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REVIEW

Understanding and Preventing Recurring Bacterial Vaginosis: Important Considerations for Clinicians

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Abstract: Bacterial vaginosis (BV) is the most common vaginal infection worldwide. It is associated with an increased risk of acquisition of HIV and other sexually transmitted infections (STIs) as well as pelvic inflammatory disease and adverse birth outcomes. During BV, a polymicrobial biofilm forms on the surface of the vaginal mucosa. However, the exact etiology of BV remains controversial which has impeded significant advances in diagnosis, treatment, and prevention. Despite 30-day cure rates approaching 80% in BV-infected women treated with 7 days of oral metronidazole, recurrence within 12 months is common. This article provides a current review of the epidemiology, pathogenesis, diagnosis, and treatment of recurrent BV for practicing clinicians who commonly see women with this recurrent vaginal infection. Regarding management, we focus primarily on antimicrobial measures that may be effective. Future areas of research in this field are also discussed.

Keywords: bacterial vaginosis, recurrent, vaginal infection

Introduction

Bacterial vaginosis (BV) is the most common vaginal infection in reproductive-age women.[1] It is associated with an increased risk of acquisition of HIV[2] and other sexually transmitted infections (STIs),[3] pelvic inflammatory disease (PID),[4] and adverse birth outcomes (ie, spontaneous abortion and preterm delivery of low-birth-weight infants).[5,6] The etiology of BV remains controversial and is an area of active research.[7–10] While it is known that BV is a dysbiosis characterized by a shift in the vaginal microbiota from *Lactobacillus* species (spp.) dominance to a dramatic increase in facultative (ie, *Gardnerella vaginalis*) and strict anaerobic bacteria (ie, *Prevotella bivia, Fannyhessea vaginae* (previously *Atopobium vaginae), Megasphaera* spp., *Sneathia* spp., etc.), the inciting agent(s) are not yet clearly defined.[11] This knowledge gap significantly impedes advances in BV diagnosis, treatment, and prevention.

During BV, a polymicrobial biofilm containing *G. vaginalis* and other BV-associated bacteria (BVAB) forms on vaginal epithelial cells, providing an antibiotic impregnable sanctuary to protect this pathologic community of micro-organisms. Desquamation of these cells coated with biofilm results in clue cells seen on wet mount of vaginal fluid.[12] Despite 30-day cure rates approaching 80% in BV-infected women treated with 7 days of oral metronidazole (MTZ),[13] BV recurrence within 12 months is common (ie, 58% in one study).[14] While the BV biofilm likely contributes to high recurrence rates after therapy by reducing antimicrobial penetration,[15–17] antimicrobial resistance in BV-associated bacteria (BVAB), both within the biofilm and the vaginal canal, may also play a role.[18]

This article provides a current review of the epidemiology, pathogenesis, diagnosis, and management of recurrent BV for clinicians who commonly see women with this recurrent vaginal infection. Regarding management, we primarily focus on antimicrobial measures that may be effective. Other reviews of non-antibiotic approaches have recently been published.[19] For the purpose of this article, recurrent BV is defined as ≥3 annual episodes of symptomatic BV requiring antimicrobial therapy.[18] This is in contrast to refractory BV, in which short-term conventional antimicrobial therapy fails

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to achieve a positive response in terms of resolution of vaginal symptoms and/or resolution of a BV Nugent score ≥7[20] or Amsel criteria ≥3/4,[21] respectively.[18]

Epidemiology

The prevalence and incidence of recurrent BV among women in the United States (US) and worldwide is not known; BV is not a reportable disease in any country. In addition, the definition of recurrent BV has varied across studies,[22] making it difficult to summarize prevalence and incidence estimates among women with this infection. The majority of studies published on the overall prevalence of BV among women primarily include clinic-based populations, with a smaller number of studies providing population-based estimates.[1] In a recent systematic review and meta-analysis of global BV prevalence and incidence among reproductive-aged women in the general population which included data from 122 publications,[1] BV prevalence ranged from 23% to 29% across regions. Within North America, black (33%), and Hispanic (31%) women were found to have a significantly higher prevalence of BV compared to other racial groups (ie, white 23% and Asian 11% women, respectively) ($p < 0.01$). Of the reviewed studies, only five provided estimates of BV incidence, which ranged from 1.6 to 74.2 per 100 woman-years.[1] Among post-menopausal women, BV prevalence ranges between 2% and 57% with an overall prevalence of 16.9%, per a recent systematic review and meta-analysis including 13 English-language studies.[23]

A large majority of women (50–80%) with BV are asymptomatic.[24] Routine screening for BV among asymptomatic women (including pregnant women) is controversial[25] and is not currently recommended,[26] as treatment has not been found to reduce adverse outcomes in multiple studies.[27–30]

Other studies have found that women with an intra-uterine device (IUD) have an increased risk of BV,[31] particularly those with copper IUDs (a non-hormonal long-acting reversible contraceptive device).[32] Two hypotheses have been proposed for the elevated risk of BV among woman with copper IUDs.[32] The first is that the presence of an IUD, a foreign body, in the female genital tract may facilitate the overgrowth of BVAB.[33] In addition, use of a copper IUD can be associated with an increased volume and duration of menses,[34] potentially allowing heme-stimulated growth of *G. vaginalis*, a common, key BVAB, to the point of vaginal dysbiosis and development of BV.[33]

Pathogenesis

The pathogenesis of recurrent BV is likely multi-factorial and complex and remains incompletely understood. While persistence of the BV biofilm and/or antimicrobial resistance among key BVAB (ie, *G. vaginalis, F. vaginae*) are thought to play a role in recurrent and refractory infection,[18] a significant body of data also suggests that ongoing exchange of pathogenic BVAB between sexual partners occurs which may also be contributing. In a study of 400 women ages 18–50 with symptomatic BV based on Amsel criteria and Nugent score, recurrent BV occurred in 28% of women at 6 months after treatment and was significantly associated with sex with the same partner pre/post treatment [(adjusted HR [AHR]) =1.9; 95% CI, 1.2–3.0] and inconsistent condom use [AHR = 1.9; 95% CI, 1.0–3.3], adjusting for age and sexual frequency.[35] Additional studies provide further support of sexual exchange of BVAB between partners. The penile microbiota of male sexual partners has been found to be significantly more similar to the vaginal microbiota of their female sexual partners compared to the vaginal microbiota of non-partner women.[36] In addition, uncircumcised men with community state types 4–7 (ie, those associated with a high prevalence and abundance of BVAB) in their penile microbiota were significantly more likely to have a female sexual partner with a high Nugent score ($p = 0.03$).[37]

Another study by Toh et al[38] recently characterized 110 urethral specimens from men (53% white, 35% black) without urethral symptoms, infection, or inflammation using shotgun metagenomic sequencing, finding that sexual behavior is an important determinant of male urethral microbiome composition. Most urethral specimens contained "core bacteria" (including lactic acid bacteria and *Corynebacterium* spp.) while multiple BVAB (*Aerococcus christensenii, G. vaginalis, F. vaginae, Veillonella montpellierensis, P. amnii, Dialister micraerophilus, Sneathia amnii* [recently renamed *Sneathia vaginalis*], *Mageeibacillus indolicus*, and *L. iners*) were only present in specimens from men reporting penile-vaginal sex (both within the past 60 days and the past year).[38] Sexual behavior was strongly associated with inter-specimen variance in urethral microbiome composition in this study. It is hypothesized that BVAB colonizes the mucin-rich penile urethra in men, while "core bacteria" colonize the urethral meatus (where oxygen may be more readily

available).[38,39] The authors of this study concluded that urogenital microbiology and sexual behavior are inexorably intertwined and that the male urethra harbors female urogenital pathobionts.

In another study of 43 BV patients (18 refractory, 16 recurrent, and 11 remission patients) by Turner et al, persistently high titers of Gardnerella Gsp07 were associated with refractory treatment responses while persistently low abundance of Gardnerella Gsp07, *G. swidsinskii*, and *G. leopoldii* were associated with remission.[40] The abundance of *Lactobacillus* spp. rose 4–14 days after initiation of treatment in most but not all women with recurrent infection and those in remission; increases were more sustained in those with remission. Thus, the presence of Gardnerella Gsp07, *G. swidsinskii*, and *G. leopoldii* may be markers of a poor clinical outcome among women with BV and future therapies targeting these strains could improve treatment outcomes.

Diagnosis

BV can present clinically as sporadic, recurrent, refractory, acute, chronic, symptomatic, or asymptomatic. Diagnostic methods in BV are identical whether the clinical state is acute or chronic, sporadic or recurrent, refractory or responsive. BV is most commonly diagnosed clinically using the Amsel criteria (ie, homogeneous, thin vaginal discharge smoothly coating the vaginal walls, vaginal pH >4.5, presence of a fishy odor of the vaginal discharge before and/or after addition of 10% KOH [whiff test], and >20% clue cells [vaginal epithelial cells with a grainy border and speckled appearance due to adherent bacteria] present per high power field on microscopic examination).[21] At least three of these criteria must be met to establish a diagnosis of BV. BV is microscopically diagnosed by determining the Nugent score from a vaginal Gram stain.[20] This score is obtained by determining the relative concentration of lactobacilli (ie, long, Gram-positive rods), small Gram-negative and Gram-variable rods (*G. vaginalis* or *Bacteroides* spp.), and curved Gram-negative rods (*Mobiluncus* spp.). A score of 0–3 is consistent with a *Lactobacillus*-dominant vaginal microbiota, 4–6 with intermediate vaginal microbiota, and 7–10 with BV. The Nugent score, although more specific in research than the Amsel criteria, is rarely used clinically and provides no useful information to the practitioner when faced with the challenge of recurrent BV. More recently, a molecular diagnosis of BV has become available through the use of multiple commercially available nucleic acid amplification tests (NAATs) in symptomatic women,[41] all of which have excellent sensitivity and specificity.[42] However, more traditional methods of BV diagnosis (ie, Amsel criteria) remain useful due to their lower cost and ability to provide a rapid diagnosis.[41] Nevertheless, BV NAATs and even quantitative molecular methods (ie, quantitative polymerase chain reaction [qPCR]) have not yet facilitated or enhanced the clinical management of patients with recurrent BV as they are unable to predict prognosis in the symptomatic pre-treatment patient nor in the post-treatment asymptomatic patient.

Diagnosing the severity of BV (ie, mild vs moderate vs severe) also remains poorly defined. Clinicians commonly refer to the severity of BV with signs and symptoms that are not reflected in the Amsel criteria,[21] Nugent score,[20] or NAAT definition of BV.[42] The clinical picture that emerges with each BV recurrence may be profoundly different from the microbiologic or molecular picture. Moreover, the pattern of recurrent BV may vary considerably when analyzed longitudinally over time. Clinicians recognize these differences both cyclically and longitudinally in that patients may respond clinically to antimicrobial therapy reaching normal or near-normal Amsel scores yet may still have an abnormal vaginal microbiota per microbiological or molecular methods. While molecular methods undoubtedly demonstrate variations in the vaginal microbiota during BV, the clinician is still left with the reality of BV recurrence by patient report of symptoms, most frequently vaginal discharge and odor, driving the need to re-initiate therapy. It is of our opinion that the Amsel criteria, Nugent score, and BV NAAT tests are still useful, if not valuable, in diagnosing recurrent BV but are unable to show specific differences between successive individual recurring episodes (ie, identifying a specific or different BV fingerprint). Likewise, these tests do not allow differentiation between BV relapse and reinfection.

In general, following conventional antimicrobial therapy in women with recurrent BV, when women are asymptomatic and no longer considered to be "infected", demonstration of persistent vaginal dysbiosis by microscopy and/or elevated vaginal pH predicts a higher likelihood of future BV relapse/recurrence. However, even patients with a normal vaginal pH and perceived "eubiosis" frequently relapse, confirming that the currently available "point-of-cure" diagnostic methods do not predict cure. What is needed is a rapid point-of-care (POC) test to indicate that future BV recurrence is likely, justifying early and immediate additional therapeutic measures. Such tests will in all likelihood be molecular in

nature and, even if not available soon, will play a key role in the specific nature of the additional therapeutic interventions.

Principles of Management

It is essential to identify patients with recurrent BV, as opposed to refractory BV, as treatment regimens can be different.[18] A refractory response differs from relapse in that the major causative pathogens are not eradicated but persist in high numbers. Providing oral or intra-vaginal probiotics in either of these situations is not recommended.[41] In cases of refractory BV, switching of the class of therapeutic drug should be performed (ie, 5-nitroimidazole to clindamycin or vice versa) as should the route of therapy (ie, oral to vaginal therapy or vice versa).[41] Change in drug class exploits subtle differences in antimicrobial activity and pathogen susceptibility. It is unlikely that a switch from oral MTZ to other oral 5-nitroimidazoles (ie, tinidazole or secnidazole) will have a significant impact on the refractory nature of the BV episode.[18] If treatment failure continues despite these measures, a 7-day trial of high-dose intra-vaginal metronidazole 750 mg-1000 mg daily is reasonable.[43] It is also reasonable to use combination therapy with an oral 5-nitroimidazole for 7 days in addition to daily intra-vaginal boric acid 600 mg for 7–21 days.[18] Unfortunately, randomized controlled trials have not yet been performed including women with refractory BV. The above recommendations are thus based upon our clinical experience and extrapolated from studies targeting women with recurrent BV.[18] It is also important to recognize that patient tolerance and toxicity set a low ceiling for the use of high 5-nitroimidazole doses.

In the management of women with recurrent BV (Figure 1), reversible factors should initially be considered. First, identifying reinfection from a sexual partner as a cause of recurrence should be investigated. The role of ongoing sexual transmission of BVAB between partners has been previously discussed and is difficult to prevent outside of consistent condom use or the patient embracing celibacy. No validated data are available as to the role of oral-genital sexual contact in causing BV recurrence. Accordingly, for women diagnosed with recurrent BV, we recommend mandatory and consistent use of condoms for a period of at least 3–4 months after treatment, aimed at preventing reinfection from an asymptomatic partner who may be colonized with BVAB. Condom use has been shown to provide some benefit in prior studies[44] although the optimal duration of use is unknown. It is hypothesized that BVAB residing in the male urethra or under the prepuce might resolve over time without additional re-population from a female sexual partner. A similar concern applies to female sexual partners of women with recurrent BV, in which celibacy is advocated together with female partner evaluation and avoidance of sex toys and other sexual practices which may transmit infected vaginal fluids.[26] Unfortunately, partner treatment trials of women with BV have yet to show a significant effect[44,45] although an additional trial is currently enrolling (Australia New Zealand Clinical Trials Registry number 12619000196145).

Data from a prior literature review and meta-analysis of 55 studies found that hormonal contraceptive use was significantly associated with a reduced risk of BV, regardless of the type of contraception (ie, combined estrogen-progesterone, progesterone-only, or unspecified).[46] However, this effect may be due to confounding factors influencing contraceptive choices, including sexual partner type (regular partner vs casual partners).[47] Vodstrcil et al subsequently conducted a pilot, randomized controlled trial of combined (estrogen-progesterone) oral contraceptive pill (COCP) use to examine its effect on BV recurrence following first-line antimicrobial therapy compared to antimicrobial therapy alone in women with symptomatic BV. In this study, 95 women were randomized to COCP or non-hormonal contraceptive practices and followed monthly for 6 months or until BV recurrence. Unfortunately, BV recurrence rates were similar in the COCP arm (primary/Amsel-outcome: 10/100PY, 95% CI: 6.19/100PY) compared to the control arm (14/100PY, 95% CI: 9, 21/100PY, $p = 0.471$). However, this study did find that sex with a regular partner and a history of BV were associated with BV recurrence.[47] Overall, these data suggest that re-infection from an untreated regular sexual partner and persistence of BVAB are integral to the pathogenesis of recurrent BV and may overwhelm the potential benefits of COCP on the vaginal microbiota.[47]

Given the association between copper IUD use and BV recurrence,[32] we advocate for removal of copper IUDs among women with recurrent BV (Figure 1). Whether or not other types of IUDs should also be removed in this setting is controversial due to lack of data. Other behavioral risk factors for BV recurrence undoubtedly exist (ie, chronic smoking)[48] but are not part of the currently recommended management process.

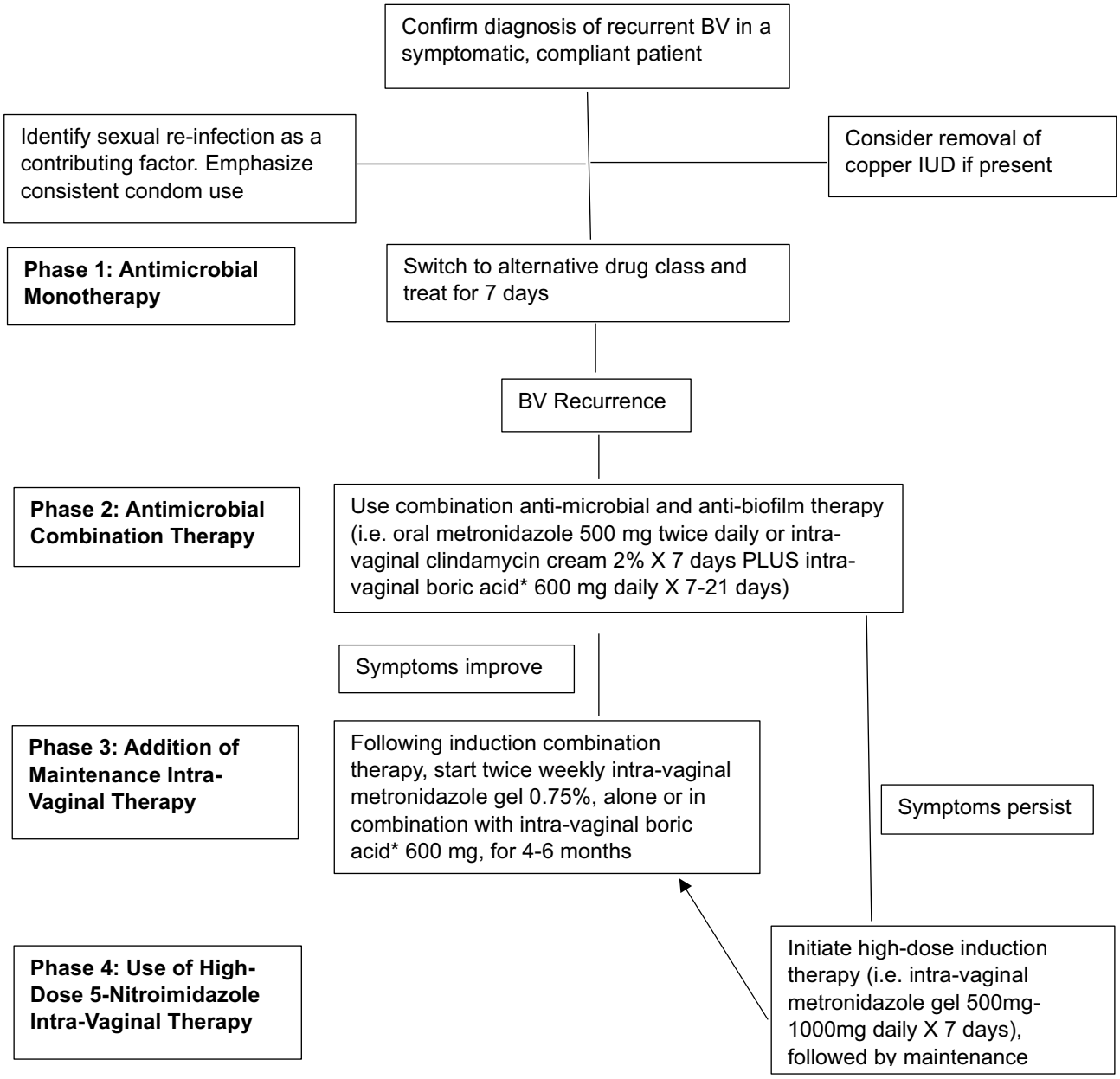

Figure 1 Recommended, sequential treatment algorithm for women with recurrent BV. Bolded text represents different phases of anti-microbial treatment during recurrent BV. *Intra-vaginal boric acid may not be available and/or is forbidden in some countries.

With regard to specific therapeutic measures for women with recurrent BV (Figure 1), following switching of antibiotic drug classes similar to what is recommended for refractory BV, we generally proceed to the use of combination therapy with oral metronidazole 500 mg twice daily for 7 days in combination with an anti-biofilm agent such as intra-vaginal boric acid 600 mg daily, to be used for 7–30 days.[18] The best data for this recommendation are based on an uncontrolled study.[49] The rationale for use of combination therapy with an oral 5-nitroimidazole and intra-vaginal boric acid is based on the presence of the polymicrobial BV biofilm.[12] As previously mentioned, this biofilm can persist and remain adherent to the vaginal epithelial surface in the face of and following administration of effective antimicrobial therapy which fails to penetrate it and eliminate the responsible pathogens.[16] Boric acid, a widely available antiseptic agent, serves as a potent method of biofilm removal in addition to providing broad-spectrum bacteriostatic activity. First administered by Reichman et al following 7 days of oral 5-nitroimidazole therapy,[50] it was shown to be more effective if

administered simultaneously with an oral 5-nitroimidazole to maintain an in vivo synergistic effect.[49] However, the optimal duration of intra-vaginal boric acid use remains unknown. Boric acid monotherapy, in spite of its dual activity, does not exert the same successful antimicrobial therapeutic efficacy.[51]

Another advance in the understanding of recurrent BV pathogenesis is that acquired antimicrobial resistance constitutes a major contributor to recurrent BV. Given the non-cultivatable nature of many BVAB,[52] conventional in vitro laboratory tests of antibiotic susceptibility were not forthcoming and resistance was underappreciated and poorly investigated, although resistance genes have been reported.[53] A clue to the possibility of antibiotic resistance was suggested more than a decade ago, when Sanchez et al reported improved therapeutic outcomes following the use of a higher dose of antimicrobials.[54] A limited clinical study performed by Aguin et al showed the clinical advantage of higher doses of intra-vaginal metronidazole achieving larger in vivo local concentrations of this agent.[55] Dose of antimicrobial does appear to matter,[43] but additional controlled studies are needed and surprisingly missing. Comparative studies need to be performed correlating in vitro activity of all members of the 5-nitroimidazole drug class and clinical outcome in so called "dose-range" studies. Schwebke et al previously demonstrated that simply prolonging standard antibiotic therapy beyond 1 week afforded little advantage.[56] Accordingly, next steps in managing women with recurrent BV should include higher drug dose and drug combination therapy.

Another step in the management of recurrent BV is the introduction of long-term maintenance suppressive or prophylactic antibiotic regimens following a course of induction or short-term antibiotic therapy. In the first maintenance therapy trial utilizing twice weekly intra-vaginal metronidazole gel 0.75%, protective efficacy was shown but overall cure was not apparent.[15] The rationale for this regimen was based upon creating a continuous vaginal micro-environment that suppressed the re-growth of pathogenic BVAB, allowing re-establishment of normally protective *Lactobacillus* spp. Miscellaneous prophylactic regimens are now widely used, including intermittent twice weekly intra-vaginal boric acid, but not compared scientifically or subject to robust evaluation. Results are only modestly successful, with occasional breakthrough recurrences of symptomatic BV, much to the distress and disappointment of patients. Unfortunately, reoccurrences also occur following cessation of the maintenance regimen. Overall, this approach is not a major step in achieving long-term BV cure. Better, more effective, bactericidal agents are needed or alternatively innovation in achieving recolonization of the vagina. Another problem is that frequent episodes of symptomatic vulvovaginal candidiasis (VVC) complicate the use of maintenance antibiotic regimens and can occur in excess of 50% of women.[49] In the short term, until there is progress with new concepts, combination treatment regimens (ie, oral 5-metronidazole plus intra-vaginal boric acid), may also be used, as needed, during the maintenance period.

Needless to say, faced with the formidable challenge of curing recurrent BV, practitioners, if not led by frustrated patients, invariably turn to a variety of expensive probiotics, both oral and vaginal. A plethora of choices exist, enthusiastically embraced by the public with good intentions at hand. Unfortunately, most of these probiotics have no efficacy and some better-studied products remain with controversial efficacy.[57] The use of Lactin V (*L. crispatus* CTV-05) remains a future possibility[58] but is not yet available commercially. Similarly, the use of vaginal microbiota transfer (VMT)[59] requires further validation and is currently not available outside of rare research centers (ie, NCT04046900).

Future Areas of Research

A solution to the problem of recurrent BV is an urgent unmet need affecting millions of women worldwide with no promising new antimicrobials in the existing pipeline or expected in the near future. The delay in arrival of new drugs reflects the continued lack of understanding of the pathogenesis of both sporadic, isolated episodes of BV as well as a clear understanding of the pathogenesis of recurrent BV. Advances in laboratory diagnostics have provided us with a more detailed description of the consortium of micro-organisms associated with BV, linked to the disappearance of normally present and protective microbiota, especially *Lactobacillus* spp. However, to date, the same molecular advances, although facilitating diagnosis, have not transformed therapy. Perhaps the next step will entail that these techniques will influence drug treatment strategy. In this context, next-generation sequencing (ie, 16S rRNA gene sequencing, shotgun metagenomic sequencing, etc.) has the capacity to provide critical details of the vaginal microbiota that will allow clinicians to prognosticate and select the optimal type of antimicrobial both individually and in combination for a given patient. Laboratory studies are now revealing that the vaginal microbiota during florid BV

and following antimicrobial therapy is uniquely different in women with refractory and recurrent disease than in those patients who enter a phase of prolonged remission or cure.[60] Apart from rapid recognition of microbial pattern in a given patient that guides the clinician in selecting drugs or duration of therapy, what is also needed is quickly available data on antibiotic resistance in the vaginal microbiota. New methods for commonly observed bacterial susceptibility or resistance should emerge, as recently suggested by Swidsinski et al.[61] Not all women with recurrent BV are the same. In some women, there is a failure to eradicate some, if not all, BVAB. Other women appear unable to reestablish normal vaginal microbiota. Future therapy may be dictated based upon the mechanism of relapse. Currently we do not individualize the treatment of patients with recurrent BV and apply treatment strategies independent of the mechanism of recurrence. Eradicating the BV biofilm is an important next step forward in this line of research, but it is not enough. As mentioned above, progress will require not only attention to detail and optimal use of molecular diagnostic methods but also innovative research related to BV pathogenesis. Needless to say, recognizing the frequency and severity of recurrence in women with BV and its impact on their lives is still a step in the right direction.

Conclusion

Progress in the treatment of women with recurrent BV has been disappointing, with minimal availability of new, more efficacious antimicrobials. The worldwide problem of frequent BV recurrence and chronicity remains neglected and underappreciated. The list of BV complications grows longer but little attention has been directed to the actual problem of recurrent BV, which often continues for many years. Drug and probiotic therapy have largely been disappointing to date, mainly the consequence of our incomplete knowledge of the pathogenesis of this common vaginal infection. In addition, we are still unable to clinically differentiate BV relapse from reinfection. Any therapeutic progress is the consequence of enlightened drug use strategy in the absence of new drugs. Unfortunately, while progress has been made with new commercially available BV molecular diagnostic tests, these remain distant from POC use. Only recently has antimicrobial resistance within BVAB become recognized, and the solution has only produced higher dose and drug combination regimens but no breakthrough advances. One may predict that any hope for future breakthrough resides with new data that may become available using next-generation sequencing to allow individualization of treatment options based upon patient-specific vaginal microbiota.

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