Gynecologic health and disease in relation to the microbiome of the female reproductive tract

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It is well established that the vagina is colonized by bacteria that serve important roles in homeostasis. Imbalances in the proportion of bacteria may lead to a predisposition to infection or reproductive complications. Molecular-based approaches demonstrated a greater degree of microbial diversity both within and between women than previously recognized. The vaginal microbiome may fluctuate during various states of health, such as during the menstrual cycle or after menopause, and there may be differences in the vaginal microbiome between women of different ethnicities. Furthermore, the specific composition of the vaginal microbiome may influence the predisposition to dysbiosis and the transmission of sexually transmitted infections. An understanding of the diversity of the vaginal microbial environment during states of health is essential for the identification of risk factors for disease and the development of appropriate treatment. (Fertil Steril® 2015;■:■–■. ©2015 by American Society for Reproductive Medicine.)

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The maintenance of human health is dependent on a symbiotic relationship between humans and associated bacteria. There are approximately 10 times as many microbes associated with a human as there are human cells in the body (1). Despite recognition of the importance of the interactions between the host human body and the bacteria it supports, there remain many unanswered questions regarding how the microbial environment varies within and among individuals in healthy and diseased states. Historically, bacteria have been identified using Gram stain or culture-based techniques. However, as few as 20% of bacteria closely associated with the human body can be cultivated via culture-based techniques (2, 3). Culture-based methods may therefore underestimate the diversity of the microbiome.

Over the past decade there has been an explosion of interest in molecular-based, culture-independent techniques to study the microbiome. Molecular-based techniques primarily involve analysis of 16S ribosomal RNA (rRNA) with polymerase chain reaction (PCR), DNA hybridization or fingerprinting, and next-generation sequencing (4–11). Recognizing the potential of molecular techniques to further our understanding of human bacterial communities, the National Institutes of Health initiated the Human Microbiome Project (HMP) in 2007 (12–15). In this consortium, samples from approximately 300 healthy human subjects from body sites including the skin, nose, mouth, lower gastrointestinal tract, and vagina were analyzed in an effort to more accurately characterize the normal human microbial environment (12–15).

The HMP targeted the genitourinary system because it has been established for more than a century that bacteria are present within the vagina and that an imbalance within this microbial environment may be associated with disease (16–19). Research has demonstrated that alterations in the vaginal microbiome

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affect susceptibility to gynecologic infections, including cervicovaginitis, postoperative infections, and human immunodeficiency virus (HIV) infection (20–26). Nevertheless, data from molecular-based techniques suggest that many of the differences in the vaginal microbiome may represent normal variation and may not necessarily indicate disease.

An important consideration when interpreting molecular-based microbiome studies is that molecular techniques primarily rely on the sequencing of bacterial RNA. Unless specific focus is placed on defining relative proportions of bacteria, the presence of a particular organism's genetic material does not imply that it is dominant or even present in significant concentration. Information obtained from both culture- and molecular-based methods are presented in this review, because a combination of the two methods may clarify both the role of bacteria in maintaining gynecologic health and how changes to the microbiome impact susceptibility to disease.

VAGINAL MICROBIOME IN THE HEALTHY STATE

It is well established that the normal vaginal microbiome is dominated by *Lactobacilli* species (16, 27, 28). *Lactobacilli* help prevent vaginal infection by producing lactic acid, hydrogen peroxide, bacteriocins, or through competitive exclusion of other bacteria (29–32). Studies utilizing 16S rRNA PCR have demonstrated that the relative proportions of specific vaginal bacteria may vary between healthy, asymptomatic women (6, 33). These genomic studies demonstrated that the vaginal microbial environment is usually dominated by one or two *Lactobacilli* species, most frequently *Lactobacillus iners, Lactobacillus crispatus, Lactobacillus gasseri*, or *Lactobacillus jensenii* (6, 34). However, a portion of asymptomatic, healthy women, particularly black and Hispanic women, host a polymicrobial vaginal environment dominated by bacteria other than *Lactobacilli*, including *Prevotella*, *Gardnerella*, *Atopobium*, and *Megasphaera* species (6, 33).

Of the 73% of women with a *Lactobacilli*-dominant environment, the most frequently detected organism was *L. iners*, which was the predominant organism in 34% of women sampled. The second most common *Lactobacilli*-dominant environment was one in which *L. crispatus* was most prevalent (26.2% of women) (6). The identification of an *L. iners*-dominant microbial environment in the majority of healthy women is in contrast to findings from culture-dependent and early molecular-based studies, which suggested other dominant *Lactobacilli* species, including *Lactobacillus acidophilus*, *L. crispatus*, and *L. jensenii* (35, 36). The species of *Lactobacilli* that dominate the vaginal environment may have implications for gynecologic health: it seems that various species may differentially predispose to dysbiosis (37, 38). For example, it has been suggested that an *L. crispatus*-dominant vaginal microbiome is more stable and less likely to transition to bacterial vaginosis (BV) than an *L. iners* or mixed-*Lactobacilli* environment (39, 40). However, recent studies have suggested that the increased proportion of *L. iners* in women with BV may be related to an inherent ability of *L. iners* to tolerate the conditions of BV (such as elevated pH) more than other species of *Lactobacilli* (41).

Microscopy and culture-dependent methods demonstrated that the composition of normal vaginal flora may also fluctuate within an individual woman; for example, throughout the menstrual cycle or as a result of sexual activity (42–44). Molecular-based studies support these earlier findings that a woman's vaginal microbial environment is dynamic and may change in response to normal life stages or activities (40, 45). During menses there is a decrease in *Lactobacilli* and a relative increase in the proportion of bacteria associated with higher Nugent scores, though women may not report BV symptoms (40, 45). Recent sexual activity may also affect the microbial composition of the vagina by decreasing the proportion of *Lactobacilli* species present (40, 46), which may predispose to dysbiosis with the loss of the protective effects of *Lactobacilli*. Decreased *Lactobacilli*, particularly *L. crispatus*, as well as decreased bacterial diversity, have also been observed in postmenopausal women, specifically those with vaginal dryness or atrophy (47–49). It is important to note that studies may differ in the sampling and sequencing methods used, and specific molecular-based methods vary in the data produced. For example, whole-genome amplification yields species and strains, whereas 16S rRNA PCR provides information on genus and may yield species (6,13–15). Therefore, variations in design and analysis should be considered before forming conclusions based on the direct comparison of different studies.

The observed fluctuations throughout the menstrual cycle may be explained by evidence that high levels of E_2 may favor a *Lactobacilli*-dominant environment, especially *L. crispatus* (40, 50, 51). In states of relatively low estrogen, such as the beginning of a menstrual cycle or in postmenopausal women, *L. crispatus* levels may also be low (40, 45, 47, 48). However, despite evidence from both culture-dependent and -independent methods supporting the dynamic nature of the vaginal microbiome, some molecular-based studies suggest that the microbiome is relatively stable through periods of hormonal fluctuation, such as puberty or the menstrual cycle (52, 53). There have been a number of studies that have evaluated the vaginal microbiota in tandem by both culture-based and molecular techniques. These studies demonstrate that culture-dependent and -independent methods have a moderate level of concordance, thus providing similar but not identical vaginal microbiome profiles (54, 55).

The quantity and proportion of specific microorganisms in the vagina may vary between women of different ethnic origins. African American women may have increased *L. iners* and decreased *L. crispatus* levels compared with Caucasian or Asian women (40). This distinction is important because, as mentioned above, an *L. iners* dominated environment may predispose to BV (39, 40). Molecular studies have also demonstrated that African American and Hispanic women are also more likely to harbor a vaginal microbiome dominated by bacteria other than *Lactobacilli* species compared with Caucasian women. These studies suggest that African American women may have higher levels of