

The vagina represents a distinct ecosystem whereby homeostasis of environmental conditions is maintained by the resident microflora. This homeostasis is a dynamic process influenced by several factors, including age, menstrual cycle, pregnancy, birth control, infections, sexual frequency and number of partners, and hygienic habits. Lactobacilli are believed to constitute the majority of the normal flora and are postulated to regulate the vaginal environment through the production of hydrogen peroxide, lactic acid, bacteriocins, and other antimicrobial agents that prevent the overgrowth of commensal bacteria with *L. crispatus* or *L. jensenii* accounting for 95% and 94% of the hydrogen peroxide-producing normal flora of Caucasian women, respectively (1). Despite the predominance of several bacterial species; *Staphylococcus*, *Escherichia*, *Gardnerella*, *Streptococcus* and *Peptostreptococcus* are also present within the normal flora. Fluctuations within the levels of vaginal lactobacilli result in a disruption of this delicate balance and are believed to be the causative factor behind Bacterial Vaginosis (BV).

Though not regarded as a sexually transmitted disease, sexual habits, including increased numbers of sexual partners and the use of intrauterine devices as a method of birth control, serve as predisposing factors for the onset and transmission of BV (6).



Figure 1. Vaginal epithelial cells demonstrating *A. vaginae* and *G. vaginalis* Biofilm formation in BV. Red stain specifically identifies *A. vaginae* and *G. vaginalis*. Arrow identifies a clue cell (2).

Bacterial Vaginosis

Bacterial Vaginosis (BV) presents as the most common cause of homeostatic disruption in women of reproductive age. The etiology of this syndrome remains unknown despite a better understanding of its pathogenesis. BV is characterized by disturbed vaginal microflora where the predominant lactobacilli are depleted and there is an overgrowth of *Gardnerella vaginalis* and other anaerobic bacteria, such as *Bacteroides fragilis*, *Mobiluncus mulieris* and *Mobiluncus curtisii* (2-5). Often, the initial symptom of BV is increased vaginal odor that may only be apparent following sexual intercourse. Less common symptoms include increased vaginal discharge, vulvar irritation and painful urination (6). Diagnosis is dependent upon patient history, vaginal examination, microscopic evaluation and clinical evaluation. The presence of “clue” cells, vaginal epithelial cells having bacteria adherent to their surface edges, upon microscopic evaluation of a vaginal smear is considered to be a specific criterion for the diagnosis of BV (**Figure 1**) (6). Treatment consists of antibiotic courses (metronidazole or clindamycin) as well as topically and orally administered lactobacilli cultures to aid their re-establishment within the vagina. Left untreated, BV may lead to adverse conditions such as pelvic inflammatory disease, preterm birth, and postpartum endometritis (7-9). Abnormal vaginal microflora has also been associated with increased susceptibility to HIV (10-12), *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections (13).

Atopobium Vaginae

A recent Australian publication reported increased incidences of both *Gardnerella vaginalis* and *Atopobium vaginae* in the vaginas of virgin women, with transmission occurring through oral sex or hand-genital contact (14). Statistically, there has been no predilection for the occurrence of BV in one racial group over another (6).

Mounting evidence has demonstrated a direct link between the presence of *A. vaginae* and BV. The genus *Atopobium* is a relatively new designation created to account for the phylogenetically distinct *Lactobacillus minutus*, *Lactobacillus rimae* and *Streptococcus parvulus* bacterial species. All strains within the genus are anaerobic, Gram-positive elliptical cocci-shaped bacteria. Prior to the isolation of the novel *Atopobium* isolate, *A. vaginae*, from the vaginal flora of a healthy woman in Sweden in 1999, members of this genus had heretofore only been detected in dental and pelvic abscesses, abdominal wounds and human feces (8,10). The clinical significance of *Atopobium* had been ill-defined until 2003, when the bacterium was isolated from a tuboovarian abscess from a woman in Germany. The inability to designate pathological roles for this bacterial species is, in large part, due to the fastidious nature of its growth, which severely impedes detection by standard culturing methods.

Table 1. Concomitant infection with *A. vaginae* and *G. vaginalis* in BV (15).

Recurrence, Nugent score	<i>G. vaginalis</i> detected (n = 89)		P	<i>G. vaginalis</i> not detected (n = 7)		P
	<i>A. vaginae</i> detected (n = 52)	<i>A. vaginae</i> not detected (n = 37)		<i>A. vaginae</i> detected (n = 1)	<i>A. vaginae</i> not detected (n = 6)	
BV						
7-10	43 (83)	14 (38)	<.001	0	0	...
0-6	9 (17)	23 (62)		1	6	
Abnormal vaginal flora	46 (89)	19 (51)	<.001	1	1	.29
0-3	6 (11)	8 (49)		0	5	

The presence of *Atopobium vaginae* has recently been demonstrated to serve as a useful diagnostic marker for BV (Table 1) (15-17). A study comprised of 358 women experiencing either abnormal vaginal odor or discharge evaluated the roles of *Gardnerella vaginalis* and *Atopobium vaginae* in BV recurrence following metronidazole therapy (15). Of this group, 50% were found to have normal vaginal flora, 10% were intermediate and 39% had BV as assessed by the Nugent Score criteria. Real-Time PCR analyses demonstrated the presence of *Gardnerella vaginalis*, often viewed as a diagnostic marker of BV, in 99% of the BV group. However, 66% of the women with normal flora were also found to harbor *G. vaginalis*; therefore, questioning its negative predictive power and its use as a diagnostic marker (Table 2). *Atopobium vaginae*, however, was determined to be highly specific for BV affected individuals, with 96% detection levels observed within the BV group and only 12% within the normal flora group (Table 2) (15).

Table 2. Incidence of *Atopobium vaginae* in BV. The presence of *Atopobium vaginae* serves as a better indicator of BV than does *Gardnerella vaginalis*.

Statistics	<i>Atopobium vaginae</i>	<i>Gardnerella vaginalis</i>
Sensitivity with relation to BV	96%	99%
Specificity with relation to BV	77%	35%
Prevalence associated with abnormal vaginal flora	88%	41%
NPV	96%	99%
PPV	74%	50%

An inextricable link between *G. vaginalis* and *A. vaginae* has become apparent from the analyses of these BV cases in that *A. vaginae* is rarely ever present in the absence of *G. vaginalis* regardless of Nugent Score, suggesting a synergistic effect exists between the two bacteria (15-16). This association was also made apparent through the analysis of vaginal biofilms, whereby *G. vaginalis* and *A. vaginae* were found to comprise greater than 90% of the film (Figure 1). The detection of high levels of both species correlates with a significant increase in antibiotic failure and the recurrence of BV, while the absence of either species is highly predictive of a negative BV diagnosis (15).

Treatment

Due to the large number of bacterial species present within the vagina, treatment courses for BV-affected individuals must be broad-based. Currently, clindamycin and metronidazole are the two most commonly prescribed antibiotics. Metronidazole has been chosen as the preferred treatment because of its ability to increase the colonization rate of hydrogen peroxide-producing lactobacilli (17). However, some cases do not resolve with a single course of treatment (6). This may in part be due to a preference in prescribing metronidazole and the decreased level of metronidazole-susceptibility observed with the few strains of *A. vaginae* which have been isolated (Figure 2) (6, 18).

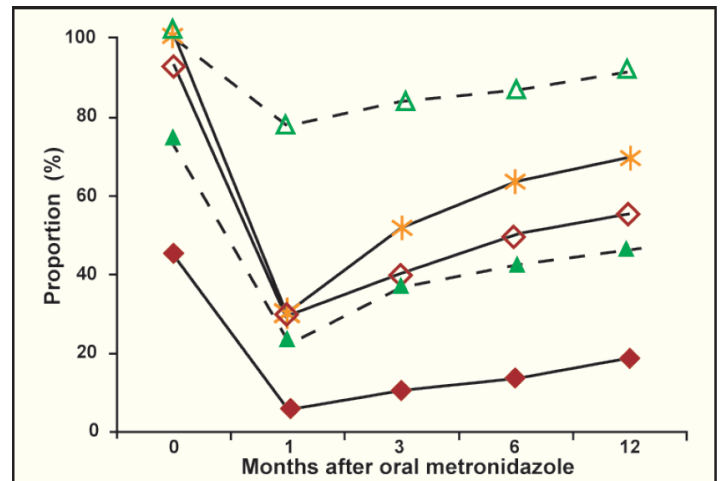


Figure 2. Incidence of metronidazole failure and recurrence of BV. Proportion of women with detectable *Atopobium vaginae* (AV) and *Gardnerella vaginalis* (GV) over a twelve month course following oral metronidazole therapy. Legend: Nugent Score of 4-10 (*); detectable AV (◇); high AV load (◆); detectable GV (△); high GV load (▲).

Table 3. Treatment Recommendations. CDC's 2015 Sexually Transmitted Diseases Summary of 2015 CDC Treatment Guidelines (20).

Recommended Regimens

Metronidazole oral^a 500 mg orally twice a day for 7 days **OR**

Metronidazole gel 0.75%^a one applicator (5 g) intravaginally, once a day for 5 days **OR**

Clindamycin cream 2%^{a, b} one applicator (5 g) intravaginally at bedtime for 7 days

Recommended Regimens (Alternatives)

Tinidazole 2 g orally once daily for 2 days **OR**

Tinidazole 1 g orally once daily for 5 days **OR**

Clindamycin 300 mg orally twice daily for 7 days **OR**

Clindamycin ovules 100 mg intravaginally once at bedtime for 3 days

★ Treatment is recommended for all symptomatic pregnant women

^a The recommended regimens are equally efficacious.

^b These creams are oil-based and may weaken latex condoms and diaphragms. Refer to product labeling for further information.

With the increased rate of drug therapy failure in mind, screening patients for the presence of *G. vaginalis* and *A. vaginae* both pre- and post-therapy might be advantageous. Medical Diagnostic Laboratories, L.L.C., (MDL) has developed a highly sensitive Real-Time PCR assay for the detection of *A. vaginae*: **Test 142 Atopobium vaginae by Real-Time PCR**. This testing is currently available utilizing the **OneSwab®** and ThinPrep® specimen collection platforms.

Exhaustive analyses confirmed the specificity of the Real-Time Assay for *A. vaginae* with no observed cross-reactivity against a panel of 51 other human pathogens of bacterial, fungal, and viral origin, including other members of the *Atopobium* genus and organisms closely related by 16S rDNA sequences. This Real-Time PCR assay was used to analyze 96 cervicovaginal swab samples submitted to our clinical laboratory for detection of organisms associated with BV. Of those samples, 28 (29%) were positive for *A. vaginae*. Of the 28 positive samples, 23 were concomitantly infected with *G. vaginalis*, two with both *G. vaginalis* and *M. mulieris* or *M. curtisii*, one with both *G. vaginalis* and *B. fragilis*, and two contained *A. vaginae* alone (**Table 4**) (19). This test provides an accurate and expeditious evaluation of human cervicovaginal samples for the presence of *A. vaginae* that will dramatically aid the diagnosis of BV.

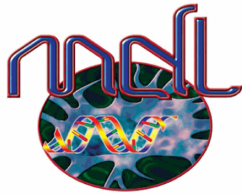
Table 4. Incidence of Atopobium vaginae in BV. The presence of *Atopobium vaginae* serves as a better indicator of BV than does *Gardnerella vaginalis*.

	BV
No pathogens detected	59
<i>Atopobium vaginae</i>	2
<i>Gardnerella vaginalis</i>	7
<i>Mobiluncus mulieris</i>	2
<i>Atopobium vaginae</i> + <i>Gardnerella vaginalis</i>	23
<i>Atopobium vaginae</i> + <i>Gardnerella vaginalis</i> + <i>Mobiluncus mulieris</i>	2
<i>Atopobium vaginae</i> + <i>Gardnerella vaginalis</i> + <i>Bacteroides fragilis</i>	1
TOTAL	96

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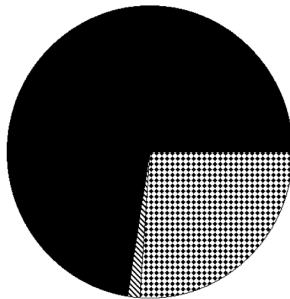
* Test Results

Physician Copy

Patient Information: SSN: XXX-XX-1111 DOB: 1/1/1987 (Age:27) DOE, JANE 56 LIBERTY DRIVE DAYTON, NJ 08810	Ordering Physician/Lab: NPI: 1234567890 JOHN DOE, MD 202 ANY STREET DAYTON, NJ 08810 Tel: 555-555-5551 Fax: 555-555-5555
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Patient ID: _____ Date Processed: 9/30/2015 Date Reported: 10/1/2015

Test	Specimen	Date Collected Comment	Results		Reference/Units/Comments
			Normal	Abnormal	
Gardnerella vaginalis by Real-Time PCR 132 Verified 10/1/2015	* Swab - 1	9/28/2015 Vag/Cerv/End		Positive	
Atopobium vaginae by Real-Time PCR 142 Verified 10/1/2015	* Swab - 1	9/28/2015 Vag/Cerv/End		Positive	
Bacterial Vaginosis Associated Bacteria 2 (BVAB2) by Real-Time PCR 164 Verified 9/30/2015	* Swab - 1	9/28/2015 Vag/Cerv/End	Negative		
Megasphaera species (Type 1 and Type 2) by Real-Time PCR 165 Verified 10/1/2015	* Swab - 1	9/28/2015 Vag/Cerv/End	Negative (Type2)	Positive (Type1)	Type1:Positive; Type2:Negative.
Lactobacillus (BVPanel) by Real Time PCR 179 Verified 10/1/2015	* Swab - 1	9/28/2015 Vag/Cerv/End			L.crispatus : Negative; L.jensenii : Negative; L.gasseri : Negative; L.iners : Negative.



Gardnerella vaginalis (26.0%)
 Atopobium vaginae (1.3%)
 Megasphaera Type 1 (72.8%)

Indicative of Abnormal microflora.
Suggestive of Bacterial Vaginosis.

View: M

Mail:	Yes	Overnight
	All	Yes

Fax:	Yes	Manual
	All	No

Medical Director, Dante A. Ragasa, MD.