Evaluation of the vaginal microbiome in clinical diagnosis and management of vaginal infectious diseases

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Introduction

When patients attend with medical complaints such as abnormal leucorrhea, the clinician should consider the possibility of an infection in the lower genital tract, such as vulvovaginal candidiasis (VVC). At that point, it is appropriate to then further assess the typical clinical manifestation, diagnosis, and routine management of the infection. However, sometimes, it is not possible to detect any pathogen at all through standard diagnostic techniques. In such a scenario, what happens to these patients?

It has been suggested that the human microbiome may be thought of as the "second human genome," and recent data published in the field have revealed that the urogenital site contributes approximately 9% of the entire human microbiome. This compares with the gastrointestinal tract, which comprises 29%.[1] The vaginal microbiome is an intricate and dynamic system of bacteria flora where various microbial communities exhibit considerable diversity and density. Any imbalance in the naturally occurring bacterial flora may result in infections such as VVC, bacterial vaginitis (BV), or aerobic vaginitis (AV).[2,3] In order to navigate and explore this exciting microecosystem and its inhabitants, we need a valid roadmap. Changes to several microecological indicators of the vaginal microbiome providing a unique "signature" for lower genital tract infections are usually identified by clinical microbiology laboratories through vaginal swab cultures and microscopic examinations. A multicenter epidemiological study used the Vaginal Microecology Evaluation System (VMES) as a tool to analyze the vaginal microbiomes in most areas of China.[4] The aim of this article is to introduce knowledge on changes to the vaginal microecological environment and inform users of the VMES in diagnosing and managing the broad range of vaginal infections that can occur.

What is the vaginal microecology evaluation system?

The vaginal microbiome is in a dynamic and complex environment. The diversity and bacterial species within the vagina can change rapidly in response to numerous endogenous and exogenous influences, reflecting the progression of vaginal infections. In healthy women, the vaginal microbiome changes according to age, pregnancy, menstruation, injury, and direct bacterial flora destruction (antibiotic usage, sexually transmitted infections, and vaginal irrigation).[5] Previous studies have supported a causal link between vaginal dysbiosis and carcinogenesis.[6]

In recent years, molecular, microscopic, and culture evaluating methods have been used to confirm changes in the vaginal flora. With progress in basic and clinical research and the increasingly prevalent use of probiotics, vaginal microecology has attracted increasing attention in China. In 2016, the Committee of Infectious Disease collaborative group was published, which assessed the standard VMES and provided an opinion [Table 1].[7]

Wet preparations of vaginal swab samples that are examined microscopically and stained by Gram staining are investigated using the VMES.[4] This tool is mainly composed of morphological and functional microecological indicators.[4] The former diagnostic modality includes bacterial density, flora diversity, dominant bacterial flora, indicators of inflammation [such as white blood cell (WBC) count], and pathogenic microorganisms. The system also assesses Nugent score for BV,[8] and AV score for AV,[9] which might provide the clinician with very valuable information that could increase their understanding of the

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000000211

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Chinese Medical Journal 2019;132(9)
Received: 12-12-2018 **Edited by:** Qiang Shi

Table 1: Vaginal microecology evaluation system.

Items	Normal	Abnormal
Morphological indicators		
Bacterial density	II (10–100/field) III (100–1000/field)	I (1–10/field) IV (>1000/field)
Flora diversity	II (4–6 types/field) III (7–9 types/field)	I (1–3 types/field) IV (>10 types/field)
Predominant flora*	Large Gram-positive rods	Gram-positive cocci Large Gram-negative rods Small Gram-negative rods
Nugent score	1–3	≥7
AV score	<3	≥3
Vaginal pH	≥3.8 and <4.5	<3.8 or ≥4.5
Pathogen	Negative	Fungus (spore, budding yeast, or hyphal forms) and/or trichomonads
Functional indicators		
H_2O_2	Negative	Positive
Enzymes	Negative	Positive for sialidase, β-glucuronidase, leukocyte esterase, and acetylglucosaminidase

Increased numbers of other morphotypes are not part of the pattern of the normal vaginal flora. *Large Gram-positive rods (*Lactobacillus*); Gram-positive cocci (*Staphylococcus aureus, Staphylococcus epidermidis*); Gram-negative rods (*Escherichia coli*). AV: Aerobic vaginitis.

vaginal microecological status and propensity for infection. Functional indicators reflect microbial functional status, consisting of three main components: abiotic factors, metabolites, and microbial enzymes such as vaginal pH value, H_2O_2, and activity of several enzymes such as sialidase, β-glucuronidase, leukocyte esterase, and acetylglucosaminidase [Table 1]. It should be noted that if the functional indicators are inconsistent with the morphological indicators, the morphological indicators should be taken as reference indicators. Furthermore, an evaluation of the vaginal microecosystem can help the clinician to make a prompt diagnosis and improve treatment regimens for vaginal infectious diseases.

Vaginal cleanliness is used to judge inflammation status, as it can also cause further inflammation.[4] Degree II to III represents normal vaginal microecological status, while degree III to IV represents abnormal status. The most common cause of abnormal status (degree III–IV) is likely due to vaginal infectious diseases. Bacterial density refers to the intensive degree of bacterial distribution, reflecting the total biomass of the vaginal flora. Flora diversity refers to the total bacterial species in the vaginal flora, reflecting the variety of vaginal flora present.[4] Vaginal H_2O_2 is mainly produced by the lactobacilli, such as *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus jensenii*, and *Lactobacillus acidophilus*. Thus, as these lactobacilli are often the predominant bacteria in healthy women, H_2O_2 levels may reflect the function of lactobacilli. Leukocyte esterase activity indicates the presence of inflammation in the vagina. Sialidase is a specific marker of BV,[10] whereas β-glucuronidase and coagulase activity tend to represent AV.[11]

Recognizing the vaginal microecology of health women

The initial issue for the clinician is the definition of normal microecological status and how it changes to a diseased state; the second issue relates to the presenting individual and the appropriate clinical management of that patient. The Human Microbiome Project demonstrates that the microflora of a healthy vagina is present when bacteria are at a level of 10^8 to 10^9 colony-forming units per gram (CFU/g). The "normal" microbiome of the vagina in non-pregnant, healthy women predominantly includes a variety of *Lactobacillus* species, dominating this ecosystem at a concentration of 10^7 to 10^8 CFU/g of vaginal fluid. The vaginal microbiome of a normal, asymptomatic woman of reproductive age also includes multiple aerobic or facultative species, as well as obligate anaerobic species.

More than 20 species of lactobacilli have been detected in the vagina, and different molecular-based techniques have confirmed that most healthy women contain one or two lactobacilli species from a range of three or four species.[12] The most common species are *L. crispatus*, *Lactobacillus iners*, *L. jensenii*, and *L. gasseri*.[12] Nugent scoring has traditionally specified lactobacilli-dominant microbiota as a normal vaginal ecosystem. Lactobacilli enable the metabolism of glycogen, which is liberated by vaginal epithelial cells, to glucose and lactic acid, and the resulting lower vaginal pH (pH $\leq$ 4.5, range 3.8–4.4) creates an unfavorable environment for the growth of bacteria including pathogens.[13] In addition, lactobacilli may prevent the adherence of pathogenic microorganisms to vaginal epithelial cells through "competitive exclusion" and "bacterial interference." Furthermore, these lactobacilli produce various metabolites, such as lactic acid, bacteriocins, and H_2O_2, which may help stimulate the immune response during vaginal infections, given that lactobacilli reduce local production of interleukin (IL)-1β, IL-6, and IL-8. In contrast, these organisms increase anti-inflammatory cytokines such as IL-2 and IL-17.

According to laboratory results, patients with the following data are considered to have a normal microecological

status: pH values ranging from 3.8 to 4.5, bacterial density degree II to III, flora diversity degree II to III, large Gram-positive rods as predominant flora, Nugent and AV score ≤3, and absence of pathogens and negative specific enzymes [Table 1].

The vaginal microbiome may be classified into five community state types (CSTs), which vary by race/ethnicity as follows[14]: CST I (26.2%), characterized by a dominance of *L. crispatus*, while CST II (6.3%), III (34.1%), and V (5.3%) were identified by a dominance of *L. gasseri*, *L. iners*, and *Lactobacillus jenseii*, respectively. CST IV is the diversity state with lower levels of Lactobacillus, and has been further stratified into two sub-states. CST IV-A contains a number of anaerobic species in the genera *Anaerococcus*, *Peptoniphilus*, *Prevotella*, and *Streptococcus*, while CST IV-B is predominated by *Atopobium* and *Megasphera*. Age, pregnancy, sexual activity, menstruation, and exogenous hormones are some of the factors that impact upon the bacterial community at this mucosal site.

Management of vulvovaginal candidiasis

Differences between the vaginal microbiome of individuals should be taken into account in the assessment of disease risk, diagnosis, and treatment. Comprehensive therapies should be used to manage women with abnormal vaginal microecology, including specific antimicrobial treatments that aim to recover the normal vaginal microecology disrupted by vaginal infections.

VVC is estimated to be the second most common form of infection after BV.[15] Females suspected of having VVC are assessed by VMES according to microscopical examination of spores, hyphae, and blastospores. The first-line treatments for VVC are azoles or polyene drugs. The alternatives include products that can reduce the infectious agent, restoring the balance of the microbiota, promoting repopulation of lactobacilli, and re-establishing normal pH.[16] Recently, the use of probiotics has been shown to have evidence-based clinical benefit with supplementation of antimicrobial treatment to improve cure rates and prevent recurrences. The use of lactobacilli could be regarded as a good alternative for the prevention and treatment of *Candida* infections, showing that this intervention could relieve symptoms and rapidly eradicate the fungal infection.[17] The protective role of lactobacilli appears to be exerted through different mechanisms, including the production of various antibacterial compounds (hydrogen peroxide, lactic acid, bacteriocins, and biosurfactants), competitive exclusion for epithelial adhesion, and immunomodulation.

Several studies supported the hypothesis that VVC or recurrent VVC was associated with an immunopathogenic response, whereby therapeutic strategies that stimulate the host defense response against *Candida* infections may reduce fungal colonization.

Management of bacterial vaginosis

According to the literature, the clinical syndrome due to BV is represented by a milky, homogeneous, malodorous vaginal discharge that causes vulvovaginal discomfort and vulvar irritation. In most instances, it is likely to be caused by disrupted or dysbiotic vaginal microbiota.[3] Females suspected of having BV and who attend examination of the microbiome are assessed by VMES according to the combined results of the Nugent score and a signature feature of clue cells. The Nugent scores range from 0 to 10, with 0 to 3 considered BV negative, 4 to 6 intermediate, and 7 to 10 representing BV positive.[8] Sialidase secreted by anaerobic Gram-negative bacterial rods is a specific marker of BV.[10]

The standard therapy for BV-positive patients is topical clindamycin cream, metronidazole gel, or oral metronidazole.[3] Metronidazole therapy combined with lactobacilli has been shown to be more effective than metronidazole alone. Vaginal administration of probiotic *Lactobacillus* sp. not only restores the normal vaginal microbiota but also reduces BV recurrence.[18] The normal pH value in the vagina ranges from 3.8 to 4.4, while the optimal pH for BV ≥4.7.[3] Treatment of BV patients through normalization of vaginal pH by administration of intravaginal boric acid has been demonstrated in many studies.[19] However, some patients still experience uncomfortable symptoms because of biofilm formation. Therefore, to completely cure the infection, clinicians should attempt to achieve full recovery of the vaginal microecological environment after treatment.

Management of aerobic vaginitis

The published literature on AV or desquamative inflammatory vaginitis is still limited. Clinical manifestations of AV include purulent vaginal discharge and a strong inflammatory reaction.[3] Females with AV are assessed by VMES according to the AV score. β-glucuronidase production related to *Escherichia coli* and Group B streptococcus infection is a specific marker of AV.[11] Coagulase activity reflects infection with *Staphylococcus aureus*, *Enterococcus faecalis*, and *E. coli*, and is another specific marker of AV.[11] Official treatment guidelines for AV have not been developed or implemented. Most proposed non-antibiotic therapies for vaginal dysbacteriosis are either vaginal or oral probiotics. Women with AV characterized by a heavy parabasal-cell component may benefit from recovery of the vaginal mucosa, which is typically through intravaginal application of estrogens as maintenance therapy.[20] Sufficient estrogen levels are essential in maintaining an intact, mature vaginal epithelium.

Conclusion

The establishment of VMES has a high clinical value in guiding the selection of treatment for vaginal infections. VMES could provide new opportunities for the comprehensive management of dysbacteriosis when such infections occur. The final goal of clinical management of vaginal infections is complete recovery of the normal vaginal microecology.

Conflicts of interest

None.

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How to cite this article: Li T, Liu ZH, Li K, Bai HH. Evaluation of the vaginal microbiome in clinical diagnosis and management of vaginal infectious diseases. Chin Med J 2019;132:1100–1103. doi: 10.1097/CM9.0000000000000211