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Original Article

Diagnosis and microecological characteristics of aerobic vaginitis in outpatients based on preformed enzymes

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ABSTRACT

Objective: *Aerobic vaginitis* (AV) is a recently proposed term for genital tract infection in women. The diagnosis of AV is mainly based on descriptive diagnostic criteria proposed by Donders and co-workers. The objective of this study is to report AV prevalence in southwest China using an objective assay kit based on preformed enzymes and also to determine its characteristics.
Materials and methods: A total of 1948 outpatients were enrolled and tested by a commercial diagnostic kit to investigate the AV prevalence and characteristics in southwestern China. The study mainly examined the vaginal ecosystem, age distribution, *Lactobacillus* amount, and changes in pH. Differences within groups were analyzed by Wilcoxon two-sample test.
Results: The AV detection rate is 15.40%. The AV patients were usually seen in the sexually active age group of 20–30 years, followed by those in the age group of 30–40 years. The vaginal ecosystems of all the patients studied were absolutely abnormal, and diagnosed to have a combined infection [aerobic vaginitis (AV) + bacterial vaginitis (BV) 61.33%; 184/300]. Aerobic bacteria, especially *Staphylococcus aureus* and *Escherichia coli*, were predominantly found in the vaginal samples of these women.
Conclusion: AV is a common type of genital infection in southwestern China and is characterized by sexually active age and combined infection predominated by the AV and BV type.

Introduction

Human microbiome is an intricate ecosystem that varies substantially between individuals and across the body, where different microbial communities (e.g., vaginal, oral, skin, gastrointestinal, nasal, urethral) inhabit [1]. However, only very recently our knowledge on vaginal microbiome improved considerably [2]. Researchers from the Human Microbiome Project have confirmed that the most stable microbiome community of the body is observed in the stool and vagina [1]. The equilibrium among microbe–microbe and microbe–host interactions is crucial for maintaining a healthy microenvironment in the human vagina [3]. Any imbalance of the naturally occurring bacterial flora may result in infections such as *Candida* vaginitis, atrophic vaginitis, bacterial vaginitis (BV), or *Trichomonas* vaginitis etc. Donders and co-workers [4,5] identified a nonclassifiable pathology that is neither specific vaginitis nor bacterial vaginosis according to bacterial, immunologic, and clinic characteristics, and termed it as "aerobic vaginitis" (AV).

As proposed by Donders and co-workers [4,5], the diagnosis of AV is primarily based on microscopic examinations (400× magnification; phase-contrast microscope). For a more accurate diagnosis of AV, it was recommended to consider *Lactobacillus* grade, number of leukocytes, proportion of toxic leukocytes, background flora, and proportion of parabasal epitheliocytes [2,4,5]. A score ranging from 0 to 2 is assigned to the aforementioned five parameters. AV was then diagnosed according to the composite score as follows: a score of 1–4 represents normal microbiota (no signs of AV), a score between 3 and 4 indicates slight signs of AV, a score between 5 and 6 represents moderate AV, and a score between 6 and 10 represents severe AV [5].

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Nevertheless, the most reliable methods for identification of the composition and ecology of the vaginal microbial ecosystem are culture-independent molecular approaches based on the cloning and sequencing of 16S ribosomal RNA genes or polymerase chain reaction amplification of 16S ribosomal DNA [6]. Given the limitation of high cost and low throughput, these approaches are used only in a minimal number of studies and only small numbers of samples have usually been analyzed. Obviously, neither the microscopic method nor the gene method is suitable for application in developing countries, especially in China, where the average health care resource for citizens is limited [7] (including medical resources and inquiry time with doctors).

Thus, in this study, using a commercial diagnostic kit primarily based on preformed enzymes in combination with microscopic examinations, we retrospectively investigated the vaginal microflora in 1948 outpatients. Among these, we analyzed the characteristics of 300 patients diagnosed with AV.

Materials and methods

After obtaining oral consent, vaginal samples were taken from 1948 women presenting at the Department of Gynecology and Obstetrics, the Second Affiliated Hospital of Chongqing Medical University, Chongqing, China, from July to December in 2011. The study was reviewed and approved by the Ethical Committee of the Second Affiliated Hospital of Chongqing Medical University. Women (age range 17–71; 34 ± 9.4 years) who mainly presented with vaginitis symptoms without treatment outside the hospital were included in the study. We excluded women presenting at the hospital for hormonal replacement therapy, genital prolapse, or overt genital bleeding.

Vaginal secretions were obtained on two sterile cotton swabs at the upper one-third of the lateral vaginal wall after sterile speculum had been inserted. The specimens were obtained prior to vaginal operation. The samples obtained were sent to two rigorously trained professional technicians for testing within 15 minutes. A brief schematic diagram of the standard operation procedure is shown in Figure 1.

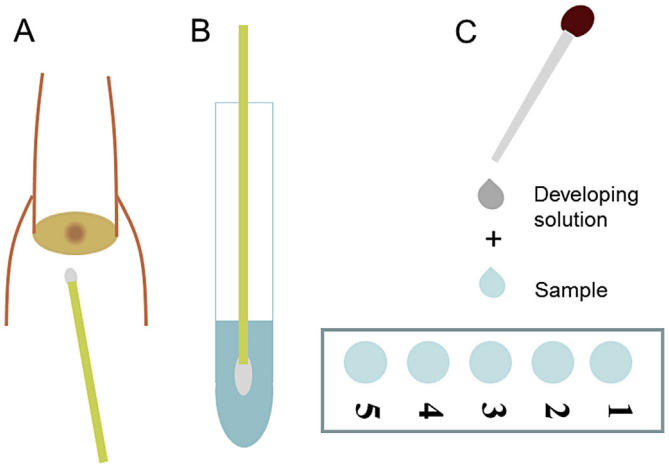

Figure 1. A brief schematic diagram of the standard operation procedure for analysis of vaginal secretion. A. (A) Vaginal secretions were taken on a sterile cotton swab at the upper one third of the lateral vaginal wall. (B) The sample was then placed in a tube to which 400 μL of diluent was added. The swab was then repeatedly squeezed against the tube wall to dissolve the sample as much as possible. (C) A drop of sample (about 35 μL) was added into each well. After incubating the mixture for 10 minutes at 37°C, a drop of color development solution A was added to the sialidase well followed by the addition of a drop of color development solution B to the coagulase reaction well.

One of the swabs was spread onto a glass slide and saline was added. The specimen (secretion squeezed on the slide) was then closed with a cover slip and microscopic examination was performed immediately. Vaginal pH was measured on the glass slide after microscopy, using color strips with a pH range of 3.8–5.4. Then, 10% KOH was added to perform the amine test (potassium hydroxide odor test). Another glass slide was spread onto a glass slide, heated, and Gram stained to count the number of *Lactobacillus*, observe clue cells, *Neisseria gonorrhoeae*, vulvovaginal yeast (including spores and hyphae), etc. under an oil-immersion microscope.

Another swab was diluted to perform AV and BV diagnostic strip sets test to identify whether there was an AV infection. According to the kit instructions, the samples were placed in a tube and 400 μL of a diluent was added. The swab was then repeatedly squeezed against the tube wall to dissolve the sample as much as possible. A drop of sample (about 35 μL) was added into each well. After incubating the mixture for 10 minutes at 37°C, a drop of color development solution A was added to the sialidase well followed by the addition of a drop of color development solution B to the coagulase reaction well. According to the technicians' instruction, the results were interpreted.

AV and BV diagnostic strip sets (Beijing ZhongSheng JinYu Diagnosis Technology Co., Ltd, Beijing, China.), a commercial kit, was used to diagnose AV and discriminate the vaginal ecosystem. AV was diagnosed based on the following five indicators in which four of them are preformed enzymes: (1) hydrogen peroxide (H2O2) concentration, which reflects the growth status of probiotic lactobacilli; (2) leukocyte esterase (LE) activity, which indicates the presence of inflammation in relation to the predominating bacterial morphotypes in the vagina [8]; (3) sialidase activity, which is exhibited by BV-associated bacteria, such as *Gardnerella vaginalis* and *Prevotella bivia* [9]; (4) β-glucuronidase (Gus) activity, which is considered to be related to *Escherichia coli* and Group B streptococcus infection in vaginal fluid; and (5) *coagulase* activity, which shows the existence of *Staphylococcus aureus, Enterococcus faecalis,* and *E. coli* according to the product development background and description. If the samples were positive for H2O2, LE, Gus or coagulase, or both Gus and coagulase (Table 1), AV is diagnosed.

According to the laboratory results, patients with pH value ranging from 3.8 to 4.5, *Lactobacillus* cell counts above 95%, and absence of pathogens were identified as having normal vaginal microecological status. Patients were classified as being in the vaginal microecological intermediate status if their pH value showed an increase (pH > 5) and there is increased detection of other bacterial pathogens on wet smear, in combination with decreased *Lactobacillus* cell count, but not severe enough to be diagnosed as a case of BV in clinic. Patients with the absence of *Lactobacillus* and with the detection of *Gardnerella, Mobiluncus* morphotypes and/or clue cells on fresh wet mounts were diagnosed to have an abnormal vaginal microecology.

Table 1
Reference standard for aerobic vaginitis/bacterial vaginitis diagnostic strip sets test.

Test items	Positive(+)	Negative (−)	Aerobic vaginitis
Hydrogen peroxide (H_2O_2)	Lavender	Blue	+
Sialidase	Purple	Colorless	−
Leukocyte esterase (LE)	Blue	Colorless	+
β-Glucuronidase (Gus)	Blue	Colorless	+/−[a]
Coagulase	Purple	Buff	−/+[a]

According to the product's technical parameters, the minimal detectable amount of the aforementioned five test items is ≥2 μmol/L, ≥7 U/L, ≥9 U/L, ≥15 U/L, and ≥20 U/L, respectively.

[a] Aerobic vaginitis is diagnosed if the samples were positive for H2O2, LE, Gus or coagulase, or both Gus and coagulase.