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Current Concepts of *Gardnerella vaginalis* Biofilm: Significance in Bacterial Vaginosis

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Abstract

Gardnerella vaginalis (GV) has been implicated in BV development. Further, biofilm is accepted as one, if not the principle reason, for recurrent or recalcitrant BV. GV has defined virulence factors that contribute to biofilm, though more may be discovered within genomic information. Key players in genital tract microecology include GV, other species of the microbiome, and the epithelial base on which microbial interactions occur. The epithelium is influenced by various forces such as douching, smoking, diet, and estrogen: other potential factors are yet unidentified. All of these factors may contribute to bacterial vaginosis. Further, biofilms usually contain microbial species in addition to GV, and the mechanisms for supporting roles of these other species provide an opportunity for elucidation. Gaps in knowledge still exist in effective therapeutics aimed at biofilm, and better understanding of the process of bacterial quiescence, persistence, and biofilm formation is a key step in future research. **Purpose:** This review examines current literature for information about biofilm significance in relation to GV and bacterial vaginosis. **Methods:** Structured literature review.

Keywords

Gardnerella, Bacterial Vaginosis, Virulence Factors, Biofilm, Microbiome

1. Introduction—Biofilm and Virulence

Bacterial Vaginosis (BV) is the most common vaginal bacterial condition presenting with discharge. The condition is characterized as a dysbiosis with abundant anaerobic bacteria, strongly linked with a substantial *Gardnerella vaginalis* (GV) population. This is coupled with a paucity of *Lactobacillus*, especially Lactobacilli that are able to produce hydrogen peroxide [1]. These conditions are also associated with an elevated pH [2]. GV is capable of adhering to vaginal ep-

ithelium and forming biofilms. Coupled with these microenvironmental changes, GV is able to elicit symptoms [3]. These conditions prime for, or result from, bacterial growth changes. Such conditions likely arise with the help of environmental influences, such as douching, sexual intercourse, or even cigarette smoking [4].

Understanding BV is particularly important because the condition is associated with health risks that go beyond vaginal discharge, including adverse pregnancy outcomes, increased sexually transmitted disease and HIV infection risk, pelvic inflammatory disease, and increased complications with vaginal procedures [5]. Further, while BV can be addressed with antibiotics, many cases are refractory or recur frequently.

A great deal of basic and clinical research has been published regarding the natural history of BV within the past 10 years. **Figure 1** provides a brief bibliometric overview of the history of interest in this topic.

There have been two surges of interest in GV: first around 1980 and then again after 2007. The use of the term BV and related clinical criteria (Amsel's criteria emerging in 1983) and Gram stain diagnostics (Nugent scoring dates to 1991) may have fueled the first wave. The second may have been related to the NIH Human Microbiome Project kicking off late 2007 early 2008 and the emerging concerns about the relationship of BV to other clinical conditions. This review examines current research (a majority of which has been published within the past decade) focusing on the importance of GV biofilm formation in BV. Further, this review explores new treatment ideas that target these biofilms specifically.

2. Central Concepts

Gardnerella and BV have been topics of investigation for decades. However, recent research has provided a clearer understanding of the role of biofilm in mucosal infections and how microorganisms operate in the phenotypic state of

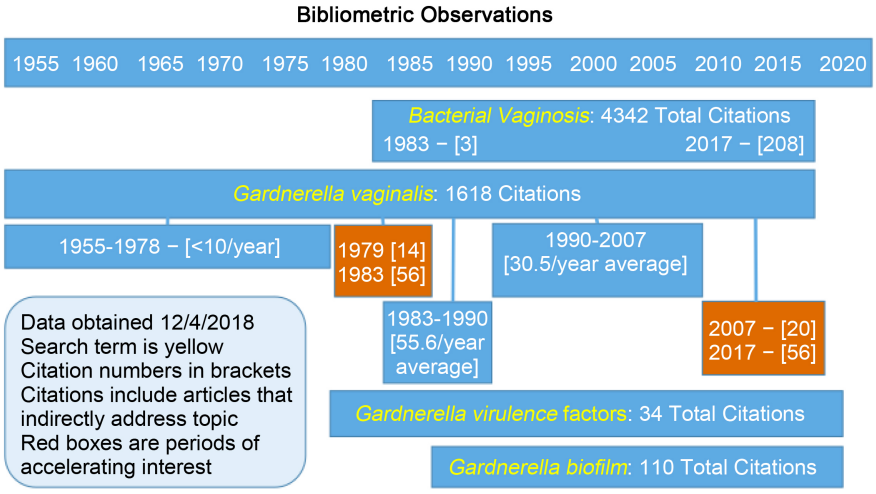

Figure 1. Bibliometric analysis of GV/BV biofilm.

biofilm growth. Subsequently, new insight into these topics has greatly influenced understanding of BV development.

G. vaginalis biofilm production is considered to be a major step in BV development and persistence. *G. vaginalis* forms a biofilm that supports an environment conducive to bacterial growth. These bacteria, and not necessarily *Gardnerella vaginalis* alone, may contribute significantly to BV symptoms. For example, pelvic infections are linked to lower tract bacteria ascending to the upper tract [6]. This may explain why metronidazole alone often fails as a treatment option. Antibiotics used in primary therapy for BV may allow other bacteria to survive, and may also fail to eradicate *G. vaginalis* biofilm. This could also help explain recrudescent symptoms, making targeting of biofilm specifically an essential step in future treatment of BV.

3. Methods

This review is largely based on literature findings from PubMed results using the search term "*Gardnerella vaginalis* biofilm". On July 5, 2018, this search term yielded 76 results. 36 were excluded for one of the following reasons: the content was not relevant to *G. vaginalis* biofilm specifically, the result was a review that did not aid in providing new information, or the material was outdated. Other sources were found via PubMed by searching articles cited within those results, via slightly altered search terms, or terms specific for subtopics relevant to this review.

For example, other search terms augmenting this review included "*Lactobacillus Gardnerella vaginalis*", "Bacterial vaginosis biofilm", and "*Gardnerella vaginalis* biofilm treatment". Further terms searched were involved in supportive subsections, such as "bacteriocins" and "quorum sensing *Gardnerella*".

4. GV Biofilm and BV

Biofilm represents a complex phenotype for bacteria. In the case of GV, there seems to be an important interplay between other microorganisms sharing the vaginal microenvironment and the products of GV expressed under various environmental influences. Thus, not only do other organisms substantially affect GV biofilm, but products of GV growth such as hemolysin (vaginolysin) and sialidase may also contribute to biofilm formation. It is first important to establish the significance of GV itself in BV, and then explore its relationship with *Lactobacillus*, as the interaction between GV and *Lactobacillus* may influence biofilm formation.

4.1. Probiotic Bacteria

The interplay between GV in BV and probiotic bacteria, such as *Lactobacillus*, that colonize the vaginal epithelium is complex. The inverse relationship between GV and *Lactobacillus* has been well studied, if not completely explained. In healthy (asymptomatic) women, gram-positive *Lactobacillus species* frequently

dominate. In BV, however, obligate and facultative anaerobes (including GV) are increased while beneficial *Lactobacillus species* show decreased abundance. Thus, BV typically exists in an environment with diminished *Lactobacillus* and abundant anaerobic species. Generally this is described as an increase in microbial diversity, under conditions of diminished *Lactobacillus* colonization, which comprises various (and not always identical) species. 16S rRNA gene PCR and pyrosequencing have found that women who test positive for BV, both via the Amsel criteria (clinical categorization) and through Nugent scoring (gram stain categorization), had more diverse bacterial flora. On the other hand, women who were negative for BV tended to have flora heavily dominated by *Lactobacillus*, especially *Lactobacillus crispatus*. Strong negative correlations were noted between most *Lactobacillus species* and typical BV organisms. GV, in particular, has been widely implicated as integral in BV because it has been found to be present in up to 98% of BV cases [7]. As described later, detailed microbiome assessments reveal a subset of women who harbor a diverse flora but do not have BV symptoms.

However, *Lactobacillus species* (notably *L. crispatus*) are usually capable of withstanding GV virulence factors. In a study by Breshears *et al.*, an *ex-vivo* porcine vaginal mucosal model was used to co-culture *L. crispatus* with GV. *L. crispatus* alone was grown for 48 hours, which allowed for acidification of the medium (pH < 4.0), after which GV was added. Under these conditions, GV growth was inhibited. It was further noted that lactic acid, acetic acid, and hydrochloric acid (at a pH of 4.0) had similar effects on GV when used alone in similar models. Thus, one attribute of probiotic strains of bacteria is producing conditions lowering pH (including *L. crispatus*), negatively affecting *G. vaginalis* growth [8].

L. crispatus also decreases the cytotoxicity of GV on epithelium in conditions typical of the vaginal microenvironment. In a study in which HeLa cell cultures were exposed to GV obtained from women with or without BV symptoms, the effect of *L. crispatus* pre-treatment was examined. Under these conditions, in addition to acidification of the culture by *L. crispatus*, the investigators were able to demonstrate differences in the gene expression of GV vaginolysin, and sialidase. Moreover, this experiment distinguished between planktonic and HeLa cell-associated GV. Establishing GV infection without *L. crispatus* induced greater damage to the HeLa cells; as precoating HeLa cells with *L. crispatus* reduced cytotoxicity. Vaginolysin transcript levels were elevated in GV from BV positive patients compared to BV negative patients. Both vaginolysin and sialidase were found to be higher in cell-associated (biofilm cultures) than in planktonic bacteria [9].

4.2. Virulence Factors

Sialidase has been associated with GV biofilm. A BV diagnosis based on Nugent score is significantly more likely with elevated GV sialidase A gene expression

[10]. Vaginolysin is frequently implicated in GV cytotoxicity as well, but studies have indicated that *L. crispatus* is capable of reducing vaginolysin expression in BV [9]. Though cytotoxicity and biofilm both seem to have an association with sialidase and vaginolysin, further investigation is needed to establish a mechanism that might further explain how these factors interact. While it has been suggested that sialidase and vaginolysin aid in biofilm formation, the mechanism whereby this occurs is not clear. Theoretically cytolysin could disrupt cell membranes and sialidase could disturb tissue ground substance. Further, it is possible that cytolysin could disrupt mucin stability, although data by Wiggens *et al.* suggest otherwise [11]. Perhaps more interesting is the possibility that some factor, apart from organic acids produced by *Lactobacillus*, may inhibit virulence factors and biofilm in *Gardnerella* and may suggest novel therapies.

4.3. Non-GV Microbiome

With the advent of the human microbiome project, more detail is known about the various Lactobacilli that inhabit the vaginal microenvironment. Despite the attention that has been paid to *L. crispatus*, it appears that *L. jensenii* and *L. gasseri* may play a similar role to *L. crispatus* in the microbiome [7] [12]. Interestingly, another *Lactobacillus*, *Lactobacillus iners*, seems to have a different role in the microbiome. In general, *Lactobacilli* seem to represent species that are useful in establishing community-state types in the microbiome. Ravel and co-workers indicated that, in addition to community state types associated with *Lactobacillus species*, there is an additional community state type (the diversity type) that is more typical of BV. The diversity state type contains fewer Lactobacilli and more varied organisms [12].

While *in-vitro* studies have been useful in characterizing GV biofilms, it is clear that *in-vivo* this ecological niche is shared by a variety of other organisms, especially in the case of symptomatic BV. Contemporary methods have identified the BV microenvironment as having a diversity of species: BV associated anaerobic organisms include *A. vaginae*, *Prevotella bivia*, *Mobiluncus mulieris*, *Veillonella sp.*, *Peptostreptococcus sp.*, *Peptoniphilus sp.* and *Fusobacterium nucleatum*. *A. vaginae* is of particular interest in this context, as it is often found with GV [13] [14]. However, among these species GV was the only one capable of lysing ME-180 vaginal epithelial cells in *in-vitro* assays attributed to vaginolysin [15]. It was also found that only GV was able to compete for adherence and was best able to form a biofilm [15].

Specific BV-associated organisms are found to occur together, which could suggest metabolic co-dependencies [7]. This relationship has been found in many different studies, indicating that other BV organisms, and not just GV, thrive in environments with decreased Lactobacilli. This may imply that GV and *Lactobacillus* require similar nutrients or similar receptors, addressing competition with each other. This leads to an important question: in cases of BV, how does GV establish dominance when it often cannot resist the protective mechan-

isms of *Lactobacillus*?

Two competing concepts would be that different strains of *Gardnerella* have different propensity to cause symptoms, vs. environmental factors that allow *Gardnerella* to become dominant, though there may be some convergence of the two. In terms of environmental factors, epidemiologic observations have been helpful. A change in the micro-environment of the lower female genital tract may result from environmental factors such as sexual intercourse, changes secondary to smoking or douching, and a variety of others which have not been as well-studied [5]. For example, the support of an abundant *Lactobacillus* population seems to be abetted by adequate levels of estrogen. Further, systemic antibiotics may also alter the composition of the microbiome.

5. Extrinsic Effects on BV and GV

Smoking has been associated with BV in epidemiological studies, but it has also been linked to metabolic changes in the vaginal microbiome. In a study by Nelson *et al.*, metabolomic profiles of smokers and non-smokers were compared. Cultures were separated into *L. crispatus*-dominated, *L. iners*-dominated, and low-*Lactobacillus* groups. The study found that bacterial composition was the most important aspect of metabolomic changes associated with smoking (with the low-*Lactobacillus* group showing the most significant changes). In women from the low-*Lactobacillus* group, those who smoked had significantly higher biogenic amines, including agmatine, cadaverine, putrescine, tryptamine and tyramine; while dipeptides were lower. Biogenic amines are known to have a role in anaerobic bacterial growth. Thus, smoking could make significant changes in vaginal metabolites, allowing for greater GV virulence [16].

It has also been suggested that smoking decreases estrogen, which is supportive of *Lactobacillus* colonization [17] [18]. An alternative explanation is related to the immune system, in which smoking induces cytokine response. IL-10, an anti-inflammatory interleukin, is found to increase in the mucosa of smokers. This may exacerbate infective conditions, as increased IL-10 levels are also found with an increase in mucosal pH [19]. Overall, it seems that certain conditions (such as direct contact with epithelial cells or changes in the epithelial environment), coupled with low *Lactobacillus* counts, may be required for strains to become virulent and create biofilms [7] [20].

Lactobacillus iners is a notable exception. *L. iners* is frequently found in both symptomatic BV infection and non-BV flora [7] [21]. This may be because *L. iners* produces less lactic acid than other Lactobacilli [22]. It has also been suggested that *L. iners*-colonized hosts may move between BV and non-BV states [7]. Interestingly, *L. iners* differentially expresses cytolysin, mucin transport, glycerol transport, and other related metabolic enzymes up to 10% greater in dysbiotic vs healthy states. These point to metabolic end products other than lactic acid (such as short-chain fatty acids), subsequently leading to a less acidic pH [23]. This is unlike asymptomatic/healthy flora, where the metabolic end

products tend to be predominantly lactic acid. It is plausible that *L. iners* has the potential to change genomic expression to fit pathologic states, subsequently elevating the pH. It remains to be discovered what modulates expression of these metabolic factors in *L. iners* and whether it relates to BV symptoms.

Historically, many reports related to biofilm note its formation on abiotic surfaces including medical devices. In the present context, biofilm has been discussed as occurring on vaginal epithelial cells. However, one important abiotic entity in the female genital tract is the IUD. In a recent report by Adam *et al.*, IUDs were recovered from 100 women for various reasons and for varying durations after implantation. Bacteria released by sonication from these specimens were subjected to PCR evaluation for GV, STI organisms, and organisms the authors designated as BV "signal" organisms. Signaling organisms were defined as GV, *Atopobium*, *Mobiluncus* and *Ureaplasma*. They found that 76% of IUDs had at least one of the 4 signal organisms. Interestingly, the most common pair of signal organisms was *Atopobium* with GV. The co-colonization of IUDs did not appear to be different from what would be expected based on the prevalence of the individual species. The authors identified other organisms that may be of greater significance for the upper tract, including *Streptococcus agalactiae* and Actinomycetes [24].

6. Durability of GV Biofilm

All BV patients have GV colonization, and 90% of all BV patients also have GV-dominated biofilms [25]. To better study the significance of these biofilms, Patterson *et al.* developed an *in-vitro* model for pure GV biofilm. After biofilms were formed, lactic acid and H_2O_2 were added and were compared to planktonic cultures. Biofilms were more resistant to killing by H_2O_2 and lactic acid than planktonic cells, which support resistance of biofilms to Lactobacilli [26].

Further, GV biofilms are thicker and are more resistant to *Lactobacillus* than biofilms produced by the other BV-associated bacteria. When tested against 30 other BV-associated species, GV was better able to produce biofilm and exert cytotoxic properties than comparator organisms. For example, it was more capable than other BV-associated bacteria to adhere to HeLa cells [27]. Other anaerobes, including *A. vaginae*, *M. mulieris*, *P. bivia*, *Veillonella*, *Peptostreptococcus*, and *Peptoniphilus* formed significantly less abundant biofilm than *G. vaginalis* [9]. Moreover, *in-vitro* models showed *G. vaginalis* biofilms were more resistant to disruption through washing [9].

6.1. Polymicrobial Biofilms

While GV produces robust biofilm, other BV-related bacteria seem to function synergistically in this environment. Polymicrobial biofilm confers additional resistance to antibiotics through increased overall bacterial survival. Systematic reviews, noted that increased bacterial diversity is associated with higher Nugent scores and vaginal pH [28]. Thus, it is important to understand that synergistic

relationships exist between these common BV-associated organisms, however mechanisms involved in their synergy remains obscure.

In-vitro studies showed that biofilm formation benefitted from the presence of a second organism. GV biofilm grew better with any BV-associated anaerobe, regardless of species (with the exception of *Lactobacillus*). Further, *G. vaginalis* enhanced the growth of *P. bivia* and *F. nucleatum* [29]. This supports the idea that *G. vaginalis* acts synergistically, supporting proliferation of other bacteria, and perhaps some cross-feeding activity is at play.

It is likely that certain bacteria are better able to become incorporated with specific group compositions within the biofilm. In FISH (Fluorescent *in-situ* hybridization) studies, *A. vaginae* was frequently incorporated with *G. vaginalis* in biofilms and was present with *G. vaginalis* in 99.5% of samples. The probability of having a higher Nugent score was increased when both bacteria were found together in biofilm [13] [14]. *Prevotella bivia* and *Streptococcus anginosus* may also be elements of polymicrobial biofilm. In sequencing studies with BV status based on Nugent scores, *P. bivia* and *S. anginosus* were found largely in the BV group and were believed to aid in GV biofilm formation. The non-BV group, dominated by *Lactobacillus crispatus* and *iners*, (*L. iners* is considered by some to be part of an intermediate or transitional rather than non-BV flora) and the intermediate group dominated by other lactic-acid producing species, such as *Lactobacillus gasseri*, showed less involvement of *P. bivia* and *S. anginosus* [30] [31]. Such observations suggest synergy among non-*Lactobacillus* species present in BV. However, associations do not reveal the underlying mechanism for synergy.

6.2. The Importance of Polymicrobial Colonization

Identifying bacterial species is also important because symptoms have been reported to change based on which bacteria are present with GV. Different bacteria identified by sequencing from vaginal samples showed different whiff test results and different pH levels, which were associated with specific commensal bacteria. Thus, some symptoms of BV relate to the distribution of species in biofilm, such as *A. vaginae*, as opposed to being entirely associated with GV itself [7].

Because there is no clear correlation between increased loads of *G. vaginalis* in persistent or recurrent infection, other bacteria present in biofilm formed by *G. vaginalis* may account for such persistence [32]. For example, it has been proposed that bacteria belonging to *Clostridiales* and *Mobiluncus* have greater resistance to metronidazole, the drug most commonly used to treat BV [32] [33]. Other anaerobes may also contribute to such resistance. For example, *Atopobium vaginae* is also suggested to be involved in BV infections and has been found to be capable of metronidazole resistance [34].

6.3. Resistance to Animicrobials

Drug-refractory BV could be due to persister phenotypes within BV-associated

organisms rather than genotypic antimicrobial resistance. Though not well-studied in GV, persister phenotypes (those able to display temporary phenotypic resistance to antibiotics) represent another possible explanation for antimicrobial refractoriness. Persister cells are more abundant in biofilm than in planktonic growth and are found in both gram-positive and gram-negative species [35]. This suggests a need for evaluation of the persister phenotype in GV. Little study has been done on persister cells in GV up until this point, but represents a rich and potentially significant area of study, especially when combined with studies of conventional resistance mechanisms.

Phenotypic changes (such as metabolic activity, stress responses, and antimicrobial drug efflux) within biofilms may play a major role in BV. For example, transcriptome sequencing shows an association between phenotypic changes and biofilm production. When GV biofilms form, increased transcription of genes coding for antimicrobial resistance were noted [36]. Further, genes involved in glucose and carbon metabolism were decreased, indicating lower energy needs (cell density increases in biofilms, so there is likely decreased nutrient availability). Genes upregulated in biofilms included those involved in amino acid biosynthesis, DNA repair, efflux transporters, TadE-like protein (involved in adhesion to vaginal epithelial cells), and those aiding in survival [36]. These changes would theoretically allow GV biofilm to create a more favorable environment for persistent infection even in the case of decreased nutrient availability. According to some sources, it is nutrient scarcity that actually promotes persistership [37].

Following the previously mentioned phenotypic changes with low nutrient availability, persister phenotypes are typically more abundant in bacterial populations with nutrient depletion [38]. Again, it may be important to focus future study on genesis of these phenotypes in *G. vaginalis* and nutrient availability *in-vivo*.

Overall, the ability to change phenotype from vegetative to biofilm growth and to integrate other commensal bacteria indicates that *G. vaginalis* biofilms, once established, become stable and capable of maintaining an environment primed for support of a diverse flora.

7. Novel Strategies for Preventing and Disrupting GV Biofilms

Biofilms in general are known to aid in antibiotic resistance and recurrence of infection. The same has been found in BV. In follow-up from an interventional study, it was noted that patients who did not respond to metronidazole maintained a GV and *A. vaginae* biofilm after treatment [39]. Treatments aimed at destroying biofilms directly are now considered to be an important goal in conjunction with effective antibiotics, especially in light of the weak response of biofilms to antibiotics. The persister phenotype is characterized by decreased metabolic activity (dormancy), as antimicrobials that depend on bacterial metabol-

ism for efficacy have limited activity against persisters in biofilm.

7.1. Disruption

A safe and effective method of disrupting biofilm would be advantageous, but at this point we only have theoretical approaches. One option for defeating biofilms is to target the preformed biofilm directly. There have been multiple approaches proposed as adjuncts to current antimicrobial treatment. Some examples of techniques developed to attempt disruption of biofilm include DNAse, amphiphiles, and bacteriocins.

In a study by Hymes *et al.*, it was noted that biofilms contain extracellular DNA, which is necessary for biofilm integrity. This study indicated that DNAse enzymes specifically targeting biofilms could be a potential treatment for BV [40]. However, this has not been tested in the clinic.

Cationic amphiphiles have also been proposed as potential anti-biofilm agents. These complexes are able to decrease development of GV biofilms specifically, with morphological and structural changes in these biofilms demonstrated by scanning electron microscopy. Further, amphiphiles furnished activity against biofilm without harming *Lactobacillus* [41]. Thus, this treatment is theoretically able to prevent biofilm formation without disrupting *Lactobacillus* strains. A surfactant, the amphoteric tenside, sodium cocoamphoacetate, has also been used to disrupt biofilms [42].

7.2. Interdicting Biofilm Phenotype Generation

A highly studied option for preventing biofilm formation employs bacteriocins, which may interfere with quorum sensing. Quorum sensing refers to changes in gene expression resulting from interactions with other bacteria, and is a well-known aspect of transition from planktonic to biofilm mode of growth. Autoinducer-2, involved in extracellular signaling molecules produced by bacteria, aids in this alteration of gene expression. As the bacterial population increases, autoinducers will increase in concentration, allowing bacteria to monitor and make changes in gene expression accordingly [43]. Autoinducer-2 in particular has been implicated, as it has been suggested to communicate between species [44]. Thus, Autoinducer-2 may be an extremely important factor in quorum sensing and efforts to interdict its effect would be an interesting approach, as has been done with brominated furanones in the case of gram negative bacteria such as *E. coli* [45].

The luxS/autoinducer is involved in quorum sensing and Yeoman and co-workers reported the presence of the cognate gene in GV (S-ribosylhomocysteine lyase) that would indicate the possibility of targeting this system [46]. There are additional targets for future therapies suggested by this work.

Benzoyl peroxide is also noted to decrease quorum sensing and biofilm formation of GV. In this study, though highest tested concentrations of benzoyl peroxide did not inhibit GV growth, it did notably prevent biofilm formation.

Reactive oxygen species (ROS) were suggested as an influential aspect of benzoyl peroxide quorum sensing inhibition, as ROS have been shown to be released from benzoyl peroxide. ROS have also been shown to negatively influence biofilm formation in several species [47].

Clue cells, used in diagnosis of BV, are noted to contain heavy localized aggregations of GV [48]. Such microcolonies are consistent with a role for quorum sensing in BV development. The bacteriocin subtilosin, a product of *Bacillus subtilis*, was found to significantly disrupt *G. vaginalis* as well as gram-negative biofilms, ostensibly decreasing Autoinducer-2 production [49]. A similar study also tested Plantaricin A in a similar manner—however, this bacteriocin supported protective *Lactobacillus plantarum* biofilms [50]. As discussed previously, the possibility that *Lactobacilli* may elaborate factors that have therapeutic effect of biofilm formation may be an opportunity for future investigation. The work of Yeoman *et al.* also identified genes for lantibiotic extrusion in addition to other toxin-antitoxin systems, which are part of the system that promotes persistership and biofilm along with additional factors that promote GV competition with other organisms [46]. Thus, by either targeting pathogenic biofilms or by promoting protective biofilms, treatment options for BV can benefit by utilizing a multi-modal approach, instead of solely relying on antibiotics. Other bacteriocins, (including ε-Poly-L-Lysine and Lauramide Arginine Ethyl Ester), have been noted with similar effects (with LAE showing significant bactericidal effects on GVbiofilms) [49] [50] [51] [52].

7.3. Virulence Modulation

Additional treatments aimed at preventing biofilm formation include inhibition of virulence factors such as GV sialidase. In a recent *in-vitro* study by Govinden *et al.*, it was found that the sialidase inhibitor Zanamivir, an antiviral drug used in influenza as a neuraminidase inhibitor, could reduce sialidase activity and decrease ability to adhere to human cells [53]. Targeting vaginolysin via retrocyclins has also been proposed to inhibit biofilm formation. However, this was found to not affect planktonic cells [54].

Lysozyme, with its ability to induce bacterial autolysis can affect biofilm production through its activity of cleaving bacterial peptidoglycan. One study found that in the absence of lysozyme, GV was able to produce a moderate amount of biofilm. Of the 5 tested bacteria (including MRSA, MSSA, *S. pyogenes*, and *P. aeruginosa*), *GV* was the most sensitive to lysozyme. With as little as 2.5 μg/ml, lysozyme was capable of completely inhibiting GV biofilm formation [55]. Lysozyme may also inhibit quorum sensing, significantly decreasing infectious properties [55]. It is interesting, however, that sequencing studies have indicated that GV possesses the gene for lysozyme, which complicates the findings of susceptibility to lysozyme.

This could be a potential treatment option, as lysozyme retains activity in the increased pH that accompanies BV infection. Though there is already lysozyme

in cervical mucus, perhaps greater concentrations are needed to prevent biofilm formation in the lower vaginal segment. Demonstrating differences in various portions of the lower genital tract may be informative in the use of lysozyme.

An additional proposed approach to BV management is the use of essential oils or other botanicals. While this concept has limited support, especially in the form of detailed clinical studies, interest in natural products and other botanicals by consumers would be commercially attractive if these prove efficacious. Further exploration of this topic will be addressed in a later review.

8. Summary

BV is especially challenging because it is multifactorial, involving a complex interplay between organisms and their ecological niche. An abundance of confusion has been attached to this condition over the half century that it has been studied. Advances have been made in understanding BV, and new treatments that rely on a clearer understanding of the molecular details of biofilm formation and maintenance may lead to better approaches to BV management. This review presented the following points:

Biofilm is important if not essential for BV and GV has defined virulence factors that contribute to biofilm. Key players in genital tract microecology include GV, other species of the microbiome, and the epithelial base on which microbial interactions occur which are influenced by various forces such as douching, smoking, diet, estrogen with other potential factors as yet unidentified.

Biofilms usually contain microbial species in addition to *Gardnerella* and the mechanisms for supporting roles of these other species provide an opportunity for elucidation and inverse relationships between some species of lactobacilli and GV generally hold true but are dependent on specific species and strains.

Biofilm, accepted as one, if not the principle reason, for recurrent or recalcitrant BV, depends on the linked phenomena of quorum sensing, stress response, biofilm development, microbial persistership, and drug resistance providing opportunities for discovery of new therapeutics.

Sequence data for the GV genome coupled with metabolomics data opens new opportunities for understanding one player within the complexity of BV. The assembly of information about metabolic activities of various microbial species may unlock the intricacies of polymicrobial synergy in BV and inform development of therapies.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

BV = bacterial vaginosis