Better Detection Methods More Accurately Define the Vaginal Microbiota

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Bacterial vaginosis (BV) is a highly prevalent condition in women. It is associated with poor reproductive and health outcomes, including preterm birth and the acquisition of sexually transmitted diseases such as HIV infection. The current methods for diagnosing BV may not correctly define the vaginal microbiota because identification of bacterial species are typically made using cultivation techniques and not all bacteria present can be cultivated. This misidentification can result in an inadequate diagnosis of a patient's vaginal health and less than optimal recommendations for treatment. In a recent study published in *PLOS One*, researchers from the laboratory of Dr. David Fredricks (Vaccine and Infectious Disease Division) utilized cultivation-independent molecular methods to obtain a highly precise characterization of the vaginal microbiota in study subjects. Surprisingly, the researchers found that the predominant bacterial species that is typically detected in some patients with BV via traditional methods is actually an altogether different species that has been misidentified.

As the most common cause of vaginal discharge, BV is thought to be the result of a shift in the vaginal bacterial community. Healthy women without BV show the predominant presence of lactobacilli, bacteria believed to promote a healthy ecosystem. When lactobacilli are replaced with other bacteria species, such as Gram-negative rods, this can lead to poorer health, the diagnosis of BV, and symptoms such as discharge and irritation. However, many women with BV report no symptoms.

"The gold standard method for diagnosing BV relies on detection of particular bacterial shapes and their abundances on microscopic examination of vaginal fluid smears," explains lead author and staff scientist, Dr. Sujatha Srinivasan. "One indicator of BV is the presence of curved Gram-negative rods that were previously thought to represent *Mobiluncus* species." She and her collaborators sought to characterize the vaginal microbiota by detecting bacterial nucleic acids, which would allow for better identification of bacterial species through detection of genetic sequences as well as a more precise quantification of each species through quantitative polymerase chain reaction (qPCR). They

compared their genetic-based results with the bacterial morphotypes as determined by the traditional Gram staining of vaginal fluid smears.

Strikingly, samples containing curved rods that were previously identified as *Mobiluncus* through Gram staining were shown by molecular methods to be a different bacterium named BV-associated bacterium 1 (BVAB1). In fact, BVAB1 DNA was 100-fold more abundant than *Mobiluncus* DNA (p<0.001) in BV patient samples. This analysis was further corroborated by fluorescence in situ hybridization (FISH) of vaginal fluid smears (see figure).

According to Drs. Srinivasan and Fredricks, "this study raises the question of whether women who have BVAB1-dominant vaginal bacterial communities have distinct risks for the adverse health outcomes associated with BV." Additionally, the molecular detection techniques utilized "highlight the limitations of using microscopy with Gram stain as a proxy to infer the composition of complex microbial communities, and illuminates the increased taxonomic resolution obtained from use of molecular tools."

Indeed, the ability to further improve diagnostic methods will ultimately improve health outcomes. The researchers are optimistic that "a better understanding of the precise composition of the vaginal microbiota in women will allow researchers to better assess how particular microbes affect disease risk, and will pave the way to manipulate the microbiota to optimize health and reproductive outcomes."

Srinivasan S, Morgan MT, Liu C, Matsen FA, Hoffman NG, Fiedler TL, Agnew KJ, Marrazzo JM, and Fredricks DN. 2013. More than meets the eye: associations of vaginal bacteria with Gram stain morphotypes using molecular phylogenetic analysis. *PLoS One*. 8(10), e78633.

See also: Srinivasan S and Fredricks DN. 2008. The Human Vaginal Bacterial Biota and Bacterial Vaginosis. *Interdisciplinary Perspectives on Infectious Diseases* 2008:750479
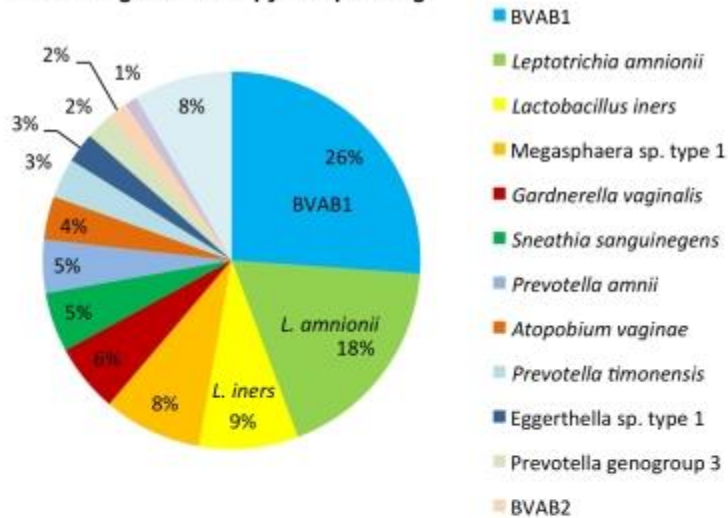
Broad-range PCR with pyrosequencing

Legend:
- BVAB1
- *Leptotrichia amnionii*
- *Lactobacillus iners*
- Megasphaera sp. type 1
- *Gardnerella vaginalis*
- *Sneathia sanguinegens*
- *Prevotella amnii*
- *Atopobium vaginae*
- *Prevotella timonensis*
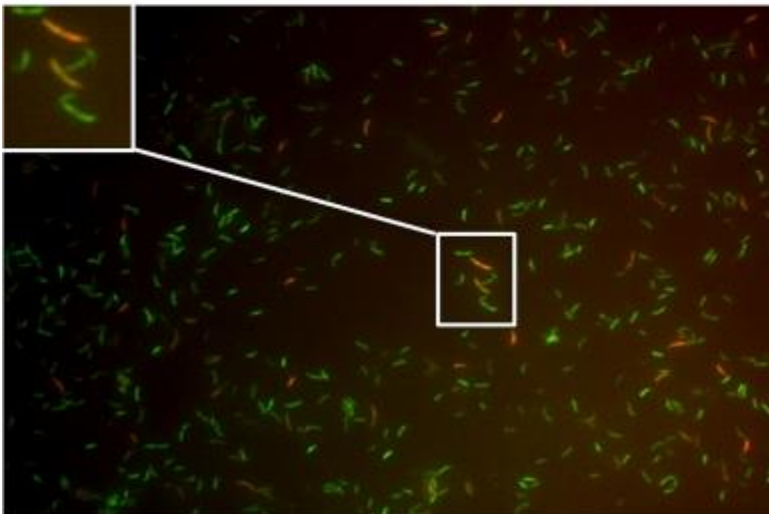- Eggerthella sp. type 1
- Prevotella genogroup 3
- BVAB2

FISH with probes targeting BVAB1 (green) and *Mobiluncus* (red)

Image provided by Dr. Sujatha Srinivasan.

Figure Legend: BVAB1, a recently discovered novel bacterium, is the dominant member of vaginal bacterial communities in some women with BV. Above: Rank abundance plot showing percentages of sequence reads obtained using broad-range PCR with high-throughput sequencing in women with curved Gram-negative rods in Gram stains of vaginal fluid. BVAB1 is the most abundant member of the vaginal bacterial community in these women. Below: Vaginal fluid smears from a representative participant shows that both BVAB1 (green) and Mobiluncus (red) are curved rods. Hence, these bacteria cannot be distinguished in Gram stains.