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Why screen the vaginal microbiome?

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Introduction

Bacterial infections occurring in both the obstetrical and gynecologic patients have greater than 90 to 95% of their origin in the patient's vaginal microbiome or indigenous vaginal bacterial community. These infections range from mild infections such as vaginitis and vaginosis to severe life threating infections, e.g., severe sepsis and necrotizing infections that are associated with significant morbidity and mortality. There is a plethora of literature that suggests and supports the causality of the pathogenic bacteria that reside within the vaginal microbiome. Three of the most common infections that illustrates these findings are urinary tract infections, postpartum endometritis, and post hysterectomy pelvic infection. Thus, the question is, in addition to the administration of antibiotics for surgical prophylaxis, what can be done to reduce the patient's risk for developing a post-surgical infection?

Vaginal micriome

The vaginal microbiome (VM) is a significant part of the vaginal ecosystem. The VM is dynamic community that exist in six basic communities (Table 1). These VMs can be subdivided into sub communities. Several investigators have established, basically, five Community States (CST); CST I, dominated by *Lactobacillus crispatus*, CST II, dominated by *L. gasseri*, CST III, dominated by *L. iners*, CST V, dominated by *L. jensenii* and CST IV, dominated by obligate anaerobes.[1] Further investigation demonstrated that CST IV could be divided into CST IVA and CST IVB. CST IVA appears to be a heterogenous community characterized by either *L. crispatus*, *L. iners*, other lactobacilli species and a low proportion of anaerobic species, such as *Anaerococcus, Corynebacterium, Finegoldia*, or *Streptococcus*. CST IVB Appeared to be resemble Bacterial Vaginosis (BV) and is characterized by a preponderance of Atopobium, Prevotella, Parsimonies, *Sneathia, Gardnerella, Mobiluncus*, or *Peptoniphilus* (formerly *Peptostreptococcus*).[2] The important fact to consider, the VM as the source of infection, associated with any invasive procedure performed through the vagina, is that the VM is a polymicrobial community containing bacteria of low-pathogenicity (non-pathogens) and high pathogenicity. In this context the VM can be categorized into the following groups:

A. Group 1: dominated by one or more species of *Lactobacillus; L. crispatus, L. gasseri*, or L. jensenii. Additional species that may be significant *L. rhamnosus* and *L. vaginalis.*

B. Group 2: contains a significant concentration of *L. iners*.

C. Group 3: absence or very low concentration of Lactobacillus, a high concentration of facultative anaerobic bacteria.

Table 1 Vaginal dysbiosis is associated with infections in the obstetric and gynecologic patient

Obstetric Infections	Infection common to both	Gynecologic infections
Septic abortion	Cystitis	Cervicitis
Abortion (not septic)	Urethritis	Endometritis
Chorioamnionitis	Pyelonephritis	Salpingitis
PPROM	Vulva cellulitis	Tubo-ovarian abscess
Preterm labor	Abdominal incision infection	Post hysterectomy:
Post-partum endometritis	Bartholin's gland abscess	Vaginal cuff cellulitis
	Skene's gland abscess	Pelvic cellulitis
	Necrotizing Soft Tissue infection	
	Bacteremia	
	Sepsis	
	Septic shock	
	Toxic Shock Syndrome	

The VM may be made up of one or more of these highly virulent bacteria, e.g., *Streptococcus agalactiae, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae,* and *Enterobacter cloacae.* Other bacteria that may be of concern to the obstetrician/gynecologists are *Staphylococcus saprophyticus, Staphylococcus epidermidis*, and *Streptococcus dysgalactiae.* The obstetrician/gynecologist must always consider the possibility of other highly virulent bacteria such as Streptococcus pyogenes, methicillin staphylococci, and other gram-negative facultative anaerobic bacteria.

1) Group 4: obligate anaerobic bacteria, basically Bacterial Vaginosis

2) Group 5: presence of *Trichomonas vaginalis* which is almost always associated with vaginal dysbiosis.

3) Group 6: *Candida* vaginitis which can be associated with a Lactobacillus dominated VM or vaginal dysbiosis.

These various vaginal communities, probably rarely exist in a pure form, but exists as a complex polymicrobial community. Mixed communities, i.e., Aerobic Vaginitis (AV) + BV, may be more common than is recognized; molecular microbiology will help answer this question. The presence of facultative anaerobic bacteria increases the virulence of the vaginal microbiome. This is because the bacteria that make-up AV reproduce every 30 to40 minutes, whereas the obligate anaerobes (BV) reproduce approximately every 4 hours. In addition, the AV community can grow in both an aerobic and anaerobic environment. Therefore, AV can cause serious infection but can set the stage for a complex polymicrobial infection involving both facultative and obligate anaerobic bacteria.

Aerobic vaginitis is characterized by a decrease or absence of *Lactobacillus*, a vaginal discharge that ranges from gray to dirty

gray to purulent. There is no odor associated with the discharge. The number of leukocytes can range from <10 to >50.[3] The number of WBCs present differentiates an inflammatory state from a non-inflammatory state. The prevalent bacteria are *Escherichia coli, Streptococcus agalactiae, Staphylococcus aureus,* and *Enterococcus faecalis.* The use of molecular microbiology techniques will increase the number of bacteria that make-up AV.

Significance of a polymicrobial vaginal microbiome

Each vaginal microbiome is a polymicrobial community, consisting of a variety of bacteria. This bacterial niche is adapted to an acidic environment that functions as "friend or foe" or the "yin and yang" and exists in a delicate balance. When this balance is upset vaginal dysbiosis develops. Once vaginal dysbiosis develops the bacteria of high virulence become dominant and pose a threat to the patient's well=being. Vaginal dysbiosis has been shown to be associated with, if not the cause, a variety of infections (Table 1).

Therefore, the vaginal microbiome when shifted, by the loss of *Lactobacillus* dominance, to vaginal dysbiosis becomes a significant threat to the well-being of the patient. Therefore, the patient with vaginal dysbiosis is a significant challenge to the obstetrician and gynecologist because this bacterial community, be it AV or BV or a mixed bacterial community (AV/BV) or trichomoniasis contains many pathogenic bacteria, some will contain strains that are resistant to antibiotics.

In addition to bacterial strains possessing antibiotic resistant, strains of Gram-negative bacteria, e.g., *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter cloacae, Proteus mirabilis, Pseudomonas aeruginosa*, may possess beta-lactamases.[4] Bet-lactamases afford the bacteria the ability to resist the activity of beta-lactam antibiotics, e.g., extended spectrum penicillins and cephalosporins. These bacteria can acquire and transmit the genes necessary to produce extended spectrum beta-lactamases plasmids (ESBL) and can pass genes conferring resistant to other antibiotics such as tetracyclines and fluroquinolones. The ability to produce a variety ESBLs makes the administration of antibiotics empirically somewhat precarious, because administering antibiotics empirically can lead to the establishment of resistant strains as dominant members of the vaginal microbiome. ESBLs hydrolyze extended spectrum penicillins, cephalosporins and monobactams.[5] *Escherichia coli, Klebsiella oxytoca*, and *Klebsiella pneumoniae* also produce carbapenemase making these Gram-negative bacteria uniquely challenging because these bacteria are frequent inhabitants of the vagina.[6,7] *Escherichia coli, Klebsiella oxytoca*, and *Klebsiella pneumoniae* are commonly found in bacterial communities termed aerobic vaginitis.[7] The significance of this is that the genetic transfer of genes conferring resistance to beta-lactam antibiotics is frequently associated with genes producing resistance to fluoroquinolone, aminoglycoside, and trimethoprim/sulfamethoxazole.[8,9] Therefore, it behooves obstetricians and gynecologist to become familiar with their hospitals antibiogram because the traditional empiric administration of clindamycin or metronidazole plus ampicillin plus gentamicin is becoming less effective. In addition, the empiric administration of gentamicin has become almost ineffectual because of the increased resistance of the Gram-negative facultative anaerobic bacteria, e.g., especially Escherichia coli with rates exceeding 20% in some hospitals.[9–11]

In addition, to the presence of a variety of pathogenic bacteria being present in the vaginal microbiome, other factors than antibiotic resistance pose a significant threat. These are the concentration or potential inoculum of bacteria, and whether these bacteria have initiated a cytokine response. The ratio of Lactobacillus to pathogenic bacteria is critical to the VM ability to induce an infection. In a VM where Lactobacillus is dominant, the concentration is typically $>10^{6\text{ to }8}$ bacteria/ml versus the concentration of pathogenic bacteria, $<10^3$ bacteria/ml or 1,000: 1 to 100,000: 1. The relative low numbers of pathogenic bacteria is not sufficient to cause infection in an immunocompetent patient. Conversely, when vaginal dysbiosis is present this ratio is reverse, e.g., Lactobacillus may be absent or present in a concentration of $<10^3$ and pathogenic bacteria $>10^{6\text{ to }8}$ bacteria/ml, yielding ratios of 1: 1,000 to 1: 100, 000 bacteria/ml. Concentration or inoculums of pathogenic bacteria of this magnitude can over-come a single dose of antibiotic administered for surgical prophylaxis. The presence of pathogenic bacteria and depending upon their concentration will result in a concentration of endotoxin and exotoxin release into the vaginal ecosystem. The concentration will depend upon the numbers of pathogenic bacteria present.[12–14]

The presence and concentration of lipopolysaccharide (LPS) is dependent upon the number (concentration) of Gram-negative bacteria residing in the vagina. LPS, an endotoxin, is found on the outer membrane of Gram-negative bacteria and binds to the CD14/TLR4/MD7 receptor complex on a variety of cells, e.g., monocytes, dendritic cells, macrophages and B cells which stimulates the production and secretion of and array of proinflammatory cytokines, nitric oxide and eicosanoids.[15] The presence of Gram-negative bacteria in the vagina, e.g., Prevotella bivia, can stimulate the cytokine system and increase the concentration of TNF-alpha.[16]

In a study examining the potential of lipopolysaccharides from different Gram-negative bacteria isolated from the vagina demonstrated that *Escherichia coli* and *Enterobacter aerogenes* exhibited a stronger stimulating immune response than *Klebsiella pneumoniae,* Proteus mirabilis, Acinetobacter colcoaceticus, Citrobacter freundii, and Pseudomonas aeruginosa.[14] Seelbach-Goebel et al.18 reported on the isolation of a variety of Gram-negative bacteria from women with PROM and preterm labor (Table 2).

Table 2 Gram-negative and Gram -positive isolates from the vagina's women with PROM

Gram-positive	Gram-negative
Enterococcus 64%	Escherichia coli 90%
Streptococcus agalactiae 24%	Klebsiella spp. 12%
Staphylococcus aureus 10%	Proteus spp. 9%
	Pseudomonas spp. 5%
	Morganella morganii 2%
	Enterobacter spp. (2%)
	Citrobacter (2%)
	Acinetobacter (2%)

(N = 231 pregnant women) [Adapted from Seelbach-Goebel B. 201315].

Thus, the presence of pathogenic bacteria, especially the Gram-negative can, if present in sufficient numbers, stimulate an inflammatory response or infection? One of the initial responses is to upregulate the innate immune response resulting in cytokine release, complement activation and phagocytosis. This response is initiated via the production of T and B cells, either as a primary response or memory cellular response. There is no doubt that vaginal dysbiosis results in proinflammatory response, stimulating a host immune response in the vagina. This response is marked by the production of IL-1ß. IL-1alpha, IL-6, IL-8, 12p70, and TNF-alpha.[17–23] Interestingly, Lactobacillus dominance is associated with an increase in gamma-induced protein 10(IP-10). IP-10 was initially referred to as IFN-gamma-inducible chemokine, which activates and recruits white blood cells, e.g., T cells, eosinophils, monocytes, and NK cells through binding to C-X-C motif and receptor 3 (CXCR03).[23,24] Gardnerella vaginalis and Atopobium vaginae dominance result in a decline in IP-10 and an increase in IL-1 alpha, IL-1 beta, IL-8 and IL-12.[25] Prevotella is associated with an increase in IL-1beta, IL-8, IFN-gamma, and TNF-alpha.[12,22,23]

There is no doubt that vaginal dysbiosis initiates an immunological response and therefore can be considered an infection. The association of vaginal dysbiosis with the variety of infections that occur in

both obstetric and gynecologic infections leaves little doubt there is need for screening women routinely for the presence of vaginal dysbiosis. Thus, the polymicrobial community that constitutes the vaginal microbiome, not only presents several challenges to the clinician treating patients with vaginal dysbiosis but, has the burden of minimizing the evolution of resistant bacteria within this polymicrobial community. There is an additional burden, the effect of the administered antimicrobial agent on all the other microbiomes within and on the patient.

There is no doubt that infectious diseases will not be eliminated because of the on -going evolution and the ability of microbes to adapt to their environment. Understanding the vaginal microbiome and its role in the individual's health, as well as the potential for causing local and systemic infection is crucial to reduce the morbidity and mortality of the obstetric and gynecologic patients. There is a significant void in our understanding the complex relationships between the various members of this polymicrobial community and how these interplay with treatment. Eschenbach concisely stated the problems associated with treating BV, but this applies to vaginal dysbiosis in general:

1) Is BV an infection,
2) The microbial cause of BV, and
3) Is there an effective treatment.[26]

The current discussion has demonstrated that vaginal dysbiosis is an infection, as characterized by stimulation of the innate and adaptive immune system. This is not infection in the classic sense, one microbe being responsible for the infection. Vaginal dysbiosis presents as either a symptomatic or asymptomatic state, but the underlying activity is stimulation of the immune system, migration of WBCs and upregulation of the cytokine system. These findings have significance because of the local inflammatory response which

a) Facilitates acquisition of microbes from the external environment
b) Encourages the administration of antibiotics
c) Increases the risk of post procedure infection when the vagina is part of the procedure.

Treatment of vaginal dysbiosis is far from satisfactory, e.g., the primary antibiotic for treating BV is metronidazole. Initially, metronidazole had a cure rate of 90% but this suddenly decreased to 50% to 80% depending on the study conditions.[27] Verwijs et al found that administering metronidazole. 500 mg orally, twice a day for 7 days achieve a cure rate of 54.5% based on the Nugent score (26). Interestingly, these investigators found the mean total vaginal bacteria count decreased from 6.59 to 5.85 $\log_{10}/\mu L$ ($P<0.001$), due mostly to a reduction in obligate anaerobes. Only 16.4% of the women with BV had a reduction in anaerobes of 50% and only 3/68 women experienced complete eradication. Importantly, the mean concentration of lactobacilli species increased from 4.98 to 5.56 $\log_{10}/\mu L$ ($P=0.017$). In their study population Lactobacillus iners was the most common species pre- and post-treatment.[28] Although the failure to eradicate vaginal dysbiosis with antibiotic treatment may be that there are significant s aerobic vaginitis and bacterial vaginosis, other than the microbial constituents. However,the known or suspectedcommon factors are (1) which species of Lactobacillus is dominant, (2) the presence of L. iners may well indicate that the bacterial community is unstable, and (3) differences in amylase concentration in the vagina.[29,30]

Why screen the vaginal microbiome?

Vaginal dysbiosis:

1) can exist in a state of subclinical or subtle infection.
2) Can set the stage for clinical infection in the obstetric and gynecologic patient,
3) is associated with an increased risk of poor pregnancy outcome.
4) induces inflammation of the endocervical and squamous epithelium, enhancing the acquisition of STDs.
5) Increases the risk for the development of postoperative infection.
6) Provides a bacterial population that is more likely to contain antibiotic resistant strains.

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Conflicts of interest

Authors declare that there is no conflict of interest.

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