

## A Cluster Analysis of Bacterial Vaginosis-associated Microflora and Pelvic Inflammatory Disease

Roberta B. Ness<sup>1,2</sup>, Kevin E. Kip<sup>1</sup>, Sharon L. Hillier<sup>1,2</sup>, David E. Soper<sup>3</sup>, Carol A. Stamm<sup>4</sup>, Richard L. Sweet<sup>5</sup>, Peter Rice<sup>6</sup>, and Holly E. Richter<sup>7</sup>

<sup>1</sup> Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA.

<sup>2</sup> Department of Obstetrics, Gynecology, and Reproductive Sciences, Magee-Womens Hospital and Magee-Womens Research Institute, Pittsburgh, PA.

<sup>3</sup> Department of Obstetrics and Gynecology, Medical University of South Carolina, Charleston, SC.

<sup>4</sup> Department of Obstetrics and Gynecology, Denver Health Medical Center, Denver, CO.

<sup>5</sup> Center for Women's Health, University of California at Davis, San Diego, CA.

<sup>6</sup> Division of Infectious Disease, Boston Medical Center, Maxwell Finland Laboratory, Boston, MA.

<sup>7</sup> Department of Obstetrics and Gynecology, University of Alabama School of Medicine, Birmingham, AL.

Received for publication January 7, 2005; accepted for publication April 28, 2005.

Controversy surrounds the association between bacterial vaginosis (BV) and pelvic inflammatory disease (PID). Women ( $N = 1,140$ ) were ascertained at five US centers, enrolled (1999–2001), and followed up for a median of 3 years. Serial vaginal swabs were obtained for Gram's stain and cultures. PID was defined as 1) histologic endometritis or 2) pelvic pain and tenderness plus oral temperature  $>38.8^{\circ}\text{C}$ , leukorrhea or mucopus, erythrocyte sedimentation rate  $>15$  mm/hour, white blood cell count  $>10,000$ , or gonococcal/chlamydial lower genital infection. Exploratory factor analysis identified two discrete clusters of genital microorganisms. The first correlated with BV by Gram's stain and consisted of the absence of hydrogen peroxide-producing lactobacillus, *Gardnerella vaginalis*, *Mycoplasma hominis*, anaerobic Gram-negative rods, and, to a lesser degree, *Ureaplasma urealyticum*. The second, unrelated to BV by Gram's stain, consisted of *Enterococcus* species and *Escherichia coli*. Being in the highest tertile in terms of growth of BV-associated microorganisms increased PID risk (adjusted rate ratio = 2.03, 95% confidence interval: 1.16, 3.53). Carriage of non-BV-associated microorganisms did not increase PID risk. Women with heavy growth of BV-associated microorganisms and a new sexual partner appeared to be at particularly high risk (adjusted rate ratio = 8.77, 95% confidence interval: 1.11, 69.2). When identified by microbial culture, a combination of BV-related microorganisms significantly elevated the risk of acquiring PID.

chlamydia; gonorrhea; pelvic inflammatory disease; sexually transmitted disease; vaginitis

Abbreviations: BV, bacterial vaginosis; PID, pelvic inflammatory disease.

Bacterial vaginosis (BV) is characterized by a disequilibrium in vaginal microflora in which the normally predominant hydrogen peroxide-producing strains of lactobacilli are overgrown by facultative and anaerobic vaginal microorganisms (1). BV has been associated with lower genital tract *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection and with upper genital tract infection/inflammation of the endometrium (endometritis) and fallopian tubes (salpingitis), together termed pelvic inflammatory disease (PID)

(2–8). However, the use of cross-sectional or retrospective study designs in relating BV to PID has raised questions about whether BV-associated bacteria cause PID a priori or whether they are carried into the upper genital tract during ascension of *N. gonorrhoeae* and *C. trachomatis*.

To examine whether BV increases the risk of developing incident PID, we conducted a multicenter study in which we followed, for a median of 3 years, predominantly young, African-American women at risk of acquiring sexually

transmitted infections. Unexpectedly, we have not yet demonstrated an association between BV, characterized by findings on microscopy, and development of PID (9). In the present study, we examined whether bacteriologic cultures identify a cluster of BV-associated vaginal microorganisms that relate to the risk of PID.

## MATERIALS AND METHODS

### Patient selection

The methods used for subject enrollment, data collection, and follow-up have been reported in detail elsewhere (9, 10). Briefly, women 13–36 years of age were recruited into the GYN Infections Follow-through (GIFT) Study from five US sites between May 1999 and June 2001. Human subjects approval was obtained at each participating institution, and all women signed informed consent. Women enrolled were at high risk of chlamydial cervicitis (11) if they scored at least three points on an algorithm wherein points were derived as follows: aged 24 years or less = 1; Black race = 2; never pregnant = 1; two or more sexual partners = 1; douching at least once per month = 2; and any prior sexually transmitted infection, including *N. gonorrhoeae*, *C. trachomatis*, and *Trichomonas vaginalis* = 2. Women were excluded if they were currently pregnant, married, virginal, or using antibiotics at baseline. Of the 1,628 women eligible for the study, the 1,140 (70 percent) who completed a questionnaire, had their vaginal flora evaluated at baseline, and had follow-up and nonmissing covariate data were the focus of these analyses.

### Microbiologic methods for evaluation of the vaginal flora

At baseline and every 6–12 months thereafter, each subject obtained her own vaginal specimens with a cotton swab (12). Smears from these swabs were Gram stained, and a microscopy score of 0–10 was assigned by laboratory staff using the standardized method described by Nugent et al. (13). 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 BV.

Two swabs, placed in an anaerobic transport vial, were also shipped to the microbiology laboratory for characterization of the following: *Lactobacillus* species, anaerobic Gram-negative rods, *Gardnerella vaginalis*, group B streptococcus, *Enterococcus* species, *Escherichia coli*, *Candida* species, *Mycoplasma hominis*, and *Ureaplasma urealyticum*. Lactobacilli were identified to the genus level on the basis of Gram's-stain morphology and production of lactic acid. All lactobacilli were further tested for production of hydrogen peroxide by using a qualitative assay on a tetramethylbenzidine agar plate, as previously described (14).

### DNA amplification for *N. gonorrhoeae* and *C. trachomatis*

DNA amplification for *N. gonorrhoeae* and *C. trachomatis* was performed by using a strand displacement DNA

Amplification (SDA) Assay (Becton Dickinson, Sparks, Maryland) 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.

### Follow-up

For the 1,140 women in this analysis, median length of follow-up was 3.0 years (interquartile range: 2.4–3.4 years). In addition, 88 percent of the women were interviewed at their final, regularly scheduled contact.

### Categorization of PID

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. PID was categorized upon finding 1) endometritis, a histologic diagnosis based on a modification (15) of the criteria proposed by Kiviat et al. (16) involving identification of at least five neutrophils in the endometrial surface epithelium in the absence of menstrual endometrium and/or at least two 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 (17): a complaint of pelvic discomfort of less than 4 weeks' duration; a pelvic tenderness score, using the McCormack scale (18), of 1 or more; and the presence of an oral temperature of higher than 101°F (>38.3°C), leukorrhea or mucopus, erythrocyte sedimentation rate of more than 15 mm/hour, white blood cell count of more than 10,000, and *N. gonorrhoeae* or *C. trachomatis* genital infection. Of the women categorized as having incident PID, two thirds met the clinical criteria and one third met the histologic (endometritis) criteria.

### Other data collection

Women were asked about demographic factors and lifestyle, sexual, and contraceptive behaviors at baseline and then every 6 months afterward.

### Statistical analysis

Exploratory factors analysis was used to investigate clustering of the microflora measured. Scree plots from this analysis were inspected, and factors (clusters) with a minimum eigenvalue of 1.0 were retained (19). Because approximately 20 percent of the data were missing for *U. urealyticum* and *M. hominis*, and factor analysis requires complete data for all variables in the analysis, multiple regression was used to impute values for missing microflora, as derived from non-missing data on other microflora. This method of imputation compares favorably with more sophisticated methods when the overall percentage of missing data is low (20). For each cluster identified, a factor score (microflora score) was derived from a linear combination of the individually measured microorganisms (19, 21). Specifically, the observed value of

**TABLE 1. Factor loadings for exploratory factor analysis of microorganisms from the vaginal environment of women recruited from five US centers, 1999–2001**

Microbes and higher-level factor (cluster) identified	Complete-case analysis (n = 3,153)*				Regression-imputed analysis (n = 3,882)*			
	Orthogonal rotation†		Oblique rotation‡		Orthogonal rotation§		Oblique rotation‡	
	Factor 1	Factor 2	Factor 1	Factor 2	Factor 1	Factor 2	Factor 1	Factor 2
Factor 1: BV¶-associated microflora								
<i>Lactobacillus</i> H <sub>2</sub> O <sub>2</sub> +¶	0.71	0.04	0.72	0.03	0.70	0.04	0.71	0.02
<i>Gardnerella vaginalis</i>	0.81	–0.08	0.79	–0.09	0.80	–0.11	0.77	–0.14
<i>Mycoplasma hominis</i>	0.76	–0.09	0.75	–0.10	0.79	–0.09	0.70	–0.06
Anaerobic GNR,¶ nonpigmented	0.83	0.08	0.84	0.07	0.82	0.09	0.82	0.06
Anaerobic GNR, pigmented	0.76	–0.01	0.76	–0.02	0.76	–0.02	0.75	–0.06
<i>Ureaplasma urealyticum</i>	0.35	–0.08	0.34	–0.08	0.38	–0.08	0.34	–0.01
Factor 2: non-BV-associated microflora								
<i>Enterococcus</i> species	–0.15	0.76	–0.07	0.76	–0.15	0.76	–0.08	0.77
<i>Escherichia coli</i>	0.08	0.79	0.17	0.79	0.08	0.79	0.15	0.79

\* The analysis includes the baseline and all follow-up visits of the 1,140 study subjects.

† The eigenvalues for factors 1 and 2 were 3.16 and 1.21, respectively.

‡ The correlation between factor 1 and factor 2 was –0.10.

§ The eigenvalues for factors 1 and 2 were 3.17 and 1.22, respectively.

¶ BV, bacterial vaginosis; H<sub>2</sub>O<sub>2</sub>+, hydrogen peroxide producing; GNR, Gram-negative rods.

each microorganism (range, 0–4) within the cluster was multiplied by a specific weight and then summed to obtain a theoretical (unobserved) microflora score. Pearson correlation coefficients were also calculated between each microorganism and the overall BV Gram's stain score (0–10) to aid in interpretation of the clusters identified.

Discrete-time proportional hazards models (22) fit by pooled logistic regression (23) were used to assess the time-varying impact of the microflora-derived cluster scores (categorized into tertiles) on acute risk of PID. The “critical exposure” visit to estimate an acute effect from microflora status was considered the visit that most immediately preceded the diagnosis of PID and that occurred within 9 months of the diagnosis. All models were adjusted for time of visit during follow-up, age, race, education, and history of PID. Analyses were conducted by using the SAS System for Windows, version 8.02 (SAS Institute, Inc., Cary, North Carolina).

## RESULTS

Participants were predominantly 19–24 years of age (67 percent), were Black (75 percent), and had a household income of less than \$20,000 (74 percent). Forty-six percent of the women had a history of gonococcal/chlamydial infection, and 14 percent had a history of PID at study entry. At baseline, 40 percent (458 women) had BV by Gram's stain.

Two independent clusters of microorganisms were determined by the exploratory factor analysis (table 1). Because results were essentially identical between the complete-case and regression-imputed methods, and between the orthogonal and oblique rotation methods, we report here and based further analyses on the regression-imputed method and orthogonal rotation. The factor 1 cluster consisted of the mi-

croorganisms *G. vaginalis* and *M. hominis*, both types of anaerobic Gram-negative rods, the absence of hydrogen peroxide-producing lactobacillus (factor loadings ( $r$ ) = 0.70–0.82), and, to a lesser degree, *U. urealyticum* ( $r$  = 0.38). This cluster was labeled “BV-associated microorganisms” because of consistent individual Pearson correlations with BV by Gram's stain ( $r$  = 0.73,  $r$  = 0.60,  $r$  = 0.66,  $r$  = 0.57,  $r$  = 0.61, and  $r$  = 0.22, respectively). The second cluster, factor 2, was loaded heavily and uniquely on *Enterococcus* species and *E. coli* (factor loadings ( $r$ ) = 0.76–0.79) and was largely unrelated to BV by Gram's stain (individual Pearson correlations = 0.22 and 0.03, respectively). This cluster was labeled “non-BV-associated microorganisms.”

Eighty-three women experienced incident PID and had had a full microbiologic assessment within the prior 9 months. After we adjusted for age, race, education, history of PID, and timing of PID diagnosis, we found that women in the highest tertile for growth of BV-associated microorganisms were significantly more likely than those in the lowest tertile to experience incident PID (adjusted rate ratio = 2.03, 95 percent confidence interval: 1.16, 3.53) (table 2). The higher the BV-associated microorganism score, the greater the risk of PID ( $p$  for trend = 0.008). A high non-BV-associated microorganism score within the prior 9 months did not affect the risk of PID (adjusted rate ratio = 0.83, 95 percent confidence interval: 0.50, 1.37).

In modestly sized subgroups of women, a high BV-associated microorganism score was particularly strongly associated with the development of PID among women who reported two or more sexual partners in the 2 months prior to baseline (adjusted rate ratio = 3.63, 95 percent confidence interval: 0.99, 13.3) and women who reported a new sexual partner in the 2 months prior to baseline (adjusted rate ratio = 8.77, 95 percent confidence interval: 1.11,

**TABLE 2. Cohort analysis of the time-varying effect of BV\*-associated microflora scores and subsequent risk of PID\* within 9 months, by potential modifying characteristics, for women recruited from five US centers, 1999–2001**

Tertile of BV-associated microflora score	Exposure visits for subjects with PID† (no.)	All other clinic visits (no.)	Adjusted rate ratio‡	95% CI*
All subjects				
Lower tertile	19	1,401	1.0	
Middle tertile	17	865	1.20	0.61, 2.34
Upper tertile	47	1,363	2.03	1.16, 3.53
<i>p</i> for trend			0.008	
Subgroup analyses§				
≤1 sexual partner in the past 2 months				
Lower tertile	16	1,166	1.0	
Middle tertile	16	730	1.37	0.67, 2.79
Upper tertile	34	1,160	1.76	0.94, 3.29
<i>p</i> for trend			0.07	
≥2 sexual partners in the past 2 months				
Lower tertile	3	235	1.0	
Middle tertile	1	135	0.45	0.04, 4.45
Upper tertile	13	203	3.63	0.99, 13.3
<i>p</i> for trend			0.02	
No recent new sexual partner				
Lower tertile	15	904	1.0	
Middle tertile	12	578	1.15	0.53, 2.49
Upper tertile	30	957	1.69	0.88, 3.23
<i>p</i> for trend			0.10	
Recent new sexual partner				
Lower tertile	1	254	1.0	
Middle tertile	4	143	4.01	0.43, 37.4
Upper tertile	13	238	8.77	1.11, 69.2
<i>p</i> for trend			0.02	
Absence of GC/CT* at study entry				
Lower tertile	14	1,270	1.0	
Middle tertile	12	757	1.18	0.54, 2.59
Upper tertile	29	1,109	1.83	0.94, 3.56
<i>p</i> for trend			0.06	
Presence of GC/CT at study entry				
Lower tertile	4	104	1.0	
Middle tertile	5	92	1.39	0.35, 5.50
Upper tertile	15	233	1.72	0.54, 5.48
<i>p</i> for trend			0.35	

\* BV, bacterial vaginosis; PID, pelvic inflammatory disease; CI, confidence interval; GC/CT: gonococcal cervicitis, chlamydial cervicitis, or both.

† Clinic visit that preceded the diagnosis of PID and occurred within 9 months of the diagnosis.

‡ Adjusted for time of visit during follow-up, age, race, education, and history of PID.

§ Based on measurement at study entry.

69.2). The association between BV-associated microorganisms and PID was not particularly strong among women in whom gonococcal/chlamydial cervicitis was detected at baseline (adjusted rate ratio = 1.72, 95 percent confidence interval: 0.54, 5.48).

Regarding the robustness of our findings, measurement of microorganisms in a 6-month rather than a 9-month window prior to the outcome of PID had little impact on observed rate ratios. Moreover, categorization of sexual partnerships and gonococcal/chlamydial acquisition based on reports

from the period immediately prior to PID rather than from baseline data had little impact on our results. Finally, BV-associated microorganism score was related to the histologic definition of PID (endometritis), with a rate ratio similar to, yet slightly higher than, that for PID overall (adjusted rate ratio = 2.49 vs. 2.03).

## DISCUSSION

Why might these observations contrast with our previous finding from the same data that BV by Gram's stain did not predict development of PID (9)? BV as determined by microscopy is a good (14), but not absolute surrogate for aberrant vaginal microecology, as demonstrated by high, but not perfect correlations between various microorganisms and BV in our study. Moreover, BV Gram's stain scoring comprises a weighted combination of lactobacilli, *G. vaginalis* or bacteroides, and curved Gram-variable rods (13) yet does not capture an important component of our BV-associated cluster, anaerobic Gram-negative rods.

We observed that women in whom BV-associated microorganisms were most strongly related to PID had a new sexual partner or more than one recent sexual partner but no prior gonococcal/chlamydial cervicitis. This finding suggests the possibility that a sexually transmitted cofactor may strengthen the relation between BV-associated microorganisms and the development of PID. The recently identified *M. genitalium* has been isolated from women with cervicitis endometritis, and clinically suspected PID, and would be a consideration as such a cofactor (24–27). Alternatively, a subset of anaerobic Gram-negative rods may themselves be sexually transmitted infections. However, because these interaction effects were based on a limited number of observations, replication is needed.

Strengths of our study include the large number of women analyzed; 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 assessment of vaginal microflora at various points in time. Weaknesses include the observational nature of the study, making it impossible to exclude unmeasured confounding. Furthermore, the relatively long intervals between vaginal microbiologic assessments allowed for a somewhat gross assessment of the impact of variation in vaginal flora over time.

In summary, among predominantly young, African-American women followed longitudinally, we found that a cluster of BV-associated microorganisms cultured from the vagina increased the risk of developing PID.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the funding source: grant AI44151-01 from the National Institute of Allergy and Infectious Diseases.

The authors thank the following persons, whose dedication to working with the women enrolled in the GIFT Study made this study possible: Susie Alagasarmy, Julie Beuler, Debbie Carr, Hope Cohen-Webb, Leslie Curll, Christine Donahue, Amanda Farmer, Janice French, Melissa Girman, Alice Howell, Juliette Hunt, Ellen Klein, Faye LeBoeuf, April Lehman, Rosalyn Liu, Ingrid Macio, Kathleen McKenna, Kim Miller, Megan Mundy, Anne Rideout, Jacqueline Travasso, Jennifer Watts, Casey Zuckerman, Lori Paladino, and Barbara Kolodziej.

Conflict of interest: none declared.

## REFERENCES

1. Sweet RL. Role of bacterial vaginosis in pelvic inflammatory disease. *Clin Infect Dis* 1995;20:S271–5.
2. Hillier SL, Kiviat NB, Hawes SE, et al. Role of bacterial vaginosis-associated microorganisms in endometritis. *Am J Obstet Gynecol* 1996;175:435–41.
3. Peipert JR, Montagna AB, Cooper AS, et al. Bacterial vaginosis as a risk factor for upper genital tract infection. *Am J Obstet Gynecol* 1997;177:1184–7.
4. Korn AP, Bolan G, Padian N, et al. Plasma cell endometritis in women with symptomatic bacterial vaginosis. *Obstet Gynecol* 1995;85:387–90.
5. Wiesenfeld HC, Hillier SL, Krohn MA, et al. Lower genital tract infection and endometritis: insight into subclinical pelvic inflammatory disease. *Obstet Gynecol* 2002;100:456–63.
6. Eschenbach DA, Buchanan TM, Pollock TM, et al. Polymicrobial etiology of acute pelvic inflammatory disease. *N Engl J Med* 1975;293:166–71.
7. Sweet RL, Mills J, Hadley KW, et al. Use of laparoscopy to determine the microbiologic etiology of acute salpingitis. *Am J Obstet Gynecol* 1979;134:68–74.
8. Paavonen J, Teisala K, Heinonen P. Microbiological and histopathological findings in acute pelvic inflammatory disease. *Br J Obstet Gynecol* 1987;94:454–60.
9. Ness RB, Hillier SL, Kip KE, et al. Bacterial vaginosis and risk of pelvic inflammatory disease. *Obstet Gynecol* 2004;104:1–9.
10. Ness RB, Hillier SL, Richter HE, et al. Douching in relation to bacterial vaginosis, lactobacilli, and facultative bacteria in the vagina. *Obstet Gynecol* 2002;100:765–72.
11. Stergachis A, Scholes D, Heidrich FE, et al. Selective screening for *Chlamydia trachomatis* infection in a primary care population of women. *Am J Epidemiol* 1993;138:143–53.
12. Schwabke JR, Hillier SL, Sobel JD, et al. Validity of the vaginal Gram stain for the diagnosis of bacterial vaginosis. *Obstet Gynecol* 1996;88:573–6.
13. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standard method of Gram stain interpretation. *J Clin Microbiol* 1991;29:297–301.
14. Hillier SL, Krohn MA, Rabe LK, et al. The normal vaginal flora, H<sub>2</sub>O<sub>2</sub>-producing lactobacilli, and bacterial vaginosis in pregnant women. *Clin Infect Dis* 1993;16(suppl4):S273–81.
15. Ness RB, Keder LM, Soper DE, et al. Oral contraception and the recognition of endometritis. *Am J Obstet Gynecol* 1997;176:580–5.
16. Kiviat NB, Wolner-Hanssen P, Eschenbach DA, et al. Endometrial histopathology in patients with culture-proved upper genital tract infection and laparoscopically diagnosed acute salpingitis. *Am J Surg Pathol* 1990;14:167–75.

17. 1993 sexually transmitted disease treatment guidelines. Centers for Disease Control and Prevention. MMWR Recomm Rep 1993;42(RR-14):1–102.
18. McCormack WM, Nowroozi K, Alpert S, et al. Acute pelvic inflammatory disease: characteristics of patients with gonococcal and nongonococcal infection and evaluation of their response to treatment with aqueous procaine penicillin G and spectinomycin hydrochloride. Sex Transm Dis 1977;4: 125–31.
19. Manly BFJ. Factor analysis. In: Manly BFJ, ed. Multivariate statistical methods. A primer. London, United Kingdom: Chapman & Hall, 1994:93–106.
20. Nunnally JC. Fundamentals of factor analysis. In: Nunnally JC, ed. Psychometric theory, 2nd edition. New York, NY: McGraw-Hill Book Company, 1978:327–404.
21. Barzi F, Woodward M. Imputations of missing values in practice: results from imputations of serum cholesterol in 28 cohort studies. Am J Epidemiol 2004;160:34–45.
22. Cox DR, Oakes D. Analysis of survival data. London, United Kingdom: Chapman & Hall, 1984.
23. D'Agostino RB, Lee ML, Belanger AJ, et al. Relation of pooled logistic regression to time dependent Cox regression analysis: the Framingham Heart Study. Stat Med 1990;9: 1501–15.
24. Uno M, Deguchi T, Komeda H, et al. *Mycoplasma genitalium* in the cervixes of Japanese women. Sex Transm Dis 1997; 24:284–6.
25. Manhart LE, Crichtlow CW, Holmes KK, et al. Mucopurulent cervicitis and *Mycoplasma genitalium*. J Infect Dis 2003;187: 650–7.
26. Simms I, Mallinson H, Peeling RW, et al. Risk factors associated with pelvic inflammatory disease: a UK study. Int J STD AIDS 2002;13:18.
27. Cohen CR, Manhart LE, Bukusi EA, et al. Association between *Mycoplasma genitalium* and acute endometritis. Lancet 2002;359:765–6.