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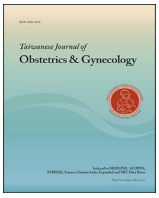

Original Article

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.

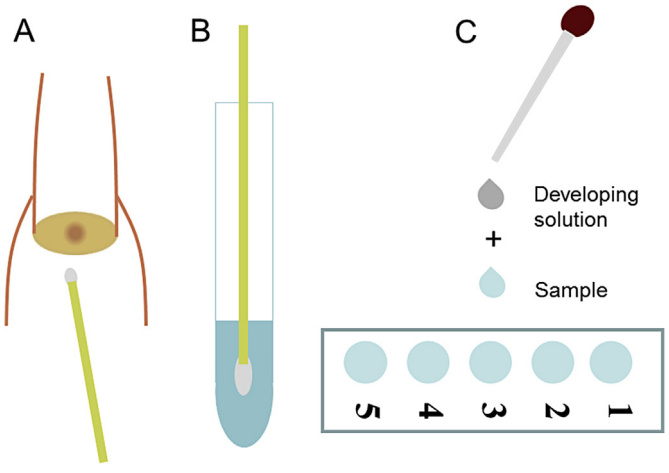

Figure 1. A brief schematic diagram of the standard operation procedure for analysis of vaginal secretion. A. (A) Vaginal secretions were taken on a sterile cotton swab at the upper one third of the lateral vaginal wall. (B) The sample was then placed in a tube to which 400 μL of diluent was added. The 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.

BV, vulvovaginal candidiasis (VVC), and trichomonal vaginitis were diagnosed according to standardized definitions.

Numerical data of patients in the two different infection statuses were analyzed by Wilcoxon two-sample test. Analyses were performed using SPSS version 19.0 (SPSS, Inc., Chicago, IL, USA). Values of $p < 0.05$ were considered statistically significant.

Results

In this study, 292 of the 1948 patients (14.99%) were found to have normal vaginal microecology; among the 1948 patients, for 826 patients (42.40%), the vaginal microecology was in the intermediate status and for 830 patients (42.61%), it was in the abnormal status. Figure 2 shows the results of microecological evaluation. A total of 300 patients (300/1948, 15.40%) were verified as having AV. Moreover, none of these patients was in the normal microecology status. Vaginal infection was most common in three of the seven age groups studied, with prevalence rates of 37.33% (112/300), 32.00% (96/300), and 20.33% (61/300) for those in their 20s, 30s, and 40s, respectively (Figure 3).

With respect to the primary pathogens in the vagina of AV patients, an indirect inference was made based on the comprehensive analysis of the involved marker. Among the 300 patients with AV, 198 were Gus negative and coagulase positive (198/300; 66.00%), 100 were positive for both Gus and coagulase (100/300; 33.33%), and two were only Gus positive (2/300; 0.67%). Taken together, our data showed that the detection of *S. aureus*, *E. coli*, and *E. faecalis* was obviously higher (99.33%) than other flora, whereas there was a low detection of Group B streptococcus (0.67%).

Of the 300 patients with AV, 116 (38.67%) were found to have pure aerobic infections. The remaining 184 patients had mixed infections (61.33%). The most frequent infection combination was that of AV and BV (101/184, 54.89%), followed by AV and VVC (48/184, 26.09%). Table 2 presents the detailed results of the mixed infections. A comparison between pure and mixed aerobic infections is shown in Table 3 (based on Wilcoxon two-sample test).

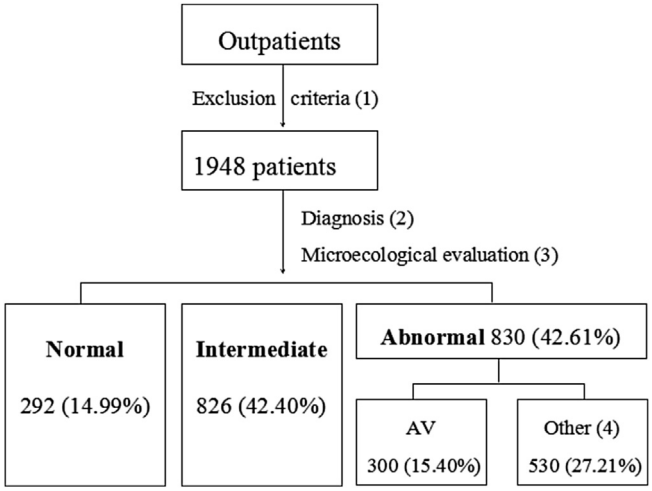

Figure 2. Flowchart of patients' enrolment and results of microecological evaluation. [a]Women presenting at hospital for hormonal replacement therapy, genital prolapse, or overt genital bleeding were excluded. [b]All the 1948 patients were diagnosed by the preformed enzymes method in combination with microscopic examinations. [c]*Normal*: pH value ranging from 3.8 to 4.5, *Lactobacillus* count above 95%, and no detection of pathogens; *intermediate status*: increase in pH value (pH > 5) and increased detection of other bacteria on wet smear, in combination with decrease in the counts of *Lactobacillus*, but not severe enough to be diagnosed as a case of bacterial vaginitis (BV) in clinic; *abnormal status*: absence of *Lactobacillus*, and the detection of *Gardnerella*, *Mobiluncus* morphotypes and/or clue cells on fresh wet mounts. [d]Other conditions included BV, trichomonal vaginitis, vulvovaginal candidiasis, which were diagnosed according to standardized definitions.

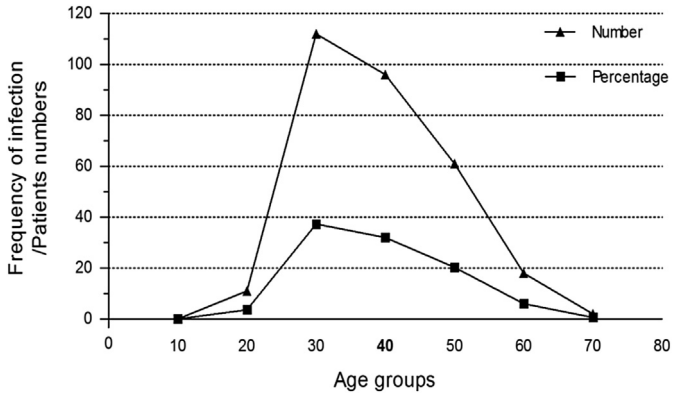

Figure 3. Age distribution of aerobic vaginitis infection. The highest frequency of infection was observed for women in the sexually active age groups.

Table 2
Infection status in the AV patients studied ($N = 300$).

Status	Case(s)	%
Pure aerobic infections	116	38.67
Mixed infections	184	61.33
AV + BV	101	33.67
AV + VVC	48	16.00
AV + TV	15	5.00
AV + BV + VVC	11	3.66
AV + BV + TV	9	3.00
Total	300	100

AV = aerobic vaginitis; BV = bacterial vaginitis; TV = trichomonal vaginitis; VVC = vulvovaginal candidiasis.

Discussion

As a recently identified type of vaginitis, AV is poorly diagnosed in clinical practice. Moreover, it is worth noting that AV is linked to miscarriage, chorioamnionitis, premature rupture of membranes, preterm delivery, infertility, and pelvic inflammatory disease (PID) [10–12], which is a polymicrobial infection involving sexually transmitted organisms as well as anaerobic and aerobic vaginal microbes. PID is the main cause of tubal infertility in northern China. More importantly, nonspecific PID including aerobic infections has been the main etiology of PID [13]. Unfortunately, because of the limited diagnostic tools available in underdeveloped regions, AV is poorly recognized in clinical practice. To date, the diagnosis of AV mainly depends on microscopic examinations and a somewhat complex scoring system (score ≥ 3) [5]. Although this scoring system is efficient and has been wildly accepted, to some extent, it is susceptible and time consuming because microscopic examination performed by a professional technician takes a long time. Thus, there is a need to identify methods to quickly diagnose AV, such as using a convenient and fast diagnosis strip, similar to a pH strip or pregnancy test strip.

Given the unequal distribution of health care resources in our country [7], a quick diagnosis of AV is a simple but important way to shorten the patients' residence time in polyclinics. This would also facilitate access to health care service for more patients. Thus, the China Food and Drug Administration has censored and approved a fast diagnose strip for AV/BV diagnosis. The strip was developed by ZhongSheng JinYu Diagnosis Technology Co., Ltd, and its accuracy and sensitivity have been evaluated by the Chinese government.

Table 3
Comparison between pure and mixed aerobic infections.

Items		Single aerobic infections (n = 116), %	Mixed infections (n = 184), %	p (Wilcoxon two-sample test)
pH	≤4.5	38 (32.76)	28 (15.22)	0.271
	4.6–5.0	19 (16.38)	67 (36.41)	
	>5.0	59 (50.86)	89 (48.37)	
Lactobacillus grade counts	I + IIa	9 (7.76)	1 (0.54)	<0.001
	IIb	25 (21.56)	10 (5.43)	
	III	82 (70.68)	173 (94.03)	

Grade I = indicated by a predominance of *Lactobacillus* morphotypes of variable size, and indicates a *healthy microflora*; Grade Type IIa = indicated by a mixed flora, but still lactobacilli predominate; Grade Type IIb = indicated by a mixed flora, but the proportion of lactobacilli is severely reduced due to the increased number of other bacteria. Grade Type III = indicated by severely decreased or absence of lactobacilli because of the overgrowth of other bacteria.

The manufacturer's claim that both accuracy and sensitivity of this strip are no less than 90%. As explained in the "Materials and Methods" section, diagnosis of AV/BV using this strip is based on color changes in the test strip. This inspiring and promising diagnostic method is expected to be utilized in the crowded polyclinics in China. Our study here reports the characteristics of AV in outpatients using this test strip.

To our knowledge, the prevalence of AV is still largely unknown both at home and abroad. In this study, we found that among 1948 outpatients, 300 (15.40%) had AV. More importantly, these 300 women mainly belonged to the sexually active age groups of 21–50 years (269/300, 89.67%), and this rate was higher than those reported previously by Zodzika et al (15/139, 10.8%) [14] and Donders et al (8.3%) [15]. Apart from some objective factors such as age, race or ethnicity, education, and poverty, the fact that all the patients studied were symptomatic or had requirements for medical intervention must be taken into account for deducing differences in the prevalence rates.

In this study, we found that the pH value increased as the *Lactobacillus* amount decreased. The elevated pH value reflected the increased alteration of the microbial flora, which was in accordance with some previous studies [16,17]. The ecosystems of all the 300 AV patients in this study were abnormal, which suggests that the predominant flora had changed. What makes AV distinct from BV is the predominance of aerobic pathogens in the vagina, especially *S. aureus* and *E. coli*, and these results are in accordance with those reported by Mumtaz [18], who reported that of 731 isolates, *S. aureus* was the most prevalent organism [337/731 (46%)], followed by *E. coli* [100/731 (13.7%)]. Tempera et al [19] studied a sample of 30 women with a clinical and microbiological diagnosis of AV. In their study, *E. coli* was the most frequently isolated pathogen (n = 29), followed by *E. faecalis* (n = 15). Similar result was observed 2 years later in another study involving 81 patients with AV [20]. Murphy and Edwards [21] showed that AV involves colonization mainly by group B streptococci and Enterobacteriaceae in the vagina. However, *S. aureus*, *E. coli*, and *E. faecalis* were the commonly detected pathogens (99.33%) in our study. The main pathogen and the infection status differ from one study to another, as they are not only associated with factors such as age, race or ethnicity, education, and poverty, but are also associated with some intern disease (VVC is related to diabetes mellitus [22]) prevalent in different regions.

In addition, mixed or combined infections are considered to be highly prevalent, as they accounted for nearly 30% of all the cases [23,24]. In another study [25] in Northern China, mixed infections were found in 170/657 AV patients (25.88%); among these 170 patients, 84 (49.41%) had mixed AV infections. Although our study showed AV/BV to be the most frequently detected mixed infections (101/300, 33.67%), environmental and lifestyle differences between the two cities may have contributed to the divergence of incidence. Statistical analysis showed that individuals are prone to acquire mixed infections if their *Lactobacillus* counts decrease; however, it does not show the same tendency among different pH levels. These facts remind our physicians to pay more attention to some "atypical BV" witnessed in clinical practice. The exact mechanism of this tendency needs further in-depth study and elaboration.

In conclusion, our study shows that AV is a common female genital infection, and is usually seen in sexually active women aged between 21 years and 50 years, whose vaginal ecosystems demonstrate an abnormal status of the vaginal flora. Aerobic flora such as *S. aureus* and *E. coli* were primarily detected in this study. In patients with BV infection, the pH value increases while the *Lactobacillus* cell count decreases. Patients with AV are prone to have mixed infections with decreasing *Lactobacillus* grade, especially the AV/BV type. Thus, there is a need to pay increasing attention to the diagnosis of AV in clinic, in particular the mixed aerobic infections. All the aforementioned analyses are based on a commercial diagnostic kit, and our results are in accordance with current findings in this area. Hopefully, this is a promising way to diagnose AV in polyclinics in underdeveloped regions of China. However, because all study patients in our study were symptomatic or have requirements for medical intervention, the precise prevalence of AV should be identified in an in-depth study involving a large sample size. Furthermore, as a new diagnosis system, it is still indispensable to compare the strip with Donders' criterion or sequencing technological results, which have greatly contributed to the current knowledge of human vaginal microbiome in recent years [26].

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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