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Utilization of molecular methods to identify prognostic markers for recurrent bacterial vaginosis

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ABSTRACT

Background: Recurrent bacterial vaginosis (BV) after antimicrobial therapy is a major problem, affecting >50% of patients within 1 year. The objective of this study was to determine if prospective identification of patients at risk for recurrence using molecular methods is feasible.
Methods: Women were evaluated for BV by Amsel criteria and Nugent score. Vaginal specimens were analyzed using a panel of quantitative real-time polymerase chain reactions (qPCRs) at three times: pre-treatment, 7–10 days post-treatment and 40–45 days post-treatment. The PCRs quantified DNA of the following organisms: *Gardnerella vaginalis*; *Atopobium vaginae*; Bacterial Vaginosis–Associated Bacteria-1 (BVAB1), -2 (BVAB2) and -3 (BVAB3); *Leptotrichia/Sneathia*; *Megasphaera* Phylotypes 1 and 2; and *Lactobacillus spp.* (*L. crispatus*, *L. gasseri*, *L. iners* and *L. jensenii*).
Results: Out of 84 women diagnosed with BV (Amsel ≥3 and Nugent ≥4), 77 (91.7%) were successfully treated after 7-10 days (asymptomatic and Amsel of either 0 or 1 with elevated vaginal pH and Nugent ≤6). Of these 77 women, 46 (59.7%) remained cured after 40–45 days and 31 (40.3%) developed recurrent BV. In univariate analysis, we found that women who would have recurrent BV during the study had greater concentrations of *Megasphaera* Phylotype 2 ($P = 0.001$) and BVAB2 ($P = 0.015$) at initial diagnosis and greater vaginal pH ($P = 0.030$), higher Nugent score ($P = 0.043$) and a greater concentration of *G. vaginalis* ($P = 0.012$) post-treatment, when compared to women who were cured during the study. These differences largely remained when cure was defined as Nugent ≤3 or when only women treated with intravaginal metronidazole were evaluated.
Conclusion: Molecular analysis of BV is a useful adjunct to clinical and microscopic analysis to prospectively identify patients at high risk for recurrent BV.

1. Introduction

Bacterial vaginosis (BV) is characterized by a shift in the vaginal flora from commensal *Lactobacillus spp.* dominant to diverse fastidious gram-negative and variable, anaerobic and facultative species (Sobel, 2000). It is the most common gynecological infection in the United States, affecting 29% of women (Koumans et al., 2007), and is associated with serious complications, including increased susceptibility to sexually transmitted diseases (Balkus et al., 2014; Martin et al., 1999), post-operative infections (Larsson et al., 1992; Persson et al., 1996) and pre-term labor (Hillier et al., 1995). Diagnosis of BV is often based upon the presence of at least three of the four following clinical signs and symptoms (Amsel criteria) (i) vaginal pH > 4.5, (ii) homogenous white/gray vaginal discharge coating the vaginal walls, (iii) the presence of clue cells (vaginal epithelial cells covered in bacteria) and (iv) the whiff test (fishy odor after addition of 10% KOH) (Amsel et al., 1983). However, diagnosis based on signs and symptoms alone suffers from low specificity (Landers et al., 2004) and the gold-standard method is enumeration of bacterial morphotypes on a Gram-stained vaginal smear (*i.e.* Nugent scoring) (Nugent et al., 1991). Specimens are categorized with a score from 0 to 10, with 0–3 indicating *Lactobacillus*-dominated normal vaginal flora, 4–6 indicating the presence intermediate vaginal flora with *Gardnerella vaginalis* present and 7–10 indicating abnormal vaginal flora indicative of BV where *Lactobacillus spp.* are absent and large numbers of *G. vaginalis* and strict anaerobes are present. Although BV has traditionally been associated with *G. vaginalis* (Gardner & Dukes, 1955) the use of culture-based (Ferris et al., 2004; Spiegel et al.,

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Table 1
Study population characteristics and clinical characteristics at Visits #1 and #2. Abbreviation: IQ (interquartile).

	Complete and Partial Responders to Any Therapy			Complete Responders to Any Therapy			Complete and Partial Responders to Intravaginal Metronidazole Therapy		
	Cured (N = 43)	Recurrent BV (N = 31)	*P* Value	Cured (N = 32)	Recurrent BV (N = 24)	*P* Value	Cured (N = 28)	Recurrent BV (N = 22)	*P* Value [c]
Characteristic									
Race [a]									
African-American	24 (55.8)	24 (77.4)		18 (56.3)	21 (87.5)	0.018	17 (60.7)	17 (77.2)	
Caucasian	18 (41.9)	6 (19.4)		13 (40.6)	3 (12.5)		11 (39.3)	4 (18.2)	
Hispanic	1 (2.3)	0 (0.00)		1 (3.1)	0 (0.0)		0 (0.0)	0 (0.0)	
Asian	0 (0.0)	1 (3.2)		0 (0.0)	0 (0.0)		0 (0.0)	1 (4.5)	
Age[b]	38.0 (31.5–41.5)	32.0 (29.0–36.0)		30.0 (30.8–41.0)	32.0 (29. - 36.0)		37.5 (31.8–41.0)	32.0 (29.0–35.5)	0.032
Treatment After Visit #1 [a]									
Metronidazole 750 mg gel plus miconazole 200 mg	21 (48.8)	14 (45.2)		18 (56.3)	12 (50.0)		21 (75.0)	15 (68.2)	
Metronidazole 750 mg suppository	7 (16.3)	7 (22.6)		4 (12.5)	4 (16.7)		7 (25.0)	7 (31.8)	
Metronidazole 500 mg bid po	2 (4.7)	0 (0.00)		2 (6.3)	0 (0.0)		0 (0.0)	0 (0.0)	
Clindamycin 2% cream	6 (14.0)	5 (16.1)		4 (12.5)	5 (20.8)		0 (0.0)	0 (0.0)	
Tinidazole 500 mg bid po	3 (7.0)	2 (6.5)		2 (6.3)	2 (8.3)		0 (0.0)	0 (0.0)	
No treatment information	4 (9.3)	3 (9.7)		2 (6.3)	1 (4.2)		0 (0.0)	0 (0.0)	
1st Visit (Pre-treatment)									
Amsel[a]									
pH > 4.5	43 (100)	31 (100)		32 (100)	24 (100)		28 (100)	22 (100)	
Discharge	43 (100)	30 (96.8)		32 (100)	23 (95.8)		28 (100)	21 (95.5)	
Clue Cells	40 (93.0)	29 (93.5)		30 (94)	22 (91.7)		25 (89.3)	20 (90.9)	
Amines	43 (100)	31 (100)		32 (100)	24 (100)		28 (100)	22 (100)	
Nugent									
Median (IQ range)	8.0 (7.0–10.0)	9.0 (8.0–10.0)		8.0 (7.0–9.0)	10.0 (7.3–10.0)	0.023	8.0 (7.0–9.0)	8.5 (8.0–10.0)	
4–6 [a]	7 (16.3)	1 (3.2)		6 (18.8)	1 (4.2)		6 (21.4)	0 (0.0)	0.028
7–10 [a]	36 (85.7)	30 (96.8)		26 (81.3)	23 (95.8)		22 (78.6)	22 (100)	
2nd Visit (7–10 Day Follow-Up)									
Amsel[a]									
pH > 4.5	2 (4.7)	7 (22.6)	0.030	1 (3.1)	4 (16.7)		1 (3.6)	4 (18.2)	
Discharge	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Clue Cells	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Whiff Test	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Nugent									
Median (IQ range)	0.0 (0.0–0.0)	0.0 (0.0–3.0)	0.043	0.0 (0.0–0.0)	0.0 (0.0–0.0)		0.0 (0.0–0.0)	0.0 (0.0–4.0)	0.036
0–3 [a]	40 (93.0)	24 (78.1)		32 (100)	24 (100)		27 (96.4)	16 (72.8)	
4–6 [a]	3 (7.0)	7 (21.9)		0 (0.0)	0 (0.0)		1 (3.6)	6 (27.2)	
3rd Visit (40–45 Day Follow-Up)									
Amsel [a]									
pH > 4.5	6 (14.0)	31 (100)	<0.001	2 (6.3)	24 (100)	<0.001	5 (17.9)	22 (100)	<0.001
Discharge	0 (0.0)	29 (93.5)	<0.001	0 (0.0)	22 (91.7)	<0.001	0 (0.0)	19 (86.3)	<0.001
Clue Cells	0 (0.0)	24 (77.4)	<0.001	0 (0.0)	21 (87.5)	<0.001	0 (0.0)	18 (81.8)	<0.001
Amines	0 (0.0)	30 (96.8)	<0.001	0 (0.0)	24 (100)	<0.001	0 (0.0)	22 (100)	<0.001
Nugent									
Median (IQ range)	0.0 (0.0–2.0)	8.0 (7.0–9.0)	<0.001	0.0 (0.0–0.8)	7.0 (4.0–8.0)	<0.001	0.0 (0.0–2.8)	7.0 (5.0–9.0)	<0.001
0–3 [a]	35 (81.4)	0 (0.0)		36 (100)	0 (0.0)		23 (82.1)	3 (13.6)	
4–6 [a]	8 (18.6)	2 (6.5)		0 (0.0)	3 (12.5)		5 (17.9)	19 (86.3)	
7–10 [a]	0 (0.0)	29 (93.5)		0 (0.0)	21 (87.5)				

[a] n (%).
[b] Median (IQ range).
[c] Only *P* values <0.05 are listed.

1980) and molecular methods (Fredricks et al., 2005) have identified numerous additional microbes associated with the disease, including *Atopobium vaginae;* Bacterial Vaginosis Associated Bacteria 1, 2 and 3 (BVAB1, -2, -3); *Megasphaera* Phylotype 1 and 2; and *Leptotrichia aminionii*. As these organisms are largely unculturable, molecular methods, such as polymerase chain reactions (PCRs), including quantitative real-time PCRs (qPCRs), as well as next-generation DNA sequencing, have emerged as technologies to monitor the presence and quantity of these organisms in BV patients (Fredricks et al., 2007; Ravel et al., 2011; Zozaya-Hinchliffe et al., 2010).

BV is treated with oral or intravaginal antimicrobial therapy, usually metronidazole or clindamycin. Cure rates at ~1 month follow-up range from 100% to as low as 60% (Koumans et al., 2002), and >50% of patients experience recurrent disease within one year (Bradshaw et al., 2006). The discovery of easily identifiable prognostic markers for subsequent relapse or recurrence following therapy could allow clinicians to select repeated high-dose, longer-term or adjunctive therapies for high-risk patients that may improve cure rates over conventional therapy (Chavoustie et al., 2015; Reichman et al., 2009; Sobel et al., 2006). The objective of our study was to determine if women at high risk of recurrent BV could be identified prospectively through molecular analysis of their vaginal microbiota, at either initial diagnosis and/or immediately after treatment. Women were evaluated at initial diagnosis, a 7–10 day follow-up visit and a 40–45 day follow-up visit by Amsel's criteria and their vaginal flora characterized by Nugent scoring as well as by a panel of qPCR assays that quantify BV-