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Cultivation-Independent Analysis of Changes in Bacterial Vaginosis Flora Following Metronidazole Treatment

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PCR was used to survey bacterial vaginosis flora before and after metronidazole treatment. The species composition for pretreatment patients was variable. Lactobacillus iners was prominent in all patients post-treatment. Atopobium vaginae concentrations were highest for patients who failed or responded incompletely to treatment and lowest for patients who were cured.

Bacterial vaginosis (BV) is the most common cause of vaginal irritation and is associated with adverse pregnancy outcomes (4, 13) and an increased risk of human immunodeficiency virus infection (14, 15). BV results when the normal, characterized by a predominance of Lactobacillus species, is replaced by a variety of anaerobic organisms. However, no specific pathogen has been identified, and the cause of BV is unknown (6).

Metronidazole is the most commonly prescribed antibiotic for treatment of BV, but failure and recurrence rates are high (7). Recent cultivation-independent analyses of PCR-amplified 16S rRNA gene sequences reveal that there are bacterial genera associated with BV that were not previously recognized, including a metronidazole-resistant anaerobe, Atopobium vaginae (8, 9, 17, 19). We examined the species composition of the vaginal flora of BV patients before and 1 month after metronidazole treatment by using PCR assays directed toward a broad range of bacterial genera and a quantitative PCR assay targeting A. vaginae.

Clinical assessments of BV were made just prior to treatment and at 4 weeks posttreatment. All six BV patients in the study met all Amsel criteria (2) and had Nugent scores of 8. At the 1-month follow-up visit, one patient, No. 500, had a normal Nugent score but was judged a “complete” treatment failure, since neither the Amsel criteria nor the Nugent score had improved at the follow-up visit. The remaining five patients were judged complete cures.

Quantitative PCR indicated that pretreatment A. vaginae concentrations were highest in patients who completely or 507 Cure (0) Cure No 23.1 28.8

Patient 499 failed treatment by Nugent’s criterion with a score of 8 but was clinically cured, having none of Amsel’s criteria. Patient 500 had a normal Nugent score but was judged a treatment failure based on a persistently elevated vaginal pH. The three remaining patients were judged complete cures.

Quantitative PCR indicated that pretreatment A. vaginae concentrations were highest in patients who completely or

Table 1. Metronidazole treatment outcome and detection of A. vaginae

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Gram stain outcome (score)</th>
<th>Clinical outcomea</th>
<th>A. vaginae in pretreatment clone library</th>
<th>Quantitative PCR result (Ct)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>498 Failure (8) Failurec</td>
<td>Yes</td>
<td>16.2</td>
<td>14.7</td>
<td></td>
</tr>
<tr>
<td>499 Failure (8) Cure</td>
<td>Yes</td>
<td>18.5</td>
<td>27.0</td>
<td></td>
</tr>
<tr>
<td>500 Cure (0) Failurec</td>
<td>Yes</td>
<td>17.7</td>
<td>30.2</td>
<td></td>
</tr>
<tr>
<td>505 Cure (0) Cure</td>
<td>No</td>
<td>21.3</td>
<td>31.9</td>
<td></td>
</tr>
<tr>
<td>506 Cure (0) Cure</td>
<td>No</td>
<td>19.7</td>
<td>34.8</td>
<td></td>
</tr>
<tr>
<td>507 Cure (0) Cure</td>
<td>No</td>
<td>23.1</td>
<td>28.8</td>
<td></td>
</tr>
</tbody>
</table>

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b PCR cycle at which the specimen crossed the positivity threshold. Lower values indicate higher organism concentrations.

c Cure, none of the Amsel criteria met.

d Based on persistent clue cells and discharge.

e Based on a pH of >4.7.
partially failed treatment (Table 1), and *A. vaginae* sequences were detected in pretreatment clone libraries of these patients (Table 1; Fig. 1). In contrast, quantitative PCR indicated that *A. vaginae* concentrations were lowest in patients who were cured, and no *A. vaginae* sequences were detected in pretreatment clone libraries of any of these cases. It is possible that posttreatment specimens could include DNA from nonviable organisms; however, we think it unlikely that nonviable-organism DNA would persist for a month following treatment. Clearly, more-extensive studies are necessary to assess whether high pretreatment concentrations of individual species, such as *A. vaginae*, are predictive of adverse treatment outcomes for BV patients, since *G. vaginalis* and, to a lesser extent, *A. vaginae* are detectable among patients without BV by species-specific PCR (8). However, it is of interest that quantitative PCR studies of *Gardnerella vaginalis* and *Mycoplasma hominis*...
have already provided evidence that concentrations of individual vaginal species may be more predictive of adverse sequelae than the diagnosis of BV alone (14).

After treatment, sequence analyses indicated that a single species, *Lactobacillus iners*, was predominant in all patients, except for the patient who was a complete treatment failure, for whom *L. iners* sequences were prevalent but not predominant (Fig. 1). Since we routinely detect predominantly *Lactobacillus crispatus* sequences in patients classified as normal by Nugent’s score and Amsel’s criteria (data not shown), and normal *L. crispatus*-dominant vaginal flora is commonly described in the literature (8, 17–19), the predominance of *L. iners* in “cured” patients was unexpected. Recently, more-refined Gram stain subcategories of vaginal flora have been proposed (16, 18). In this system, *L. crispatus* is prevalent in specimens with a grade Ia Gram stain and the flora is predominantly *L. crispatus* as determined by culture. *L. iners* is rare in grade Ia specimens; however, it is prevalent in grade Ib, a variant of normal, and in grade III, representing BV. The “protective” role of individual vaginal *Lactobacillus* species is unclear (18). We speculate that *L. iners* is a transitional species and that an *L. crispatus*-predominant species composition represents a stable normal flora.

Clone library analyses indicated that, prior to treatment; each BV patient harbored a unique complement of bacterial species (Fig. 1). Evidence of high variability in species composition among BV patients has been well documented by recent extensive PCR analyses of thousands of 16S rRNA gene sequences from dozens of patients (8, 9). We noted that almost all (32 of 35) phylotypes detected in our study were highly related (≥99% sequence similarity) to sequences in GenBank and that most (31 of 35) were from studies of vaginal flora (8, 9, 19). It may be that most, if not all, of the novel species commonly inhabiting the vagina have been described in the published literature. With the exception of *Leptotrichia, Sneathia*, and *Porphyromonas*-like sequences, all phylotypes in this study clade within the phylum *Firmicutes* or *Fusobacteria*, which contain traditional gram-positive actinomyecetes and *Clostridium-Bacillus* species, including *Lactobacillus* species, respectively. However, some cultivated members of these phyla, such as *Megasphaera* spp., which are commonly detected in tRNA gene libraries of BV patients, are known to have atypical cell walls, resulting in negative Gram reactions (11). Thus, descriptions of BV as an increase in gram-“negative” species might exaggerate perceptions of phylogenic distance between normal and BV-associated bacterial communities.

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