Review Article

Bacterial Vaginosis—Vaginal Polymicrobial Biofilms and Dysbiosis

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Summary

Background: Bacterial vaginosis (BV) is the most common genital disease worldwide in women of sexually active age, with a prevalence of 23–29%. Its traditional definition as dysbiosis, i.e., a disruption of the normal balance of the vaginal microbiota, with a massive increase of facultative and obligate anaerobic bacteria (mainly Gardnerella spp.) and a loss of lactobacilli, accurately describes the change in the vaginal microbiota, but does not explain the underlying pathophysiology.

Methods: This review is based on information in pertinent articles retrieved by a selective literature search and on the authors' own research findings.

Results: Fluorescent in situ hybridization (FISH) has revealed Gardnerella spp.–dominated polymicrobial vaginal biofilm as a cause of ascending gynecologic and pregnancy-related infections, preterm birth, and infertility in patients with BV. The biofilm-induced disturbance of epithelial homeostasis favors co-infection with pathogens of sexually transmitted infection (STI). Standard antibiotic therapy is ineffective against biofilms, and there is thus a recurrence rate above 50%. The characteristic biofilm can be followed as a diagnostic marker and is considered evidence of sexual transmission when heterosexual couples and ejaculate samples are examined. FISH studies have shown that, in addition to biofilm-related vaginosis, there are other dysbiotic changes in the vaginal microbiota that have not yet been characterized in detail. It is therefore justified to speak of a "bacterial vaginosis syndrome."

Conclusion: The simplistic view of BV as dysbiosis, characterizable by microscopic reference methods, has so far led to inadequate therapeutic success. An evaluation of molecular genetic testing methods that would be suitable for routine use and the development of therapeutic agents that are effective against biofilms are urgently needed if the "bacterial vaginosis syndrome" is to be effectively treated.

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Bacterial vaginosis (BV) is the most common genital disease in women of sexually active age. Its prevalence worldwide is put at 23–29%, while in Germany it was detected in 20% of women in preterm-birth prevention programs (1, 2). Dysbiosis is defined as disruption of the vaginal microbiota that primarily causes increased vaginal discharge with a fishy odor but without signs of inflammation. In addition to local disorders in the vulvovaginal region, complications occur mainly due to ascending genital tract infections *(eTable)*. For example, BV patients are at a 1.53-fold higher risk for pelvic inflammatory disease (PID) and a 3.32-fold higher risk for infertility (3, 4). In pregnancy, BV increases the risk for preterm birth by a factor of 2.16 and for late miscarriage by a factor of 6.32 as a result of ascending infection (5). Furthermore, BV promotes co-infections with STI pathogens (STI, sexually transmitted infections), such as *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, human papillomaviruses (HPV) and human immunodeficiency virus (HIV) (6–8).

The BV treatment failure rate is unacceptably high. More than 50% of patients treated according to the guidelines experience a recurrence within 1 year (9). Treatment-refractory or recurrent BV involving at least three episodes per year causes impaired quality of life in > 65% of affected individuals (10). BV is associated with a high burden of disease that is often underestimated by the general public. Approaches to reliable prevention and treatment are urgently needed but require am understanding of BV pathogenesis.

Pathomechanisms

Gardnerella spp.-dominated polymicrobial vaginal biofilms

BV is traditionally defined as dysbiosis, that is, a disruption of the normal balance of the vaginal microbiota *(Table 1)* (11). In contrast to healthy women who have lactobacilli dominance and low bacterial diversity, BV patients exhibit a 1000-fold higher number of bacteria, greater diversity of facultative and obligate anaerobic bacteria, as well as suppressed lactobacilli (12). The factors that trigger dysbiosis have not been conclusively identified to date (13). A classic pathogen in the sense of Koch's postulates can also not be detected.

Using fluorescence in situ hybridization (FISH), it was possible to demonstrate that not only is there a far higher occurrence of bacteria on the vaginal

TABLE 1

Paradigm shift in the assessment of BV pathogenesis

Year of description			
1955	1982	2005	2022
Source/investigation technique			
Gardner und Dukes (18) • Gram stain: Clue cells: vaginal epithelial cells coated with short rod bacteria	Kristiansand conference (11) • Gram stain: Disruption of the normal balance of the vaginal microbiota of unknown etiology	Swidsinski (14) • FISH: *Gardnerella* spp.-dominated polymicrobial vaginal biofilms	Swidsinski (17) • FISH : *Gardnerella* spp.-dominated polymicrobial vaginal biofilms (clue cells) and other dysbiotic changes in the vaginal microbiota that have not yet been characterized in detail (pseudo clue cells)
Pathomechanism			
Monoinfection	Dysbiosis	Polymicrobial infection	Various nosologies: biofilm vaginosis and dysbioses
Name			
Haemophilus vaginalis vaginitis	Bacterial vaginosis	Biofilm vaginosis	Bacterial vaginosis syndrome

BV, bacterial vaginosis; FISH, fluorescence in situ hybridization

epithelium of patients with BV but that these bacteria are also present as a characteristic, confluent biofilm lying directly on epithelial cells (14). *Figure 1a* shows that this biofilm is primarily comprised of densely packed *Gardnerella* spp. lying adjacent to one another, as well as other bacterial species.

Until recently, *Gardnerella vaginalis* was considered to be the only species in the genus *Gardnerella*. However, genetic differences within this species have now been identified, on the basis of which 13 *Gardnerella* species have been differentiated: *G. vaginalis*, *G. piotii*, *G. leopoldii*, and *G. swidsinskii*, as well as nine further, hitherto unnamed species (15). According to recent research, several *Gardnerella* spp. co-occur in the BV biofilm (16).

In addition to *Fannyhessea vaginae*, formerly *Atopobium vaginae*, the species most commonly found *(eFigure 1)*, a broad spectrum of taxonomically widely differing bacterial species are found in the *Gardnerella* spp. biofilm scaffold. The vaginal biofilm explains the changes to the vaginal microbiota hitherto interpreted as dysbiosis, as well as the pronounced impairments to epithelial homeostasis in BV (12).

Clue cells

Figures 1a, b also illustrate that epithelial cells released in vaginal discharge during the process of natural desquamation are coated with the intact biofilm in BV patients. In vaginal wet mount specimens, these appear as cells covered—indeed, literally coated—by the polymicrobial biofilm *(Figure 2b* (17).

As early on as 1955, Gardner and Dukes found what they described as vaginal epithelial cells densely covered with short rod bacteria in women with vulvovaginal symptoms. Since these cells could not be detected in healthy women, the investigators regarded them as a diagnostic clue for bacterial vaginal infection and named them "clue cells" (18). Detection was by means of Gram stain, a staining method that enables only a differentiation of bacterial morphotypes (cocci/rod bacteria) and orientational information (Gram-positive/-negative), but no taxonomic identification of the pathogens. Therefore, it is not surprising that they failed to recognize the bacteria they detected on microscopy as a pathogenic species, *Haemophilus vaginalis* (later renamed *Gardnerella vaginalis*). Since this pathogen was also detected in the bacterial culture results of > 50% of healthy women in subsequent investigations and an abundance of accompanying anaerobic bacteria were additionally found in BV patients in the absence of signs of inflammation, a paradigm shift from bacterial monoinfection to dysbiosis took place in 1982 *(Table 1)*.

In 2005, FISH studies using a panel of different bacteria-specific probes showed that the bacteria described by Gardner and Dukes do not overlie vaginal epithelial cells as a "monoculture." *Gardnerella* spp. form the scaffold of a polymicrobial biofilm that can include any bacterial species in the vaginal microbiome. Thus, 50 years after clue cells were first described, the FISH method identified them as biofilm-coated vaginal epithelial cells, recognized the *Gardnerella* spp.-dominated vaginal biofilm as the crucial pathogenic agent in BV, and brought about yet another paradigm shift *(Table 1)* (19).

Pseudo clue cells

Recent FISH studies further revealed that in addition to biofilm vaginosis, dysbiotic changes without adherence to the vaginal mucosa also occur. These are diffuse accumulations *(Figure 2c)* or clusters of bacteria

(Figure 2d) irregularly distributed in the specimen and dominated by *Lactobacillus iners*, *Enterobacterales*, *Prevotella* spp., or *Fannyhessea vaginae*. What is notable is that this distinction is not made in routine laboratory testing of smear samples and that an assessment as "clue cell-positive, indicative of bacterial vaginosis" is made based on Gram staining. The rate of false results with pseudo clue cells is between 30 and 60% depending on the source/sender.

The already long-debated hypothesis that BV is a syndrome—comprising various nosologies—can thus be confirmed (13, 20). According to current knowledge, bacterial vaginosis syndrome includes not only vaginal epithelium-adherent "biofilm vaginosis" but also other non-cell-adherent dysbiotic changes in the vaginal microbiota that are detectable only in cervicovaginal discharge and that have yet to be characterized in more detail (17).

Complications

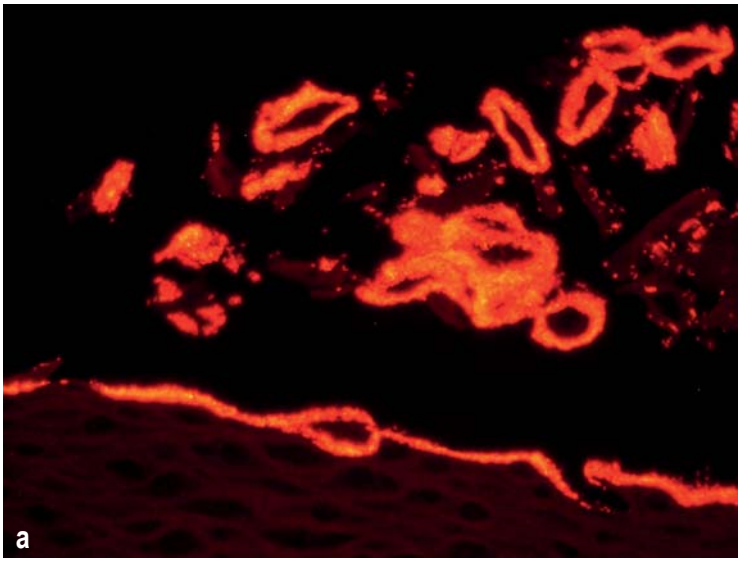
The *Gardnerella* spp.-dominated polymicrobial biofilm alters epithelial homeostasis of the vagina by reducing the viscosity of the cervicovaginal discharge and impairing the mucosal barrier, thereby promoting co-infections and ascending infections in the upper genital tract. In BV patients, FISH was also able to detect the characteristic biofilms outside the vagina in endometrial samples, fallopian tube samples, and spontaneous abortion material. These explain the increased risk for endometritis, salpingitis, tubo-ovarian abscess, pregnancy-related infections, and neonatal complications *(eFigure 2)*(21).

Complex interactions involving co-aggregation and metabolic cooperation occur between the species in the polymicrobial biofilm in BV, resulting in increased resistance to antibiotics or host immune defenses. STI pathogens also benefit from ecological interactions with the BV biofilm (12). In the case of exposure to *C. trachomatis*, *M. genitalium*, *N. gonorrhoeae*, *T. vaginalis*, HIV, and HPV, BV patients are twice as likely to develop disease compared to women without BV (6–8).

It has been demonstrated that treatment failure and recurrent disease in BV patients can be attributed to the insufficient effect of current therapeutic agents, such as metronidazole, moxifloxacin, and octenisept, on the biofilm *(eFigure 3)* (22–25).

Sexual transmission of biofilm vaginosis

STIs known to date are explained through transmission of a single pathogen. BV, on the other hand, is not caused by a single pathogen, but rather by a polymicrobial biofilm as a whole. For its transmission with all necessary microbial components, biofilm-coated epithelial cells (clue cells) are an ideal vector and can at the same time be used as a diagnostic marker to follow chains of infection. Clue cells in BV patients are found by means of the FISH technique in vaginal swabs as well as in urine samples, into which a high number of vaginal epithelial cells are always washed.



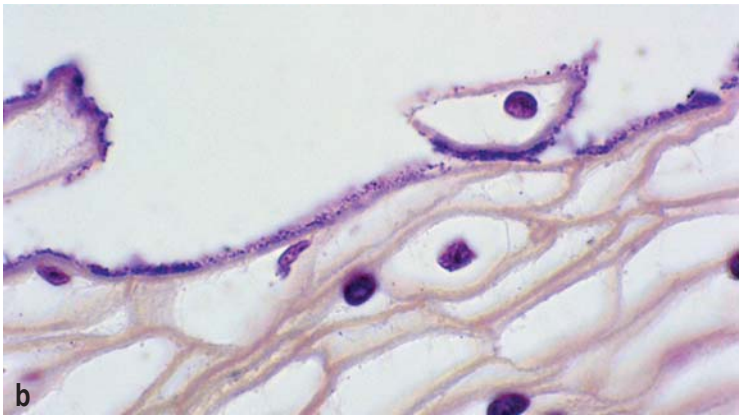


Figure 1:

a) Confluent *Gardnerella* spp.-dominated biofilm on the vaginal epithelium of a patient with bacterial vaginosis (*Gardnerella* spp. Cy5 probe [red fluorescence] × 400). One can discern the formation of clue cells through desquamation of vaginal epithelial cells with adherent biofilm.

b) Gram stain of a vaginal biopsy showing the formation of clue cells × 1000. The Gram stain also reveals how the clue cells are coated with the entire biofilm, thereby becoming a vector of infection transmission.

The examination of couples presenting for prenatal care showed high concordance between the detection of biofilm-coated clue cells in vaginal swabs and urine samples of pregnant women and the urine sample of the sexual partner. In all cases of clue cell-negative women, the partners' samples were also negative (26). Sequencing studies confirm that asymptomatic male partners of BV patients have an abundance of BV-associated bacteria (BVAB) in the subpreputial space and distal urethra, which serve as pathogen reservoirs for infections or re-infections (27). The detection of *Gardnerella* spp. biofilm-coated epithelial cells in three of 20 cryopreserved semen samples also showed that these cells can be sexually transmitted by asymptomatic male partners (28).

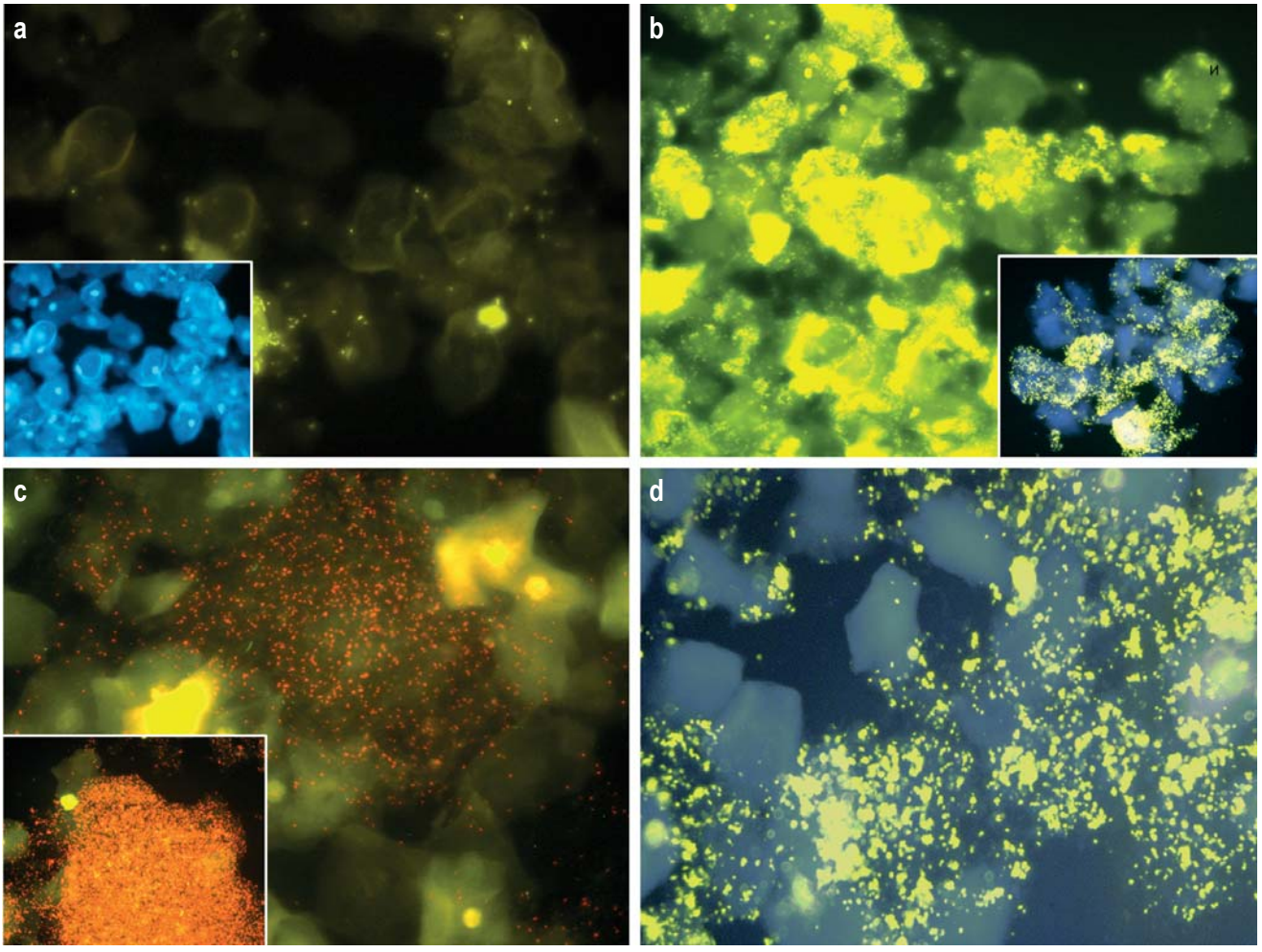


Figure 2:

a) Healthy premenopausal woman
Main image: Vaginal epithelial cells and isolated lactobacilli, *Lactobacillus* Cy3 probe (yellow fluorescence) × 400
Inset: Same microscopic field—clear contours of epithelial cells visible, stained with DAPI (nonspecific DNA stain, blue fluorescence) × 400

b) Biofilm vaginosis: clue cells
Main image: Biofilm-coated vaginal epithelial cells (clue cells), *Gardnerella* spp. Cy3 probe (yellow fluorescence) × 400
Inset: Clue cells with low bacterial density in a different patient with bacterial vaginosis (BV), *Gardnerella* spp. Cy3 probe (yellow fluorescence) and DAPI counterstain (blue fluorescence) × 400. Despite lower bacterial density, the biofilm on the epithelial cells can be clearly seen.

c) Dysbiotic change in the vaginal microbiota: pseudo clue cells
Main image: diffusely distributed bacteria (pseudo clue cells) and epithelial cells with surfaces free of bacteria, enterobacteria Cy5 probe (red fluorescence) × 1 000
Inset: massive accumulation of enterobacteria not adhering to epithelial cells in a different field of view of the same specimen (pseudo clue cells)

d) Dysbiotic changes in the vaginal microbiota: pseudo clue cells
Islands of growth/clusters of bacteria without adherence to epithelial cells (pseudo clue cells), *Lactobacillus iners* Cy3 probe (yellow fluorescence), DAPI counterstain (blue fluorescence) × 400

Taken as a whole, these results in heterosexual partners point to sexual transmission of biofilm vaginosis and confirm the epidemiological facts that have already been circulating in publications for many years, such as the occurrence of BV only in sexually active women, following a change of sexual partner, in the case of frequent changes of sexual partner, as well as the option of contraception using condoms (29, 30). Women who have sex with women (WSW) are as a matter of principle at greater risk for BV. In this risk group, sexual transmission is regarded as confirmed due to the association between incident BV and first sexual contact, change of sexual partner, contact to a BV-positive partner, or frequent change of sexual partner (31).

Clinical presentation and diagnosis

Symptomatic BV is characterized by increased, thin vaginal discharge, which is perceived as a sensation of moistness in the vulvar region as well as producing an unpleasant fishy odor. This increased vaginal discharge can cause vulvar irritation, dyspareunia, and dysuria. Signs of inflammation such as redness, swelling, and

TABLE 2

A comparison of testing methods for the diagnosis of BV

Test method	Criteria	Advantages	Disadvantages	Performance data
Reference method				
Amsel criteria (35, e11, e12)	Clinical/microscopy-based (wet mount microscopy/phase contrast) 1. Genital discharge 2. pH value 3. Odor of the discharge 4. Clue cells	Rapid point-of-care test	• Subjective criteria • Time-consuming	Comparison with Nugent score Sensitivity: 37–70% Specificity: 94–99%
Nugent score (36)	Microscopy-based laboratory method (Gram stain) 0–3: No indication of BV 4–6: No conclusive indication of BV 7–10: Indication of BV	Objective criteria	• Delayed reporting of findings • Complex evaluation • Intermediate range 4–6 unclear • Determination only of morphotypes/Gram behavior • No consideration of clue cells	Comparison with Amsel criteria: Sensitivity: 89% Specificity: 83%
Hay/Ison criteria (37)	Microscopy-based method (Gram stain) 0: No bacteria 1: No indication of BV 2: No conclusive indication of BV 3: Indication of BV 4: Gram-positive cocci	Objective criteria	• Delayed reporting of findings • Less complex evaluation • Intermediate range (2) inconclusive • Determination only of morphotypes/Gram behavior • No consideration of clue cells	Comparison with Amsel criteria Sensitivity: 97.5% Specificity: 96% PPV: 94% NPV: 96%
Molecular genetic methods				
FISH (38)	Fluorescence microscopy-based analysis of polymicrobial structures using 16S rRNA probes	Biofilm visualization in situ/ dysbiosis visualization	• Higher cost • Elaborate equipment • Manual evaluation	Comparison with reference methods: Sensitivity: 100% Specificity: 100%
Sequencing (39)	Gene sequencing (NGS)	Quantitative determination of the vaginal microbiome	• Very high cost • Elaborate equipment	Comparison with reference methods: Sensitivity: 100% Specificity: 95%
Multiplex qPCR (13, 40, e1)	Multiplex quantitative PCR	Commercially available automated test, indirect biofilm detection possible	• Higher cost • Limited evidence • Direct comparison with biofilm detection lacking	Comparison with reference methods: Sensitivity: 91–97% Specificity: 77–91%

BV, bacterial vaginosis; NGS, next generation sequencing; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value

pain are generally absent. Symptoms are tolerated by individual patients to a highly varying degree, with approximately 50% of women reporting no symptoms despite changes to the vaginal microbiota typical for BV (11).

Treatment-resistant or recurrent BV involving at least three episodes per year are problematic, with > 65% of affected women experiencing a negative impact on their sex lives, relationships, resilience, and mental health (10). For diagnosis, microscopy-based reference methods as well as molecular genetic methods are available, the advantages of which are presented in *Table 2*.

Reference methods

Guidelines developed by the International Union against Sexually Transmitted Infections (IUSTI) and by the American College of Obstetricians and Gynecologists (ACOG), as well as the current Association of the Scientific Medical Societies in Germany (AWMF) guideline "*Bakterielle Vaginose*" (bacterial vaginosis) of the German Society of Gynecology and Obstetrics (*Deutsche Gesellschaft für Gynäkologie und Geburtshilfe*, DGGG), recommend wet mount microscopy or Gram stain as reference methods (32–34). These methods are only able to to determine bacterial morphotypes or Gram stain behavior and do not provide any information on the taxonomy of the detectable pathogens. It is not possible to reliably differentiate between biofilm-coated epithelial cells (clue cells) and other types of dysbiotic changes in the vaginal microbiota (pseudo clue cells). Bacterial vaginosis syndrome is recorded.

Amsel criteria

The Amsel criteria (35) are assessed during gynecological examinations and point to BV if three out of four of the following features are present:

- Homogeneous, grayish-white vaginal discharge
- pH value > 4.5
- Fishy amine odor (upon addition of 10% potassium hydroxide)
- Detection of clue cells by wet mount microscopy.

TABLE 3

Current treatment recommendations

Active agent	Dose	Application	Duration	Reference
Standard therapy (first-line)				
Metronidazole	2 × 400–500 mg	p. o.	5–7 Days	WHO, CDC, IUSTI, DGGG, DSTIG
Clindamycin	2 × 300 mg	p. o.	7 Days	WHO, CDC, IUSTI, DGGG, DSTIG
Metronidazole	1–2 × 0.75% Gel (5 g)	Intravaginal	5–7 Days	WHO, CDC, IUSTI, DGGG
Clindamycin	1 × 2% Cream (5 g)	Intravaginal	7 Days	CDC, IUSTI, DGGG, DSTIG
Clindamycin	1 × 100 mg Ovule	Intravaginal	3 Days	DGGG
Alternatives				
Metronidazole	1 × 2 g	p. o.	1–2 Days	WHO, IUSTI, DGGG
Tinidazole	1 × 2 g	p. o.	1–2 Days	CDC, IUSTI, DGGG
Tinidazole	1 × 1 g	p. o.	5 Days	CDC, IUSTI, DGGG
Dequalinium chloride	10 mg Vaginal tablet	Intravaginal	6 Days	IUSTI, DGGG
Octenidine	Day 1: 2 × 1 Spray Day 2–7: 1 × 1 Spray	Intravaginal	7 Days	DGGG

CDC, Centers for Disease Control and Prevention; DGGG, German Society of Gynecology and Obstetrics (*Deutsche Gesellschaft für Gynäkologie und Geburtshilfe*); DSTIG, German STI Society (*Deutsche STI-Gesellschaft*; a medical society for the promotion of sexual health); IUSTI, The International Union Against Sexually Transmitted Infections; p. o., per os; WHO, World Health Organization

Nugent score

Microscopy-based determination of the Nugent score (36) is generally performed as a laboratory test independently of clinical information. By assessing Gram staining, three morphotypes are quantitatively evaluated according to a predetermined protocol:

- *Lactobacillus*: long Gram-positive rods
- *Gardnerella*/anaerobic rods: short Gram-negative or Gram-variable rods
- *Mobiluncus*: curved Gram-variable rods.

The sum of the determined scores corresponds to the categories 0–3: "no indication of BV," 4–6: "no clear indication," and 7–10: "indication of BV."

Hay/Ison criteria

The Hay/Ison criteria (37) enable a simpler assessment of Gram-stained vaginal smears since the evaluation of morphotypes is semiquantitative. Additional categories for smears with no bacteria or with only Gram-positive cocci are also taken into consideration.

Molecular genetic methods

In the last 20 years, polymerase chain reaction (PCR) tests and sequencing techniques, alongside FISH, have been evaluated for BV diagnosis. In contrast to the reference methods, these techniques are able to map changes to the vaginal microbiota both quantitatively and on the taxonomic level. Due to the higher costs involved, they are not yet established in routine diagnostics.

Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) is based on the detection of 16S rRNA, which is present in a high copy number of 10^3–10^5 in the ribosomes of bacterial cells and contains species-specific as well as group-specific or universal bacterial segments. For the diagnosis of bacterial vaginosis syndrome, a panel of fluorescently labeled probes is used that encompasses the relevant bacteria/bacterial group in the vaginal microbiota. From a microscopy point of view, probes labeled with various fluorescent dyes can be viewed simultaneously (multicolor FISH), thereby making "mixed cultures" transparent. FISH is the only method for the direct detection of polymicrobial biofilms. It simultaneously enables the taxonimc identification and assessment of the spatial arrangement of microorganisms, and with this technique, one is additionally able to determine the morphology of the material under analysis thanks to background fluorescence (38).

Sequencing

Results of gene sequencing of vaginal discharge samples demonstrate a significant association with clinically/microscopically confirmed BV for *Gardnerella* spp. and *F. vaginae*, as well as *Megasphaera* spp., *Sneathia sanguinegens*, *Candidatus Lachnocurva vaginae*, *Mageeibacillus indolicus*, *Mobiluncus* spp., *Leptotrichia amnionii*, and *Eggerthella* spp. None of these bacterial vaginosis-associated bacteria (BVAB) has sufficient sensitivity or specificity for diagnostic purposes. High concordance with the reference methods is achieved when a combined quantitative determination of positive and negative predictors is performed. For example, high copy numbers of *Gardnerella* spp. ($\geq 10^9$ copies/mL) and *F. vaginae* ($\geq 10^8$ copies/mL) combined with a low proportion of *Lactobacillus* DNA are suggestive for the presence of BV (39).

Quantitative multiplex PCR

In Germany, four CE-IVD-marked PCR tests, that is, tests that are officially approved for medical products and in vitro diagnostics, are available from a number of manufacturers for the diagnosis of BV. They follow the sequencing-based algorithm and quantitatively determine *Gardnerella* spp., *F. vaginae*, and *Lactobacillus* spp. DNA, as well as additionally providing a qualitative assessment of other BVAB DNA where necessary (13, 40, e1). Quantitative multiplex PCR (qPCR) can make it possible to indirectly detect a biofilm (38), thereby differentiating biofilm vaginosis from other dysbiotic changes in bacterial vaginosis syndrome. Corresponding comparative studies using FISH are still awaited. This diagnostic method would be particularly beneficial for pregnant women and women wishing to start a family, as well as in the case of in vitro fertilization (IVF) and risk of STI, since it provides an indication of the problems to be expected in antibiotic therapy.

Treatment

For decades, representative guidelines have been recommending broad-spectrum antibiotics that act against anaerobic bacteria such as metronidazole and clindamycin as standard therapy for BV *(Table 3)*. A Cochrane analysis shows identical 4-week cure rates for the two drugs, irrespective of the mode of administration/application, of approximately 70–85% (combined relative risk [RR] 0.91; 95% confidence interval: [0.70; 1.18]). Clindamycin tended to produce fewer side effects compared to metronidazole (RR 0.75; [0.56; 1.02]) (e2).

However, antibiotic therapy did not lead to long-term freedom from symptoms. Despite the favorable pharmacodynamics, > 50% of treated patients experience recurrence (e3). A prospective study in which oral metronidazole was administered reported a recurrence rate of 58% at 1 year [49; 66] (e4). All previously investigated modifications, such as extended treatment duration, long-term suppressive treatments, combined oral and vaginal antibiotic therapy, and adjunctive intravaginal or oral probiotic treatment, confer no significant benefit in terms of long-term treatment success (e5).

The causes of this high treatment failure rate are thought to be the insufficient therapeutic effect of antibiotics on the *Gardnerella* spp.-dominated polymicrobial biofilm, an intrinsic resistance of *Gardnerella* species to metronidazole, failure to recolonize the vagina with lactobacilli, as well as reinfection occurring from sexual partners (22, e6, e7).

In view of the frequency of BV, the severity of its complications, and the high treatment failure rate, there is an urgent need for the development of new therapeutic approaches (9, e5). Alternative therapeutic agents that are effective on the biofilm (antiseptics, natural antimicrobial agents, plant extracts, probiotics, and prebiotics) are undergoing trials as monotherapies or adjuncts to antibiotic BV therapy (e8). At present, the use of phage therapy appears to be promising. The genetically engineered endolysin PM-477, a cell wall-degrading enzyme originally found in bacteriophages, exhibits a highly selective bactericidal effect on *Gardnerella* spp. when used on the vaginal discharge of BV patients (e6).

Therapeutic considerations are also focused on treatment of the partner. Epidemiological data demonstrate that incident BV is associated with a change of partner. In contrast, women with BV in a steady sexual relationship with an untreated partner are at an approximately two- to three-fold higher risk for BV recurrence. A pilot study that combined oral metronidazole therapy of female BV patients with oral metronidazole and topical clindamycin gel applied to the penile skin of male partners demonstrated a reduction in recurrence rates (e9). However, no significant progress can be expected until testing systems have been established that have the lytic action of anti-infective agents on polymicrobial communities such as biofilms or dysbiosis, thereby enabling more targeted treatment (e10).

Conflict of interest statement
The authors declare that no conflict of interest exists.

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► **TSupplementary material**

eReferences, eTable, eFigures:
www.aerzteblatt-international.de/m2023.0090

Supplementary material to:

Bacterial Vaginosis—Vaginal Polymicrobial Biofilms and Dysbiosis

by Sonja Swidsinski, Wiltrud Maria Moll, and Alexander Swidsinski

Dtsch Arztebl Int 2023; 120: 347–54. DOI: 10.3238/arztebl.m2023.0090

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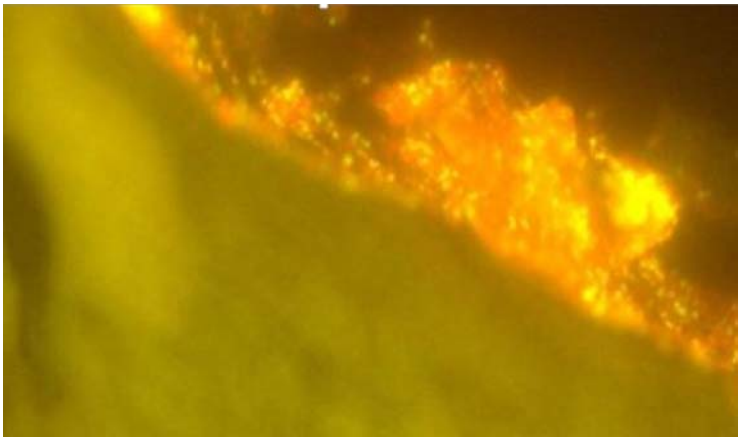
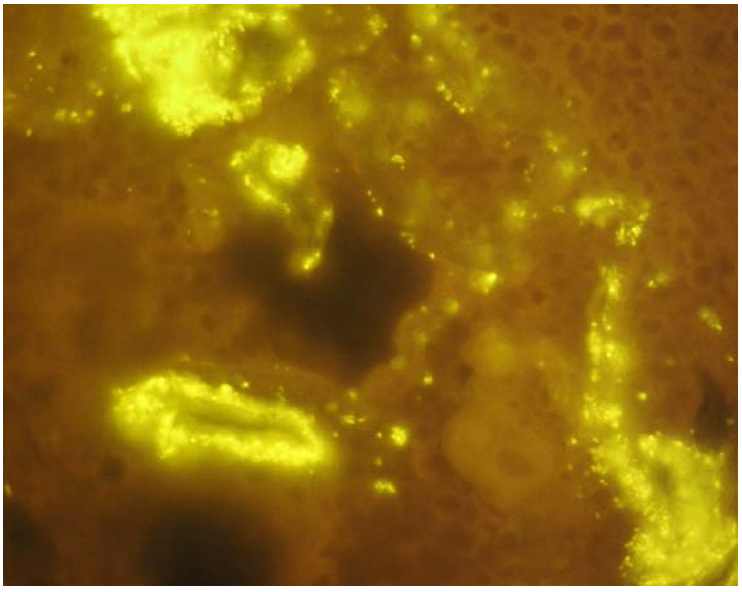
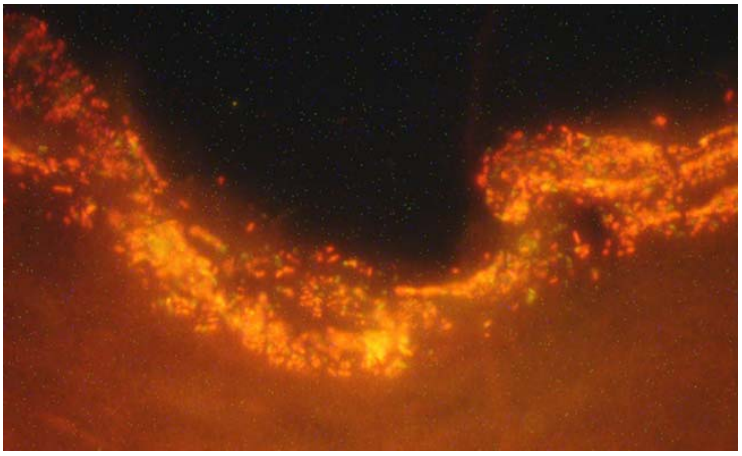


eFigure 1: Polymicrobial composition of the biofilm, illustrated using the example of *Fannyhessia vaginae* bacterial cells integrated in the *Gardnerella* spp. biofilm (*Fannyhessia vaginae* probe, Cy3 [yellow fluorescence] and *Gardnerella* spp. probe, Cy5 [red fluorescence] × 1000)



eFigure 2: *Gardnerella* spp. biofilm in the endometrium (luteal); (*Gardnerella* spp. Cy3 probe, yellow fluorescence × 1000)



eFigure 3: Proliferating *Gardnerella* spp.-dominated polymicrobial biofilm on the vaginal epithelium at day 35 following completion of standard metronidazole therapy (*Gardnerella* spp. Cy5 probe, red fluorescence, *Fannyhessia vaginae* Cy3 probe, yellow fluorescence × 400)

eTABLE

Infection-related complications in patients with bacterial vaginosis

	Risk [95% CI]	Study size	Study design (reference)
Complications			
PID	aHR 1.53 [1.05; 2.21]	N = 2956	Prospective cohort study over 12 months (3)
Infertility	Women with infertility compared to antenatal women in the same population: OR 3.32 [1.53; 7.20]	N = 3229	Meta-analysis, 12 studies (4)
Pregnancy complications	Risk of preterm birth: OR 2.16 [1.56; 3.00] Risk for late miscarriage: OR 6.32 [3.65; 10.94]	N = 30,158	Meta-analysis, 32 studies (5)
STI co-infections			
Chlamydia trachomatis *Neisseria gonorrhoeae* *Trichomonas vaginalis*	Incident *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* infection: aHR 1.73 [1.42; 2.11]	N = 3620	Prospective multicenter study, 12 centers over 12 months (6)
Mycoplasma genitalium	*Mycoplasma genitalium* infection: aOR 3.49 [1.86; 6.56]	N = 280	Cohort study, sex workers in Kenya (7)
HIV	Incident HIV infection: RR 1.61 [1.21; 2.13]	N = 30,739	Meta-analysis, 23 HIV incidence studies (8)
Human papillomaviruses (HPV)	Significant association between BV and HPV infection: $p < 0.001$	N = 10,456	Retrospective study (e13)

Reference category is women without BV;
aHR, adjusted hazard ratio; aOR, adjusted odds ratio; BV, bacterial vaginosis; CI, confidence interval; OR, odds ratio; PID, pelvic inflammatory disease; RR, relative risk; STI, sexually transmitted infections

Questions on the article in issue 20/2023:

Bacterial Vaginosis—Vaginal Polymicrobial Biofilms and Dysbiosis

cme plus

The submission deadline is 18 May 2024. Only one answer is possible per question.
Please select the answer that is most appropriate.

Question 1

In a German preterm birth prevention program, how high was the proportion of pregnant women in whom bacterial vaginosis was identified?

a) An eighth
b) Three quarters
c) Two thirds
d) A fifth
e) A tenth

Question 2

The biofilm that forms directly on the epithelium in bacterial vaginosis primarily consists of which bacteria?

a) *Lactobacillus gasseri*
b) Döderlein's lactobacillus
c) *Gardnerella* spp.
d) Group B streptococci
e) *Neisseria gonorrhoeae*

Question 3

What type of cells are the "clue cells" described in the text?

a) Bacteria
b) Intraepithelial lymphocytes
c) Erythrocytes
d) Keratinocytes
e) Vaginal epithelial cells

Question 4

What change to vaginal homeostasis as a result of bacterial vaginosis does the text describe?

a) Greenish discoloration of cervicovaginal discharge
b) Vaginal pH value of less than 4.0
c) Increased mucosal barrier
d) Reduced viscosity of cervicovaginal discharge
e) Increased perfusion of the vaginal mucosa

Question 5

Which if the following criteria/techniques is reported to have both a sensitivity and a specificity of 100% in the diagnosis of bacterial vaginosis?

a) Amsel criteria
b) Nugent score
c) Quantitative multiplex PCR (qPCR)
d) Sequencing
e) FISH technique

Question 6

Which of the following statements regarding clue cells is true?

a) Clue cells can also be sexually transmitted by asymptomatic males.
b) Clue cells get their name from fact that they are particularly adherent.
c) Clue cells were first described in 2005 in the context of FISH testing.
d) Clue cells are not considered to be a vector for the transmission of bacterial vaginosis infection.
e) Clue cells are generally not coated with a biofilm.

Question 7

In which setting do pseudo clue cells occur?

a) Biofilm vaginosis
b) Previous bacterial vaginosis
c) Dysbiotic changes in the vaginal microbiota
d) Healthy vaginal microbiome
e) Pre-menarche girls

Question 8

Which of the following symptoms is <u>not</u> mentioned in the text as being a typical sign of bacterial vaginosis?

a) Pain during sexual intercourse
b) Feeling of dryness in the vulvar region
c) Dysuria
d) Increased vaginal discharge
e) Fishy odor

Question 9

When calculating the microscopy-based Nugent score, a number of different morphotypes are described. Which bacterial morphology is referred to as *Mobiluncus*?

a) Gram-positive straight rods
b) Gram-negative short rods
c) Gram-positive cocci
d) Gram-negative cocci
e) Gram-variable curved rods

Question 10

Which antibiotics are currently recommended by a number of guidelines?

a) Metronidazole and clindamycin
b) Tinidazole and gentamicin
c) Penicillin and tinidazole
d) Octenidine and amoxicillin
e) Dequalinium chloride and vancomycin