

*Bacterial Vaginosis:
Historical stigmas and diagnostic methods influence modern day perceptions*

**Dettle SK, Sieber JR
Department of Biology, University of Minnesota - Duluth**

ABSTRACT

For over a century research on the composition of the vaginal microbiome and its correlation to symptoms and disease has been of interest. Early studies have identified key *Lactobacillus* bacteria that are abundant in healthy women (Doderlein 1891), while other studies identified the possible pathogenic microbe, *Gardnerella vaginalis*, abundant in women with vaginal discomfort (Gardner 1957). The term used to describe **the shift from the “healthy” bacteria to a different composition of bacteria in the vaginal microbiota is *bacterial vaginosis* (BV)**. This shift is seen as a dysbiosis that can cause vaginal discomfort. The method that is universally accepted for diagnosing BV scores vaginal bacterial compositions morphologically by the amount of Gram-positive *Lactobacillus* versus the amount of Gram-negative *Gardnerella* and other Gram-variable present in the canal (Nugent 1991). Recent studies have indicated there are multiple unique bacterial community compositions in women asymptomatic for BV (Ravel 2011). Some vaginal communities lack a dominating *Lactobacillus* species, and are then classified as having BV based on Nugent score criteria. Women who lack *Lactobacillus* species often are of different ethnic groups (Anukam 2006), leading researchers to question the reason and find correlations for this phenomenon. The present analysis highlights the skewed diagnosis techniques of BV that establish a social stigma against certain ethnic groups.

DEFINING BACTERIAL VAGINOSIS FOR DIAGNOSTIC PURPOSES

In literature, bacterial vaginosis has been defined by Zhou, et al as “events that lead to *decreased numbers of lactic acid bacteria* in the vagina and *the concomitant increase in the abundances of other bacteria* (as retold from Thorsen et al 1998, Koumans and Kendrick 2001, by Zhou et al 2007). Zhou and colleagues add to the definition that “BV is not an infectious disease but rather *a set of symptoms that reflect an ecological disturbance* that is accompanied by changes in the relative abundance of autochthonous organisms in the vagina.”

These definitions match the diagnostic criteria established decades earlier. The BV diagnostic criteria was implemented in response to inconsistent diagnostic methods in research and in clinic. Amsel criteria used physical symptoms, biochemical tests, and microscopy to develop standard clinical bacterial vaginosis diagnostic criteria (Amsel et

al, 1983). A patient was diagnosed with having BV if they possessed one of the four following symptoms:

- (1) Vaginal pH above 4.5
- (2) presence of white adherent discharge
- (3) Positive whiff test (fishy odor) after addition of KOH
- (4) Clue cells with a granular appearance and who are gram-variable coccobacilli

Less than one decade later, the Nugent score was created to improve intercenter reliability when diagnosing bacterial vaginosis (Nugent, 1991), a system that provided the option of diagnosing bacterial vaginosis on a spectrum of severity, rather than on its presence or absence. This technique used laboratory Gram staining to identify bacterial morphotypes from vaginal discharge. It scored a microbiome based on the number of gram-positive rod shaped *Lactobacilli* versus the number of gram-negative curved shaped *Gardnerella* present in a vaginal discharge sample. Research has supported that the abundance of *Lactobacilli* in a vaginal microbiome constitutes a healthy individual (Doderlein 1891), while the abundance of *Gardnerella* in a vaginal microbiome is associated with a microbiome in dysbiosis (Gardner & Dukes 1955).

There was a need to define BV with a matched set of symptoms for accurate diagnosis. Yet the definition as well as the universally accepted diagnostic criteria fail to consider naturally occurring compositional changes as discovered during a regular menses (Gajer et al 2012). As scientific methods continue to be refined, researchers have discovered that the vaginal canal plays host to a microbiome unique to each woman.

METHODS THROUGHOUT HISTORY

1891, Germany: Morphology and Gram-stain technique. Isolation of microorganisms from sterile swab. Single organisms viewed at a time. Doderlein discovered novel *Lactobacillus* species that was named Doderlein's bacillus (Doderlein 1891).

1928, USA: Simple enzymatic assays characterized Doderlein's bacillus as *Lactobacillus acidophilus* (Thomas 1928)

1955-1960 Texas, USA: Gardner and Dukes identify *Gardnerella* as a "pathogenic" microbe present in symptomatic women. 1957: Gardner and Dukes compared BV incidence between White and Black women in Texas.

1977: Carl Woese founded 16srRNA characterization (Woese et al 1977)

1980: A taxonomy study divided *Lactobacillus acidophilus* into six homology groups (Johnson et al 1980)

1983: Amsel criteria

1991: Nugent scoring using Gram staining technique

1999: Recommended treatment for BV is clindamycin or metronidazole antibiotic cream.

Antibiotic treatment is recommended even if moderate BV is discovered.

2012: Literature review by Ma et al suggested a bacterial function versus residence approach to researching the vaginal microbiome.

2018: Malawi on the continent of Africa: Metagenomic analysis of vaginal microbiomes of women post-partum. Metagenomics considers associations between bacteria within a community (Doyle R et al)

2018 (preprint): A metatranscriptomic analysis discovered which bacteria were transcriptionally active in subjects known to have had antibiotic treatment for BV (Deng, ZL)

FIVE UNIQUE BACTERIAL COMMUNITY STATE TYPES

Jacques Ravel and his colleagues sampled 394 nonpregnant, reproductive age women who were constitutionally healthy, to compare and contrast vaginal bacterial Community State Types between women of a variety of ethnic backgrounds (Ravel, 2011). Subjects were from the United States and identified as one of four ethnic groups: White, Black, Asian, or Hispanic. Community State Types were divided into 5 common supergroups called Community State Types (CST): CST I, CST II, CST III, CST IV and CST V. After the categorization, each supergroup was compared using the Nugent score method. Results revealed that CST 4, which hosted a diverse microbiome with a low abundance of *Lactobacillus*, was characterized as a microbiome with bacterial vaginosis.

MENSES AND THE MICROBIOME

A temporal study on 39 reproductive-aged nonpregnant women from the United States revealed the dynamic microbial changes of vaginal microbiome (Gajer et al, 2012). For example, the vaginal microbiome of Subject 13 is dominated by *L. iners*, which is the most abundant organism of the previously categorized CST III (Ravel 2011). The study also discovered that a vaginal microbiome can change Community State Types. Subject 26 had a microbiome that changed from CST III to CST I, and later to CST IV. (C) The vaginal microbiome of subject 26. Yet Subject 26 was ultimately dominated by *Lactobacillus crispatus* from CST I. Depending on when subjects were tested, the Nugent scoring varied. Based on results from Gajer and his colleagues, the Nugent score might influence the diagnosis of BV differently in the same patient, depending on the time of the sample.

COMMENTS AND FUTURE DIRECTION

As long as symptoms persist, there is still a medical need to continue research on the vaginal microbiome. Current investigators are aware there is a statistically significant difference in vaginal bacterial communities between groups of women. This difference has caused medical workers and researchers to reconsider the diagnostic method of

bacterial vaginosis. Although some microbiomes could be more prone to obtaining BV and its health implications, there must also be an evolutionary benefit to each CST because of their high prevalence in women. The tone of research should celebrate women's differences. This celebratory tone should drive the introductory paragraphs of future published papers.