

# Bacterial Vaginosis and Risk of Pelvic Inflammatory Disease

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**BACKGROUND:** Bacterial vaginosis commonly is found in women with pelvic inflammatory disease (PID), but it is unclear whether bacterial vaginosis leads to incident PID.

**METHODS:** Women (n = 1,179) from 5 U.S. centers were evaluated for a median of 3 years. Every 6–12 months, vaginal swabs were obtained for gram stain and culture of microflora. A vaginal microflora gram stain score of 7–10 was categorized as bacterial vaginosis. Pelvic inflammatory disease was diagnosed by presence of either histologic endometritis or pelvic pain and tenderness plus one of the following: oral temperature greater than 38.3°C; sedimentation rate greater than 15 mm/hour; white blood count greater than 10,000; or lower genital tract detection of leukorrhea, mucopus, or *Neisseria gonorrhoeae* or *Chlamydia trachomatis*.

**RESULTS:** After adjustment for relevant demographic and lifestyle factors, baseline bacterial vaginosis was not associated with the development of PID (adjusted hazard ratio 0.89, 95% confidence interval 0.55–1.45). Carriage of bacterial vaginosis in the previous 6 months before a diagnosis (adjusted risk ratio 1.31, 95% confidence interval 0.71–2.42) also was not significantly associated with PID. Similarly, neither absence of hydrogen peroxide-producing *Lactobacillus* nor high levels of *Gardnerella vaginalis* significantly increased the risk of PID. Dense growth of pigmented, anaerobic gram-negative rods in the 6 months before diagnosis did significantly increase a woman's risk of PID ( $P =$

.04). One subgroup of women, women with 2 or more recent sexual partners, demonstrated associations among bacterial vaginosis, *Gardnerella vaginalis*, anaerobic gram-negative rods, and PID.

**CONCLUSION:** In this cohort of high-risk women, after adjustment for confounding factors, we found no overall increased risk of developing incident PID among women with bacterial vaginosis. (Obstet Gynecol 2004;104:761–9. © 2004 by The American College of Obstetricians and Gynecologists.)

**LEVEL OF EVIDENCE: II-2**

Bacterial vaginosis is an imbalance in the vaginal microflora thought to increase susceptibility to sexually transmitted pathogens.<sup>1</sup> In the healthy vagina, hydrogen peroxide-producing lactobacilli inhibit other endogenous bacteria (such as the anaerobic gram-negative rods *Bacteroides* and *Prevotella*, genital mycoplasmas, and *Gardnerella vaginalis*) by producing bacteriocins, as well as hydrogen peroxide and lactic acid, all of which lower the vaginal pH to a level that is inhospitable to many other bacteria.<sup>2–4</sup> When the flora is disrupted, the hydrogen peroxide-producing lactobacilli decrease in concentration and are replaced by an overgrowth of anaerobic and facultative aerobic bacteria, a microecology termed bacterial vaginosis.<sup>5,6</sup>

Bacterial vaginosis is common among women with upper genital tract inflammation and pelvic inflammatory disease (PID).<sup>7–12</sup> The ascent of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* from the lower to the upper genital tract is thought to be the most common precipitant of PID.<sup>13</sup> The presence of these sexually transmitted pathogens frequently is accompanied by the presence, in the upper genital tract, of the anaerobic and facultative bacteria that comprise bacterial vaginosis.<sup>14</sup> Whether anaerobes and facultative bacteria can cause PID a priori or whether they ascend from the lower genital tract, where they normally reside, into the upper tract as a consequence of *N. gonorrhoeae* or *C. trachomatis* infections, is unclear. However, bacterial vaginosis-associated bac-

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teria in the upper genital tract have been associated with salpingitis and endometritis independent of *N. gonorrhoeae* or *C. trachomatis* infections.<sup>15–17</sup> We conducted a multicenter, prospective observational cohort study designed to examine whether, among women at high risk for sexually transmitted infections, bacterial vaginosis increases the risk for developing PID.

## METHODS

Women aged 13 to 36 years were recruited into the GYN Infections Follow-through Study from family-planning clinics, university health clinics, gynecology clinics, and sexually transmitted disease (STD) units at each of 5 clinical sites located throughout the eastern, southern, and western regions of the United States between May 1999 and June 2001. Approval for the use of human subjects was obtained at each participating institution, and all women gave informed consent. Women were eligible for the GYN Infections Follow-through Study if they were not specifically seeking care for an STD yet, on the basis of a previous risk-stratification paradigm for chlamydial cervicitis,<sup>18</sup> they were considered to be at high risk for acquiring a sexually transmitted bacterial infection. Specifically, to be enrolled in the study, a woman had to have a score of 3 points or more on an algorithm wherein points were derived as follows: aged 24 or younger = 1 point; black race = 2; never pregnant = 1; 2 or more sexual partners = 1; douche at least once per month = 2; and any prior sexually transmitted infection, including *N. gonorrhoeae*, *C. trachomatis*, or *Trichomonas vaginalis* = 2. Of 2,740 women screened for study entry, 853 (31.1%) did not meet these inclusion criteria. An additional 259 (9.5%) women were excluded on the basis of a priori criteria, including currently pregnant by  $\beta$ -hCG testing; currently married; never having had sexual intercourse; having pelvic tenderness on examination at baseline; having had a prior hysterectomy, salpingectomy, or tubal ligation; or being on antibiotics at baseline. Among the 1,628 women who were eligible for the study, 1,179 (72.4%) completed a questionnaire and evaluation of their vaginal flora at baseline and are the focus of these analyses.

Enrolled versus eligible, nonenrolled women were more likely to have risk factors for PID, including being 24 years of age or younger (74.8% versus 67.1%;  $P = .002$ ) and having 2 or more sexual partners in the past 2 months (53.8% versus 36.7%;  $P < .001$ ). They were less likely to be African American (76.1% versus 79.9%;  $P = .001$ ) and did not differ significantly in number of previous pregnancies or douching status.

Using a directed, validated method, all subjects collected self-obtained vaginal specimens with a cotton

swab at baseline and on a standard schedule of every 6–12 months.<sup>19</sup> Vaginal swabs were smeared onto slides by study staff, and these slides were air dried and later gram-stained at a centralized microbiology laboratory under the direction of one of us (S.L.H.). A score of 0–10 was assigned by laboratory staff, who were masked to any subject characteristics, in light of the relative proportions of large gram-positive rods (lactobacilli), small gram-negative or gram-variable rods (*Bacteroides* or *Gardnerella*), and curved gram-variable rods (*Mobiluncus*).<sup>20</sup> The results were scored by a standardized method as described by Nugent et al.<sup>20</sup> A score of 0–3 was interpreted as consistent with normal vaginal flora; a score of 4–6, corresponding to disturbed flora, was designated as intermediate; and a score of 7–10 was considered to be bacterial vaginosis. Relative to clinical signs, the scoring system has been shown to have a specificity of 83% and a sensitivity of 89% for detecting bacterial vaginosis in nonpregnant women.<sup>19</sup>

Two swabs, which were placed in an anaerobic transport vial, also were shipped to the microbiology laboratory for characterization of the following: *Lactobacillus* species, anaerobic gram-negative rods, *G. vaginalis*, group B *Streptococcus*, *Enterococcus* species, *Escherichia coli*, *Candida* species, *Mycoplasma hominis*, and *Ureaplasma urealyticum*. The growth of each of these microorganisms was recorded on a semiquantitative scale from 0 to 4. All lactobacilli were tested for production of hydrogen peroxide ( $H_2O_2$ ) using a qualitative assay on a tetramethylbenzidine agar plate as described previously.<sup>21</sup>

DNA amplification for *N. gonorrhoeae* and *C. trachomatis* was performed using a strand-displacement DNA Amplification Assay (Becton Dickinson, Sparks, MD) from self-obtained vaginal swabs. All positive test results for gonococcal or chlamydial infection were reported to the clinical sites within 1 week of enrollment where infected subjects were treated.

Among the 1,179 subjects who had a baseline assessment of bacterial vaginosis, 26 (2.2%) had a baseline visit only, and 19 (1.6%) had missing data on covariates used for statistical adjustment (see statistical analysis). Among the remaining 1,134 subjects, the median length of follow-up was 3.0 years (interquartile range, 2.4 years to 3.4 years), and 88% of women were interviewed at their final, regularly scheduled contact.

To detect PID, women who experienced pelvic pain during follow-up and women who tested positive on *N. gonorrhoeae* or *C. trachomatis* screening were scheduled for an additional visit involving a pelvic examination and an endometrial biopsy. Pelvic inflammatory disease was categorized upon finding: 1) endometritis, a histologic diagnosis based on a modification<sup>22</sup> of the criteria proposed by Kiviat et al<sup>23</sup> involving identification of at least



5 neutrophils in the endometrial surface epithelium, in the absence of menstrual endometrium, and/or at least 2 plasma cells in the endometrial stroma on a hematoxylin and eosin-stained and methyl green pyronine-stained endometrial tissue slide and/or 2) the presence of all of the following:<sup>24</sup> a complaint of pelvic discomfort of less than 4 weeks in duration; a pelvic tenderness score, using the McCormack scale,<sup>25</sup> of 1 or more; and the presence of oral temperature greater than 101°F (>38.3°C), leukorrhea or mucopus, erythrocyte sedimentation rate greater than 15 mm/hour, white blood cell count greater than 10,000, or *N. gonorrhoeae* or *C. trachomatis* genital infection. Of women who met our categorization for incident PID, two thirds met the clinical criteria and one third met the histologic criteria. Results relating bacterial vaginosis to PID were almost identical in the clinical and histologic subgroups.

Women were asked during each 6-month follow-up about demographic factors, including age, race, education, and income. They also reported relevant lifestyle behaviors, such as tobacco smoking, number of sexual partners in the past 2 months, acquisition of a new partner in the past 2 months, contraception use, and sex with menses. Furthermore, they were requested to recall past episodes of sexually transmitted infections, including PID and gonococcal and/or chlamydial genital infections.

The impact of bacterial vaginosis among study participants was based on 2 types of exposure classification: 1) initial baseline status and 2) time-varying acute effect. For initial status, analyzed at the subject level, differences in the carriage of bacterial vaginosis status (normal flora, intermediate flora, or bacterial vaginosis) by baseline characteristics were compared by  $\chi^2$  tests. Incidence rates of PID during the course of 4 years of follow-up by initial bacterial vaginosis status were estimated by the Kaplan-Meier method and tested for association with failure time (PID) as a 3-level ordinal variable.<sup>26</sup> Furthermore, bacterial vaginosis was characterized and assessed as a continuous variable (score 0–10). Subjects who did not experience PID during follow-up were censored at the last date of follow-up. Cox regression analysis was used to estimate adjusted hazard ratios of PID by initial bacterial vaginosis status, including within clinically relevant subgroups. Covariates selected for adjustment included those that resulted in a 10% or greater change in parameter estimates from unadjusted models<sup>27</sup> and those considered to be biologically relevant. The proportional hazards assumption of invariant relative risk during follow-up was assessed and found to be satisfactory. Clinical site was assessed and found not to be an effect modifier, ie, there was no statistically significant differ-

ence in the bacterial vaginosis and PID association between sites.

Discrete-time proportional hazards models<sup>28</sup> fit by pooled logistic regression<sup>29</sup> were used to assess the time-varying impact of bacterial vaginosis status on acute risk of PID. The “critical exposure” visit to estimate an acute effect from bacterial vaginosis was considered to be the visit that immediately preceded the diagnosis of PID and that occurred within 6 months of the diagnosis. In instances when multiple visits were conducted within 6 months of PID diagnosis, the visit more proximal to the diagnosis was considered to be the critical exposure period.

All of the above fixed and time-varying analyses were repeated for H<sub>2</sub>O<sub>2</sub> producing *Lactobacillus*, *G. vaginalis*, and pigmented, anaerobic gram-negative rods as the exposure variables.

With an estimated cumulative incidence rate of approximately 9% for PID among women with normal vaginal flora, the study cohort had 80% power to detect hazard ratios of 1.99 and 1.80 associated with intermediate flora and bacterial vaginosis, respectively, assuming 2-sided  $\alpha = .05$  and pair-wise comparisons. Tests of bacterial vaginosis as a 3-3-level ordinal variable and as a continuous variable in Cox regression analyses afforded greater power.<sup>30</sup>

## RESULTS

At baseline, the 1,179 women from the GYN Infections Follow-through Study were predominantly 19–24 years of age, African American, and of low socioeconomic status (Table 1). Four hundred twenty-eight women (36.3%) had normal vaginal flora at baseline, 280 (23.7%) had intermediate flora, and 471 (39.9%) had bacterial vaginosis. Detection of bacterial vaginosis at baseline was significantly more common among women of black race; those with less than a high school education and a low family income; current smokers; women who had past PID and gonococcal/chlamydial infections; and those who had sex during menses. Baseline identification of gonococcal cervicitis, chlamydial cervicitis, or both was more common among women with intermediate vaginal flora than women with normal flora and was most common among women with bacterial vaginosis.

Overall, the incidence of PID during the 4 years of follow-up was similar between women with normal flora (9.5%), those with intermediate flora (10.0%), and those with bacterial vaginosis (12.0%;  $P = .28$ ). Similarly, after adjustment for race, education, income, current smoking, sex during menses, condom use consistency, history of gonococcal/chlamydial infection, and history of PID, the presence of bacterial vaginosis at baseline did not



**Table 1.** Descriptive Characteristics Among Women by Baseline Bacterial Vaginosis Status

Baseline Characteristic	Bacterial vaginosis status at baseline			P
	Normal (N = 428)	Intermediate (N = 280)	Bacterial vaginosis (N = 471)	
Age (y)				.91
13 to 18	25 (5.8)	17 (6.1)	34 (7.2)	
19 to 24	288 (67.3)	188 (67.1)	304 (64.5)	
25 to 29	73 (17.1)	52 (18.6)	90 (19.1)	
30 and older	42 (9.8)	23 (8.2)	43 (9.1)	
Race				< .001
Black	275 (64.2)	222 (79.3)	389 (82.6)	
White	124 (29.0)	50 (17.9)	58 (12.3)	
Other	29 (6.8)	8 (2.9)	24 (5.1)	
Education				< .001
Less than high school	65 (15.2)	58 (20.7)	111 (23.6)	
High school graduate	99 (23.1)	74 (26.4)	155 (32.9)	
More than high school	264 (61.7)	148 (52.9)	205 (43.5)	
Annual household income				< .001
Less than \$10,000	153 (39.2)	135 (52.3)	225 (53.2)	
\$10,000 to \$19,999	95 (24.4)	73 (28.3)	113 (26.7)	
\$20,000 or more	142 (36.4)	50 (19.4)	85 (20.1)	
Smoking status				.001
Never	241 (56.3)	168 (60.0)	238 (50.6)	
Former	53 (12.4)	25 (8.9)	35 (7.5)	
Current	134 (31.3)	87 (31.1)	197 (41.9)	
History of pelvic inflammatory disease				.002
No	382 (90.3)	244 (87.1)	386 (82.1)	
Yes	41 (9.7)	36 (12.9)	84 (17.9)	
History gonorrhea or chlamydia				.007
No	254 (59.9)	154 (55.2)	229 (49.3)	
Yes	170 (40.1)	125 (44.8)	235 (50.7)	
No. of sexual partners in the past 2 mo				.06
None	76 (17.8)	31 (11.1)	68 (14.4)	
One	289 (67.5)	194 (69.3)	334 (70.9)	
Two or more	63 (14.7)	55 (19.6)	69 (14.7)	
New sexual partner in the past 2 mo				.92
No	281 (79.8)	197 (79.1)	317 (78.7)	
Yes	71 (20.2)	52 (20.9)	86 (21.3)	
Sex during menses				.008
No	325 (92.3)	210 (85.0)	345 (86.0)	
Yes	27 (7.7)	37 (15.0)	56 (14.0)	
Condom use consistency				.04
None	219 (51.2)	118 (42.1)	225 (47.8)	
≤ 5 per 10 times	58 (13.5)	56 (20.0)	94 (20.0)	
6 to 9 per 10 times	59 (13.8)	37 (13.2)	68 (14.4)	
10 out of 10 times	92 (21.5)	69 (24.6)	84 (17.8)	
Gonococcal/Chlamydial infection				< .001
None	387 (92.8)	240 (87.0)	372 (80.2)	
Gonorrhea only	7 (1.7)	8 (2.9)	17 (3.7)	
Chlamydia only	22 (5.3)	24 (8.7)	63 (13.6)	
Gonorrhea and chlamydia	1 (0.2)	4 (1.4)	12 (2.6)	

Data are presented as n (%).

elevate the risk for PID (adjusted hazard ratio [HR] 0.89, 95% confidence interval [CI] 0.55–1.45; Table 2). Bacterial vaginosis score (0–10), measured continuously, also was not significantly associated with PID after covariate adjustment (adjusted HR 0.99, 95% CI 0.93–1.05). In selected subgroups of women, including younger/older women; black/white women; women with/without a his-

tory of PID; women with 1 or fewer sexual partners in the past 2 months; and women with/without gonococcal/chlamydial genital infection at baseline, there was no apparent relationship between baseline bacterial vaginosis or bacterial vaginosis score and PID. The only subgroup in which bacterial vaginosis score significantly related to PID was among women who at baseline



**Table 2.** Adjusted Hazard Ratios for Incident Pelvic Inflammatory Disease by Baseline Bacterial Vaginosis Status and Stratified by Potentially Modifying Characteristics

Bacterial vaginosis status at baseline and potential modifying characteristic	Risk of incident pelvic inflammatory disease		
	No. of events	Adjusted hazard ratio	95% confidence interval
Overall (n = 1,134)			
Normal	29	1.0	...
Intermediate	24	1.07	0.62–1.85
Bacterial vaginosis	42	0.89	0.55–1.45
Bacterial vaginosis as continuous variable (0 to 10)	95	0.99	0.93–1.05
Age 13 to 24 years (n = 828)			
Normal	20	1.0	...
Intermediate	18	1.12	0.59–2.12
Bacterial vaginosis	33	1.02	0.58–1.80
Bacterial vaginosis as continuous variable (0 to 10)	71	1.00	0.93–1.08
Age 25 years and older (n = 306)			
Normal	9	1.0	...
Intermediate	6	0.97	0.34–2.81
Bacterial vaginosis	9	0.65	0.24–1.78
Bacterial vaginosis as continuous variable (0 to 10)	24	0.97	0.85–1.11
Race: black (n = 857)			
Normal	23	1.0	...
Intermediate	18	0.89	0.48–1.65
Bacterial vaginosis	39	0.92	0.55–1.56
Bacterial vaginosis as continuous variable (0 to 10)	80	1.00	0.93–1.07
Race: white (n = 222)			
Normal	5	1.0	...
Intermediate	5	1.84	0.47–7.08
Bacterial vaginosis	3	0.55	0.11–2.79
Bacterial vaginosis as continuous variable (0 to 10)	13	0.93	0.76–1.13
History of pelvic inflammatory disease: no (n = 975)			
Normal	20	1.0	...
Intermediate	16	1.07	0.55–2.10
Bacterial vaginosis	26	1.04	0.57–1.90
Bacterial vaginosis as continuous variable (0 to 10)	62	1.02	0.94–1.10
History of pelvic inflammatory disease: yes (n = 159)			
Normal	9	1.0	...
Intermediate	8	1.26	0.47–3.37
Bacterial vaginosis	16	0.71	0.31–1.64
Bacterial vaginosis as continuous variable (0 to 10)	33	0.95	0.85–1.05
Sexual partners past in the 2 mo: $\geq 2$ (n = 182)			
Normal	4	1.0	...
Intermediate	3	0.77	0.17–3.48
Bacterial vaginosis	12	2.73	0.84–8.89
Bacterial vaginosis as continuous variable (0 to 10)	19	1.19	1.01–1.40
Sexual partners in the past 2 mo: $\leq 1$ (n = 952)			
Normal	25	1.0	...
Intermediate	21	1.12	0.62–2.01
Bacterial vaginosis	30	0.69	0.40–1.18
Bacterial vaginosis as continuous variable (0 to 10)	76	0.95	0.89–1.02
Absence of gonococcal/chlamydial infection at baseline (n = 959)			
Normal	24	1.0	...
Intermediate	18	0.97	0.52–1.80
Bacterial vaginosis	24	0.63	0.35–1.13
Bacterial vaginosis as continuous variable (0 to 10)	66	0.96	0.89–1.04
Presence of gonococcal/chlamydial infection at baseline (n = 153)			
Normal	5	1.0	...
Intermediate	5	0.82	0.22–2.98
Bacterial vaginosis	17	1.18	0.42–3.33
Bacterial vaginosis as continuous variable (0 to 10)	27	0.98	0.86–1.12

The analysis excludes subjects with baseline data only and includes adjustment for race (white/nonwhite), years of education (continuous), annual household income (3-level ordinal variable: < \$10,000, \$10,000 to < \$20,000,  $\geq$  \$20,000), current smoker (yes/no), sex during menses (yes/no), condom use consistency (4-level ordinal variable: none,  $\leq 5$  per 10 times, 6 to 9 per 10 times, 10 out of 10 times), history of gonococcal/chlamydial infection (yes/no), history of pelvic inflammatory disease (yes/no).



**Table 3.** Adjusted Hazard Ratios for Incident Pelvic Inflammatory Disease by Baseline Status of H<sub>2</sub>O<sub>2</sub> Lactobacillus, *Gardnerella vaginalis*, Anaerobic Gram Negative Rod–Pigmented Status, and Gonorrhea or Chlamydia

Microbial status at baseline	All subjects*			< 2 sexual partners in the past 2 months†			≥ 2 sexual partners in months‡		
	No. of events	Adjusted hazard ratio§	95% confidence interval	No. of events	Adjusted hazard ratio§	95% confidence interval	No. of events	Adjusted hazard ratio§	95% confidence interval
H <sub>2</sub> O <sub>2</sub> + Lactobacillus (positive)	44	1.0	...	37	1.0	...	7	1.0	...
H <sub>2</sub> O <sub>2</sub> + Lactobacillus (negative)	51	0.86	0.57–1.30	39	0.76	0.47–1.20	12	1.30	0.48–3.48
H <sub>2</sub> O <sub>2</sub> + scaled (1 = positive, 5 = negative)	95	0.97	0.86–1.10	76	0.94	0.82–1.08	19	1.04	0.78–1.40
<i>Gardnerella vaginalis</i> growth = 0	36	1.0	...	34	1.0	...	2	1.0	...
<i>Gardnerella vaginalis</i> growth = 1 to 3+	39	1.21	0.76–1.91	31	0.98	0.59–1.61	8	5.48	1.12–26.84
<i>Gardnerella vaginalis</i> growth = 4+	20	0.62	0.36–1.09	11	0.37	0.18–0.73	9	4.81	1.01–23.00
<i>Gardnerella vaginalis</i> as continuous (0 to 4)	95	0.93	0.83–1.05	76	0.85	0.75–0.98	19	1.39	1.00–1.94
Gram-negative rod growth = 0	41	1.0	...	33	1.0	...	8	1.0	...
Gram-negative rod growth = 1 to 3+	45	1.06	0.69–1.62	38	1.07	0.66–1.72	7	1.02	0.36–2.87
Gram-negative rod growth = 4+	9	1.00	0.48–2.08	5	0.67	0.27–1.72	4	3.06	0.83–11.22
Gram-negative rod as continuous (0 to 4)	95	1.04	0.90–1.20	76	0.97	0.82–1.15	19	1.39	1.01–1.91
Gonorrhea or chlamydia absent	66	1.0	...	53	1.0	...	13	1.0	...
Gonorrhea or chlamydia present	27	2.47	1.56–3.89	21	2.52	1.50–4.23	6	2.20	0.82–5.90

\* The effective N ranges from 1,114 to 1,132.

† The effective N ranges from 934 to 951.

‡ The effective N ranges from 180 to 181.

§ The analysis excludes subjects with baseline data only and includes adjustment for race (white/nonwhite), years of education (continuous), annual household income (3-level ordinal variable: < \$10,000, \$10,000 to < \$20,000, ≥ \$20,000), current smoker (yes/no), sex during menses (yes/no), condom use consistency (4-level ordinal variable: none, ≤ 5 per 10 times, 6 to 9 per 10 times, 10 out of 10 times), history of gonococcal/chlamydial infection (yes/no), history of pelvic inflammatory disease (yes/no).

reported 2 or more sexual partners in the previous 2 months.

Considering key microbial patterns (Table 3), women with an absence, versus a presence, of hydrogen peroxide–producing *Lactobacillus* at baseline did not have significant elevations in PID (adjusted HR 0.86, 95% CI 0.57–1.30) either overall or within the aforementioned subgroups. Similarly, women with greater growth on semiquantitative *G. vaginalis* cultures (value of 4) were not significantly more likely than those with no *G. vaginalis* growth to acquire PID (adjusted HR 0.62, 95% CI 0.36–1.09), with the exception of an association to PID among women with 2 or more recent sexual partners. A greater growth of pigmented, anaerobic gram-negative rods (growth 4+) also was not associated with PID (adjusted HR 1.00, 95% CI 0.48–2.08), except in

the subgroup of women with 2 or more recent sexual partners.

In contrast, baseline infection with *N. gonorrhoeae* or *C. trachomatis* substantially elevated the risk of PID (adjusted HR 2.47, 95% CI 1.56–3.89; Table 3). Within subgroups of younger/older women; black/white women; women with/without a history of PID; women with 1 or fewer/2 or more sexual partners in the past 2 months, the adjusted HRs associating baseline gonococcal/chlamydial genital infection with PID ranged from 2.11 to 3.97 and, in 6 of 8 subgroups, the association was significant. Among women with normal vaginal flora (398 women who experienced 29 PID events) and intermediate vaginal flora (269 women who experienced 23 PID events) at baseline, the risks of PID associated with gonococcal/chlamydial genital infection were 1.91 (95% CI 0.69–



**Table 4.** Cohort Analysis of Time-Varying Effect of Bacterial Vaginosis or Anaerobic Gram-Negative Rod–Pigmented Status and Subsequent Risk of Pelvic Inflammatory Disease

Time varying exposure status	Exposure visits for subjects with pelvic inflammatory disease*	All other clinic visits	Adjusted rate ratio†	95% confidence interval
<b>Bacterial vaginosis status</b>				
None	18	1461	1.0	...
Intermediate	14	902	1.06	0.51–2.19
Bacterial vaginosis	34	1426	1.31	0.71–2.42
<i>P</i> (3-level variable)			.36	.36
<b>Anaerobic gram-negative rods–pigmented</b>				
Growth = 0	21	1932	1.0	...
Growth = 1 to 2+	26	1105	1.93	1.05–3.53
Growth = 3 to 4+	19	722	1.78	0.91–3.47
<i>P</i> (3-level variable)			.04	.04

\* Clinic visit that preceded diagnosis of pelvic inflammatory disease and occurred within 6 months of the diagnosis.

† Adjusted for time of visit during follow-up, history of pelvic inflammatory disease, gonococcal/chlamydial infection at baseline, and education. For assessment of bacterial vaginosis status, N = 3,803; 52 cases deleted due to missing covariate data, including 3 incident events. For assessment of pigmented anaerobic gram-negative rod, N = 3,714; 111 cases deleted due to missing covariate data, including 3 incident events.

5.27) and 1.83 (95% CI 0.66–5.07), respectively. Among women with bacterial vaginosis (445 women who experienced 41 PID events), the presence of gonococcal/chlamydial genital infection was associated with a significantly elevated 3.1-fold risk of PID (95% CI 1.67–5.87), suggesting a higher risk among women with concurrent vaginal microflora alterations and gonococcal/chlamydial infections.

Among women with a documented occurrence of PID, bacterial vaginosis was not significantly more common than lack of bacterial vaginosis at the visit immediately preceding the diagnosis of PID (adjusted risk ratio 1.31, 95% CI 0.71–2.42) and, similarly, level of bacterial vaginosis assessed as a 3-level ordinal variable (comparing none/intermediate/bacterial vaginosis) was not acutely related to PID ( $P = .36$ ; Table 4). The presence of denser growth of pigmented, anaerobic gram-negative rods in the period immediately preceding PID was associated, albeit nonsignificantly, with incident PID (adjusted risk ratio 1.78, 95% CI 0.91–3.47), a finding that achieved statistical significance when evaluated as a 3-3-level variable (no growth/1–3+ growth/4+ growth;  $P = .04$ ).

## DISCUSSION

In this large cohort of high-risk women, after adjustment for relevant demographic and behavioral risk factors, we found no significant association between baseline or acute bacterial vaginosis carriage and PID. Similarly, absence of hydrogen peroxide-producing *Lactobacillus* and high levels of *G. vaginalis* did not elevate risk of developing PID overall. Pigmented, anaerobic gram-negative rods were associated with PID when assessed acutely but not as a fixed baseline factor.

A number of cross-sectional studies have found abnormal vaginal flora to be more common among women with PID or its surrogate, histologic endometritis.<sup>7–12</sup> Other studies have detected bacterial vaginosis-related bacteria in the upper genital tract of women with PID.<sup>7,16,31</sup> However, these research designs cannot exclude the possibility that aberrancies within the vaginal flora follow, rather than precede, the exposures or infections that initiate PID. They also cannot exclude the possibility that ascension of gonococcal/chlamydial lower tract infections may be facilitated by bacterial vaginosis, whereas in the absence of bacterial STDs, bacterial vaginosis may not enhance PID risk.<sup>31</sup> Our data are consistent with the latter possibility in that gonococcal/chlamydial genital infection elevated the risk of PID, particularly in women who had bacterial vaginosis at baseline. Finally, previous studies did not consistently adjust for the many risk factors common to bacterial vaginosis and PID (see Table 1). Bacterial vaginosis may mark women at high risk rather than itself being a cause of PID.

Among subtypes of vaginal microflora, the strongest risk found in our data was for pigmented, anaerobic gram-negative rods. This category typically includes microorganisms in the species *Porphyromonas*, *Prevotella*, and *Bacteroides*. This observation is consistent with a previous study in which Hillier et al<sup>8</sup> demonstrated an association between anaerobic gram-negative rods and histologic endometritis that was independent of bacterial vaginosis. Our data demonstrated that acute but not baseline carriage of these organisms significantly elevated the risk for PID, suggesting that a new infection may trigger PID. Anaerobic gram-negative rods may represent the most



significant bacterial pathogen among the microflora that characterize bacterial vaginosis. Further research into this question includes replication of these findings, understanding the transmission characteristics of anaerobic gram-negative rods, and determining whether, in clinical trials, the prevention of anaerobic gram-negative rods reduces the risk of PID.

We examined a series of subgroup effects and found one to consistently suggest an association between bacterial vaginosis/related flora-disrupting microbes and PID: having 2 or more sexual partners in the 2 months before enrollment. Women with multiple sexual partners may represent a singularly high-risk group in whom surveillance for both gonococcal/chlamydial cervicitis and specific vaginal microflora is warranted.

Strengths of our study include the large number of women studied, enrollment of a high-risk population, which enhanced study power, use of consistent and standardized enrollment and data collection protocols, collection of biomarkers of effect, and relatively long-term and complete longitudinal data collection, which permitted the assessment of bacterial vaginosis status as a time-varying, as well as a fixed, baseline biologic exposure. Weaknesses include the observational nature of the study, making it impossible to exclude unmeasured confounding. Furthermore, the relatively long intervals between vaginal microbiologic assessments allow for a somewhat gross assessment of the impact of variation of vaginal flora over the course of time. Finally, screening and timely treatment may have diminished the observed association between abnormal vaginal flora and PID. However, this is unlikely, given that bacterial vaginosis and the triggers for a symptomatic PID visit occurred contemporaneously, ie, treatment occurred after each observed association. Furthermore, a modifying effect from treatment would be inconsistent with the relatively high rates of PID observed.

In summary, among predominantly young, African-American women who were studied longitudinally, we did not demonstrate an independent effect of bacterial vaginosis on the development of PID. High-risk women with multiple sexual partners may be at increased risk for PID from bacterial vaginosis and pigmented, anaerobic gram-negative rods may be a particularly virulent subset of bacterial vaginosis-related organisms. Nonetheless, on the basis of these data, screening and treatment for bacterial vaginosis cannot be recommended in women, overall, for the prevention of PID.

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