

Aerobic Vaginitis:

Abnormal Vaginal Flora That Is Distinct From Bacterial Vaginosis.

Aerobic vaginitis (AV) is a state of abnormal vaginal flora that is distinct from the more common bacterial vaginosis (BV) (**Table 1**). AV is caused by a displacement of the healthy vaginal *Lactobacillus* species with aerobic pathogens such as *Escherichia coli*, Group B Streptococcus (GBS), *Staphylococcus aureus*, and *Enterococcus faecalis* that trigger a localized vaginal inflammatory immune response. Clinical signs and symptoms include vaginal inflammation, an itching or burning sensation, dyspareunia, yellowish discharge, and an increase in vaginal pH > 4.5, and inflammation with leukocyte infiltration. (1) Severe, persistent, or chronic forms of AV can also be referred to as desquamative inflammatory vaginitis (DIV). (2, 3)

BV is a common vaginal disorder associated with the overgrowth of anaerobic bacteria, a distinct vaginal malodorous discharge, but is not usually associated with a strong vaginal inflammatory immune response. Like AV, BV also includes an elevation of the vaginal pH > 4.5 and a depletion of healthy *Lactobacillus* species. BV is treated with traditional metronidazole therapy that targets anaerobic bacteria. However, approximately 10% to 20% of women diagnosed with BV and treated with metronidazole will fail

to respond to therapy at one week and will experience persistent symptoms. (4, 5) It is believed that a subset of these patients may have been misdiagnosed and actually suffer from AV, which requires an antibiotic therapy with intrinsic activity against specific aerobic bacteria. AV has been implicated in complications of pregnancy such as ascending chorioamnionitis, premature rupture of the membranes, and preterm delivery.

Epidemiology

In a study of 631 patients attending routine prenatal care from a vaginitis clinic, 7.9% had moderate to severe AV signs and symptoms and 6% had 'full-blown' BV. (1)

In a study of 3,000 women, 4.3% were found to have severe AV, also called DIV. Furthermore, 49.5% of the women with DIV were peri- or postmenopausal. A reported hypothesis is that a drop in estrogen may trigger the development of AV in the aforementioned menopausal women, as well as postpartum nursing women. (3)

Table 1. A Comparison of Bacterial Vaginosis and Aerobic Vaginitis.

Clinical Characteristics	Bacterial Vaginosis	Aerobic Vaginitis (1)
Lactobacilli	Displaced	Displaced
Pathogen	<i>Gardnerella vaginalis</i> , <i>Atopobium vaginae</i> , <i>Megasphaera</i> species, BVAB2	<i>Escherichia coli</i> , Group B Streptococcus, <i>Staphylococcus aureus</i> , <i>Enterococcus faecalis</i>
Vaginal epithelial inflammation	None	Present
Elevation of pro-inflammatory cytokines (IL-1 β , IL-6, IL-8)	Moderate elevation	High elevation
Immune reaction (cytotoxic leukocyte)	Non-reactive	Reactive
pH [Normal = 3.8 – 4.2]	T= 4.2-4.5 BV \geq 4.5	> 4.5; usually >6
Shed vaginal epithelial cells	Clue cells	Parabasal cells
Vaginal discharge characteristic	White, homogenous	Yellowish
10% KOH Whiff Test (fishy amine odor)	Positive	Negative
Treatment	Metronidazole ^b Clindamycin ^b	Kanamycin ovule. (5) 2% clindamycin topical. (3) Fluoroquinolones are reported to have clinical success. (5) GBS is uniformly sensitive to penicillin, ampicillin, amoxicillin, amoxicillin/ clavulanic acid. (7) <i>E. faecalis</i> is traditionally treated with ampicillin. (8)

References are provided for treatment information; Fluoroquinolones, such as ciprofloxacin, ofloxacin, and levofloxacin, are contraindicated in pregnant women. Levofloxacin has improved efficacy against Streptococci compared to ciprofloxacin. T= Transitional.



In a more recent study of 215 women, 19.1% were found to have 'common vaginitis' caused by BV, vulvovaginal candidiasis (VVC), or trichomoniasis (TV), whereas 12.6% were found to have 'inflammatory vaginitis' (IV). Of the IV group, 77.8% were characterized as having DIV. (11) In fact, 42.9% of the women with DIV were found to be GBS positive, a 5-fold increase over the healthy patients (17.7% positive). (11) This study was similar to an earlier study that found 43% of DIV patients were GBS positive. (2)

Pathogenesis

AV is associated with an increase in vaginal pH (> 4.5), depletion of vaginal healthy Lactobacilli, and an overgrowth of aerobic or facultative anaerobic bacteria, usually the Gram-negative bacilli *E. coli* or Gram-positive cocci GBS, and occasionally *S. aureus* and *E. faecalis*. The high concentration of these aerobic bacteria and the absence of healthy vaginal Lactobacilli results in triggering the immune system as evidenced by vaginal inflammation, high levels of proinflammatory cytokine production, recruitment of leukocytes, and the generation of toxic leukocytes and parabasal cells. The patient may present with all or some of these signs and symptoms of AV: yellowish discharge, itching or burning sensation, dyspareunia, absence of the fishy odor (negative amine test) typically associated with BV, inflammation (**Figure 1**), toxic leukocyte infiltration, and the presence of parabasal cells and naked rounded vaginal epithelial cells (**Figure 2**).

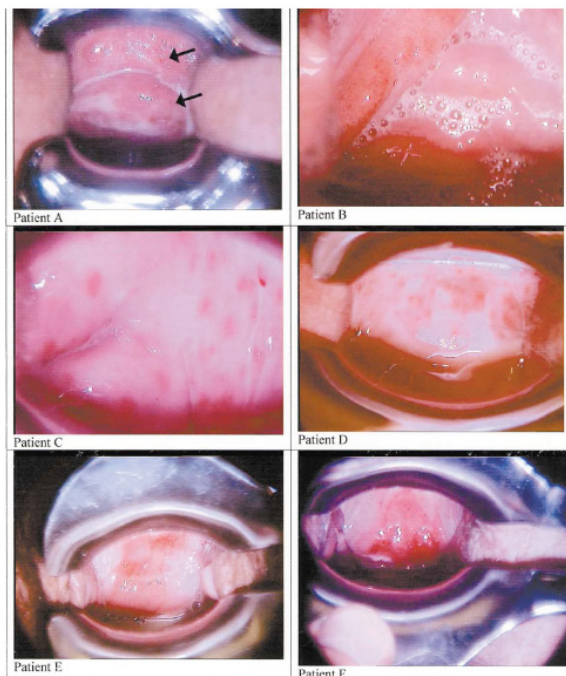


Figure 1. Aerobic vaginitis inflammation. Clinical pictures adopted from Donders *et al*, 2002, demonstrates patients with moderate to severe AV. Discrete (Patients A & B), moderate (Patients C & D), and severe ulcerations (Patients E & F) are observed along with yellowish discharge and inflammation of the vagina. (1)

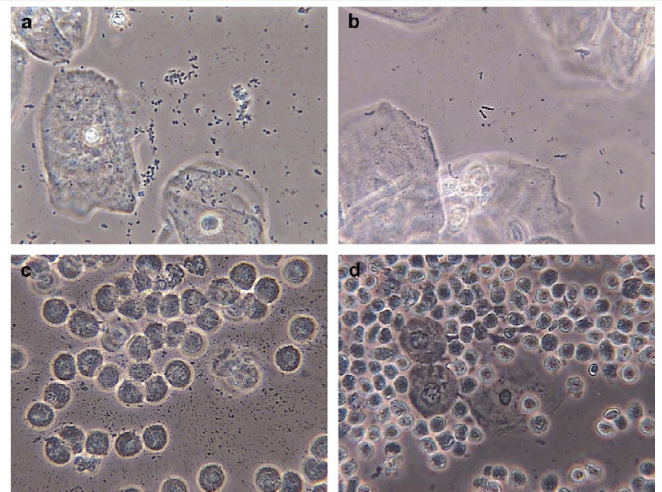


Figure 2: Aerobic Vaginitis microscopy. Images of phase-contrast microscopy (x400) adopted from Donders *et al.*, 2002, of vaginal fluid from patients with AV. The vaginal Lactobacilli are displaced with coccoid bacteria (a) or chains of cocci typical for GBS (b). Leukocytes and 'toxic' leukocytes (full of lysozymic granules) are present in high numbers (c). Parabasal cells or rounded-up vaginal epithelia, are present (d). (1)

Clinical Significance

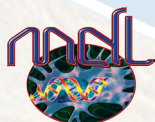
Patients with AV present with distinct clinical signs and symptoms of abnormal vaginal flora that can be confused with common vaginitis etiologies such as BV, VVC, and TV (**Table 1**).

AV is treated with an antibiotic course of therapy characterized by an intrinsic activity against the majority of bacteria of fecal origin, which is different than the metronidazole (BV, TV) and antifungal (VVC) antimicrobial agents used to treat common vaginitis (**Table 1**).

In addition to the clinical symptoms of vaginal discharge, dyspareunia, itching and burning sensation, and a strong inflammatory response, AV was shown to have an association with miscarriage and preterm labor and delivery. (12, 13, 14) Inflammation derived from the cervical-vaginal environment (vaginitis) and urinary tract infections are known to be associated with triggering labor. Cellular components of GBS such as peptidoglycan and hemolysin and *E. coli* lipopolysaccharide (LPS), known mediators that trigger the inflammatory response, are proposed to be the causative agents that can initiate preterm labor. Additionally, GBS and *E. coli* are also major bacterial species involved in neonatal sepsis.

Laboratory Diagnosis

In 2002, Donders *et al.* published guidelines to characterize the presence and severity of aerobic vaginitis. This was based on a similar Nugent scoring method used for bacterial vaginosis determination, which is based on a Gram-stained microscopic evaluation that enumerates specific bacterial morphotypes. The presence and number of the different bacterial morphotypes, such as healthy Gram-positive



Lactobacilli and anaerobic BV associated Gram-negative and Gram-variable rods, contribute to the overall Nugent Score. A Nugent score of 0 to 3 indicates normal flora, 4 to 6 intermediate flora, and 7 to 10 bacterial vaginosis.

The determination of AV is also established by an 'AV' score. The score is calculated with the use of high-power field microscopy to evaluate the presence or absence of healthy Lactobacilli, number of leukocytes, number of toxic leukocytes, type of vaginal flora, and parabasal epithelial cells (**Table 2**). Here, the presence of the healthy Gram-positive Lactobacilli is compared to the presence of aerobic or facultative anaerobic Gram-positive cocci (such as Streptococci, Staphylococci, or Enterococci) and Gram-negative bacilli (*E. coli* and *Klebsiella* species).

Table 2. Criteria for the microscopic diagnosis of Aerobic Vaginitis (AV) (400X magnification, phase contrast microscopy). (1, 14)

AV score	Lactobacillary grades (LBG)	Number of leukocytes	Proportion of toxic leukocytes	Background flora	Proportion of parabasal epitheliocytes (PBCs)
0	I and IIa	≤ 10/hpf	None or sporadic	Unremarkable or cytotoxic	None or <1%
1	IIb	> 10/hpf and ≤ 10/epithelial cell	≤ 50% of leukocytes	Small coliform bacilli	≤ 10%
2	III	> 10/epithelial cell	> 50% of leukocytes	Cocci or chains	>10%

LBGI, numerous pleomorphic lactobacilli, no other bacteria; LBGIIa, mixed flora, but predominantly lactobacilli; LBGIIb, mixed flora, but proportion of lactobacilli severely decreased due to increased number of other bacteria; LBGIII, lactobacilli severely depressed or absent because of overgrowth of other bacteria; hpf, high-power field (400 times magnification).

AV score of <3 corresponds to 'no signs of aerobic vaginitis (AV)', 3-4 to 'light AV', 5-6 to moderate AV, and >6 to 'severe AV'. The latter group corresponds well to the entity 'desquamative inflammatory vaginitis'

The Aerobic Vaginitis (AV) Panel by PCR developed and validated by Medical Diagnostic Laboratories, L.L.C. (MDL) utilizes four qPCR reactions to detect the four most common AV-associated bacteria (*E. coli*, *GBS*, *S. aureus*, and *E. faecalis*). Along with the clinical signs and symptoms (**Table 1**), this assay, which correlates with the AV flora discussed in the clinical AV scoring characterization (**Table 2**), can identify for healthcare providers the AV pathogens involved in the inflammatory vaginitis. (1, 14)

Treatment for Aerobic Vaginitis

The therapy for aerobic vaginitis should include an antibiotic with an intrinsic activity against the majority of bacteria of fecal origin.

To increase the safety and compliance, it is best to use a topical formulation which has slow or little absorbency, but is able to maintain the correct pharmaceutical concentration *in situ*. (8)

The optimal treatment includes antibiotics that have little effect on the normal flora, commonly *Lactobacillus* species, while effectively eradicating the Gram-negative enterics

such as *E. coli*, and Gram-positive GBS, *S. aureus*, and *E. faecalis*. In a study that measured the minimum inhibitory concentrations (MIC) of prulifloxacin, ciprofloxacin, ofloxacin, erythromycin, doxycycline, clindamycin, ampicillin, kanamycin, and vancomycin antibiotics for 73 vaginal *Lactobacillus* species, the MICs for kanamycin, ciprofloxacin, and ofloxacin were reported to be the greatest and in a concentration range categorized as intermediate or resistant for the AV pathogens.

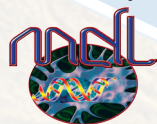
In a study by Tempera *et al.*, topical kanamycin ovules (100 mg, corresponding to 83 mg of active compound; one ovule per day for 6 days) was shown to have clinical success for the treatment of AV. (7, 8)

Fluoroquinolones, such as ciprofloxacin and ofloxacin, have also been reported to have clinical success. These fluoroquinolones were reported to have little effect on the normal flora allowing for a rapid recovery. (8) A study measuring MICs of the four most common vaginal *Lactobacillus* species found that the three healthy *Lactobacillus* species, *L. crispatus*, *L. gasseri*, and *L. jensenii*, were resistant to ciprofloxacin, while *L. iners*, a Lactobacilli not associated with a healthy vaginal flora, was susceptible. (15)

In severe cases of aerobic vaginitis, also referred to as DIV, a successful treatment is 4 to 5 grams of 2% clindamycin cream daily for 4 to 6 weeks, which has coverage for Gram-positive GBS and also has been reported to reduce inflammation (5). Although all women experienced improvement using this therapy, it was reported that 32.1% of patients relapsed after 6 weeks and 43.4% of patients relapsed after 23 weeks. (5) However, GBS vaginitis case reports have demonstrated clindamycin treatment failures due to clindamycin resistant isolates. (16) Approximately 21% (17) to 38% (18) of GBS clinical isolates were reported to be clindamycin resistant; furthermore, clindamycin is not effective against *E. coli*.

Group B Streptococci are uniformly susceptible to penicillin, ampicillin, amoxicillin, amoxicillin-clavulanic acid, and cefuroxime axetil and all were therefore reported to be appropriate treatment for GBS vaginitis. (19) For penicillin allergic patients, clindamycin is an acceptable alternative. Fluoroquinolones (levofloxacin) appear to have efficacy against isolates of Group B Streptococci; resistance to fluoroquinolones has only recently been reported. (20)

E. faecalis infections can be treated with ampicillin. The combination of ampicillin and an aminoglycoside, such as gentamicin or spectinomycin, has been shown to have a synergistic effect on this bacterium, which is effective for severe infections (10). Although rare, strains with β -lactamase activity or increased MIC for gentamicin can be treated with ampicillin-sulbactam or high-dose gentamicin, respectively.



Summary of Treatment

- Kanamycin ovules (100 mg, corresponding to 83 mg of active compound) one ovule per day for 6 days. (7, 8)
- 2% topical clindamycin. 4 to 5 grams of 2% clindamycin cream daily for 4 to 6 weeks. (desquamative inflammatory vaginitis). (5)
- Ciprofloxacin or ofloxacin (8, 15).
- Fluoroquinolones (ciprofloxacin, ofloxacin, and levofloxacin) are contraindicated in pregnant women.
- Group B Streptococcus is uniformly susceptible to penicillin, ampicillin, amoxicillin, amoxicillin-clavulanic acid, and cefuroxime axetil. Alternatives are clindamycin and levofloxacin.
- *E. faecalis* is traditionally treated with ampicillin. A combination of ampicillin plus an aminoglycoside (gentamicin or spectinomycin) is used for severe infections. (10)

Clinical Benefits of Testing

MDL offers highly sensitive and specific quantitative Real-Time PCR (qPCR)-based assays for the detection of AV-associated pathogens utilizing the **OneSwab®** platform, **Test 182: Aerobic Vaginitis (AV) by Real-Time PCR**. Benefits of this system include:

Real-Time PCR

- Simple and convenient sample collection.
- No refrigeration is required before or after collection.
- Specimen stable for up to five days.
- Test additions are available up to 30 days after receipt of the specimen.
- 24 - 48 hour turnaround time.
- High diagnostic specificity and sensitivity.
- One vial, multiple pathogens.

References

1. **Donders GGG, Vereecken A, Bosmans E, Dekeersmaecker A, Salembier G, Spitz B.** 2002. Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis: aerobic vaginitis. *Br J Obstet Gynecol* **109**: 34-43.
2. **Sobel JD.** 1994. Desquamative inflammatory vaginitis: a new subgroup of purulent vaginitis responsive to topical 2% clindamycin therapy. *Am J Obstet Gynecol* **171**: 1215-1220.
3. **Sobel JD, Reichman O, Misra D, Yoo W.** 2011. Prognosis and Treatment of Desquamative Inflammatory Vaginitis. *Obstet Gynecol* **117**: 850-855.
4. **Wilson J.** 2004. Managing recurrent bacterial vaginosis. *Sex Transm Infect* **80**: 8-11.
5. **Larsson PG.** 1992. Treatment of bacterial vaginosis. *Int J STD AIDS* **3**: 239-247.
6. **Centers for Disease Control and Prevention (CDC).** 2010. Sexually Transmitted Diseases Treatment Guidelines. *MMWR* **59**: 1-110.
7. **Tempera G, Bonfiglio G, Comparata E, Corsello S, Cianci A.** 2004. Microbiological/clinical characteristics and validation of topical therapy with kanamycin in aerobic vaginitis: a pilot study. *Int J Antimicrob Agents* **24**: 85-88.
8. **Tempera G, Furneri PM.** 2010. Management of Aerobic Vaginitis. *Gynecol Obstet Invest* **70**: 244-249.
9. **Clinical and Laboratory Standards Institute.** 2010. Performance standards for antimicrobial susceptibility testing: 21st informational supplement. M100-S21, Vol. 31 (1), Clinical and Laboratory Standards Institute, Wayne, PA.
10. **Arias CA, Contreras GA, Murray BE.** 2010. Management of multidrug-resistant enterococcal infections. *Clin Microbiol Infect* **16**: 555-562.
11. **Leclair CM, Hart AE, Goetsch MF, Carpentier H, Jensen JT.** 2010. Group B Streptococcus: Prevalence in a non-obstetric population. *J Low Genit Tract Dis* **14**: 162-166.
12. **Donders G, Van Calsteren K, Bellen G, Reybrouck R, Van den Bosch T, Riphagen I, Van Lierde S.** 2009. Predictive value for preterm birth of abnormal vaginal flora, bacterial vaginosis and aerobic vaginitis during the first trimester of pregnancy. *Br J Obstet Gynecol* **116**: 1315-1324.
13. **Donati L, Di Vico A, Nucci M, Quagliozzi L, Spagnuolo T, Labianca A, Bracaglia M, Ianniello F, Caruso A, Paradisi G.** 2010. Vaginal microbial flora and outcome of pregnancy. *Arch Gynecol Obstet* **281**: 589-600.
14. **Donders G, Bellen G, Rezeberga D.** 2011. Aerobic vaginitis in pregnancy. *Br J Obstet Gynecol* **118**: 1163-1170.
15. **De Backer E, Verhelst R, Verstraeten H, Claeys G, Verschraegen G, Temmerman M, Vanechoutte M.** 2006. Antibiotic susceptibility of *Atopobium vaginae*. *BMC Infect Dis* **6**: 51.
16. **Honig E, Mouton JW, van der Meijden WI.** 1999. Can Group B Streptococci cause symptomatic vaginitis? *Infect Dis Obstet Gynecol* **7**: 206-209.
17. **Gygax SE, Schuyler JA, Kimmel LE, Trama JP, Mordechai E, Adelson ME.** 2006. Erythromycin and clindamycin resistance in Group B Streptococcal clinical isolates. *Antimicrob Agents Chemother* **50**: 1875-1877.
18. **Back EE, O'Grady EJ, Back JD.** 2012. High rates of perinatal Group B Streptococcus clindamycin and erythromycin resistance in an upstate New York hospital. *Antimicrob Agents Chemother* **56**: 739-742.
19. **Clark LR, Atendido M.** 2005. Group B Streptococcal vaginitis in postpubertal adolescent girls. *J Adolescent Health* **36**: 437-440.
20. **Wu HM, Janapatla RP, Ho YR, Hung KH, Wu CW, Yan JJ, et al.** 2008. Emergence of fluoroquinolone resistance in group B streptococcal isolates in Taiwan. *Antimicrob Agents Chemother* **52**: 1888-1890.

