



The biofilm in bacterial vaginosis: implications for epidemiology, diagnosis and treatment: 2018 update

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Purpose of review

Bacterial vaginosis is conventionally appreciated as a temporary community disturbance of the vaginal microbiota, though really involving self-organization as a resilient biofilm community. We will briefly review here how recent findings on this matter may affect practice and research in this field.

Recent findings

The rapidly expanding literature base on the vaginal microbiome is largely based on 16S ribosomal ribonucleic acid (rRNA) and *cpn60* gene sequencing, with few studies accounting thus far for spatial microbiota organization. The putative sexually transmitted disease profile of bacterial vaginosis inferred from epidemiologic research, may concur with its biofilm nature, notably involving spread of dispersed cells or cell aggregates between hosts. De novo biofilm formation in response to prolonged vaginal ecosystem pressure should be considered a pathogenetic model as well. Biofilm assays may not only aid epidemiologic research, but also add to monitoring therapeutic efficacy of novel treatments. Therapeutic research thus far is largely confined to inhibition of in-vitro biofilm formation, though a recent innovative clinical trial involved a combinational approach of metronidazole and a surfactant, specifically aiming for the biofilm.

Summary

Bacterial vaginosis research will further benefit from biofilm assays complementing taxonomy-based data, and this already translates in a novel treatment paradigm.

Keywords

bacterial vaginosis, biofilm, *Gardnerella vaginalis*, vaginal dysbiosis, vaginal microbiome

INTRODUCTION

Self-organizing as a biofilm community, is an evolutionary conserved, ubiquitous mode of growth in bacteria, that markedly enhances their odds of survival and propagation, notably also by cooperatively withstanding environmental pressures [1]. Human microbiota in contrast, selected and assembled under tight control of their host [2], primarily engage in host-microbe symbiosis as planktonic communities. Under some conditions, however, human-associated bacterial consortia still may seek refuge in biofilm formation [3], which is increasingly understood as a mechanism underlying chronic infectious disease, as well as other conditions, including cancer [1,4]. Sheltering from environmental threats is indeed what also clinically defines bacterial biofilms, as these elicit marked resistance to host defence mechanisms as well as to high concentrations of antimicrobial agents even over prolonged periods of time [5^{***}]. Bacterial vaginosis, hitherto broadly understood as anaerobic

vaginal dysbiosis, is one such prevalent condition in which a polymicrobial biofilm takes central stage [6,7]. Though still as elusive a condition as ever, with wide-ranging impact on reproductive health [8^{*,9}], the unveiling of the biofilm nature of bacterial vaginosis does offer a novel avenue to vaginal microbiome research. We will briefly discuss here, how our current understanding of the polymicrobial, *Gardnerella*-led vaginal biofilm [6] may affect

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KEY POINTS

- As much as a temporary ecosystem disbalance or dysbiosis, bacterial vaginosis may well be considered a resilient, and hence more persistent, biofilm community.
- Concordance between heterosexual partners of genital *Gardnerella vaginalis*-dominated biofilms is suggestive of sexual between-host transmission, but firm proof thereof is lacking at present.
- *G. vaginalis* clade diversity is presumably huge and of clinical importance; however, the most marked difference in *Gardnerella* phenotype thus far observed, was revealed through comparison between the planktonic and biofilm-type transcriptome.
- Biofilm assessment offers a valuable adjunct to conventional assessment criteria in monitoring short and long-term therapeutic efficacy of bacterial vaginosis treatment.
- Antibiotic therapy alone is unlikely to have a future role in the treatment of bacterial vaginosis, but combinational approaches that include novel agents are being explored.

practice and research in the epidemiology, diagnosis and treatment of bacterial vaginosis.

IMPLICATIONS FOR THE EPIDEMIOLOGY OF BACTERIAL VAGINOSIS

Although bacterial vaginosis has been found a risk factor for a number of aspects of reproductive health and human reproduction [8[•]], the epidemiology of bacterial vaginosis as a vaginal microbiota community state remains poorly understood, if not contentious. Of all risk factors explored, arguably, African descent and sexual behaviour-related characteristics have most commonly emerged from epidemiological research [10]. Kenyon *et al.* [10,11,12[•]] on that account, recently elaborated on sexual network-level factors in which individual-level risk factors, such as ethnicity or lifetime number of sex partners, fail to explain broader differences in bacterial vaginosis prevalence patterns between populations or population groups. From their continued work, sexual network connectivity, and partnership concurrency in particular, emerges as a key explanatory variable to sexually transmitted infection and bacterial vaginosis epidemiology [10,11,12[•]]. Although this fuels the long-standing debate over its putative sexually transmitted nature [13], the STI-like disease profile of bacterial vaginosis may well concur with its biofilm mode of growth. Detachment and dispersal of bacterial cells from a sessile community is integral to the biofilm life-cycle [14,15]. This in turn,

may allow for horizontal transmission of dispersed biofilm cells that establish novel, satellite biofilm communities in infected hosts, as a previously unrecognized infection mode. Notably, in a *Pseudomonas aeruginosa* biofilm model, dispersed biofilm cells were found to elicit a unique, virulent phenotype, consistent with their purported role in biofilm spread [16]. No comparable data for *Gardnerella*, or on bacterial vaginosis for that matter, are available at present. Nonetheless, almost absolute concordance among heterosexual couples in the presence of adherent *Gardnerella vaginalis*-dominated biofilm communities retrieved from genital epithelia has been observed, as well as the absence thereof, if *G. vaginalis* was part of planktonic vaginal communities [6]. Although these observations are definitely suggestive of some mode of transmission of spatially structured, *Gardnerella*-dominated communities in at least one direction between sexual partners, it should be acknowledged that concordance between partners was observed in a cross-sectional study set-up, and hence there is no firm proof as yet of sexually mediated, between-host biofilm dispersal. Moreover, as plausible as sexual transmission between women and their male or female sexual partners may seem, it may not be the sole mechanism involved in bacterial vaginosis pathogenesis and epidemiology [13]. Clearly, biofilm formation is not necessarily dependent on such interhost dispersal mechanics. Albeit involving a wide variety of mechanisms in different species and strains, biofilm transitioning is generally a highly regulated, programmed process that may occur in response to a host of ecological stressors and/or opportunities [1,17]. Accordingly, bacterial vaginosis may conceivably emerge from de novo biofilm formation, possibly driven by sexual intercourse and other reproductive phenomena, although through ecological mechanisms that we ignore at present. Both cooperative [18] as well as competitive [19] ecological mechanisms have been found to drive biofilm formation. As previously suggested, *G. vaginalis* presumably is the first species to adhere to the vaginal epithelium and then becomes the scaffolding to which other species adhere [7]. Common co-occurrence of *G. vaginalis* with *Atopobium vaginae*, both typically present in large numbers as the biofilm core, may further reflect the cooperative involvement of both species in biofilm formation [6,20], though other synergistic relationships are presumably also at play [21[•],22]. In fact, a vast number of other bacterial vaginosis-associated species were found to elicit biofilm formation in an in-vitro model system [23]. Of note, in this respect, did a recent sequencing study point at the pivotal role of *Prevotella* spp. abundance in the ecology of the

vaginal microbiota [24], a finding that definitely deserves further scrutiny in the context of biofilm formation. Regardless of species-level synergistic networking, ecological competition for strain dominance, as had been documented in an in-vitro *P. aeruginosa* biofilm model [19], is yet another mechanism to consider in bacterial vaginosis epidemiology and pathogenesis. *G. vaginalis*, a monophyletic group within the *Bifidobacterium* genus, consisting of a single species within a single genus, has been scrutinized since the 1980s for its subspecies diversity, initially through phenotypical typing, until recent whole genome sequencing and related phylogenetic studies revealed clade diversity at the genomic level, up to the upcoming proposal of subdividing the current taxonomy in at least four distinct *Gardnerella* species [21[■],25[■]]. Along the same lines, numerous studies have been devoted to distinguish between *Gardnerella* clades with regard to their virulence potential or with regard to their specificity to bacterial vaginosis and nonbacterial vaginosis communities [21[■]], albeit with no consistent findings as yet. Rather, a pioneering transcriptome study, though culture-based, concluded that *G. vaginalis* is capable of drastically adjusting its phenotype through an extensive change of gene expression leading to biofilm formation [26[■]]. Accordingly, environmental pressures or ecological disturbances of the vaginal niche might be a more determining factor in biofilm formation and development of bacterial vaginosis in a given woman, than *Gardnerella* genotype alone.

IMPLICATIONS FOR THE DIAGNOSIS OF BACTERIAL VAGINOSIS

Diagnosis of bacterial vaginosis has a convoluted history, which has led to rather different diagnostic approaches that are still broadly used, albeit in different settings. Composite clinical criteria, commonly referred to as Amsel's criteria, has been the mainstay for clinical diagnosis, that is in-office diagnosis when bacterial vaginosis is suspected or needs to be ruled out. Despite some concern over its validity, which has also resulted in various modifications, this time-honoured approach is particularly well suited to the purpose [27]. Nonetheless, as clinicians are getting less familiar with wet mount microscopy and are facing time constraints, molecular approaches make their way to the clinician's office and will presumably become first in line as point-of-care tests [28]. In research settings, a quite different approach is generally handled, notably Gram-stain-based microbiological diagnosis of bacterial vaginosis, most commonly through use of Nugent's criteria, which is based on enumerating

a limited set of bacterial cell morphotypes on a Gram-stained smear. Despite concern over the internal and external validity of this approach (for review of this issue, see [27]), this technique has been found fairly reliable in terms of repeatability and interobserver agreement. Hence, although both approaches clearly have their merits in their respective settings, counterintuitively, Amsel's criteria and Nugent's criteria are notoriously discordant, as typically expressed by low kappa statistic values in cross-validation studies. Although this may result from an overall lack of validity of at least one of both approaches indeed, it may also result from case heterogeneity, especially in such different settings. Nugent's approach is basically an assessment of where a vaginal community state relates to a spectrum ranging from absolute *Lactobacillus* dominance to overt anaerobic dysbiosis, and hence purely based on community composition in terms of bacterial cell morphotypes. Amsel's approach on the other hand does not directly target vaginal community assembly, but rather markers thereof, including biofilm formation. Indeed, in the original study of Swidsinski *et al.* [29], it was revealed that 'clue cells', the defining criterion described by Gardner and Dukes [30] in their seminal article, actually represent desquamated cells covered with the *G. vaginalis*-dominated biofilm. Although Amsel's approach may therefore be expected to better reflect the presence of the bacterial vaginosis biofilm relative to the Nugent method, this warrants further scrutiny. Similarly, analysis and categorization of 16S rRNA or cpn60 sequencing studies thus far, is primarily based on compositional dissimilarity approaches by accounting for taxonomy and taxonomy-specific relative abundances, whereas these approaches do not account for spatial community structure *per se*, although of defined importance to community phenotype, and hence for human health. Several follow-up studies on various antibiotic and antiseptic treatment approaches for bacterial vaginosis have also highlighted the potential importance of assessing spatial community architecture in monitoring short and long-term therapeutic efficacy [6,31,32[■]]. It is therefore conceivable, that this approach might become a compulsory complement to current clinical trial guidelines [27].

IMPLICATIONS FOR THE TREATMENT OF BACTERIAL VAGINOSIS

Treatment of bacterial vaginosis with currently recommended antibiotics (metronidazole, tinidazole and clindamycin) falls notoriously short, with, regardless of initial treatment success, high rates of recurrence at mid-term and long-term follow-up

[27]. This is generally suspected to relate to the resistance conferred by the multispecies bacterial vaginosis biofilm [6,7,21¹]. Stand-alone therapy with antibiotics is therefore unlikely to have a future role in the treatment of bacterial vaginosis. A number of alternative approaches are being considered at present to target human-associated biofilms, as recently comprehensively reviewed by Koo *et al.* [33¹]. Briefly, as detailed in the former overview [33¹], novel approaches are expected to inhibit biofilm formation either to degrade established biofilms through one of four mechanisms of action: first, targeting the extracellular polymeric substance (EPS) matrix by inhibiting EPS build-up, blocking EPS adhesion to the host surface, or by EPS degrading enzymes in established biofilms; second, inducing biofilm dispersal and biofilm self-disassembly by targeting the cyclic-di-GMP pathway (the intracellular, secondary messenger c-di-GMP has a key role in the biofilm life-cycle) or through agents that inhibit quorum sensing systems or hence cell-cell communication in the biofilm community; third, altering biofilm community structure and formation through interference with biofilm community metabolism; and fourth, targeting dormant or so-called persister cells, known to have a key role in biofilm drug tolerance, by mechanically or chemically disrupting biofilm cells, rather than targeting cellular processes, through agents that include antimicrobial peptides and antiseptics. Given the intrinsic complexity of biofilm formation and the multifaceted bacterial survival strategies involved, no single agent is expected to provide a definite therapeutic answer however, but rather different combinational approaches at distinct biofilm life-cycle stages are envisaged [33¹]. Only recently, several of these approaches have been reported to inhibit *Gardnerella* biofilm formation in in-vitro or animal models, including DNase targeting EPS extracellular deoxyribonucleic acid [34], the quorum sensing inhibitors subtilisin (a bacteriocin) [35] and benzoyl peroxide [36], the multifunctional innate immune factor lysozyme [37–39], and bacterial cell membrane-disrupting cationic amphiphilic agents [40]. Mathur *et al.* [41¹] very recently specifically reviewed the available data on bacteriocins (including the aforementioned subtilisin), antimicrobial peptides which are ribosomally synthesized by bacteria and which often are more potent than their antibiotic counterparts, in targeting biofilms, including the bacterial vaginosis biofilm. It should be acknowledged however, that many of aforementioned in-vitro studies, as biofilm research in general, have primarily focused on tackling biofilm formation in its initial stages, and less so on

disassembly of established biofilms. Koo *et al.* [33¹] in this respect pointed at the perspective, in the light of advancing technology, of nanomedical approaches and of a wide array of potential physical and physicochemical strategies, specifically that interfere with surfaces and surface adhesion interactions. The nano approach in particular offers a broad opportunity for bioactive particles as well drug-delivering nanocarriers in effectuating combinational therapy targeting mature and dispersing biofilms [42]. Gottschick *et al.* [37] recently reported on comprehensive study, in which a wide variety of agents were screened for their potential in an in-vitro *Gardnerella* biofilm model, which included antibiotics (metronidazole and tobramycin), enzymes (lysozyme and proteinase K), the antibacterial peptide OP-145, antiseptics (chlorocresol and polyhexamethylene biguanide), but also surface-active agents (tensides), notably lecithin and the amphoteric tenside sodium cocoamphacetate. From this study, the same research group took the tenside cocoamphopropionate to a randomized clinical trial [32¹], in which after initial metronidazole treatment of bacterial vaginosis, patients received either cocoamphopropionate or lactic acid, both administered as a pessary. This innovative work is unique in several respects, and, for one thing, did appear to have overcome our defined lack of validated in-vitro or animal models, as we have previously indicated [7]. Despite this continuing impediment to bacterial vaginosis research, the latter study does herald a shift-of-paradigm in developing novel treatment options for bacterial vaginosis, by specifically accounting for its biofilm nature.

CONCLUSION

The discovery of bacterial vaginosis as a polymicrobial biofilm condition may significantly aid epidemiological research on the vaginal microbiota and dysbiosis in particular, but this has not been really exploited to significant extent as yet. At the same time does recognition of the *Gardnerella*-dominated biofilm challenge our conventional understanding of antimicrobial treatment; however, now also allows for the development of tailored approaches, despite the lack of validated in-vitro or animal models for bacterial vaginosis.

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Conflicts of interest

There are no conflicts of interest.

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