *Candida* spp. and *Trichomonas vaginalis*.[21]

Nucleic acid amplification tests (NAATs), in turn, include an amplification step in which a specific nucleic acid sequence is enzymatically exponentially multiplied, before being detected by DNA probes. Hence, NAATs have very low detection limits and are theoretically capable of detecting as little as one organism in a sample.[21] Several such tests have been marketed. These NAATs for BV diagnosis will typically target multiple BV-related species (positive predictors), and most one or more vaginal *Lactobacillus* spp., as negative predictors. Some of these test have data validating the use of self sampling. (Table 3.2)

Differential diagnosis

An increased pH is not specific of BV; it can also be found in cases of trichomoniasis, vaginal atrophy and aerobic vaginitis/desquamative inflammatory vaginitis (AV/DIV).

Moreover, patients with trichomoniasis, atrophy and AV/DIV frequently have symptoms and findings of vaginal inflammation and dyspareunia. Women with BV usually lack these inflammatory symptoms and signs. Furthermore, parabasal cells are often increased in vaginal atrophy or AV/DIV and it can be easily detected in a wet mount preparation.

At times, patients can present with mixed "infections", such as, for example BV and *Trichomonas vaginalis* or *Candida* spp..

TABLE 3.2 Commercial nucleic acid amplification tests (NAATs) for BV (adapted from Coleman *et al.* and Muzny C *et al.*[21, 117]). Not all available tests are listed and not all listed tests widely available.

BVAB – Bacterial Vaginosis Associated Bacteria. [a] Compared with a combination of Nugent score and Amsel's criteria; [b] Compared with Nugent score; [c] Clinician collected sample (similar data for self-collected samples); [d] Compared with the BD Max™ Vaginal Panel; [e] personal communication from Barbara Van Der Pol

	Gardnerella spp.	*F. vaginae*	*Mobilluncus* spp.	*Megasphaera*	BVAB	*Lactobacillus*	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
NuSwab® (Laboratory Corporation of America Holdings, NC, USA)		x		x	x	x	96.7[a]	92.2[a]
BD Max™ Vaginal Panel (Becton Dickinson, MD, USA)	x	x		x	x	x	90.5[a] (88.3-92.2)	85.8 [a] (83-88.3)
MDL BV Panel (Medical Diagnostic Laboratory, NJ, USA)	x	x		x	x	x	99[a]	94[a]
Allplex™ Vaginitis (Seegene, Seoul, Korea)	x	x	x			x	91.7[b] (86.49-95.40)	86.6[b] (83.57-89.24)
Aptima® BV (Hologic, MA, USA)	x	x				x	95.0a[c] (93.1–96.4)	89.6[a,c] (87.1–91.6)
Xpert® Xpress MVP (Cepheid, CA, USA)[e]		x		x	x		93.8[c,d] (91.5-95.5%)	93.8% [c,d] (92.0-95.3)

3.8 Treatment

The treatment targets include symptom alleviation, infection prevention and control following surgery, and reduction of STIs. Regarding non-pregnant women, the established advantages of treatment include alleviating vulvo vaginal symptoms. Currently, there is no evidence to recommend the treatment for asymptomatic non-pregnant women.[81] BV can spontaneously clear without treatment in both pregnant and non-pregnant women.[118, 119] Screening and treatment may, nevertheless, be explored in high-risk groups for STIs since it

has been shown to increase the risk of infection with HIV, HPV, herpes simplex virus (HSV) 2, *T. vaginalis, Chlamydia trachomatis, Neisseria gonorrhea*, and *Mycoplasma genitalium*.

The principles of treatment for non-pregnant women; drug selection, dosing, adverse effects, and efficacy

The prescribing rational should be based on cost-effectiveness, availability of alternatives, adverse effects, and patient factors (request, previous response history). Oral and topical antibiotics (metronidazole, clindamycin, tinidazole, and secnidazole) and antiseptics (dequalinium chloride) are available to treat BV. (Table 3.3) Although the cure rates are about 80% for all medications and methods, relapses are frequent.[120] When metronidazole or clindamycin are not available, dequalinium chloride, tinidazole or secnidazole are acceptable alternatives.

Oral versus vaginal treatment

Both metronidazole and clindamycin are available in oral and vaginal forms. Oral treatment produces greater systemic adverse effects, including headache, nausea, abdominal pain, and diarrhea. The vaginal levels attained with topical treatment can be up to 30 times those of oral medication. This results in cure rates comparable to or slightly greater than those obtained by the oral route, with the added benefit of fewer side effects. [120]

Metronidazole

Dosing: Oral 500 mg metronidazole twice daily for seven days or vaginal metronidazole 0.75% gel once daily for five days.[116, 121] A novel single-dose 1.3% metronidazole gel is available.[122] However, we suggest the multiday treatments because it remains unknown if the 1.3% single-day dose is as effective as the multiday oral or vaginal regimens.

Adverse effects: A metallic taste, nausea, neutropenia, elevated international normalized ratio in patients receiving vitamin K antagonists (i.e. warfarin), peripheral neuropathy, and candidiasis are all possible side effects of oral and vaginal metronidazole.[123] Metronidazole allergy is uncommon, presenting as a rash, urticaria, and pruritus. Compared to clindamycin, metronidazole is less frequently related to *Clostridioides difficile* infection.[124]

Efficacy: The majority of comparative trials utilizing divided-dose oral regimens for one week obtained cure rates greater than 90% in the first week and up to 80% in the fourth week (based on Amsel criteria).[120, 125-127]

Special considerations:

- There is less evidence that continuing treatment beyond seven days is beneficial.[81]
- A novel metronidazole-loaded solid lipid nanoparticles vaginal emulgel showed a substantial therapeutic benefit for BV treatment in a randomized controlled trial.[128]

Clindamycin

Physicians should be aware of the potential for drug interactions and ensure the antibiotic is effective; drug interactions may occur when any form of clindamycin is used with medi-

cations that impact the functioning of CYP3A4 (clarithromycin, erythromycin, rifampin, tamoxifen, glucocorticoids, etc.).

Dosing: The recommended regimen is 5 g of 2% clindamycin cream intravaginally for seven days. Alternatives include clindamycin 300 mg twice a day orally for seven days or clindamycin 100 mg vaginal suppositories for three days.[116, 129] In some countries a formulation of clindamycin phosphate vaginal cream 2% is available; it is a sustained release formulation, used as a single dose.[130]

Adverse effects: Overgrowth of *Candida* spp. and gastrointestinal side effects are the most frequently reported adverse effects, and pseudomembranous colitis has been rarely reported.

Efficacy: A meta-analysis of randomized studies, both comparative and placebo-controlled, revealed the effectiveness of oral and vaginal clindamycin regimens.[120]

Special considerations:

- Clindamycin cream is oil-based and has the potential to weaken latex condoms and diaphragms for five days following application.

Overview of second-line and alternative treatments

Dequalinium chloride

Dosing: One 10 mg vaginal tablet daily for six days is the recommended regimen.

Adverse effects: The majority of adverse effects were local reactions, including vulvovaginal pruritus, vaginal discharge and burning sensation. Dequalinium chloride, unlike antibiotics, is less toxic to lactobacilli and does not increase the risk of candidiasis.[131]

Efficacy: In one report the cure rate was non-inferior to those attained with clindamycin.[132] It is not anticipated that bacteria can acquire resistance to it, and it is effective against causes of vaginitis other than BV, making it at least partially beneficial for "mixed" infections. Nevertheless, long-term research on recurrences is currently limited. Dequalinium is not available worldwide, including in the US.

Tinidazole

Tinidazole is a second-generation nitroimidazole that may be used in place of metronidazole or clindamycin if they are not accessible or tolerated.[133] It has an extended half-life (12 to 14 hours).

Dosing: We recommend taking 1 g orally once a day for five days. The effectiveness is somewhat higher, and the adverse effects are slightly lower than with tinidazole 2 g orally daily for two days.[134]

Adverse effects: The most often reported symptoms included a metallic taste, nausea, and fatigue and are comparable to oral metronidazole.[135]

Efficacy: Similar to metronidazole.

Secnidazole

Secnidazole is a nitroimidazole antibiotic with a longer half-life (17-19h) than metronidazole that is used as a substitute for metronidazole in BV treatment.

Dosing: Secnidazole is administered orally in a single 2 g packet of granules, which can be dissolved in a serving of pudding, applesauce, or yogurt.[136, 137]

Adverse effects: Secnidazole treatment is associated with an increased risk of candidiasis, nausea, diarrhea, and abdominal pain.[138]

Efficacy: Although single-dose secnidazole was superior to placebo and similar to metronidazole, there is no indication that it is superior to multidose metronidazole treatment.[138, 139]

Focused assessments of experimental/investigational treatments

Triple-sulfa creams, tetracycline, erythromycin, azithromycin, ampicillin, amoxicillin, 5% monolaurin vaginal gel, vaginal boric acid, lactic acid or acetic acid gel are not recommended. They are significantly less effective than metronidazole or clindamycin.[81, 140-148]

Although there is limited research on the use of lactobacilli, estriol, and sucrose gel in addition to antibiotics, there is not adequate evidence to include these approaches in treatment guidelines.[149]

A recent meta-analysis of three randomized controlled trials indicated that the novel drug Astodrimer 1% gel is superior to placebo and safe for BV treatment.[150] Future studies should compare it to antibiotic treatment.

Efficacy of probiotics for bacterial vaginosis treatment

Probiotics as a supplement to medication may be beneficial in the short term for treating recurrent vaginal infections in women. In some studies, probiotics decreased the recurrence rate of BV and the frequency of adverse effects and increased the cure rate of BV compared to antibiotics.[151] However, there is inadequate evidence that probiotics alone effectively treat acute symptomatic BV.[152, 153]

In pregnant women, oral probiotic preparations do not prevent BV.[154] Vaginal probiotics, including lactobacilli, offer the potential to treat and prevent BV.[155] However, vaginal probiotic capsules do not increase BV cure rates nor reduce recurrence.[156]

Follow-up

If symptoms resolve, follow-up is not indicated following sporadic infections.

Treatment regimens during pregnancy and lactation

Metronidazole 500 mg orally twice daily for seven days, metronidazole 250 mg orally three times daily for seven days, or clindamycin 300 mg orally twice daily for seven days are all efficacious and have been related with no significant fetal or obstetric complications.[157-159]

Topical regimens are not inferior to oral medication at treating or preventing adverse BV outcomes. Topical therapy includes metronidazole 0.75% gel intravaginally once daily for five days or clindamycin cream 2% intravaginally for seven nights.

For breastfeeding women, we recommend primarily oral metronidazole 500 mg twice a day for seven days or metronidazole 0.75% gel 5 g once daily intravaginally for five days. Clindamycin may have an adverse effect on the gastrointestinal microbiota of breastfed infants.

In animal models the use of high doses of dequalinium chloride was not detected in the blood stream. Studies using other quaternary ammonium compounds showed no embryofetal toxicity. Thus, it is assumed to be safe both during pregnancy and breastfeeding, but data are limited.[132]

Special considerations:

- Although some authors reported teratogenicity concerns in the past regarding metronidazole use during the first trimester, a meta-analysis concluded that there is no relationship between metronidazole exposure during the first trimester and congenital malformations.[160]
- The rate of vaginal lactobacilli colonization was low in pregnant women with normal vaginal microbiota at risk for preterm delivery following two months of oral *L. reuteri* RC-14 and *L. rhamnosus* GR-1 supplementation.[161]

Counseling-supportive management in infertile women attending fertility treatment

In individuals with tubal infertility, the prevalence of BV is considerably increased, and BV has been related to early spontaneous abortion. Nevertheless, the data was of extremely poor quality, and the inconclusive results suggest the need for more studies.[162]
Currently, a recommendation for screening of BV prior to fertility treatment cannot be made.[163, 164]

Screening and treatment of asymptomatic bacterial vaginosis in pregnancy

To avoid PTB and its associated complications, we do not recommend routine screening or treatment of pregnant women with asymptomatic BV. Although early diagnosis and treatment of asymptomatic pregnant women with a history of preterm delivery may have advantages, there is insufficient evidence to advocate this as a standard.[88, 89, 157, 165-168]

Approache to preoperative screening strategies for bacterial vaginosis

We recommend antibiotic therapy before transvaginal surgery for women with verified BV. The treatment alternatives are identical to those available to symptomatic non pregnant women.[169-174]

Management of sexual partners

A meta-analysis demonstrated that antibiotic treatment of male sexual partners did not increase the rate of clinical or symptomatic recovery, nor did it decrease the recurrence rate

throughout a four-week research period.[175] However, sexual activity with an untreated regular sexual partner following BV treatment was related to the development of a suboptimal vaginal microbiome.[176] In a large recent, randomized control study treatment of male sexual partners of women with recurrent BV, the use of oral metronidazole failed to reduce the recurrence rate.[177] Recently, Plummer *et al*. showed that the concomitant treatment of male partners of women with recurrent BV, using oral metronidazole and 2% clindamycin cream applied topically to penile skin, both twice daily for seven days, was not only well tolerated but also lead to higher than expected rates of cure.[178]

Despite the scarcity of data, treatment of female partners of women with BV may be considered, as there is high agreement rate concerning the BV status, even if asymptomatic.[179]

Management of recurrent and refractory bacterial vaginosis

Recurrent BV is defined as a confirmed diagnosis of BV occurring three or more times within one year.[180]

Within 12 months, following a successful treatment of BV, more than half of women experience a recurrence.[181] There is a lack of guidance for the optimal treatment of women with recurrent BV.[182] We recommend preventing symptomatic relapses with metronidazole 0.75% gel twice a week for 4–6 months, immediately following successful induction therapy. It is one of the most widely utilized regimens, with one study demonstrating a 70% success rate while on this maintenance prophylactic regimen. However, recurrence may still occur when medication is discontinued, and candidiasis is common throughout this treatment regimen.[183]

In women living with HIV 1, monthly treatment consisting of 2 g of oral metronidazole and 150 mg of oral fluconazole was useful in reducing recurrence, and the risk of candidiasis was also lower than in the placebo group.[184] In another study, in HIV negative women, with recurrent BV, a triple phase regimen consisting of oral induction metronidazole or tinidazole, followed by 30 consecutive days of vaginal boric acid, and then twice weekly vaginal metronidazole has been demonstrated to have a therapeutic efficacy of 65% at 28 weeks, but a 50% failure rate following cessation of drugs when followed at 36 weeks.[185] This triple phase therapy was further improved upon by using vaginal boric acid simultaneously with oral nitroimidazole and then followed by twice weekly vaginal metronidazole gel.[186]

BV recurrence and therapeutic failures may be linked to the failure to eradicate the biofilm. Vaginal boric acid is one of the drugs able to eradicate the biofilm; others include tobramycin, octenidine, and retrocyclin.[187] When prescribing boric acid women need to be warned of toxicity if ingested; ideally, the boric acid should be administered from a compounded formulation, not bought over the counter. Following effective treatment of BV, using 250 mg ascorbic acid vaginal tablets six times per month for six months in one study decreased the probability of recurrence from 32.4% to 16.2% (p=0.024).[188] More data however are needed. Mechanical removal of the biofilm by acidic, antiseptic or disinfectant vaginal washes has been proposed, but has not been adequately studied.[186, 189]

The utilization of *L. crispatus* CTV-05 (Lactin-V) following vaginal metronidazole treatment resulted in a substantially reduced BV recurrence compared to placebo at 12 weeks but, although encouraging, is not commercially available.[190]

The notion of utilizing probiotics to restore vaginal health is intuitive and tempting, but it has not been confirmed by adequate evidence. It does not affect the cure rate, although it may prolong the period between recurrences in up to 50%.[191, 192] Failure to restore the vaginal microbiota with probiotics may be related to the use of insufficient species and the failure of exogenous lactobacilli to colonize the vagina. Oral consumption of yogurt preparation was also suggested but has not been studied adequately.[193] Combined oral contraceptive pill alone does not lower the likelihood of BV recurrence; vaginal contraceptive ring use may promote a favorable microbiome after successful treatment.[194] Other risk factors worth addressing include cessation of smoking, use of condoms, and removal of intrauterine devices.

Refractory BV is a less common problem and is more likely due do antibiotic microbial resistance. It is more common in non-compliant women and with the use of single- dose therapy. Currently, there is a lack of guidelines for the management of refractory BV. Possible strategies may include ensuring compliance, changing the original route of treatment and always using a multidose scheme. In case of failure, changing the drug class should be tried. If the response is still inadequate, increasing dose (usually of vaginal formulations, as with the oral route there may be tolerance and safety issues) or combination therapy including vaginal boric acid can be tried.[182]

TABLE 3.3 BV treatment algorithm for first-line, second-line, and alternative medications in the current clinical practice

First-line	Metronidazole tablets	500 mg oral twice daily for 7 days
	Metronidazole 0.75% gel	5 g intravaginally once daily for 5 days
	Clindamycin cream 2%	5 g intravaginally once daily for 7 days
Second-line	Tinidazole	1 g oral once daily for 5 days
	Tinidazole	2 g oral once daily for 2 days
	Clindamycin	300 mg oral 2 once daily for 7 days
	Clindamycin	100 mg vaginal suppositories once daily for 3 days
	Secnidazole	2 g oral, single dose (dissolved in a serving of pudding, applesauce, or yogurt)
Alternatives	Dequalinium chloride	10 mg tablets intravaginally once daily for 6 days
	Clindamycin phosphate 2% cream	Single vaginal dose
	Metronidazole 1.3% gel	Single vaginal dose
Recurrent BV	Metronidazole 0.75% gel	2 times/week for 4–6 months
	Triple phase regimen: oral nitroimidazole, vaginal boric acid, and vaginal metronidazole	Oral nitroimidazole once daily for 7 days Vaginal boric acid once daily for 3 weeks Vaginal metronidazole gel twice a week for 16 weeks
	Metronidazole 2 g + fluconazole 150 mg	Once a month
BV during pregnancy and lactation	Metronidazole tablets	500 mg oral twice daily for 7 days 250 mg oral 3 times daily for 7 days
	Clindamycin capsules	300 mg oral twice daily for 7 days
	Metronidazole 0.75% gel	5 g intravaginally once daily 5 days
	Clindamycin cream 2%	5 g intravaginally once daily 7 days

3.9
Special situations

Infancy

An extensive review of the literature did not identify any specific information pertaining to BV in infancy. This is not unexpected as BV is a disruption of the normal post-menarchal VMB, which has not yet been established in infancy. As such, BV is not a condition usually associated with infancy.

Postmenopausal women

An extensive review of the literature identified a paucity of reliable data pertaining to the incidence of BV in postmenopausal women. This is not unexpected as BV is a disruption of the normal VMB, which is often not present in postmenopausal women, especially those who are not receiving estrogen therapy. The absence of local vaginal estrogen, especially in those women who are not newly postmenopausal, will create conditions such as increased pH, loss of lactobacilli, and vaginal dryness which will result in a dysbiotic picture in both wet mount and Nugent's score. In addition, it may take many years after onset of amenorrhea before developing changes in vaginal microbiota, regardless of age of onset of menopause or its duration. Also important are changes in well recognized lifestyle factors which influence the incidence of BV such as sexual activity, frequency of coitus, number of partners, and absence of contraception.

In postmenopausal women with a normal microbiome with or without use of estrogen supplementation, BV would be anticipated to occur at a similar rate as premenopausal women. However, in a systemic review and meta-analysis of BV in postmenopausal women by Stewart L *et al*., prevalence estimates ranged from 2.0 to 57.1% and the overall summary prevalence estimate was 16.93% (95% CI 8.45–27.4%; I2 = 97.9%; $p < 0.01$) but with marked heterogeneity. This was based on a total of 328 full-text articles assessed for eligibility, with only 13 studies found to be eligible for inclusion in the review. In addition, only three studies focused on postmenopausal women, with all other studies including adult women of all ages and none of the studies reported any sample size calculations. Also, only one population-based study was identified, and the pooled estimate had marked heterogeneity. All these limitations reinforce the scarcity of reliable BV prevalence data in the postmenopausal population.[65]

Finally, we must consider the tools used to diagnose BV as well. Nugent scores and Amsel's criteria were developed to diagnose BV in premenopausal women and whether or not these tests are reliable for the diagnosis of BV in postmenopausal women has not been validated. As such, it is uncertain if studies using these tests to diagnose BV provide accurate rates in the postmenopausal population. Molecular studies in postmenopausal women which can assess quantity and identity of suspected pathogens have not been conducted.[195]

Immunosuppression

Immunosuppression is a widely used term which is nonspecific and not frequently quantified, thus standardizing study criteria to assess its impact on disease states is challenging. Other than the complex relationship between BV and HIV, there are limited data discussing BV in the immunosuppressed population, however, BV is not a common problem in immunocompromised patients in general. A small study by Demirbilek M *et al*., using the Nugent score, diagnosed BV in 42% of kidney transplant recipients compared with 9% of healthy women.[196] Murphy *et al*. reviewed the relationship of host immunity, environment and the risk of BV and concluded that individuals with genetic variations which lowers their mucosal innate immune response are at a higher risk of developing BV.[84] It has long been known that women infected with HIV and in whom the disease is not well controlled have an increased risk of BV due to alterations in mucosal immunity.[72] In a review of the vaginal microbiome's relationship to various urogenital disorders de Seta *et al*. noted that multiple cross sectional studies have shown that independent of behavioral variables, HIV is often correlated with the presence of BV. They proposed that hydrogen peroxide producing lactobacilli were protective against HIV acquisition due to reduced recruitment of CD4+ cells to the vaginal mucosa. In addition, increased HIV-1 replication has been noted in a dysbiotic VMB due to the presence of HIV-inducing factor (HIF) in vaginal secretions.[5] Onderdonk AB *et al*. reviewed the human microbiome during BV and noted an increased risk of HIV acquisition in women with BV due to lower levels of antiviral factors such as secretory leukocyte protease inhibitor (SLPI). They also reported that women with BV, compared to controls, had cervicovaginal secretions which were lower in innate anti-HIV activity. [8]

Despite these associations between BV and HIV, the presence of BV or even recurrent BV is not considered an indication to screen for HIV.

Bacterial vaginosis in pregnancy

A dysbiotic vaginal microbiota has been linked to poor pregnancy outcomes, including PTB, PROM, fetal growth restriction/low birth weight, abortion, stillbirth, as well as to neonatal and puerperal infection.[4, 86] Nevertheless, a dysbiotic microbiota cannot be assumed as synonymous of BV.

The dominance by lactobacilli and consequent low pH out of pregancy is a unique feature of women, that is not shared with other mammals, including other primates. In these species, dominance by lactobacilli is seen merely during pregnancy, which led to the theory that this feature is needed for the success of pregnancy (leaving unanswered the question of why most women during their fertile years have dominance by lactobacilli, even out of pregnancy).[197]

Pregnancies with good outcomes tend to be dominated from an early phase by lactobacilli, have a stable microbiota, and low diversity during the whole pregnancy.[198-200] This profile is most probably a consequence of the marked increase in circulating estrogens during this phase. The shift from a less to a more favorable microbiota is more evident in women of African descendent, who when non-pregnant more often have non-lactobacilli dominated vaginal microbiota.[200] The success of pregnancy is associated with lactobacilli dominance, and not necessarily with a specific species within the genus.[201]

Nevertheless, the evidence of a relationship between PTB and BV has not been proved, with meta-analysis in different populations not showing a clear association.[86, 87]

The same is true for low birth weight, infants which in a 2020 meta-analysis of studies performed in Sub-Saharan women, was reported in two out of six studies; PROM was reported in two out of four studies and none showed an association with pregnancy loss.[86]

In 2020, based on the available evidence (review of 44 studies), the US Preventive Services Task Force issued a recommendation on the screening of BV in pregnant adolescents and women. They concluded that, in a general population, there was no benefit of screening and treating asymptomatic BV; however, in women with a previous PTB, the results were inconsistent, with three studies showing benefit, while two failed to do so. The question of whether to screen for BV in asymptomatic women remains unanswered.[87]

Part of the inconsistencies may be due to the diagnostic criteria and tests used for the diagnosis of BV (i.e. increased pH used as a surrogate of BV), the gestational age at the time of diagnosis, and the outcome evaluated (i.e. early or late PTB).

3.10
Future perspectives

BV is a field in which much work is still needed, including a better understanding of its etiology and complications. For instance, BV is a condition clearly sex-related but not defined as a STI. One of the interesting theories worth exploring in the future is that a phage may be the cause of BV and would explain its "transmission".[202]

There is a clear idea that, while common, BV is not an ideal or optimal type of microbiota. Most women with BV are asymptomatic, but it may constitute a disadvantage anyway. A better understanding of the relation between BV and STIs (including HPV infection and consequent cervical dysplasia), infertility, and obstetrical complications is essential to establish recommendations on eventual screening and treatment in asymptomatic populations.[163] An association may not necessarily be a cause-effect relationship. Also, even if indeed it is a cause-effect type of relationship, the direction of such may not always be obvious: for instance, it still is not clear if dysbiosis is a risk factor for HPV infection or if HPV infection leads to changes in the cervical and vaginal microbiota.[203]

As with other "vaginitis" in general, the assumption that an empirical diagnosis is easy and that no exams are needed must be challenged and changed.[204] This approach must be moved to the standard use of wet mount microscopy in office or, in the lack of expertise, the use the Amsel criteria or of Gram stain and Nugent score (despite the delay in obtaining the diagnosis). While point-of-care tests seem a reasonable intermediate option, molecular tests likely will be a major part of the future of the diagnosis of vaginitis. These tests have good performance and are already commercially available, despite lack of general access and having a significant associated cost. These open new perspectives, including "profiling" BV (i.e. the risk associated with BV may be different according to the specific bacteria – or even clades - present) and in the future antibiotic resistance testing is very likely to be pos-

sible.[205, 206] Molecular tests may become the gold standard for the diagnosis of BV. However, before that can be assumed, agreement on a bacterial profile (or profiles) of "molecular BV" will have to be established.[207]

The available treatments are very effective in the treatment of acute episodes, but recurrence is common. New therapeutic approaches, probably targeting the biofilm, are needed to improve the rates of recurrence. While probiotics use seems, from a theoretical point of view, a logical approach, the available results are not promising (for further details see chapter 10).[208]

A very promising area in the field of treatment is the vaginal microbiome transplant. The concept of transplanting vaginal microbiome from healthy women to women with intractable BV is still investigational, but the results are encouraging.[209, 210]

The establishment of core outcome sets to be evaluated in studies is also a topic that deserves attention in the near future. This will allow direct comparison between studies in the short term and in the medium and long term, the performance of meta-analysis with lower heterogeneity.

Recommendations

Recommendation	Quality of evidence	Strength of recommendation
Screening and treatment of bacterial vaginosis to prevent preterm birth is currently not recommended.	1a	A
The Amsel criteria may be useful in clinical practice, in the absence of expertise or availability of a microscope or other tests.	1b	A
The Amsel criteria are not suitable for the screening of bacterial vaginosis.	2b	C
The Nugent score is the gold standard for the diagnosis of bacterial vaginosis.	2a	B
The Ison-Hay criteria can be used as an alternative to the Nugent score.	4	C
Wet mount microscopy is a good tool for in office diagnosis of bacterial vaginosis.	2b	B
Cultures should not be used for the diagnosis of bacterial vaginosis.	4	D
Point of care tests, such as the OSOM BV Blue test and the FexExam card, can be used for the diagnosis of bacterial vaginosis.	3b	C
Direct DNA probe assays (Affirm VP) can be used for the diagnosis of bacterial vaginosis (as well as of candidiasis and trichomononisis).	2b	B
Nucleic acid amplification tests (Allplex vaginitis, BD Max vaginal panel, NuSwab, MDL BV panel) are recommended for the diagnosis of bacterial vaginosis.	2b	B
There is no evidence to recommend the treatment of bacterial vaginosis in asymptomatic non-pregnant women.	2b	B

There is no evidence to recommend screening and treatment of bacterial vaginosis prior to fertility treatments.	2b	B
Screening and treatment of bacterial vaginosis can be considered in women at high risk for sexually transmitted infections.	4	C
Treatment of asymptomatic bacterial vaginosis is recommended prior to transvaginal surgery.	2b	B
Topical or oral metronidazole or clindamycin are considered first line treatments for bacterial vaginosis.	1b	A
Tinidazole or secnidazole are acceptable oral alternatives.	2a	B
Vaginal dequalinium chloride can be considered as as option for the treatment of bacterial vaginosis.	2b	B
Women using vaginal clindamycin must be warned that it weakens condoms for up to 5 days after finishing the treatment.	5	D
Astodrimer 1% vaginal gel may be useful in the treatment of vaginal bacteriosis.	3a	B
Probiotics alone are not recommended as a treatment for bacterial vaginosis.	1a	A
Probiotics may decrease the rate of recurrence of bacterial vaginosis.	2a	B
The first line treatment options used in non-pregnant women can be used during pregnancy.	2a	B
In breastfeeding women, metronidazole may be preferable to clindamycin.	5	D
Treatment of partners is currently not recommended.	2a	B

References

1. Moosa, Y.; Kwon, D.; de Oliveira, T.; Wong, E. B., Determinants of Vaginal Microbiota Composition. *Front Cell Infect Microbiol* 2020, 10, 467.
2. Verstraelen, H.; Vieira-Baptista, P.; De Seta, F.; Ventolini, G.; Lonnee-Hoffmann, R.; Lev-Sagie, A., The Vaginal Microbiome: I. Research Development, Lexicon, Defining "Normal" and the Dynamics Throughout Women's Lives. *J Low Genit Tract Dis* 2022, 26, (1), 73-78.
3. Anahtar, M. N.; Gootenberg, D. B.; Mitchell, C. M.; Kwon, D. S., Cervicovaginal Microbiota and Reproductive Health: The Virtue of Simplicity. *Cell Host Microbe* 2018, 23, (2), 159-168.
4. Ventolini, G.; Vieira-Baptista, P.; De Seta, F.; Verstraelen, H.; Lonnee-Hoffmann, R.; Lev-Sagie, A., The Vaginal Microbiome: IV. The Role of Vaginal Microbiome in Reproduction and in Gynecologic Cancers. *J Low Genit Tract Dis* 2022, 26, (1), 93-98.
5. De Seta, F.; Lonnee-Hoffmann, R.; Campisciano, G.; Comar, M.; Verstraelen, H.; Vieira-Baptista, P.; Ventolini, G.; Lev-Sagie, A., The Vaginal Microbiome: III. The Vaginal Microbiome in Various Urogenital Disorders. *J Low Genit Tract Dis* 2022, 26, (1), 85-92.
6. Lev-Sagie, A.; De Seta, F.; Verstraelen, H.; Ventolini, G.; Lonnee-Hoffmann, R.; Vieira-Baptista, P., The Vaginal Microbiome: II. Vaginal Dysbiotic Conditions. *J Low Genit Tract Dis* 2022, 26, (1), 79-84.
7. Zozaya-Hinchliffe, M.; Lillis, R.; Martin, D. H.; Ferris, M. J., Quantitative PCR assessments of bacterial species in women with and without bacterial vaginosis. *J Clin Microbiol* 2010, 48, (5), 1812-9.
8. Onderdonk, A. B.; Delaney, M. L.; Fichorova, R. N., The Human Microbiome during Bacterial Vaginosis. *Clin Microbiol Rev* 2016, 29, (2), 223-38.
9. Boskey, E. R.; Telsch, K. M.; Whaley, K. J.; Moench, T. R.; Cone, R. A., Acid production by vaginal flora in vitro is consistent with the rate and extent of vaginal acidification. *Infect. Immun.* 1999, 67, (10), 5170-5.

10. Boskey, E. R.; Cone, R. A.; Whaley, K. J.; Moench, T. R., Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source. *Hum. Reprod.* 2001, 16, (9), 1809-13.
11. Fredricks, D. N.; Fiedler, T. L.; Marrazzo, J. M., Molecular identification of bacteria associated with bacterial vaginosis. *N. Engl. J. Med.* 2005, 353, (18), 1899-911.
12. Ravel, J.; Gajer, P.; Abdo, Z.; Schneider, G. M.; Koenig, S. S.; McCulle, S. L.; Karlebach, S.; Gorle, R.; Russell, J.; Tacket, C. O.; Brotman, R. M.; Davis, C. C.; Ault, K.; Peralta, L.; Forney, L. J., Vaginal microbiome of reproductive-age women. *Proc. Natl. Acad. Sci. U. S. A.* 2011, 108 Suppl 1, 4680-7.
13. McKinnon, L. R.; Achilles, S. L.; Bradshaw, C. S.; Burgener, A.; Crucitti, T.; Fredricks, D. N.; Jaspan, H. B.; Kaul, R.; Kaushic, C.; Klatt, N.; Kwon, D. S.; Marrazzo, J. M.; Masson, L.; McClelland, R. S.; Ravel, J.; van de Wijgert, J.; Vodstrcil, L. A.; Tachedjian, G., The Evolving Facets of Bacterial Vaginosis: Implications for HIV Transmission. *AIDS Res Hum Retroviruses* 2019, 35, (3), 219-228.
14. Nugent, R. P.; Krohn, M. A.; Hillier, S. L., Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol* 1991, 29, (2), 297-301.
15. Hillier, S. L., Diagnostic microbiology of bacterial vaginosis. *Am. J. Obstet. Gynecol.* 1993, 169, (2 Pt 2), 455-9.
16. Srinivasan, S.; Hoffman, N. G.; Morgan, M. T.; Matsen, F. A.; Fiedler, T. L.; Hall, R. W.; Ross, F. J.; McCoy, C. O.; Bumgarner, R.; Marrazzo, J. M.; Fredricks, D. N., Bacterial communities in women with bacterial vaginosis: high resolution phylogenetic analyses reveal relationships of microbiota to clinical criteria. *PLoS One* 2012, 7, (6), e37818.
17. Gajer, P.; Brotman, R. M.; Bai, G.; Sakamoto, J.; Schütte, U. M.; Zhong, X.; Koenig, S. S.; Fu, L.; Ma, Z. S.; Zhou, X.; Abdo, Z.; Forney, L. J.; Ravel, J., Temporal dynamics of the human vaginal microbiota. *Sci Transl Med* 2012, 4, (132), 132ra52.
18. Muzny, C. A.; Taylor, C. M.; Swords, W. E.; Tamhane, A.; Chattopadhyay, D.; Cerca, N.; Schwebke, J. R., An updated conceptual model on the pathogenesis of bacterial vaginosis. *J. Infect. Dis.* 2019, 220, (9), 1399-1405.
19. Muzny, C. A.; Schwebke, J. R., Gardnerella vaginalis: Still a Prime Suspect in the Pathogenesis of Bacterial Vaginosis. *Curr. Infect. Dis. Rep.* 2013, 15, (2), 130-5.
20. Schwebke, J. R.; Muzny, C. A.; Josey, W. E., Role of Gardnerella vaginalis in the pathogenesis of bacterial vaginosis: A conceptual model. *J. Infect. Dis.* 2014, 210, (3), 338-343.
21. Coleman, J. S.; Gaydos, C. A., Molecular Diagnosis of Bacterial Vaginosis: an Update. *J Clin Microbiol* 2018, 56, (9).
22. Vaneechoutte, M.; Guschin, A.; Van Simaey, L.; Gansemans, Y.; Van Nieuwerburgh, F.; Cools, P., Emended description of Gardnerella vaginalis and description of Gardnerella leopoldii sp. nov., Gardnerella piotii sp. nov. and Gardnerella swidsinskii sp. nov., with delineation of 13 genomic species within the genus Gardnerella. *Int. J. Syst. Evol. Microbiol.* 2019, 69, (3), 679-687.
23. Konschuh, S.; Jayaprakash, T.; Dolatabadi, A.; Dayo, E.; Ramay, H.; Sycuro, L., O02.3 Reclassification of Atopobium vaginae as three novel Fannyhessea species: implications for understanding their role in bacterial vaginosis. *Sexually Transmitted Infections* 2021, 97, (Suppl 1), A18-A18.
24. Swidsinski, A.; Mendling, W.; Loening-Baucke, V.; Ladhoff, A.; Swidsinski, S.; Hale, L. P.; Lochs, H., Adherent biofilms in bacterial vaginosis. *Obstet. Gynecol.* 2005, 106, (5 Pt 1), 1013-23.
25. Patterson, J. L.; Stull-Lane, A.; Girerd, P. H.; Jefferson, K. K., Analysis of adherence, biofilm formation and cytotoxicity suggests a greater virulence potential of Gardnerella vaginalis relative to other bacterial-vaginosis-associated anaerobes. *Microbiology* 2010, 156, (Pt 2), 392-9.
26. Alves, P.; Castro, J.; Sousa, C.; Cereija, T. B.; Cerca, N., Gardnerella vaginalis outcompetes 29 other bacterial species isolated from patients with bacterial vaginosis, using in an in vitro biofilm formation model. *J. Infect. Dis.* 2014, 210, (4), 593-6.
27. Udayalaxmi, J.; Bhat, G.; Kotigadde, S.; Kotian, S., Effect of pH on the adherence, surface hydrophobicity and the biofilm formation of Gardnerella Vaginalis. *Journal of Clinical and Diagnostic Research* 2012, 6, (6), 967-969.
28. Pybus, V.; Onderdonk, A. B., Evidence for a commensal, symbiotic relationship between Gardnerella vaginalis and Prevotella bivia involving ammonia: potential significance for bacterial vaginosis. *J. Infect. Dis.* 1997, 175, (2), 406-13.
29. Hardy, L.; Jespers, V.; Dahchour, N.; Mwambarangwe, L.; Musengamana, V.; Vaneechoutte, M.; Crucitti, T., Unravelling the bacterial vaginosis-associated biofilm: a multiplex Gardnerella vaginalis and Atopobium vaginae fluorescence in situ hybridization assay using peptide nucleic acid probes. *PLoS One* 2015, 10, (8), e0136658.
30. Briselden, A. M.; Moncla, B. J.; Stevens, C. E.; Hillier, S. L., Sialidases (neuraminidases) in bacterial vaginosis and bacterial vaginosis-associated microflora. *J. Clin. Microbiol.* 1992, 30, (3), 663-6.
31. Hardy, L.; Jespers, V.; Van den Bulck, M.; Buyze, J.; Mwambarangwe, L.; Musengamana, V.; Vaneechoutte, M.; Crucitti, T., The presence of the putative Gardnerella vaginalis sialidase A gene in vaginal specimens is associated with bacterial vaginosis biofilm. *PLoS One* 2017, 12, (2), e0172522.
32. Muzny, C. A.; Laniewski, P.; Schwebke, J. R.; Herbst-Kralovetz, M. M., Host-vaginal microbiota interactions in the pathogenesis of bacterial vaginosis. *Curr. Opin. Infect. Dis.* 2019.

33. Amsel, R.; Totten, P. A.; Spiegel, C. A.; Chen, K. C.; Eschenbach, D.; Holmes, K. K., Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. *Am J Med* 1983, 74, (1), 14-22.
34. Brand, J. M.; Galask, R. P., Trimethylamine: the substance mainly responsible for the fishy odor often associated with bacterial vaginosis. *Obstet. Gynecol.* 1986, 68, (5), 682-5.
35. Chen, K. C.; Forsyth, P. S.; Buchanan, T. M.; Holmes, K. K., Amine content of vaginal fluid from untreated and treated patients with nonspecific vaginitis. *J. Clin. Invest.* 1979, 63, (5), 828-35.
36. Srinivasan, S.; Morgan, M. T.; Fiedler, T. L.; Djukovic, D.; Hoffman, N. G.; Raftery, D.; Marrazzo, J. M.; Fredricks, D. N., Metabolic signatures of bacterial vaginosis. *mBio* 2015, 6, (2).
37. Muzny, C. A.; Schwebke, J. R., Pathogenesis of Bacterial Vaginosis: Discussion of Current Hypotheses. *J. Infect. Dis.* 2016, 214 Suppl 1, S1-5.
38. Mehta, S. D.; Zhao, D.; Green, S. J.; Agingu, W.; Otieno, F.; Bhaumik, R.; Bhaumik, D.; Bailey, R. C., The microbiome composition of a man's penis predicts incident bacterial vaginosis in his female sex partner with high accuracy. *Front Cell Infect Microbiol* 2020, 10, 433.
39. Vodstrcil, L. A.; Walker, S. M.; Hocking, J. S.; Law, M.; Forcey, D. S.; Fehler, G.; Bilardi, J. E.; Chen, M. Y.; Fethers, K. A.; Fairley, C. K.; Bradshaw, C. S., Incident bacterial vaginosis (BV) in women who have sex with women is associated with behaviors that suggest sexual transmission of BV. *Clin. Infect. Dis.* 2015, 60, (7), 1042-53.
40. Zozaya, M.; Ferris, M. J.; Siren, J. D.; Lillis, R.; Myers, L.; Nsuami, M. J.; Eren, A. M.; Brown, J.; Taylor, C. M.; Martin, D. H., Bacterial communities in penile skin, male urethra, and vaginas of heterosexual couples with and without bacterial vaginosis. *Microbiome* 2016, 4, 16.
41. Muzny, C. A.; Lensing, S. Y.; Aaron, K. J.; Schwebke, J. R., Incubation period and risk factors support sexual transmission of bacterial vaginosis in women who have sex with women. *Sex. Transm. Infect.* 2019, 0, (0), 1-5.
42. Bradshaw, C. S.; Vodstrcil, L. A.; Hocking, J. S.; Law, M.; Pirotta, M.; Garland, S. M.; De Guingand, D.; Morton, A. N.; Fairley, C. K., Recurrence of bacterial vaginosis is significantly associated with posttreatment sexual activities and hormonal contraceptive use. *Clin. Infect. Dis.* 2013, 56, (6), 777-86.
43. Bradshaw, C. S.; Walker, J.; Fairley, C. K.; Chen, M. Y.; Tabrizi, S. N.; Donovan, B.; Kaldor, J. M.; McNamee, K.; Urban, E.; Walker, S.; Currie, M.; Birden, H.; Bowden, F.; Garland, S.; Pirotta, M.; Gurrin, L.; Hocking, J. S., Prevalent and incident bacterial vaginosis are associated with sexual and contraceptive behaviours in young Australian women. *PLoS One* 2013, 8, (3), e57688.
44. Muzny, C. A.; Schwebke, J. R., Suspected heterosexual transmission of bacterial vaginosis without seminal fluid exposure. *Sex. Transm. Dis.* 2014, 41, (1), 58-60.
45. Marrazzo, J. M.; Thomas, K. K.; Fiedler, T. L.; Ringwood, K.; Fredricks, D. N., Risks for acquisition of bacterial vaginosis among women who report sex with women: a cohort study. *PLoS One* 2010, 5, (6), e11139.
46. Fethers, K. A.; Fairley, C. K.; Hocking, J. S.; Gurrin, L. C.; Bradshaw, C. S., Sexual risk factors and bacterial vaginosis: a systematic review and meta-analysis. *Clin Infect Dis* 2008, 47, (11), 1426-35.
47. Larsson, P. G.; Platz-Christensen, J. J.; Sundstrom, E., Is bacterial vaginosis a sexually transmitted disease? *Int. J. STD AIDS* 1991, 2, (5), 362-4.
48. Fethers, K. A.; Fairley, C. K.; Morton, A.; Hocking, J. S.; Hopkins, C.; Kennedy, L. J.; Fehler, G.; Bradshaw, C. S., Early sexual experiences and risk factors for bacterial vaginosis. *J. Infect. Dis.* 2009, 200, (11), 1662-70.
49. Hellberg, D.; Nilsson, S.; Mardh, P. A., Bacterial vaginosis and smoking. *Int. J. STD AIDS* 2000, 11, (9), 603-6.
50. Bradshaw, C. S.; Walker, S. M.; Vodstrcil, L. A.; Bilardi, J. E.; Law, M.; Hocking, J. S.; Fethers, K. A.; Fehler, G.; Petersen, S.; Tabrizi, S. N.; Chen, M. Y.; Garland, S. M.; Fairley, C. K., The influence of behaviors and relationships on the vaginal microbiota of women and their female partners: the WOW Health Study. *J Infect Dis* 2014, 209, (10), 1562-72.
51. Brotman, R. M.; He, X.; Gajer, P.; Fadrosh, D.; Sharma, E.; Mongodin, E. F.; Ravel, J.; Glover, E. D.; Rath, J. M., Association between cigarette smoking and the vaginal microbiota: a pilot study. *BMC Infect Dis* 2014, 14, 471.
52. Mehta, S. D.; Donovan, B.; Weber, K. M.; Cohen, M.; Ravel, J.; Gajer, P.; Gilbert, D.; Burgad, D.; Spear, G. T., The vaginal microbiota over an 8- to 10-year period in a cohort of HIV-infected and HIV-uninfected women. *PLoS One* 2015, 10, (2), e0116894.
53. Ness, R. B.; Hillier, S. L.; Richter, H. E.; Soper, D. E.; Stamm, C.; McGregor, J.; Bass, D. C.; Sweet, R. L.; Rice, P., Douching in relation to bacterial vaginosis, lactobacilli, and facultative bacteria in the vagina. *Obstet Gynecol* 2002, 100, (4), 765.
54. Hutchinson, K. B.; Kip, K. E.; Ness, R. B.; Gynecologic Infection Follow-Through, I., Vaginal douching and development of bacterial vaginosis among women with normal and abnormal vaginal microflora. *Sex. Transm. Dis.* 2007, 34, (9), 671-5.
55. Brotman, R. M.; Klebanoff, M. A.; Nansel, T. R.; Andrews, W. W.; Schwebke, J. R.; Zhang, J.; Yu, K. F.; Zenilman, J. M.; Scharfstein, D. O., A longitudinal study of vaginal douching and bacterial vaginosis--a marginal structural modeling analysis. *Am. J. Epidemiol.* 2008, 168, (2), 188-96.

56. Van der Veer, C.; Bruisten, S. M.; van Houdt, R.; Matser, A. A.; Tachedjian, G.; van de Wijgert, J.; de Vries, H. J. C.; van der Helm, J. J., Effects of an over-the-counter lactic-acid containing intra-vaginal douching product on the vaginal microbiota. *BMC Microbiol* 2019, 19, (1), 168.
57. Peebles, K.; Velloza, J.; Balkus, J. E.; McClelland, R. S.; Barnabas, R. V., High Global Burden and Costs of Bacterial Vaginosis: A Systematic Review and Meta-Analysis. *Sex Transm Dis* 2019, 46, (5), 304-311.
58. Torrone, E. A.; Morrison, C. S.; Chen, P. L.; Kwok, C.; Francis, S. C.; Hayes, R. J.; Looker, K. J.; McCormack, S.; McGrath, N.; van de Wijgert, J.; Watson-Jones, D.; Low, N.; Gottlieb, S. L.; Group, S. W., Prevalence of sexually transmitted infections and bacterial vaginosis among women in sub-Saharan Africa: An individual participant data meta-analysis of 18 HIV prevention studies. *PLoS Med.* 2018, 15, (2), e1002511.
59. Chico, R. M.; Mayaud, P.; Ariti, C.; Mabey, D.; Ronsmans, C.; Chandramohan, D., Prevalence of malaria and sexually transmitted and reproductive tract infections in pregnancy in sub-Saharan Africa: a systematic review. *JAMA* 2012, 307, (19), 2079-86.
60. Nyemba, D. C.; Haddison, E. C.; Wang, C.; Johnson, L. F.; Myer, L.; Davey, D. J., Prevalence of curable STIs and bacterial vaginosis during pregnancy in sub-Saharan Africa: a systematic review and meta-analysis. *Sex. Transm. Infect*. 2021.
61. Peebles, K.; Kiweewa, F. M.; Palanee-Phillips, T.; Chappell, C.; Singh, D.; Bunge, K. E.; Naidoo, L.; Makanani, B.; Jeenarain, N.; Reynolds, D.; Hillier, S. L.; Brown, E. R.; Baeten, J. M.; Balkus, J. E.; team, M. T. N. A. s., Elevated Risk of Bacterial Vaginosis among Users of the Copper Intrauterine Device: A Prospective Longitudinal Cohort Study. *Clin. Infect. Dis.* 2020.
62. Sabour, S.; Arzanlou, M.; Vaez, H.; Rahimi, G.; Sahebkar, A.; Khademi, F., Prevalence of bacterial vaginosis in pregnant and non-pregnant Iranian women: a systematic review and meta-analysis. *Arch. Gynecol. Obstet.* 2018, 297, (5), 1101-1113.
63. Takemoto, M. L. S.; Menezes, M. O.; Polido, C. B. A.; Santos, D. S.; Leonello, V. M.; Magalhaes, C. G.; Cirelli, J. F.; Knobel, R., Prevalence of sexually transmitted infections and bacterial vaginosis among lesbian women: systematic review and recommendations to improve care. *Cad. Saude Publica* 2019, 35, (3), e00118118.
64. Skafte-Holm, A.; Humaidan, P.; Bernabeu, A.; Lledo, B.; Jensen, J. S.; Haahr, T., The Association between Vaginal Dysbiosis and Reproductive Outcomes in Sub-Fertile Women Undergoing IVF-Treatment: A Systematic PRISMA Review and Meta-Analysis. *Pathogens* 2021, 10, (3).
65. Stewart, L. L.; Vodstrcil, L. A.; Coombe, J.; Bradshaw, C. S.; Hocking, J. S., Prevalence of bacterial vaginosis in postmenopausal women: a systematic review and meta-analysis. *Sex Health* 2022, 19, (1), 17-26.
66. Allsworth, J. E.; Peipert, J. F., Prevalence of bacterial vaginosis: 2001-2004 National Health and Nutrition Examination Survey data. *Obstet Gynecol* 2007, 109, (1), 114-20.
67. Fethers, K.; Marks, C.; Mindel, A.; Estcourt, C. S., Sexually transmitted infections and risk behaviours in women who have sex with women. *Sex Transm Infect* 2000, 76, (5), 345-9.
68. Marrazzo, J. M.; Antonio, M.; Agnew, K.; Hillier, S. L., Distribution of genital Lactobacillus strains shared by female sex partners. J Infect Dis 2009, 199, (5), 680-3.
69. Marrazzo, J. M.; Koutsky, L. A.; Eschenbach, D. A.; Agnew, K.; Stine, K.; Hillier, S. L., Characterization of vaginal flora and bacterial vaginosis in women who have sex with women. *J Infect Dis* 2002, 185, (9), 1307-13.
70. Evans, A. L.; Scally, A. J.; Wellard, S. J.; Wilson, J. D., Prevalence of bacterial vaginosis in lesbians and heterosexual women in a community setting. *Sex Transm Infect* 2007, 83, (6), 470-5.
71. Esber, A.; Vicetti Miguel, R. D.; Cherpes, T. L.; Klebanoff, M. A.; Gallo, M. F.; Turner, A. N., Risk of Bacterial Vaginosis Among Women With Herpes Simplex Virus Type 2 Infection: A Systematic Review and Meta-analysis. *J Infect Dis* 2015, 212, (1), 8-17.
72. Jamieson, D. J.; Duerr, A.; Klein, R. S.; Paramsothy, P.; Brown, W.; Cu-Uvin, S.; Rompalo, A.; Sobel, J., Longitudinal analysis of bacterial vaginosis: findings from the HIV epidemiology research study. *Obstet Gynecol* 2001, 98, (4), 656-63.
73. Myer, L.; Denny, L.; Telerant, R.; Souza, M.; Wright, T. C., Jr.; Kuhn, L., Bacterial vaginosis and susceptibility to HIV infection in South African women: a nested case-control study. *J Infect Dis* 2005, 192, (8), 1372-80.
74. Atashili, J.; Poole, C.; Ndumbe, P. M.; Adimora, A. A.; Smith, J. S., Bacterial vaginosis and HIV acquisition: a meta-analysis of published studies. *Aids* 2008, 22, (12), 1493-501.
75. Brotman, R. M.; Klebanoff, M. A.; Nansel, T. R.; Yu, K. F.; Andrews, W. W.; Zhang, J.; Schwebke, J. R., Bacterial vaginosis assessed by gram stain and diminished colonization resistance to incident gonococcal, chlamydial, and trichomonal genital infection. *J Infect Dis* 2010, 202, (12), 1907-15.
76. Abbai, N. S.; Reddy, T.; Ramjee, G., Prevalent bacterial vaginosis infection - a risk factor for incident sexually transmitted infections in women in Durban, South Africa. *Int J STD AIDS* 2016, 27, (14), 1283-1288.
77. Lokken, E. M.; Balkus, J. E.; Kiarie, J.; Hughes, J. P.; Jaoko, W.; Totten, P. A.; McClelland, R. S.; Manhart, L. E., Association of Recent Bacterial Vaginosis With Acquisition of Mycoplasma genitalium. *Am J Epidemiol* 2017, 186, (2), 194-201.

78. Brusselaers, N.; Shrestha, S.; van de Wijgert, J.; Verstraelen, H., Vaginal dysbiosis and the risk of human papillomavirus and cervical cancer: systematic review and meta-analysis. *Am J Obstet Gynecol* 2019, 221, (1), 9-18.e8.
79. Schwebke, J. R.; Desmond, R. A.; Oh, M. K., Predictors of bacterial vaginosis in adolescent women who douche. *Sex Transm Dis* 2004, 31, (7), 433-6.
80. Ness, R. B.; Kip, K. E.; Soper, D. E.; Stamm, C. A.; Rice, P.; Richter, H. E., Variability of bacterial vaginosis over 6- to 12-month intervals. *Sex Transm Dis* 2006, 33, (6), 381-5.
81. Schwebke, J. R.; Desmond, R. A., A randomized trial of the duration of therapy with metronidazole plus or minus azithromycin for treatment of symptomatic bacterial vaginosis. *Clin Infect Dis* 2007, 44, (2), 213-9.
82. Klebanoff, M. A.; Nansel, T. R.; Brotman, R. M.; Zhang, J.; Yu, K. F.; Schwebke, J. R.; Andrews, W. W., Personal hygienic behaviors and bacterial vaginosis. *Sex Transm Dis* 2010, 37, (2), 94-9.
83. Brookheart, R. T.; Lewis, W. G.; Peipert, J. F.; Lewis, A. L.; Allsworth, J. E., Association between obesity and bacterial vaginosis as assessed by Nugent score. *Am J Obstet Gynecol* 2019, 220, (5), 476.e1-476.e11.
84. Murphy, K.; Mitchell, C. M., The Interplay of Host Immunity, Environment and the Risk of Bacterial Vaginosis and Associated Reproductive Health Outcomes. *J Infect Dis* 2016, 214 Suppl 1, (Suppl 1), S29-35.
85. Peebles, K.; Kiweewa, F. M.; Palanee-Phillips, T.; Chappell, C.; Singh, D.; Bunge, K. E.; Naidoo, L.; Makanani, B.; Jeenarain, N.; Reynolds, D.; Hillier, S. L.; Brown, E. R.; Baeten, J. M.; Balkus, J. E., Elevated Risk of Bacterial Vaginosis Among Users of the Copper Intrauterine Device: A Prospective Longitudinal Cohort Study. *Clin Infect Dis* 2021, 73, (3), 513-520.
86. Juliana, N. C. A.; Suiters, M. J. M.; Al-Nasiry, S.; Morré, S. A.; Peters, R. P. H.; Ambrosino, E., The Association Between Vaginal Microbiota Dysbiosis, Bacterial Vaginosis, and Aerobic Vaginitis, and Adverse Pregnancy Outcomes of Women Living in Sub-Saharan Africa: A Systematic Review. *Front Public Health* 2020, 8, 567885.
87. Kahwati, L. C.; Clark, R.; Berkman, N.; Urrutia, R.; Patel, S. V.; Zeng, J.; Viswanathan, M., Screening for Bacterial Vaginosis in Pregnant Adolescents and Women to Prevent Preterm Delivery: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. *JAMA* 2020, 323, (13), 1293-1309.
88. Owens, D. K.; Davidson, K. W.; Krist, A. H.; Barry, M. J.; Cabana, M.; Caughey, A. B.; Donahue, K.; Doubeni, C. A.; Epling, J. W., Jr.; Kubik, M.; Ogedegbe, G.; Pbert, L.; Silverstein, M.; Simon, M. A.; Tseng, C. W.; Wong, J. B., Screening for Bacterial Vaginosis in Pregnant Persons to Prevent Preterm Delivery: US Preventive Services Task Force Recommendation Statement. *JAMA* 2020, 323, (13), 1286-1292.
89. Thinkhamrop, J.; Hofmeyr, G. J.; Adetoro, O.; Lumbiganon, P.; Ota, E., Antibiotic prophylaxis during the second and third trimester to reduce adverse pregnancy outcomes and morbidity. *Cochrane Database Syst Rev* 2015, 2015, (6), Cd002250.
90. Eason, E.; Wells, G.; Garber, G.; Hemmings, R.; Luskey, G.; Gillett, P.; Martin, M., Antisepsis for abdominal hysterectomy: a randomised controlled trial of povidone-iodine gel. *Bjog* 2004, 111, (7), 695-9.
91. Russo, J. A.; Achilles, S.; DePineres, T.; Gil, L., Controversies in family planning: postabortal pelvic inflammatory disease. *Contraception* 2013, 87, (4), 497-503.
92. Haggerty, C. L.; Hillier, S. L.; Bass, D. C.; Ness, R. B., Bacterial vaginosis and anaerobic bacteria are associated with endometritis. *Clin Infect Dis* 2004, 39, (7), 990-5.
93. Wiesenfeld, H. C.; Hillier, S. L.; Krohn, M. A.; Amortegui, A. J.; Heine, R. P.; Landers, D. V.; Sweet, R. L., Lower genital tract infection and endometritis: insight into subclinical pelvic inflammatory disease. *Obstet Gynecol* 2002, 100, (3), 456-63.
94. Abbai, N. S.; Nyirenda, M.; Naidoo, S.; Ramjee, G., Prevalent Herpes Simplex Virus-2 Increases the Risk of Incident Bacterial Vaginosis in Women from South Africa. *AIDS Behav* 2018, 22, (7), 2172-2180.
95. Schwebke, J. R.; Desmond, R., A randomized trial of metronidazole in asymptomatic bacterial vaginosis to prevent the acquisition of sexually transmitted diseases. *Am J Obstet Gynecol* 2007, 196, (6), 517.e1-6.
96. Wiesenfeld, H. C.; Hillier, S. L.; Krohn, M. A.; Landers, D. V.; Sweet, R. L., Bacterial vaginosis is a strong predictor of Neisseria gonorrhoeae and Chlamydia trachomatis infection. *Clin Infect Dis* 2003, 36, (5), 663-8.
97. Verstraelen, H.; Verhelst, R., Bacterial vaginosis: an update on diagnosis and treatment. *Expert Rev Anti Infect Ther* 2009, 7, (9), 1109-24.
98. Olmsted, S. S.; Meyn, L. A.; Rohan, L. C.; Hillier, S. L., Glycosidase and proteinase activity of anaerobic gram-negative bacteria isolated from women with bacterial vaginosis. *Sex Transm Dis* 2003, 30, (3), 257-61.
99. Spence, D.; Melville, C., Vaginal discharge. *BMJ* 2007, 335, (7630), 1147-51.
100. Nelson, T. M.; Borgogna, J. L.; Brotman, R. M.; Ravel, J.; Walk, S. T.; Yeoman, C. J., Vaginal biogenic amines: biomarkers of bacterial vaginosis or precursors to vaginal dysbiosis? *Front Physiol* 2015, 6, 253.
101. Anderson, M. R.; Klink, K.; Cohrssen, A., Evaluation of vaginal complaints. *JAMA* 2004, 291, (11), 1368-79.
102. Benyas, D.; Sobel, J. D., Mixed Vaginitis Due to Bacterial Vaginosis and Candidiasis. *J Low Genit Tract Dis* 2022, 26, (1), 68-70.
103. Thomason, J. L.; Gelbart, S. M.; Anderson, R. J.; Walt, A. K.; Osypowski, P. J.; Broekhuizen, F. F., Statistical evaluation of diagnostic criteria for bacterial vaginosis. *Am J Obstet Gynecol* 1990, 162, (1), 155-60.

104. Gutman, R. E.; Peipert, J. F.; Weitzen, S.; Blume, J., Evaluation of clinical methods for diagnosing bacterial vaginosis. *Obstet Gynecol* 2005, 105, (3), 551-6.
105. Vieira-Baptista, P.; Silva, A. R.; Costa, M.; Figueiredo, R.; Saldanha, C.; Sousa, C., Diagnosis of bacterial vaginosis: Clinical or microscopic? A cross-sectional study. *Int J Gynaecol Obstet* 2022, 156, (3), 552-559.
106. Eschenbach, D. A.; Hillier, S.; Critchlow, C.; Stevens, C.; DeRouen, T.; Holmes, K. K., Diagnosis and clinical manifestations of bacterial vaginosis. *Am J Obstet Gynecol* 1988, 158, (4), 819-28.
107. Sha, B. E.; Chen, H. Y.; Wang, Q. J.; Zariffard, M. R.; Cohen, M. H.; Spear, G. T., Utility of Amsel criteria, Nugent score, and quantitative PCR for Gardnerella vaginalis, Mycoplasma hominis, and Lactobacillus spp. for diagnosis of bacterial vaginosis in human immunodeficiency virus-infected women. *J Clin Microbiol* 2005, 43, (9), 4607-12.
108. Vieira-Baptista, P.; Grincevičienė, Š.; Oliveira, C.; Fonseca-Moutinho, J.; Cherey, F.; Stockdale, C. K., The International Society for the Study of Vulvovaginal Disease Vaginal Wet Mount Microscopy Guidelines: How to Perform, Applications, and Interpretation. *J Low Genit Tract Dis* 2021, 25, (2), 172-180.
109. Srinivasan, S.; Morgan, M. T.; Liu, C.; Matsen, F. A.; Hoffman, N. G.; Fiedler, T. L.; Agnew, K. J.; Marrazzo, J. M.; Fredricks, D. N., More than meets the eye: associations of vaginal bacteria with gram stain morphotypes using molecular phylogenetic analysis. *PLoS One* 2013, 8, (10), e78633.
110. Holm, J. B.; France, M. T.; Ma, B.; McComb, E.; Robinson, C. K.; Mehta, A.; Tallon, L. J.; Brotman, R. M.; Ravel, J., Comparative Metagenome-Assembled Genome Analysis of "Candidatus Lachnocurva vaginae", Formerly Known as Bacterial Vaginosis-Associated Bacterium-1 (BVAB1). *Front Cell Infect Microbiol* 2020, 10, 117.
111. Ison, C. A.; Hay, P. E., Validation of a simplified grading of Gram stained vaginal smears for use in genitourinary medicine clinics. *Sex Transm Infect* 2002, 78, (6), 413-5.
112. Forsum, U.; Larsson, P. G.; Spiegel, C., Scoring vaginal fluid smears for diagnosis of bacterial vaginosis: need for quality specifications. *Apmis* 2008, 116, (2), 156-9.
113. Forsum, U.; Jakobsson, T.; Larsson, P. G.; Schmidt, H.; Beverly, A.; Bjørnerem, A.; Carlsson, B.; Csango, P.; Donders, G.; Hay, P.; Ison, C.; Keane, F.; McDonald, H.; Moi, H.; Platz-Christensen, J. J.; Schwebke, J., An international study of the interobserver variation between interpretations of vaginal smear criteria of bacterial vaginosis. *Apmis* 2002, 110, (11), 811-8.
114. Larsson, P. G.; Carlsson, B.; Fåhraeus, L.; Jakobsson, T.; Forsum, U., Diagnosis of bacterial vaginosis: need for validation of microscopic image area used for scoring bacterial morphotypes. *Sex Transm Infect* 2004, 80, (1), 63-7.
115. Taylor-Robinson, D.; Morgan, D. J.; Sheehan, M.; Rosenstein, I. J.; Lamont, R. F., Relation between Gram-stain and clinical criteria for diagnosing bacterial vaginosis with special reference to Gram grade II evaluation. *Int J STD AIDS* 2003, 14, (1), 6-10.
116. Workowski, K. A.; Bachmann, L. H.; Chan, P. A.; Johnston, C. M.; Muzny, C. A.; Park, I.; Reno, H.; Zenilman, J. M.; Bolan, G. A., Sexually Transmitted Infections Treatment Guidelines, 2021. *MMWR Recomm Rep* 2021, 70, (4), 1-187.
117. Muzny, C. A.; Balkus, J.; Mitchell, C.; Sobel, J. D.; Workowski, K.; Marrazzo, J.; Schwebke, J. R., Diagnosis and Management of Bacterial Vaginosis: Summary of Evidence Reviewed for the 2021 Centers for Disease Control and Prevention Sexually Transmitted Infections Treatment Guidelines. *Clin Infect Dis* 2022, 74, (Suppl_2), S144-s151.
118. Klebanoff, M. A.; Hauth, J. C.; MacPherson, C. A.; Carey, J. C.; Heine, R. P.; Wapner, R. J.; Iams, J. D.; Moawad, A.; Miodovnik, M.; Sibai, B. M.; vanDorsten, J. P.; Dombrowski, M. P., Time course of the regression of asymptomatic bacterial vaginosis in pregnancy with and without treatment. *Am J Obstet Gynecol* 2004, 190, (2), 363-70.
119. Sherrard, J.; Wilson, J.; Donders, G.; Mendling, W.; Jensen, J. S., 2018 European (IUSTI/WHO) International Union against sexually transmitted infections (IUSTI) World Health Organisation (WHO) guideline on the management of vaginal discharge. *Int J STD AIDS* 2018, 29, (13), 1258-1272.
120. Oduyebo, O. O.; Anorlu, R. I.; Ogunsola, F. T., The effects of antimicrobial therapy on bacterial vaginosis in non-pregnant women. *Cochrane Database Syst Rev* 2009, (3), Cd006055.
121. Livengood, C. H., 3rd; Soper, D. E.; Sheehan, K. L.; Fenner, D. E.; Martens, M. G.; Nelson, A. L.; Ismail, M.; Thorp, J. M.; Lappin, M.; Long, B. J.; Blackwelder, T.; Sweet, R. L.; Sagov, S., Comparison of once-daily and twice-daily dosing of 0.75% metronidazole gel in the treatment of bacterial vaginosis. *Sex Transm Dis* 1999, 26, (3), 137-42.
122. Schwebke, J. R.; Marrazzo, J.; Beelen, A. P.; Sobel, J. D., A Phase 3, Multicenter, Randomized, Double-Blind, Vehicle-Controlled Study Evaluating the Safety and Efficacy of Metronidazole Vaginal Gel 1.3% in the Treatment of Bacterial Vaginosis. *Sex Transm Dis* 2015, 42, (7), 376-81.
123. Retamal-Valdes, B.; Tavares, A. P. L.; Monique, S.; Pereira da Silva, H. D.; Mestnik, M. J.; Duarte, P. M.; Miranda, T. S.; Borges, I.; Soares, G. M. S.; Faveri, M.; Castro Dos Santos, N.; Graças, Y. T. D.; Souto, M. L. S.; Giudicissi, M.; Romito, G. A.; Saraiva, L.; Pannuti, C. M.; Figueiredo, L. C.; Feres, M., Adverse events of metronidazole and amoxicillin: Retrospective analysis of a large data set of five randomized clinical trials. *J Clin Periodontol* 2022.

124. Slimings, C.; Riley, T. V., Antibiotics and hospital-acquired Clostridium difficile infection: update of systematic review and meta-analysis. *J Antimicrob Chemother* 2014, 69, (4), 881-91.
125. Hanson, J. M.; McGregor, J. A.; Hillier, S. L.; Eschenbach, D. A.; Kreutner, A. K.; Galask, R. P.; Martens, M., Metronidazole for bacterial vaginosis. A comparison of vaginal gel vs. oral therapy. *J Reprod Med 2000*, 45, (11), 889-96.
126. Joesoef, M. R.; Schmid, G. P.; Hillier, S. L., Bacterial vaginosis: review of treatment options and potential clinical indications for therapy. *Clin Infect Dis* 1999, 28 Suppl 1, S57-65.
127. Joesoef, M. R.; Schmid, G. P., Bacterial vaginosis: review of treatment options and potential clinical indications for therapy. *Clin Infect Dis* 1995, 20 Suppl 1, S72-9.
128. Badawi, N. M.; Elkafrawy, M. A.; Yehia, R. M.; Attia, D. A., Clinical comparative study of optimized metronidazole loaded lipid nanocarrier vaginal emulgel for management of bacterial vaginosis and its recurrence. *Drug Deliv* 2021, 28, (1), 814-825.
129. Paavonen, J.; Mangioni, C.; Martin, M. A.; Wajszczuk, C. P., Vaginal clindamycin and oral metronidazole for bacterial vaginosis: a randomized trial. *Obstet Gynecol* 2000, 96, (2), 256-60.
130. Faro, S.; Skokos, C. K., The efficacy and safety of a single dose of Clindesse vaginal cream versus a seven-dose regimen of Cleocin vaginal cream in patients with bacterial vaginosis. *Infect Dis Obstet Gynecol* 2005, 13, (3), 155-60.
131. Donders, G.; Bellen, G.; Donders, F.; Pinget, J.; Vandevelde, I.; Michiels, T.; Byamughisa, J., Improvement of abnormal vaginal flora in Ugandan women by self-testing and short use of intravaginal antimicrobials. *Eur J Clin Microbiol Infect Dis* 2017, 36, (4), 731-738.
132. Mendling, W.; Weissenbacher, E. R.; Gerber, S.; Prasauskas, V.; Grob, P., Use of locally delivered dequalinium chloride in the treatment of vaginal infections: a review. *Arch Gynecol Obstet* 2016, 293, (3), 469-84.
133. Tinidazole (Tindamax)--a new option for treatment of bacterial vaginosis. *Med Lett Drugs Ther* 2007, 49, (1269), 73-4.
134. FDA Tindamax® (tinidazole) tablets for oral use. https://www.accessdata.fda.gov/drugsatfda_docs/label/2007/021618s003lbl.pdf
135. Schwebke, J. R.; Desmond, R. A., Tinidazole vs metronidazole for the treatment of bacterial vaginosis. *Am J Obstet Gynecol* 2011, 204, (3), 211.e1-6.
136. Abd El Aziz, M. A.; Sharifipour, F.; Abedi, P.; Jahanfar, S.; Judge, H. M., Secnidazole for treatment of bacterial vaginosis: a systematic review. *BMC Womens Health* 2019, 19, (1), 121.
137. Pentikis, H.; Adetoro, N.; Tipping, D.; Levy, S., An Integrated Efficacy and Safety Analysis of Single-Dose Secnidazole 2 g in the Treatment of Bacterial Vaginosis. *Reprod Sci* 2020, 27, (2), 523-528.
138. Elghazaly, S. M.; Hamam, K. M.; Badawy, M. M.; Yakoub Agha, N. A.; Samy, A.; Abbas, A. M., Efficacy and safety of single dose of oral secnidazole 2 g in treatment of bacterial vaginosis: A systematic review and meta-analysis. *Eur J Obstet Gynecol Reprod Biol* 2019, 238, 125-131.
139. Schwebke, J. R.; Morgan, F. G., Jr.; Koltun, W.; Nyirjesy, P., A phase-3, double-blind, placebo-controlled study of the effectiveness and safety of single oral doses of secnidazole 2 g for the treatment of women with bacterial vaginosis. *Am J Obstet Gynecol* 2017, 217, (6), 678.e1-678.e9.
140. Plummer, E. L.; Bradshaw, C. S.; Doyle, M.; Fairley, C. K.; Murray, G. L.; Bateson, D.; Masson, L.; Slifirski, J.; Tachedjian, G.; Vodstrcil, L. A., Lactic acid-containing products for bacterial vaginosis and their impact on the vaginal microbiota: A systematic review. *PLoS One* 2021, 16, (2), e0246953.
141. McCormack, W. M.; Covino, J. M.; Thomason, J. L.; Eschenbach, D. A.; Mou, S.; Kapernick, P.; McGregor, J.; Rein, M. F.; Hillier, S. L., Comparison of clindamycin phosphate vaginal cream with triple sulfonamide vaginal cream in the treatment of bacterial vaginosis. *Sex Transm Dis* 2001, 28, (10), 569-75.
142. Wathne, B.; Holst, E.; Hovelius, B.; Mårdh, P. A., Erythromycin versus metronidazole in the treatment of bacterial vaginosis. *Acta Obstet Gynecol Scand* 1993, 72, (6), 470-4.
143. Piot, P., Bacterial vaginosis. An evaluation of treatment. *Scand J Urol Nephrol Suppl* 1984, 86, 229-35.
144. Wewalka, G.; Stary, A.; Bosse, B.; Duerr, H. E.; Reimer, K., Efficacy of povidone-iodine vaginal suppositories in the treatment of bacterial vaginosis. *Dermatology* 2002, 204 Suppl 1, 79-85.
145. Duff, P.; Lee, M. L.; Hillier, S. L.; Herd, L. M.; Krohn, M. A.; Eschenbach, D. A., Amoxicillin treatment of bacterial vaginosis during pregnancy. *Obstet Gynecol* 1991, 77, (3), 431-5.
146. Mancuso, A. C.; Widdice, L. E.; Hughes, B. L.; Schlievert, P.; Swamy, G. K.; Stockdale, C. K.; Bernstein, D. I.; Winokur, P. L., Five Percent Monolaurin Vaginal Gel for the Treatment of Bacterial Vaginosis: A Randomized Placebo-Controlled Trial. *J Low Genit Tract Dis* 2020, 24, (3), 277-283.
147. Schoeman, J.; Steyn, P. S.; Odendaal, H. J.; Grové, D., Bacterial vaginosis diagnosed at the first antenatal visit better predicts preterm labour than diagnosis later in pregnancy. *J Obstet Gynaecol* 2005, 25, (8), 751-3.
148. Armstrong-Buisseret, L.; Brittain, C.; Kai, J.; David, M.; Anstey Watkins, J.; Ozolins, M.; Jackson, L.; Abdali, Z.; Hepburn, T.; Griffiths, F.; Montgomery, A.; Daniels, J.; Manley, A.; Dean, G.; Ross, J. D., Lactic acid gel versus metronidazole for recurrent bacterial vaginosis in women aged 16 years and over: the VITA RCT. *Health Technol Assess* 2022, 26, (2), 1-170.

149. Tidbury, F. D.; Langhart, A.; Weidlinger, S.; Stute, P., Non-antibiotic treatment of bacterial vaginosis-a systematic review. *Arch Gynecol Obstet* 2021, 303, (1), 37-45.
150. Abu-Zaid, A.; Alshahrani, M. S.; Bakhsh, H.; Miski, N. T.; Abuzaid, M.; Alomar, O.; Jabrah, E.; Jamjoom, M. Z.; Salem, H.; Al-Badawi, I. A.; Baradwan, S., Astodrimer gel for treatment of bacterial vaginosis: A systematic review and meta-analysis of randomized controlled trials. *Int J Clin Pract* 2021, 75, (7), e14165.
151. Liu, H. F.; Yi, N., A systematic review and meta-analysis on the efficacy of probiotics for bacterial vaginosis. *Eur Rev Med Pharmacol Sci* 2022, 26, (1), 90-98.
152. Jeng, H. S.; Yan, T. R.; Chen, J. Y., Treating vaginitis with probiotics in non-pregnant females: A systematic review and meta-analysis. *Exp Ther Med* 2020, 20, (4), 3749-3765.
153. Vieira-Baptista, P.; De Seta, F.; Verstraelen, H.; Ventolini, G.; Lonnee-Hoffmann, R.; Lev-Sagie, A., The Vaginal Microbiome: V. Therapeutic Modalities of Vaginal Microbiome Engineering and Research Challenges. *J Low Genit Tract Dis* 2022, 26, (1), 99-104.
154. Husain, S.; Allotey, J.; Drymoussi, Z.; Wilks, M.; Fernandez-Felix, B. M.; Whiley, A.; Dodds, J.; Thangaratinam, S.; McCourt, C.; Prosdocimi, E. M.; Wade, W. G.; de Tejada, B. M.; Zamora, J.; Khan, K.; Millar, M., Effects of oral probiotic supplements on vaginal microbiota during pregnancy: a randomised, double-blind, placebo-controlled trial with microbiome analysis. *Bjog* 2020, 127, (2), 275-284.
155. Van de Wijgert, J.; Verwijs, M. C., Lactobacilli-containing vaginal probiotics to cure or prevent bacterial or fungal vaginal dysbiosis: a systematic review and recommendations for future trial designs. *Bjog* 2020, 127, (2), 287-299.
156. Marcotte, H.; Larsson, P. G.; Andersen, K. K.; Zuo, F.; Mikkelsen, L. S.; Brandsborg, E.; Gray, G.; Laher, F.; Otwombe, K., An exploratory pilot study evaluating the supplementation of standard antibiotic therapy with probiotic lactobacilli in south African women with bacterial vaginosis. *BMC Infect Dis* 2019, 19, (1), 824.
157. Brocklehurst, P.; Gordon, A.; Heatley, E.; Milan, S. J., Antibiotics for treating bacterial vaginosis in pregnancy. *Cochrane Database Syst Rev* 2013, (1), Cd000262.
158. Leitich, H.; Brunbauer, M.; Bodner-Adler, B.; Kaider, A.; Egarter, C.; Husslein, P., Antibiotic treatment of bacterial vaginosis in pregnancy: a meta-analysis. *Am J Obstet Gynecol* 2003, 188, (3), 752-8.
159. Riggs, M. A.; Klebanoff, M. A., Treatment of vaginal infections to prevent preterm birth: a meta-analysis. *Clin Obstet Gynecol* 2004, 47, (4), 796-807; discussion 881-2.
160. Caro-Patón, T.; Carvajal, A.; Martin de Diego, I.; Martin-Arias, L. H.; Alvarez Requejo, A.; Rodríguez Pinilla, E., Is metronidazole teratogenic? A meta-analysis. *Br J Clin Pharmacol* 1997, 44, (2), 179-82.
161. Yefet, E.; Colodner, R.; Strauss, M.; Gam Ze Letova, Y.; Nachum, Z., A Randomized Controlled Open Label Crossover Trial to Study Vaginal Colonization of Orally Administered Lactobacillus Reuteri RC-14 and Rhamnosus GR-1 in Pregnant Women at High Risk for Preterm Labor. *Nutrients* 2020, 12, (4).
162. Haahr, T.; Zacho, J.; Bräuner, M.; Shathmigha, K.; Skov Jensen, J.; Humaidan, P., Reproductive outcome of patients undergoing in vitro fertilisation treatment and diagnosed with bacterial vaginosis or abnormal vaginal microbiota: a systematic PRISMA review and meta-analysis. *Bjog* 2019, 126, (2), 200-207.
163. Vieira-Baptista, P.; Silva-Soares, S.; Lyra, J.; Falcão, V.; Póvoa, A. M.; Calejo, L.; Sousa, S., Wet Mount Microscopy of the Vaginal Milieu Does Not Predict the Outcome of Fertility Treatments: A Cross-sectional Study. *J Low Genit Tract Dis* 2022.
164. Van Oostrum, N.; De Sutter, P.; Meys, J.; Verstraelen, H., Risks associated with bacterial vaginosis in infertility patients: a systematic review and meta-analysis. *Hum Reprod* 2013, 28, (7), 1809-15.
165. Nygren, P.; Fu, R.; Freeman, M.; Bougatsos, C.; Klebanoff, M.; Guise, J. M., Evidence on the benefits and harms of screening and treating pregnant women who are asymptomatic for bacterial vaginosis: an update review for the U.S. Preventive Services Task Force. *Ann Intern Med* 2008, 148, (3), 220-33.
166. Rebouças, K. F.; Eleutério, J., Jr.; Peixoto, R. C.; Costa, A. P. F.; Cobucci, R. N.; Gonçalves, A. K., Treatment of bacterial vaginosis before 28 weeks of pregnancy to reduce the incidence of preterm labor. *Int J Gynaecol Obstet* 2019, 146, (3), 271-276.
167. Subtil, D.; Brabant, G.; Tilloy, E.; Devos, P.; Canis, F.; Fruchart, A.; Bissinger, M. C.; Dugimont, J. C.; Nolf, C.; Hacot, C.; Gautier, S.; Chantrel, J.; Jousse, M.; Desseauve, D.; Plennevaux, J. L.; Delaeter, C.; Deghilage, S.; Personne, A.; Joyez, E.; Guinard, E.; Kipnis, E.; Faure, K.; Grandbastien, B.; Ancel, P. Y.; Goffinet, F.; Dessein, R., Early clindamycin for bacterial vaginosis in pregnancy (PREMEVA): a multicentre, double-blind, randomised controlled trial. *Lancet* 2018, 392, (10160), 2171-2179.
168. Yudin, M. H.; Money, D. M., No. 211-Screening and Management of Bacterial Vaginosis in Pregnancy. *J Obstet Gynaecol Can* 2017, 39, (8), e184-e191.
169. Soper, D. E., Bacterial vaginosis and surgical site infections. *Am J Obstet Gynecol* 2020, 222, (3), 219-223.
170. Penney, G. C.; Thomson, M.; Norman, J.; McKenzie, H.; Vale, L.; Smith, R.; Imrie, M., A randomised comparison of strategies for reducing infective complications of induced abortion. *Br J Obstet Gynaecol* 1998, 105, (6), 599-604.

171. Larsson, P. G.; Platz-Christensen, J. J.; Dalaker, K.; Eriksson, K.; Fåhraeus, L.; Irminger, K.; Jerve, F.; Stray-Pedersen, B.; Wölner-Hanssen, P., Treatment with 2% clindamycin vaginal cream prior to first trimester surgical abortion to reduce signs of postoperative infection: a prospective, double-blinded, placebo-controlled, multicenter study. *Acta Obstet Gynecol Scand* 2000, 79, (5), 390-6.
172. Miller, L.; Thomas, K.; Hughes, J. P.; Holmes, K. K.; Stout, S.; Eschenbach, D. A., Randomised treatment trial of bacterial vaginosis to prevent post-abortion complication. *Bjog* 2004, 111, (9), 982-8.
173. Crowley, T.; Low, N.; Turner, A.; Harvey, I.; Bidgood, K.; Horner, P., Antibiotic prophylaxis to prevent post-abortal upper genital tract infection in women with bacterial vaginosis: randomised controlled trial. *Bjog* 2001, 108, (4), 396-402.
174. Larsson, P. G.; Platz-Christensen, J. J.; Thejls, H.; Forsum, U.; Påhlson, C., Incidence of pelvic inflammatory disease after first-trimester legal abortion in women with bacterial vaginosis after treatment with metronidazole: a double-blind, randomized study. *Am J Obstet Gynecol* 1992, 166, (1 Pt 1), 100-3.
175. Amaya-Guio, J.; Viveros-Carreño, D. A.; Sierra-Barrios, E. M.; Martinez-Velasquez, M. Y.; Grillo-Ardila, C. F., Antibiotic treatment for the sexual partners of women with bacterial vaginosis. *Cochrane Database Syst Rev* 2016, 10, (10), Cd011701.
176. Ratten, L. K.; Plummer, E. L.; Murray, G. L.; Danielewski, J.; Fairley, C. K.; Garland, S. M.; Hocking, J. S.; Tachedjian, G.; Chow, E.; Bradshaw, C. S.; Vodstrcil, L. A., Sex is associated with the persistence of non-optimal vaginal microbiota following treatment for bacterial vaginosis: a prospective cohort study. *Bjog* 2021, 128, (4), 756-767.
177. Schwebke, J. R.; Lensing, S. Y.; Lee, J.; Muzny, C. A.; Pontius, A.; Woznicki, N.; Aguin, T.; Sobel, J. D., Treatment of Male Sexual Partners of Women With Bacterial Vaginosis: A Randomized, Double-Blind, Placebo-Controlled Trial. *Clin Infect Dis* 2021, 73, (3), e672-e679.
178. Plummer, E. L.; Vodstrcil, L. A.; Doyle, M.; Danielewski, J. A.; Murray, G. L.; Fehler, G.; Fairley, C. K.; Bulach, D. M.; Garland, S. M.; Chow, E. P. F.; Hocking, J. S.; Bradshaw, C. S., A Prospective, Open-Label Pilot Study of Concurrent Male Partner Treatment for Bacterial Vaginosis. *mBi*o 2021, 12, (5), e0232321.
179. Muzny, C. A.; Schwebke, J. R., Asymptomatic Bacterial Vaginosis: To Treat or Not to Treat? *Curr Infect Dis Rep* 2020, 22, (12).
180. Bilardi, J. E.; Walker, S. M.; Temple-Smith, M. J.; McNair, R. P.; Mooney-Somers, J.; Vodstrcil, L. A.; Bellhouse, C. E.; Fairley, C. K.; Bradshaw, C. S., Women view key sexual behaviours as the trigger for the onset and recurrence of bacterial vaginosis. *PLoS One* 2017, 12, (3), e0173637.
181. Bradshaw, C. S.; Morton, A. N.; Hocking, J.; Garland, S. M.; Morris, M. B.; Moss, L. M.; Horvath, L. B.; Kuzevska, I.; Fairley, C. K., High recurrence rates of bacterial vaginosis over the course of 12 months after oral metronidazole therapy and factors associated with recurrence. *J Infect Dis* 2006, 193, (11), 1478-86.
182. Muzny, C. A.; Sobel, J. D., The Role of Antimicrobial Resistance in Refractory and Recurrent Bacterial Vaginosis and Current Recommendations for Treatment. *Antibiotics (Basel)* 2022, 11, (4).
183. Sobel, J. D.; Ferris, D.; Schwebke, J.; Nyirjesy, P.; Wiesenfeld, H. C.; Peipert, J.; Soper, D.; Ohmit, S. E.; Hillier, S. L., Suppressive antibacterial therapy with 0.75% metronidazole vaginal gel to prevent recurrent bacterial vaginosis. *Am J Obstet Gynecol* 2006, 194, (5), 1283-9.
184. McClelland, R. S.; Richardson, B. A.; Hassan, W. M.; Chohan, V.; Lavreys, L.; Mandaliya, K.; Kiarie, J.; Jaoko, W.; Ndinya-Achola, J. O.; Baeten, J. M.; Kurth, A. E.; Holmes, K. K., Improvement of vaginal health for Kenyan women at risk for acquisition of human immunodeficiency virus type 1: results of a randomized trial. *J Infect Dis* 2008, 197, (10), 1361-8.
185. Reichman, O.; Akins, R.; Sobel, J. D., Boric acid addition to suppressive antimicrobial therapy for recurrent bacterial vaginosis. *Sex Transm Dis* 2009, 36, (11), 732-4.
186. Surapaneni, S.; Akins, R.; Sobel, J. D., Recurrent Bacterial Vaginosis: An Unmet Therapeutic Challenge. Experience With a Combination Pharmacotherapy Long-Term Suppressive Regimen. *Sex Transm Dis* 2021, 48, (10), 761-765.
187. Unemo, M.; Bradshaw, C. S.; Hocking, J. S.; de Vries, H. J. C.; Francis, S. C.; Mabey, D.; Marrazzo, J. M.; Sonder, G. J. B.; Schwebke, J. R.; Hoornenborg, E.; Peeling, R. W.; Philip, S. S.; Low, N.; Fairley, C. K., Sexually transmitted infections: challenges ahead. *Lancet Infect Dis* 2017, 17, (8), e235-e279.
188. Krasnopolsky, V. N.; Prilepskaya, V. N.; Polatti, F.; Zarochentseva, N. V.; Bayramova, G. R.; Caserini, M.; Palmieri, R., Efficacy of vitamin C vaginal tablets as prophylaxis for recurrent bacterial vaginosis: a randomised, double-blind, placebo-controlled clinical trial. *J Clin Med Res* 2013, 5, (4), 309-15.
189. Verstraelen, H.; Verhelst, R.; Roelens, K.; Temmerman, M., Antiseptics and disinfectants for the treatment of bacterial vaginosis: a systematic review. *BMC Infect Dis* 2012, 12, 148.
190. Cohen, C. R.; Wierzbicki, M. R.; French, A. L.; Morris, S.; Newmann, S.; Reno, H.; Green, L.; Miller, S.; Powell, J.; Parks, T.; Hemmerling, A., Randomized Trial of Lactin-V to Prevent Recurrence of Bacterial Vaginosis. *N Engl J Med* 2020, 382, (20), 1906-1915.
191. Heczko, P. B.; Tomusiak, A.; Adamski, P.; Jakimiuk, A. J.; Stefański, G.; Mikołajczyk-Cichońska, A.; Suda-Szczurek, M.; Strus, M., Supplementation of standard antibiotic therapy with oral probiotics for bacterial vaginosis and aerobic vaginitis: a randomised, double-blind, placebo-controlled trial. *BMC Womens Health* 2015, 15, 115.

192. Xie, H. Y.; Feng, D.; Wei, D. M.; Mei, L.; Chen, H.; Wang, X.; Fang, F., Probiotics for vulvovaginal candidiasis in non-pregnant women. *Cochrane Database Syst Rev* 2017, 11, (11), Cd010496.
193. Laue, C.; Papazova, E.; Liesegang, A.; Pannenbeckers, A.; Arendarski, P.; Linnerth, B.; Domig, K. J.; Kneifel, W.; Petricevic, L.; Schrezenmeir, J., Effect of a yoghurt drink containing Lactobacillus strains on bacterial vaginosis in women - a double-blind, randomised, controlled clinical pilot trial. *Benef Microbes* 2018, 9, (1), 35-50.
194. Vodstrcil, L. A.; Plummer, M. E.; Fairley, C. K.; Tachedjian, G.; Law, M. G.; Hocking, J. S.; Worthington, M. K.; Grant, M. M.; Okoko, N.; Bradshaw, C. S., Combined oral contraceptive pill-exposure alone does not reduce the risk of bacterial vaginosis recurrence in a pilot randomised controlled trial. *Sci Rep* 2019, 9, (1), 3555.
195. Vieira-Baptista, P.; Silva, A. R.; Costa, M.; Aguiar, T.; Saldanha, C.; Sousa, C., Clinical validation of a new molecular test (Seegene Allplex™ Vaginitis) for the diagnosis of vaginitis: a cross-sectional study. *Bjog* 2021, 128, (8), 1344-1352.
196. Demirbilek, M.; Can, F.; Güleç, A. T.; Kuşçu, E.; Kayhan, Z.; Haberal, M., Incidence of bacterial vaginosis in renal transplant recipients. *Transplant Proc* 2003, 35, (7), 2696-7.
197. Miller, E. A.; Beasley, D. E.; Dunn, R. R.; Archie, E. A., Lactobacilli Dominance and Vaginal pH: Why Is the Human Vaginal Microbiome Unique? *Front Microbiol* 2016, 7, 1936.
198. Bayar, E.; Bennett, P. R.; Chan, D.; Sykes, L.; MacIntyre, D. A., The pregnancy microbiome and preterm birth. *Semin Immunopathol* 2020, 42, (4), 487-499.
199. Gupta, S.; Kakkar, V.; Bhushan, I., Crosstalk between Vaginal Microbiome and Female Health: A review. *Microb Pathog* 2019, 136, 103696.
200. Serrano, M. G.; Parikh, H. I.; Brooks, J. P.; Edwards, D. J.; Arodz, T. J.; Edupuganti, L.; Huang, B.; Girerd, P. H.; Bokhari, Y. A.; Bradley, S. P.; Brooks, J. L.; Dickinson, M. R.; Drake, J. I.; Duckworth, R. A., 3rd; Fong, S. S.; Glascock, A. L.; Jean, S.; Jimenez, N. R.; Khoury, J.; Koparde, V. N.; Lara, A. M.; Lee, V.; Matveyev, A. V.; Milton, S. H.; Mistry, S. D.; Rozycki, S. K.; Sheth, N. U.; Smirnova, E.; Vivadelli, S. C.; Wijesooriya, N. R.; Xu, J.; Xu, P.; Chaffin, D. O.; Sexton, A. L.; Gravett, M. G.; Rubens, C. E.; Hendricks-Muñoz, K. D.; Jefferson, K. K.; Strauss, J. F., 3rd; Fettweis, J. M.; Buck, G. A., Racioethnic diversity in the dynamics of the vaginal microbiome during pregnancy. *Nat Med* 2019, 25, (6), 1001-1011.
201. MacIntyre, D. A.; Chandiramani, M.; Lee, Y. S.; Kindinger, L.; Smith, A.; Angelopoulos, N.; Lehne, B.; Arulkumaran, S.; Brown, R.; Teoh, T. G.; Holmes, E.; Nicoholson, J. K.; Marchesi, J. R.; Bennett, P. R., The vaginal microbiome during pregnancy and the postpartum period in a European population. *Sci Rep* 2015, 5, 8988.
202. Hay, P., Bacterial vaginosis. F1000Res 2017, 6, 1761.
203. Kyrgiou, M.; Moscicki, A. B., Vaginal microbiome and cervical cancer. *Semin Cancer Biol* 2022, 86, (Pt 3), 189-198.
204. Vieira-Baptista, P.; Eleutério Jr., J., Diagnosis of vaginitis: time to improve and move on. DST - *J bras Doenças Sex Transm* 2020, 32, (e203214), 1-3.
205. Castro, J.; Jefferson, K. K.; Cerca, N., Genetic Heterogeneity and Taxonomic Diversity among Gardnerella Species. *Trends Microbiol* 2020, 28, (3), 202-211.
206. Le Roy, C.; Bébéar, C.; Pereyre, S., Performance of Three Commercial Molecular Diagnostic Assays for the Simultaneous Detection of Mycoplasma genitalium and Macrolide Resistance. *J Clin Microbiol* 2021, 59, (6).
207. Turpin, R.; Slopen, N.; Borgogna, J. C.; Yeoman, C. J.; He, X.; Miller, R. S.; Klebanoff, M. A.; Ravel, J.; Brotman, R. M., Perceived Stress and Molecular Bacterial Vaginosis in the National Institutes of Health Longitudinal Study of Vaginal Flora. *Am J Epidemiol* 2021, 190, (11), 2374-2383.
208. Li, C.; Wang, T.; Li, Y.; Zhang, T.; Wang, Q.; He, J.; Wang, L.; Li, L.; Yang, N.; Fang, Y., Probiotics for the treatment of women with bacterial vaginosis: A systematic review and meta-analysis of randomized clinical trials. *Eur J Pharmacol* 2019, 864, 172660.
209. Lev-Sagie, A.; Goldman-Wohl, D.; Cohen, Y.; Dori-Bachash, M.; Leshem, A.; Mor, U.; Strahilevitz, J.; Moses, A. E.; Shapiro, H.; Yagel, S.; Elinav, E., Vaginal microbiome transplantation in women with intractable bacterial vaginosis. *Nat Med* 2019, 25, (10), 1500-1504.
210. Yockey, L. J.; Hussain, F. A.; Bergerat, A.; Reissis, A.; Worrall, D.; Xu, J.; Gomez, I.; Bloom, S. M.; Mafunda, N. A.; Kelly, J.; Kwon, D. S.; Mitchell, C. M., Screening and characterization of vaginal fluid donations for vaginal microbiota transplantation. *Sci Rep* 2022, 12, (1), 17948.

CANDIDIASIS

4

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4.1 Introduction

Vulvovaginal candidiasis or candidosis (VVC) is a common condition. Frequently trivialized and as a result mismanaged, VVC has significant adverse effects on women's overall health. When diagnosed and managed correctly, these ill effects can be minimized. This document represents our current understanding of the condition and highlights the best practices for diagnosis and treatment of uncomplicated and complicated VVC.

4.2 Etiology and pathophysiology

VVC is an inflammatory disease caused by *Candida* spp. that affects the female lower genital tract. Less commonly, there may be other kinds of fungal organisms causing disease in these organs which together with VVC represent female lower genital tract mycosis. Among the various species, *Candida albicans* is by far the most frequent infecting organism, causing more than 80% of cases. However, a broad range of other species may cause VVC, including *C. glabrata, C. guilliermondii* and *C. tropicalis*.[1-4] Finally, variants of *C. albicans* such as *C. africana* and *C. dubliniensis* also play a role in producing VVC.[5] Geographical and population diversity may explain the differences in relative prevalence of the more common species. Furthermore, reported experiences from tertiary care centers, which tend to treat more difficult cases, may exaggerate the contributions of non-*albicans Candida* species to the overall burden of VVC. On a final note, it should be mentioned that, due to taxonomic changes, some clinically important causes of VVC, including *C. glabrata*, are now being classified as other species.[6] For example, *C. glabrata* is now known as *Nakaseomyces glabrata*. To avoid confusion and since these name changes have not been instituted in clinical settings, we will be using the more widely used nomenclature.

In order to cause disease, *Candida* spp. must first colonize the vaginal epithelium. With colonization, the presence of yeast may be temporary and seems to frequently be followed by its elimination by normal vaginal defense mechanisms involving neutrophils and macrophages.[7] In the absence of pro-inflammatory mediators, long-standing asymptomatic colonization may take place. At some point, in women with symptomatic VVC, the infecting organism becomes pathogenic. It is not fully understood what induces *Candida* spp. to cause an actual infection. At some point, yeasts may express greater virulence factors through different mechanisms, like morphologic changes (dimorphism), proteinase secretion and cell surface composition changes.[8] Histologic evaluation of the vaginal wall in women with acute VVC has demonstrated evidence of superficial tissue invasion but has failed to show any sign of biofilm formation.[9] In some women, symptoms and signs may occur with a low burden of *Candida*; it is thought that symptoms in these cases may be due to an allergic or inflammatory response to the presence of the yeast, mediated by an immunologic, perhaps allergic mechanism.[10]

In women with repeated episodes of VVC, the source of the organism remains controversial. Proposed sources include an intestinal reservoir, reinfection from a sexual partner, or failure to fully clear the organism after initial infection (the vaginal relapse theory). For women with recurrent VVC where vaginal relapse seems to play a very large role, evaluating and treating an intestinal or partner source remain controversial and seems unhelpful.

4.3
Prevalence and epidemiology

The estrogenized vagina is colonized by *Candida* spp. in at least 20% of pregnant women and 30% of immunocompromised patients, if examined by culture.[11] When sequencing methods are used, vaginal fungal colonization can be found in >60% of all premenopausal women, of which the most abundant species is *C. albicans* in >80%.[12] It is estimated, that 30 – 50 % of all women at least once during their life suffer from VVC and that most of these women are in their reproductive years.[13, 14]

Without a correct diagnosis of VVC, it is difficult to estimate the prevalence and incidence of this disease in different populations. Further complicating these estimates, VVC is not a reportable disease and treatment is often given based on symptoms. In some studies, more than 60% of US women who receive treatment for VVC are diagnosed without either microscopy or culture.[15] This finding is probably similar in all countries. Thus, the historical estimate that 75% of women will suffer at least one episode of VVC during her lifetime has been based on very little data.[16] Nevertheless, recent studies seem to confirm the historical estimations.[17]

Worldwide, recurrent vulvovaginal candidiasis (RVVC), which is currently defined as three or more confirmed episodes per year, affects about 138 million women annually (range 103–172 million) and causes substantial morbidity and economic burden, with a global annual prevalence of 3871 per 100,000 women and 372 million women affected by RVVC over their lifetime.[18] The 19–35 year age group has the highest prevalence (9%). A qualified online survey questionnaire with responses from 284 women between 2016 und 2018, in three

university-affiliated gynecological clinics in the US, revealed at least one lifetime episode in 77.5%, 1-3 in 29.0%, 4–10 in 28.4% and >10 episodes in 43.6% of the participants; 44.3% with an age of 26–40 years suffered from >3 episodes per year at some point.[17] The quality of life of affected women is heavily impaired, especially in recurrent cases, with impacts similar to those seen in women with chronic obstructive lung disease or asthma.[19, 20]

4.4 Risk factors

A series of conditions are considered to favor the establishment of symptomatic VVC. Among them are non-pathological (pregnancy, estrogen-containing contraceptives, and menopause hormone therapies), behavioral (frequent sexual intercourse or multiple partners), or disease-related events and comorbidities (antibiotic use, innate reduced cellular immunity, iatrogenic or spontaneous immunosuppressive conditions, poorly controlled diabetes *mellitus*).[4] With antibiotic use specifically, it is important to note that most women who take antibiotics will not get VVC.[21] When they do, the mechanism is thought to be either from the induction of growth of *Candida* spp. in either the intestine or the vagina. In diabetics the use of sodium-glucose cotransporter 2 (SGLT2) inhibitors (i.e. canaglifozin, dapaglifozin, empaglifozin) to control the disease do promote VVC.[22]

Suggesting that certain women are simply more prone to VVC, a history of episodes of VVC seems to put women at higher risk of new ones. In women with persistence of VVC, the culture-proven presence of yeast in the vagina, particularly during treatment or immediately after the end of treatment, suggests clinical resistance, whereas clinical recurrence after an asymptomatic and culture negative episode may represent re-infection.

4.5 Classification of vulvovaginal candidiasis

Because episodes of VVC can vary between affected women and because these variations may affect treatment outcomes, providers should make every effort to classify infection. Of the various systems in use, perhaps the most widely known is the one first proposed in 1998[23] and still recommended by the US Centers for Disease Control and Prevention.[24] (Table 4.1)

TABLE 4.1 Classification of vulvovaginal candidiasis and its clinical implications.

Type of infection	Clinical implications
Uncomplicated	All treatments with similar efficacy Choice can be individualized
Complicated Severe or predisposing factors Recurrent or chronic Non-*albicans Candida* vulvovaginal candidiasis	More likely to fail short course therapy 50% idiopathic Usually requires maintenance therapy More likely to fail azole therapy

This system distinguishes between uncomplicated and complicated VVC. In general, uncomplicated VVC affects women with no predisposing factors for yeast infections, such as diabetes or immunosuppressive conditions, are sporadic (two or less per year) episodes, with mild or moderate symptoms, and the infection is caused by *C. albicans*. In general, women with uncomplicated VVC will respond to pretty much any of the treatment options available. Complicated VVC consists of women with any one of the following: 1) severe infection, 2) recurrent episodes (defined as three or more episodes in the previous year), 3) conditions such as diabetes, underlying immunodeficiency, or immunosuppressive therapy, or 4) infections due to a non-*albicans Candida*. In general, women with complicated VVC are less likely to respond to standard regimens of antifungal therapy and will require closer follow-up and more aggressive treatments. However, it is important to emphasize that each category of complicated VVC deserves its own individualized approach to management.

Severe infections

VVC-related symptoms and signs can be assessed on a semi-quantitative basis.[25] Women with severe VVC are more likely to fail standard treatment for VVC and should receive more prolonged courses of treatment.

Recurrent infections

Based largely on consensus expert opinion, the current definition of RVVC is three or more episodes in the prior 12 months. In most women with RVVC, there are no underlying known predisposing factors to infection, and no further work-up is indicated. In most affected women, *C. albicans* is the causative organism. Women with chronic VVC may possibly be a separate group from those with RVVC, but should also be considered complicated.[26] Recently, fluconazole-resistant *C. albicans* organisms seem to be an emerging and particularly problematic cause of RVVC.[27] It is well accepted that certain conditions, including diabetes, treatments for diabetes such as SGLT2 inhibitors and immunosuppressive conditions and medications increase *Candida* spp. colonization and infection.[22] HIV is not considered a predisposing condition since HIV-positive and HIV-negative women have similar responses to treatment.[28]

Non-*albicans Candida* infections

In general, it is felt that 90-95% of women with VVC have infections due to *C. albicans*. However, other organisms such as *C. glabrata, C. parapsilosis, C. krusei, C. tropicalis* and *Saccharomyces cerevisiae* can sometimes be found in symptomatic women. There exists some controversy into whether these species can cause true vulvovaginal infections, and the extent to which their presence may simply represent asymptomatic colonization in a woman who has a different cause for her symptoms. In these cases, treating the organism and having the patient return after the organism has been suppressed may be the only way to decide whether this organism is contributing to the patient's symptoms.[29]

4.6

Signs and symptoms

The symptoms and signs of VVC can be relatively nonspecific. In the classic description of VVC, affected women will complain of an abnormal thick white discharge, itching, irritation and burning. They may also complain of external dysuria.[30] If sexually active, they may note dyspareunia. The presentation will often be relatively acute, but it may occur on a repeated basis in women with recurrent yeast infection or simply be chronic if they have chronic VVC, either because of a missed diagnosis or an organism which is resistant to antifungal therapy. It should be emphasized that the symptoms attributed to VVC can have many other potential causes, including vaginal infections and vulvar dermatoses. The presence of a discharge described as "cheesy" and of itching, which occurs in 70-90% of women with VVC, increases the likelihood of VVC; the absence of itching or irritative symptoms makes it less likely.[31] On physical examination, women with VVC may have erythema of the *labia majora*, *minora* or vestibule. (Figure 4.1)

They may have swelling of these structures or fissures. If itching is severe, excoriations may be noted. (Figure 4.2)

On speculum examination, there may be vaginal enanthema. Thrush, which is an adherent white discharge on the sidewalls of the vagina, may be noted. [31] (Figure 4.3)

Many women with symptomatic VVC will have no significant physical findings.

With both symptoms and signs, VVC represents a spectrum. Affected women may have few or all of them, and the symptoms and signs may affect the vulva, the vagina, or both.

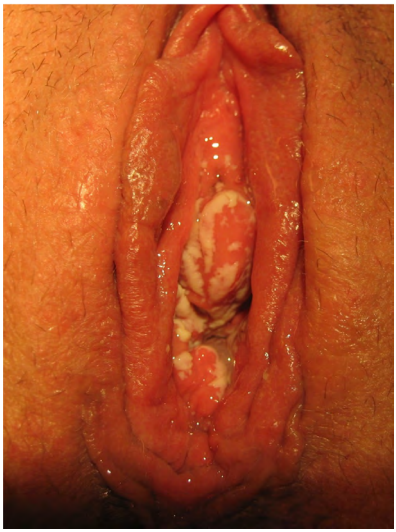

Figure 4.1 Acute vulvovaginal candidiasis. Exteriorization of white "cheesy" discharge, vulvar erythema and edema.

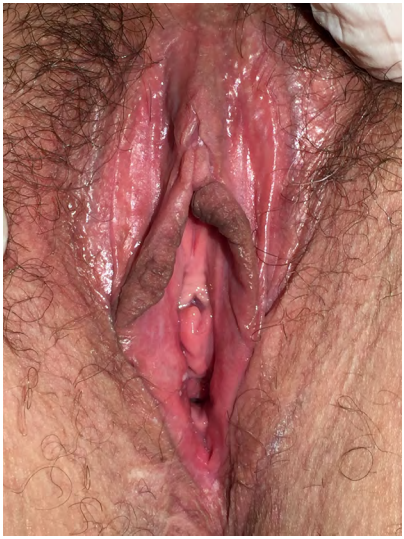

Figure 4.2 Acute vulvovaginal candidiasis. Erythema and fissures of the intelabial *sulci*.

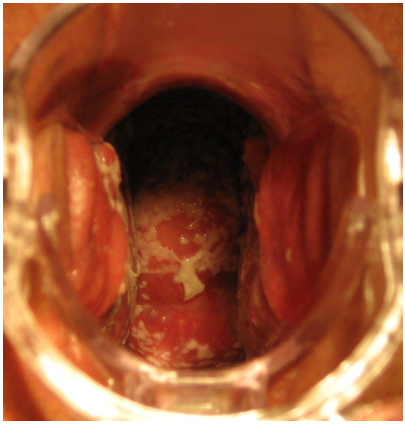

Figure 4.3 Acute vulvovaginal candidiasis. Adherent white discharge on the sidewalls of the vagina and cervix.

4.7 Diagnosis

Since asymptomatic colonization with *Candida* spp. is a common life event, accurate diagnosis relies on obtaining an appropriate history, as well as the detection of yeast through some sort of testing modality. Any sort of diagnosis which does not attempt to detect the organism runs the risk of misdiagnosis. In a study of women about to self-treat for acute VVC, only 33.7% had VVC alone.[32] Similarly, diagnosis of vaginal infections over the telephone by a nurse showed poor agreement beyond chance for diagnosing VVC.[33] Thus, in cases where patients are treated without an examination and obtaining vaginal samples to exclude other infections and detect yeast, it is important to recognize that the likelihood of misdiagnosis is quite high.

In general, it is recommended that office laboratory testing, composed of checking the vaginal pH, mixing the vaginal secretions with 10% KOH to detect amines (the whiff test) and performing microscopic examinations of the discharge mixed with saline and, separately, 10% KOH (important if phase contrast is not being used), be performed in all women with vulvovaginal symptoms. In women with VVC, the pH will be often normal, the amine test will

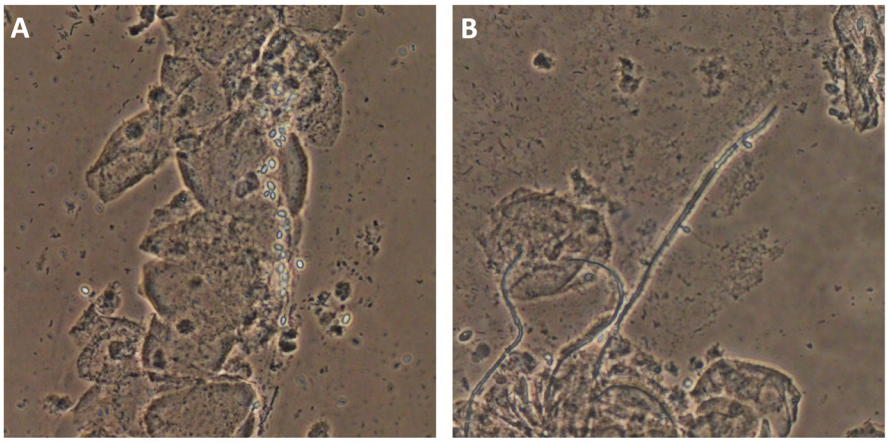

Figure 4.4 Wet mount microscopy (400x, phase contrast).

A– Blastospores (culture positive for *C. krusei*) B– Hyphae and blastospores (culture positive for *C. albicans*)

be negative, and microscopic examination will reveal blastospores, pseudohyphae or hyphae, or other fungal elements. (Figure 4.4 and 4.5)

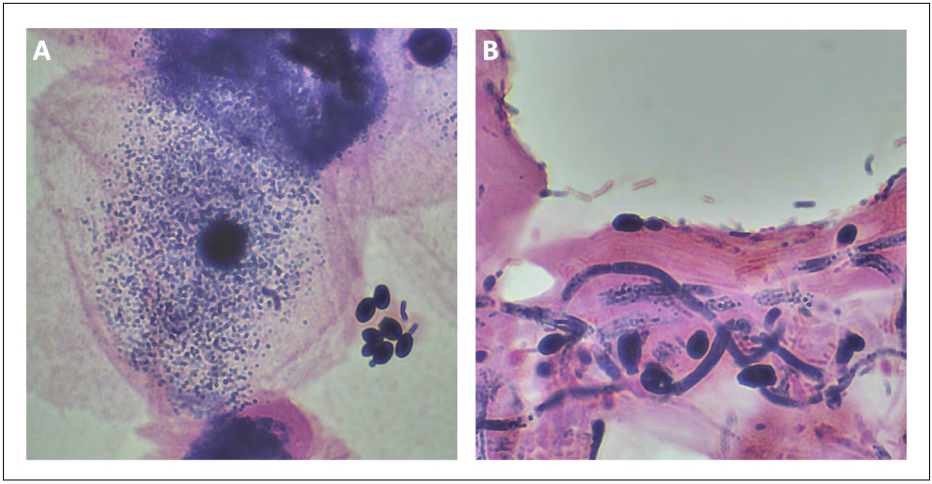

Figure 4.5 Gram stain (1000x, oil immersion).

A– Blastospores B– Hyphae and blastospores

Microscopy, which by its nature is provider-specific, has two main limitations, underdiagnosis and overdiagnosis. In general, the estimated sensitivity of microscopy, performed in research settings, is about 56%; in community settings, the sensitivity is lower.[34] There remain significant concerns with false positive rates for detecting VVC, which may be as high as 49%.[35] Phase contrast microscopy and training courses can significantly improve the diagnostic competence.[36]

Nucleic acid amplifications tests (NAATs) for yeast have become widely available and can be used in symptomatic women. Depending on the country, these tests may be either cleared by governmental authorities or they may have only undergone validation by a local laboratory. In general, the NAATs are quite sensitive (>90%) for *C. albicans*.[34, 37] When it comes to non-*albicans Candida* species, performance data for NAAT tests may be difficult to measure given the low rates of such infections in clinical studies.[34, 37] Thus, if providers use NAATs for diagnosing VVC, they should be aware of the performance characteristics of the specific lab they are using and also realize that NAATs may miss yeast infections by less common organisms.

Yeast culture remains the gold standard for confirming the diagnosis for VVC. In a woman with uncomplicated VVC, culture is probably not necessary as most patients will get better with treatment. However, in a woman with complicated VVC, culture will help to confirm the diagnosis, permit speciation of the infecting organism, and make the organism available for susceptibility testing. In cases where drug resistance is suspected, susceptibility testing can be considered, but providers should be aware that susceptibility tests done at a pH of 7, the standard for most clinical laboratories, may vary dramatically from those at lower pHs.[38] Thus, susceptibility testing will only add a little insight into which drugs may be of use. In comparing NAATs to culture, NAATs offers the advantages of sometimes wider availability and a quicker result, but they are usually more expensive and may miss non-*albicans Candida*.

4.8
Treatment of vulvovaginal candidiasis

Asymptomatic colonization

Although asymptomatic colonization represents the first step to developing symptomatic disease, asymptomatic women should not be screened for VVC and those who happen to have a positive culture or its identification in a Pap test do not require any sort of treatment.[39]

Uncomplicated vulvovaginal candidiasis

Azole antifungals are the treatment of choice in uncomplicated cases of VVC. They come in a large variety of formulations, such as topical vaginal creams, ointments, and suppositories. Some of the many options available internationally are shown in Table 4.2; it should be stressed that this list is not exhaustive. The most common treatments, such as local clotrimazole, miconazole, or econazole or oral single dose fluconazole resolve up to 80-85% of cases.[40-42] Topical azoles are well tolerated, although side-effects such as slight burning have been reported in 1-10% of cases; allergic reactions are rare.[41] The Centers for Disease Control and Prevention (CDC) also recommends formulations of tioconazole, butaconazole, and terconazole; these seem to be less available outside of the US. In general, since one can expect similar efficacy with all of the available options, treatment should be individualized depending on drug availability, tolerability, price and patient preference.

TABLE 4.2 Commonly available treatment options for patients with uncomplicated vulvovaginal candidiasis. The list does not include all options and the options listed may not be available in all countries

Local treatment (mild symptoms)	
Clotrimazole	200 mg vaginal tablets, once daily (3 days) 100 mg vaginal tablets, once daily (7 days) 1% cream, 5 g vaginally once daily (7 days) 500 mg vaginal tablet, once daily (1 day) 1% cream, once daily for 7 days
Econazole	150 mg vaginal suppository, twice daily (1 day) 150 mg vaginal suppository, once daily (3 days)
Fenticonazole	600 mg vaginal capsule, once daily (1 day)
Isoconazole	150 mg vaginal suppository, twice daily (1 day) 150 mg vaginal suppository, once daily (3 days) 600 mg vaginal suppository, once daily (1 day)
Alternative treatment (severe symptoms)	
Fluconazole	150 mg orally, single dose 50 mg orally, once daily (7–14 days) 100 mg orally, once daily (14 days) (if immunocompromised)
Itraconazole	100 mg orally 2×2 capsules daily (1 day) 100 mg orally 1×2 capsules daily (3 days)
Nystatin	100,000 units vaginal tablets (14 days) 200,000 units vaginal tablets (6 days)

Recurrent vulvovaginal candidiasis due to *C. albicans*

Treatments of RVVC differ from country to country, depending on traditions and the importance of dominating research fields, as well as regulatory factors. The treatment options in RVVC are complex and include local and oral antifungals, as well as vaginal boric acid. No matter the chosen treatment, a positive yeast culture is crucial prior to initiating any plan. Yeast cultures help to establish the diagnosis, determine the species of the infecting organism, and makes it available for susceptibility testing. Susceptibility testing, however, does not correlate with clinical outcome, and the result of such testing depends on the pH at which is the testing is performed.[38]

For women with RVVC, some form of maintenance therapy is the standard approach for treating women with *C. albicans* infections. Standard treatment in culture-verified chronic or recurrent vulvovaginal *C. albican*s is accepted in most countries. The regimen adopted by many experts is oral fluconazole 150 mg every three days for three doses, followed by 150 mg weekly for six months. When on this regimen, 90% of women are well controlled[43], and this straightforward approach has been shown to improve quality of life in 96% of women.[44] However, it is uncommonly curative and recurrence occurs frequently, with one study finding that more than 63% of women who had completed maintenance therapy continued to have ongoing infections.[45]

As discussed later, recent concerns about fluconazole use in women who are either attempting to become or are pregnant limit its use in this population. Similarly, a small proportion of women may be intolerant or allergic to fluconazole. Because fluconazole is a potent inhibitor of cytochrome P450, there are a large number of possible drug interactions. Since even maintenance fluconazole is a low dose, it is unclear whether these interactions are more than theoretical. As a separate concern, fluconazole may prolong the QT interval. However, QT effects seem to be dose related and low relative to prevalence in the general population.[46] In women where fluconazole is ruled out as a course of treatment, options include clotrimazole 1% cream 5 grams nightly for 14 days, followed by 5 grams vaginally twice a week for 6 months. One would expect that similar topical maintenance regimens would also be successful at controlling RVVC. No matter the maintenance regimen, re-evaluating the patient after therapy is initiated, preferably with repeated yeast cultures is very helpful to determine the response to treatment.

Ancillary measures can be considered to improve patient outcome. Treatment of the asymptomatic sexual partner does not seem to be beneficial.[39] Removal of intrauterine contraceptives should also be considered in women with chronic RVVC, as *C. albicans* may be more likely to attach to it, possibly due to formation of biofilm on the intrauterine device.[47] After removal of the intrauterine device and treatment with fluconazole, affected women are more likely to stay recurrence-free for a longer time period. In a small pilot study of administration of oral or depot formulations of medroxyprogesterone acetate (MPA) for RVVC, treated women described a reduction of symptoms in the second year of therapy.[48] For women who have fewer than three annual episodes of VVC but are otherwise complicated (e.g. diabetics with a severe infection), they should be treated in a manner similar to someone with RVVC. Finally, it should be noted that azole-resistant *C. albicans* infections are being encountered with increasing frequency by many tertiary care centers. Resistant infections are a clinical

definition, i.e. a women who remains symptomatic and culture positive while taking antifungal treatment. Evidence for treatment options is relatively sparse.[27] Since many non-*albicans Candida* spp. are inherently azole resistant, providers should consider the treatment options in the section on non-*albicans Candida*.

Non-*albicans Candida* vulvovaginitis

When a non-*albicans Candida* grows on a culture from a woman with vulvovaginal symptoms, providers should keep in mind that vulvovaginal symptoms are often non-specific and that the patient 's symptoms may be due to some other cause than the cultured organism (i.e. vulvodynia, lichen sclerosus). For example, it is estimated that 50% of women with growth of non-*albicans Candida* are asymptomatic.[49] Thus, it is recommended that treatment only be offered to symptomatic women with no other identifiable cause. Treatment with azoles is frequently unsuccessful in symptomatic *C. glabrata*; most of the options will consist of compounded medications. Local administration of nystatin (100,000 units vaginally nightly for a month), boric acid (600 mg capsules vaginally nightly for three weeks)[50] or amphotericin B (50 mg suppositories vaginally nightly for two weeks)[51] have been suggested as reasonable options. In particularly refractory cases, a compounded cream with 1 gram flucytosine and 100 mg of amphotericin formulated in lubricating jelly base in a total 8 g delivered dose, inserted nightly for 14 days, was effective in two cases.[52] Flucytosine is both difficult to obtain and, in many countries, expensive.

C. krusei, C. tropicalis and *S. cerevisiae* are almost always inherently resistant to fluconazole. Topical clotrimazole 100 mg daily for two weeks, nystatin[53] or vaginal boric acid are the treatments of choice in symptomatic women. Vaginal boric acid is suggested if azoles do not work.[29, 54] Providers should be aware that the European Chemicals Agency has issued a warning against the use of boric acid, as it feels that it may impair fertility and might be embryotoxic.[55] On the other hand, the CDC do not mention any significant safety concerns with boric acid.[24] As a result of this controversy, boric acid is not readily available in many countries, despite the evidence that it may represent the best option for treating azole-resistant yeast infections. Clearly, there remains a need for better treatment options for non-*albicans* yeast and *C. albicans* infections resistant than azoles.

4.9 Special situations

Prepubertal children

It is assumed that the likelihood of term neonates to develop oral thrush or "diaper dermatitis" during the first year of life is increased in those who are colonized through maternal-to-neonatal transmission during vaginal delivery.[56, 57] In these earlier papers, prophylactic antimycotic treatment was suggested during the last weeks of pregnancy in women with asymptomatic colonization to prevent transmission to the newborn during vaginal delivery. However, given the absence of high-quality data and the difficulties involved with institut-

ing a huge screening program to identify women colonized with *C. albicans*, there are no countries apart from Germany, to our knowledge, who have implemented such a program.[11] After the initial postnatal period, due to reduced estrogenization of the vagina, premenstrual girls are much less likely to develop *Candida* spp. colonization and symptomatic VVC.[14] Although yeast cultures may be obtained in premenarchal girls with vulvovaginal complaints, it should be expected that the vast majority will not have VVC and that antifungal treatment will not be helpful.

Pregnancy

Pregnancy is a known risk factor for development of VVC, likely due to pregnancy-related factors, including increased estrogen levels, increased vaginal glycogen, and alterations in the immune system. Vaginal treatment with topical azoles, preferably clotrimazole, is recommended during pregnancy. We feel that it is reasonable to consider a longer course of 7-14 days, according to studies. Given the absence of safety data with regard to use in pregnancy, boric acid should not be used. Although there are few clinical studies on the use of dequalinium chloride as an alternative treatment during pregnancy, available data suggest good tolerability and effectiveness. Therefore, dequalinium chloride may be considered as a therapeutic option for VVC during pregnancy in the countries where it is available.[58-60]

Oral fluconazole may be associated with malformations such as transposition of large vessels and cleft palate and also with miscarriages. The US National Birth Defects Prevention Study analyzed data from 43,257 women and found a significant association between low-dose fluconazole use during the first trimester and incidence of fetal cleft lip and palate and transposition of the large vessels[61]; similar results have been reported in a Danish registry and a Canadian study.[62, 63] Thus, oral fluconazole is not recommended in early pregnancy.
A more controversial question is whether treatment of VVC can improve pregnancy outcome. Although some studies suggest that colonization with *C. albicans* is associated with preterm birth and one prospective randomized study suggested that treatment with clotrimazole might decrease the risk, the data are insufficient to recommend screening or treating for VVC in asymptomatic pregnant women.[64, 65] Additional higher quality studies are needed to further investigate a possible relationship between VVC and pregnancy outcome.

Postpartum and breastfeeding mothers

In general, women with symptomatic VVC who are postpartum or breastfeeding can be treated similarly to other healthy women. Fluconazole is considered safe to use in breastfeeding women.

Menopause

In general, because estrogen is thought to be the hormone associated with symptomatic VVC, menopausal women are less likely to get VVC.[66] Menopausal women who take menopause hormone therapy seem to be more likely to get VVC than those who do not and are more likely to recur.[67]

Immunosuppression

Women with immune deficiencies are more likely to be colonized by yeast and less likely to clear infection. As such, consideration should be given to using regimens which are longer in duration than those for uncomplicated infection and to recommend follow-up to make sure that the treatment was effective.

4.10
Future perspectives

The current guidelines recommend fluconazole to suppress RVVC for most women, but emerging resistance and therapeutic failure are creating a need for better treatment options. New medications may be on the horizon which may change current treatment algorithms. Ibrexafungerp, an oral agent which affects the cell wall instead of the cell membrane (the target of azoles) was recently approved as a single day two-dose regimen for acute VVC in the US but is not available in Europe. Published data suggest that it is as effective as one would expect with single dose fluconazole.[68] Its role and the optimal dosing for managing RVVC and resistant yeast infections remain to be elucidated. For RVVC, oteseconazole has shown better results in reducing recurrences of *C. albicans*. In a double-blind placebo-controlled randomized controlled trial of women with RVVC, patients treated with VT-1161 at either a high or low dose for 12 or 24 weeks showed remarkably lower rates of recurrence than those on placebo at the 48-week study time point (4 *vs.* 52%).[69] Oteseconazole is soon to be registered in Europe and was recently approved by the FDA in the US. However, the FDA warning that it should not be used in women of reproductive potential because of the combination of ocular abnormalities in the offspring of pregnant rats and a drug exposure window of 690 days may sharply limit its use. Neither drug is approved for use in pregnancy. As an alternative to antifungal medications, vaccination against *C. albicans* in a phase 2 study suggested that it was capable of reducing the frequency of symptomatic VVC for up to 12 months, but only in a subset of women under 40 years of age.[70]

Beyond the ever present need for new therapeutics, there are many important research questions which remain unanswered. Many of them relate to basic questions of pathophysiology, such as the virulence or adherence factors of *Candid*a spp. which play a role in causing an infection, or the exact mechanisms which make certain women more prone to getting VVC than others. Quick, easy and accurate methods of diagnosis, preferably available directly to patients, would greatly improve the care of affected women. Finally, understanding the potential role of immune modulating medications and how they affect patient response may shed further light on optimal patient management.

Despite these ongoing needs, it should be emphasized that applying our current state of knowledge to women with VVC can frequently lead to excellent patient outcome and satisfaction.

Recommendations

Recommendation	Quality of evidence	Strength of recommendation
For women with recurrent vulvovaginal candidiasis, evaluation and treatment of an intestinal or partner source are not recommended.	1a	A
Providers should classify candidiasis (complicated *vs.* uncomplicated, and according to severity).	5	D
Asymptomatic colonization should not prompt treatment.	2a	B
Self-diagnosis or empirical diagnosis are not recommended.	2b	B
pH measurement, whiff test and wet mount microscopy are recommended for evaluation of women with possible acute vulvovaginal candidiasis.	3a	B
Validated nucleic acid amplification tests can be used for the diagnosis of vulvovaginal candidiasis in symptomatic women.	1a	A
Yeast culture remains the gold standard for confirming the diagnosis of vulvovaginal candidiasis.	1a	A
In women with uncomplicated vulvovaginal candidiasis, culture is usually not necessary.	5	D
In women with suspected complicated vulvovaginal candidiasis, culture is recommended.	5	D
In cases where drug resistance is suspected, susceptibility testing can be considered.	5	D
Azole antifungals, including local clotrimazole, miconazole, or econazole or oral single dose fluconazole, are the treatment of choice in uncomplicated cases of vulvovaginal candidiasis.	1a	A
A positive yeast culture is crucial prior to initiating suppressive treatment for recurrent vulvovaginal candidiasis.	2b	B
Susceptibility testing is not needed to start a suppressive treatment for recurrent vulvovaginal candidiasis.	5	D
For recurrent vulvovaginal candidiasis, oral fluconazole 150 mg every three days for three doses, followed by 150 mg weekly for six months is the most commonly recommended regimen.	1a	A
For recurrent vulvovaginal candidiasis, if fluconazole cannot be used, clotrimazole 1% cream 5 grams nightly for 14 days, followed by 5 grams vaginally twice a week for 6 months can be considered.	1a	A
Ancillary measures can be considered to improve patient outcome.	5	D
Removal of intrauterine contraceptives should be considered in women with chronic or recurrent vulvovaginal candidiasis.	4	C
Medroxyprogesterone acetate can be considered in recurrent vulvovaginal candidiasis.	5	D
Women who have fewer than three annual episodes of vulvovaginal candidiasis but are otherwise complicated should be treated in a manner similar to someone with recurrent vulvovaginal candidiasis.	1b	A

In a symptomatic women in whom a non-*albicans Candida* is identified, it is recommended that treatment should only be offered if no other causes are identified.	5	D
For *Candida glabrata* most of the options will consist of compounded medications.	4	C
For *Candida krusei, C. tropicalis* and *Saccharomyces cerevisiae* topical clotrimazole 100 mg daily for two weeks, nystatin or boric acid are the treatments of choice in symptomatic women.	4	C
Vaginal boric acid may represent the best option for treating azole-resistant yeast infections, despite safety concerns.	4	C
Vaginal treatment with longer courses of topical azoles, preferably clotrimazole, is recommended during pregnancy.	1a	A
Boric acid should not be used during pregnancy.	5	D
Oral fluconazole is not recommended in early pregnancy.	1a	A
Women with symptomatic vulvovaginal candidiasis who are breastfeeding can be treated similarly to other healthy women, including with fluconazole.	1b	A
Immunosuppressed women with vulvovaginal candidiasis should be given longer regimens and followed-up to make sure that the treatment was effective.	5	D
Ibrexafungerp and oteseconazole are new drugs that may change the algorithms in the future, but are contra-indicated in pregnant women or in those who may become pregnant in the near (ibrexafungerp) or distant (oteseconazole) future.	1b	A

References

1. Rolo, J.; Faria-Gonçalves, P.; Barata, T.; Oliveira, A. S.; Gaspar, C.; Ferreira, S. S.; Palmeira-de-Oliveira, R.; Martinez-de-Oliveira, J.; Costa-de-Oliveira, S.; Palmeira-de-Oliveira, A., Species Distribution and Antifungal Susceptibility Profiles of Isolates from Women with Nonrecurrent and Recurrent Vulvovaginal Candidiasis. *Microb Drug Resist* 2021, 27, (8), 1087-1095.
2. Sasani, E.; Rafat, Z.; Ashrafi, K.; Salimi, Y.; Zandi, M.; Soltani, S.; Hashemi, F.; Hashemi, S. J., Vulvovaginal candidiasis in Iran: A systematic review and meta-analysis on the epidemiology, clinical manifestations, demographic characteristics, risk factors, etiologic agents and laboratory diagnosis. *Microb Pathog* 2021, 154, 104802.
3. Sherrard, J.; Wilson, J.; Donders, G.; Mendling, W.; Jensen, J. S., 2018 European (IUSTI/WHO) International Union against sexually transmitted infections (IUSTI) World Health Organisation (WHO) guideline on the management of vaginal discharge. *Int J STD AIDS* 2018, 29, (13), 1258-1272.
4. Sobel, J. D., Genital candidiasis. *Medicine* 2014, 42, (7), 364-368.
5. Gharehbolagh, S. A.; Fallah, B.; Izadi, A.; Ardestani, Z. S.; Malekifar, P.; A, M. B.; Mahmoudi, S., Distribution, antifungal susceptibility pattern and intra-Candida albicans species complex prevalence of Candida africana: A systematic review and meta-analysis. *PLoS One* 2020, 15, (8), e0237046.
6. Borman, A. M.; Johnson, E. M., Name Changes for Fungi of Medical Importance, 2018 to 2019. *J Clin Microbiol* 2021, 59, (2).
7. Yano, J.; Peters, B. M.; Noverr, M. C.; Fidel, P. L., Jr., Novel Mechanism behind the Immunopathogenesis of Vulvovaginal Candidiasis: "Neutrophil Anergy". *Infect Immun* 2018, 86, (3).
8. De Bernardis, F.; Graziani, S.; Tirelli, F.; Antonopoulou, S., Candida vaginitis: virulence, host response and vaccine prospects. *Med Mycol* 2018, 56, (suppl_1), 26-31.

9. Swidsinski, A.; Guschin, A.; Tang, Q.; Dörffel, Y.; Verstraelen, H.; Tertychnyy, A.; Khayrullina, G.; Luo, X.; Sobel, J. D.; Jiang, X., Vulvovaginal candidiasis: histologic lesions are primarily polymicrobial and invasive and do not contain biofilms. *Am J Obstet Gynecol* 2019, 220, (1), 91.e1-91.e8.
10. Oliveira, A. S.; Rolo, J.; Gaspar, C.; Palmeira de Oliveira, R.; Martinez de Oliveira, J.; Palmeira de Oliveira, A., Allergic vulvovaginitis: a systematic literature review. *Arch Gynecol Obstet* 2022, 306, (3), 593-622.
11. Farr, A.; Effendy, I.; Frey Tirri, B.; Hof, H.; Mayser, P.; Petricevic, L.; Ruhnke, M.; Schaller, M.; Schaefer, A. P. A.; Sustr, V.; Willinger, B.; Mendling, W., Guideline: Vulvovaginal candidosis (AWMF 015/072, level S2k). *Mycoses* 2021, 64, (6), 583-602.
12. Drell, T.; Lillsaar, T.; Tummeleht, L.; Simm, J.; Aaspõllu, A.; Väin, E.; Saarma, I.; Salumets, A.; Donders, G. G.; Metsis, M., Characterization of the vaginal micro- and mycobiome in asymptomatic reproductive-age Estonian women. *PLoS One* 2013, 8, (1), e54379.
13. Foxman, B.; Muraglia, R.; Dietz, J. P.; Sobel, J. D.; Wagner, J., Prevalence of recurrent vulvovaginal candidiasis in 5 European countries and the United States: results from an internet panel survey. *J Low Genit Tract Dis* 2013, 17, (3), 340-5.
14. Dennerstein, G. J.; Ellis, D. H., Oestrogen, glycogen and vaginal candidiasis. *Aust N Z J Obstet Gynaecol* 2001, 41, (3), 326-8.
15. Benedict, K.; Lyman, M.; Jackson, B. R., Possible misdiagnosis, inappropriate empiric treatment, and opportunities for increased diagnostic testing for patients with vulvovaginal candidiasis-United States, 2018. *PLoS One* 2022, 17, (4), e0267866.
16. Hurley, R., Candidal vaginitis. *Proc R Soc Med* 1977, 70 Suppl 4, (Suppl 4), 1-2.
17. Blostein, F.; Levin-Sparenberg, E.; Wagner, J.; Foxman, B., Recurrent vulvovaginal candidiasis. *Ann Epidemiol* 2017, 27, (9), 575-582.e3.
18. Denning, D. W.; Kneale, M.; Sobel, J. D.; Rautemaa-Richardson, R., Global burden of recurrent vulvovaginal candidiasis: a systematic review. *Lancet Infect Dis* 2018, 18, (11), e339-e347.
19. Aballéa, S.; Guelfucci, F.; Wagner, J.; Khemiri, A.; Dietz, J. P.; Sobel, J.; Toumi, M., Subjective health status and health-related quality of life among women with Recurrent Vulvovaginal Candidosis (RVVC) in Europe and the USA. *Health Qual Life Outcomes* 2013, 11, 169.
20. Zhu, Y. X.; Li, T.; Fan, S. R.; Liu, X. P.; Liang, Y. H.; Liu, P., Health-related quality of life as measured with the Short-Form 36 (SF-36) questionnaire in patients with recurrent vulvovaginal candidiasis. Health Qual Life Outcomes 2016, 14, 65.
21. Shukla, A.; Sobel, J. D., Vulvovaginitis Caused by Candida Species Following Antibiotic Exposure. *Curr Infect Dis Rep* 2019, 21, (11), 44.
22. Nyirjesy, P.; Sobel, J. D.; Fung, A.; Mayer, C.; Capuano, G.; Ways, K.; Usiskin, K., Genital mycotic infections with canagliflozin, a sodium glucose co-transporter 2 inhibitor, in patients with type 2 diabetes mellitus: a pooled analysis of clinical studies. *Curr Med Res Opin* 2014, 30, (6), 1109-19.
23. Sobel, J. D.; Faro, S.; Force, R. W.; Foxman, B.; Ledger, W. J.; Nyirjesy, P. R.; Reed, B. D.; Summers, P. R., Vulvovaginal candidiasis: epidemiologic, diagnostic, and therapeutic considerations. *Am J Obstet Gynecol* 1998, 178, (2), 203-11.
24. Workowski, K. A.; Bachmann, L. H.; Chan, P. A.; Johnston, C. M.; Muzny, C. A.; Park, I.; Reno, H.; Zenilman, J. M.; Bolan, G. A., Sexually Transmitted Infections Treatment Guidelines, 2021. *MMWR Recomm Rep* 2021, 70, (4), 1-187.
25. Sobel, J. D.; Kapernick, P. S.; Zervos, M.; Reed, B. D.; Hooton, T.; Soper, D.; Nyirjesy, P.; Heine, M. W.; Willems, J.; Panzer, H.; Wittes, H., Treatment of complicated Candida vaginitis: comparison of single and sequential doses of fluconazole. *Am J Obstet Gynecol* 2001, 185, (2), 363-9.
26. Hong, E.; Dixit, S.; Fidel, P. L.; Bradford, J.; Fischer, G., Vulvovaginal candidiasis as a chronic disease: diagnostic criteria and definition. *J Low Genit Tract Dis* 2014, 18, (1), 31-8.
27. Sobel, J. D.; Sobel, R., Current treatment options for vulvovaginal candidiasis caused by azole-resistant Candida species. *Expert Opin Pharmacother* 2018, 19, (9), 971-977.
28. Pappas, P. G.; Kauffman, C. A.; Andes, D. R.; Clancy, C. J.; Marr, K. A.; Ostrosky-Zeichner, L.; Reboli, A. C.; Schuster, M. G.; Vazquez, J. A.; Walsh, T. J.; Zaoutis, T. E.; Sobel, J. D., Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. *Clin Infect Dis* 2016, 62, (4), e1-50.
29. Powell, A. M.; Gracely, E.; Nyirjesy, P., Non-albicans Candida Vulvovaginitis: Treatment Experience at a Tertiary Care Vaginitis Center. *J Low Genit Tract Dis* 2016, 20, (1), 85-9.
30. Eckert, L. O.; Hawes, S. E.; Stevens, C. E.; Koutsky, L. A.; Eschenbach, D. A.; Holmes, K. K., Vulvovaginal candidiasis: clinical manifestations, risk factors, management algorithm. *Obstet Gynecol* 1998, 92, (5), 757-65.
31. Anderson, M. R.; Klink, K.; Cohrssen, A., Evaluation of vaginal complaints. *JAMA* 2004, 291, (11), 1368-79.
32. Ferris, D. G.; Nyirjesy, P.; Sobel, J. D.; Soper, D.; Pavletic, A.; Litaker, M. S., Over-the-counter antifungal drug misuse associated with patient-diagnosed vulvovaginal candidiasis. *Obstet Gynecol* 2002, 99, (3), 419-25.
33. Allen-Davis, J. T.; Beck, A.; Parker, R.; Ellis, J. L.; Polley, D., Assessment of vulvovaginal complaints: accuracy of telephone triage and in-office diagnosis. *Obstet Gynecol* 2002, 99, (1), 18-22.

34. Schwebke, J. R.; Gaydos, C. A.; Nyirjesy, P.; Paradis, S.; Kodsi, S.; Cooper, C. K., Diagnostic Performance of a Molecular Test versus Clinician Assessment of Vaginitis. *J Clin Microbiol* 2018, 56, (6).
35. Ledger, W. J.; Polaneczky, M. M.; Yih, M. C.; Jeremias, J.; Tolbert, V.; Witkin, S. S., Difficulties in the Diagnosis of Candida Vaginitis. *Infectious Diseases in Clinical Practice* 2000, 9, (2), 66-69.
36. Donders, G. G.; Marconi, C.; Bellen, G.; Donders, F.; Michiels, T., Effect of short training on vaginal fluid microscopy (wet mount) learning. *J Low Genit Tract Dis* 2015, 19, (2), 165-9.
37. Vieira-Baptista, P.; Silva, A. R.; Costa, M.; Aguiar, T.; Saldanha, C.; Sousa, C., Clinical validation of a new molecular test (Seegene Allplex™ Vaginitis) for the diagnosis of vaginitis: a cross-sectional study. *Bjog* 2021, 128, (8), 1344-1352.
38. Danby, C. S.; Boikov, D.; Rautemaa-Richardson, R.; Sobel, J. D., Effect of pH on in vitro susceptibility of Candida glabrata and Candida albicans to 11 antifungal agents and implications for clinical use. *Antimicrob Agents Chemother* 2012, 56, (3), 1403-6.
39. Sobel, J. D., Vulvovaginal candidosis. *Lancet* 2007, 369, (9577), 1961-71.
40. Pitsouni, E.; Iavazzo, C.; Falagas, M. E., Itraconazole vs fluconazole for the treatment of uncomplicated acute vaginal and vulvovaginal candidiasis in nonpregnant women: a metaanalysis of randomized controlled trials. *Am J Obstet Gynecol* 2008, 198, (2), 153-60.
41. Mendling, W.; Krauss, C.; Fladung, B., A clinical multicenter study comparing efficacy and tolerability of topical combination therapy with clotrimazole (Canesten, two formats) with oral single dose fluconazole (Diflucan) in vulvovaginal mycoses. *Mycoses* 2004, 47, (3-4), 136-42.
42. Nurbhai, M.; Grimshaw, J.; Watson, M.; Bond, C.; Mollison, J.; Ludbrook, A., Oral versus intra-vaginal imidazole and triazole anti-fungal treatment of uncomplicated vulvovaginal candidiasis (thrush). *Cochrane Database Syst Rev* 2007, (4), Cd002845.
43. Sobel, J. D.; Wiesenfeld, H. C.; Martens, M.; Danna, P.; Hooton, T. M.; Rompalo, A.; Sperling, M.; Livengood, C., 3rd; Horowitz, B.; Von Thron, J.; Edwards, L.; Panzer, H.; Chu, T. C., Maintenance fluconazole therapy for recurrent vulvovaginal candidiasis. *N Engl J Med* 2004, 351, (9), 876-83.
44. Nguyen, Y.; Lee, A.; Fischer, G., Quality of life in patients with chronic vulvovaginal candidiasis: A before and after study on the impact of oral fluconazole therapy. *Australas J Dermatol* 2017, 58, (4), e176-e181.
45. Crouss, T.; Sobel, J. D.; Smith, K.; Nyirjesy, P., Long-Term Outcomes of Women With Recurrent Vulvovaginal Candidiasis After a Course of Maintenance Antifungal Therapy. *J Low Genit Tract Dis* 2018, 22, (4), 382-386.
46. Berger, F. A.; Monadian, N.; de Groot, N. M. S.; Santbergen, B.; van der Sijs, H.; Becker, M. L.; Broers, A. E. C.; van Gelder, T.; van den Bemt, P., QTc prolongation during ciprofloxacin and fluconazole combination therapy: prevalence and associated risk factors. *Br J Clin Pharmacol* 2018, 84, (2), 369-378.
47. Cakiroglu, Y.; Caliskan, S.; Doger, E.; Ozcan, S.; Caliskan, E., Does removal of CU-IUD in patients with biofilm forming candida really maintain regression of clinical symptoms? *J Obstet Gynaecol* 2015, 35, (6), 600-3.
48. Špaček, J.; Kestřánek, J.; Jílek, P.; Leško, D.; Plucnarová, S.; Buchta, V., Comparison of two long-term gestagen regimens in the management of recurrent vulvovaginal candidiasis: A pilot study. Mycoses 2017, 60, (4), 260-265.
49. Kennedy, M. A.; Sobel, J. D., Vulvovaginal Candidiasis Caused by Non-albicans Candida Species: New Insights. *Curr Infect Dis Rep* 2010, 12, (6), 465-70.
50. Sobel, J. D.; Chaim, W.; Nagappan, V.; Leaman, D., Treatment of vaginitis caused by Candida glabrata: use of topical boric acid and flucytosine. *Am J Obstet Gynecol* 2003, 189, (5), 1297-300.
51. Phillips, A. J., Treatment of non-albicans Candida vaginitis with amphotericin B vaginal suppositories. *Am J Obstet Gynecol* 2005, 192, (6), 2009-12; discussion 2012-3.
52. White, D. J.; Habib, A. R.; Vanthuyne, A.; Langford, S.; Symonds, M., Combined topical flucytosine and amphotericin B for refractory vaginal Candida glabrata infections. *Sex Transm Infect* 2001, 77, (3), 212-3.
53. Singh, S.; Sobel, J. D.; Bhargava, P.; Boikov, D.; Vazquez, J. A., Vaginitis due to Candida krusei: epidemiology, clinical aspects, and therapy. *Clin Infect Dis* 2002, 35, (9), 1066-70.
54. Sobel, J. D.; Suprapaneni, S., Candida parapsilosis Vaginal Infection-a New Site of Azole Drug Resistance. *Curr Infect Dis Rep* 2018, 20, (11), 43.
55. Felix, T. C.; de Brito Röder, D. V. D.; Dos Santos Pedroso, R., Alternative and complementary therapies for vulvovaginal candidiasis. *Folia Microbiol (Praha)* 2019, 64, (2), 133-141.
56. Blaschke-Hellmessen, R., [Epidemiological studies of the occurrence of yeasts in children and their mothers]. *Mykosen* 1968, 11, (9), 611-6.
57. Schnell, J. D., Epidemiology and the prevention of peripartal mycoses. *Chemotherapy* 1982, 28 Suppl 1, 66-72.
58. Mendling, W.; Weissenbacher, E. R.; Gerber, S.; Prasauskas, V.; Grob, P., Use of locally delivered dequalinium chloride in the treatment of vaginal infections: a review. *Arch Gynecol Obstet* 2016, 293, (3), 469-84.
59. Frey Tirri, B., Antimicrobial topical agents used in the vagina. *Curr Probl Dermatol* 2011, 40, 36-47.
60. Della Casa, V.; Noll, H.; Gonser, S.; Grob, P.; Graf, F.; Pohlig, G., Antimicrobial activity of dequalinium chloride against leading germs of vaginal infections. *Arzneimittelforschung* 2002, 52, (9), 699-705.

61. Howley, M. M.; Carter, T. C.; Browne, M. L.; Romitti, P. A.; Cunniff, C. M.; Druschel, C. M., Fluconazole use and birth defects in the National Birth Defects Prevention Study. *Am J Obstet Gynecol* 2016, 214, (5), 657.e1-9.
62. Mølgaard-Nielsen, D.; Svanström, H.; Melbye, M.; Hviid, A.; Pasternak, B., Association Between Use of Oral Fluconazole During Pregnancy and Risk of Spontaneous Abortion and Stillbirth. *JAMA* 2016, 315, (1), 58-67.
63. Bérard, A.; Sheehy, O.; Zhao, J. P.; Gorgui, J.; Bernatsky, S.; de Moura, C. S.; Abrahamowicz, M., Associations between low- and high-dose oral fluconazole and pregnancy outcomes: 3 nested case-control studies. *Cmaj* 2019, 191, (7), E179-e187.
64. Farr, A.; Kiss, H.; Holzer, I.; Husslein, P.; Hagmann, M.; Petricevic, L., Effect of asymptomatic vaginal colonization with Candida albicans on pregnancy outcome. *Acta Obstet Gynecol Scand* 2015, 94, (9), 989-96.
65. Holzer, I.; Farr, A.; Kiss, H.; Hagmann, M.; Petricevic, L., The colonization with Candida species is more harmful in the second trimester of pregnancy. *Arch Gynecol Obstet* 2017, 295, (4), 891-895.
66. Nyirjesy, P.; Leigh, R. D.; Mathew, L.; Lev-Sagie, A.; Culhane, J. F., Chronic vulvovaginitis in women older than 50 years: analysis of a prospective database. *J Low Genit Tract Dis* 2012, 16, (1), 24-9.
67. Fischer, G.; Bradford, J., Vulvovaginal candidiasis in postmenopausal women: the role of hormone replacement therapy. *J Low Genit Tract Dis* 2011, 15, (4), 263-7.
68. Schwebke, J. R.; Sobel, R.; Gersten, J. K.; Sussman, S. A.; Lederman, S. N.; Jacobs, M. A.; Chappell, B. T.; Weinstein, D. L.; Moffett, A. H.; Azie, N. E.; Angulo, D. A.; Harriott, I. A.; Borroto-Esoda, K.; Ghannoum, M. A.; Nyirjesy, P.; Sobel, J. D., Ibrexafungerp Versus Placebo for Vulvovaginal Candidiasis Treatment: A Phase 3, Randomized, Controlled Superiority Trial (VANISH 303). *Clin Infect Dis* 2022, 74, (11), 1979-1985.
69. Brand, S. R.; Degenhardt, T. P.; Person, K.; Sobel, J. D.; Nyirjesy, P.; Schotzinger, R. J.; Tavakkol, A., A phase 2, randomized, double-blind, placebo-controlled, dose-ranging study to evaluate the efficacy and safety of orally administered VT-1161 in the treatment of recurrent vulvovaginal candidiasis. *Am J Obstet Gynecol* 2018, 218, (6), 624.e1-624.e9.
70. Edwards, J. E., Jr.; Schwartz, M. M.; Schmidt, C. S.; Sobel, J. D.; Nyirjesy, P.; Schodel, F.; Marchus, E.; Lizakowski, M.; DeMontigny, E. A.; Hoeg, J.; Holmberg, T.; Cooke, M. T.; Hoover, K.; Edwards, L.; Jacobs, M.; Sussman, S.; Augenbraun, M.; Drusano, M.; Yeaman, M. R.; Ibrahim, A. S.; Filler, S. G.; Hennessey, J. P., Jr., A Fungal Immunotherapeutic Vaccine (NDV-3A) for Treatment of Recurrent Vulvovaginal Candidiasis-A Phase 2 Randomized, Double-Blind, Placebo-Controlled Trial. *Clin Infect Dis* 2018, 66, (12), 1928-1936.

TRICHOMONIASIS

5

(alphabetical order)
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5.1 Introduction

Trichomonas vaginalis is estimated to be the most common curable non-viral sexually transmitted infection (STI) worldwide.[1] It is associated with multiple adverse health outcomes including adverse birth outcomes,[2] increased risk of acquisition and transmission of human immunodeficiency virus (HIV) and other STIs,[3-5] pelvic inflammatory disease (PID),[6, 7] infertility,[8, 9] and cervical cancer.[10] A predominant health disparity exists for *T. vaginalis* infection, as African Americans are significantly more likely to be infected than persons of other races.[11] Beyond screening recommendations at entry to care and annually thereafter for HIV-infected women,[12] there are no established screening, surveillance, or control programs for this infection. Due to this limited public health response, *T. vaginalis* is frequently considered a "neglected" STI.[13] This chapter reviews the etiology, pathophysiology, epidemiology, clinical manifestations, diagnosis, and treatment recommendations for this common infection.

5.2 Etiology and pathophysiology

Trichomoniasis is caused by the parasitic pathogen, *T. vaginalis*, which primarily infects the squamous epithelium of the genital tract and causes damage to host epithelial cells. (Figure 5.1)

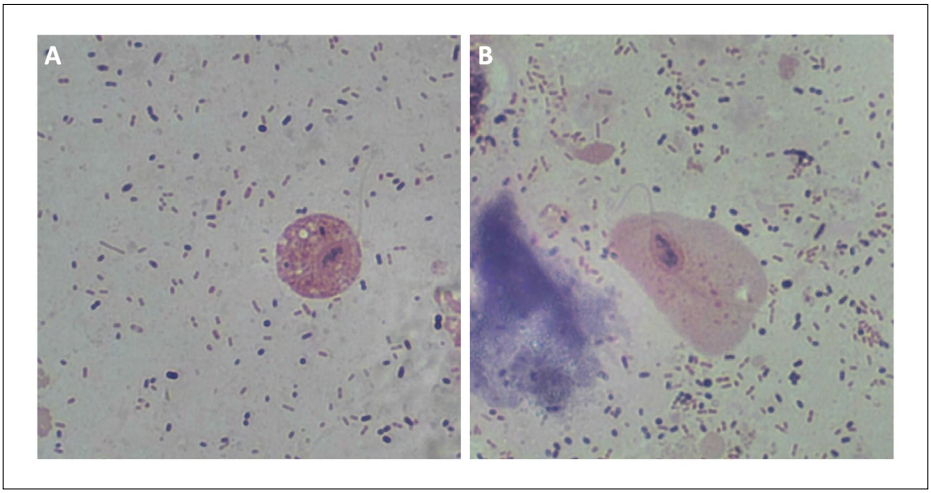

Figure 5.1 A and B– Trichomonads seen with Gram stain (1000x, oil immersion)

It typically infects the female lower genital tract (vagina, urethra, and endocervix) and the male urethra and prostate. It is transmitted among humans, its only known natural host.[14, 15] While transmission by fomites has been occasionally reported,[16-19] transmission occurs primarily by sexual contact.[20] Based on *in vitro* studies, its incubation period is 4-28 days.[21] *T. vaginalis* does not exist in a cyst form and does not survive well in the environment, but has been identified outside the human body in warm and wet locations (i.e. moist towels) for >3 hours.[16] It has its own microbiota, harboring two *Mycoplasma* species and a double-stranded RNA virus, *T. vaginalis* virus (TVV), which can contribute to its pathogenesis.[22, 23] Of the four known TVV viruses, TVV1 and TVV2 have been linked to genital symptom severity[24] and TVV2 and TVV3 to surface expression of an immunogenic protein P270 (associated with cytotoxicity, cytoadherence, and host immune evasion);[25] the role of TVV4 is not yet elucidated. However, in a study of 355 US *T. vaginalis* isolates from women participating in a clinical trial, of which 40% were positive for TVV, there was no association between TVV positivity and genital symptoms, repeated infections, or metronidazole resistance, suggesting that TVV may be commensal to *T. vaginalis*.[26]

T. vaginalis infection is more common in women than in men, which may be due to the anatomy of the female genital tract.[27-31] Other possibilities could be due to spontaneous resolution in men (which may occur in 36-69% of cases)[32, 33] or less effective testing among asymptomatic men.[11, 29, 34, 35]

Notably, *T. vaginalis* may persist for long periods of time in women (months or years)[36] while persistence in men is typically shorter (less than a month in some cases).[32] The greater likelihood of persistence in women has been linked to the greater availability of iron, an essential nutrient for the parasite.[15, 31, 37-40] Additionally, menstrual blood creates a rich growth medium which, when combined with the high concentration of iron during menstruation, promotes vaginal attachment and parasite growth.[27, 28]

5.3
Prevalence and epidemiology

While not a reportable STI, global estimates indicate that, among women and men, there are 156 million new cases per year.[1] In addition, the global prevalence of *T. vaginalis* among women (5.3%) is higher than that of chlamydia (3.8%), gonorrhea (0.9%), and syphilis (0.5%) combined.[1] In the US, the prevalence of *T. vaginalis* by urine nucleic acid amplification testing (NAAT) in a recent population-based study was 1.8% in women and 0.5% in men.[11] African Americans had a 4-fold higher prevalence than other racial groups, constituting a dramatic health disparity.[11] Unlike many STIs, *T. vaginalis* prevalence can be higher among older persons with rates ranging from 0.2-21.4% among persons >45 years of age.[41] In addition, population-based studies have found that *T. vaginalis* rates are highest among those 25 years or older.[42] The prevalence of urethral *T. vaginalis* in men who have sex with men (MSM) is extremely low to non-existent.[43] Although extra-genital (oral, rectal) *T. vaginalis* occasionally occurs, it is much less common than genital infection;[44, 45] testing is not recommended.

5.4
Risk factors

Table 5.1 lists common risk factors for *T. vaginalis* infection. While present in all races,[11] infection is more common in African American women engaging in high-risk sexual behaviors including having multiple sexual partners,[46, 47] inconsistent condom use, illicit drug use during

TABLE 5.1 Risk factors for *T. vaginalis* infection.
BV – bacterial vaginosis, HIV - human immunodeficiency virus

Female sex
African American race
Multiple sexual partners
Early coitarche
Older age
Inconsistent condom use
Illicit drug use
Sex with partners using illicit drugs
Transactional sex
History of incarceration
Having less than a high school education
Living below the national poverty level
Concurrent BV
Concurrent HIV

sex,[46] sex with partners using illicit drugs,[29, 46, 48, 49] and transactional sex.[50-53] Other risk factors include early coitarche,[54] older age,[29, 48, 55] history of incarceration,[56, 57] having less than a high school education,[11] and living below the national poverty level.[12, 47]

Women with bacterial vaginosis (BV) are at higher risk for acquiring *T. vaginalis*.[12, 20, 58] While vaginal dysbiosis has been associated with increased pathogenicity of *T. vaginalis*,[59] it is not clear if the presence of BV interferes with *T. vaginalis* treatment. In two prior randomized controlled trials (RCTs), BV was found to increase metronidazole treatment failure in HIV-infected women[60] but not in HIV-uninfected women.[61] This difference may be due to impaired immunity in HIV-infected women,[60] altered pharmacokinetics and pharmacodynamics of metronidazole,[62] or inadequate power in the studies conducted.[61] Additionally, women with HIV are at higher risk for *T. vaginalis*.[50, 63] Several studies have also shown that women who have sex with women and men are at higher risk for *T. vaginalis* than women who have sex with women and women who have sex with men.[29, 64]

5.5 Complications

Table 5.2 lists major complications associated with *T. vaginalis* infection among women, which are further detailed below.

Adverse birth outcomes

In a meta-analysis of 19 peer-reviewed studies, significant associations were found between *T. vaginalis* and preterm delivery (odds ratio [OR] 1.27; 95% CI 1.08-1.50), pre-labor rupture of membranes (OR 1.87; 95% CI 1.53-2.29), and low birth weight (OR 2.12; 95% CI 1.15-3.91).[2] The physiological mechanisms linking trichomoniasis and adverse birth outcomes are not well understood. One hypothesis is that preterm delivery and premature rupture of membranes in *T. vaginalis*-infected pregnant women are related to maternal innate immune inflammatory responses to the parasite, which involve elevated cervical interleukin-8 (IL-8) and vaginal defensin levels.[8, 65] These cytokines are markers of neutrophil activation, which has been associated with preterm delivery and other adverse birth events. For example, cervical IL-8 is thought to trigger cervical ripening and dilatation.[66, 67] Additionally, one study has shown an association between maternal *T. vaginalis* infection and intellectual disability in children born to infected mothers.[68]

TABLE 5.2 Complications of *T. vaginalis* infection in women.
CI - confidence interval, HIV - human immunodeficiency virus, OR - odds ratio, PID - pelvic inflammatory disease, RCT- randomized controlled trial, RR - relative risk, STI - sexually transmitted infection

Outcome	Author (Year)	Study Design	Study Findings
Adverse birth outcomes	Van Gerwen *et al*, 2021 [2]	Meta-Analysis	Among 19 studies, significant associations were found between *T. vaginalis* and preterm delivery (OR 1.27; 95% CI 1.08-1.50), prelabor rupture of membranes (OR 1.87; 95% CI 1.53-2.29) and low birthweight (OR 2.12; 95% CI 1.15-3.91).
HIV acquisition	Masha *et al*, 2018 [69]	Meta-Analysis	Among 19 studies, *T. vaginalis*-infected individuals were 1.5 times more likely to acquire HIV than non-infected individuals (95% CI 1.3-1.7; $p<0.001$).
	Barker *et al,* 2022 [70]	Meta-Analysis	Of 32 studies reporting k=97 effect size estimates of HIV acquisition risk due to non-viral STI infections, HIV acquisition risk was statistically significant for *T. vaginalis*-infected women (RR 1.54; 95% CI 1.31-1.82; k = 17).
Pelvic inflammatory disease	Moodley *et al*, 2002 [6]	Cross-sectional study	*T. vaginalis* was associated with PID in HIV-infected women but not HIV-uninfected women ($p=0.002$).
	Wiringa *et al*, 2020 [7]	Secondary analysis of RCT data	The odds of endometritis at baseline were twice as high among *T. vaginalis*-infected women compared to uninfected women (adjusted OR 1.9, 95% CI 1.0-3.3).
Infertility	Zhang *et al*, 2022 [77]	Meta-Analysis	Among 8 studies, *T. vaginalis* was associated with a 1.7 times greater risk of infertility in women (95% CI 1.25-2.31).
Cervical cancer	Yang *et al*, 2018 [10]	Meta-Analysis	Among 17 studies, the odds of cervical cancer for *T. vaginalis*-infected women was 2.06 (95% CI 1.77-2.39).

Risk of HIV

A meta-analysis of 19 peer-reviewed studies found that persons infected with *T. vaginalis* were 1.5 times more likely to acquire HIV than non-infected individuals (95% CI 1.3-1.7; $p<0.001$).[69] In another meta-analysis of 32 peer-reviewed studies reporting k=97 effect size estimates of HIV acquisition risk due to non-viral STI infections among high-risk heterosexuals diagnosed with chlamydia, gonorrhea, syphilis, *Mycoplasma genitalium*, and/or *T. vaginalis*, HIV acquisition risk was statistically significant for *T. vaginalis*-infected women (relative risk [RR] 1.54; 95% CI 1.31-1.82; k=17).[70] The greater susceptibility to HIV among *T. vaginalis*-infected individuals is biologically plausible for several reasons: (1) *T. vaginalis* damages epithelial cell membranes which act as a structural barrier to HIV, (2) the host immune response to *T. vaginalis* stimulates an increased number of HIV target cells in the genital tract mucosa, and (3) *T. vaginalis* alters the normal vaginal microbiota, rendering it more permissive to the development of BV, which, in turn, increases HIV acquisition risk.[69]

There is less direct evidence suggesting that HIV-infected individuals with *T. vaginalis* are more likely to transmit HIV. A review paper found that only seven of 14 studies demonstrated a higher likelihood of HIV shedding in the genital fluids of *T. vaginalis* coinfected individuals compared to HIV-infected individuals without coinfection.[71] In other studies, vaginal shedding of HIV-1 RNA was decreased after *T. vaginalis* treatment in a cohort of women from Kenya[72] and New Orleans, LA.[73] However, in the Kenyan cohort, the prevalence of vaginal HIV-1 DNA remained unchanged despite *T. vaginalis* treatment.

Risk of other sexually transmitted infections

Concomitant infection with *T. vaginalis* has been associated with a higher incidence of genital herpes simplex virus (HSV) 2 infection[74] as well as genital HSV2 shedding.[75] It has also been associated with the presence of other STIs including chlamydia, gonorrhea, and human papillomavirus (HPV).[4, 76]

Pelvic inflammatory disease

T. vaginalis is not traditionally considered STI associated with PID. However, in a 2002 study of 119 South African women, those infected with *T. vaginalis* had a significantly higher risk of PID than those without (p=0.03).[6] When women were stratified according to their HIV status, the risk of PID in HIV-infected women with *T. vaginalis* increased significantly (p=0.002); no association was found in women without HIV.[6] More recently, among 647 women in the PID Evaluation and Clinical Health (PEACH) study, *T. vaginalis* was frequently isolated from the vagina in 12.8% and the odds of having endometritis at baseline was twice as high among women with *T. vaginalis* compared to those without (adjusted OR 1.9; 95% CI 1.0-3.3). Infertility and recurrent PID were also more common among women with *T. vaginalis.*[7]

Infertility

A meta-analysis of eight peer-reviewed studies found that *T. vaginalis* was associated with a 1.7 times greater risk of infertility in women (95% CI 1.25-2.31).[77] Similarly, a meta-analysis of five peer-reviewed studies found that *T. vaginalis* was associated with a 1.91 times greater risk of infertility in men (95% CI 1.02-3.58).[77] This is thought to be due to inflammatory damage of female reproductive organs and changes in the vaginal environment which may result in a decrease or loss of reproductive function in women.[77] In men, *T. vaginalis* itself or the induced inflammatory response can impair sperm cells, causing a decrease in cell viability or death, which may result in a decrease or loss of reproductive function.[77]

Risk of cervical cancer

A study of Tanzanian women found that those infected with *T. vaginalis* were 6.5 times more likely to have high-risk HPV, suggesting an indirect link between *T. vaginalis* and cervical neoplasia.[4] In addition, a meta-analysis of 17 peer-reviewed studies found that *T. vaginalis*-infected women had a higher risk of cervical neoplasia (OR 2.06, 95% CI 1.77-2.39), with HPV co-infection playing a central role.[10]

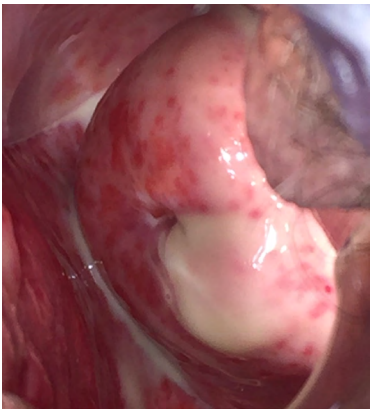

Figure 5.2 Strawberry cervix

5.6 Signs and symptoms

The "classic" symptoms of *T. vaginalis* infection include vaginal odor and a yellow-green, frothy, malodorous vaginal discharge.[12, 27, 48, 78, 79] However, a large number of infected women have minimal or no symptoms, with only 11-17% presenting with typical symptoms.[80] Half of asymptomatic women infected with *T. vaginalis* may become symptomatic within six months.[14] Infected women can also develop cyclic symptoms that are worse during menstruation.[27] Symptomatic women with *T. vaginalis* may note a wide range of additional symptoms including genital pruritus, dysuria, and dyspareunia.

On exam, signs may include vaginal enanthema, malodorous, frothy, vaginal discharge ranging from clear to yellow-green, colpitis *macularis* or "strawberry cervix" (present in <5% of women;[81, 82] rises to nearly 50% with colposcopy[82]), and elevated vaginal pH >4.5.[20] (Figure 5.2)

Infection may also be present in the setting of a normal vaginal pH.[20]

5.7 Diagnosis

T. vaginalis has been traditionally diagnosed at the point-of-care (POC) by wet mount microscopy (WMM) of vaginal discharge for motile trichomonads (sensitivity 44-68%; specificity 100%).[83] (Figure 5.3)

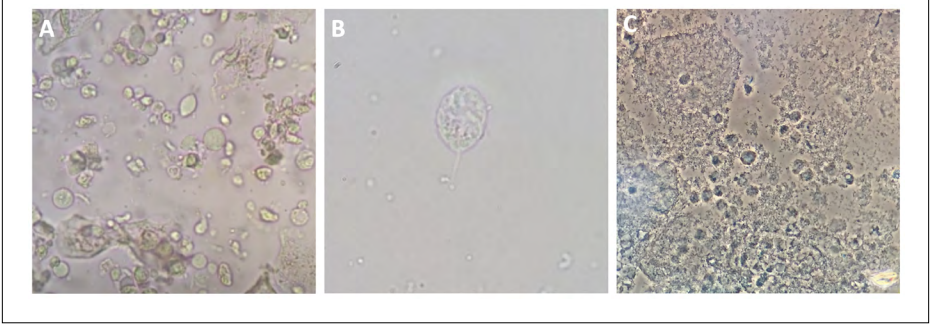

Figure 5.3 Wet mount microscopy.

A– Several trichomonads and inflammation (https://www.youtube.com/watch?v=pTL-_Q4S1Og) (200x)
B– Trichomonad with its typical structures: flagella on the outside and hydrogenosomes on the inside (400x)
C– Several trichomonads, inflammation and bacterial vaginosis (400x, phase contrast)

Ideally, this test must be performed within 10-20 minutes after collection or the trichomonads will lose their motility, increasing the likelihood of a false negative test. The OSOM® rapid test (Sekisui Diagnostics, California) is another POC test (results ≤10 minutes) that uses antibodies to detect *T. vaginalis* protein antigens in vaginal discharge (sensitivity 82-95% and specificity 97-100%, compared to WMM and culture). It is a qualitative test that should primarily be used in symptomatic women or contacts to *T. vaginalis.*[83] When present, *T. vaginalis* antigens bind the antibodies resulting in the formation of a blue line on the test strip. This test does not require microscopy, however is more expensive than WMM.

Trichomonas culture (InPouch® system [BioMed Diagnostics, White City, OR]) has previously been the gold standard for diagnosis (sensitivity 44-96%; specificity 100%).[83-85] Specimens from women (vaginal swabs) or men (urethral swabs, urine sediment, and/or semen; multiple specimens recommended to increase yield) should be used to inoculate the culture medium <1 hour after collection.[83] This test is categorized by the Clinical Laboratory Improvement Amendments (CLIA) as moderately complex, as it requires incubation at 37°C and reading over multiple days.[86]

Over the past decade, the availability of highly sensitive and specific *T. vaginalis* molecular diagnostic assays has grown rapidly. These assays can be sub-divided into molecular amplified assays (i.e. AmpliVue™ and Solana® assays),[87, 88] instrument-based assays (i.e. Hologic Aptima® *T. vaginalis* NAAT, Becton Dickinson [BD] ProbeTec™ Qx *T. vaginalis* NAAT, BD Max™ CT/GC/TV2 NAAT, Cepheid GeneXpert® *T. vaginalis* NAAT, Roche Cobas® MG/TV NAAT, and the Abbott Alinity m STI assay [including *T. vaginalis* NAAT testing],[89-93] and instrument-free assays (i.e. Visby GC/CT/TV NAAT testing device).[94] These assays, with their respective sensitivities and specificities, specimen types in women, complexity, and time to results, are detailed in Table 5.3. Several of these molecular assays can provide testing results within one hour or less (i.e. AmpliVue™ assay [results in 45-50 minutes]; Solana® assay [results in <40 minutes]; Cepheid GeneXpert® *T. vaginalis* NAAT [results in 40-63 minutes], and Visby GC/CT/TV NAAT testing device [results in 25 minutes]).

TABLE 5.3 *T. vaginalis* diagnostic tests in women.

CLIA - Clinical Laboratory Improvement Amendments, CT - *Chlamydia trachomatis*, GC - *Neisseria gonorrhoeae*, MG - *Mycoplasma genitalium*, NAAT - nucleic acid amplification test, POC - point-of-care. STI - sexually transmitted infection, TV - *Trichomonas vaginalis*.

***FDA-approved 5/4/22; https://www.molecular.abbott/int/en/products/infectious-disease/alinity-m-sti-assay**

Test	Sample	Sensitivity/Specificity for *T. vaginalis*	Complexity/Time
Wet mount microscopy[83]	Vaginal specimens	Sensitivity: 44-68%; Specificity: 100%	CLIA waived; POC test (results in ≤10 minutes).
OSOM®[83]	Vaginal specimens (Most useful in symptomatic women)	Sensitivity: 83-92%; Specificity: 99-100%	CLIA waived; POC test (results in ≤ 10 minutes).

BD Affirm™ VPIII[83]	Vaginal specimens	Sensitivity: 91-100%; Specificity: 93-96%	Moderate complexity. Results <1 hour.
Culture[83, 85]	Vaginal specimens	Sensitivity: 44-81%; Specificity: 100%	Moderate complexity. Requires incubation at 37°C; should be read for 5 days over a 7 day period. [86]
AmpliVue™ [87]	Vaginal specimens from symptomatic and asymptomatic women	Sensitivity 90.7%; Specificity 98.9%	Results in 45-50 minutes.
Solana® [88]	Vaginal specimens from symptomatic and asymptomatic women; urine specimens	Sensitivity/specificity 98.6%-100%/98.5%-98.9% for vaginal specimens and 92.9%-98%/97.9%-98.4% for urine specimens	Results in <40 minutes.
Hologic Aptima® *T. vaginalis* NAAT[89]	Vaginal, endocervical, ThinPrep®, and urine specimens from symptomatic and asymptomatic women	Sensitivity: 95.2%-100%; Specificity: 98.9%-99.6%	High complexity. Requires Panther, Viper, or Tigris system. Results in <8 hours.
BD ProbeTec™ Qx *T. vaginalis* NAAT[90]	Vaginal, endocervical, and urine specimens from symptomatic and asymptomatic women	Sensitivity: 98%-100%; Specificity: 98%-100%	High complexity. Requires Viper system. Results in <8 hours.
BD Max™ CT/GC/TV2 NAAT[93]	Vaginal, endocervical, and urine specimens from symptomatic and asymptomatic women	Sensitivity: 86.6%-100%; Specificity: 99.2%-99.8%	High complexity.
Cepheid GeneXpert® [91]	Self-collected vaginal, clinician-collected endocervical, and urine specimens from symptomatic and asymptomatic women	Sensitivity: 99.5%-100%; Specificity: 99.4-99.9%	Moderate complexity. Results in 40-63 minutes.
Roche Cobas® MG/TV NAAT [92]	Vaginal and endocervical specimens from symptomatic and asymptomatic women	Sensitivity: 96.4%-100%; Specificity: 96.5%-98.8%	High complexity. For use on Cobas 6800/8800 systems.
Abbott Alinity m STI assay*	Vaginal, endocervical, ThinPrep® and urine specimens from symptomatic and asymptomatic women	Sensitivity, specificity not yet published; refer to Abbott Molecular website*	Results in <115 minutes.
Visby GC/CT/TV NAAT Testing Device[94]	Self-collected vaginal specimens	Sensitivity: 99.2%; Specificity 96.9%	CLIA waived; POC test (results in 25 minutes).

5.8
Treatment and follow-up

The primary drug class used to treat *T. vaginalis* is the 5-nitroimidazoles (metronidazole, tinidazole, and secnidazole). For decades, the Centers for Disease Control and Prevention (CDC) and World Health Organization have recommended single dose 2 g oral metronidazole as the preferred treatment for *T. vaginalis*, with oral metronidazole 400-500 mg twice daily for seven days or single-dose 2 g oral tinidazole as alternative therapies. The recommended treatment was changed to the seven-day oral metronidazole dose for HIV-infected women over a decade ago in response to a multi-center RCT demonstrating superiority of the seven day oral metronidazole dose over singe-dose.[60] A subsequent meta-analysis[95] and multi-center RCT[61] found similar results in HIV-uninfected women. *In vivo* pharmacokinetic and pharmacodynamic effects of metronidazole may be playing a role in treatment failure with the use of the single 2 g oral dose, necessitating a longer treatment regimen in women.[62] Two hypotheses for this finding are: (1) competition for oral metronidazole by BV-associated bacteria in the vaginal microbiota of *T. vaginalis*-infected women and (2) inadequate accumulation of the active metabolites of metronidazole when only a single oral dose is given.[62] Thus, the seven day oral metronidazole regimen has since become the recommended treatment regimen for all women with single dose 2 g oral tinidazole remaining as an alternative;[12, 96] single dose 2 g oral metronidazole is no longer recommended in women. Given the lack of a comparable RCT in men, single dose 2 g oral metronidazole remains the recommended therapy for men with single dose 2 g oral tinidazole as an alternate until additional studies are conducted.[12]

If a woman is still infected with *T. vaginalis* after multi-dose oral metronidazole and has been re-exposed to an untreated sexual partner, she should be re-dosed with the same seven day treatment regimen. If she has not been re-exposed, she should be re-treated with either 2 g of oral metronidazole or tinidazole daily for seven days.[12] If a male is still infected with *T. vaginalis* after treatment with single dose 2 g oral metronidazole and has been re-exposed to an untreated sexual partner, he should be re-dosed with another single dose 2 g oral metronidazole. If he has not been re-exposed, he should be given a course of oral metronidazole 500 mg twice daily for seven days.[12]

Most recently, a randomized, double-blind, placebo controlled, delayed-treatment study evaluating the efficacy and safety of a single 2 g dose of oral secnidazole, a second generation 5-nitroimidazole with a longer half-life (17-19 hours), in 147 women with trichomoniasis was conducted.[97] At the test-of-cure visit 6–12 days after randomization, the microbiologic cure rate was 92.2% (95% CI 82.7-97.4) in the secnidazole group and 1.5% (95% CI 0.0-8.0) in the placebo group ($p<0.001$).[97] For women who received placebo at baseline, the opposite treatment was given at test-of-cure to ensure all participants were treated per standard of care. Overall, secnidazole was well tolerated. The most frequent adverse events were vulvovaginal candidiasis and nausea (2.7% each); no serious adverse events were observed. Secnidazole has since been FDA approved for *T. vaginalis* treatment in adolescent and adult women and men aged ≥12 years. It is also FDA approved for BV treatment in women[12] and is the only oral single-dose treatment currently available for both BV and trichomoniasis.[98]

Re-testing for *T. vaginalis* is recommended, preferably by NAAT, for all sexually active women between three weeks to three months after the end of treatment, regardless of whether their sexual partner(s) were treated or not.[12] The optimal time for repeat *T. vaginalis* NAAT testing after completion of multi-dose oral metronidazole was three weeks or greater in a recent study, informing this recommendation;[99] repeat NAAT testing before this time carries the risk of detecting remnant *T. vaginalis* nucleic acid that can still exist even if no viable organism persists. If re-testing by three months is not possible, women should be re-tested whenever they next seek medical care <12 months after treatment.[12]

5.9 Special situations

Infants

T. vaginalis has been documented to be transmitted perinatally in case reports,[100] although this is rare. In female newborns, *T. vaginalis* acquisition during birth may cause vaginal discharge during the first week of life.[101] Respiratory infection in newborns is also possible.[102]

Pregnant and lactating women

Several meta-analyses have found metronidazole to be safe for use in pregnant women in all stages of pregnancy;[103, 104] this is supported by current US guidelines.[12] Tinidazole use should be avoided in pregnant women based on preclinical data suggesting that it poses a moderate risk.[12] Limited data are available on the use of secnidazole in pregnant women however there is no evidence of adverse developmental outcomes in animal studies.[98]

In lactating women who are administered metronidazole, withholding breastfeeding during treatment and for 12–24 hours after the last dose will reduce the exposure of the infant to metronidazole. For women treated with tinidazole, interruption of breastfeeding is recommended during treatment and for three days after the last dose.[105]

5-nitroimidazole hypersensitivity

The most common reactions associated with 5-nitroimidazoles (primarily metronidazole) are immediate, type I IgE-mediated hypersensitivity reactions, occurring within 1-2 hours of drug exposure. This includes urticaria and hives with potential life-threatening manifestations such as angioedema, bronchospasm, and anaphylaxis.[106] Type II and IV hypersensitivity reactions have also been described, although less commonly.[107-110] The prevalence of metronidazole hypersensitivity has been found to be approximately 0.15% in a study of 2,375,424 Kaiser Permanente health plan members (a representative sample of 1% of the US population).[111]

Although uncommon, treatment for *T. vaginalis*-infected patients with a history of 5-nitroimidazole hypersensitivity is challenging.[112] If a prior IgE-mediated hypersensitivity reaction has been confirmed based on patient history and/or graded oral challenge (drug provocation test),[112] desensitization to the 5-nitroimidazole performed by an allergist is the first

line of treatment.[12] Oral[107, 113] and intravenous[114] protocols for desensitization have been published, primarily involving metronidazole. Intensive monitoring is required during the desensitization process due to the need for frequent drug administration and close monitoring for reactions; thus, it should typically be performed in the inpatient setting.[112] After completion of a desensitization protocol, patients are able to safely take oral metronidazole for 4-5 half-lives of the drug (half-life = 7-8 hours), approximately two days.[111] If the drug is not continued at regular intervals after successful completion of the desensitization protocol, it will need to be restarted from the beginning to avoid breakthrough of a type I hypersensitivity reaction.[111]

For patients in which metronidazole desensitization is not an option, use of other 5-nitroimidazoles such as tinidazole or secnidazole is not recommended because of the risk of cross-reactivity within the same drug class.[109] In this case, alternative treatment options outside of the 5-nitroimidazoles should be used.[12, 112] Use of these alternative treatments is anecdotal, limited to vaginal formulations (the majority of which have to be compounded), and may not reach all sites infected with *T. vaginalis* (i.e. Bartholin's and Skene's glands).[12, 112] One option based on case reports is a prolonged course of vaginal boric acid 600 mg twice daily for 60 days, either alone[115, 116] or in combination with vaginal clotrimazole.[117] Another option based on case reports and case series data is vaginal paromomycin 6.25% cream daily for 8-14 days;[118-120] topical use of this medication can result in painful vulvar ulcers that are self-limited and resolve once treatment is discontinued. Use of lubricating jelly to the vulva before use has been successful in preventing the development of these ulcers in some women.[120]

Persistent *T. vaginalis* infection

For patients who are experiencing persistent infection not due to sexual re-exposure, clinicians in the US can request a trichomonas culture kit from the CDC to perform drug resistance testing (404-718-4141; (https://www.cdc.gov/laboratory/specimen-submission/detail.html?CDCTestCode=CDC-10239). CDC has experience with susceptibility testing for 5-nitroimidazole-resistant *T. vaginalis* as well as management of infected patients and can provide guidance on treatment in these cases. Based on resistance testing results, an alternative treatment regimen may be recommended. Resistance rates of *T. vaginalis* for metronidazole and tinidazole have ranged from 4.3-10%,[121] although these data are not contemporary; resistance rates of secnidazole among clinical *T. vaginalis* isolates are unknown. *In vitro* resistance may not always correlate with clinical treatment failure,[122] especially in pregnant women,[53] but use of alternative treatment regimens following drug resistance testing results in cure of resistant infections in >80% of cases, suggesting that there is a benefit to drug resistance testing.[123]

Alternative treatment regimens for infections demonstrating *in vitro* drug resistance may include 2 g oral metronidazole or tinidazole daily for seven days.[12] If a patient fails the seven day regimen of high dose oral metronidazole or tinidazole, two additional treatment options have had successful results in women. The first is high dose oral tinidazole 2 g daily plus vaginal tinidazole 500 mg twice daily for 14 days.[124] If this fails, high dose oral tinidazole (1 g three times daily) plus vaginal paromomycin (4 g of 6.25% cream nightly) for 14 days could be considered.[125]

HIV-infected women

In a RCT of HIV-infected women co-infected with *T. vaginalis*, seven day oral metronidazole was found to be superior to single dose 2 g oral metronidazole.[60] Further analysis revealed that this superiority only occurred in the presence of BV.[126] Studies have also found that protease inhibitors used for the treatment of HIV may interfere with the efficacy of single-dose 2 g oral metronidazole among HIV-infected women.[127, 128]

As previously mentioned, *T. vaginalis* screening (and treatment for positive cases) at entry to care and annually is recommended for HIV-infected women.[12] It has been estimated that if this recommendation for *T. vaginalis* screening and treatment among HIV-infected women were followed, the lifetime cost of new HIV infections prevented would approximate U.S. $159,264,000 and could potentially prevent new HIV cases secondary to female-to-male transmission.[129]

Partner management

Sexual partners of patients with *T. vaginalis* infection should be treated. Commonly, patients are told by their providers to tell their partners to seek testing and treatment. Providers should consider treating the partner(s) of positive patients presumptively. One method of presumptive partner treatment is expedited partner therapy (EPT). EPT is the clinical practice of treating sexual partner(s) of patients diagnosed with an STI by providing prescriptions or medications to the patient to take to his/her partner(s) without the health care provider first examining the partner(s).

One RCT demonstrated that partner treatment with single dose 2 g oral tinidazole resulted in a >4 fold reduction in repeat infections among *T. vaginalis*-infected index women.[130] Two other studies using single dose 2-gram oral metronidazole for male partners of *T. vaginalis*-infected women found no effect of EPT[131] or a borderline effect.[132] While it is possible that the two studies that used metronidazole were either underpowered or did not use a correct control arm, it is also possible that oral tinidazole is a better treatment for men.

5.10
Future perspectives

Given that most studies have examined outcomes of symptomatic *T. vaginalis* infection, additional studies are needed to examine the importance of asymptomatic infection. This is particularly important given the proliferation of *T. vaginalis* molecular diagnostic tests, including those available at the POC.[94] Additional investigation of the role of *T. vaginalis* in the etiology of PID is also needed, especially among HIV-uninfected women. Regarding treatment, contemporary data on rates of *T. vaginalis* resistance among 5-nitroimidazoles, including secnidazole, are needed. In addition, the role of oral secnidazole in the treatment of persistent *T. vaginalis* infection should be elucidated. Finally, far less is known about *T. vaginalis* infection in men, particularly the most optimal treatment.

Recommendations

Recommendation	Quality of evidence	Strength of recommendation
Annual screening is recommended in women living with HIV.	4	C
Testing for non-genital trichomoniasis is not recommended.	5	D
A normal pH is not enough to exclude *T. vaginalis* infection.	2b	B
Wet mount microscopy should be performed when trichomoniasis is suspected, but a negative result does not exclude the diagnosis.	1b	A
Molecular tests are currently the gold standard for the diagnosis of trichomoniasis.	1a	A
Oral metronidazole 400-500 mg twice daily for 7 days is the recommended standard treatment for trichomoniasis in women, regardless of the HIV status.	1a	A
Single dose 2 g oral tinidazole or secnidazole can be considered as an alternative.	2b	B
Single dose 2 g oral metronidazole is no longer recommended in women for treatment for trichomoniasis.	1a	A
If a male is still infected with *T. vaginalis* after treatment with single dose 2 g oral metronidazole and has been re-exposed to an untreated sexual partner, he should be re-dosed with another single dose 2 g oral metronidazole.	5	D
If a male is still infected with *T. vaginalis* and he has not been re-exposed, he should be given a course of oral metronidazole 500 mg twice daily for 7 days.	5	D
Re-testing for *T. vaginalis* is recommended, preferably by nucleic acid amplification test, for all sexually active women between 3 weeks to 3 months after the end of treatment regardless of whether or not their sexual partner(s) was/were treated.	4	C
If re-testing by 3 months is not possible, women should be re-tested whenever they next seek medical care <12 months after treatment.	5	D
Metronidazole is safe for use in pregnant women in all stages of pregnancy.	1a	A
Tinidazole use should be avoided in pregnant women.	4	C
Limited data are available on the use of secnidazole in pregnant women, but there is no evidence of adverse developmental outcomes in animal studies.	4	C
In lactating women who are administered metronidazole, withholding breastfeeding during treatment and for 12–24 hours after the last dose is recommended.	4	C
In lactating women who are administered tinidazole, interruption of breastfeeding is recommended during treatment and for 3 days after the last dose.	4	C

In case of 5-nitroimidazoles hypersensitivity, desensitization is the first line option of treatment.	4	C
For patients in which metronidazole desensitization is not an option, use of other 5-nitroimidazoles such as tinidazole or secnidazole is not recommended because of the risk of cross-reactivity within the same drug class.	5	D
For patients who are experiencing persistent infection not due to sexual re-exposure, culture and drug resistance testing are recommended.	5	D
Sexual partners of patients with *T. vaginalis* infection should be treated.	5	D
Providers should consider treating partner(s) of positive patients presumptively, without the need of observing or testing them.	5	D
A single dose 2 g oral tinidazole as expedited partner therapy may be superior to single dose metronidazole for male partners of infected women.	1b	A

References

1. Rowley, J.; Vander Hoorn, S.; Korenromp, E.; Low, N.; Unemo, M.; Abu-Raddad, L. J.; Chico, R. M.; Smolak, A.; Newman, L.; Gottlieb, S.; Thwin, S. S.; Broutet, N.; Taylor, M. M., Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates, 2016. *Bull World Health Organ* 2019, 97, (8), 548-562P.
2. Van Gerwen, O. T.; Craig-Kuhn, M. C.; Jones, A. T.; Schroeder, J. A.; Deaver, J.; Buekens, P.; Kissinger, P. J.; Muzny, C. A., Trichomoniasis and adverse birth outcomes: a systematic review and meta-analysis. *BJOG* 2021, 128, (12), 1907-1915.
3. Sorvillo, F.; Smith, L.; Kerndt, P.; Ash, L., Trichomonas vaginalis, HIV, and African-Americans. *Emerg Infect Dis* 2001, 7, (6), 927-32.
4. Lazenby, G. B.; Taylor, P. T.; Badman, B. S.; McHaki, E.; Korte, J. E.; Soper, D. E.; Young Pierce, J., An association between Trichomonas vaginalis and high-risk human papillomavirus in rural Tanzanian women undergoing cervical cancer screening. *Clin Ther* 2014, 36, (1), 38-45.
5. Allsworth, J. E.; Ratner, J. A.; Peipert, J. F., Trichomoniasis and other sexually transmitted infections: results from the 2001-2004 National Health and Nutrition Examination Surveys. *Sex Transm Dis* 2009, 36, (12), 738-44.
6. Moodley, P.; Wilkinson, D.; Connolly, C.; Moodley, J.; Sturm, A. W., Trichomonas vaginalis is associated with pelvic inflammatory disease in women infected with human immunodeficiency virus. *Clin Infect Dis* 2002, 34, (4), 519-22.
7. Wiringa, A. E.; Ness, R. B.; Darville, T.; Beigi, R. H.; Haggerty, C. L., Trichomonas vaginalis, endometritis and sequelae among women with clinically suspected pelvic inflammatory disease. *Sex Transm Infect* 2020, 96, (6), 436-438.
8. Mielczarek, E.; Blaszkowska, J., Trichomonas vaginalis: pathogenicity and potential role in human reproductive failure. *Infection* 2016, 44, (4), 447-58.
9. Van Gerwen, O. T.; Camino, A. F.; Sharma, J.; Kissinger, P. J.; Muzny, C. A., Epidemiology, Natural History, Diagnosis, and Treatment of Trichomonas vaginalis in Men. *Clin Infect Dis* 2021, 73, (6), 1119-1124.
10. Yang, S.; Zhao, W.; Wang, H.; Wang, Y.; Li, J.; Wu, X., Trichomonas vaginalis infection-associated risk of cervical cancer: A meta-analysis. *European journal of obstetrics, gynecology, and reproductive biology* 2018, 228, 166-173.
11. Patel, E. U.; Gaydos, C. A.; Packman, Z. R.; Quinn, T. C.; Tobian, A. A. R., Prevalence and Correlates of Trichomonas vaginalis Infection Among Men and Women in the United States. *Clin Infect Dis* 2018, 67, (2), 211-217.
12. Workowski, K. A.; Bachmann, L. H.; Chan, P. A.; Johnston, C. M.; Muzny, C. A.; Park, I.; Reno, H.; Zenilman, J. M.; Bolan, G. A., Sexually Transmitted Infections Treatment Guidelines, 2021. *MMWR Recomm Rep* 2021, 70, (4), 1-187.
13. Muzny, C. A., Why Does Trichomonas vaginalis Continue to be a "Neglected" Sexually Transmitted Infection? *Clin Infect Dis* 2018, 67, (2), 218-220.
14. Petrin, D.; Delgaty, K.; Bhatt, R.; Garber, G., Clinical and microbiological aspects of Trichomonas vaginalis. *Clin Microbiol Rev* 1998, 11, (2), 300-17.
15. Lizarraga, A.; Munoz, D.; Strobl-Mazzulla, P. H.; de Miguel, N., Toward incorporating epigenetics into regulation of gene expression in the parasite Trichomonas vaginalis. *Mol Microbiol* 2021, 115, (5), 959-967.
16. Burch, T. A.; Rees, C. W.; Reardon, L. V., Epidemiological studies on human trichomoniasis. *Am J Trop Med Hyg* 1959, 8, (3), 312-8.

17. Muzny, C. A.; Rivers, C. A.; Mena, L. A.; Schwebke, J. R., Genotypic characterization of Trichomonas vaginalis isolates among women who have sex with women in sexual partnerships. *Sex Transm Dis* 2012, 39, (7), 556-8.
18. Crucitti, T.; Jespers, V.; Mulenga, C.; Khondowe, S.; Vandepitte, J.; Buve, A., Non-sexual transmission of Trichomonas vaginalis in adolescent girls attending school in Ndola, Zambia. *PLoS One* 2011, 6, (1), e16310.
19. Charles, S. X., Epidemiology of trichomonas vaginalis (TV) in rural adolescent and juvenile children. *J Trop Pediatr* 1991, 37, (2), 90.
20. Kissinger, P. J.; Gaydos, C. A.; Sena, A. C.; Scott McClelland, R.; Soper, D.; Secor, W. E.; Legendre, D.; Workowski, K. A.; Muzny, C. A., Diagnosis and Management of Trichomonas vaginalis: Summary of Evidence Reviewed for the 2021 Centers for Disease Control and Prevention Sexually Transmitted Infections Treatment Guidelines. *Clin Infect Dis* 2022, 74, (Supplement_2), S152-S161.
21. Schwebke, J. R.; Burgess, D., Trichomoniasis. *Clin Microbiol Rev* 2004, 17, (4), 794-803, table of contents.
22. Fichorova, R.; Fraga, J.; Rappelli, P.; Fiori, P. L., Trichomonas vaginalis infection in symbiosis with Trichomonasvirus and Mycoplasma. *Res Microbiol* 2017, 168, (9-10), 882-891.
23. Mercer, F.; Johnson, P. J., Trichomonas vaginalis: Pathogenesis, Symbiont Interactions, and Host Cell Immune Responses. *Trends Parasitol* 2018, 34, (8), 683-693.
24. Fraga, J.; Rojas, L.; Sariego, I.; Fernandez-Calienes, A.; Nunez, F. A., Species typing of Cuban Trichomonas vaginalis virus by RT-PCR, and association of TVV-2 with high parasite adhesion levels and high pathogenicity in patients. *Arch Virol* 2012, 157, (9), 1789-95.
25. Bessarab, I. N.; Nakajima, R.; Liu, H. W.; Tai, J. H., Identification and characterization of a type III Trichomonas vaginalis virus in the protozoan pathogen Trichomonas vaginalis. *Arch Virol* 2011, 156, (2), 285-94.
26. Graves, K. J.; Ghosh, A. P.; Schmidt, N.; Augostini, P.; Secor, W. E.; Schwebke, J. R.; Martin, D. H.; Kissinger, P. J.; Muzny, C. A., Trichomonas vaginalis Virus Among Women With Trichomoniasis and Associations With Demographics, Clinical Outcomes, and Metronidazole Resistance. *Clin Infect Dis* 2019, 69, (12), 2170-2176.
27. Bouchemal, K.; Bories, C.; Loiseau, P. M., Strategies for Prevention and Treatment of Trichomonas vaginalis Infections. *Clin Microbiol Rev* 2017, 30, (3), 811-825.
28. Harp, D. F.; Chowdhury, I., Trichomoniasis: evaluation to execution. *European journal of obstetrics, gynecology, and reproductive biology* 2011, 157, (1), 3-9.
29. Bassey, G. B.; Clarke, A. I. L.; Elhelu, O. K.; Lee, C. M., Trichomoniasis, a new look at a common but neglected STI in African descendance population in the United States and the Black Diaspora. A review of its incidence, research prioritization, and the resulting health disparities. *J Natl Med Assoc* 2022, 114, (1), 78-89.
30. Ryan, C. M.; de Miguel, N.; Johnson, P. J., Trichomonas vaginalis: current understanding of host-parasite interactions. *Essays Biochem* 2011, 51, 161-75.
31. Secor, W. E.; Meites, E.; Starr, M. C.; Workowski, K. A., Neglected parasitic infections in the United States: trichomoniasis. *Am J Trop Med Hyg* 2014, 90, (5), 800-804.
32. Krieger, J. N.; Verdon, M.; Siegel, N.; Holmes, K. K., Natural history of urogenital trichomoniasis in men. *J Urol* 1993, 149, (6), 1455-8.
33. Schwebke, J. R.; Rompalo, A.; Taylor, S.; Sena, A. C.; Martin, D. H.; Lopez, L. M.; Lensing, S.; Lee, J. Y., Re-evaluating the treatment of nongonococcal urethritis: emphasizing emerging pathogens--a randomized clinical trial. *Clin Infect Dis* 2011, 52, (2), 163-70.
34. Van der Pol, B., Trichomonas vaginalis infection: the most prevalent nonviral sexually transmitted infection receives the least public health attention. *Clin Infect Dis* 2007, 44, (1), 23-5.
35. Poole, D. N.; McClelland, R. S., Global epidemiology of Trichomonas vaginalis. *Sex Transm Infect* 2013, 89, (6), 418-22.
36. Van Der Pol, B.; Williams, J. A.; Orr, D. P.; Batteiger, B. E.; Fortenberry, J. D., Prevalence, incidence, natural history, and response to treatment of Trichomonas vaginalis infection among adolescent women. *J Infect Dis* 2005, 192, (12), 2039-44.
37. Figueroa-Angulo, E. E.; Rendon-Gandarilla, F. J.; Puente-Rivera, J.; Calla-Choque, J. S.; Cardenas-Guerra, R. E.; Ortega-Lopez, J.; Quintas-Granados, L. I.; Alvarez-Sanchez, M. E.; Arroyo, R., The effects of environmental factors on the virulence of Trichomonas vaginalis. *Microbes Infect* 2012, 14, (15), 1411-27.
38. Beltran, N. C.; Horvathova, L.; Jedelsky, P. L.; Sedinova, M.; Rada, P.; Marcincikova, M.; Hrdy, I.; Tachezy, J., Iron-induced changes in the proteome of Trichomonas vaginalis hydrogenosomes. *PLoS One* 2013, 8, (5), e65148.
39. Lehker, M. W.; Alderete, J. F., Iron regulates growth of Trichomonas vaginalis and the expression of immunogenic trichomonad proteins. *Mol Microbiol* 1992, 6, (1), 123-32.
40. Lehker, M. W.; Arroyo, R.; Alderete, J. F., The regulation by iron of the synthesis of adhesins and cytoadherence levels in the protozoan Trichomonas vaginalis. *J Exp Med* 1991, 174, (2), 311-8.
41. Lindrose, A. R.; Htet, K. Z.; O'Connell, S.; Marsh, J.; Kissinger, P. J., Burden of trichomoniasis among older adults in the United States: a systematic review. *Sex Health* 2022, 19, (3), 151-156.

42. Lewis, F. M. T.; Spicknall, I. H.; Flagg, E. W.; Papp, J. R.; Kreisel, K. M., Incidence and Prevalence of Trichomonas vaginalis Infection Among Persons Aged 15 to 59 Years: United States, 2018. *Sex Transm Dis* 2021, 48, (4), 232-237.
43. Kelley, C. F.; Rosenberg, E. S.; O'Hara, B. M.; Sanchez, T.; del Rio, C.; Sullivan, P. S., Prevalence of urethral Trichomonas vaginalis in black and white men who have sex with men. *Sex Transm Dis* 2012, 39, (9), 739.
44. Carter-Wicker, K.; Utuama, O.; Omole, F., Can trichomoniasis cause pharyngitis? A case report. *SAGE Open Med Case Rep* 2016, 4, 2050313X16682132.
45. Francis, S. C.; Kent, C. K.; Klausner, J. D.; Rauch, L.; Kohn, R.; Hardick, A.; Gaydos, C. A., Prevalence of rectal Trichomonas vaginalis and Mycoplasma genitalium in male patients at the San Francisco STD clinic, 2005-2006. *Sex Transm Dis* 2008, 35, (9), 797-800.
46. Miller, M.; Liao, Y.; Gomez, A. M.; Gaydos, C. A.; D'Mellow, D., Factors associated with the prevalence and incidence of Trichomonas vaginalis infection among African American women in New York city who use drugs. *J Infect Dis* 2008, 197, (4), 503-9.
47. Rogers, S. M.; Turner, C. F.; Hobbs, M.; Miller, W. C.; Tan, S.; Roman, A. M.; Eggleston, E.; Villarroel, M. A.; Ganapathi, L.; Chromy, J. R.; Erbelding, E., Epidemiology of undiagnosed trichomoniasis in a probability sample of urban young adults. *PLoS One* 2014, 9, (3), e90548.
48. Sutton, M.; Sternberg, M.; Koumans, E. H.; McQuillan, G.; Berman, S.; Markowitz, L., The prevalence of Trichomonas vaginalis infection among reproductive-age women in the United States, 2001-2004. *Clin Infect Dis* 2007, 45, (10), 1319-26.
49. Shafir, S. C.; Sorvillo, F. J.; Smith, L., Current issues and considerations regarding trichomoniasis and human immunodeficiency virus in African-Americans. *Clin Microbiol Rev* 2009, 22, (1), 37-45, Table of Contents.
50. Cu-Uvin, S.; Ko, H.; Jamieson, D. J.; Hogan, J. W.; Schuman, P.; Anderson, J.; Klein, R. S.; Group, H. I. V. E. R. S., Prevalence, incidence, and persistence or recurrence of trichomoniasis among human immunodeficiency virus (HIV)-positive women and among HIV-negative women at high risk for HIV infection. *Clin Infect Dis* 2002, 34, (10), 1406-11.
51. Kissinger, P., Trichomonas vaginalis: a review of epidemiologic, clinical and treatment issues. *BMC Infect Dis* 2015, 15, 307.
52. Najafi, A.; Chaechi Nosrati, M. R.; Ghasemi, E.; Navi, Z.; Yousefi, A.; Majidiani, H.; Ghaneialvar, H.; Sayehmiri, K.; Galvan-Ramirez, M. L.; Fakhar, M., Is there association between Trichomonas vaginalis infection and prostate cancer risk?: A systematic review and meta-analysis. *Microb Pathog* 2019, 137, 103752.
53. Lazenby, G. B.; Thompson, L.; Powell, A. M.; Soper, D. E., Unexpected High Rates of Persistent Trichomonas vaginalis Infection in a Retrospective Cohort of Treated Pregnant Women. *Sex Transm Dis* 2019, 46, (1), 2-8.
54. Ijasan, O.; Okunade, K. S.; Oluwole, A. A., The prevalence and risk factors for Trichomonas vaginalis infection amongst human immunodeficiency virus-infected pregnant women attending the antenatal clinics of a university teaching hospital in Lagos, South-Western, Nigeria. *Niger Postgrad Med J* 2018, 25, (1), 21-26.
55. Muzny, C. A.; Blackburn, R. J.; Sinsky, R. J.; Austin, E. L.; Schwebke, J. R., Added benefit of nucleic acid amplification testing for the diagnosis of Trichomonas vaginalis among men and women attending a sexually transmitted diseases clinic. *Clin Infect Dis* 2014, 59, (6), 834-41.
56. Sutcliffe, S.; Newman, S. B.; Hardick, A.; Gaydos, C. A., Prevalence and correlates of Trichomonas vaginalis infection among female US federal prison inmates. *Sex Transm Dis* 2010, 37, (9), 585-90.
57. Freeman, A. H.; Katz, K. A.; Pandori, M. W.; Rauch, L. M.; Kohn, R. P.; Liska, S.; Bernstein, K. T.; Klausner, J. D., Prevalence and correlates of Trichomonas vaginalis among incarcerated persons assessed using a highly sensitive molecular assay. *Sex Transm Dis* 2010, 37, (3), 165-8.
58. Balkus, J. E.; Richardson, B. A.; Rabe, L. K.; Taha, T. E.; Mgodi, N.; Kasaro, M. P.; Ramjee, G.; Hoffman, I. F.; Abdool Karim, S. S., Bacterial Vaginosis and the Risk of Trichomonas vaginalis Acquisition Among HIV-1-Negative Women. *Sex Transm Dis* 2014, 41, (2), 123-8.
59. Hinderfeld, A. S.; Simoes-Barbosa, A., Vaginal dysbiotic bacteria act as pathobionts of the protozoal pathogen Trichomonas vaginalis. *Microb Pathog* 2020, 138, 103820.
60. Kissinger, P.; Mena, L.; Levison, J.; Clark, R. A.; Gatski, M.; Henderson, H.; Schmidt, N.; Rosenthal, S. L.; Myers, L.; Martin, D. H., A randomized treatment trial: single versus 7-day dose of metronidazole for the treatment of Trichomonas vaginalis among HIV-infected women. *J Acquir Immune Defic Syndr* 2010, 55, (5), 565-71.
61. Kissinger, P.; Muzny, C. A.; Mena, L. A.; Lillis, R. A.; Schwebke, J. R.; Beauchamps, L.; Taylor, S. N.; Schmidt, N.; Myers, L.; Augostini, P.; Secor, W. E.; Bradic, M.; Carlton, J. M.; Martin, D. H., Single-dose versus 7-day-dose metronidazole for the treatment of trichomoniasis in women: an open-label, randomised controlled trial. *Lancet Infect Dis* 2018, 18, (11), 1251-1259.
62. Legendre, D.; Muzny, C. A.; Kissinger, P., Pharmacokinetic and Pharmacodynamic Effects of Metronidazole May Account for the Superior Efficacy of Multidose Therapy Among Women With Trichomoniasis. *Sex Transm Dis* 2019, 46, (11), 751-752.
63. Balkus, J. E.; Richardson, B. A.; Mochache, V.; Chohan, V.; Chan, J. D.; Masese, L.; Shafi, J.; Marrazzo, J.; Farquhar, C.; McClelland, R. S., A prospective cohort study comparing the effect of single-dose 2 g metronidazole on Trichomonas

vaginalis infection in HIV-seropositive versus HIV-seronegative women. *Sex Transm Dis* 2013, 40, (6), 499-505.
64. Muzny, C. A.; Sunesara, I. R.; Martin, D. H.; Mena, L. A., Sexually transmitted infections and risk behaviors among African American women who have sex with women: does sex with men make a difference? *Sex Transm Dis* 2011, 38, (12), 1118-25.
65. Simhan, H. N.; Anderson, B. L.; Krohn, M. A.; Heine, R. P.; Martinez de Tejada, B.; Landers, D. V.; Hillier, S. L., Host immune consequences of asymptomatic Trichomonas vaginalis infection in pregnancy. *Am J Obstet Gynecol* 2007, 196, (1), 59 e1-5.
66. Fichorova, R. N., Impact of T. vaginalis infection on innate immune responses and reproductive outcome. *J Reprod Immunol* 2009, 83, (1-2), 185-9.
67. Tanaka, Y.; Narahara, H.; Takai, N.; Yoshimatsu, J.; Anai, T.; Miyakawa, I., Interleukin-1beta and interleukin-8 in cervicovaginal fluid during pregnancy. *Am J Obstet Gynecol* 1998, 179, (3 Pt 1), 644-9.
68. Mann, J. R.; McDermott, S.; Barnes, T. L.; Hardin, J.; Bao, H.; Zhou, L., Trichomoniasis in pregnancy and mental retardation in children. *Ann Epidemiol* 2009, 19, (12), 891-9.
69. Masha, S. C.; Cools, P.; Sanders, E. J.; Vaneechoutte, M.; Crucitti, T., Trichomonas vaginalis and HIV infection acquisition: a systematic review and meta-analysis. *Sex Transm Infect* 2019, 95, (1), 36-42.
70. Barker, E. K.; Malekinejad, M.; Merai, R.; Lyles, C. M.; Sipe, T. A.; DeLuca, J. B.; Ridpath, A. D.; Gift, T. L.; Tailor, A.; Kahn, J. G., Risk of Human Immunodeficiency Virus Acquisition Among High-Risk Heterosexuals With Nonviral Sexually Transmitted Infections: A Systematic Review and Meta-Analysis. *Sex Transm Dis* 2022, 49, (6), 383-397.
71. Kissinger, P.; Adamski, A., Trichomoniasis and HIV interactions: a review. *Sex Transm Infect* 2013, 89, (6), 426-33.
72. Wang, C. C.; McClelland, R. S.; Reilly, M.; Overbaugh, J.; Emery, S. R.; Mandaliya, K.; Chohan, B.; Ndinya-Achola, J.; Bwayo, J.; Kreiss, J. K., The effect of treatment of vaginal infections on shedding of human immunodeficiency virus type 1. *J Infect Dis* 2001, 183, (7), 1017-22.
73. Kissinger, P.; Amedee, A.; Clark, R. A.; Dumestre, J.; Theall, K. P.; Myers, L.; Hagensee, M. E.; Farley, T. A.; Martin, D. H., Trichomonas vaginalis treatment reduces vaginal HIV-1 shedding. *Sex Transm Dis* 2009, 36, (1), 11-6.
74. Gottlieb, S. L.; Douglas, J. M., Jr.; Foster, M.; Schmid, D. S.; Newman, D. R.; Baron, A. E.; Bolan, G.; Iatesta, M.; Malotte, C. K.; Zenilman, J.; Fishbein, M.; Peterman, T. A.; Kamb, M. L.; Project, R. S. G., Incidence of herpes simplex virus type 2 infection in 5 sexually transmitted disease (STD) clinics and the effect of HIV/STD risk-reduction counseling. *J Infect Dis* 2004, 190, (6), 1059-67.
75. Boselli, F.; Chiossi, G.; Bortolamasi, M.; Gallinelli, A., Prevalence and determinants of genital shedding of herpes simplex virus among women attending Italian colposcopy clinics. *European journal of obstetrics, gynecology, and reproductive biology* 2005, 118, (1), 86-90.
76. Ginocchio, C. C.; Chapin, K.; Smith, J. S.; Aslanzadeh, J.; Snook, J.; Hill, C. S.; Gaydos, C. A., Prevalence of Trichomonas vaginalis and coinfection with Chlamydia trachomatis and Neisseria gonorrhoeae in the United States as determined by the Aptima Trichomonas vaginalis nucleic acid amplification assay. *J Clin Microbiol* 2012, 50, (8), 2601-8.
77. Zhang, Z.; Li, Y.; Lu, H.; Li, D.; Zhang, R.; Xie, X.; Guo, L.; Hao, L.; Tian, X.; Yang, Z.; Wang, S.; Mei, X., A systematic review of the correlation between Trichomonas vaginalis infection and infertility. *Acta Trop* 2022, 236, 106693.
78. Sena, A. C.; Miller, W. C.; Hobbs, M. M.; Schwebke, J. R.; Leone, P. A.; Swygard, H.; Atashili, J.; Cohen, M. S., Trichomonas vaginalis infection in male sexual partners: implications for diagnosis, treatment, and prevention. *Clin Infect Dis* 2007, 44, (1), 13-22.
79. Swygard, H.; Sena, A. C.; Hobbs, M. M.; Cohen, M. S., Trichomoniasis: clinical manifestations, diagnosis and management. *Sex Transm Infect* 2004, 80, (2), 91-5.
80. Landers, D. V.; Wiesenfeld, H. C.; Heine, R. P.; Krohn, M. A.; Hillier, S. L., Predictive value of the clinical diagnosis of lower genital tract infection in women. *Am J Obstet Gynecol* 2004, 190, (4), 1004-10.
81. Edwards, T.; Burke, P.; Smalley, H.; Hobbs, G., Trichomonas vaginalis: Clinical relevance, pathogenicity and diagnosis. *Crit Rev Microbiol* 2016, 42, (3), 406-17.
82. Wolner-Hanssen, P.; Krieger, J. N.; Stevens, C. E.; Kiviat, N. B.; Koutsky, L.; Critchlow, C.; DeRouen, T.; Hillier, S.; Holmes, K. K., Clinical manifestations of vaginal trichomoniasis. *JAMA* 1989, 261, (4), 571-6.
83. Hobbs, M. M.; Sena, A. C., Modern diagnosis of Trichomonas vaginalis infection. *Sex Transm Infect* 2013, 89, (6), 434-8.
84. Vieira-Baptista, P.; Bornstein, J., Candidiasis, Bacterial Vaginosis, Trichomoniasis and Other Vaginal Conditions Affecting the Vulva. *In Vulvar Disease: Breaking the Myths, Bornstein, J., Ed. Springer International Publishing: Cham,* 2019; pp 167-205.
85. Ohlemeyer, C. L.; Hornberger, L. L.; Lynch, D. A.; Swierkosz, E. M., Diagnosis of Trichomonas vaginalis in adolescent females: InPouch TV culture versus wet-mount microscopy. *The Journal of adolescent health : official publication of the Society for Adolescent Medicine* 1998, 22, (3), 205-8.
86. Rivers, C. A.; Muzny, C. A.; Schwebke, J. R., Diagnostic rates differ on the basis of the number of read days with the use of the InPouch culture system for Trichomonas vaginalis screening. *J Clin Microbiol* 2013, 51, (11), 3875-6.

87. Gaydos, C. A.; Hobbs, M.; Marrazzo, J.; Schwebke, J.; Coleman, J. S.; Masek, B.; Dize, L.; Jang, D.; Li, J.; Chernesky, M., Rapid Diagnosis of Trichomonas vaginalis by Testing Vaginal Swabs in an Isothermal Helicase-Dependent AmpliVue Assay. *Sex Transm Dis* 2016, 43, (6), 369-73.
88. Gaydos, C. A.; Schwebke, J.; Dombrowski, J.; Marrazzo, J.; Coleman, J.; Silver, B.; Barnes, M.; Crane, L.; Fine, P., Clinical performance of the Solana(R) Point-of-Care Trichomonas Assay from clinician-collected vaginal swabs and urine specimens from symptomatic and asymptomatic women. *Expert Rev Mol Diagn* 2017, 17, (3), 303-306.
89. Schwebke, J. R.; Hobbs, M. M.; Taylor, S. N.; Sena, A. C.; Catania, M. G.; Weinbaum, B. S.; Johnson, A. D.; Getman, D. K.; Gaydos, C. A., Molecular testing for Trichomonas vaginalis in women: results from a prospective U.S. clinical trial. *J Clin Microbiol* 2011, 49, (12), 4106-11.
90. Van Der Pol, B.; Williams, J. A.; Taylor, S. N.; Cammarata, C. L.; Rivers, C. A.; Body, B. A.; Nye, M.; Fuller, D.; Schwebke, J. R.; Barnes, M.; Gaydos, C. A., Detection of Trichomonas vaginalis DNA by use of self-obtained vaginal swabs with the BD ProbeTec Qx assay on the BD Viper system. *J Clin Microbiol* 2014, 52, (3), 885-9.
91. Schwebke, J. R.; Gaydos, C. A.; Davis, T.; Marrazzo, J.; Furgerson, D.; Taylor, S. N.; Smith, B.; Bachmann, L. H.; Ackerman, R.; Spurrell, T.; Ferris, D.; Burnham, C. A.; Reno, H.; Lebed, J.; Eisenberg, D.; Kerndt, P.; Philip, S.; Jordan, J.; Quigley, N., Clinical Evaluation of the Cepheid Xpert TV Assay for Detection of Trichomonas vaginalis with Prospectively Collected Specimens from Men and Women. *J Clin Microbiol* 2018, 56, (2).
92. Van Der Pol, B., A profile of the cobas(R) TV/ MG test for the detection of Trichomonas vaginalis and Mycoplasma genitalium. *Expert Rev Mol Diagn* 2020, 20, (4), 381-386.
93. Van Der Pol, B.; Torres-Chavolla, E.; Kodsi, S.; Cooper, C. K.; Davis, T. E.; Fife, K. H.; Taylor, S. N.; Augenbraun, M. H.; Gaydos, C. A., Clinical Performance of the BD CTGCTV2 Assay for the BD MAX System for Detection of Chlamydia trachomatis, Neisseria gonorrhoeae, and Trichomonas vaginalis Infections. *Sex Transm Dis* 2021, 48, (2), 134-140.
94. Morris, S. R.; Bristow, C. C.; Wierzbicki, M. R.; Sarno, M.; Asbel, L.; French, A.; Gaydos, C. A.; Hazan, L.; Mena, L.; Madhivanan, P.; Philip, S.; Schwartz, S.; Brown, C.; Styers, D.; Waymer, T.; Klausner, J. D., Performance of a single-use, rapid, point-of-care PCR device for the detection of Neisseria gonorrhoeae, Chlamydia trachomatis, and Trichomonas vaginalis: a cross-sectional study. *Lancet Infect Dis* 2021, 21, (5), 668-676.
95. Howe, K.; Kissinger, P. J., Single-Dose Compared With Multidose Metronidazole for the Treatment of Trichomoniasis in Women: A Meta-Analysis. *Sex Transm Dis* 2017, 44, (1), 29-34.
96. Committee on Practice, B.-G., Vaginitis in Nonpregnant Patients: ACOG Practice Bulletin, Number 215. *Obstet Gynecol* 2020, 135, (1), e1-e17.
97. Muzny, C. A.; Schwebke, J. R.; Nyirjesy, P.; Kaufman, G.; Mena, L. A.; Lazenby, G. B.; Van Gerwen, O. T.; Graves, K. J.; Arbuckle, J.; Carter, B. A.; McMahon, C. P.; Eder, S.; Shaw, J.; Pandey, B.; Chavoustie, S. E., Efficacy and Safety of Single Oral Dosing of Secnidazole for Trichomoniasis in Women: Results of a Phase 3, Randomized, Double-Blind, Placebo-Controlled, Delayed-Treatment Study. *Clin Infect Dis* 2021, 73, (6), e1282-e1289.
98. Muzny, C. A.; Van Gerwen, O. T.; Legendre, D., Secnidazole: a treatment for trichomoniasis in adolescents and adults. *Expert Rev Anti Infect Ther* 2022.
99. Craig-Kuhn, M. C.; Granade, C.; Muzny, C. A.; Van Der Pol, B.; Lillis, R.; Taylor, S. N.; Schmidt, N.; Martin, D. H.; Kissinger, P., Optimal Timing for Trichomonas vaginalis Test of Cure Using Nucleic Acid Amplification Testing. *Sex Transm Dis* 2019, 46, (5), 312-316.
100. Schwandt, A.; Williams, C.; Beigi, R. H., Perinatal transmission of Trichomonas vaginalis: a case report. *J Reprod Med* 2008, 53, (1), 59-61.
101. Trintis, J.; Epie, N.; Boss, R.; Riedel, S., Neonatal Trichomonas vaginalis infection: a case report and review of literature. *Int J STD AIDS* 2010, 21, (8), 606-7.
102. Carter, J. E.; Whithaus, K. C., Neonatal respiratory tract involvement by Trichomonas vaginalis: a case report and review of the literature. *Am J Trop Med Hyg* 2008, 78, (1), 17-9.
103. Burtin, P.; Taddio, A.; Ariburnu, O.; Einarson, T. R.; Koren, G., Safety of metronidazole in pregnancy: a meta-analysis. *Am J Obstet Gynecol* 1995, 172, (2 Pt 1), 525-9.
104. Caro-Patón, T.; Carvajal, A.; Martin de Diego, I.; Martin-Arias, L. H.; Alvarez Requejo, A.; Rodríguez Pinilla, E., Is metronidazole teratogenic? A meta-analysis. *Br J Clin Pharmacol* 1997, 44, (2), 179-82.
105. Evaldson, G. R.; Lindgren, S.; Nord, C. E.; Rane, A. T., Tinidazole milk excretion and pharmacokinetics in lactating women. *Br J Clin Pharmacol* 1985, 19, (4), 503-7.
106. Garcia-Rubio, I.; Martinez-Cocera, C.; Santos Magadan, S.; Rodriguez-Jimenez, B.; Vazquez-Cortes, S., Hypersensitivity reactions to metronidazole. *Allergol Immunopathol (Madr)* 2006, 34, (2), 70-2.
107. Gendelman, S. R.; Pien, L. C.; Gutta, R. C.; Abouhassan, S. R., Modified oral metronidazole desensitization protocol. *Allergy Rhinol (Providence)* 2014, 5, (2), 66-9.

108. Madsen, J. T.; Thormann, J.; Kerre, S.; Andersen, K. E.; Goossens, A., Allergic contact dermatitis to topical metronidazole - 3 cases. *Contact Dermatitis* 2007, 56, (6), 364-6.
109. Mishra, D.; Mobashir, M.; Zaheer, M. S., Fixed drug eruption and cross-reactivity between tinidazole and metronidazole. *Int J Dermatol* 1990, 29, (10), 740.
110. Kanwar, A. J.; Sharma, R.; Rajagopalan, M.; Kaur, S., Fixed drug eruption due to tinidazole with cross-reactivity with metronidazole. *Dermatologica* 1990, 180, (4), 277.
111. Macy, E.; Romano, A.; Khan, D., Practical Management of Antibiotic Hypersensitivity in 2017. *J Allergy Clin Immunol Pract* 2017, 5, (3), 577-586.
112. Van Gerwen, O. T.; Camino, A. F.; Bourla, L. N.; Legendre, D.; Muzny, C. A., Management of Trichomoniasis in the Setting of 5-Nitroimidazole Hypersensitivity. *Sex Transm Dis* 2021, 48, (8), e111-e115.
113. Kurohara, M. L.; Kwong, F. K.; Lebherz, T. B.; Klaustermeyer, W. B., Metronidazole hypersensitivity and oral desensitization. *J Allergy Clin Immunol* 1991, 88, (2), 279-80.
114. Pearlman, M. D.; Yashar, C.; Ernst, S.; Solomon, W., An incremental dosing protocol for women with severe vaginal trichomoniasis and adverse reaction to metronidazole. *Am J Obstet Gynecol* 1996, 174, (3), 934-6.
115. Muzny, C.; Barnes, A.; Mena, L., Symptomatic Trichomonas vaginalis infection in the setting of severe nitroimidazole allergy: successful treatment with boric acid. *Sex Health* 2012, 9, (4), 389-91.
116. Backus, K. V.; Muzny, C. A.; Beauchamps, L. S., Trichomonas vaginalis Treated With Boric Acid in a Metronidazole Allergic Female. *Sex Transm Dis* 2017, 44, (2), 120.
117. Aggarwal, A.; Shier, R. M., Recalcitrant Trichomonas vaginalis infections successfully treated with vaginal acidification. *J Obstet Gynaecol Can* 2008, 30, (1), 55-58.
118. Nyirjesy, P.; Sobel, J. D.; Weitz, M. V.; Leaman, D. J.; Gelone, S. P., Difficult-to-treat trichomoniasis: results with paromomycin cream. *Clin Infect Dis* 1998, 26, (4), 986-8.
119. Helms, D. J.; Mosure, D. J.; Secor, W. E.; Workowski, K. A., Management of trichomonas vaginalis in women with suspected metronidazole hypersensitivity. *Am J Obstet Gynecol* 2008, 198, (4), 370 e1-7.
120. Keating, M. A.; Nyirjesy, P., Trichomonas vaginalis Infection in a Tertiary Care Vaginitis Center. *Sex Transm Dis* 2015, 42, (9), 482-5.
121. Kirkcaldy, R. D.; Augostini, P.; Asbel, L. E.; Bernstein, K. T.; Kerani, R. P.; Mettenbrink, C. J.; Pathela, P.; Schwebke, J. R.; Secor, W. E.; Workowski, K. A.; Davis, D.; Braxton, J.; Weinstock, H. S., Trichomonas vaginalis antimicrobial drug resistance in 6 US cities, STD Surveillance Network, 2009-2010. *Emerg Infect Dis* 2012, 18, (6), 939-43.
122. Schwebke, J. R.; Barrientes, F. J., Prevalence of Trichomonas vaginalis isolates with resistance to metronidazole and tinidazole. *Antimicrob Agents Chemother* 2006, 50, (12), 4209-10.
123. Bosserman, E. A.; Helms, D. J.; Mosure, D. J.; Secor, W. E.; Workowski, K. A., Utility of antimicrobial susceptibility testing in Trichomonas vaginalis-infected women with clinical treatment failure. *Sex Transm Dis* 2011, 38, (10), 983-7.
124. Sobel, J. D.; Nyirjesy, P.; Brown, W., Tinidazole therapy for metronidazole-resistant vaginal trichomoniasis. *Clin Infect Dis* 2001, 33, (8), 1341-6.
125. Nyirjesy, P.; Gilbert, J.; Mulcahy, L. J., Resistant trichomoniasis: successful treatment with combination therapy. *Sex Transm Dis* 2011, 38, (10), 962-3.
126. Gatski, M.; Martin, D. H.; Levison, J.; Mena, L.; Clark, R. A.; Murphy, M.; Henderson, H.; Schmidt, N.; Kissinger, P., The influence of bacterial vaginosis on the response to Trichomonas vaginalis treatment among HIV-infected women. *Sex Transm Infect* 2011, 87, (3), 205-8.
127. Adamski, A.; Clark, R. A.; Mena, L.; Henderson, H.; Levison, J.; Schmidt, N.; Gebrekristos, H. T.; Martin, D. H.; Kissinger, P., The influence of ART on the treatment of Trichomonas vaginalis among HIV-infected women. *Clin Infect Dis* 2014, 59, (6), 883-7.
128. Kissinger, P.; Adamski, A.; Clark, R. A.; Mena, L.; Levison, J.; Martin, D. H., Does Antiretroviral Therapy Interfere With the Treatment of Trichomonas vaginalis Among HIV+ Women? *Sex Transm Dis* 2013, 40, (6), 506-7.
129. Lazenby, G. B.; Unal, E. R.; Andrews, A. L.; Simpson, K., Cost-effectiveness analysis of annual Trichomonas vaginalis screening and treatment in HIV-positive women to prevent HIV transmission. *Sex Transm Dis* 2014, 41, (6), 353-8.
130. Lyng, J.; Christensen, J., A double-blind study of the value of treatment with a single dose tinidazole of partners to females with trichomoniasis. *Acta Obstet Gynecol Scand* 1981, 60, (2), 199-201.
131. Kissinger, P.; Schmidt, N.; Mohammed, H.; Leichliter, J. S.; Gift, T. L.; Meadors, B.; Sanders, C.; Farley, T. A., Patient-delivered partner treatment for Trichomonas vaginalis infection: a randomized controlled trial. *Sex Transm Dis* 2006, 33, (7), 445-50.
132. Schwebke, J. R.; Desmond, R. A., A randomized controlled trial of partner notification methods for prevention of trichomoniasis in women. *Sex Transm Dis* 2010, 37, (6), 392-6.

CYTOLYTIC VAGINOSIS, LACTOBACILLOSIS AND LEPTOTHRIX

6

(alphabetical order)
Roni Kraut
Pedro Vieira-Baptista

6.1 Introduction

Traditionally, lactobacilli have been considered the "good" and "protective" bacteria of the vagina and thus excess or abnormal lactobacilli has typically not been considered a concern. Cytolytic vaginosis (CV), lactobacillosis and "leptothrix" are conditions characterized by abundant or oversized lactobacilli. **It should be acknowledged that these conditions are controversial and some experts feel that they are so poorly defined that their existence is unclear.**[1] Nevertheless, other experts report them in up to 5% of all the cases of "vaginitis".[2] Lactobacilli have long been considered as a marker of vaginal health, which may contribute to the skepticism of some authors towards these entities.

CV is characterized by an excessive number of lactobacilli and cytolysis. Lactobacillosis and leptothrix are often used interchangeably and the definitions are evolving. The current approach is to consider lactobacillosis as an increased number of lactobacilli without cytolysis, likely on the spectrum of CV, and to consider "leptothrix" as a separate entity characterized by elongated, serpiginous, bacteria thought to be lactobacilli, without cytolysis.[3, 4]

6.2 Cytolytic vaginosis

CV has been described in studies as early as 1961.[5] In 1991, Cibley *et al*. published a pivotal paper of this condition based on their experience in clinical practice.[6] They hypothesized the existence of CV, coined the term CV, provided clinical diagnostic criteria and suggested treatment options. However, their paper has been critiqued for lack of rigor and CV has remained a little known, understudied, and controversial condition.

Prevalence and epidemiology

Available studies suggest that the prevalence of CV in symptomatic women may be around 5%.[2, 7-14] However, this remains unclear given the small number of studies, their overall low quality, the different diagnostic techniques used, and the lack of standardized criteria.

A cross-sectional study found pregnant women with CV to have a decreased odds of group B streptococci colonization.[15]

Risk factors

Evidence suggests that a cytolytic pattern may occur more commonly in pregnancy and women <40 years; it seems to be less common in women with frequent intercourse.[15-17] It is currently unclear if prevalence varies according to geography or ethnic factors.

Complications

There is a paucity of studies that have examined the possible complications of CV and most have significant risk of bias. Most studies focus on pregnancy[5, 7, 15] and cervical dysplasia.[18-20] A case-control study concluded that women with CV have an increased odds for vulvodynia.[21]

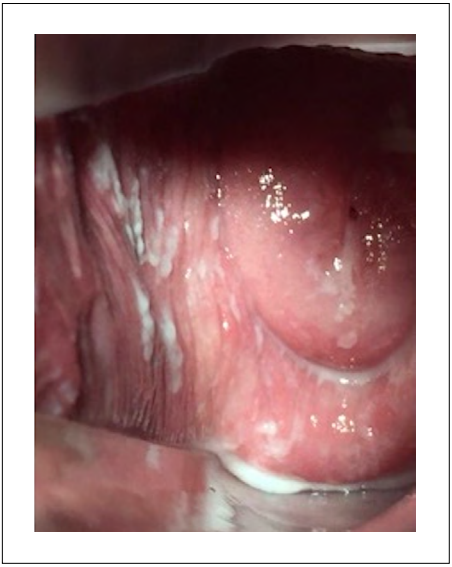

Figure 6.1 Typical discharge associated with cytolytic vaginosis

Signs and symptoms

CV may present without any signs or it may include erythema, swelling, and erosions. Symptoms include excess discharge (Figure 6.1), pruritus, burning, dysuria, pain, and dyspareunia. The symptoms tend to be cyclical, worsening after ovulation and improving with the onset of menses. The signs and symptoms of CV overlap with those of vulvovaginal candidiasis, making it difficult to differentiate between these two conditions based on signs and symptoms alone.[16]

Diagnosis

The diagnosis can be made with the use of wet mount microscopy.[3] It can also be achieved using vaginal Gram or Pap stain.[22] (Figure 6.2)

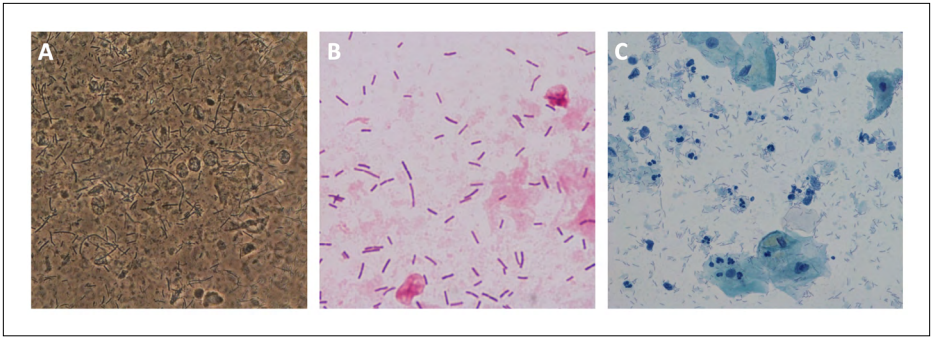

Figure 6.2 Cytolytic vaginosis.

A– Wet mount microscopy (400x, phase contrast) B– Gram stain (1000x, oil immersion) C– Pap smear (conventional) (400x)

On saline microscopy the presence of abundant lactobacilli, with length variation, is noted; other bacteria are typically scarce or, most often, absent; epithelial cells are fragmented (bare nuclei and cytoplasmatic debris), and inflammation is absent. (Table 6.1) (Figure 6.2 A) Cultures for *Candida* spp. are essential, but both entities may coexist.

The pH is low (often around 3.6) and the whiff test is negative. To date, there are no molecular tests commercially available for the diagnosis.

TABLE 6.1 Diagnostic criteria of cytolytic vaginosis.
* Donders criteria I or IIa or Ison-Hay criteria grade I or II[23, 24]

Criteria (all needed)	Method
1. Abundant pleomorphic lactobacilli 2. Other bacteria scarce/absent* 3. Fragmented epithelial cells 4. Inflammation absent	Wet mount, Pap or vaginal Gram stain

The differential diagnosis primarily includes vulvovaginal candidiasis, especially non-*albicans* candidiasis in which burning may predominate.

Treatment

The incidental diagnosis of a cytolytic pattern in asymptomatic women should not prompt treatment.

The most commonly used treatment is sodium bicarbonate, either as irrigations or sitz baths. (Table 6.2) Usually, the symptomatic relief is better achieved if treatment is used during the morning. According to our clinical experience, while in some cases two weeks of treatment are enough, most women will need to use it for several months or years, on demand. For some women, it can be useful to record their symptoms in a calendar, so they can establish a pattern and predict when they should resort to the prophylactic use of sodium bicarbonate. The treatment usually does not cure CV (microscopically or clinically), but rather allows control of the symptoms.

Vaginal antibiotics have been suggested as second line options if sodium bicarbonate proves to be insufficient, but data are scarce, and the effect appears to be transient.

TABLE 6.2 Treatments for cytolytic vaginosis

Treatment		
First line	Sodium bicarbonate 30-40 g/L (sitz baths or irrigations)	Once a day for 2 weeks
	Discontinue tampon use	Until symptoms resolve
	Discontinue antifungal treatment, antibiotics and probiotics	
	Use only water and soap to wash genital area	
Alternatives	Clindamycin vaginal cream (2%)	Once a day 5 days
	Amoxicillin 500 mg	3 times a day, orally, for 7 days

According to our clinical experience, changing the contraceptive method does not have an impact in the presence of a cytolytic pattern or in symptom control.

If CV coexists with *Candida* spp., we recommend starting by adequately treating the latter, and if symptoms persist, check if the microorganism was eliminated.

Special situations (infancy, pregnancy, postpartum/breastfeeding, menopause, immunosuppression)

A cytolytic pattern is common during pregnancy and usually asymptomatic – it is likely that such pattern is protective. We do not usually recommend treatment during this phase; if treatment is required, only sitz baths should be used and never irrigations or antibiotics.

Future perspectives

CV is a little-known, under-researched condition. It has been suggested that the symptoms and signs are physiological,[25] and it is not typically considered in the differential diagnosis for women presenting with vulvovaginal concerns.[26] A gold-standard objective diagnostic technique needs to be established and then used to further delineate this still equivocal condition.

6.3 Leptothrix

The first description of these bacteria was made in 1861[27] and has since been described in Pap smear samples. The oldest clinical reference to this comes from a paper by Horowitz *et al*., published in 1994 where it was referred to as lactobacillosis.[28] They described women with cyclical symptoms (irritation, burning and discharge), usually starting 7-10 days before menses, in whom long and serpiginous anaerobic bacilli were identified. It is not yet proven whether "leptothrix" can be a sole cause of vulvovaginal symptoms and it often is present with other conditions.[29] While usually assumed that leptothrix are lactobacilli, to date it remains unresolved which species it belongs to. One theory is that these may be indeed com-

mon lactobacilli that due to pressure of the vaginal milieu (i.e. antibiotic or antifungal use) acquire these characteristics.[4]

Prevalence and epidemiology

There are few data on the prevalence of the condition. A study from 1952 reported the presence of "leptothrix" in 15.2% of pregnant Black women, contrasting with 0.5% in White women.[27]

A Russian series from 1997 reported a prevalence of these bacteria in genital discharge (of both males and females) of 4%.[30] In 2016, a similar rate was found by Meštrović *et al*. in Pap smear samples.[29] More recently, in a study involving 3620 women, a rate of 2.8% was established.[4]

The original study by Horowitz *et al*. found the mean age of affected women to be of 33 years (range 24-59 years) and reported it also in postmenopausal and hysterectomized women.[28] In this study, the most commonly identified species of lactobacilli were *L. acidophilus* and *L. casei* and most were strong hydrogen peroxide producers. However, it is unclear if these species corresponded or not to leptothrix. In a more recent study, the mean age of affected women was 38.8±10.65 years (range 18–76).[31]

Risk factors

No risk factors have been clearly identified. One study showed a higher prevalence of leptothrix in women living with human immunodeficiency virus (HIV) infection (relative risk 3.0, 95% CI, 1.6–5.7), however no explanation for such was established.[4]

Some theories suggest that the previous use of antibiotics may be associated with the appearance of these long forms, similarly to what happens with other bacterial species.[28] While most women report previous episodes of candidiasis and antifungal treatments[32], it is not clear if those are causally associated with the presence of these bacteria or if it was an unconfirmed diagnosis and empirical treatment.

In one observational study, in which a clear distinction between CV and lactobacillosis was not made, it was suggested that symptoms' worsening could be associated with the ingestion of dairy products.[33]

While the nature of the association is unknown, one study correlated the presence of leptothrix with that of *T. vaginalis*, leading some authors to recommend excluding the presence of the latter when the first is identified.[29] More recent studies do not support the need to exclude trichomoniasis when leptothrix is identified.[4]

Complications

A study presented in 2013 showed a prevalence of 13% of lactobacillosis/leptothrix in women with vulvar pain, but it is not clear whether or not it distinguished this condition from CV.[33]

In one study there was no association between the presence of leptothrix and adverse outcomes of fertility treatments or higher risk of cervical dysplasia.[4]

Signs and symptoms

Most women in whom leptothrix is identified are asymptomatic.[4, 29]

Horowitz *et al* reported the following symptoms: thick, white, curdy or creamy discharge (83.3%), vulvar irritation (20.0%), burning (63.3%) or itching (86.7%), usually cyclical and peaking immediately before the menses and waning once it starts.[28] The vulvar exam is usually unremarkable, but there may be a discrete erythema and edema of the vulva and vaginal enanthema. The aspect of the cervix is unremarkable.[27, 34] Given these symptoms and signs overlap with vulvovaginal candidiasis, women often present after a lengthy duration of symptoms (average 22.9 months, range 1-84 months) and have already been submitted to several ineffective treatments.[28] In the Vieira-Baptista *et al*. study, the average duration of symptoms was 12.8±9.36 months and was significantly shorter than if women had another explanation for their vulvovaginal symptoms.[4]

Diagnosis

The diagnosis is usually accomplished using wet mount microscopy, by identifying elongated lactobacilli in the absence of cytolysis. These lactobacilli in some cases are very long (60 µm, range 40-75 µm) serpiginous, non-motile, non-branching and sometimes appearing segmented.[28] Leptothrix can be found associated with different background microbiota types, inflammation and other conditions. (Figure 6.3)

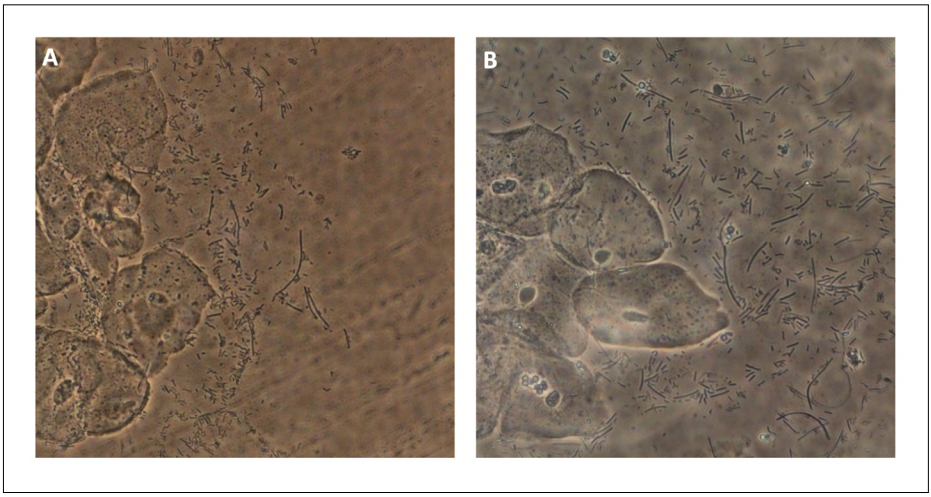

Figure 6.3 Leptothrix seen in wet mount microscopy (400x, phase contrast).
A– Leptothrix and normal background microbiota B– Leptothrix and *Candida* spp. blastospores

In one study, *T. vaginalis* was also present in 18% of cases and *Candida* spp. in 2%,[27, 35, 36] but more recent studies did not confirm an association with trichomoniasis. In fact, leptothrix was more often found with a normal background microbiota (in 63.7% of cases) and associated with a higher risk of candidiasis and a lower risk of BV and CV.[4]

When seen on Pap smears, these bacteria tend to stain blue.[37] The diagnosis is easier in conventional smears than in liquid-based cytology.[29] Using Gram stain, they are seen as Gram positive rods.[29] (Figure 6.4)

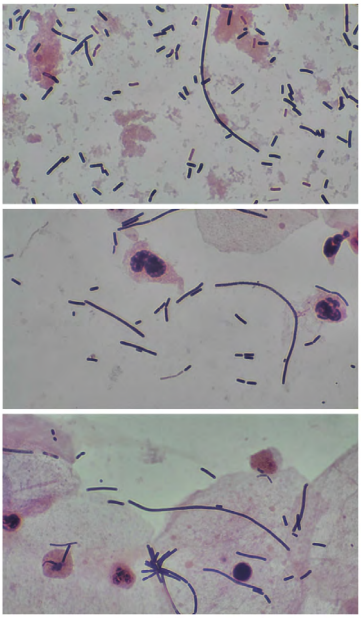

Figure 6.4 Leptothrix seen using Gram stain (1000x, oil immersion)

The pH has been described to be within low to normal range (3.6-4.7).[4, 28, 38]

The differential diagnosis include vulvodynia[32] and the presence of *Actinomyces* spp., which usually branch at acute angles and are more often found in women using intrauterine contraception.[37]

Treatment

If other changes are present (BV, *Candida* spp., trichomoniasis, cytolytic pattern) these should be assumed to be the cause of the symptoms.

The initial studies on the topic found the involved lactobacilli to be sensitive *in vitro* to penicillin, ampicillin, tetracycline, clindamycin and doxycycline and resistant to metronidazole, trimethoprim, gentamicin, amikacin, tobramycin, cefalexin, and ofloxacin.

In the Horowitz *et al*. report, expectant management for up to 3-4 months was not effective. The use of amoxicillin and clavulanate led to clearance of symptoms in 86.3% of cases; doxycycline was effective in all six cases in which it was prescribed. There were no clinical or microscopic relapses during the 18 month follow-up period.[28] No control studies were performed.

The use of sodium bicarbonate douches may be helpful in some women, but data are scarce.[4, 34]

TABLE 6.3 Treatment options for leptothrix

First options	Amoxicillin + clavulanate 500/125	3 times a day, per mouth, for 7 days	
Alternatives	Doxycycline 100 mg	2 times a day, per mouth, for 10 days	If allergic to penicillin or failure of amoxicillin + clavulanate
	Nifuratel 200 mg and Nifuratel 500 mg + nystatin 200000 IU	3 times a day, per mouth 7 days and once a day vaginally 7 days	Scarce evidence
	Sodium bicarbonate 30-40 g/L (sitz baths or irrigations)	Once a day for 2 weeks	Scarce evidence of limited results

Special situations (infancy, pregnancy, postpartum/breastfeeding, menopause, immunosuppression)

No data available.

6.4 Future perspectives

More rigorous epidemiological studies are needed to fully understand the role of these bacteria in health and disease.

To date, no 16S rRNA sequencing studies have been reported. A better understanding of the exact species involved and its physiology could help clarify their role, the risks associated with their presence (if any) and, if necessary, to more rationally treat.[29]

Recommendations

Recommendation	Quality of evidence	Strength of recommendation
The diagnosis of cytolytic vaginosis can be made with the use of wet mount microscopy, Gram or Pap stain.	4	C
Cultures for *Candida* spp. are recommended in all cases.	5	D
An increased pH excludes the diagnosis of cytolytic vaginosis.	4	C
The incidental diagnosis of a cytolytic pattern in asymptomatic women should not prompt treatment.	5	D
If cytolytic vaginosis coexists with *Candida* spp. and the woman is symptomatic, antifungals should be prescribed first.	5	D
Sodium bicarbonate (douche or sitz bath) is the recommended first line treatment for cytolytic vaginosis.	4	D
Treatment of cytolytic vaginosis is not recommended during pregnancy.	5	D
Leptothrix should only be considered a possible cause of symptoms in the absence of any other explanation.	4	C
There is no recommendation to exclude the presence of *Trichomonas vaginalis* when leptothrix is identified.	4	C

References

1. Voytik, M.; Nyirjesy, P., Cytolytic Vaginosis: a Critical Appraisal of a Controversial Condition. *Current Infectious Disease Reports* 2020, 22, (10), 26.
2. Wathne, B.; Holst, E.; Hovelius, B.; Mårdh, P. A., Vaginal discharge--comparison of clinical, laboratory and microbiological findings. *Acta Obstet Gynecol Scand* 1994, 73, (10), 802-8.
3. Vieira-Baptista, P.; Grincevičienė, Š.; Oliveira, C.; Fonseca-Moutinho, J.; Cherey, F.; Stockdale, C. K., The International Society for the Study of Vulvovaginal Disease Vaginal Wet Mount Microscopy Guidelines: How to Perform, Applications, and Interpretation. *J Low Genit Tract Dis* 2021, 25, (2), 172-180.
4. Vieira-Baptista, P.; Lima-Silva, J.; Preti, M.; Sousa, C.; Caiano, F.; Stockdale, C. K.; Bornstein, J., Vaginal Leptothrix: An Innocent Bystander? *Microorganisms* 2022, 10, (8).
5. Zidovsky, J., The significance of parabasal ("postnatal") cells in the vaginal smear in prolonged pregnancy. *Acta Cytol* 1961, 5, 393-398.

6. Cibley, L. J.; Cibley, L. J., Cytolytic vaginosis. *Am J Obstet Gynecol* 1991, 165, (4 Pt 2), 1245-9.
7. Akgun, I.; Yaziei Ensari, L., Cytolytic vaginosis: May cause infertility? . *Virchows Arch* 2012, 461, S1-S332.
8. Azevedo, S.; Lima-Silva, J.; Vieira-Baptista, P., Impact of the Sampling Site in the Result of Wet Mount Microscopy. *J Low Genit Tract Dis* 2019, 23, (2), 176-181.
9. Batashki, I.; Markova, D.; Milchev, N., [Frequency of cytolytic vaginosis--examination of 1152 patients]. *Akush Ginekol (Sofiia)* 2009, 48, (5), 15-6.
10. Cerikcioglu, N.; Beksac, M. S., Cytolytic vaginosis: misdiagnosed as candidal vaginitis. *Infect Dis Obstet Gynecol* 2004, 12, (1), 13-6.
11. Demirezen, S., Cytolytic vaginosis: examination of 2947 vaginal smears. *Cent Eur J Public Health* 2003, 11, (1), 23-4.
12. Fan, A. P.; Xue, F. X., [Clinical characteristics of aerobic vaginitis and its mixed infections]. *Zhonghua Fu Chan Ke Za Zhi* 2010, 45, (12), 904-8.
13. Moghaddam, N.; Rajabi, P., The relationship between symptomatic vaginal candidiasis and lactobacillus flora, using methenamine silver staining method. . *RMJ* 2009, 34, 82-85.
14. Raykova, V.; Baykushev, R.; Milanova, K.; Mitov, I., Prevalence of cytolytic vaginosis in symptomatic Bulgarian women – need for microbiological study. *Acta Microbiol Bulg* 2018, 34, 95-99.
15. Rocchetti, T. T.; Marconi, C.; Rall, V. L.; Borges, V. T.; Corrente, J. E.; da Silva, M. G., Group B streptococci colonization in pregnant women: risk factors and evaluation of the vaginal flora. *Arch Gynecol Obstet* 2011, 283, (4), 717-21.
16. Yang, S.; Zhang, Y.; Liu, Y.; Wang, J.; Chen, S.; Li, S., Clinical Significance and Characteristic Clinical Differences of Cytolytic Vaginosis in Recurrent Vulvovaginitis. *Gynecol Obstet Invest* 2017, 82, (2), 137-143.
17. Giraldo, P.; Amara, I. R.; Goncalves, A.; Vicentini, R.; Martins, C.; Giraldo, H.; Fachini, A., [Influence of frequency of vaginal intercourses and the use of doushing on vaginal microbiota] *Rev Bras Ginecol Obstet* 2005, 27, 257-262.
18. Nasielli, K.; Dudkiewicz, J.; Nasiell, M.; Hjerpe, A.; Silfverswärd, C., The occurrence of Bacillus vaginalis Döderlein and cytolysis in dysplasia, carcinoma in situ, and invasive carcinoma of the uterine cervix. *Acta Cytol* 1972, 16, (1), 21-5.
19. Silva, C.; Almeida, E. C.; Côbo Ede, C.; Zeferino, V. F.; Murta, E. F.; Etchebehere, R. M., A retrospective study on cervical intraepithelial lesions of low-grade and undetermined significance: evolution, associated factors and cytohistological correlation. *Sao Paulo Med J* 2014, 132, (2), 92-6.
20. Vieira-Baptista, P.; Lima-Silva, J.; Tavares, S.; Beires, J.; Donders, G., Cytolytic vaginosis does not have an impact on human papilloma virus (HPV) infection and cervical dysplasia. J Low Genit Tract Dis 2017, 21, (S27).
21. Vieira-Baptista, P.; Lima-Silva, J.; Xavier, J.; Beires, J.; Donders, G., Vaginal flora influences the risk of vulvodynia. *J Low Genit Tract Dis* 2017, 21, S26.
22. Garg, K.; Khare, A.; Bansal, R.; Sharma, S.; Chaudhary, N., Effects of Different Contraceptive Methods on Cervico-Vaginal Cytology. *J Clin Diagn Res* 2017, 11, (7), Ec09-ec11.
23. Ison, C. A.; Hay, P. E., Validation of a simplified grading of Gram stained vaginal smears for use in genitourinary medicine clinics. *Sex Transm Infect* 2002, 78, (6), 413-5.
24. Donders, G. G. G.; Bellen, G.; Grinceviciene, S.; Ruban, K.; Vieira-Baptista, P., Aerobic vaginitis: no longer a stranger. *Res Microbiol* 2017, 168, (9-10), 845-858.
25. Kaufman, R.; Friedrich, E.; Gardner, H., Benign diseases of the vulva and vagina. 3rd ed ed.; *Year Book Medical Publishers: Chicago,* 1989.
26. Workowski, K. A.; Bachmann, L. H.; Chan, P. A.; Johnston, C. M.; Muzny, C. A.; Park, I.; Reno, H.; Zenilman, J. M.; Bolan, G. A., Sexually Transmitted Infections Treatment Guidelines, 2021. *MMWR Recomm Rep* 2021, 70, (4), 1-187.
27. Feo, L. G.; Dellette, B. R., Leptotrichia (Leptothrix) vaginalis. *Am J Obstet Gynecol* 1952, 64, (2), 382-6.
28. Horowitz, B. J.; Mårdh, P. A.; Nagy, E.; Rank, E. L., Vaginal lactobacillosis. Am J Obstet Gynecol 1994, 170, (3), 857-61.
29. Meštrović, T.; Profozić, Z., Clinical and microbiological importance of Leptothrix vaginalis on Pap smear reports. *Diagn Cytopathol* 2016, 44, (1), 68-9.
30. Pliutto, A. M., [Laboratory diagnosis of bacterial vaginosis]. *Klin Lab Diagn* 1997, (3), 16-8.
31. Vieira-Baptista, P.; Bornstein, J., Candidiasis, Bacterial Vaginosis, Trichomoniasis and Other Vaginal Conditions Affecting the Vulva. *In Vulvar Disease: Breaking the Myths, Bornstein, J., Ed. Springer International Publishing: Cham,* 2019; pp 167-205.
32. Paavonen, J., Vulvodynia--a complex syndrome of vulvar pain. *Acta Obstet Gynecol Scand* 1995, 74, (4), 243-7.
33. Ricci, P.; Troncoso, J., Lactobacillosis and Chronic Vulvar Pain: Looking for High-Risk Factors as Precursors in Women Who Developed Vulvodynia. *Journal of Minimally Invasive Gynecology* 2013, 20, (6).

34. Hills, R. L., Cytolytic vaginosis and lactobacillosis. Consider these conditions with all vaginosis symptoms. *Adv Nurse Pract* 2007, 15, (2), 45-8.
35. Von Maseela, T., [Leptothrix vaginalis. Morphological studies]. *Fortschr Med* 1976, 94, (16), 295-8.
36. McLellan, R.; Spence, M. R.; Brockman, M.; Raffel, L.; Smith, J. L., The clinical diagnosis of trichomoniasis. *Obstet Gynecol* 1982, 60, (1), 30-4.
37. Fitzhugh, V. A.; Heller, D. S., Significance of a diagnosis of microorganisms on pap smear. *J Low Genit Tract Dis* 2008, 12, (1), 40-51.
38. Bibbo, M.; Harris, M. J., Leptothrix. *Acta Cytol* 1972, 16, (1), 2-4.

AEROBIC VAGINITIS/ DESQUAMATIVE INFLAMMATORY VAGINITIS

7

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7.1 Introduction

Desquamative inflammatory vaginitis (DIV) made its debut in the medical literature in 1965, by the hands of Gray and Barnes. In that paper, they presented their findings on 478 women complaining of vaginal discharge; among six of them "the vaginas were thin, quite reddened, with numerous pus cells and with oval and round parabasal cells in the secretions".[1] Three years later, Gardner described eight cases with similar features, among 3,000 women with vaginitis. He summarized it by pointing the similarities between these findings and those of atrophic vaginitis, despite the normal levels of estrogens in the affected women. Given the lack of microbiological pattern in these women, he assumed that infection was likely a secondary phenomenon.[2]

Despite the condition being known for almost 60 years, there has been no significant improvements in terms of understanding its etiology, diagnostic criteria or treatment, and it still is omitted from most textbooks.

In 2002, Donders *et al*. described a new entity, referred to as aerobic vaginitis (AV). This term emphasized the clear contrast with the far more common and acknowledged form of dysbiosis: bacterial vaginosis (BV). These women, microscopically, presented with different degrees of lactobacilli depletion, overgrowth of aerobic bacteria (mainly group B streptococci [GBS], *Escherichia coli* and *Staphylococcus aureus*), inflammation, and parabasal cells. The authors proposed a scoring system, in which the highest scores match DIV.[3] (Table 7.1)

TABLE 7.1 Aerobic vaginitis score, after Donders G *et al*..[4]
LbG – lactobacillary grade; hpf – high power field; EC – epithelial cell; PBC – parabasal cell
A score <3 corresponds to "no AV", score 3 - 4 to "light AV", score 5 – 6 to "moderate AV" and scores >6 to "severe AV" or DIV

Score	LbG	Number of leucocytes	Proportion of toxic leucocytes	Background microbiota	Proportion of PBC
0	I or IIa	≤10/hpf	None or sporadic	Unremarkable or cytolysis	<1%
1	IIb	>10/hpf and ≤10/EC	≤50% of total leucocytes	Small coliform bacteria	1-10%
2	III	>10/EC	>50% of total leucocytes	Cocci or chains of cocci	>10%

While it is not clear if these are two different entities or represent different aspects of the same spectrum, for practical purposes, we opt to refer to it as AV/DIV.[5, 6] Nevertheless, the distinction between AV and DIV may be relevant in clinical practice, not only because of the differences in severity and possible associated complications but mostly because treatment regimens differ slightly between the two conditions. However, the distinction is not always clear cut, as some overlapping in the clinical behavior exists.

Acknowledging this entity (or entities) is of uttermost importance not only for the proper diagnosis and management of symptomatic women, but also because of its potential role in obstetrical and non-obstetrical complications.[4, 7, 8]

7.2 Etiology and physiopathology

AV is characterized by moderate to severe colonization by facultative aerobic bacteria, depletion of lactobacilli, and moderate to severe inflammatory reaction of the vulvovaginal mucosa. Nevertheless an infectious etiology is unproven. It is assumed that this microbiota shift may be secondary to a harsh milieu resulting in loss of lactobacilli species, and thus allowing other bacteria to thrive.[4]

DIV (corresponding to severe AV), is characterized by colonization by aerobic facultative bacteria, absence of lactobacilli and signs of severe inflammation of the vaginal mucosa.[1, 9] It may be postulated that it happens due to a systemic inflammatory condition that produces vaginal inflammation resulting in abnormal vaginal microbiota, rather than the opposite.[9] DIV is frequently a chronic condition, with most women reporting symptoms for more than a year, and requiring treatment for a long period.[4]

Data showing similar effectiveness of vaginal steroids and 2% clindamycin in the treatment of AV/DIV suggest that the presence of aerobic bacteria is not a primary cause but rather the consequence of lactobacilli depletion and mucosal inflammation.[9] The most commonly reported bacteria isolated in women with AV/DIV are GBS, *E. coli, S. aureus, Enterococcus faecalis* and *Klebsiella pneumoniae*.[10-12]

Vitamin D deficiency has been postulated as a possible cause, but the correction of its level did not lead to improvement.[9] Pereira *et al*. hypothesized, based upon two cases, that a toxic

shock reaction to *S. aureus* in the vagina could lead to the development of the condition.[13] Despite the increased proportion of parabasal epithelial cells in the vagina, lack of estrogens has been excluded as the etiology of AV/DIV. Serum estradiol levels are usually within normal range and isolated topical estrogens are usually insufficient for symptomatic improvement.[9, 14]

A genetic predisposition toward autoimmune processes has also been considered a possible risk factor. Researchers reported an association of AV/DIV with other autoimmune conditions, such as thyroiditis and Crohn's disease.[4, 9, 15]

7.3 Prevalence and epidemiology

Prevalence of the condition is largely unknown, mainly due to lack of awareness and recognition of the disease by clinicians.[16] Available data point to a prevalence rate of 2-25% worldwide.[4, 17] The lowest percentage was reported in South American countries (Brazil, Chile) in which it was reported to be of only 2-3% in both pregnant and non-pregnant women.[18, 19] The highest rates have been described in sub-Saharan countries (11-25%); in a study conducted in Ethiopia, including only pregnant women, a slightly lower rate was reported (8%).[20-22] In Europe the range of disease prevalence is 8-12%, without any trend to be lower among pregnant women.[11, 23-27]

The AV prevalence in pregnant women is reported to be 4.1-10.8%. [10-12] DIV has been reported to be more common in perimenopausal White women.[9]

7.4 Risk factors

The risk factors for development of the disease are unknown. One study of AV found an association with vaginal douching, long term antibiotic use, presence of an intrauterine device, and condom use.[28] Most cases of DIV are idiopathic or primary, whereas secondary DIV may complicate other non-genital tract autoinflammatory diseases (i.e. Crohn's disease or systemic lupus erythematous) or be associated with rituximab use.[29, 30]

7.5 Complications

Vaginal dysbiosis is acknowledged as a risk factor for several gynecological and obstetrical complications.[6-8] As in BV and trichomoniasis, the risks seem to be independent of the presence of symptoms.

AV/DIV has been associated with an increased risk for sexually transmitted infections, including human immunodeficiency virus (HIV),[20, 31] *Chlamydia trachomatis*[32, 33] and possibly *T. vaginalis*.[3] In one study, the rate of *C. trachomatis* was more than three times higher in women with AV, when compared to those with a normal vaginal microbiota (71.4 *vs*. 21.7%, p=0.018).[32]

Given that AV/DIV leads to the development of erosions and increased leukocytes in the

vaginal mucosa, in theory it may also increase the risk of transmission and acquisition of herpes and human papillomavirus (HPV) infection. Some studies have shown a possible role of AV/DIV in the development of abnormal Pap tests and cervical dysplasia.[25, 34, 35] There are no studies showing benefit in the treatment of AV/DIV to promote the clearance of the HPV infection or regression of dysplasia.

Other possible non-obstetrical complications include infertility,[20] pelvic inflammatory disease, and toxic shock syndrome.[36]

Obstetrical complications have been reported to be associated with AV, including abortion, preterm labor, premature rupture of membranes (PROM), chorioamnionitis and funisitis (inflammation of the umbilical cord), puerperal sepsis and possibly neonatal sepsis.[12, 37-40] In one study, if AV was present in the first trimester the odds ratio (OR) of abortion (<25 weeks) was of 5.2 (interval of confidence [IC] 95% 1.5–17.7) and that of preterm delivery (<35 weeks) was of 3.2 (IC 95% 1.2–9.1).[12] In another study, the presence of severe AV in the first trimester was correlated with a shorter cervical length at 20-24 weeks.[41] A recent study conducted in Vietnam showed an OR of 8.65 (IC 95% 1.41-53.16, p=0.020) of puerperal sepsis.

Bacterial colonization and infection of the lower genital tract may induce cytokines and chemokines production, including interleukin (IL)-1β, IL-6 and IL-8, thus enhancing uterine contractibility.[42]

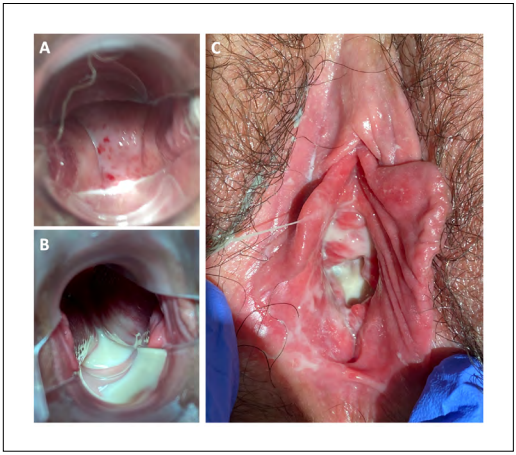

Figure 7.1 Severe aerobic vaginitis/desquamative inflammatory vaginitis.

A– Vaginal and cervical petechiae B– Copious discharge
C– Vestibular involvement

7.6 Signs and symptoms

Many cases of AV/DIV are asymptomatic, especially its mild forms. When symptomatic, the most characteristic clinical manifestation is an intense inflammatory reaction of the vaginal mucosa. This results in remarkable tenderness, dyspareunia, stinging and burning. Itching may also be present in some cases. Vaginal and cervical enanthema and submucosal petechiae can be noted, and in the most severe cases, the vestibule may also be involved. The vaginal discharge is purulent, sometimes copious, green or yellow, and can be stained with small amounts of blood.[1, 9, 43] (Figure 7.1)

The symptoms are often long lasting and of fluctuating intensity.[4] These manifestations are strikingly different from those of the far more common causes of vaginal discharge, namely BV.

7.7
Diagnosis

The diagnosis should be suspected based on the aforementioned symptoms and a compatible vulvovaginal examination. The gold standard for diagnosis is wet mount microscopy (WMM), preferably using phase contrast. (Figure 7.2)

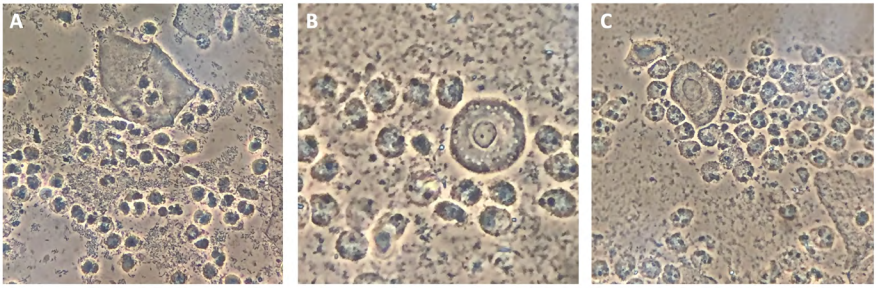

Figure 7.2 Aerobic vaginitis/desquamative inflammatory vaginitis in wet mount microscopy (400x, phase contrast).
A– Moderate aerobic vaginitis B and C– Severe aerobic vaginitis/desquamative inflammatory vaginitis

The diagnosis can be established in the presence of:

1. reduced or absent *Lactobacillus* morphotypes;
2. presence of other bacteria (small rods or cocci – the latter sometimes in chains);
3. a significant amount of inflammatory cells;
4. presence of parabasal epithelial cells;
5. elevated pH and;
6. negative whiff test.[4, 9]

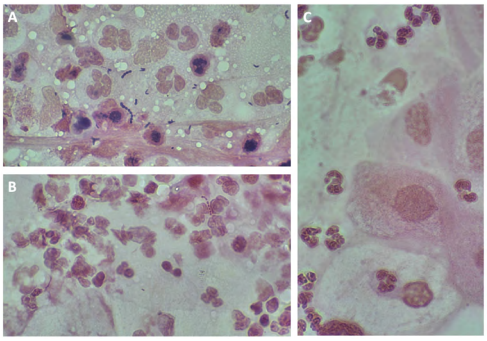

Figure 7.3 Aerobic vaginitis/desquamative inflammatory vaginitis aspects with Gram stain (1000x, oil immersion). Chains of cocci seen in A and B

Table 7.1 shows an AV scoring system, that can be used to diagnose and grade its severity. The AV score is a calculated sum of all sub-scores (lactobacillary grade [LbG], number of leucocytes, proportion of toxic leucocytes, background microbiota, and proportion of parabasal cells). A score of less than 3 is normal, a score 3 - 4 corresponds to "light AV", a score of 5 – 6 to "moderate AV" and if higher than 6 to "severe AV".[4]
Gram-stain preparation is currently not validated as a diagnostic tool for AV/DIV, due to lack of criteria.[5] (Figure 7.3)

Also, it is believed that lactobacillary grades are more accurately evaluated using WMM.[44] The pH is typically increased.

Routine bacterial cultures of the vaginal discharge are not recommended. These may, however, be used to rule out group A streptococci infection.[45]

The exclusion of the presence of *T. vaginalis*, using a nucleic acid amplification test is recommended, especially in the most severe cases, as the presentation of both conditions can be very similar. In postmenopausal women, differential diagnosis from atrophic vaginitis is not straightforward, but DIV does not respond to isolated estrogen replacement therapy.[5] The distinction between AV/DIV and vaginal involvement by erosive lichen planus may be hard to establish. However, some features of the latter can help in the differential diagnosis, including the presence of well-demarcated erosions or glazed erythema at the vaginal introitus and the involvement of other mucosal sites.[46]

7.8 Treatment

Recommended treatment options are shown in Table 7.2. No randomized clinical trials on the treatment of AV/DIV exist and the few recommendations that exist are based on limited observational studies and expert opinions.[9, 16, 47, 48]

The treatment regimen is guided by the microscopic findings: the presence of a disturbed microbiota, inflammation and atrophy are treated, respectively, with topical antibiotics or antiseptics, topical steroids, and topical estrogens.[4] Usually, in severe AV/DIV, it is useful to use a combination of all the three components at the beginning of the treatment. Both clindamycin and hydrocortisone have anti-inflammatory effect. Since severe AV/DIV is a chronic condition, maintenance therapy, for a two to six months period, is recommended.[16]

In moderate AV conditions, when there is no suspicion of an underlying immune-inflammatory condition, treatment with a single course of clindamycin or dequalinium chloride may be successful.[4, 49] In cases with only slightly or moderately disturbed microbiota (lactobacillary grade IIa or IIb) and without severe signs of inflammation (AV scores less than 5) treatment with only topical antibiotics or antiseptics may be effective.

Kanamycin has good effect against Gram-negative bacilli, does not disrupt vaginal lactobacilli and has also proven effective in AV treatment, used in a regimen of 100 mg vaginally for six consecutive days.[50]

Oral moxifloxacin has shown some efficacy in AV treatment. Almost two thirds of the patients treated with a single six day course of 400 mg moxifloxacin once daily, and 85% of those who received a second course, were cured.[51] Nevertheless, there is no reason to expose the woman to a systemic antibiotic when the condition can be managed with a topical regimen.

It should be noted that metronidazole (vaginal or oral) is not a drug of choice, because bacteria associated with AV/DIV are not anaerobic species.

When a condition such as Crohn's disease underlies DIV, the adequate treatment of the former with immunomodulators seems to effectively control the latter.[4, 9, 15]

In postmenopausal women, given the difficult distinction between AV/DIV and atrophic vaginitis, treatment with vaginal estrogens or prasterone alone may be tried.[9] It can also be liberally used in perimenopausal women, as it supports the natural vaginal lactobacilli-rich microbiota.[16]

Despite the theoretical benefits of the use of pro and prebiotics, data showing benefit are scarce.[52] In a randomized double-blind placebo-controlled trial, Heczko *et al*. demonstrated that supplementation of standard antibiotic therapy with oral probiotics lengthened remission in patients with recurrent AV/BV, and improved clinical and microbiological parameters.[53]

TABLE 7.2 Recommended treatments for severe aerobic vaginitis/desquamative inflammatory vaginitis
[(a)] Patients who are at risk of developing a yeast infection
[(b)] Peri- and postmenopausal women

Recommended treatments for severe AV/DIV		**Regimen**
Clindamycin	Clindamycin 2% cream	5 g vaginally daily at bedtime for 2-4 weeks; consider maintenance therapy twice a week for 2-6 months[16, 47]
	Clindamycin 100 mg suppository	2 suppositories vaginally daily at bedtime for 2-4 weeks; consider maintenance therapy twice a week for 2-6 months[16, 48]
Corticosteroids	Hydrocortisone 300 – 500 mg	Vaginally daily at bedtime for 2-4 weeks; consider maintenance therapy twice a week for 2-6 months[48]
	Cortisone acetate suppository 25 mg	Vaginally daily at bedtime for 2-4 weeks; consider maintenance therapy twice a week for 2-6 months[47]
Ancillary treatments for DIV		
Fluconazole[(a)]	Fluconazole 150 mg	Orally once weekly suppression for 2-6 months
Estradiol or estriol[(b)]	Estradiol or estriol cream or suppository	Vaginally twice a week for 2-6 months
Recommended treatments for moderate AV		
Dequalinium chloride	Dequalinium chloride 10 mg suppository	10 mg daily at bedtime for 6 days[43, 49]
Clindamycin	Clindamycin 2% cream	5 g vaginally daily at bedtime for 7 days[4]

7.9
Special situations (pregnancy, postpartum/breastfeeding)

AV, as previously referred, is associated with adverse pregnancy outcomes, such as miscarriage, preterm delivery, PROM and stillbirth, intra-amniotic aerobic infection and chorioamniotitis.[10] Nevertheless, there is no recommendation for systematic screening in pregnancy. The authors opt to treat the condition when diagnosed and recommend, despite the absence of good quality data, that it should be screened in women with prior adverse obstetrical outcomes possibly associated with AV/DIV.

GBS, *E. coli* and *S. aureus* are often associated with AV/DIV and also with negative obstetrical outcomes. Nevertheless, not all women colonized by these bacteria have AV/DIV criteria.

It is estimated that 7 to 25% of pregnant women between 35 and 37 weeks of gestation are positive for GBS.[54-56] Universal screening of GBS is recommended, as it is the first cause of neonatal mortality and morbidity worldwide[57] and ascending vaginal infection can lead to chorioamnionitis, PROM and endometritis,[58-60] resulting in neonatal sepsis and stillbirth.

E. coli causing AV seems to be a separate strain from those isolated from the gut, bladder or other sites of infection and thus specific strains may cause maternal disease.[61] *E. coli* is associated with adverse pregnancy outcomes and may cause frequent infections in pregnant women, mainly of the urinary tract and vagina, especially in the third trimester.

S. aureus is able to secrete exotoxins capable of inducing a cascade upregulating proinflammatory genes transcription, and is reported to be present in 4-22% of pregnant women.[62, 63] It is a leading cause responsible of late-onset sepsis in newborn[64] and a major pathogen in pediatric intensive care units.[65]

Despite limited data, it is not clear if there is an advantage in screening for AV/DIV and other bacteria beyond GBS in the 3rd trimester of pregnancy.[17]

Clindamycin is a broad spectrum antibiotic and its use in pregnant women is reported to lower the incidence of premature delivery.[66-70] It is considered a category B drug according to the FDA Pregnancy and Lactation Labeling Rule.[69] Data on its use in all trimesters is reassuring.[71, 72] The route of administration is vaginal, either as 2% cream or 100 mg suppositories. Clindamycin use during breastfeeding is unlikely to cause newborn side effects and the vaginal route of administration is preferred.[73]

Moxifloxacin and kanamycin both have shown efficacy in AV/DIV treatment in non-pregnant women. As potential hazards to the fetus, use of these compounds should be avoided during pregnancy.[74, 50, 75]

The use of oral or vaginal probiotics can be considered and has limited efficacy in improving conditions of the vaginal microbiota, but no clear impact on pregnancy outcomes have emerged.[53, 76-78]

7.10
Future perspectives

AV/DIV remains a poorly understood condition. More information is clearly needed in order to allow better management of women with vaginitis, but also to reduce the associated complications.

The complete understanding of the etiology of the condition would allow the rational development of adequate and effective treatments. Animal models, based on bacteria inoculation have been attempted, but it still remains unproven if AV/DIV is purely an infectious condition.[79]

The importance of screening and treatment of AV/DIV during pregnancy is an area that urgently needs to be studied.

Improvement is needed in terms of definition of the condition and consequent development of diagnostic tools. The development and validation of criteria for the diagnosis using Gram stain may be helpful in increasing the accuracy of diagnosis.[80] While some attempts have been made to develop molecular tests, these still need further advancement and validation. PCR-based methods targeted to detect bacteria that commonly associate with AV/DIV may be of some usefulness in the future, especially in settings where microscopy is not available.[16, 24, 47] Artificial intelligence is likely to be a game-changer in this field.[80] Meanwhile, more education and training in the practice of WMM by clinicians is needed.

Recommendations

Recommendation	Quality of evidence	Strength of recommendation
There is no recommendation to treat asymptomatic aerobic vaginitis/desquamative inflammatory vaginitis to improve HPV clearance.	5	D
The gold standard for diagnosis of aerobic vaginitis/desquamative inflammatory vaginitis is wet mount microscopy.	3b	C
The "AV score" can be used for the grading of aerobic vaginitis.	4	C
Routine bacterial cultures of the vaginal discharge are not recommended.	5	D
In severe cases of suspected severe aerobic vaginitis/desquamative inflammatory vaginitis the presence of *T. vaginalis* should be excluded using a molecular test.	5	D
The treatment regimen is guided by the microscopic findings.	4	C
A combination of topical antibiotics or antiseptics, topical steroids, and topical estrogens is usually recommended.	4	C
In moderate forms of aerobic vaginitis, a single course of topical clindamycin or dequalinium chloride can be attempted.	5	D
In severe forms of aerobic vaginitis, maintenance therapy, for a two to six months period, is recommended.	5	D

When there is an underlying condition for desquamative inflammatory vaginitis (i.e. Crohn's disease or rituximab treatment) it should be controlled first.	4	C
In postmenopausal women with suspected aerobic vaginitis/desquamative inflammatory vaginitis, treatment with topical estrogens should be attempted initially.	5	D
There is no recommendation to use pre or probiotics.	4	C
There is no recommendation to screen for aerobic vaginitis/desquamative inflammatory vaginitis during pregnancy.	5	D

References

1. Gray, L. A.; Barnes, M. L., VAGINITIS IN WOMEN, DIAGNOSIS AND TREATMENT. Am J Obstet Gynecol 1965, 92, 125-36.
2. Gardner, H. L., Desquamative inflammatory vaginitis: a newly defined entity. *Am J Obstet Gynecol* 1968, 102, (8), 1102-5.
3. Donders, G. G.; Vereecken, A.; Bosmans, E.; Dekeersmaecker, A.; Salembier, G.; Spitz, B., Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis: aerobic vaginitis. *Bjog* 2002, 109, (1), 34-43.
4. Donders, G. G. G.; Bellen, G.; Grinceviciene, S.; Ruban, K.; Vieira-Baptista, P., Aerobic vaginitis: no longer a stranger. *Res Microbiol* 2017, 168, (9-10), 845-858.
5. Vieira-Baptista, P.; Grincevičienė, Š.; Oliveira, C.; Fonseca-Moutinho, J.; Cherey, F.; Stockdale, C. K., The International Society for the Study of Vulvovaginal Disease Vaginal Wet Mount Microscopy Guidelines: How to Perform, Applications, and Interpretation. *J Low Genit Tract Dis* 2021, 25, (2), 172-180.
6. Lev-Sagie, A.; De Seta, F.; Verstraelen, H.; Ventolini, G.; Lonnee-Hoffmann, R.; Vieira-Baptista, P., The Vaginal Microbiome: II. Vaginal Dysbiotic Conditions. *J Low Genit Tract Dis* 2022, 26, (1), 79-84.
7. De Seta, F.; Lonnee-Hoffmann, R.; Campisciano, G.; Comar, M.; Verstraelen, H.; Vieira-Baptista, P.; Ventolini, G.; Lev-Sagie, A., The Vaginal Microbiome: III. The Vaginal Microbiome in Various Urogenital Disorders. *J Low Genit Tract Dis* 2022, 26, (1), 85-92.
8. Ventolini, G.; Vieira-Baptista, P.; De Seta, F.; Verstraelen, H.; Lonnee-Hoffmann, R.; Lev-Sagie, A., The Vaginal Microbiome: IV. The Role of Vaginal Microbiome in Reproduction and in Gynecologic Cancers. *J Low Genit Tract Dis* 2022, 26, (1), 93-98.
9. Reichman, O.; Sobel, J., Desquamative inflammatory vaginitis. *Best Pract Res Clin Obstet Gynaecol* 2014, 28, (7), 1042-50.
10. Ma, X.; Wu, M.; Wang, C.; Li, H.; Fan, A.; Wang, Y.; Han, C.; Xue, F., The pathogenesis of prevalent aerobic bacteria in aerobic vaginitis and adverse pregnancy outcomes: a narrative review. *Reprod Health* 2022, 19, (1), 21.
11. Zodzika, J.; Rezeberga, D.; Jermakova, I.; Vasina, O.; Vedmedovska, N.; Donders, G., Factors related to elevated vaginal pH in the first trimester of pregnancy. *Acta Obstet Gynecol Scand* 2011, 90, (1), 41-6.
12. Donders, G. G.; Van Calsteren, K.; Bellen, G.; Reybrouck, R.; Van den Bosch, T.; Riphagen, I.; Van Lierde, S., Predictive value for preterm birth of abnormal vaginal flora, bacterial vaginosis and aerobic vaginitis during the first trimester of pregnancy. *Bjog* 2009, 116, (10), 1315-24.
13. Pereira, N.; Edlind, T. D.; Schlievert, P. M.; Nyirjesy, P., Vaginal toxic shock reaction triggering desquamative inflammatory vaginitis. *J Low Genit Tract Dis* 2013, 17, (1), 88-91.
14. Zaino, R. J.; Nucci, M. R.; Kurman, R. J., Diseases of the Vagina. In Blaustein's Pathology of the Female Genital Tract, Kurman, R. J.; Hedrick Ellenson, L.; Ronnett, B. M., Eds. *Springer US: New York, NY,* 2018; pp 1-63.
15. Shukla, A.; Surapaneni, S.; Sobel, J. D., Desquamative Inflammatory Vaginitis as an Extraintestinal Manifestation of Crohn's Disease. *Current Infectious Disease Reports* 2020, 22, (9), 24.
16. Paavonen, J.; Brunham, R. C., Bacterial Vaginosis and Desquamative Inflammatory Vaginitis. *N Engl J Med* 2018, 379, (23), 2246-2254.
17. Nguyen, A. T. C.; Le Nguyen, N. T.; Hoang, T. T. A.; Nguyen, T. T.; Tran, T. T. Q.; Tran, D. N. T.; Nguyen, A. T. K.; Tran, L. M.; Nguyen, D. H. C.; Le, T. M.; Ho, B. D.; Rööp, T.; Kõljalg, S.; Štšepetova, J.; Van Le, A.; Salumets, A.; Mändar, R., Aerobic vaginitis in the third trimester and its impact on pregnancy outcomes. *BMC Pregnancy Childbirth* 2022, 22, (1), 432.
18. Gondo, D. C.; Duarte, M. T.; da Silva, M. G.; de Lima Parada, C. M., Abnormal vaginal flora in low-risk pregnant women cared for by a public health service: prevalence and association with symptoms and findings from gynecological exams. *Rev Lat Am Enfermagem* 2010, 18, (5), 919-27.
19. Villaseca, R.; Ovalle, A.; Amaya, F.; Labra, B.; Escalona, N.; Lizana, P.; Montoya, M. J.; Lillo, E.; Martínez, M. A., [Vaginal infections in a Family Health Clinic in the Metropolitan Region, Chile]. *Rev Chilena Infectol* 2015, 32, (1), 30-6.
20. Donders, G. G.; Gonzaga, A.; Marconi, C.; Donders, F.; Michiels, T.; Eggermont, N.; Bellen, G.; Lule, J.; Byamugishisa, J., Increased vaginal pH in Ugandan women: what does it indicate? *Eur J Clin Microbiol Infect Dis* 2016, 35, (8), 1297-303.

21. Vieira-Baptista, P.; Grinceviciene, S.; Bellen, G.; Sousa, C.; Saldanha, C.; Broeck, D. V.; Bogers, J. P.; Donders, G., Genital Tract Infections in an Isolated Community: 100 Women of the Príncipe Island. *Infect Dis Obstet Gynecol* 2017, 2017, 3058569.
22. Yalew, G. T.; Muthupandian, S.; Hagos, K.; Negash, L.; Venkatraman, G.; Hagos, Y. M.; Meles, H. N.; Weldehaweriat, H. H.; Al-Dahmoshi, H. O. M.; Saki, M., Prevalence of bacterial vaginosis and aerobic vaginitis and their associated risk factors among pregnant women from northern Ethiopia: A cross-sectional study. *PLoS One* 2022, 17, (2), e0262692.
23. Tibaldi, C.; Cappello, N.; Latino, M. A.; Polarolo, G.; Masuelli, G.; Cavallo, F.; Benedetto, C., Maternal risk factors for abnormal vaginal flora during pregnancy. *Int J Gynaecol Obstet* 2016, 133, (1), 89-93.
24. Rumyantseva, T. A.; Bellen, G.; Savochkina, Y. A.; Guschin, A. E.; Donders, G. G., Diagnosis of aerobic vaginitis by quantitative real-time PCR. *Arch Gynecol Obstet* 2016, 294, (1), 109-14.
25. Vieira-Baptista, P.; Lima-Silva, J.; Pinto, C.; Saldanha, C.; Beires, J.; Martinez-de-Oliveira, J.; Donders, G., Bacterial vaginosis, aerobic vaginitis, vaginal inflammation and major Pap smear abnormalities. *Eur J Clin Microbiol Infect Dis* 2016, 35, (4), 657-64.
26. Tomusiak, A.; Heczko, P. B.; Janeczko, J.; Adamski, P.; Pilarczyk-Zurek, M.; Strus, M., Bacterial infections of the lower genital tract in fertile and infertile women from the southeastern Poland. *Ginekol Pol* 2013, 84, (5), 352-8.
27. Donders, G. G., The prevalence of bacterial vaginosis and aerobic vaginitis in young Finish women. *Apmis* 2011, 119, (3), 224-5; author reply 226.
28. Geng, N.; Wu, W.; Fan, A.; Han, C.; Wang, C.; Wang, Y.; Xue, F., Analysis of the Risk Factors for Aerobic Vaginitis: A Case-Control Study. *Gynecol Obstet Invest* 2015.
29. Vempati, Y. S.; Sobel, J. D., Desquamative Inflammatory Vaginitis as an Expression of Systemic Lupus Erythematosus. *J Low Genit Tract Dis* 2022, 26, (4), 345-346.
30. Yockey, L.; Dowst, S.; Zonozi, R.; Huizenga, N.; Murphy, P.; Laliberte, K.; Rosenthal, J.; Niles, J. L.; Mitchell, C. M., Inflammatory vaginitis in women on long-term rituximab treatment for autoimmune disorders. *BMC Womens Health* 2021, 21, (1), 285.
31. Mascellino, M. T.; Iona, E.; Iegri, F.; Catania, S.; Trinchieri, V.; Oliva, P.; Amenta, L.; Revérberi, L.; Sorice, F., Evaluation of vaginal microflora in patients infected with HIV. *Microbiologica* 1991, 14, (4), 343-9.
32. Marconi, C.; Donders, G. G.; Martin, L. F.; Ramos, B. R.; Duarte, M. T.; Parada, C. M.; Tristão, A. R.; Silva, M. G., Chlamydial infection in a high risk population: association with vaginal flora patterns. *Arch Gynecol Obstet* 2012, 285, (4), 1013-8.
33. Donders, G.; De Wet, H. G.; Hooft, P.; Desmyter, J., Lactobacilli in Papanicolaou smears, genital infections, and pregnancy. *Am J Perinatol* 1993, 10, (5), 358-61.
34. Jahic, M.; Mulavdic, M.; Hadzimehmedovic, A.; Jahic, E., Association between aerobic vaginitis, bacterial vaginosis and squamous intraepithelial lesion of low grade. *Med Arch* 2013, 67, (2), 94-6.
35. Plisko, O.; Zodzika, J.; Jermakova, I.; Pcolkina, K.; Prusakevica, A.; Liepniece-Karele, I.; Donders, G. G. G.; Rezeberga, D., Aerobic Vaginitis-Underestimated Risk Factor for Cervical Intraepithelial Neoplasia. *Diagnostics (Basel)* 2021, 11, (1).
36. MacPhee, R. A.; Miller, W. L.; Gloor, G. B.; McCormick, J. K.; Hammond, J. A.; Burton, J. P.; Reid, G., Influence of the vaginal microbiota on toxic shock syndrome toxin 1 production by Staphylococcus aureus. *Appl Environ Microbiol* 2013, 79, (6), 1835-42.
37. Donders, G.; Bellen, G.; Rezeberga, D., Aerobic vaginitis in pregnancy. *Bjog* 2011, 118, (10), 1163-70.
38. Rezeberga, D.; Lazdane, G.; Kroica, J.; Sokolova, L.; Donders, G. G., Placental histological inflammation and reproductive tract infections in a low risk pregnant population in Latvia. *Acta Obstet Gynecol Scand* 2008, 87, (3), 360-5.
39. Vedmedovska, N.; Rezeberga, D.; Teibe, U.; Polukarova, S.; Donders, G. G., Fetal growth restriction in Latvia. *Int J Gynaecol Obstet* 2010, 111, (2), 185-6.
40. Yang, S.; Zhang, Y.; Liu, Y.; Wang, J.; Chen, S.; Li, S., Clinical Significance and Characteristic Clinical Differences of Cytolytic Vaginosis in Recurrent Vulvovaginitis. *Gynecol Obstet Invest* 2017, 82, (2), 137-143.
41. Donders, G. G.; Van Calsteren, C.; Bellen, G.; Reybrouck, R.; Van den Bosch, T.; Riphagen, I.; Van Lierde, S., Association between abnormal vaginal flora and cervical length as risk factors for preterm birth. *Ultrasound Obstet Gynecol* 2010.
42. Cauci, S.; Culhane, J. F.; Di Santolo, M.; McCollum, K., Among pregnant women with bacterial vaginosis, the hydrolytic enzymes sialidase and prolidase are positively associated with interleukin-1beta. *Am J Obstet Gynecol* 2008, 198, (1), 132.e1-7.
43. Nyirjesy, P.; Peyton, C.; Weitz, M. V.; Mathew, L.; Culhane, J. F., Causes of chronic vaginitis: analysis of a prospective database of affected women. *Obstet Gynecol* 2006, 108, (5), 1185-91.
44. Donders, G. G.; Vereecken, A.; Dekeersmaecker, A.; Van Bulck, B.; Spitz, B., Wet mount microscopy reflects functional vaginal lactobacillary flora better than Gram stain. *J Clin Pathol* 2000, 53, (4), 308-13.
45. Donders, G.; Greenhouse, P.; Donders, F.; Engel, U.; Paavonen, J.; Mendling, W., Genital Tract GAS Infection ISIDOG Guidelines. *J Clin Med* 2021, 10, (9).
46. Simpson, R. C.; Thomas, K. S.; Leighton, P.; Murphy, R., Diagnostic criteria for erosive lichen planus affecting the vulva: an international electronic-Delphi consensus exercise. *Br J Dermatol* 2013, 169, (2), 337-43.

47. Sobel, J. D.; Reichman, O.; Misra, D.; Yoo, W., Prognosis and treatment of desquamative inflammatory vaginitis. *Obstet Gynecol* 2011, 117, (4), 850-855.
48. Sobel, J. D., Desquamative inflammatory vaginitis: a new subgroup of purulent vaginitis responsive to topical 2% clindamycin therapy. *Am J Obstet Gynecol* 1994, 171, (5), 1215-20.
49. Mendling, W.; Weissenbacher, E. R.; Gerber, S.; Prasauskas, V.; Grob, P., Use of locally delivered dequalinium chloride in the treatment of vaginal infections: a review. *Arch Gynecol Obstet* 2016, 293, (3), 469-84.
50. Tempera, G.; Furneri, P. M., Management of aerobic vaginitis. *Gynecol Obstet Invest* 2010, 70, (4), 244-9.
51. Wang, C.; Han, C.; Geng, N.; Fan, A.; Wang, Y.; Yue, Y.; Zhang, H.; Xue, F., Efficacy of oral moxifloxacin for aerobic vaginitis. *Eur J Clin Microbiol Infect Dis* 2016, 35, (1), 95-101.
52. Borges, S.; Silva, J.; Teixeira, P., The role of lactobacilli and probiotics in maintaining vaginal health. *Arch Gynecol Obstet* 2014, 289, (3), 479-89.
53. Heczko, P. B.; Tomusiak, A.; Adamski, P.; Jakimiuk, A. J.; Stefański, G.; Mikołajczyk-Cichońska, A.; Suda-Szczurek, M.; Strus, M., Supplementation of standard antibiotic therapy with oral probiotics for bacterial vaginosis and aerobic vaginitis: a randomised, double-blind, placebo-controlled trial. *BMC Womens Health* 2015, 15, 115.
54. Rocchetti, T. T.; Marconi, C.; Rall, V. L.; Borges, V. T.; Corrente, J. E.; da Silva, M. G., Group B streptococci colonization in pregnant women: risk factors and evaluation of the vaginal flora. *Arch Gynecol Obstet* 2011, 283, (4), 717-21.
55. Zhou, X.; Brotman, R. M.; Gajer, P.; Abdo, Z.; Schüette, U.; Ma, S.; Ravel, J.; Forney, L. J., Recent advances in understanding the microbiology of the female reproductive tract and the causes of premature birth. *Infect Dis Obstet Gynecol* 2010, 2010, 737425.
56. Romero, R.; Chaiworapongsa, T.; Espinoza, J., Micronutrients and intrauterine infection, preterm birth and the fetal inflammatory response syndrome. *J Nutr* 2003, 133, (5 Suppl 2), 1668s-1673s.
57. Seale, A. C.; Bianchi-Jassir, F.; Russell, N. J.; Kohli-Lynch, M.; Tann, C. J.; Hall, J.; Madrid, L.; Blencowe, H.; Cousens, S.; Baker, C. J.; Bartlett, L.; Cutland, C.; Gravett, M. G.; Heath, P. T.; Ip, M.; Le Doare, K.; Madhi, S. A.; Rubens, C. E.; Saha, S. K.; Schrag, S. J.; Sobanjo-Ter Meulen, A.; Vekemans, J.; Lawn, J. E., Estimates of the Burden of Group B Streptococcal Disease Worldwide for Pregnant Women, Stillbirths, and Children. *Clin Infect Dis* 2017, 65, (suppl_2), S200-s219.
58. Tsolia, M.; Psoma, M.; Gavrili, S.; Petrochilou, V.; Michalas, S.; Legakis, N.; Karpathios, T., Group B streptococcus colonization of Greek pregnant women and neonates: prevalence, risk factors and serotypes. *Clin Microbiol Infect* 2003, 9, (8), 832-8.
59. Vornhagen, J.; Armistead, B.; Santana-Ufret, V.; Gendrin, C.; Merillat, S.; Coleman, M.; Quach, P.; Boldenow, E.; Alishetti, V.; Leonhard-Melief, C.; Ngo, L. Y.; Whidbey, C.; Doran, K. S.; Curtis, C.; Waldorf, K. M. A.; Nance, E.; Rajagopal, L., Group B streptococcus exploits vaginal epithelial exfoliation for ascending infection. *J Clin Invest* 2018, 128, (5), 1985-1999.
60. Shabayek, S.; Spellerberg, B., Group B Streptococcal Colonization, Molecular Characteristics, and Epidemiology. *Front Microbiol* 2018, 9, 437.
61. Lobos, O.; Padilla, C., Phenotypic characterization and genomic DNA polymorphisms of Escherichia coli strains isolated as the sole micro-organism from vaginal infections. *Microbiology (Reading)* 2009, 155, (Pt 3), 825-830.
62. Top, K. A.; Buet, A.; Whittier, S.; Ratner, A. J.; Saiman, L., Predictors of Staphylococcus aureus Rectovaginal Colonization in Pregnant Women and Risk for Maternal and Neonatal Infections. *J Pediatric Infect Dis Soc* 2012, 1, (1), 7-15.
63. Bourgeois-Nicolaos, N.; Lucet, J. C.; Daubié, C.; Benchaba, F.; Rajguru, M.; Ruimy, R.; Andremont, A.; Armand-Lefèvre, L., Maternal vaginal colonisation by Staphylococcus aureus and newborn acquisition at delivery. *Paediatr Perinat Epidemiol* 2010, 24, (5), 488-91.
64. Grass, B.; Leone, A., Severe complications in preterm infant with late-onset Staphylococcus aureus sepsis. *Swiss Soc. Neonatol* 2013.
65. Lazenby, G. B.; Soper, D. E.; Beardsley, W.; Salgado, C. D., Methicillin-resistant Staphylococcus aureus colonization among women admitted for preterm delivery. *Am J Obstet Gynecol* 2012, 206, (4), 329.e1-5.
66. Larsson, P. G.; Fåhraeus, L.; Carlsson, B.; Jakobsson, T.; Forsum, U., Late miscarriage and preterm birth after treatment with clindamycin: a randomised consent design study according to Zelen. *Bjog* 2006, 113, (6), 629-37.
67. Subramaniam, A.; Abramovici, A.; Andrews, W. W.; Tita, A. T., Antimicrobials for preterm birth prevention: an overview. *Infect Dis Obstet Gynecol* 2012, 2012, 157159.
68. Schmitz, T.; Sentilhes, L.; Lorthe, E.; Gallot, D.; Madar, H.; Doret-Dion, M.; Beucher, G.; Charlier, C.; Cazanave, C.; Delorme, P.; Garabédian, C.; Azria, E.; Tessier, V.; Sénat, M. V.; Kayem, G., Preterm premature rupture of the membranes: Guidelines for clinical practice from the French College of Gynaecologists and Obstetricians (CNGOF). *Eur J Obstet Gynecol Reprod Biol* 2019, 236, 1-6.

69. Ugwumadu, A.; Manyonda, I.; Reid, F.; Hay, P., Effect of early oral clindamycin on late miscarriage and preterm delivery in asymptomatic women with abnormal vaginal flora and bacterial vaginosis: a randomised controlled trial. *Lancet* 2003, 361, (9362), 983-8.
70. Kiss, H.; Petricevic, L.; Husslein, P., Prospective randomised controlled trial of an infection screening programme to reduce the rate of preterm delivery. *Bmj* 2004, 329, (7462), 371.
71. Workowski, K. A.; Bachmann, L. H.; Chan, P. A.; Johnston, C. M.; Muzny, C. A.; Park, I.; Reno, H.; Zenilman, J. M.; Bolan, G. A., Sexually Transmitted Infections Treatment Guidelines, 2021. *MMWR Recomm Rep* 2021, 70, (4), 1-187.
72. Lamont, R. F.; Nhan-Chang, C. L.; Sobel, J. D.; Workowski, K.; Conde-Agudelo, A.; Romero, R., Treatment of abnormal vaginal flora in early pregnancy with clindamycin for the prevention of spontaneous preterm birth: a systematic review and metaanalysis. *Am J Obstet Gynecol* 2011, 205, (3), 177-90.
73. Drugs and Lactation Database (LactMed) [Internet]. Bethesda (MD): National Library of Medicine (US); 2006-. Clindamycin. [Updated 2021 Feb 15]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK501208/. 2006.
74. Von Keutz, E.; Rühl-Fehlert, C.; Drommer, W.; Rosenbruch, M., Effects of ciprofloxacin on joint cartilage in immature dogs immediately after dosing and after a 5-month treatment-free period. *Arch Toxicol* 2004, 78, (7), 418-24.
75. Wang, Z.; Liou, L., Auditory effect of kanamycin given to newborn guinea pigs whose mothers received kanamycin during pregnancy. *Ann Otol Rhinol Laryngol* 1994, 103, (12), 983-5.
76. Russo, R.; Edu, A.; De Seta, F., Study on the effects of an oral lactobacilli and lactoferrin complex in women with intermediate vaginal microbiota. *Arch Gynecol Obstet* 2018, 298, (1), 139-145.
77. Samuel, T. M.; Sakwinska, O.; Makinen, K.; Burdge, G. C.; Godfrey, K. M.; Silva-Zolezzi, I., Preterm Birth: A Narrative Review of the Current Evidence on Nutritional and Bioactive Solutions for Risk Reduction. Nutrients 2019, 11, (8).
78. Othman, M.; Neilson, J. P.; Alfirevic, Z., Probiotics for preventing preterm labour. *Cochrane Database Syst Rev* 2007, (1), Cd005941.
79. Fatahi Dehpahni, M.; Chehri, K.; Azadbakht, M., Therapeutic effects of silver nanoparticle and L-carnitine on aerobic vaginitis in mice: an experimental study. *Bioimpacts* 2022, 12, (1), 33-42.
80. Dong, M.; Wang, C.; Li, H.; Yan, Y.; Ma, X.; Li, H.; Li, X.; Wang, H.; Zhang, Y.; Qi, W.; Meng, K.; Tian, W.; Wang, Y.; Fan, A.; Han, C.; Donders, G. G. G.; Xue, F., Aerobic Vaginitis Diagnosis Criteria Combining Gram Stain with Clinical Features: An Establishment and Prospective Validation Study. *Diagnostics (Basel)* 2022, 12, (1).

VULVOVAGINAL ATROPHY

8

(alphabetical order)
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Isabel do Val

8.1 Introduction

The reduction of estrogen production associated with menopause leads to genital and systemic changes. One of the most common and more uncomfortable consequences of menopause is vulvovaginal atrophy (VVA).[1] Several other terms are used to refer to this condition, including atrophic vaginitis, urogenital atrophy, urogenital syndrome, and genitourinary syndrome of menopause (GSM).[2] The term atrophic vaginitis may be used when inflammation is present, along with atrophy.[3] While hot flashes usually subside with time, VVA often persists and might worsen if left untreated.[4]

8.2 Etiology and physiopathology

The vaginal wall has estrogen, progesterone, and androgen receptors. During the reproductive years, the female genital tract maintains its trophism under the stimulation of estrogens and progesterone. The estrogen receptor density is higher in the vagina and lower in the external genitalia. Progesterone receptors are found in the vagina and the transitional epithelium of the vulvovaginal junction. Androgens also play a significant role in lower genital tract trophism. The density of androgen receptors is low in the vagina and higher in the external genitalia.[5-11] With the decline of ovarian function after menopause, the entire genital tract becomes atrophic.

The vaginal microbiome (VMB) varies throughout a woman's life. Levels of sex hormones, glycogen content in the vaginal epithelium, menstrual cycle, vaginal pH, intercourse, and immune responses influence these changes. *Lactobacillus* spp. dominance in the vaginal niche is generally driven by the availability of glycogen, which accumulates in an estrogen-dependent manner in the cervicovaginal environment.[12-14] The VMB has been primarily studied in reproductive-age women. While 20 species of lactobacilli have been found in the vagina, it is usually dominated by a single species, more often *L. crispatus or L. iners*.[14, 15]

During the reproductive years, the "normal" pH is usually lower than 4.5 in White and Asian women, and slightly higher in Black and Hispanic women.[16] Lactobacilli in the vagina play an important protective role, counteracting the overgrowth of other microorganisms which may compete for nutrients and tissue adherence. This function is accomplished by modulating the local immune system, reducing the vaginal pH, producing organic acids (mainly lactic acid), and antimicrobial substances, such as bacteriocins. The glycogen content of the vaginal epithelium alters with the estrogen levels and, in general, high estradiol levels favor a lactobacilli-dominant environment.[14, 15]

Circulating estrogen decreases drastically in menopause, leading to a reduction of *Lactobacillus* spp. dominance and a concomitant increase in the diversity of species. Despite some contradictory findings, some studies show that moderate to severe atrophy and dryness can be associated with community state type IV-A (diversity group, not dominated by *Fannyhessea [Atopobium]* spp. and/or *Gardnerella* spp.), while the state type IV-B (corresponding to bacterial vaginosis) is less symptomatic.[14, 15]

Hypoestrogenism induces a decline in the vaginal epithelium glycogen level, which is the substrate for *Lactobacillus* spp. The resulting depletion of lactobacilli leads to a pH increase, which is typical of VVA. Nevertheless, some menopausal women still have a strong presence of lactobacilli in their VMB.[15] The connection between the vaginal microbiota and estrogens demonstrates the importance of its use to prevent or treat VVA. In postmenopausal women with VVA, vaginal or oral low dose estrogen therapy effectively increases the level of *Lactobacillus* spp., decreases *Gardnerella* spp. and vaginal pH, and also leads to a significant improvement in the Vaginal Maturation Index (VMI).[17] The transition from a lactobacilli dominated to a non-dominated VMB is neither abrupt nor time predictable.

Hypoestrogenism affects the normal structure and function of the genital tissues, largely contributing to the loss of mucosal elasticity, and inducing the fusion and hyalinization of collagen fibers, and the fragmentation of elastin fibers. Estrogen receptor (ER)-α is present in the vaginal tissues of both pre and postmenopausal women, whereas ER-β appears to have no or low expression in postmenopausal vaginal tissue.

There is a decrease in the hydration of the vaginal mucosa in the dermal layer, with a reduction of mucopolysaccharides and intercellular hyaluronic acid, which generates a thin stratified epithelium with only the basal and parabasal layers.[18]

8.3
Prevalence and epidemiology

The self-reported prevalence of symptoms of VVA ranges from 4% in the early postmenopausal years to 50% among late postmenopausal women (>10 years of menopause).[19, 20]

In addition to menopause, VVA can be a physiological finding during breastfeeding due to the transient but significant hypoestrogenism seen during that period. Non-physiological conditions such as immunological disorders, premature ovarian failure, oophorectomy, radiotherapy, and chemotherapy can also cause VVA. Additionally, some endocrine treatments, such as tamoxifen, aromatase inhibitors, progestins, and gonadotropin-releasing hormone analogs can induce symptoms of VVA.[1, 21]

8.4
Complications

The symptoms of VVA impact the quality-of-life, sexual function, social or mental health (anxiety and/or depressive symptoms, isolation, etc.). Loss of estrogen predisposes to urinary symptoms such as urgency, dysuria, and nocturia, in addition to recurrent urinary tract infections. Estrogens play an important role in urinary continence through several mechanisms, including its effect on the vessels of the periurethral region, on the striated and smooth muscles and on the pelvic connective tissue, thus, hypoestrogenism can potentiate stress urinary incontinence.[1]

There may be progressive loss of elasticity, thinning of the vaginal walls, shortening of the vaginal barrel and disappearance of the mucosal rugae. Consequently, the mucosa may become friable and easily damaged, leading to petechiae, dyspareunia and bleeding upon contact, creating more sexual difficulties.[1] (Figure 8.1)

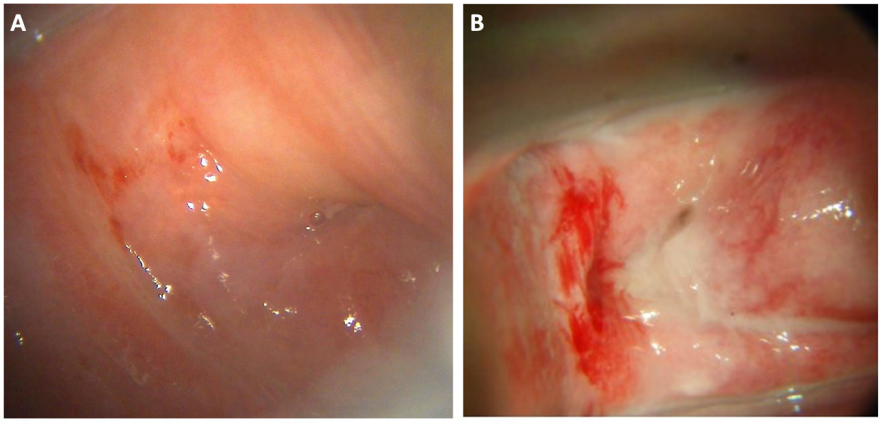

Figure 8.1 A and B– Colposcopic aspect of the vagina of a postmenopausal woman. Loss of vaginal rugae, petechiae, and easy bleeding.

Sexual dysfunction can be potentiated by other conditions that are prevalent during the postmenopausal years, such as: depressive symptoms, trauma, decreased mobility, previous hysterectomy, hot flashes, sleep disorders, use of multiple drugs, overweight, and chronic diseases (including metabolic syndrome).[1]

8.5
Signs and symptoms

The most frequent symptoms are vaginal dryness, burning, pain, itching, and vulvar irritation. Upon clinical examination, signs of vaginal inflammation with hyperemia in addition to yellowish discharge may be present. These may be associated with sexual discomfort, including dyspareunia or post-coital bleeding.[20] As the urethra and the bladder trigone are estrogen-dependent tissues, its deficiency in postmenopausal women can contribute to urinary incontinence, urgency, and recurrent urinary tract infections.[1]

8.6
Diagnosis

The diagnosis of VVA is based on symptoms, complemented with clinical examination. The provider must rule out possible clinical conditions that are part of the differential diagnosis, such as aerobic vaginitis/desquamative inflammatory vaginitis, trichomoniasis, and dermatosis (lichen sclerosus, erosive lichen planus, lichen simplex chronicus, etc.).[1] (Table 8.1)

TABLE 8.1 Differential diagnosis of genital diseases or conditions during the identification of atrophic vaginitis.
Adapted from Pérez-López *et al*.[1]

Disease or condition	Clinical characteristics
Vaginal atrophy	Associated with hypoestrogenism states; thin and fragile vaginal epithelium, but inflammation absent.
Atrophic vaginitis	Term used when inflammation is present, along with atrophy.
Desquamative inflammatory vaginitis	A syndrome that is frequently unrecognized, characterized by vaginal enanthema, pethechiae and purulent discharge (see chapter 7).
Trichomoniasis	A sexually transmitted infection caused by the protozoan *Trichomonas vaginalis* (see chapter 5).
Erosive lichen planus	Inflamed painful red plaques or erosions that can affect the skin, nails, and mucous membranes, including the genital area.

Clinical criteria of VVA are: vaginal dryness, itching or irritation, and dyspareunia; the vulvar examination may show atrophy of the *labia minora*, pubic hair scarcity, reduction of the volume of the *labia majora*, retraction of the vestibule and the presence of an urethral caruncle. The vagina is usually pale, dry and smooth, with loss of rugae. (Figure 8.1)

In some cases, it may however, be shiny and a purulent discharge may be present. In the presence of signs of inflammation, it may be classified as atrophic vaginitis. VMI (Figure 8.2) is not usually required in clinical practice but can be a simple way of documenting clinical findings and their course.[1]

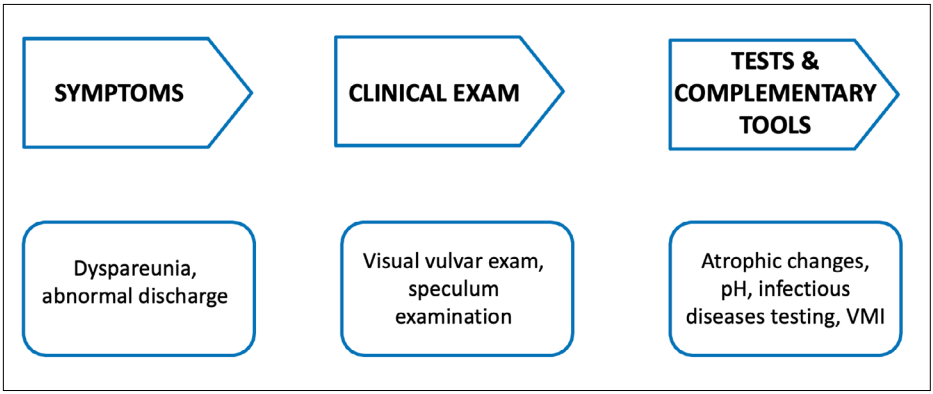

Figure 8.2 Flowchart for the clinical assessment in suspected vaginal atrophy/atrophic vaginitis. Adapted from Pérez-López *et al*. 2021.[1]

VMI– Vaginal maturation index

Wet mount microscopy allows immediate assessment of the hormonal status of the vagina.[3] Vaginal atrophy is characterized by is an increase in parabasal cells and a decrease in superficial cells.[3] Sometimes, abundant leukocytes and the presence of bacteria other than *Lactobacillus* morphotypes can be found, resembling desquamative inflammatory vaginitis. (Figure 8.3 and 8.4)

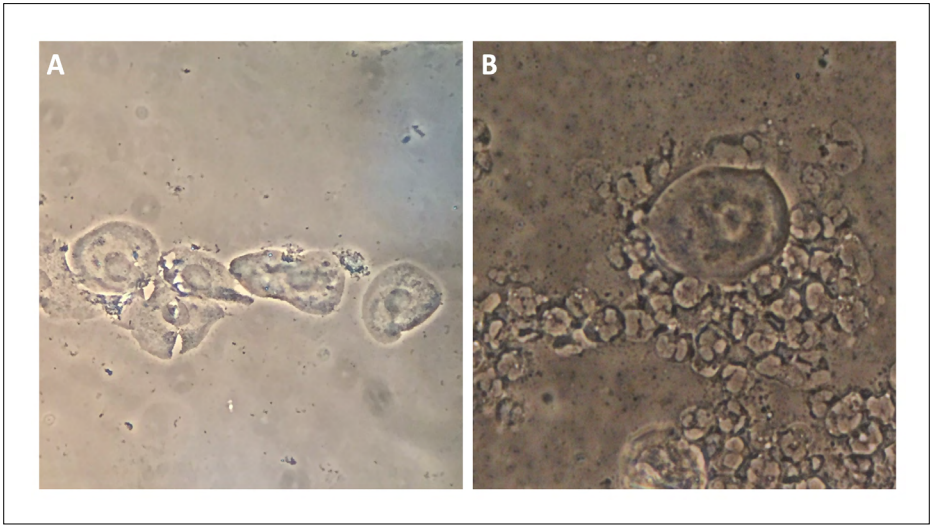

Figure 8.3 Wet mount microscopy (400x, phase contrast).

A– Vaginal atrophy B– Atrophic vaginitis

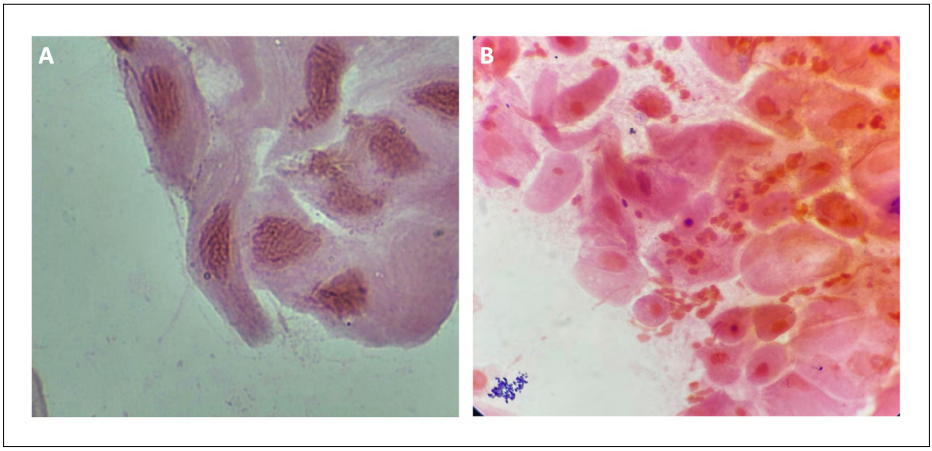

Figure 8.4 Gram stain (1000x, oil immersion), vaginal atrophy.

A– Vaginal atrophy B– Atrophic vaginitis

A vaginal pH >5.0 in the absence of other causes, such as infection or semen, is considered an indicator of vaginal atrophy.[3]

8.7 Treatment

VVA can be treated with hormonal and non-hormonal therapies. Non-hormonal treatment recommendations include vaginal lubricants and moisturizers, and continued sexual activity should be encouraged.[22] In this section both hormonal and non-hormonal therapies will be discussed.[10] (Table 8.2)

TABLE 8.2 Recommendations for the management of vulvovaginal atrophy. Adapted from Pérez-López *et al*. 2021.[10]
DHEA dehydroepiandrosterone, VMI vaginal maturation index, VVA vulvovaginal atrophy

Treatment	Recommendation
1. Low dose and ultralow dose vaginal estrogens	Estradiol, conjugated equine estrogens, estriol, and promestriene are effective for VVA, and without risk of endometrial or systemic effects.
2. Vaginal prasterone	Intravaginal prasterone reduces vaginal pH, improves VMI, and decreases dyspareunia. Circulating levels of DHEA and its metabolites (testosterone and estradiol) remain in the postmenopausal range in up to 52 weeks of use.
3. Systemic estrogens	Should not be used for the sole purpose of treating vaginal atrophy; it is an option to consider in women who also have vasomotor symptoms. Not always effective in treating VVA.
4. Vaginal testosterone	Topical testosterone reduces vaginal pH and improves VMI and the number of lactobacilli. Longer and larger studies are needed to assess safety and efficacy.
5. Lubricants and moisturizers	Lubricants and moisturizers are appropriate for those women that cannot use or do not want to receive hormone treatments.
6. Vaginal LASER	CO_2 and erbium laser treatments have been reported in women with VVA, although there is no clear evidence of the benefits as compared to hormone treatments. Currently, the ISSVD does not endorse the use of these technologies out of the setting of clinical trials.
7. Radiofrequency	Intravaginal microablative radiofrequency has been suggested as a possible alternative treatment for VVA, but data are scarce. Currently, the ISSVD does not endorse the use of these technologies out of the setting of clinical trials.

Vaginal lubricants and moisturizers

While less effective than hormonal treatments, some women and healthcare providers prefer non-hormonal therapy as the first therapeutic approach to relieve the symptoms of VVA.[23] Non-hormonal approaches are particularly beneficial in women with contraindications to the use of hormones, or for those who prefer not to use them.[24]

Lubricants can be used before intercourse to reduce friction and discomfort during penetration in sexual activity. They can be water-based, silicone, mineral oil, or herbal products applied to the vagina and vulva and/or to the partner's genitals. However, these products are not effective in the treatment of the underlying causes of VVA.[25]

Moisturizers adhere to the vaginal mucosa, promoting rehydration and mimicking normal

lubrication. These products improve the integrity, elasticity, and flexibility of the tissue. They must be used regularly (from daily to every three days). Moisturizers contain water and other substances such as hyaluronic acid or polycarbophil.[23, 26] Hyaluronic acid is a polymer found in cartilage and other soft tissues in the body. In randomized clinical trials (RCTs) comparing hyaluronic acid to placebo or vaginal estrogens, all were associated with a decrease in the severity of dryness and dyspareunia (probably because the placebo had a lubricant effect). To date, there is no evidence that products with hyaluronic acid have a greater benefit than nonhyaluronic acid moisturizers.[5] Studies with the use of moisturizers show improvement in vaginal dryness and sexual function, as well as an improvement in the vaginal epithelium maturation. Despite some mild irritation associated with its use, no serious adverse events have been reported.[26, 27]

Estrogen (systemic and vaginal) and selective estrogen receptor modulator therapy

Vaginal estrogens are effective for the management of VVA. Several low-dose formulations are available: creams, pessaries, tablets, and vaginal rings. Available active ingredient options include promestriene, estradiol, conjugated estrogens, and estriol.[19, 21] The absorption is variable depending on the degree of VVA, but plasma estrogen levels do not exceed the normal postmenopausal range.[2, 19, 28] Topical vaginal estrogens should be started with a nightly application for two to three weeks and are later reduced to two to three times a week, depending on the degree of atrophy. Women should be warned about a potential burning sensation during the first weeks and that maximum effect may take up to eight weeks to be reached.

Promestriene (3-propyl 17β -methyl diether estradiol) is a synthetic estrogen that is used vaginally in a 1% cream formulation, which appears to have intramucosal effects only and has been tested on women with gynecologic cancer. However, despite the promising results, larger and longer studies in relation to long-term safety are lacking.[29, 30]

The ultra-low-dose concentration of estriol formulations (vaginal gel containing 50 µg/gram of estriol or 30 µg associated with *L. crispatus*, formulated in vaginal pills) significantly improve both the VMI and pH when compared to placebo after 12 weeks.[31, 32] The same findings were confirmed in a double-blind randomized clinical trial comparing the use of 200 µg and 30 µg estriol pessaries: at 12 weeks, VMI and pH similarly and significantly improved. Adverse events were rare and similar among all the groups.[33]

A Cochrane systematic review evaluated randomized controlled trials comparing vaginal estrogens *vs.* placebo over 12 weeks for the treatment of VVA. The authors concluded that there were no substantial differences in the effects of the different options. However, endometrial thickness was increased in women who received estrogen cream compared to those who wore rings — likely due to exposure to a higher dose in the former. There were no differences in this aspect between users of pills or creams.[34]

Biehl *et al*. published a systematic review of 53 RCTs reporting on the efficacy and safety of different vaginal estrogens used for GSM. Compared to placebo, all vaginal estrogens, regardless of dosages and formulations, were superior in objective and subjective outcomes.

They also showed superiority over lubricants and moisturizers for improving objective, but not subjective, clinical outcomes. Doses as low as 4 µg have been shown to be effective. In a review of studies of one year of treatment with vaginal estrogen, the complication rate was overall low: vulvovaginal mycosis (0.73%), vaginal bleeding (0.75%), endometrial hyperplasia (0.06%), and there was one case of endometrial cancer (out of more than 4,500 women).[35] Another systematic review of 20 RCTs on the use of vaginal estrogen alone for 12 to 52 weeks in postmenopausal women showed that the rate of endometrial cancer and hyperplasia was 0.03 and 0.4%, respectively.[36] Finally, treatment with vaginal estrogen in women not exposed to menopausal systemic hormone therapy for more than 18 years has shown that the risk of cardiovascular disease, cancer, and hip fracture is similar to that of non-vaginal estrogen users.[37]

Systemic estrogen therapies are also available for patients with vasomotor symptoms. However, risks and benefits should be discussed. Systemic estrogen therapy should be used together with progestogens for women with an intact uterus, or alone after hysterectomy.[38] This option can be tried in women suffering from vaginal atrophy and concomitant vasomotor symptoms.[39] However, for some women, systemic hormone therapy is insufficient and further require local therapy. The Women's Health Initiative found that 74% of patients reported improvement after one year of systemic hormone therapy.[40] The fact that up to 1/4 of women using systemic hormonal therapy continue to experience symptoms of urogenital atrophy is sufficient reason to justify not recommending systemic hormonal therapy in women with vaginal symptoms only; many women initially require a combination of systemic and local estrogen therapy, especially when it is used at low doses.[41, 42]

Ospemifene is an oral tissue-selective estrogen receptor modulator (SERM).[43] It has antagonistic-antiestrogenic effects on the breast.[44] Since the ER-beta is significantly reduced in postmenopausal women, ospemifene seems to act on the ER-alpha.[6] It does not increase the risk of endometrial hyperplasia or thrombosis but improves bone density.[44] Studies show that ospemifene improves the VMI, vaginal pH, and decreases vaginal dryness, as well as dyspareunia.[44] Being an oral drug, it avoids local discomfort related to excipients of vaginal drug delivery systems and can be considered in women with a history of breast cancer.[45]

Vaginal androgen (testosterone) therapy

Intravaginal testosterone has been studied in short-term interventions (4-12 weeks). Systemic absorption of a single intravaginal dose of 2 mg in a double-blind, placebo-controlled study in premenopausal women resulted in supraphysiological serum testosterone levels, while there were no changes in estradiol.[46] In a randomized study of women between 40-70 years of age comparing vaginal treatment with conjugated estrogen, testosterone, or placebo (glycerin lubricant), applied three times a week for 12 weeks, it was shown that hormone treatments reduced the pH to <5 and increased the VMI as well as the number of lactobacilli. In addition, there was no significant difference in serum hormone levels between hormone treatments and placebo; there was also no difference in endometrial thickness among the groups.[47]

However, longer and better studies to evaluate safety and efficacy are needed before the use of vaginal testosterone can be recommended.[10, 48]

Vaginal dehydroepiandrosterone (prasterone) therapy

Dehydroepiandrosterone (DHEA; prasterone) is converted to estradiol and testosterone in the vaginal epithelium. It is an alternative to estrogens, which is administered vaginally at a daily dose of 6.5 mg with no reported risks of cancer, although there are no long-term studies. Compared to placebo, vaginal prasterone for 12 weeks was associated with improvement in dyspareunia, pH, and vaginal maturation, and there were no endometrial changes.[49] Vaginal dryness and discharge, vaginal epithelium thickness, and color improved while circulating steroid levels remained within the normal range for postmenopausal women.[50] Despite the paucity of data, it can be considered in women with an history of breast cancer.[51]

Vaginal LASER

Physical methods such as LASER in non-ablative, ablative, and microablative forms have been used for skin "rejuvenation" on the face, neck, and body. Fractional LASER is also used in the vaginal mucosa, allegedly promoting neocollagenesis and neoelastogenesis.[52-54] LASER purportedly induces morphological changes in vaginal tissue, leading to relief from vaginal dryness and dyspareunia.[24] The application of microablative fractional LASER has generated controversial opinions due to being based on poor studies and because of the use outside of the released or approved intended uses.[55]

The two main types of LASERS available are the microablative fractional carbon dioxide (CO_2) LASER and the non-ablative vaginal erbium:YAG LASER. Regarding the CO_2 LASER, it is hypothesized that the thermal energy deposited in the vaginal wall stimulates neovascularization, promotes collagen synthesis, and improves natural lubrication and leads to a significant improvement in vaginal health.[50, 52] Cruz *et al*. compared three arms: fractional CO_2 LASER, topical estriol, and CO_2 LASER with estriol for 20 weeks. The combined LASER and estrogen treatment showed the most significant change in the Vaginal Health Index (VHI), and both the LASER treatment alone arm and the combined treatment demonstrated significant improvement in dyspareunia, burning, and dryness when compared to the estrogen group. Importantly, in the LASER treatment alone arm there was an increase in pain. However, this study had some limitations, including that it was designed to detect differences only in the VHI and not in the other parameters.[56]

In 2021, a RCT comparing the effect of fractional CO_2 LASER versus sham treatment on vaginal symptom severity was conducted. Of the 85 randomized participants (mean age, 57 years), 78 (91.7%) completed the 12-month follow-up. From baseline to 12 months, there was no significant difference between the CO_2 LASER and the sham treatment groups concerning symptom´s severity, quality of life score, VHI or histology. There were 16 adverse events in the LASER group and 17 in the sham group, including vaginal pain/discomfort, spotting, discharge, and lower urinary tract symptoms. No severe adverse events were reported in either group.[57]

A recent study, on an ewe model, showed that CO_2 LASER effect in histological terms was similar to that of sham treatment, contrarily to what was noticed in the estrogen arm.[58] The same group, in a well-designed RCT, showed that there was similar improvement in terms of the most bothersome symptom in the LASER and placebo arm, highlighting the significant placebo effect, as well as that of mechanical manipulation.[59]

The recently available data, combined with the high placebo effect expected in treatments to improve sexual function, sustains the recommendation issued by the International Society for the Study of Vulvovaginal Disease (ISSVD) in 2019, that vaginal LASERs should not be used out of the setting of clinical trials.[55, 60]

Vaginal radiofrequency

Radiofrequency is performed by cutting and/or coagulating biological tissues, using a high-frequency alternating current, which instantly raises the cell temperature up to 100°C, leading to the expansion and rupture of the cell membrane. Observational studies have shown an apparent change in pH, increase in lactobacilli, VMI and VHI. These studies suffered from several limitations including the small number of enrolled participants, the lack of a control arm, and the short follow-up.[53, 61]

The available data are insufficient to demonstrate efficacy and safety as an alternative to hormonal treatments.[55, 62] Similar to LASERs, there is lack of studies including objectives and standardized measurable results, as well as follow-up of short- and long-term adverse effects.[63] Currently, the ISSVD does not endorse the use of these technologies outside the clinical trial setting.[55]

Pelvic floor rehabilitation

Pelvic floor physiotherapy with muscle training significantly reduces VVA in postmenopausal women. Mercier *et al*. showed that a 12-week program, oriented and monitored by physical therapists increases vaginal wall lubrication, thickens the vaginal epithelial surface, and improves the vaginal mucosa color.[64] Pelvic floor rehabilitation has also been used combined with intravaginal estriol for six months and compared to an estriol arm only.[65] This approach has also been examined along with the addition of *L. acidophilus* and showed that triple therapy (*L. acidophilus*, estriol and pelvic floor rehabilitation) was effective and could be considered as first-line treatment for symptoms of urogenital aging in postmenopausal women.[66]

8.8
Special situations (postpartum/breastfeeding, breast cancer)

In transient postpartum/breastfeeding situations due to the increase in prolactin, and the consequent blockage of the hypothalamic-pituitary-ovarian hormonal axis, it is not uncommon for women to experience transient hypoestrogenism and VVA. The issue should be addressed with women who, if symptomatic, may opt to be treated with a similar approach to postmenopausal women.

Women diagnosed with breast cancer may experience early menopause or worsening symptoms if already post-menopausal, due to chemotherapy, radiotherapy, and/or endocrine treatments. In breast cancer survivors, estrogens are usually avoided as they may pose a theoretical risk of cancer recurrence, possible interference with tamoxifen or aromatase

inhibitors, or fear of a patient lawsuit against the physician.[67] Moisturizers and lubricants are first-line therapy. The data on the safety of the use of vaginal estrogens in women treated with aromatase inhibitors is contradictory.[68, 69] Due to tamoxifen's receptor-blocking action, the use of vaginal estrogen therapies may be safer than in women treated with aromatase inhibitors.[10]

Some studies have addressed the safety of using ultra-low doses of vaginal estriol in women with a history of breast cancer, showing that despite an initial transient elevation, the systemic levels remain within the normal post-menopausal range.[70] A meta-analysis reported the safety of vaginal estrogen application in women with breast cancer receiving aromatase inhibitors.[71] There were no changes in serum LH and estradiol levels, while FSH almost doubled compared to baseline levels. Therefore, it can be assumed that vaginal estrogens are not significantly absorbed, which is indirect evidence of safety. Of note, the efficacy of vaginal estrogens in women receiving aromatase inhibitors is not confirmed in all studies.[72] In another study it was shown that the estradiol ring (7.5 µg/d) was effective when compared to testosterone.[73] Caution should be exercised when prescribing hormone treatments in patients with hormone-dependent cancer, as transient elevations of estradiol have been reported in women with breast cancer on aromatase inhibitors who received vaginal estradiol or testosterone.[74, 75]

Prasterone has been studied as a treatment for GSM in cancer survivors; the limited data available have demonstrated improved vaginal symptoms at 12 weeks.[74]
In general, longer and larger studies are needed to assess safety and efficacy of vaginal hormone treatments in women with a history of breast cancer.

8.9
Future perspectives

The therapeutic management of VVA should follow a sequential order, taking into consideration the woman's age, preferences, symptoms, and general health status, as well as previous treatments. Systemic hormone therapy should only be used to treat VVA in women with other menopausal symptoms and without contraindications. Lifestyle, comorbidities, and chronic diseases can also influence the choice of treatment. Vaginal options that produce benefits for VVA include lubricants and moisturizers, estrogens (estradiol, estriol, promestriene), or prasterone. Although LASER and radiofrequency procedures are currently used, the ISSVD does not currently endorse their use outside the clinical trial setting, due to the lack of evidence on safety and efficacy.[55]

There are significant limitations in publications on VVA and related issues, including heterogeneity of outcomes, with the available evidence being based on short-term interventions and small samples. Another relevant issue is that the ages of the population studied correspond to young postmenopausal women, and VVA is a progressive phenomenon requiring specific information related to treatments in women over 65 years of age. In addition, sexual needs and practices change with age, and partner capacities should also be considered in future studies.

Recommendations

Recommendation	Quality of evidence	Strength of recommendation
The diagnosis of vaginal atrophy is clinical.	5	D
Wet mount microscopy can be used to confirm the diagnosis of vaginal atrophy.	3b	C
A pH>5 in the absence of semen, infection or use of vaginal medication is suggestive of vaginal atrophy.	2b	B
Vaginal lubricants and moisturizers are particularly beneficial in women with contraindications to the use of hormones, or for those who prefer not to use them.	2a	B
Vaginal lubricants and moisturizers are the first line choice for vaginal atrophy in women with breast cancer.	5	D
Topical vaginal estrogens should be started with a nightly application for 2 to 3 weeks and are later reduced to 2 to 3 times a week.	2b	B
Systemic estrogens can be used in women without contraindication and who have vaginal atrophy and vasomotor symptoms.	2a	B
Ultralow dose vaginal estriol may be safe in women with breast cancer who are taking tamoxifen.	3a	C
Ospemifen may be an option in women who prefer an oral option.	5	D
Ospemifen can be considered in women with a history of breast cancer.	4	C
The data on vaginal testosterone is too limited to allow recommending it to treat vaginal atrophy.	4	C
Prasterone can be used to treat vaginal atrophy.	2a	B
The available data do not allow recommending LASER for the treatment of vaginal atrophy.	2a	B
The available data do not allow recommending radiofrequency for the treatment of vaginal atrophy.	2b	B
Pelvic floor physiotherapy with muscle training can be recommended for vaginal atrophy.	4	C

References

1. Pérez-López, F. R.; Vieira-Baptista, P.; Phillips, N.; Cohen-Sacher, B.; Fialho, S.; Stockdale, C. K., Clinical manifestations and evaluation of postmenopausal vulvovaginal atrophy. *Gynecol Endocrinol* 2021, 37, (8), 740-745.
2. Portman, D. J.; Gass, M. L., Genitourinary syndrome of menopause: new terminology for vulvovaginal atrophy from the International Society for the Study of Women's Sexual Health and the North American Menopause Society. *Menopause* 2014, 21, (10), 1063-8.
3. Vieira-Baptista, P.; Grincevičienė, Š.; Oliveira, C.; Fonseca-Moutinho, J.; Cherey, F.; Stockdale, C. K., The International Society for the Study of Vulvovaginal Disease Vaginal Wet Mount Microscopy Guidelines: How to Perform, Applications, and Interpretation. *J Low Genit Tract Dis* 2021, 25, (2), 172-180.
4. Palma, F.; Volpe, A.; Villa, P.; Cagnacci, A., Vaginal atrophy of women in postmenopause. Results from a multicentric observational study: The AGATA study. *Maturitas* 2016, 83, 40-4.

5. The 2020 genitourinary syndrome of menopause position statement of The North American Menopause Society. *Menopause* 2020, 27, (9), 976-992.
6. Gebhart, J. B.; Rickard, D. J.; Barrett, T. J.; Lesnick, T. G.; Webb, M. J.; Podratz, K. C.; Spelsberg, T. C., Expression of estrogen receptor isoforms alpha and beta messenger RNA in vaginal tissue of premenopausal and postmenopausal women. *Am J Obstet Gynecol* 2001, 185, (6), 1325-30; discussion 1330-1.
7. Labrie, F.; Archer, D. F.; Martel, C.; Vaillancourt, M.; Montesino, M., Combined data of intravaginal prasterone against vulvovaginal atrophy of menopause. *Menopause* 2017, 24, (11), 1246-1256.
8. Traish, A. M.; Vignozzi, L.; Simon, J. A.; Goldstein, I.; Kim, N. N., Role of Androgens in Female Genitourinary Tissue Structure and Function: Implications in the Genitourinary Syndrome of Menopause. *Sex Med Rev* 2018, 6, (4), 558-571.
9. Maseroli, E.; Vignozzi, L., Testosterone and Vaginal Function. *Sex Med Rev* 2020, 8, (3), 379-392.
10. Pérez-López, F. R.; Phillips, N.; Vieira-Baptista, P.; Cohen-Sacher, B.; Fialho, S.; Stockdale, C. K., Management of postmenopausal vulvovaginal atrophy: recommendations of the International Society for the Study of Vulvovaginal Disease. *Gynecol Endocrinol* 2021, 37, (8), 746-752.
11. Bertin, J.; Dury, A. Y.; Ouellet, J.; Pelletier, G.; Labrie, F., Localization of the androgen-synthesizing enzymes, androgen receptor, and sex steroids in the vagina: possible implications for the treatment of postmenopausal sexual dysfunction. *J Sex Med* 2014, 11, (8), 1949-61.
12. Verstraelen, H.; Vervaet, C.; Remon, J. P., Rationale and Safety Assessment of a Novel Intravaginal Drug-Delivery System with Sustained DL-Lactic Acid Release, Intended for Long-Term Protection of the Vaginal Microbiome. *PLoS One* 2016, 11, (4), e0153441.
13. Mirmonsef, P.; Hotton, A. L.; Gilbert, D.; Gioia, C. J.; Maric, D.; Hope, T. J.; Landay, A. L.; Spear, G. T., Glycogen Levels in Undiluted Genital Fluid and Their Relationship to Vaginal pH, Estrogen, and Progesterone. *PLoS One* 2016, 11, (4), e0153553.
14. Verstraelen, H.; Vieira-Baptista, P.; De Seta, F.; Ventolini, G.; Lonnee-Hoffmann, R.; Lev-Sagie, A., The Vaginal Microbiome: I. Research Development, Lexicon, Defining "Normal" and the Dynamics Throughout Women's Lives. *J Low Genit Tract Dis* 2022, 26, (1), 73-78.
15. Brotman, R. M.; Shardell, M. D.; Gajer, P.; Fadrosh, D.; Chang, K.; Silver, M. I.; Viscidi, R. P.; Burke, A. E.; Ravel, J.; Gravitt, P. E., Association between the vaginal microbiota, menopause status, and signs of vulvovaginal atrophy. *Menopause* 2014, 21, (5), 450-8.
16. Ravel, J.; Gajer, P.; Abdo, Z.; Schneider, G. M.; Koenig, S. S.; McCulle, S. L.; Karlebach, S.; Gorle, R.; Russell, J.; Tacket, C. O.; Brotman, R. M.; Davis, C. C.; Ault, K.; Peralta, L.; Forney, L. J., Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci U S A* 2011, 108 Suppl 1, (Suppl 1), 4680-7.
17. Shen, J.; Song, N.; Williams, C. J.; Brown, C. J.; Yan, Z.; Xu, C.; Forney, L. J., Effects of low dose estrogen therapy on the vaginal microbiomes of women with atrophic vaginitis. *Sci Rep* 2016, 6, 24380.
18. Archer, D. F., Efficacy and tolerability of local estrogen therapy for urogenital atrophy. *Menopause* 2010, 17, (1), 194-203.
19. Rees, M.; Pérez-López, F. R.; Ceasu, I.; Depypere, H.; Erel, T.; Lambrinoudaki, I.; Schenck-Gustafsson, K.; Simoncini, T.; van der Schouw, Y.; Tremollieres, F., EMAS clinical guide: low-dose vaginal estrogens for postmenopausal vaginal atrophy. *Maturitas* 2012, 73, (2), 171-4.
20. Sherrard, J.; Wilson, J.; Donders, G.; Mendling, W.; Jensen, J. S., 2018 European (IUSTI/WHO) International Union against sexually transmitted infections (IUSTI) World Health Organisation (WHO) guideline on the management of vaginal discharge. *Int J STD AIDS* 2018, 29, (13), 1258-1272.
21. Mac Bride, M. B.; Rhodes, D. J.; Shuster, L. T., Vulvovaginal atrophy. *Mayo Clin Proc* 2010, 85, (1), 87-94.
22. Paladine, H. L.; Desai, U. A., Vaginitis: Diagnosis and Treatment. *Am Fam Physician* 2018, 97, (5), 321-329.
23. Edwards, D.; Panay, N., Treating vulvovaginal atrophy/genitourinary syndrome of menopause: how important is vaginal lubricant and moisturizer composition? *Climacteric* 2016, 19, (2), 151-61.
24. Kamilos, M. F.; Borrelli, C. L., New therapeutic option in genitourinary syndrome of menopause: pilot study using microablative fractional radiofrequency. *Einstein (Sao Paulo)* 2017, 15, (4), 445-451.
25. Cunha, A. R.; Machado, R. M.; Palmeira-de-Oliveira, A.; Martinez-de-Oliveira, J.; das Neves, J.; Palmeira-de-Oliveira, R., Characterization of commercially available vaginal lubricants: a safety perspective. *Pharmaceutics* 2014, 6, (3), 530-42.
26. Chen, J.; Geng, L.; Song, X.; Li, H.; Giordan, N.; Liao, Q., Evaluation of the efficacy and safety of hyaluronic acid vaginal gel to ease vaginal dryness: a multicenter, randomized, controlled, open-label, parallel-group, clinical trial. *J Sex Med* 2013, 10, (6), 1575-84.
27. Vale, F.; Rezende, C.; Raciclan, A.; Bretas, T.; Geber, S., Efficacy and safety of a non-hormonal intravaginal moisturizer for the treatment of vaginal dryness in postmenopausal women with sexual dysfunction. *Eur J Obstet Gynecol Reprod Biol* 2019, 234, 92-95.
28. Phillips, N. A.; Bachmann, G. A., Genitourinary syndrome of menopause: Common problem, effective treatments. *Cleve Clin J Med* 2018, 85, (5), 390-398.

29. Del Pup, L., Management of vaginal dryness and dyspareunia in estrogen sensitive cancer patients. *Gynecol Endocrinol* 2012, 28, (9), 740-5.
30. Del Pup, L.; Postruznik, D.; Corona, G., Effect of one-month treatment with vaginal promestriene on serum estrone sulfate levels in cancer patients: a pilot study. *Maturitas* 2012, 72, (1), 93-4.
31. Cano, A.; Estévez, J.; Usandizaga, R.; Gallo, J. L.; Guinot, M.; Delgado, J. L.; Castellanos, E.; Moral, E.; Nieto, C.; del Prado, J. M.; Ferrer, J., The therapeutic effect of a new ultra low concentration estriol gel formulation (0.005% estriol vaginal gel) on symptoms and signs of postmenopausal vaginal atrophy: results from a pivotal phase III study. *Menopause* 2012, 19, (10), 1130-9.
32. Mueck, A. O.; Ruan, X.; Prasauskas, V.; Grob, P.; Ortmann, O., Treatment of vaginal atrophy with estriol and lactobacilli combination: a clinical review. *Climacteric* 2018, 21, (2), 140-147.
33. Griesser, H.; Skonietzki, S.; Fischer, T.; Fielder, K.; Suesskind, M., Low dose estriol pessaries for the treatment of vaginal atrophy: a double-blind placebo-controlled trial investigating the efficacy of pessaries containing 0.2mg and 0.03mg estriol. *Maturitas* 2012, 71, (4), 360-8.
34. Lethaby, A.; Ayeleke, R. O.; Roberts, H., Local oestrogen for vaginal atrophy in postmenopausal women. *Cochrane Database Syst Rev* 2016, 2016, (8), Cd001500.
35. Biehl, C.; Plotsker, O.; Mirkin, S., A systematic review of the efficacy and safety of vaginal estrogen products for the treatment of genitourinary syndrome of menopause. Menopause 2019, 26, (4), 431-453.
36. Constantine, G. D.; Bruyniks, N.; Princic, N.; Huse, D.; Palmer, L.; Lenhart, G.; Blumentals, W. A.; Nappi, R. E., Incidence of genitourinary conditions in women with a diagnosis of vulvar/vaginal atrophy. *Curr Med Res Opin* 2014, 30, (1), 143-8.
37. Bumphenkiatikul, T.; Panyakhamlerd, K.; Chatsuwan, T.; Ariyasriwatana, C.; Suwan, A.; Taweepolcharoen, C.; Taechakraichana, N., Effects of vaginal administration of conjugated estrogens tablet on sexual function in postmenopausal women with sexual dysfunction: a double-blind, randomized, placebo-controlled trial. *BMC Womens Health* 2020, 20, (1), 173.
38. NICE NICE: Menopause, Diagnosis and Management – from Guideline to Practice Guideline Summary. https://thebms.org.uk/wp-content/uploads/2019/04/09-BMS-TfC-NICE-Menopause-Diagnosis-and-Management-from-Guideline-to-Practice-Guideline-Summary-01-April2019.pdf (11/07/2022),
39. The 2017 hormone therapy position statement of The North American Menopause Society. *Menopause* 2018, 25, (11), 1362-1387.
40. Barnabei, V. M.; Cochrane, B. B.; Aragaki, A. K.; Nygaard, I.; Williams, R. S.; McGovern, P. G.; Young, R. L.; Wells, E. C.; O'Sullivan, M. J.; Chen, B.; Schenken, R.; Johnson, S. R., Menopausal symptoms and treatment-related effects of estrogen and progestin in the Women's Health Initiative. *Obstet Gynecol* 2005, 105, (5 Pt 1), 1063-73.
41. Palacios, S.; Mejía, A.; Neyro, J. L., Treatment of the genitourinary syndrome of menopause. *Climacteric* 2015, 18 Suppl 1, 23-9.
42. Sturdee, D. W.; Panay, N., Recommendations for the management of postmenopausal vaginal atrophy. *Climacteric* 2010, 13, (6), 509-22.
43. Del Pup, L., Ospemifene: a safe treatment of vaginal atrophy. Eur Rev Med Pharmacol Sci 2016, 20, (18), 3934-3944.
44. Berga, S. L., Profile of ospemifene in the breast. *Reprod Sci* 2013, 20, (10), 1130-6.
45. Lilue, M.; Palacios, S.; Del Carmen Pingarrón Santofimia, M., Experience with ospemifene in patients with vulvar and vaginal atrophy and a history of breast cancer: case studies. *Drugs Context* 2020, 9.
46. Apperloo, M.; Midden, M.; van der Stege, J.; Wouda, J.; Hoek, A.; Weijmar Schultz, W., Vaginal application of testosterone: A study on pharmacokinetics and the sexual response in healthy volunteers. *J Sex Med* 2006, 3, (3), 541-9.
47. Fernandes, T.; Pedro, A. O.; Baccaro, L. F.; Costa-Paiva, L. H., Hormonal, metabolic, and endometrial safety of testosterone vaginal cream versus estrogens for the treatment of vulvovaginal atrophy in postmenopausal women: a randomized, placebo-controlled study. *Menopause* 2018, 25, (6), 641-647.
48. Simon, J. A.; Goldstein, I.; Kim, N. N.; Davis, S. R.; Kellogg-Spadt, S.; Lowenstein, L.; Pinkerton, J. V.; Stuenkel, C. A.; Traish, A. M.; Archer, D. F.; Bachmann, G.; Goldstein, A. T.; Nappi, R. E.; Vignozzi, L., The role of androgens in the treatment of genitourinary syndrome of menopause (GSM): International Society for the Study of Women's Sexual Health (ISSWSH) expert consensus panel review. *Menopause* 2018, 25, (7), 837-847.
49. Archer, D. F.; Labrie, F.; Bouchard, C.; Portman, D. J.; Koltun, W.; Cusan, L.; Labrie, C.; Côté, I.; Lavoie, L.; Martel, C.; Balser, J., Treatment of pain at sexual activity (dyspareunia) with intravaginal dehydroepiandrosterone (prasterone). *Menopause* 2015, 22, (9), 950-63.
50. Labrie, F.; Archer, D. F.; Koltun, W.; Vachon, A.; Young, D.; Frenette, L.; Portman, D.; Montesino, M.; Côté, I.; Parent, J.; Lavoie, L.; AB, B. S.; Martel, C.; Vaillancourt, M.; Balser, J.; Moyneur, É., Efficacy of intravaginal dehydroepiandrosterone (DHEA) on moderate to severe dyspareunia and vaginal dryness, symptoms of vulvovaginal atrophy, and of the genitourinary syndrome of menopause. *Menopause* 2018, 25, (11), 1339-1353.

51. Sussman, T. A.; Kruse, M. L.; Thacker, H. L.; Abraham, J., Managing Genitourinary Syndrome of Menopause in Breast Cancer Survivors Receiving Endocrine Therapy. *J Oncol Pract* 2019, 15, (7), 363-370.
52. Bretas, T. L. B.; Issa, M. C. A.; Fialho, S.; Villar, E. A. G.; Velarde, L. G. C.; Pérez-López, F. R., Vaginal collagen I and III changes after carbon dioxide laser application in postmenopausal women with the genitourinary syndrome: a pilot study. *Climacteric* 2022, 25, (2), 186-194.
53. Sarmento, A. C.; Fernandes, F. S.; Marconi, C.; Giraldo, P. C.; Eleutério-Júnior, J.; Crispim, J. C.; Gonçalves, A. K., Impact of microablative fractional radiofrequency on the vaginal health, microbiota, and cellularity of postmenopausal women. *Clinics (Sao Paulo)* 2020, 75, e1750.
54. Salvatore, S.; Pitsouni, E.; Grigoriadis, T.; Zacharakis, D.; Pantaleo, G.; Candiani, M.; Athanasiou, S., CO(2) laser and the genitourinary syndrome of menopause: a randomized sham-controlled trial. *Climacteric* 2021, 24, (2), 187-193.
55. Preti, M.; Vieira-Baptista, P.; Digesu, G. A.; Bretschneider, C. E.; Damaser, M.; Demirkesen, O.; Heller, D. S.; Mangir, N.; Marchitelli, C.; Mourad, S.; Moyal-Barracco, M.; Peremateu, S.; Tailor, V.; Tarcan, T.; De, E. J. B.; Stockdale, C. K., The Clinical Role of LASER for Vulvar and Vaginal Treatments in Gynecology and Female Urology: An ICS/ISSVD Best Practice Consensus Document. *J Low Genit Tract Dis* 2019, 23, (2), 151-160.
56. Cruz, V. L.; Steiner, M. L.; Pompei, L. M.; Strufaldi, R.; Fonseca, F. L. A.; Santiago, L. H. S.; Wajsfeld, T.; Fernandes, C. E., Randomized, double-blind, placebo-controlled clinical trial for evaluating the efficacy of fractional CO2 laser compared with topical estriol in the treatment of vaginal atrophy in postmenopausal women. *Menopause* 2018, 25, (1), 21-28.
57. Li, F. G.; Maheux-Lacroix, S.; Deans, R.; Nesbitt-Hawes, E.; Budden, A.; Nguyen, K.; Lim, C. Y.; Song, S.; McCormack, L.; Lyons, S. D.; Segelov, E.; Abbott, J. A., Effect of Fractional Carbon Dioxide Laser vs Sham Treatment on Symptom Severity in Women With Postmenopausal Vaginal Symptoms: A Randomized Clinical Trial. *JAMA* 2021, 326, (14), 1381-1389.
58. Mackova, K.; Mazzer, A. M.; Mori Da Cunha, M.; Hajkova Hympanova, L.; Urbankova, I.; Kastelein, A. W.; Vodegel, E.; Vander Linden, K.; Fehervary, H.; Guler, Z.; Roovers, J. P.; Krofta, L.; Verhaeghe, J.; Deprest, J., Vaginal Er:YAG laser application in the menopausal ewe model: a randomised estrogen and sham-controlled trial. *Bjog* 2021, 128, (6), 1087-1096.
59. Page, A. S.; Verbakel, J. Y.; Verhaeghe, J.; Latul, Y. P.; Housmans, S.; Deprest, J., Laser versus sham for genitourinary syndrome of menopause: A randomised controlled trial. *Bjog* 2022.
60. Pérez-López, F. R.; Varikasuvu, S. R., Vulvovaginal atrophy management with a laser: the placebo effect or the conditioning Pavlov reflex. *Climacteric* 2022, 25, (4), 323-326.
61. Juhász, M. L. W.; Korta, D. Z.; Mesinkovska, N. A., Vaginal Rejuvenation: A Retrospective Review of Lasers and Radiofrequency Devices. *Dermatol Surg* 2021, 47, (4), 489-494.
62. Pitsouni, E.; Grigoriadis, T.; Douskos, A.; Kyriakidou, M.; Falagas, M. E.; Athanasiou, S., Efficacy of vaginal therapies alternative to vaginal estrogens on sexual function and orgasm of menopausal women: A systematic review and meta-analysis of randomized controlled trials. *Eur J Obstet Gynecol Reprod Biol* 2018, 229, 45-56.
63. Vieira-Baptista, P., Better studies needed on LASER use in urinary incontinence. *Bjog* 2020, 127, (11), 1347.
64. Mercier, J.; Morin, M.; Zaki, D.; Reichetzer, B.; Lemieux, M. C.; Khalifé, S.; Dumoulin, C., Pelvic floor muscle training as a treatment for genitourinary syndrome of menopause: A single-arm feasibility study. *Maturitas* 2019, 125, 57-62.
65. Capobianco, G.; Donolo, E.; Borghero, G.; Dessole, F.; Cherchi, P. L.; Dessole, S., Effects of intravaginal estriol and pelvic floor rehabilitation on urogenital aging in postmenopausal women. *Arch Gynecol Obstet* 2012, 285, (2), 397-403.
66. Capobianco, G.; Wenger, J. M.; Meloni, G. B.; Dessole, M.; Cherchi, P. L.; Dessole, S., Triple therapy with Lactobacilli acidophili, estriol plus pelvic floor rehabilitation for symptoms of urogenital aging in postmenopausal women. *Arch Gynecol Obstet* 2014, 289, (3), 601-8.
67. ACOG Committee Opinion No. 659: The Use of Vaginal Estrogen in Women With a History of Estrogen-Dependent Breast Cancer. *Obstet Gynecol* 2016, 127, (3), e93-e96.
68. Cold, S.; Cold, F.; Jensen, M. B.; Cronin-Fenton, D.; Christiansen, P.; Ejlertsen, B., Systemic or Vaginal Hormone Therapy After Early Breast Cancer: A Danish Observational Cohort Study. *J Natl Cancer Inst* 2022, 114, (10), 1347-1354.
69. Pavlović, R. T.; Janković, S. M.; Milovanović, J. R.; Stefanović, S. M.; Folić, M. M.; Milovanović, O. Z.; Mamillapalli, C.; Milosavljević, M. N., The Safety of Local Hormonal Treatment for Vulvovaginal Atrophy in Women With Estrogen Receptor-positive Breast Cancer Who Are on Adjuvant Aromatase Inhibitor Therapy: Meta-analysis. *Clin Breast Cancer* 2019, 19, (6), e731-e740.
70. Sánchez-Rovira, P.; Hirschberg, A. L.; Gil-Gil, M.; Bermejo-De Las Heras, B.; Nieto-Magro, C., A Phase II Prospective, Randomized, Double-Blind, Placebo-Controlled and Multicenter Clinical Trial to Assess the Safety of 0.005% Estriol Vaginal Gel in Hormone Receptor-Positive Postmenopausal Women with Early Stage Breast Cancer in Treatment with Aromatase Inhibitor in the Adjuvant Setting. *Oncologist* 2020, 25, (12), e1846-1854.
71. Hirschberg, A. L.; Sánchez-Rovira, P.; Presa-Lorite, J.; Campos-Delgado, M.; Gil-Gil, M.; Lidbrink, E.; Suárez-Almarza, J.; Nieto-Magro, C., Efficacy and safety of ultra-low dose 0.005% estriol vaginal gel for the treatment of vulvovaginal atrophy in postmenopausal women with early breast cancer treated with nonsteroidal aromatase inhibitors: a phase II, randomized, double-blind, placebo-controlled trial. *Menopause* 2020, 27, (5), 526-534.

72. Jain, A. L.; Jamy, O.; Mullins, J.; Usman, R. M.; Hare, F.; Valasareddy, P.; Chaudhry, A.; Ryder, J.; Smith, J. R.; Miller, E.; Ranganath, H.; Schwartzberg, L.; Stepanski, E.; Walker, M.; Gatwood, J.; Vidal, G. A., Usefulness of patient-reported outcomes to assess the effectiveness of topical hormonal therapy for gynecologic symptoms after antihormonal treatment for breast cancer. *Proc (Bayl Univ Med Cent)* 2020, 33, (3), 331-335.
73. Melisko, M. E.; Goldman, M. E.; Hwang, J.; De Luca, A.; Fang, S.; Esserman, L. J.; Chien, A. J.; Park, J. W.; Rugo, H. S., Vaginal Testosterone Cream vs Estradiol Vaginal Ring for Vaginal Dryness or Decreased Libido in Women Receiving Aromatase Inhibitors for Early-Stage Breast Cancer: A Randomized Clinical Trial. *JAMA Oncol* 2017, 3, (3), 313-319.
74. Barton, D. L.; Sloan, J. A.; Shuster, L. T.; Gill, P.; Griffin, P.; Flynn, K.; Terstriep, S. A.; Rana, F. N.; Dockter, T.; Atherton, P. J.; Tsai, M.; Sturtz, K.; Lafky, J. M.; Riepl, M.; Thielen, J.; Loprinzi, C. L., Evaluating the efficacy of vaginal dehydroepiandosterone for vaginal symptoms in postmenopausal cancer survivors: NCCTG N10C1 (Alliance). *Support Care Cancer* 2018, 26, (2), 643-650.
75. Reeder-Hayes, K.; Muss, H. B., Vaginal Estrogens and Aromatase Inhibitors: How Safe Is Safe Enough? *JAMA Oncol* 2017, 3, (3), 305-306.

VAGINITIS IN CHILDREN

9

(alphabetical order)
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9.1 Introduction

Vulvovaginitis is the most common gynecological problem in prepubertal females. Many providers classify vaginitis under the general heading of vulvovaginitis and include causes of vulvitis. Most causes of vaginitis are different from causes of vulvitis, which are primarily skin diseases. Vulvitis is not uncommon in children and can be due to dermatological conditions such as dermatitis which can produce scaling and weeping, psoriasis and lichen sclerosus (LS) which can be mistaken for discharge.

This section addresses vaginitis, which is uncommon in the prepubertal female and, except for infectious causes, there is little research and data. Although most parents and medical personnel think of vaginal discharge and vaginitis as being of infectious origin, especially yeast, these are nearly non-existent in the healthy child, and etiologies other than infection should be considered.

9.2 The vagina in the prepubertal child

The vestibule

Erythema of the vestibule is common in children and, in the absence of symptoms, it is of no significance. If there is an associated discharge, vaginitis should be considered. Incontinence in children, especially in babies, is far from being uncommon, causing urinary and stool leakage to be mistaken for vaginal discharge. Pooling of urine and stool in the vestibule may result in dysuria and irritation. This may be the result of abnormal voiding posture.[1]

The hymen

With very rare exceptions, all girls are born with a hymen, the shape and appearance of which is highly variable.

At birth, as a result of exposure to maternal oestrogen, the hymen is thickened, returning to a thin translucent membrane over the next two years of life.

In some cases the hymen may be imperforate, requiring surgical treatment at puberty.[2]

The vagina and vaginal discharge

Data on the normal vagina in children are limited. However, as in adults, vaginal ridges and columns are normal variants. Peri-urethral and peri-hymenal fibrous bands may form a pocket on either side of the urethra or hymen.

The *linea vestibularis* is a normal variant described as an avascular area of the posterior vestibule which appears pale and may be confused with scarring.

In utero, the vaginal epithelium of the fetus is stimulated by maternal hormones that cross the placenta into the fetal circulation. After delivery, these hormone levels fall rapidly, and a thick, greyish-white, mucoid discharge from the neonate's vagina can be observed. The discharge usually resolves in 10 days. In some baby girls, the discharge from the vagina is blood-tinged or even grossly bloody. This is a physiological endometrial response to the drop of maternal estrogens after birth.[3]

The normal length of the vagina in a newborn is 4 cm with a long cervix which is larger than the uterine *corpus*. In childhood, the vaginal length increases to about 8 cm. In the neonate, a vaginal vault smear shows polygonal epithelial cells and in a prepubertal child the epithelial cells are round (parabasal cells).[4-8] (Figure 9.1)

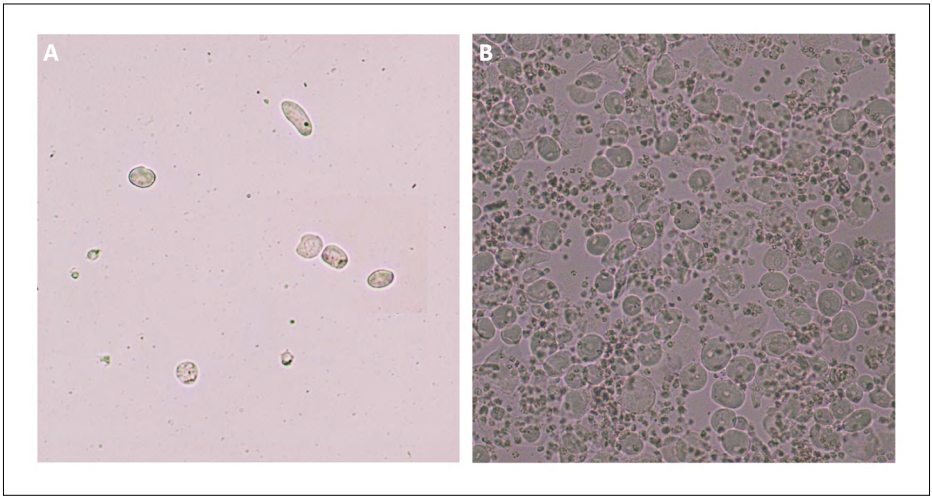

Figure 9.1 Wet mount microscopy (200x) from a prepubertal girls' vagina.
A– Exclusive presence of parabasal cells; lactobacilli absent B– Presence of inflammation in a case of bacterial vaginitis

Lactobacilli are typically absent from the prepubertal vagina, and the pH is usually higher than in adult women.

The lack of estrogens in prepubertal girls results in remarkable differences compared to the normal vaginal microbiota of post pubertal women and, by extension, to the organisms that produce vaginitis in children.[9] Normal bacteria in the prepuberal vagina include enteric microorganisms such as *Diphtheroids* spp., *Peptococcus* spp., *Bacteroides* spp., *Proteus* spp., and *Escherichia coli*, as well as those of respiratory origins such as group A *Streptococcus* and other streptococci, *Haemophilus influenzae*, and *Klebsiella* spp..[10] Other microorganisms such as *Staphylococcus aureus* and *S. epidermidis*, *Ureaplasma urealyticum*, *Gardnerella* spp., *Lactobacillus* spp., and *Candida* spp. are sometimes found in asymptomatic children.[10] Generally, data regarding both normal and pathogenic vaginal organisms in prepubertal children have not been categorized according to age or Tanner stage. Estrogen effects begin to occur at about seven years of age, so lumping all ages together is less than ideal.[11]

In the prepubertal child there is minimal vaginal discharge. However, an inoffensive, thin milky or greenish discharge which is not associated with symptoms or clinically obvious inflammation is common and harmless.

As puberty approaches, up to three years prior to menarche, a milky discharge is normal, the pH becomes lower and lactobacilli and *Gardnerella* spp. appear.[11-15]

9.3
How to conduct a vaginal examination in a child

There exists literature with recommendations for conducting a genital exam in a child and all stress the importance of creating a non-threatening environment, and distraction techniques to gain the child's and parents' trust. Each clinician will have their own method of facilitating this. Small children may be most comfortable remaining on their carer's lap, but options of either doing this or lying on the exam table should be offered.

Prepubertal children have often been told not to let anyone look at their genitals, so the exception of a medical examination needs to be explained by their parent before and during the consultation.

Adolescents are often highly embarrassed by any sort of genital examination, so preserving their modesty and asking any male relatives to leave the room is recommended.

The most often recommended position is prone knee to chest and visualisation of the vestibule can be facilitated by gently pulling the *labia majora* out and up.

The supine position with the legs in a frog-leg position is recommended to visualise the vaginal opening, however if the child is not relaxed, contraction of the perineal and gluteal muscles may render the examination difficult.

In some cases, if it is necessary to examine the upper vagina in a child (i.e. if a tumour or foreign body is suspected) an examination under general anesthesia is recommended.[1, 16, 17]

9.4
Testing for infection

If a streptococcal or *H. influenzae* infection is suspected, a saline moistened swab can be used to take a sample from the introitus. Although attempting to take a vaginal swab is unpleasant for a small child, in these cases it is necessary.

If there is a thick green discharge and/or sexual abuse is suspected, a vaginal sample is necessary to rule out *Neisseria gonorrhoeae, Chlamydia trachomatis* and *Trichomonas vaginalis*. The sample may be taken using a thin catheter and a syringe rather than inserting a swab. A urine polymerase chain reaction test can be considered rather than a vaginal sample.

9.5
Vaginal discharge

The presence of discharge in the absence of symptoms is not usually a cause of concern and should not prompt further investigation.

Although candidiasis and bacterial vaginosis (BV) are the most common causes of acute vaginal discharge or pruritus in an adult, these are rare causes of vulvovaginal symptoms in children. In fact, in some cases, what may be perceived as discharge may have other causes (i.e. an ectopic ureter or a lymphatic malformation).[11-15]

TABLE 9.1 Etiologies of vaginal discharge in children

Physiologic
Foreign Body
Infection: group A *Streptococcus*, *H. influenza*
Lymphatic malformation
Ectopic ureter
Fistula
Bacterial vaginosis (very rare)
Lichen planus (very rare)

Vaginal discharge due to infection

Bacterial vaginitis

Introduction

Bacterial vaginitis (not to be confused with BV) is an uncommon cause of vulvovaginal symptoms in the prepubertal child. However, the thin, fragile, un-estrogenized vagina of prepubertal girls, the proximity of the vagina to the perianal skin colonized with enteric organisms, and hygiene habits of small children provide a fertile environment for bacterial infection of the vagina compared to that of well estrogenized adolescent and adult women.

Prevalence

The prevalence of prepubertal bacterial vaginitis is unknown in part because many microorganisms colonizing the vagina of young girls are only occasionally pathogens, with asymptomatic girls occasionally exhibiting positive cultures for these bacteria. These issues prevent clear diagnoses, and therefore the few available data on prevalence are of poor quality.

Etiology and pathophysiology

The most common causes of symptomatic vaginitis in childhood are *S. pyogenes*, group B *Streptococcus* (*S. agalactiae), S. aureus, H. influenzae, E. coli, Enterococcus faecalis, Klebsiella pneumoniae*, and *Shigella* spp..

Risk factors

Risk factors for bacterial vaginitis are believed to include poor perineal hygiene, wiping back to front, the presence of foreign bodies, and sexual abuse.

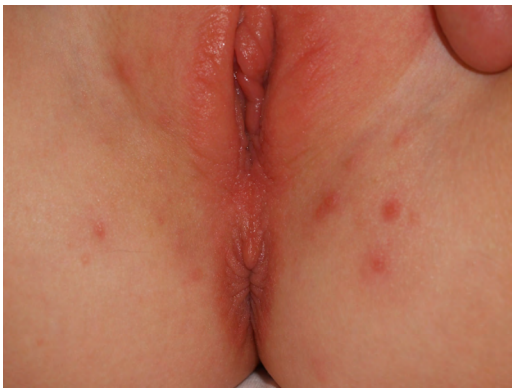

Figure 9.2 Vulvar and perianal redness in a young girl with bacterial vaginitis (*S. pyogenes*)

Signs and symptoms

The most frequent symptoms of bacterial vaginitis include vulvar itching, pain, odor, and vulvar dysuria. Redness of the introitus and, often, the modified mucous membranes of the vulva, as well as a yellow or even green vaginal discharge, are the most common signs. (Figure 9.2)

Sometimes the discharge may also be described as yellow/brown staining of the underwear. Vaginal bleeding occurs in a minority of children.

Diagnosis

There is considerable overlap between colonizers and pathogens, so a diagnosis of bacterial vaginitis by culture alone is not recommended. Cultures should be performed in the setting of clinical inflammation and symptoms, while keeping in mind that the estrogen deficient introitus is often normally strikingly erythematous.

Culture results showing a pure growth of a potential pathogen rather than mixed flora are more likely to represent true infection, and a positive response to treatment confirms the diagnosis. Generally, most bacterial vaginitis are associated with respiratory pathogens.[10, 18] *S. pyogenes* is by far the most common cause of childhood bacterial vaginitis, and often accompanies or follows streptococcal pharyngitis. *H. influenza* previously was another relevant cause,

but the increasing number of children vaccinated against this microorganism has likely resulted in a decreased prevalence of *H. influenza* bacterial vaginitis. *K. pneumoniae* and *S. aureus*, can also cause bacterial vaginitis.[10, 18, 19] Enteric organisms, especially *E. coli* and *Enterococcus* spp., are found more often in cultures from children with vaginitis symptoms compared to controls, but their role can be difficult to establish.[19, 20] Likewise, *Shigella* spp. and *Yersinia* spp. are rarely found.

Rarely, testing yields *N. gonorrhoeae* as a cause for purulent vaginitis. These children are likely to have been sexually abused; any concern for sexual abuse should prompt molecular studies for gonorrhea, chlamydia and trichomonas as causes for the vaginitis; certainly, there should be screening for other sexually transmitted diseases and appropriate referrals for these girls. However, half or more girls with symptoms and signs of vaginitis show no recognized pathogens on culture and are labeled "nonspecific vulvovaginitis".[21, 22] Alternative diagnoses should be considered in these children. For example, dermatoses such as LS, eczema, and irritant contact dermatitis can produce itching, pain, and superficial exudate that can mimic vaginitis. Urinary tract infections can also be considered.

Treatment

The management of symptomatic bacterial vaginitis consists of oral antibiotics chosen based on the culture results, as well as counseling regarding local care. The use of a mild emollient such as petroleum jelly often provides some comfort, especially if used liberally as a protective barrier before urination. Wiping back to front, particularly in children with enteric organisms on culture, irritants on delicate skin (including medicated creams), and excessive cleaning that leads to inflamed skin may play a role in the development of bacterial vaginitis in some children, so these are not recommended.

Bacterial vaginitis can be recurrent. In addition to ensuring that local care has been addressed and followed, these children should be evaluated for complicating factors, such as vaginal foreign bodies or accompanying skin disease such as LS, irritant contact dermatitis, or eczema that increases the risk of secondary infection. When enteric organisms are found recurrently and perineal hygiene has been addressed, the rare event of an enteric fistula should be considered.

Pinworms/threadworms

Introduction

Although often recognized as a common cause of perianal symptoms in young children, pinworms can also migrate from the intestines into the vagina to lay eggs. The infection occurs when eggs are ingested via contaminated fingers. Scratching the area transfers eggs to under the fingernails. Then, ingested eggs perpetuate the cycle.[23]

Etiology and pathophysiology

Pinworms/threadworms (*Enterobius vermicularis*) are common intestinal parasites.

Prevalence

Pinworms are common, particularly in overcrowded living conditions with poor personal hygiene.[24]

Risk Factors

Risk factors include crowded conditions, poor hygiene, and warm, tropical climates.

Signs and symptoms

These worms resemble short strings of white thread and cause inflammation of the fragile poorly estrogenized vaginal mucosa of young girls. Usually, children exhibit itching, irritation and, often redness and dermatitis of the perianal skin as well as abdominal pain at times and sleep disturbance. When vaginitis occurs, there is redness of the introitus and a purulent vaginal discharge.[23]

Diagnosis

The diagnosis is made by direct visualization of the worms at the anal verge, usually at night, or by identification of worms microscopically; tape is pressed against the anal verge when the child first wakes in the morning, and the tape is then affixed to a glass slide for microscopy.[25]

Treatment

Treatment consists of mebendazole, pyrantel pamoate, or albendazole. Any of these drugs is given in one dose, which is then repeated two weeks later.[26] It is, however, important to know that these drugs do not inactivate the parasite's eggs. A low potency topical corticosteroid ointment (i.e. desonide 0.05% or hydrocortisone 2.5%) applied to the areas of inflammation can hasten resolution of symptoms of itching and pain. All members of the household should be treated, and careful patient education regarding means of transmission and handwashing is important.[25, 27]

Candidiasis

Topical antifungal therapy is a common empirical management strategy for the treatment of any vulvovaginal discharge, itching or irritation. However, once a child is out of diapers, any relief is almost always from the emollient properties of the vehicle, rather than from the antifungal activity of the medication.[19, 28]

Yeasts are a rare cause of vaginitis in prepubertal children, despite positive culture of *C. albicans* from genital samples (not necessarily vaginal) reported in up to 5% of asymptomatic children.[10] The unestrogenized vagina is a hostile environment for the growth of yeasts, and unless a child is immunosuppressed, obese, diapered, diabetic, and rarely following antibiotics, the likelihood of candidiasis is exceptional. Not infrequently, in older, immediately prepubertal girls, florid vulvovaginal candidiasis may present prior to onset of menses due to the physiological increase of estrogen. Menses usually follow in weeks or months.
When candidiasis is suspected, its presence should be confirmed by culture, microscopy, or molecular tests, and underlying predisposing factors sought.

In the rare case of vaginal candidiasis in a child, oral fluconazole is indicated, with topical nystatin ointment as a less irritating medication for vulvar involvement.

Bacterial vaginosis

BV is generally a disease of post pubertal women. Despite the absence of prevalence data in children, BV is believed to be rare.

Recent data reports vaginal/introital cultures of prepubertal girls that yield bacteria associated with this condition. Almost 14% of asymptomatic girls were found to have vaginal *Gardnerella* spp., one of the bacteria involved in the development of BV.[10] Although controversial, some limited data have shown these organisms to be more common in sexually abused girls. In case of a confirmed diagnosis of BV in a child, a history of sexual abuse should be investigated.[10] However the presence of *Gardnerella* spp. alone is not synonymous with BV. As with BV, lack of lactobacilli and elevated pH are normal in children due to lack of estrogen and are not useful for the diagnosis in this population.

If treatment is needed, oral metronidazole or clindamycin are recommended, since intravaginal therapy is inappropriate.

Other infections producing vaginitis

There are several systemic infections that sometimes produce a purulent vaginitis. These include varicella, which is extremely rare in countries that vaccinate against it. Vaginal erosions from short-lived mucosal vesicles produce inflammation, which predispose to *S. pyogenes* infection, another common cause of bacterial vaginitis in children.[29] Therefore, vagini-

TABLE 9.2 Infectious causes of vaginal discharge and management

Diagnosis	Management
S. pyogenes, S. agalactiae	Penicillin 250 mg 2-3 times a day Amoxicillin 50 mg/Kg daily (maximum 1 g) Cephalexin 20 mg/Kg 2 times a day (maximum 500 mg per dose) Clindamycin 7 mg/Kg/dose, 3 times a day (maximum dose 300 mg) Duration of treatment for vaginitis has not been studied in children
S. aureus, H. influenzae, E.coli and all other causes of bacterial vaginitis	By sensitivities on culture
Bacterial vaginosis	Clindamycin 5-7 mg/Kg twice daily for 7 days (maximum daily dose 300 mg) Metronidazole 15-25 mg/Kg/day in three divided doses, maximum 2 g for 7 days
Pinworms	Mebendazole 100 mg once, repeated in 3 weeks for children over 2 years Pyrantel pamoate 1 mg/Kg in one dose, maximum 1 g; repeat dose in 2 weeks Albendazole 400 mg as a single dose; repeat dose in 2 weeks
Candidiasis	Confirm with microscopy, culture or molecular tests Fluconazole 12 mg/Kg in single dose, may repeat in 3 days Nystatin ointment 3 times a day for vulvar involvement
Systemic infections producing vaginitis	Identification and management of systemic infection

tis associated with varicella should undergo bacterial culture and antibiotic treatment when bacterial pathogens are identified. Measles, upper respiratory infections, and gastrointestinal infections are sometimes associated with vaginitis.

Vaginal foreign bodies

Introduction

A classic cause of persistent discharge in girls and prepubertal vaginal bleeding is a foreign body in the vagina. Bleeding in such a situation might be accompanied by pelvic pain and a foul-smelling discharge.[30]

Etiology and pathophysiology

Although the range of objects that find their way into the vagina of a prepubertal child can be remarkable, toilet paper is the most common finding. Toys, household items such as safety pins and pen caps occur. The most damaging foreign bodies are batteries, which can produce ulcers, scarring, become embedded into the vaginal walls, and cause fistulae.[31, 32]

Prevalence

The frequency of vaginal foreign bodies is unknown.

Signs and Symptoms

Vaginal bleeding is a more common presenting sign than a vaginal discharge, and in a recent study, foreign bodies were by far the most common cause of vaginal bleeding in 158 prepubertal girls.[33] In addition, rectal bleeding with a vaginal foreign body sometimes occurs.[34] When vaginal foreign bodies present as a purulent discharge, this is often mistaken for a primary bacterial vaginitis. However, despite initial improvement following antibiotic treatment, the discharge recurs after therapy. Other signs of an inflammatory vaginitis may be present, including erythema of the introitus as a result of either secondary infection, or simply an irritant contact dermatitis from the purulent vaginal secretions. The unidentified foreign body may lead to urinary tract infection or dermatosis and, in serious cases, to perforation into the peritoneal cavity or fistula formation.[30]

Diagnosis

The diagnosis is made by identification of the foreign body. The vagina can sometimes be visualized without instrumentation in a knee-chest position, and the foreign body seen. Occasionally, especially when the foreign body is toilet paper, it is both identified and treated by rinsing the vagina with saline.

Ultrasound can be used to identify a foreign body in some instances, although items such as toilet paper may not be evident.[35, 36] Metal and some other dense objects may be seen on a plain radiograph. The vagina can also be examined, usually under general anesthesia, using a hysteroscope inserted into the vagina.

Rarely, foreign bodies present for prolonged periods have been associated with pelvic ab-

scess, vesicovaginal fistulae, rectovaginal fistulae, and vaginal stenosis. The symptoms in these cases can include dysuria, urinary incontinence, and pelvic/abdominal pain.[37]

Treatment

The treatment consists of removing the foreign body. Occasionally, the vagina can be rinsed with a soft urinary catheter, and the foreign body washed out with the fluid. This is especially likely when the foreign body is toilet paper. When the retained object is a battery, emergent removal is indicated.

Otherwise, the foreign body is removed under general anesthesia/conscious sedation.[38]

9.6
Dermatoses and dermatitis which involve the vagina

Fixed drug eruption, toxic epidermal necrolysis, and erythema multiforme are severe cutaneous drug reactions (SCAR) which involve skin and mucosa. These conditions may include a severe erosive vaginitis which can be followed by vaginal synechiae.

Lichen planus is very rare in children but can occasionally cause vaginitis in prepubertal children. Involvement of the vulva is usually also present.

In practice, vaginitis with discharge seldom occurs in isolation as the associated discharge usually causes skin irritation and inflammation of the vulva.

Chemical irritants such as bubble bath, soaps and chlorine in swimming pools can cause inflammation of the vestibule which can result in weeping and simulate a discharge.[4, 39]

Recommendations

Recommendation	Quality of evidence	Strength of recommendation
Erythema of the vestibule is common in children and, in the absence of symptoms, it is of no significance and does not require investigation or treatment.	5	D
The presence of an inoffensive, thin milky or greenish discharge, not associated with symptoms or clinically obvious inflammation is common and harmless in prepubertal children and does not require investigation or treatment.	5	D
If it is necessary to examine the upper vagina in a child, an examination under general anesthesia is recommended.	5	D
If a streptococcal or *Haemophilus influenzae* infection is suspected, a saline moistened swab can be used to take a sample from the introitus.	5	D
If there is a thick green discharge and/or sexual abuse is suspected, a vaginal swab is necessary to rule out sexually transmitted infections.	5	D
If a vaginal sample is needed, it may be taken using a thin catheter and a syringe rather than inserting a swab.	5	D

A diagnosis of bacterial vaginitis by culture alone is not recommended.	5	D
The diagnosis of a sexually transmitted infection must prompt adequate referral due to suspected sexual abuse.	3b	B
The management of symptomatic bacterial vaginitis consists of oral antibiotics chosen on the basis of the culture results, as well as counseling regarding local care.	4	C
The treatment of pinworms consists of all members of the household with mebendazole, pyrantel pamoate, or albendazole (one dose, which is then repeated two weeks later).	3a	B
In the rare case of vaginal candidiasis in a child, oral fluconazole is indicated.	5	D
In a case of a confirmed diagnosis of bacterial vaginosis in a child, a history of sexual abuse should be investigated.	5	D

References

1. McCann, J.; Wells, R.; Simon, M.; Voris, J., Genital findings in prepubertal girls selected for nonabuse: a descriptive study. *Pediatrics* 1990, 86, (3), 428-39.
2. Abdelrahman, H. M.; Feloney, M. P., Imperforate Hymen. In StatPearls, StatPearls Publishing Copyright © 2022, StatPearls Publishing LLC.: *Treasure Island (FL),* 2022.
3. Wróblewska-Seniuk, K.; Jarząbek-Bielecka, G.; Kędzia, W., Gynecological Problems in Newborns and Infants. *J Clin Med* 2021, 10, (5).
4. Berenson, A. B., The prepubertal genital exam: what is normal and abnormal. *Curr Opin Obstet Gynecol* 1994, 6, (6), 526-30.
5. Pillai, M., Genital findings in prepubertal girls: what can be concluded from an examination? *J Pediatr Adolesc Gynecol* 2008, 21, (4), 177-85.
6. Ayson, N.; Starling, S., Normal Examination Findings and Variants. In Handbook of Interpersonal Violence and Abuse Across the Lifespan: A project of the National Partnership to End Interpersonal Violence Across the Lifespan (NPEIV), Geffner, R.; White, J. W.; Hamberger, L. K.; Rosenbaum, A.; Vaughan-Eden, V.; Vieth, V. I., Eds. *Springer International Publishing: Cham,* 2020; pp 1-14.
7. Goff, C. W.; Burke, K. R.; Rickenback, C.; Buebendorf, D. P., Vaginal opening measurement in prepubertal girls. *Am J Dis Child* 1989, 143, (11), 1366-8.
8. Elstein, M., Vaginal cytology of the newborn. *J Obstet Gynaecol Br Commonw* 1963, 70, 1050-5.
9. Chen, X.; Lu, Y.; Chen, T.; Li, R., The Female Vaginal Microbiome in Health and Bacterial Vaginosis. *Front Cell Infect Microbiol* 2021, 11, 631972.
10. Neyazi, S., Prepubertal vulvovaginitis. *Journal of Nature and Science of Medicine* 2019, 2, (1), 14-22.
11. Xiaoming, W.; Jing, L.; Yuchen, P.; Huili, L.; Miao, Z.; Jing, S., Characteristics of the vaginal microbiomes in prepubertal girls with and without vulvovaginitis. *Eur J Clin Microbiol Infect Dis* 2021, 40, (6), 1253-1261.
12. Sugar, N. F.; Graham, E. A., Common gynecologic problems in prepubertal girls. *Pediatr Rev* 2006, 27, (6), 213-23.
13. Hayes, L.; Creighton, S. M., Prepubertal vaginal discharge. *The Obstetrician & Gynaecologist* 2007, 9, (3), 159-163.
14. Hickey, R. J.; Zhou, X.; Settles, M. L.; Erb, J.; Malone, K.; Hansmann, M. A.; Shew, M. L.; Van Der Pol, B.; Fortenberry, J. D.; Forney, L. J., Vaginal microbiota of adolescent girls prior to the onset of menarche resemble those of reproductive-age women. *mBio* 2015, 6, (2).
15. Matytsina, L. A.; Greydanus, D. E.; Gurkin, Y. A., Vaginal microbiocoenosis and cytology of prepubertal and adolescent girls: their role in health and disease. *World J Pediatr* 2010, 6, (1), 32-7.
16. Jacobs, A. M.; Alderman, E. M., Gynecologic examination of the prepubertal girl. *Pediatr Rev* 2014, 35, (3), 97-104.
17. Physicians, T. R. A. C. o. Genital examinations in girls and young women: a clinical practice guideline. https://ranzcog.edu.au/wp-content/uploads/2022/05/Genital-Examinations-in-Girls-and-Young-Women-A-Clinical-Practice-Guideline.pdf
18. Kim, H. C.; Lee, M. H.; Hong, S. G., Pediatric Vulvovaginitis: A Study of Clinical and Microbiologic features and the Efficacy of Perineal Hygienic Care. *Korean J Obstet Gynecol* 1999, 42, (12), 2821-2828.
19. Loveless, M.; Myint, O., Vulvovaginitis- presentation of more common problems in pediatric and adolescent gynecology. *Best Pract Res Clin Obstet Gynaecol* 2018, 48, 14-27.
20. Gorbachinsky, I.; Sherertz, R.; Russell, G.; Krane, L. S.; Hodges, S. J., Altered perineal microbiome is associated with vulvovaginitis and urinary tract infection in preadolescent girls. *Ther Adv Urol* 2014, 6, (6), 224-9.

21. Jarienė, K.; Drejerienė, E.; Jaras, A.; Kabašinskienė, A.; Čelkienė, I.; Urbonavičienė, N., Clinical and Microbiological Findings of Vulvovaginitis in Prepubertal Girls. *J Pediatr Adolesc Gynecol* 2019, 32, (6), 574-578.
22. Baka, S.; Demeridou, S.; Kaparos, G.; Tsoutsouras, K.; Touloumakos, S.; Dagre, M.; Meretaki, S.; Chasiakou, A.; Koumaki, V.; Tsakris, A., Microbiological findings in prepubertal and pubertal girls with vulvovaginitis. *Eur J Pediatr* 2022, 181, (12), 4149-4155.
23. Serban, E. D., Perianal infectious dermatitis: An underdiagnosed, unremitting and stubborn condition. *World J Clin Pediatr* 2018, 7, (4), 89-104.
24. Rivero, M. R.; De Angelo, C.; Feliziani, C.; Liang, S.; Tiranti, K.; Salas, M. M.; Salomon, O. D., Enterobiasis and its risk factors in urban, rural and indigenous children of subtropical Argentina. *Parasitology* 2022, 149, (3), 396-406.
25. Bharti, B.; Bharti, S.; Khurana, S., Worm Infestation: Diagnosis, Treatment and Prevention. *Indian J Pediatr* 2018, 85, (11), 1017-1024.
26. Centers for Disease Control and Prevention, Parasites - enterobiasis, https://www.cdc.gov/parasites/pinworm/treatment.html
27. Weatherhead, J. E.; Hotez, P. J., Worm Infections in Children. *Pediatr Rev* 2015, 36, (8), 341-52; quiz 353-4.
28. Fischer, G. O., Vulval disease in pre-pubertal girls. *Australas J Dermatol* 2001, 42, (4), 225-34; quiz, 235-6.
29. Hasin, O.; Hazan, G.; Rokney, A.; Dayan, R.; Sagi, O.; Ben-Shimol, S.; Greenberg, D.; Danino, D., Invasive Group A Streptococcus Infection in Children in Southern Israel Before and After the Introduction of Varicella Vaccine. *J Pediatric Infect Dis Soc* 2020, 9, (2), 236-239.
30. Dwiggins, M.; Gomez-Lobo, V., Current review of prepubertal vaginal bleeding. *Curr Opin Obstet Gynecol* 2017, 29, (5), 322-327.
31. Nakib, G.; Calcaterra, V.; Pelizzo, G., Longstanding Presence of a Vaginal Foreign Body (Battery): Severe Stenosis in a 13-Year-Old Girl. *J Pediatr Adolesc Gynecol* 2017, 30, (1), e15-e18.
32. Yanoh, K.; Yonemura, Y., Severe vaginal ulcerations secondary to insertion of an alkaline battery. *J Trauma* 2005, 58, (2), 410-2.
33. Zhang, J.; Zhang, B.; Su, Y.; Guo, S.; Liu, C.; Bai, J.; Xie, X., Prepubertal Vaginal Bleeding: An Inpatient Series from a Single Center in Fujian China. *J Pediatr Adolesc Gynecol* 2020, 33, (2), 120-124.
34. Shiryazdi, S. M.; Heiranizadeh, N.; Soltani, H. R., Rectorrhagia and vaginal discharge caused by a vaginal foreign body--a case report and review of literature. *J Pediatr Adolesc Gynecol* 2013, 26, (3), e73-5.
35. Gross, I. T.; Riera, A., Vaginal Foreign Bodies: The Potential Role of Point-of-Care-Ultrasound in the Pediatric Emergency Department. *Pediatr Emerg Care* 2017, 33, (11), 756-759.
36. Yang, X.; Sun, L.; Ye, J.; Li, X.; Tao, R., Ultrasonography in Detection of Vaginal Foreign Bodies in Girls: A Retrospective Study. *J Pediatr Adolesc Gynecol* 2017, 30, (6), 620-625.
37. Ekinci, S.; Karnak, İ.; Tanyel, F. C.; Çiftçi, A., Prepubertal vaginal discharge: Vaginoscopy to rule out foreign body. *Turk J Pediatr* 2016, 58, (2), 168-171.
38. Ma, W.; Sun, Y. F.; Liu, J. H.; He, D. W.; Lin, T.; Wei, G. H., Vaginal foreign bodies in children: a single-center retrospective 10-year analysis. *Pediatr Surg Int* 2022, 38, (4), 637-641.
39. Berenson, A. B.; Heger, A. H.; Hayes, J. M.; Bailey, R. K.; Emans, S. J., Appearance of the hymen in prepubertal girls. *Pediatrics* 1992, 89, (3), 387-94.

PROBIOTICS, PREBIOTICS AND SYNBIOTICS FOR VAGINITIS

10

(alphabetical order)
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10.1 Introduction

According to consensus guidelines from the International Scientific Association for Probiotics and Prebiotics (ISAPP), a probiotic is a "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host".[1] This definition excludes microbial transplants and live cultures in food. The same organization has provided a definition for prebiotic, which states: "a substrate that is selectively utilized by host microorganisms conferring a health benefit".[2] Finally, a synbiotic is defined as "a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host". The ISAPP further divides synbiotics into synergistic and complementary. Per the publication: "A 'synergistic synbiotic' is a synbiotic in which the substrate is designed to be selectively utilized by the co-administered microorganism(s). A 'complementary synbiotic' is a synbiotic composed of a probiotic combined with a prebiotic, which is designed to target autochthonous microorganisms".[3]

Before discussing data for the use of the products in specific conditions, we must first address the available data (or lack thereof) on whether there is a difference in oral *vs.* vaginal administration. We presume that for a product to be effective for vaginal health it must actually reach the vagina. Vaginal administration directly applies the product to the target site. In the few studies that have looked for probiotic strains in the vagina and gut after oral administration, the probiotic strains were able to be cultivated from the vagina in 8-75% of women during administration, but colonization decreased after cessation of use.[4-8] However, only in one of the two studies which included a placebo arm, the probiotic was found in 45% of participants.[8] In the only study to use advanced, molecular detection methods, the probiotic strains were rarely detected in either vaginal or fecal samples.[9]

We should also acknowledge that probiotics, prebiotics and synbiotics are broad terms, encompassing a wide variety of products, and that there is significant heterogeneity in the literature with regard to dose, dosing frequency, duration and specific microbial strains, making a comprehensive and consistent summary of the data challenging.

10.2
Bacterial vaginosis

Probiotics

The desire to use probiotics in the management of bacterial vaginosis (BV) is fueled by the high rate of BV recurrence. Both providers and patients are looking desperately for something better. Some studies look at probiotics as an alternative to antibiotic therapy, while most studies evaluate the impact of post-antibiotic use of probiotics to prevent BV recurrence. As noted above, there is significant heterogeneity in the studies' methodologies.

When evaluating the available data, it is important to consider the dimensions a study would need to have to confidently prove that a therapy is either not effective, or is non-inferior to a standard regimen. When considering recurrent BV at one or six months, and two different magnitudes of reduction in that rate, the smallest randomized trial that would have enrolled a sufficient number of participants to detect a 50% reduction in BV recurrence at six months would be of at least 84 women. (Table 10.1) A study can still find a significant difference between arms when underpowered, but if no significant differences are seen between arms in a smaller trial the conclusion should not be that the arms are statistically equivalent – but that the study is underpowered and the answer remains unknown. Additionally, follow-up duration varies between studies. For clinicians and patients, long-term absence of recurrence is the primary clinical goal, thus we will focus on studies which had at least one month or more of follow up.

TABLE 10.1 Estimates of necessary sample size for a trial to be able to detect a difference between the referent and desired bacterial vaginosis incidence.

Condition	Referent	Desired	Sample size
Recurrent BV	30% at 1 month	20% at 1 month	293 per arm
		15% at 1 month	120 per arm
Recurrent BV	60% at 6 months	40% at 6 months	97 per arm
		30% at 6 months	42 per arm

Considering only randomized studies over 85 participants, with more than one month of follow up, we are left with 10 studies: four evaluating an oral probiotic[10-13] and six that used a vaginal formulation.[14-19] Three of the four studies on oral probiotics first treated participants with metronidazole, and all treated women with a probiotic for 30-120 days. Of the four studies, one did not have clear specifications for the primary analysis, which suggests significant potential for bias.[12] Of the remaining three, two showed a statistically significant reduction in BV recurrence in the probiotic arm: one using *L. rhamnosus* GR-1 and *L. reuteri* RC-14[10], and one that used a combination of *L. crispatus* LMG S-29995, *L. brevis*, and *L. acidophilus* in proportion of 60%, 20%, and 20%, respectively.[11] The study which did not show benefit also used *L. rhamnosus* GR-1 and *L. reuteri* RC-14, but in a liquid drink form.[13]

Of the six studies meeting our selection criteria evaluating vaginal probiotics, all but one first

treated participants with antibiotics and then delivered the probiotic between seven days to 11 weeks, though some included intermittent repeat dosing. Three studies showed a significant reduction in BV recurrence (one using *L. rhamnosus, L. acidophilus, S. thermophilus*[19]; one using *L. gasseri* and *L. rhamnosus*[17]; and one using *L. crispatus*[15]), and three did not (products included an *L. casei* and *L. fermentum* impregnated tampon[16]; *L. acidophilus* alone[14]; or *L. casei* alone[18]).

In 2022 Liu *et al*. included eighteen studies in a review – however, two of the included studies reported the use of fluconazole, suggesting not all studies were for BV treatment.[20] In comparison with isolated antibiotics, antibiotics plus probiotics significantly decreased the recurrence rate of BV and increased the cure/remission rate of BV at 1-3 months and overall analysis. Compared with placebo, probiotics decreased the recurrence rate of BV (at 1-3 months and overall analysis) and increased the cure/remission rate of BV (at 1-3 months). In comparison with short-term probiotics treatment (<1 month), long-term probiotics treatment (1-3 months) yielded superior beneficial outcomes and efficacy in the treatment of BV. Besides, probiotics were indeed evidently more effective than placebo, and antibiotic plus probiotics produced better results than isolated antibiotics.

In 2021 Tidbury *et al*., including 33 studies for a systematic review, focused the metanalysis on two major categories: treatment and prevention of BV.[21] The authors considered as main outcomes efficacy of treatment, cure of BV, BV recurrence rate, improvement of vaginal microbiota and/ or clinical signs and symptoms. The treatment group was categorized based on the type of intervention (oral lactobacilli, vaginal lactobacilli, lactobacilli and estriol, lactobacilli supplementary to antibiotics, lactobacilli and estriol supplementary to antibiotics, lactic acid, and sucrose). The prevention group was based on whether the intervention was administered directly after standard antibiotic treatment (prevention of persistence) or on currently healthy women with a history of recurring BV (prevention of recurrence).

In the same year Munoz-Barreno *et al*. reported a total of 57 randomized controlled trials (RCTs), comparing the effectiveness of BV treatments with different doses of antibiotics and/or probiotics through oral and local administration.[22] The highest P-scores (a scores that estimates the effect sizes of pairwise treatment comparisons) in clinical cure rate were obtained by: (1) a combined therapy of local probiotic treatment, vaginal and oral antibiotic (5-nitroimidazole and clindamycin, respectively) (P-score = 0.92); (2) a combined therapy of oral administration of 5-nitroimidazole and probiotic treatment (P-score = 0.82); and (3) a combined therapy of local administration of 5-nitroimidazole and oral probiotic treatment (P-score = 0.68). Finally, combined therapies suggested a reduction of the optimal concentration of antibiotics, and double phase treatments of antibiotics indicated an increment of clinical cure rates in BV.[22]

In 2020 Jeng *et al*. tried to clarify the efficacy of probiotics in the treatment of common vaginal infections in non-pregnant females including vulvovaginal candidiasis (VVC), BV and mixed infection (BV plus VVC). In conclusion, the authors stressed the concept that probiotics as a supplement to conventional pharmacological treatments are effective in the short term for the treatment of common vaginal infections in non-pregnant adult females.[23] However, high-quality evidence for the effectiveness of probiotics alone in recurrent or curative vaginal infections is limited.

In 2019, two studies reported that probiotic regimes are safe and may exhibit a short and long-term beneficial effect on BV treatment but there is currently no strong evidence that probiotic monotherapy is more effective than traditional antibiotics.[24, 25]

Probiotics for bacterial vaginosis in pregnancy

The failure of antibiotic treatment in pregnant women with BV to reduce preterm birth risk has led investigators to postulate that the underlying causative biologic disorder may be the absence of *Lactobacillus* spp.. Early trials randomizing patients to oral probiotic or placebo were faulted for treating with an inappropriate strain or number of species of lactobacilli, an inadequate probiotic dose, duration or delivery route, or whether the administered probiotic was identified in the vaginal microbiota.

Husain *et al*.[26] sought to determine whether a daily oral probiotic containing *L. rhamnosus* and *L. reuteri* (each at 2.5 x 10^9 colony forming units [CFU] per dose) would colonize the vagina and reduce the incidence of BV. They randomized 304 women from East London between 9-14 weeks gestational age to either probiotic or placebo from entry until delivery. The primary outcome was the rate of BV at 18-20 weeks by Nugent score. At 18-20 weeks, BV was present in 15% of the probiotic group and in 9% of the placebo group, which was not statistically significant. They concluded that the oral probiotic used in the study did not reduce the incidence of BV in pregnant women. The trial was underpowered to detect a change in the preterm birth risk.

Yang *et al*.[27] also investigated whether abnormal Nugent scores in pregnancy could be normalized by a probiotic approach. They randomized 86 asymptomatic subjects under 17 weeks of gestation to twice daily oral *L. rhamnosus* GR-1 and *L. reuteri* RC-14 (each at 2.5 x 10^9 CFU per dose) or placebo for 12 weeks and assessed the vaginal microbiota, cytokines and chemokines at 26 and 35 weeks. There was no significant reduction in Nugent score, Shannon diversity index or in cytokines at 28 or 35 weeks in either arm.

Prebiotics and synbiotics

In one randomized trial of 100 women with BV, Hakimi *et al.* reported that concurrent use of a daily prebiotic vaginal gel containing 2% red clover extract, 10% inulin and 10% fructo-oligosaccharides improved the efficacy of oral metronidazole for BV treatment compared to a placebo gel (76 *vs.* 30%, p=0.012 cure by Amsel criteria and Nugent score at 10 days).[28]

Randomization to an oral synbiotic formulation containing *L. acidophilus, L. rhamnosus* and lactoferrin (a glycoprotein found in cervical mucus), was associated with a lower rate of BV recurrence at six months in people with recurrent BV compared to placebo (29 *vs.* 58%, p<0.05).[29, 30] Several studies have been conducted with a vaginal synbiotic vaginal formulation of *L. rhamnosus* and lactose, though many of these studies were unblinded.[31]

Nasioudis *et al*. proposed that restoration of *Lactobacillus* spp. dominance requires the restoration of innate immune factors such as lactoferrin that target anaerobic bacteria and the availability of nutrients favoring proliferation of lactobacilli.[32] Based on the ability of lactoferrin to sequester iron required by anaerobic bacteria, Miranda *et al*. reviewed data prospectively collected from all consecutive patients with a history of preterm birth who screened

positive for BV before 13 weeks and administered vaginal lactoferrin 300 mg daily for 21 days.[33] The primary outcome was preterm birth (<37 weeks) in those administered lactoferrin compared to similar patients who did not receive lactoferrin. They found that those who were administered lactoferrin had a significantly lower preterm birth rate (25 *vs.* 44.6%, p=0.02). No adverse events were reported. Because the study was not randomized and lacks microbiologic outcomes, we do not at this time recommend vaginal lactoferrin treatment.[34]

Clinical recommendations

In general, results are often not comparable between studies due to differences in species, strains, dose, and route of administration. Additionally, upon critical review there is a high risk of bias in many papers. Although there is not enough evidence yet for these alternatives to be a part of formal treatment recommendations, there may be some significant benefit for some people, without reported significant adverse effects. This makes them an attractive, despite unproven option for patients with refractory, recurrent BV and may be considered in a clinical setting. Several variables (such as *Lactobacillus* species and concentration, formulation, administration route, time and phases of treatment) should be considered.

Unresolved controversies include whether probiotics more successfully decrease the recurrence rate of BV in short (one month) *vs.* long-term period (three months) and if they have to be used after standard antibiotic treatment (prevention of persistence) or can be taken by currently healthy women with a history of recurring BV (prevention of recurrence), and whether repeat maintenance dosing is necessary. Also, it must be taken into account that probiotic formulations are usually expensive and, if effective, likely to have to be used for long periods of time.

Based on Husain[26], Yang[27], and Miranda's[33] studies we recommend against probiotic and synbiotic treatment of BV in pregnancy until subsequent studies are conducted that are powered to detect a reduction of the end points of preterm birth and chorioamnionitis.

10.3 Vulvovaginal candidiasis

Probiotics

The idea that promoting vaginal lactobacilli colonization for prevention or treatment of yeast is supported by *in vitro* laboratory data showing that many *Lactobacillus* species inhibit the growth of *Candida* spp., alter the expression of *Candida* spp. virulence factors or inhibit the hyphal transformation which is thought to increase the likelihood of symptoms.[35, 36] In mouse models, vaginal application of lactobacilli decreases fungal burden.[37, 38] However, in humans, several large epidemiologic studies have shown no association between a *Lactobacillus*-dominant microbiota and a lower risk for VVC. In fact, more often there is a higher prevalence of VVC in women with high proportions of vaginal lactobacilli.[39-43]

There are few well executed randomized clinical trials of sufficient size on which to base recommendations for use of probiotics for prevention of VVC. There are only two randomized trials with a sample size over 100. One is a randomized, open label trial of a vaginal product

including *L. acidophilus, L. rhamnosus, Steptococcus thermophilus*, and *L. delbrueckii* subsp. *bulgaricus* used daily for 10 days after an antifungal. Of the 416 premenopausal women included, 5% of the intervention group (antifungal followed by probiotic) and 37% of the antifungal only arm had a positive culture for *Candida* spp. 30-45 days after antifungal treatment.[44] The second trial randomized 278 women about to receive antibiotics for a non-gynecologic infection in a multifactorial design with four arms comparing oral and vaginal probiotics (oral: *L. rhamnosus, B. longum*; vaginal: *L. rhamnosus, L. delbrueckii, L. acidophilus, S. thermophilus*). There was no preventive effect of either formulation *vs*. placebo (odds ratio for oral formulation 1.06 [0.58-1.94] and vaginal formulation 1.38 [0.75-2.54]).[45]

One smaller randomized trial (N= 48) of an oral product with *L. acidophilus* and *L. rhamnosus* demonstrated a significantly lower rate of symptomatic candidiasis after three months of maintenance dosing.[46] A slightly larger study (N = 95) of a vaginally delivered product containing *L. gasseri, L. fermentum, L. rhamnosus* and *P. acidilacti*, used after an initial antifungal treatment, did not demonstrate any reduction in symptomatic VVC recurrence one month after treatment.[47] A more comprehensive review of data can be found in a number of recent reviews on this subject. [48-50]

Prebiotics and synbiotics

A small study of 48 women with acute, culture-positive vaginal candidiasis and a history of recurrent VVC randomized participants to vaginal clotrimazole and a concurrent oral synbiotic containing *L. acidophilus, L. rhamnosus* and lactoferrin or placebo. Participants continued maintenance dosing with the study product for 10 days a month for six months. Three months after antifungal treatment, the synbiotic group had lower rates of recurrence (8.3 *vs*. 66.7%, $p<0.01$).[46]

Clinical recommendations

Given the lack of high-quality data to support the efficacy of probiotics, and the epidemiologic evidence showing *in vivo* correlations between vaginal lactobacilli and VVC, we do not recommend use of either oral or vaginal probiotics for treatment or prevention of VVC.

10.4
Aerobic vaginitis/desquamative inflammatory vaginitis

Probiotics

One study tested the inclusion of a probiotic in their approach to aerobic vaginitis (AV) therapy. Heczko *et al*. screened women with a history of recurrent BV, and treated them with oral metronidazole and 10 days of oral probiotics containing *L. gasseri, L. plantarum, L. fermentum* (prOVag, IBSS, Poland).[51] At follow-up those with aerobes (AV) or resistant *Gardnerella* spp. by culture received targeted antibiotics and were randomized to 10 days of the oral probiotic mix or placebo each month for three months, with clinical and culture testing each month preformed one week after completion of the 10-day probiotic course. The authors state that time to AV relapse was up to 76% ($p<0.05$) longer in the probiotic group. Heczko's

study is challenging to interpret: inflammatory markers were not used to define AV cases nor outcomes, and outcomes measured (recurrence of symptoms or positive culture at a follow-up visits) do not distinguish BV from AV.

Prebiotics and synbiotics

While not strictly AV, insights into probiotic approach to correct dysbiosis and thus prevent AV can be gained from studies of intermediate microbiota (IM). Women with IM have Nugent scores from 4 – 6, are largely devoid of lactobacilli but do not have BV; they are often found with itchy irritation and discharge, and many will go on to have AV. Patients with IM are at risk for the same sequelae as those with AV. Russo *et al*. randomized 40 patients with itching, irritation, discharge and IM to the oral synbiotic product containing *L. acidophilus, L. rhamnosus* and 50 mg of bovine lactoferrin once daily for 15 days. Assessment at the end of treatment found significantly less itching and discharge ($p<0.001$), synbiotic intragroup normalization of Nugent score ($p=0.0004$) and reduction in Nugent score in the synbiotic arm *vs*. placebo ($p=0.0110$). Quantitative real time polymerase chain reaction demonstrated that lactobacilli were significantly increased after 15 days of the synbiotic, however data on durability of this finding are not available.[29]

Clinical recommendations

There are few data upon which to base clinical recommendations, so we would approach this similar to BV. For patients who have refractory symptoms and have failed standard therapy, there may be biologic plausibility that a probiotic or synbiotic containing lactoferrin could be helpful.

10.5 Trichomoniasis

Probiotics

Trichomonas vaginalis is the most common pathogenic protozoan in humans in industrialized countries. 5-nitroimidazole treatment is the only effective treatment, however recurrent infections are common, on some occasions due to re-infection, on others due to antibiotic resistance. Metronidazole resistance occurs in up to 10% of cases of vaginal trichomonas.

T. vaginalis co-infection with BV is a frequent occurrence. In this setting metronidazole effectiveness in treating trichomoniasis may be reduced and explained in part by the decreased redox potential found in BV subjects.[52, 53] *In vitro* evidence suggests that addition of a probiotic to metronidazole therapy increases the cure rate of metronidazole: Sgibnev *et al*. found that co-culture of opportunistic bacteria with human derived lactobacilli, or *L. rhamnosus* LCR35 supernatants containing hydrogen peroxide, lactic acid and surfactants, increased the antibiotic sensitivity of opportunistic bacteria.[54] The authors postulate that vaginal lactobacilli administration could improve *T. vaginalis' in vivo* sensitivity to metronidazole.

In a well-conducted clinical trial, Sgibnev *et al*. randomized 90 patients with *T. vaginalis* and

BV who failed prior therapy to receive metronidazole 500 mg twice a day and either vaginal *L. rhamnosus* CR35 or vaginal placebo twice for seven days, then to continue the vaginal probiotic twice daily for seven more days.[55] Symptoms, pH, redox potential, Nugent score and presence of *T. vaginalis* were assessed before the start of therapy and on the 4th, 8th and 15th day of therapy. The authors report that women in the treatment arm found symptoms improved significantly, confirmed by vaginal examination, and a significant decrease in *T. vaginalis* positive culture rate (6.8 *vs*. 47.6%) at the completion of metronidazole. As pH decreased and redox potential increased more intensely in the probiotic arm, the authors ascribe the improved cure rate to an increase in metronidazole effectiveness secondary to physiochemical changes induced by this probiotic in the presence of BV.

Prebiotics and synbiotics

No clinical trials have been conducted to test efficacy of numerous food, marine and medicinal extracts that display strong anti-trichomonal activity *in vitro*. If *in vitro* activity translates to *in vivo* and clinical activity these extracts could provide novel strategies to combat resistant trichomonads.[56]

Clinical recommendations

When *T. vaginalis* is refractory to therapy, and when BV is present, ancillary probiotic therapy may be considered.

10.6 Conclusion

It is biologically plausible that probiotics, prebiotics and synbiotics could improve treatment and prevention of BV, especially in cases that are refractory to standard antibiotic therapy. However, there is no consensus on the appropriate species, dose, formulation, delivery route or duration of treatment. Given the out-of-pocket expense for many of these products, we encourage caution in recommending their use, and follow up to assess efficacy.

Recommendations

Recommendation	Quality of evidence	Strength of recommendation
Monotherapy with probiotics for bacterial vaginosis is not recommended.	2a	B
There is no recommendation to prophylactically use probiotics or prebiotics in pregnancy.	3b	B
There is no benefit of using probiotics in women with vulvovaginal candidiasis.	2a	B
A probiotic or synbiotic containing lactoferrin can be tried in refractory cases of aerobic vaginitis/desquamative inflammatory vaginitis.	4	C
In refractory trichomoniasis, associated with bacterial vaginosis, probiotics can be added to the treatment schemes.	4	C

References

1. Hill, C.; Guarner, F.; Reid, G.; Gibson, G. R.; Merenstein, D. J.; Pot, B.; Morelli, L.; Canani, R. B.; Flint, H. J.; Salminen, S.; Calder, P. C.; Sanders, M. E., Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* 2014, 11, (8), 506-14.
2. Gibson, G. R.; Hutkins, R.; Sanders, M. E.; Prescott, S. L.; Reimer, R. A.; Salminen, S. J.; Scott, K.; Stanton, C.; Swanson, K. S.; Cani, P. D.; Verbeke, K.; Reid, G., Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol* 2017, 14, (8), 491-502.
3. Swanson, K. S.; Gibson, G. R.; Hutkins, R.; Reimer, R. A.; Reid, G.; Verbeke, K.; Scott, K. P.; Holscher, H. D.; Azad, M. B.; Delzenne, N. M.; Sanders, M. E., The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. *Nat Rev Gastroenterol Hepatol* 2020, 17, (11), 687-701.
4. Koirala, R.; Gargari, G.; Arioli, S.; Taverniti, V.; Fiore, W.; Grossi, E.; Anelli, G. M.; Cetin, I.; Guglielmetti, S., Effect of oral consumption of capsules containing Lactobacillus paracasei LPC-S01 on the vaginal microbiota of healthy adult women: a randomized, placebo-controlled, double-blind crossover study. *FEMS Microbiol Ecol* 2020, 96, (6).
5. Strus, M.; Chmielarczyk, A.; Kochan, P.; Adamski, P.; Chelmicki, Z.; Chelmicki, A.; Palucha, A.; Heczko, P. B., Studies on the effects of probiotic Lactobacillus mixture given orally on vaginal and rectal colonization and on parameters of vaginal health in women with intermediate vaginal flora. *Eur J Obstet Gynecol Reprod Biol* 2012, 163, (2), 210-5.
6. Bohbot, J. M.; Cardot, J. M., Vaginal impact of the oral administration of total freeze-dried culture of LCR 35 in healthy women. *Infect Dis Obstet Gynecol* 2012, 2012, 503648.
7. Houng, H. S.; Noon, K. F.; Ou, J. T.; Baron, L. S., Expression of Vi antigen in Escherichia coli K-12: characterization of ViaB from Citrobacter freundii and identity of ViaA with RcsB. *J Bacteriol* 1992, 174, (18), 5910-5.
8. De Alberti, D.; Russo, R.; Terruzzi, F.; Nobile, V.; Ouwehand, A. C., Lactobacilli vaginal colonisation after oral consumption of Respecta((R)) complex: a randomised controlled pilot study. *Archives of gynecology and obstetrics* 2015, 292, (4), 861-7.
9. Chen, C.; Hao, L.; Zhang, Z.; Tian, L.; Zhang, X.; Zhu, J.; Jie, Z.; Tong, X.; Xiao, L.; Zhang, T.; Jin, X.; Xu, X.; Yang, H.; Wang, J.; Kristiansen, K.; Jia, H., Cervicovaginal microbiome dynamics after taking oral probiotics. *J Genet Genomics* 2021, 48, (8), 716-726.
10. Anukam, K. C.; Osazuwa, E.; Osemene, G. I.; Ehigiagbe, F.; Bruce, A. W.; Reid, G., Clinical study comparing probiotic LactobacillusGR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic bacterial vaginosis. *Microbes Infect* 2006, 8, (12-13), 2772-6.
11. Reznichenko, H.; Henyk, N.; Maliuk, V.; Khyzhnyak, T.; Tynna, Y.; Filipiuk, I.; Veresniuk, N.; Zubrytska, L.; Quintens, J.; Richir, K.; Gerasymov, S., Oral Intake of Lactobacilli Can Be Helpful in Symptomatic Bacterial Vaginosis: A Randomized Clinical Study. *J Low Genit Tract Dis* 2020, 24, (3), 284-289.
12. Vujic, G.; Jajac Knez, A.; Despot Stefanovic, V.; Kuzmic Vrbanovic, V., Efficacy of orally applied probiotic capsules for bacterial vaginosis and other vaginal infections: a double-blind, randomized, placebo-controlled study. *Eur J Obstet Gynecol Reprod Biol* 2013, 168, (1), 75-9.
13. Zhang, Y.; Lyu, J.; Ge, L.; Huang, L.; Peng, Z.; Liang, Y.; Zhang, X.; Fan, S., Probiotic Lacticaseibacillus rhamnosus GR-1 and Limosilactobacillus reuteri RC-14 as an Adjunctive Treatment for Bacterial Vaginosis Do Not Increase the Cure Rate in a Chinese Cohort: A Prospective, Parallel-Group, Randomized, Controlled Study. *Front Cell Infect Microbiol* 2021, 11, 669901.
14. Bradshaw, C. S.; Pirotta, M.; De Guingand, D.; Hocking, J. S.; Morton, A. N.; Garland, S. M.; Fehler, G.; Morrow, A.; Walker, S.; Vodstrcil, L. A.; Fairley, C. K., Efficacy of oral metronidazole with vaginal clindamycin or vaginal probiotic for bacterial vaginosis: randomised placebo-controlled double-blind trial. *PLoS One* 2012, 7, (4), e34540.
15. Cohen, C. R.; Wierzbicki, M. R.; French, A. L.; Morris, S.; Newmann, S.; Reno, H.; Green, L.; Miller, S.; Powell, J.; Parks, T.; Hemmerling, A., Randomized Trial of Lactin-V to Prevent Recurrence of Bacterial Vaginosis. *N Engl J Med* 2020, 382, (20), 1906-1915.
16. Eriksson, K.; Carlsson, B.; Forsum, U.; Larsson, P. G., A double-blind treatment study of bacterial vaginosis with normal vaginal lactobacilli after an open treatment with vaginal clindamycin ovules. *Acta Derm Venereol* 2005, 85, (1), 42-6.
17. Larsson, P. G.; Stray-Pedersen, B.; Ryttig, K. R.; Larsen, S., Human lactobacilli as supplementation of clindamycin to patients with bacterial vaginosis reduce the recurrence rate; a 6-month, double-blind, randomized, placebo-controlled study. *BMC Womens Health* 2008, 8, 3.
18. Petricevic, L.; Witt, A., The role of Lactobacillus casei rhamnosus Lcr35 in restoring the normal vaginal flora after antibiotic treatment of bacterial vaginosis. *BJOG* 2008, 115, (11), 1369-74.
19. Ya, W.; Reifer, C.; Miller, L. E., Efficacy of vaginal probiotic capsules for recurrent bacterial vaginosis: a double-blind, randomized, placebo-controlled study. *Am J Obstet Gynecol* 2010, 203, (2), 120 e1-6.
20. Liu, H. F.; Yi, N., A systematic review and meta-analysis on the efficacy of probiotics for bacterial vaginosis. *Eur Rev Med Pharmacol Sci* 2022, 26, (1), 90-98.

21. Tidbury, F. D.; Langhart, A.; Weidlinger, S.; Stute, P., Non-antibiotic treatment of bacterial vaginosis-a systematic review. *Arch Gynecol Obstet* 2021, 303, (1), 37-45.
22. Munoz-Barreno, A.; Cabezas-Mera, F.; Tejera, E.; Machado, A., Comparative Effectiveness of Treatments for Bacterial Vaginosis: A Network Meta-Analysis. *Antibiotics (Basel)* 2021, 10, (8).
23. Jeng, H. S.; Yan, T. R.; Chen, J. Y., Treating vaginitis with probiotics in non-pregnant females: A systematic review and meta-analysis. *Exp Ther Med* 2020, 20, (4), 3749-3765.
24. Wang, Z.; He, Y.; Zheng, Y., Probiotics for the Treatment of Bacterial Vaginosis: A Meta-Analysis. *Int J Environ Res Public Health* 2019, 16, (20).
25. Chen, X.; Lu, Y.; Chen, T.; Li, R., The Female Vaginal Microbiome in Health and Bacterial Vaginosis. *Front Cell Infect Microbiol* 2021, 11, 631972.
26. Husain, S.; Allotey, J.; Drymoussi, Z.; Wilks, M.; Fernandez-Felix, B. M.; Whiley, A.; Dodds, J.; Thangaratinam, S.; McCourt, C.; Prosdocimi, E. M.; Wade, W. G.; de Tejada, B. M.; Zamora, J.; Khan, K.; Millar, M., Effects of oral probiotic supplements on vaginal microbiota during pregnancy: a randomised, double-blind, placebo-controlled trial with microbiome analysis. *Bjog* 2020, 127, (2), 275-284.
27. Yang, S.; Reid, G.; Challis, J. R. G.; Gloor, G. B.; Asztalos, E.; Money, D.; Seney, S.; Bocking, A. D., Effect of Oral Probiotic Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 on the Vaginal Microbiota, Cytokines and Chemokines in Pregnant Women. *Nutrients* 2020, 12, (2).
28. Hakimi, S.; Farhan, F.; Farshbaf-Khalili, A.; Dehghan, P.; Javadzadeh, Y.; Abbasalizadeh, S.; Khalvati, B., The effect of prebiotic vaginal gel with adjuvant oral metronidazole tablets on treatment and recurrence of bacterial vaginosis: a triple-blind randomized controlled study. *Arch Gynecol Obstet* 2018, 297, (1), 109-116.
29. Russo, R.; Edu, A.; De Seta, F., Study on the effects of an oral lactobacilli and lactoferrin complex in women with intermediate vaginal microbiota. *Arch Gynecol Obstet* 2018, 298, (1), 139-145.
30. Russo, R.; Karadja, E.; De Seta, F., Evidence-based mixture containing Lactobacillus strains and lactoferrin to prevent recurrent bacterial vaginosis: a double blind, placebo controlled, randomised clinical trial. *Benef Microbes* 2019, 10, (1), 19-26.
31. Baldacci, F.; Baldacci, M.; Bertini, M., Lactobacillus rhamnosus BMX 54 + Lactose, A Symbiotic Long-Lasting Vaginal Approach to Improve Women's Health. *Int J Womens Health* 2020, 12, 1099-1104.
32. Nasioudis, D.; Linhares, I. M.; Ledger, W. J.; Witkin, S. S., Bacterial vaginosis: a critical analysis of current knowledge. *Bjog* 2017, 124, (1), 61-69.
33. Miranda, M.; Saccone, G.; Ammendola, A.; Salzano, E.; Iannicelli, M.; De Rosa, R.; Nazzaro, G.; Locci, M., Vaginal lactoferrin in prevention of preterm birth in women with bacterial vaginosis. *J Matern Fetal Neonatal Med* 2021, 34, (22), 3704-3708.
34. Vieira-Baptista, P.; De Seta, F.; Verstraelen, H.; Ventolini, G.; Lonnee-Hoffmann, R.; Lev-Sagie, A., The Vaginal Microbiome: V. Therapeutic Modalities of Vaginal Microbiome Engineering and Research Challenges. *J Low Genit Tract Dis* 2022, 26, (1), 99-104.
35. MacAlpine, J.; Daniel-Ivad, M.; Liu, Z.; Yano, J.; Revie, N. M.; Todd, R. T.; Stogios, P. J.; Sanchez, H.; O'Meara, T. R.; Tompkins, T. A.; Savchenko, A.; Selmecki, A.; Veri, A. O.; Andes, D. R.; Fidel, P. L., Jr.; Robbins, N.; Nodwell, J.; Whitesell, L.; Cowen, L. E., A small molecule produced by Lactobacillus species blocks Candida albicans filamentation by inhibiting a DYRK1-family kinase. *Nat Commun* 2021, 12, (1), 6151.
36. Rose Jorgensen, M.; Thestrup Rikvold, P.; Lichtenberg, M.; Ostrup Jensen, P.; Kragelund, C.; Twetman, S., Lactobacillus rhamnosus strains of oral and vaginal origin show strong antifungal activity in vitro. *J Oral Microbiol* 2020, 12, (1), 1832832.
37. Jang, S. J.; Lee, K.; Kwon, B.; You, H. J.; Ko, G., Vaginal lactobacilli inhibit growth and hyphae formation of Candida albicans. *Sci Rep* 2019, 9, (1), 8121.
38. De Gregorio, P. R.; Silva, J. A.; Marchesi, A.; Nader-Macias, M. E. F., Anti-Candida activity of beneficial vaginal lactobacilli in in vitro assays and in a murine experimental model. *FEMS Yeast Res* 2019, 19, (2).
39. Baeten, J. M.; Hassan, W. M.; Chohan, V.; Richardson, B. A.; Mandaliya, K.; Ndinya-Achola, J. O.; Jaoko, W.; McClelland, R. S., Prospective study of correlates of vaginal Lactobacillus colonisation among high-risk HIV-1 seronegative women. *Sex Transm Infect* 2009, 85, (5), 348-53.
40. McClelland, R. S.; Richardson, B. A.; Hassan, W. M.; Graham, S. M.; Kiarie, J.; Baeten, J. M.; Mandaliya, K.; Jaoko, W.; Ndinya-Achola, J. O.; Holmes, K. K., Prospective study of vaginal bacterial flora and other risk factors for vulvovaginal candidiasis. *J Infect Dis* 2009, 199, (12), 1883-90.
41. Cotch, M. F.; Hillier, S. L.; Gibbs, R. S.; Eschenbach, D. A., Epidemiology and outcomes associated with moderate to heavy Candida colonization during pregnancy. Vaginal Infections and Prematurity Study Group. *Am J Obstet Gynecol* 1998, 178, (2), 374-80.
42. Tortelli, B. A.; Lewis, W. G.; Allsworth, J. E.; Member-Meneh, N.; Foster, L. R.; Reno, H. E.; Peipert, J. F.; Fay, J. C.; Lewis, A. L., Associations between the vaginal microbiome and Candida colonization in women of reproductive age. *Am J Obstet Gynecol* 2020, 222, (5), 471 e1-471 e9.

43. Brown, S. E.; Schwartz, J. A.; Robinson, C. K.; O'Hanlon, D. E.; Bradford, L. L.; He, X.; Mark, K. S.; Bruno, V. M.; Ravel, J.; Brotman, R. M., The Vaginal Microbiota and Behavioral Factors Associated With Genital Candida albicans Detection in Reproductive-Age Women. *Sex Transm Dis* 2019, 46, (11), 753-758.
44. Kovachev, S. M.; Vatcheva-Dobrevska, R. S., Local Probiotic Therapy for Vaginal Candida albicans Infections. *Probiotics Antimicrob Proteins* 2015, 7, (1), 38-44.
45. Pirotta, M.; Gunn, J.; Chondros, P.; Grover, S.; O'Malley, P.; Hurley, S.; Garland, S., Effect of lactobacillus in preventing post-antibiotic vulvovaginal candidiasis: a randomised controlled trial. *BMJ* 2004, 329, (7465), 548.
46. Russo, R.; Superti, F.; Karadja, E.; De Seta, F., Randomised clinical trial in women with Recurrent Vulvovaginal Candidiasis: Efficacy of probiotics and lactoferrin as maintenance treatment. *Mycoses* 2019, 62, (4), 328-335.
47. Ehrstrom, S.; Daroczy, K.; Rylander, E.; Samuelsson, C.; Johannesson, U.; Anzen, B.; Pahlson, C., Lactic acid bacteria colonization and clinical outcome after probiotic supplementation in conventionally treated bacterial vaginosis and vulvovaginal candidiasis. *Microbes Infect* 2010, 12, (10), 691-9.
48. Van de Wijgert, J.; Verwijs, M. C., Lactobacilli-containing vaginal probiotics to cure or prevent bacterial or fungal vaginal dysbiosis: a systematic review and recommendations for future trial designs. *Bjog* 2020, 127, (2), 287-299.
49. Xie, H. Y.; Feng, D.; Wei, D. M.; Mei, L.; Chen, H.; Wang, X.; Fang, F., Probiotics for vulvovaginal candidiasis in non-pregnant women. *Cochrane Database Syst Rev* 2017, 11, CD010496.
50. Shenoy, A.; Gottlieb, A., Probiotics for oral and vulvovaginal candidiasis: A review. *Dermatol Ther* 2019, 32, (4), e12970.
51. Heczko, P. B.; Tomusiak, A.; Adamski, P.; Jakimiuk, A. J.; Stefański, G.; Mikołajczyk-Cichońska, A.; Suda-Szczurek, M.; Strus, M., Supplementation of standard antibiotic therapy with oral probiotics for bacterial vaginosis and aerobic vaginitis: a randomised, double-blind, placebo-controlled trial. *BMC Womens Health* 2015, 15, 115.
52. Gatski, M.; Martin, D. H.; Levison, J.; Mena, L.; Clark, R. A.; Murphy, M.; Henderson, H.; Schmidt, N.; Kissinger, P., The influence of bacterial vaginosis on the response to Trichomonas vaginalis treatment among HIV-infected women. *Sex Transm Infect* 2011, 87, (3), 205-8.
53. Holmes, K. K.; Chen, K. C.; Lipinski, C. M.; Eschenbach, D. A., Vaginal redox potential in bacterial vaginosis (nonspecific vaginitis). *J Infect Dis* 1985, 152, (2), 379-82.
54. Sgibnev, A.; Kremleva, E., Influence of Hydrogen Peroxide, Lactic Acid, and Surfactants from Vaginal Lactobacilli on the Antibiotic Sensitivity of Opportunistic Bacteria. *Probiotics Antimicrob Proteins* 2017, 9, (2), 131-141.
55. Sgibnev, A.; Kremleva, E., Probiotics in addition to metronidazole for treatment Trichomonas vaginalis in the presence of BV: a randomized, placebo-controlled, double-blind study. *Eur J Clin Microbiol Infect Dis* 2020, 39, (2), 345-351.
56. Friedman, M.; Tam, C. C.; Cheng, L. W.; Land, K. M., Anti-trichomonad activities of different compounds from foods, marine products, and medicinal plants: a review. *BMC Complement Med Ther* 2020, 20, (1), 271.

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