Use of Species-Directed 16S rRNA Gene PCR Primers for Detection of *Atopobium vaginae* in Patients with Bacterial Vaginosis

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Bacterial vaginosis (BV) is a syndrome associated with a shift in vaginal flora away from normal, with predominantly *Lactobacillus* spp., toward an increase in gram-negative anaerobes and other bacteria (7). BV is linked to morbidities, including increased susceptibility to and transmission of sexually transmitted infections, premature births, and low birth weight (4, 8–11, 14, 22, 23, 26). Some bacteria, for example, *Gardnerella vaginalis*, are commonly isolated from patients with BV; however, no single infectious agent has been identified as the cause of the syndrome.

Much of our present understanding of vaginal flora is based on bacteria that have been cultivated and identified using determinative characteristics. Cultivation-independent (molecular) analyses of 16S rRNA gene sequences from microbial communities suggest that only a small percentage of bacteria in nature have been identified, even in well-studied environments (25), and 16S rRNA gene sequences provide a more robust, systematic method of identification. It is likely that cultivated isolates do not represent all species in the complex vaginal ecosystem and that there may be pathogens associated with BV isolates that have yet to be identified (19, 20).

In a related study, members of our laboratory used 16S rRNA gene sequences that were PCR amplified from vaginal lavage fluid using universal bacterial primers (5'-ATGGCTGTGCAGCTCAAT-3' and 5'-CGCCCGCCGCGCCCGCCGCGCCGGCAGCCGCGCCCGCCCGCCGGCGGGGCTGTGTGTAC-3') and separated into banding patterns by denaturing gradient gel electrophoresis (DGGE) to analyze the flora of normal and BV patients (6). It was found that normal and BV flora were readily distinguishable by DGGE patterns. During the course of these studies, a recently described organism, *Atopobium vaginae* (21), was detected in a significant portion (55%) of the 20 BV-positive patients in the cohort (6). In contrast, this organism was present in only 2 of 24 women with normal vaginal Gram stains. Two *A. vaginae* strains were isolated from two BV-positive patients, and it was confirmed that this organism is a strict anaerobe and is highly resistant to metronidazole, the antibiotic most commonly used to treat BV. Other recent studies also suggest that the association of *A. vaginae* with BV warrants further investigation (3, 24, 27).

We suspected that template competition for universal bacterial primers during the PCR might be limiting researchers’ ability to detect *A. vaginae* as a band on denaturing gradient gels, raising the possibility that *A. vaginae* is harbored by more BV and/or normal patients than the study by Ferris et al. revealed. We explored this possibility by designing a PCR primer set specific for the 16S rRNA gene of *A. vaginae* and reanalyzing vaginal flora of normal and BV-positive patients whose DGGE profiles lacked DNA fragments typically representing sequences with high homology to *A. vaginae*. Nucleic acids were obtained from vaginal lavage samples using a bead-beating cell lysis treatment and DNA extraction protocol similar to that described by Moré (15) as presented previously (5). Normal and BV flora were assessed using Nugent scoring (16), and the presence or absence of *A. vaginae* was noted for DGGE profiles as previously described (6). PCR conditions were as follows: 95°C for 4 min followed by 25 cycles of 95, 55, and 72°C for 1 min each, and then 72°C for 7 min. PCR was performed using Taq polymerase and a commercially available kit as previously described (6).

A PCR primer set targeting *A. vaginae* was designed with the aid of the freeware package PRIMROSE (2). Using *A. vaginae* genomic DNA, the primers, AV1F 5'-TCATGGCCCAGAAGACCGTGCAGCTCAAT-3' and AV3R 5'-TCATGGCCCAGAAGACCGTGCA-3' and separated into banding patterns by denaturing gradient gel electrophoresis (DGGE) to analyze the flora of normal and BV patients (6). It was found that normal and BV flora were readily distinguishable by DGGE patterns. During the course of these studies, a recently described organism, *Atopobium vaginae* (21), was detected in a significant portion (55%) of the 20 BV-positive patients in the cohort (6). In contrast, this organism was present in only 2 of 24 women with normal vaginal Gram stains. Two *A. vaginae* strains were isolated from two BV-positive patients, and it was confirmed that this organism is a strict anaerobe and is highly resistant to metronidazole, the antibiotic most commonly used to treat BV. Other recent studies also suggest that the association of *A. vaginae* with BV warrants further investigation (3, 24, 27).

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the presence of *A. vaginae* (6). All yielded *A. vaginae*-specific amplicons (Fig. 1B). We used the *A. vaginae*-specific primer set to analyze 19 patients who had normal vaginal flora by Gram stain criteria and no evidence of *A. vaginae* by DGGE analysis. None of these yielded PCR amplicons. We analyzed 11 patients who had BV by Gram stain criteria and DGGE analyses but no evidence of *A. vaginae* by DGGE. Four (36%) showed PCR amplicons of the correct size (Fig. 1B). Sequence analysis of the amplicons confirmed that they were *A. vaginae*.

Though the clinical significance of *A. vaginae* is unclear, the data presented here further strengthen its association with BV. Four of 11 BV cases without evidence of *A. vaginae* by DGGE analysis were positive for the organism using species-specific PCR primers. The *A. vaginae*-specific PCR primers appear to be more sensitive for the detection of *A. vaginae* than the universal bacterial primers used in our previous DGGE analyses. Based on the findings of the study by Ferris et al., plus the present study, it may be that as many as 70% of women with BV harbor *A. vaginae*. The observation of *A. vaginae* in a high percentage of BV patients is consistent with other recent independent PCR-based studies of vaginal flora (3, 24). In one study, Burton et al. (3) used a different set of *A. vaginae*-specific PCR primers to detect *A. vaginae* in 50% of Canadian BV patients. Perhaps the most significant observation in the present study and that of Burton et al. (3) is that both *A. vaginae*-specific PCR assays were negative in all women with normal vaginal Gram stains (total of 35 women). These results suggest that *A. vaginae* is rarely if ever a component of normal vaginal flora. If true, this finding is in contrast to findings for other BV-associated organisms, such as *G. vaginalis*, *Peptostreptococcus* spp., and gram-negative anaerobes, which are commonly present in normal women, though in lower concentrations than in BV cases. Additional studies are needed to explore the possibility that *A. vaginae* may play a significant role in BV pathogenesis and that it might influence the outcome of metronidazole treatment in women with BV.

### REFERENCES


