Ready or Not: The Molecular Diagnosis of Bacterial Vaginosis

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(See the article by Menard et al. on pages 33–43)

Although bacterial vaginosis (BV) is a common infection associated with significant sequelae [1], our understanding of the natural history of BV and of interventions to restore normal vaginal microflora remains remarkably deficient. BV is defined as a disruption of the ecology of the vaginal microflora and has been traditionally characterized by conventional cultivation as a shift in microbial species from Lactobacillus species to Gardnerella vaginalis and anaerobic commensals, including Prevotella species, anaerobic gram-positive cocci, Mobiluncus species, Ureaplasma urealyticum, and Mycoplasma hominis.

It has been postulated that lactobacilli play a critical role in maintaining the normal vaginal ecosystem by producing lactic acid and other antimicrobial substances that prevent overgrowth of opportunistic organisms [2]. BV is largely believed to occur as an event secondary to some other stimulus. The initiating events, which may manifest as a decrease in lactic acid concentration, a increase in pH, a decimation of lactobacilli, and an overgrowth of anaerobic organisms, remain largely unknown. Changes in the vaginal ecology may be influenced by age, menstruation, concomitant infections, stress, hormonal contraception, failure to eliminate BV-associated organisms, reinoculation with organisms from an exogenous source (i.e., sexual activities), vaginal cleansing habits, failure of the Lactobacillus species to recolonize or fully acidify the vagina, and the presence of bacteriophages that target lactobacilli [3–11]. In addition, the factors controlling the resilience of the vaginal microflora, as defined by the amount of disturbance that the vaginal ecosystem can withstand without changing its structure (i.e., microbial species composition and abundance), are unknown. Although extrinsic events are important, the ecosystem resilience could be a better predictor of outcome, such that low resilience may lead to a higher risk of BV and high resilience may lead to a lower risk.

The gold standard for laboratory diagnosis of BV, the Gram stain [12], has been used for the past 25 years and is largely based on the presence or absence of lactobacilli. However, recent investigations conducted using cultivation-independent methods have revealed that a significant proportion of apparently healthy women lack appreciable numbers of lactobacilli [13, 14]. Thus, there is an urgent need for the development of new tools for the accurate diagnosis of BV.

Ultimately, the role of Lactobacillus species in diagnostic criteria for BV may change as molecular tools replace microbiological observations to define the vaginal ecosystem [15]. These tools have already led to the identification of new BV-associated bacteria that could not be identified by traditional culture-based methods. Although the methodologies employed have been somewhat different (samples were obtained from different parts of the vagina, from women of different ethnic and geographical origins, and at undisclosed times in the menstrual cycle; and different PCR amplification conditions were present), these studies revealed a more complicated picture of the vaginal microflora than was previously recognized [3, 13, 14, [16–24].

A recent study demonstrated significant differences in vaginal microbial communities between women of 2 different ethnicities [14]. The prevalence of vaginal communities dominated by various species of Lactobacillus (protective) was lower among black women than among white women. Conversely, communities dominated by another lactic-acid producer (i.e., Atopobium species) and other strict anaerobes were 4 times more common in black women than in white women. These differences in community composition and structure may account for the observed racial predisposition to BV [1]. The mechanisms that account for these dis-
fective differences are unknown, but they are likely attributable to intrinsic host factors. Overall, the data accumulated using culture-independent methods suggest that the structure of vaginal microbial communities varies among women with respect to the number and type of numerically prominent microbial populations. Despite these differences, it is hypothesized that the ecological function of the microflora—maintenance of a low-pH environment (via lactic acid production) that precludes the colonization and growth of pathogens and other undesirable organisms—is conserved.

The lack of understanding of what defines a healthy vaginal microbial community makes it difficult to develop accurate molecular diagnostic tools. It is important for such tools to have the ability to identify and differentiate strains and functions; this is particularly relevant when considering the diversity of Lactobacillus species found in the vagina [15]. The microbial genome sequencing efforts undertaken by the National Institutes of Health–funded Human Microbiome Project [25] will undoubtedly contribute to this goal. It is critical to acquire a better understanding of the vaginal flora in healthy women as a prerequisite for comprehending the factors associated with disease susceptibility. Although epidemiologic studies have demonstrated that the vaginal microflora in healthy women help to prevent colonization with pathogenic organisms, including those responsible for BV, candidiasis, sexually transmitted infections (including HIV infection), and urinary tract infections [26–29], the mechanisms by which this is accomplished are unknown.

In this issue of Clinical Infectious Diseases, Menard et al. [30] report on the application of a quantitative molecular tool targeting BV-related microorganisms that is based on quantitative real-time PCR. The authors found that the molecular quantification of 2 microorganisms, Atopobium vaginae and G. vaginalis, had excellent sensitivity (96%) and specificity (99%) when compared with the Nugent Gram stain classification of BV. Research participants were recruited from only 2 settings in France, and all participants were pregnant. Although the study represents a major step toward a molecular diagnostic test for BV, the generalizability of the results to other populations needs to be established before widespread application of this method is advocated.

Interestingly, Menard et al. [30] reported that the molecular tool resulted in 40% of the intermediate Nugent scores being assigned to the BV category in the validation study. The clinical implications of having an intermediate Nugent score are not well defined. Whether these women will develop BV or whether their flora will regress to normal is unclear. In addition, therapy is not advocated for asymptomatic women with intermediate scores. A longitudinal component would help to determine whether these 40% of women with intermediate scores who were categorized as having BV by molecular testing were ultimately more likely to develop BV, compared with the 60% of women with intermediate scores who did not receive diagnoses of BV. In the future, molecular assessment of the longitudinal changes in vaginal microflora may help to better define which fluctuations in the vaginal microbial ecology are clinically meaningful. This, in turn, might better clarify the role of the various identified risk factors for BV.

As part of the Human Microbiome Project [25], metagenomic studies of the vaginal microbial communities are expected to lead to the development of novel molecular tools for studying the human vaginal microflora and its interaction with the human host. For example, the study of gene expression patterns (host or community associated) for specific markers could facilitate future work toward a clinical diagnostic tool for use in the management and treatment of disease. Most importantly, such tools and approaches will be critical in the identification of metabolic functions that are either required for a stable vaginal ecosystem, are lacking in some community types, and are potentially associated with diseases or are present during disease but absent in a healthy vagina. These studies will spur the development of efficient and targeted treatments or probiotics. Application of this quantification tool to determine which microbial patterns are associated with poor pregnancy outcomes [31] is another promising venue. This information may help explain why the findings of BV treatment trials to prevent preterm birth have been heterogeneous [32].

Clearly, there is a need for a reliable, nonsubjective, inexpensive, and accessible test for BV. Menard et al. [30] demonstrated the feasibility of a pared-down molecular approach to diagnosing BV—at least within a fairly homogenous population. The question is, are we ready for a molecular test? A better understanding of the vaginal microbiome, a clear definition for BV, and the short-term and long-term fluctuations in vaginal microflora will help to better define molecular tests within the broader clinical context. Furthermore, understanding of these aspects will help define whether BV is an ecological disorder caused by an underlying pathogen or by environmental stimuli, as well as help define transmissibility and other key epidemiologic questions. Until then, more work is needed. Menard et al. [30] have provided us with an encouraging start.

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References

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