The biofilm in bacterial vaginosis: implications for epidemiology, diagnosis and treatment

Hans Verstraelen[a] *and Alexander Swidsinski*[b]

Purpose of review
Recent evidence supports the view that bacterial vaginosis presents as a polymicrobial biofilm infection. This has far-reaching implications for the pathogenesis, epidemiology, diagnosis and treatment of bacterial vaginosis.

Recent findings
Gardnerella vaginalis is presumably the first species to adhere to the vaginal epithelium and then becomes the scaffolding to which other species adhere. Not all *G. vaginalis* strains do form biofilms: *G. vaginalis* can be present in the vagina in a planktonic or in a biofilm mode of growth. The presence of planktonic (dispersed) or biofilm-associated (cohesive) *G. vaginalis* can be reliably shown in urine sediments in both women and men, and there is an absolute concordance in the carriage of biofilm-associated (cohesive) *G. vaginalis* between women with bacterial vaginosis and their partners. In-vitro data suggest that selected probiotic lactobacilli might be an effective means to conquer the biofilm.

Summary
Future epidemiological research may benefit from biofilm-based urine diagnosis of bacterial vaginosis to a significant extent. The search for novel therapeutic agents can now be more directed towards the biofilm-breaking agents, but is at present hampered by the lack of a proper in-vitro model of the bacterial vaginosis biofilm.

Keywords
Atopobium vaginae, bacterial vaginosis, biofilm, *Gardnerella vaginalis*, polymicrobial infection

INTRODUCTION

Contrary to our traditional view of infectious diseases resulting from colonization with a single microbial species, it has recently been acknowledged – mostly because of the advent of molecular bacterial identification techniques – that microbes actually rarely exist as single-species planktonic forms, but rather the majority of microbes are found thriving in complex polymicrobial biofilm communities [1▪▪]. This has recently been shown to be also the case for bacterial vaginosis, a pervasive polymicrobial infestation of the vagina that affects millions of women worldwide. Swidsinski *et al.* [2▪▪] documented through fluorescence in-situ-hybridization (FISH) analysis of vaginal biopsies obtained from women with and without bacterial vaginosis that a characteristic dense biofilm in confluent or patchy layers covered at least 50% of the vaginal epithelial surface in 90% of biopsies obtained from women with bacterial vaginosis as compared to 10% of women without bacterial vaginosis. Vaginal biopsies in women without bacterial vaginosis showed mostly loosely dispersed lactic acid bacteria, including *Lactobacillus*, *Streptococcus*, and *Enterococcus* bacteria. The biofilm was found to consist primarily of *Gardnerella vaginalis*, whilst *Atopobium vaginae* was present in 80% of the cases and made up 40% of the biofilm mass. Other bacteria were found more inconsistently, including bacteria belonging to the *Bacteroides, Corynebacterium, Lactobacillus, Veillonella, Ruminococcus* and *Streptococcus* genera [2▪▪].

In the present review, we will give a brief overview of the implications of this discovery for the

[a]Department of Obstetrics and Gynaecology, Vulvovaginal Disease Clinic, Ghent University, Ghent, Belgium and [b]Laboratory for Molecular Genetics, Polymicrobial Infections and Bacterial Biofilms, Department of Medicine, Gastroenterology, Universitätsmedizin Berlin, Berlin, Germany

Correspondence to Hans Verstraelen, MD, PhD, Department of Obstetrics and Gynaecology, Ghent University Hospital, De Pintelaan 185, B-9000 Ghent, Belgium. Tel: +32 9 240 37 96; fax: +32 9 240 38 31; e-mail: hans.verstraelen@ugent.be

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KEY POINTS

- *G. vaginalis* can be present in the vagina in a planktonic or in a biofilm mode of growth.
- The presence of planktonic (dispersed) or biofilm-associated (cohesive) *G. vaginalis* can be reliably shown in urine sediments in both women and men.
- There is an absolute concordance in the carriage of biofilm-associated (cohesive) *G. vaginalis* between women with bacterial vaginosis and their partners.
- *G. vaginalis* is presumably the first species to adhere to the vaginal epithelium and then becomes the scaffolding to which other species adhere.
- No proper in-vitro model of the bacterial vaginosis film exists at present, thereby hampering the search for new therapeutic agents.

epidemiology, diagnosis and treatment of bacterial vaginosis in the light of some recent evidence.

IMPLICATIONS FOR THE EPIDEMIOLOGY OF BACTERIAL VAGINOSIS

With only a few risk factors having been identified for bacterial vaginosis, study of bacterial vaginosis epidemiology has traditionally largely focussed on the occurrence of bacterial vaginosis in relation to sexual behaviour. The literature on the epidemiology of *G. vaginalis* and bacterial vaginosis in relation to sexual behaviour has recently comprehensively been reviewed [3]. Clearly, the epidemiology of bacterial vaginosis is intricate, and from the latter review data emerged that support male-to-female transmission as well as female-to-male transmission of bacterial vaginosis.

Building further on the discovery of the bacterial vaginosis biofilm [2▪▪], Swidsinski *et al.* [4▪▪] refined the picture of bacterial vaginosis transmissibility to a significant extent. The authors first described that the presence of *G. vaginalis* can also reliably be assessed through FISH analysis of desquamated epithelial cells in urine sediments in both women and men, as further discussed below. By doing so, it was observed that *G. vaginalis* presented in two distinct ways, that is, desquamated cells in urine showed either 'dispersed' *Gardnerella*, consisting of loosely dispersed *Gardnerella* cells indicative of planktonic growth, or 'cohesive' *Gardnerella*, consisting of clustered *Gardnerella* cells adhesive to the epithelium, the latter indicative of the presence of *Gardnerella* in a biofilm mode of growth [4▪▪].

The authors then enrolled, amongst others, 20 women with symptomatic bacterial vaginosis and 10 of their partners, and 72 consecutive married pregnant women and their 72 partners [4▪▪]. It was shown that the 20 women with symptomatic bacterial vaginosis consistently presented with cohesive *Gardnerella* as did the 10 partners investigated. Amongst the 72 married pregnant women and their 72 partners, dispersed *Gardnerella* was found in 14% and cohesive *Gardnerella* in 17% of the women. Again, cohesive *Gardnerella* was consistently found amongst the partners of the women with cohesive *Gardnerella* for whom the samples were analysable. No such concordance was observed for dispersed *Gardnerella*. Hence, the previously known strong concordance of *G. vaginalis* carriage by both partners when a woman has bacterial vaginosis [3] was herewith [4▪▪] confirmed, but further refined by the almost absolute concordance of cohesive *Gardnerella* carriage. From these observations, it may be inferred that the biofilm mode of growth represents the infectious or transmissible mode of *Gardnerella* and bacterial vaginosis, whereas the mere presence of dispersed *Gardnerella* seems at present of less clinical significance. Of note in this respect is that bacterial density within the biofilm mass is within the order of 10^{10-11} cells per gram as compared to 10^{6-8} cells per gram for planktonic bacteria in vaginal fluid [5].

As mentioned above, the aforementioned approach also allows detection of cohesive *Gardnerella*, consisting of voided epithelial cells covered with the polymicrobial *Gardnerella* biofilm, in men. This is of particular interest for epidemiologic bacterial vaginosis research. For instance, Swidsinski *et al.* [6] documented the presence of cohesive *Gardnerella* covering epithelial cells in randomly selected cryopreserved semen samples, reflecting a possible mode of infection. Of note is that voided epithelial cells are only recovered in sufficient amounts in men if the prepuce rests over the glans penis when voiding and not when the prepuce is pulled back, which also seem to suggest that the voided cells are preputial pocket-lining cells [4▪▪].

It is not entirely clear from the recent genomic studies whether the dispersed and cohesive *Gardnerella* modes of growth also represent different *Gardnerella* strains. Yeoman *et al.* [7] revealed that different *Gardnerella* strains show substantial differences in metabolic and virulence potential; however, they also found that all strains investigated had exopolysaccharide biosynthetic genes that are involved in encoding biofilm formation. Harwich *et al.* [8▪▪] however conducted a genomic analysis of two *Gardnerella* strains, isolated from a woman with bacterial vaginosis and a woman without bacterial vaginosis, respectively. They found that the bacterial vaginosis-associated strain encoded a different variant of a biofilm-associated protein gene and

demonstrated greater adherence, aggregation and biofilm formation, which indeed points at differing genotypes showing difference virulence patterns, including the capability of biofilm formation.

Data obtained from a recent in-vitro study further suggest that of all bacterial vaginosis-associated bacteria, *G. vaginalis* might be the initial colonizer of the vaginal epithelium because of its adherence and biofilm-forming capacities [9▪]. It has previously been shown in oral infections that a single species may be the first to adhere to a tissue and then becomes the scaffolding to which other species adhere, a process known as coaggregation [1▪▪]. This might explain why *G. vaginalis* and *A. vaginae* are such strong indicators of bacterial vaginosis [10], whilst other bacteria, presumably secondary colonizers, are found in a less consistent manner in association with bacterial vaginosis.

IMPLICATIONS FOR THE DIAGNOSIS OF BACTERIAL VAGINOSIS

Current gold-standard diagnosis of bacterial vaginosis relies on categorizing Gram-stained vaginal smears under oil immersion microscopy through Nugent scoring [11] or modifications thereof like Hay–Ison scoring [12]. Overall, the Nugent scoring system has a high degree of accuracy, high intracentre and intercentre reliability, as well as high intraobserver and interobserver reproducibility [13]. Swidsinski *et al.* [4▪▪] have recently described an alternative method of diagnosis, based on the FISH-based visualization of the bacterial vaginosis biofilm on desquamated vaginal epithelial cells present in the urine sediment. This method is particularly suited for the epidemiological and clinical studies; urine sediments fixated in Carnoy solution [2▪▪,4▪▪] can be stored for prolonged periods of time and the aliquots can be used for repeated FISH hybridizations under standardized conditions. In addition, urine samples are easily obtained in a noninvasive manner, without the need for a physician.

In a cohort of 72 pregnant women, this method was compared with Nugent scoring [4▪▪]. Amongst the 12 women with cohesive *Gardnerella*, 10 had bacterial vaginosis according to Nugent's score and 2 had intermediate microbiota. Dispersed *Gardnerella* occurred with eight women and all had Nugent scores of 3 or less. Amongst the 52 women in whom no *Gardnerella* could be documented through FISH, 2 had a Nugent score of 7 or higher, 2 a Nugent score of 4–6, and the remainder a Nugent score of 3 or less. Accordingly, when comparing the FISH-based analysis to Nugent scoring, it may be inferred that FISH-based analysis has an accuracy for the diagnosis of bacterial vaginosis of 0.94 [95% confidence interval (CI) 0.86–0.98], sensitivity 0.83 (95% CI 0.51–0.97), specificity 0.97 (95% CI 0.87–0.99), positive predictive value 0.83 (95% CI 0.51–0.97) and negative predictive value 0.97 (95% CI 0.87–0.99).

IMPLICATIONS FOR THE TREATMENT OF BACTERIAL VAGINOSIS

Treatment of bacterial vaginosis has been a longstanding challenge. Standard regimens with metronidazole or clindamycin as recommended by the U.S. Centers for Disease Control and Prevention (CDC) are associated with fairly good short-term cure rates, but, however, also with high recurrence rates in the long run [13]. Hence, it has been questioned whether such high recurrence rates reflect antibiotic resistance, recurrence or reinfection [14].

Swidsinski *et al.* [15▪▪] then conducted a longitudinal study in which 18 patients were treated with a standard oral regime of metronidazole and followed for 5 weeks. Through repeated vaginal biopsy and FISH imaging of the vaginal biofilm, it could be shown that the vaginal polymicrobial *Gardnerella* biofilm was temporarily suppressed during metronidazole treatment, turning into some dormant state, yet that the biofilm quickly regained its activity following treatment cessation. Although time to follow-up was too short in this study to document any clinical bacterial vaginosis recurrences, the observations on biofilm strongly suggest it to be a mechanism involved in the recurrence of bacterial vaginosis, as has been known for other recalcitrant infections involving biofilm mode of growth [15▪▪].

The study was in a slightly modified setup repeated with an oral course of moxifloxacin, a quinolone agent that had not previously been assessed in the treatment of bacterial vaginosis [16]. A similar observation was made, that is, in almost half of the cases resurgence of the biofilm was observed after 10–12 weeks. We have recently evaluated lactate gel as an alternative treatment, but found that lactate also failed to eradicate the biofilm (Swidsinski *et al.*, unpublished observation). Finally, we also studied the potential of octenidine hydrochloride in the treatment of bacterial vaginosis. Surprisingly, we found that octenidine did eradicate the polymicrobial biofilm in most patients. However, 6 months after repeated treatment with octenidine hydrochloride, recurrence rates similar to those observed with metronidazole were found (Swidsinski *et al.*, unpublished observation).

Though no proper in-vitro model of the bacterial vaginosis film at present exists, it is worth mentioning that a recent series of experiments do suggest that probiotics may have a place as an adjuvant to antibiotic therapy in treating bacterial vaginosis as

has been found in clinical studies [17]. McMillan *et al.* [18▪▪] grew a 12-μm thick confluent *A. vaginae* and *G. vaginalis* biofilm, which they evaluated for changes occurring with antibiotic and probiotic treatment. It was found that metronidazole produced holes throughout the biofilm, however without eradicating the bacteria. Challenge with the probiotic lactobacilli *L. reuteri* RC-14 and *L. rhamnosus GR-1*, however, led to extensive bacterial death in the biofilm [18▪▪].

Recent studies also reported the activity of thymol [19] and of a synthetic retrocyclin [20] against a monoculture *G. vaginalis* biofilm. It is questionable, however, how well an in-vitro monoculture *G. vaginalis* biofilm reflects the in-vivo polymicrobial bacterial vaginosis biofilm.

CONCLUSION

The discovery of bacterial vaginosis as a polymicrobial biofilm infection has certainly not simplified our understanding of this complex, anaerobic overgrowth condition. It is clear that the latter discovery has far-reaching implications for the pathogenesis, epidemiology and treatment of bacterial vaginosis. Future research may now focus on the ability of novel therapeutic agents to tear down the biofilm configuration, but is hampered by the lack of a validated in-vitro model of bacterial vaginosis.

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Conflicts of interest

There are no conflicts of interest.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 103).

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