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Clinical, Microbiological, and Biochemical Factors in Recurrent Bacterial Vaginosis

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Because so little is known about the pathogenesis of recurrent bacterial vaginosis (BV), a longitudinal microbiological study was conducted on 13 women with recurrent BV treated sequentially with conventional metronidazole therapy. A rapid clinical response characterized by dissappearance of malodor and an improvement in vaginal discharge occurred in 92% of 31 clinical episodes of BV, with patients no longer satisfying the composite clinical criteria for the diagnosis of BV. However, prospective evaluation of these asymptomatic women revealed profound residual biochemical and microbial abnormalities which were best evident on Gram stain and wet mount examination of vaginal secretions. Other common residual abnormalities included mild persistent elevation of vaginal pH and polyamine and fatty acid levels and the presence of clue cells in small numbers. Residual abnormalities could be quantified to create an overall symptom code which predicted recurrence, and it was found that the severity of residual abnormalities was inversely related to the time required until the next recurrence occurred. The severity and prevalence of residual abnormalities following clinically successful therapy support the concept that BV recurrence, especially when it is early, represents a relapse rather than a reinfection. This concept may have important therapeutic implications.

Bacterial vaginosis (BV) is considered the most common vaginal infection in women; however, since this infection is not reportable, there are no accurate figures of its prevalence in the community (6). The explanation for the myriad of findings in patients with BV remains incomplete, although considerable progress has been made in understanding aspects of the pathogenic process (5, 7, 9, 25, 27, 28). BV is thought to represent a massive overgrowth of vaginal microorganisms, primarily anaerobic gram-positive cocci and gram-negative bacilli, including *Prevotella* species, *Gardnerella vaginalis*, and *Mobiluncus* species (13, 19, 23, 26, 30).

Published studies indicate that the drug of choice for therapy of BV is oral metronidazole; therapeutic success is achieved in 85 to 90% of patients with this therapy (10). Treatment with metronidazole is followed by eradication of the anaerobes and *G. vaginalis* together with alleviation of symptoms (4, 22). In 30 to 40% of women who initially respond to treatment with metronidazole, however, BV recurs within 3 months of the termination of therapy (4). At present, the reasons for the recurrence are unknown. It is unknown whether recurrence is due to sexually transmitted or endogenous (oral cavity or rectum) reinfection or to a relapse of the previous, incompletely resolved vaginal infection. It is conceivable that symptomatic relapse in this context could reflect the persistence of subclinical BV.

In the present study, we studied 13 women with recurrent BV longitudinally over a 9-month period in an attempt to identify clinical, laboratory, or vaginal microbiological factors that might clarify the pathogenesis of recurrent episodes of BV.

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MATERIALS AND METHODS

Patient selection. Women attending a vaginitis referral clinic at Wayne State University were enrolled into the study when symptoms of acute BV supervened. Patients were enrolled in the study if they had presented to the clinic with three or more attacks of acute BV in the previous year and if that they had not received antibiotic therapy within the previous 3 weeks.

BV was diagnosed by the presence of at least three of the following four criteria: adherent, grayish white, homogeneous vaginal discharge, vaginal pH of >4.5, positive amine test, and the presence of clue cells in wet-mount preparations of vaginal secretions (1). Patients with mixed infections, cervicitis, trichomoniasis, and yeasts on microscopy were excluded from the study. Thirteen women met the criteria for inclusion in the study.

Each woman was treated with a 7-day course of oral metronidazole (500 mg twice daily). A vaginal examination was performed and washings were collected at the time of the first visit, at the end of therapy (day 8), 1 month after the end of therapy, and 3 months after the end of therapy. Subjects who initially responded clinically but whose BV recurred at any time within the 3-month period were fully reevaluated, retreated with metronidazole, and followed further. The total duration of the study was 14 months, with a study range of 1 to 9 months for individual patients.

Thirty-one women without a history of recurrent vaginal infection who were clinically asymptomatic and who had not had recent antibiotic therapy were enrolled in the study as a control population. All control women were selected from both medical school and hospital staff populations. The demographics of the women were recorded, and specimens were collected during a single visit to the clinic. Childbearing age was the only specific matched factor between the control and test populations. Since no statistical comparison was performed in relation to the control group, the volunteers in the control group were not matched by race, number of

sexual partners, type of sexual practice, or method of contraception.

Specimen collection. Vaginal wash specimens were collected from all women at all visits. Three milliliters of sterile prereduced, anaerobically sterilized 0.85% physiological saline was introduced into the lower blade of an inserted vaginal speculum. The vaginal walls were washed by aspiration and swab scrub (minimum of three aspirations, with care taken to avoid excess aeration). The aspirate was transferred to a Vacutainer Anaerobic Transport Tube (Becton Dickinson Vacutainer Systems, Rutherford, N.J.) that was modified for fluid transport by aseptic removal of the swab from the plug. The plug was immediately pressed into position, and the tube was delivered to the laboratory within 10 min and was introduced into an anaerobic glove box (Coy Laboratories, Ann Arbor, Mich.).

Quantitative bacteriological methods. Within the anaerobic chamber, the washings were mixed thoroughly by multiple aspiration with a plastic Pasteur pipette. Aliquots were immediately removed for biochemical analysis and storage at −70°C. Twenty microliters of washings was diluted by 10-fold increments to 10^{-6} in prereduced, anaerobically sterilized supplemented brain heart infusion (Difco Laboratories, Detroit, Mich.) broth further supplemented with Tween 80 (Sigma Chemical Company, St. Louis, Mo.) as described in the *Anaerobe Laboratory Manual* (18). Aliquots of 10 μl of each dilution were plated onto the surfaces of duplicate reducible Columbia sheep blood agar plates (BBL Microbiology Systems, Cockeysville, Md.) by using calibrated plastic loops. All plates were incubated within the anaerobic chamber at 37°C for 72 h and were reincubated for a further 48 h, primarily to aid in the detection of *Mobiluncus* species.

Broth dilutions were removed from the anaerobic chamber for aerobic culture. Aliquots of 10 μl of each dilution were plated onto the surfaces of duplicate aerobic Columbia sheep blood agar plates (BBL) by using calibrated plastic loops. All plates were incubated in a humidified 10% CO_2 incubator at 37°C for 48 h.

Different colony morphotypes on plates from both atmospheres were enumerated as CFU and were subcultured onto respective blood agar plates and purified by standard microbiological methods. The CFU for each colony type was determined, and subculture of each colony type was performed. Quantitative results were expressed as range, mean, and median $\log_{10}$ viable bacteria per milliliter of vaginal washings. Isolates from anaerobic blood agar plates were defined as anaerobic if growth was not observed on blood agar after incubation for 48 h in a 10% CO_2 in air atmosphere. All viable isolates were stored in duplicate Protect Vials (Pro-Lab Inc., Scarborough, Ontario, Canada) at −70°C.

Identification of isolates. All bacterial isolates were identified to the genus and species levels. Anaerobic bacteria were identified by using the API 20A anaerobe identification system (Analytab Products, Plainview, N.Y.) combined with gas-liquid chromatography (GLC) for the identification of the fatty acids that were produced during growth in chopped meat glucose broth (BBL). Isolates that were difficult to identify were further identified by the techniques described in the *Anaerobe Laboratory Manual* (18).

Microaerophilic and aerobic isolates were identified by standard identification schemata (2). *G. vaginalis* was identified by Gram stain morphology, beta-hemolysis on human blood V agar, and starch hydrolysis (Biplate; Remel, Lenexa, Kans.); *Lactobacillus* species were identified by GLC and the biochemical tests described in the *Anaerobe Laboratory Manual* (18); corynebacteria were identified to the species level by recommended methods (20); members of the family *Enterobacteriaceae* and yeasts were identified by using the API 20E and API 20C Systems, respectively; members of the family *Micrococcaceae* were identified by oxidation-fermentation and coagulase reactions along with novobiocin susceptibility testing; streptococci and enterococci were not determined to the species level, but their Lancefield types were determined by using hemolysis on sheep blood agar, the CAMP test, bacitracin susceptibility, bile-esculin metabolism, and high salt tolerance criteria.

Quantitative biochemical analyses. (i) Short-chain fatty acids. The volatile fatty acids (acetic, propionic, butyric, isobutyric, valeric, isovaleric, caproic, and isocaproic acids) and the nonvolatile fatty acids (pyruvic, lactic, and succinic acids) present in the vaginal washings were assayed by quantitative GLC as described in the *Anaerobe Laboratory Manual* (18). Results were expressed in milligrams per milliliter of vaginal washings. The succinate/lactate ratio was determined by using the milligrams per milliliter determined following peak area integration.

(ii) Polyamines. The polyamines (putrescine, cadaverine) that were present in the vaginal washings were assayed by ethanethiol–*o*-phthalaldehyde precolumn-derivatized, reversed-phase, ion-exchange high-performance liquid chromatography (HPLC) (24). A Nova-Pak C18 ss. column (100 by 5 mm; Waters Chromatography, Milford, Mass.) was used for all assays. The chromatograph consisted of a Waters 820 work station, dual model 501 pumps, and model 540 fluorescence detector (Waters). Prior to assay, all specimens were centrifuged at 17,000 × *g* for 1 min at room temperature in a microcentrifuge and filtered through a HV-Millex filter (Millipore Corp., Bedford, Mass.) to remove all particulate material. Peak areas were integrated, and results were expressed as nanomoles per milliliter of vaginal washings.

Data coding scheme. The appearance of vaginal bacterial flora in wet and Gram-stained smears was coded as follows: 0, heavy (3+) presence of lactobacillus-like flora; coccobacillary flora not observed; 1, light (1+) to moderate (2+) lactobacillary flora with or without rare (one organism per several fields) or scant (one organism per field) coccobacillary flora; 2, moderate lactobacillary and coccobacillary flora; 3, light to heavy coccobacillary flora and moderate lactobacillary flora; 4, light to moderate coccobacillary flora, with or without rare to scant lactobacillary flora; 5, heavy presence of coccobacillary flora; lactobacillary flora not observed.

Similarly, side-room laboratory findings were scored as follows: for pH of vaginal secretions, 1, pH ≤ 4.5; 2, pH 4.6 to 5.0; 3, pH ≥ 5.1; for clue cells (wet mount), 0, not detected; 1, clue cells <20% of total cells; 2, clue cells 21 to 30% of total cells; 3, clue cells >30% of total cells; for vaginal discharge (patient subjective), vaginal secretions, genital malodor (patient or clinician subjective), and amine test, 0, normal or absent; 1, minimal; 2, detectably abnormal; 3, distinctly abnormal; for motile rods or curved gram-negative rods in wet (magnification, ×400) or Gram (magnification, ×1,000) smears, 0, not observed; 1, ≤1/×40 or ×100 Gram-stained field; 2, 1 to 10/×40 or ×100 Gram-stained field; 3, >10/×40 or ×100 Gram-stained field.

Ten clinical and laboratory side-room evaluable factors were identified and analyzed in the symptomatic and asymptomatic patients at each visit (Fig. 1). These factors did not include microbiological culture data. Each factor was

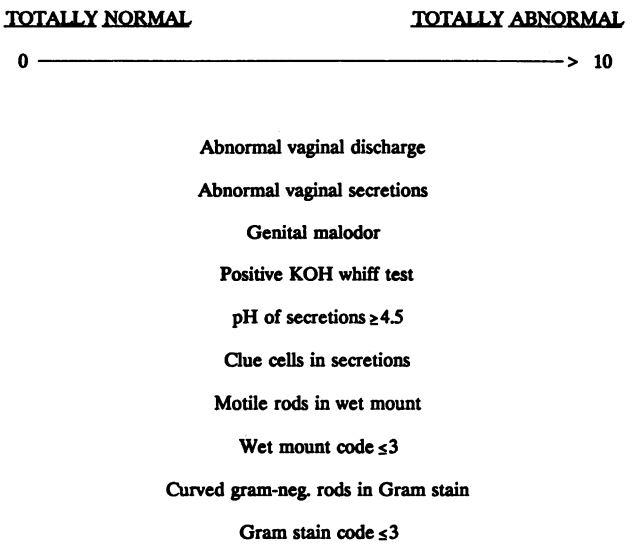

FIG. 1. Clinical code scheme for evaluation of overall symptom and laboratory criteria.

equally weighted and arbitrarily scored as 1 point. An overall clinical code was obtained by adding all points obtained for a visit in order to achieve a global index of severity of abnormality. The purpose of this scoring is relevant primarily to the evaluation of response to metronidazole therapy.

The presence of fatty acids in vaginal washings was scored by the following scheme: 0, major levels (more than 1 meq of fatty acid per ml of washings) of lactic acid only; 1, minor levels (less than 1 meq of fatty acid per ml of washings) of lactic acid only; 2, major levels of lactic acid and minor levels of acetic acid and/or minor levels of succinic acid; 3, minor levels of succinic acid, minor levels of acetic acid, and minor levels of lactic acid; 4, minor levels of succinic acid and minor levels of acetic acid; 5, major levels of succinic acid and/or major levels of acetic acid.

Statistical analysis. Statistical analysis was performed on all data by using the AbStat software package (Anderson-Bell, Arvada, Colo.). The use of repeat specimens from each woman within the acute BV and posttherapy groups was statistically validated by one-way analysis of variance. Probability values for comparisons of inter- and intraspecimen variations for all factors evaluated were >0.05. Nonparametric analyses were applied to all data, as follows: Yates-corrected chi-square analysis for testing of proportions and Mann-Whitney U test analysis for code-, concentration-, and population-level data.

RESULTS

Thirteen acutely symptomatic women with a history of recurrent BV were enrolled in the study. The subjects represented a predominantly middle-class population with a mean age of 32 years (range, 22 to 45 years) and a mean weight of 135 lb (61 kg). Eight women were white, and five women were black. Five women were married, four were divorced, and four were single. The mean number of pregnancies among the women was one. Coital frequency was twice per week, with a mean partner number of one. The type of contraception used was mixed and included tubal ligation ($n = 3$), diaphragm ($n = 4$), oral contraceptives ($n = 2$), condom ($n = 3$), and nil ($n = 1$).

Specimens were obtained from the 13 women during 31 visits for acute symptomatic BV. One patient had a single episode of vaginitis (enrollment BV), but BV did not recur within the 3-month follow-up period. Twelve women had, on average, 2.2 episodes of vaginitis as BV (31 episodes), candida vaginitis (4 episodes), mixed BV and candida vaginitis (2 episodes), or trichomoniasis (1 episode) over a maximum period of 9 months, with 8 of the 12 women having recurrent episodes of BV; there was an average of 2.3 episodes of BV per woman. The mean duration between vaginitis recurrences in the 12 patients was 34 days (range, 7 to 84 days); the mean duration between BV recurrences was 35 days (range, 14 to 84 days).

Recurrent episodes of BV included symptomatic and asymptomatic disease; all episodes satisfied the diagnostic inclusion criteria outlined in the methodology. There were 31 clinical courses of metronidazole prescribed, regardless of whether the patients were symptomatic. The mean clinical or laboratory code value for women with acute BV, using the coding outlined in Fig. 1, was 7.6, with 87% having a code of at least ≥7 and 100% having a code of ≥6 (Table 1).

The prevalence and severity of the 12 clinical or laboratory factors evaluated during the acute episodes of BV are presented in Table 1. During the acute episodes of BV, 93 and 87% of the women complained of genital malodor and of an abnormal volume and/or color of fluid discharge, respectively. Side-room laboratory findings were consistent with the diagnosis of BV in all 31 episodes. All women had a positive KOH amine whiff test, elevated pH of the vaginal secretions, and an abnormal, predominantly coccobacillary, flora as observed by wet mount and on Gram-stained smears. Similarly, clue cells were observed in the majority of women (94%), yet abnormal secretions, one of four standard diagnostic criteria for BV, were observed by the clinician in only 63% of the women. Polymorphonuclear leukocytes and motile gram-negative curved rods reminiscent of *Mobiluncus* species were infrequently observed in wet mount and Gram-stained smears.

The immediate response to metronidazole resulted in 90.3% symptomatic cure at 1 week. Evaluation of the 31 acute BV episodes at the end of 1 week of therapy revealed two patients (6%) with unchanged symptomatic BV; one patient, although symptomatic, was improved; and two patients were asymptomatic but still had BV, as defined by the presence of three of the four diagnostic criteria (asymptomatic BV). In addition, 6 women were asymptomatic, but two of the four diagnostic criteria were abnormal; 13 women were asymptomatic, with one diagnostic criterion being abnormal; and 7 women demonstrated complete resolution of all signs, symptoms, and laboratory abnormalities. Thus, complete clinical success defined by the absence of the four conventional diagnostic criteria was observed in 7 of 31 episodes (23%), partial success with one or two residual abnormalities was observed in 19 of 31 episodes (61%), and clinical failure was observed in 5 of 31 episodes (16%).

Posttreatment clinical and laboratory data for the 26 asymptomatic episodes in women with less than three of the four diagnostic criteria for BV are presented in Table 1. Under normal standards of clinical practice, these women would not be considered by their practitioners to require additional therapeutic intervention.

The successful elimination of malodor following therapy was reported for all 26 episodes. The overall symptom code decreased from a mean of 7.6 to 2.3 ($P < 0.01$). Half of the women described some abnormal discharge ($P < 0.01$), but all reported the discharge as minimal compared with that

TABLE 1. Comparison of clinical and side-room laboratory factors during 31 episodes of acute BV and following metronidazole therapy

Abnormal codes	Code (range)	Acute BV (n = 31)		Posttherapy (n = 26)[a]		Controls (n = 31)	
		Mean code	% Abnormal	Mean code	% Abnormal	Mean code	% Abnormal
Subjective discharge	≥1[b] (0–3)	1.6	87	0.7	54	0.1	3
Vaginal secretions	≥1 (0–3)	0.6	63	0.1	3	0	0
Genital malodor	≥1 (0–3)	1.8	93	0	0	0	0
KOH amine whiff test	≥1 (0–3)	1.3	100	1.5	15	0	0
pH of secretions (>4.5)	≥2 (0–3)	3.0	100	1.7	65	1.2	25
Wet mount flora	≥3 (0–5)	4.4	100	2.0	32	1.3	15
Clue cells ≥20%	≥2 (0–3)	2.7	94	0.4	31	0	0
Motile rods	≥1 (0–3)	0.3	23	0.1	8	0.1	7
PMNs[c]	≥1 (0–3)	0.2	7	0.5	22	0.2	14
Gram stain flora	≥3 (0–5)	4.4	100	1.9	24	1.4	21
Curved gram-negative rods	≥1 (0–3)	0.3	30	0.1	12	0	0
Overall clinical code	≥7 (0–10)	7.6	87	2.3	0	0.7	0
	≥6		100		0		0

[a] Asymptomatic women only.
[b] Code considered abnormal.
[c] PMNs, polymorphonuclear leukocyte/epithelial cells ratio of >1.

described during the acute BV episode ($P < 0.01$). All of the following abnormal clinical and laboratory findings observed during the acute episode prior to therapy showed dramatic improvement after therapy (values are probabilities for mean severity codes and probability value for percent abnormal): abnormal secretions, $P < 0.01$, $P < 0.001$; KOH amine whiff test, $P < 0.01$, $P < 0.01$; pH > 4.5, $P < 0.01$, $P < 0.01$; wet mount flora, $P < 0.01$, $P < 0.01$; clue cells, $P < 0.01$, $P < 0.01$; motile rods, $P = 0.03$, $P = 0.02$; Gram-stained flora, $P < 0.01$, $P < 0.01$; curved gram-negative rods, $P = 0.040$, $P < 0.01$.

Nevertheless, a wet mount with an abnormal bacteriological flora persisted in 32% of the women, clue cells persisted in 31% of the women, polymorphonuclear leukocytes persisted in 22% of the women, and motile rods reminiscent of *Mobiluncus* species persisted in 8% of the women. Similarly, 24% of the women had an abnormal Gram-stained flora, with curved gram-negative rods observed in 12% of smears. The KOH amine whiff test remained positive in 15% of the women, and the pH of the vaginal secretions remained elevated at >4.5 in 65% of the women. Nevertheless, in all women, the mean index of severity of each abnormality decreased, with the exception of the KOH amine whiff test and the presence of PMNs, which were, on average, reflected by a low positive score in pre- and posttherapy groups.

Residual clinical and side-room laboratory abnormalities (see Fig. 1), although of lesser severity, were observed in 77% (20 of 26 episodes) of the women defined as cured. The prevalence and mean code for each factor were compared between the women with recurrent BV and the control group (Table 1). The residual abnormalities were as follows: abnormal discharge as reported by the patient, KOH amine whiff test, secretion pH of >4.5 in 17 of the 20 women (pH 4.5 to 4.7 in 12 women, pH 4.8 to 5.0 in 4 women, pH of >5.0 in 1 woman), an abnormal flora observed in smears, and the presence and numbers of clue cells. Reflecting the overall incidence of residual abnormality was an elevated clinical code of 2.3 compared with a code of 0.7 for the control population.

Microbiological culture data from the 31 episodes of acute BV are presented in Table 2. Obligate anaerobes were detected in 71% of wash specimens from the vaginas of women with acute BV at a mean population level of $\log_{10}$ 6.6 CFU/ml of vaginal washings. Several genera were detected, but only two genera, *Prevotella* and *Peptostreptococcus*, were isolated from more than 10% of women. *G. vaginalis* was isolated from 87% of women at a mean population level of $\log_{10}$ 8.2 CFU/ml. *Lactobacillus* species were isolated from the vaginas of 48% of women with acute BV and at a mean population level of $\log_{10}$ 5.1 CFU/ml.

Following therapy with metronidazole (Table 2), 50% of the women previously colonized by anaerobes were no longer colonized, and the mean number of species isolated per specimen decreased from 7.3 to 6.3 species per specimen (data not shown). Population levels of total anaerobes, when they were detected, decreased by 1 $\log_{10}$ count to $\log_{10}$ 5.6 CFU/ml ($P = 0.01$). *Prevotella* and *Peptostreptococcus* species were detected in 8 and 19% of women, respectively ($P < 0.01$ and $P < 0.01$, respectively), although, when they were isolated, they were found at unchanged population levels of $\log_{10}$ 6.5 and 5.2 CFU/ml of washings, respectively ($P = 0.28$ and $P = 0.11$, respectively). Gram-negative anaerobic rods, as a whole, were detected more frequently in women with acute BV than following therapy, although the population levels did not differ ($P = 0.28$). *G. vaginalis* was isolated from 19% of women following 1 week of therapy with metronidazole ($P < 0.01$), although, when *G. vaginalis* was isolated, mean population levels remained similar to those obtained during episodes of acute BV ($\log_{10}$ 7.7 CFU/ml) ($P = 0.22$). In contrast, lactobacilli were isolated more frequently from 81% of women ($P = 0.01$) and at a mean population level of $\log_{10}$ 6.8 CFU/ml compared with $\log_{10}$ 5.1 CFU/ml of vaginal washings during acute BV ($P < 0.01$). Aerobic gram-negative rods were isolated from 50% of women following therapy. Although the prevalence of yeasts did not increase following therapy, population levels of preexisting yeasts increased.

Moderate differences were observed in both the prevalence and the population levels of vaginal bacterial species between the women with recurrent BV following therapy and the control population (Table 2); the mean number of bacterial species isolated differed at 6.3 and 3.4 per specimen, respectively. Anaerobic gram-negative rods were iso-

TABLE 2. Comparison of prevalence and population levels of vaginal microorganisms during 31 episodes of acute BV and following metronidazole therapy

Microbial species	Acute BV (n = 31)		Posttherapy (n = 26)		Controls (n = 31)	
	Mean[a]	%[b]	Mean	%	Mean	%
Obligate Anaerobes						
Total anaerobes	6.6	71	5.6	42	3.7	23
Total gram-negative rods	5.8	58	6.4	19	2.0	3
Bacteroides spp.	5.5	58	6.5	8	0	0
Fusobacterium spp.	5.8	10	6.7	8	2.0	3
Total gram-positive rods	6.4	10	3.9	8	3.2	6
Eubacterium spp.	7.0	3	3.9	8	0	0
Actinomyces spp.	6.1	6	0	0	3.2	6
Peptostreptococcus spp.	6.0	52	5.2	19	4.2	13
Veillonella spp.	6.4	6	0	0	2.5	3
Facultative anaerobes/aerobes:						
Gardnerella vaginalis	8.2	87	7.7	19	6.4	13
Lactobacillus spp.	5.1	48	6.8	81	7.1	84
Corynebacterium spp.	5.6	84	3.5	73	3.9	19
Streptococcus spp.	4.6	77	3.9	58	5.0	55
Staphylococcus spp.	3.9	65	3.8	65	3.3	39
Micrococcus spp.	4.0	33	3.5	19	3.4	10
Aerobic gram-negative rods	3.9	29	2.6	50	3.2	19
Candida albicans	1.9	10	3.0	12	2.3	13
Torulopsis glabrata	2.3	6	6.0	12	0	0

[a] Log_{10} CFU per milliliter of washings when an organism was isolated.
[b] Percentage of patients positive for organisms.

lated from 19% of the women in the posttherapy group and 3% of the women in the control group. Population levels of anaerobic gram-negative rods, when detected, and obligate anaerobes as a whole were elevated in the posttherapy group (log_{10} 6.4 and log_{10} 5.6 CFU/ml, respectively) compared with those in the women in the control group (log_{10} 2.0 [$P < 0.01$] and log_{10} 3.7 [$P = 0.01$] CFU/ml, respectively). The prevalence and mean population levels of individual anaerobic species were not different. Few differences were observed in aerobic and facultative aerobic microbiology between the two groups. The prevalence of corynebacteria, staphylococci, and aerobic gram-negative rods was higher in the posttherapy group (73, 65, and 50%, respectively) compared with that in the control population (19, 39, and 19%, respectively).

The fatty acid profile code in patients with acute BV was considered abnormal (>3) in 61% of women, with a mean code of 3.4 on a scale of 0 to 5 (Table 3). Acetic acid was predominant in 81% of women, and succinic acid was detected in 40% of women. Lactic acid was detected in only 20% of women with acute BV. The succinate/lactate ratio was 1.4. Following therapy, the fatty acid profile code was abnormal in 15% ($P < 0.01$) of women, with a mean code of 1.4 ($P < 0.01$). The predominance of fatty acids was reversed, with lactic acid detected in 69% of women ($P = 0.0001$) and acetic and succinic acids detected in 33 and 4% of women, respectively ($p < 0.01$ and $P < 0.01$, respectively). Although the mean fatty acid concentrations in the vaginal washings differed between the two groups, statistical significance was not reached. The succinate and lactate ratio decreased to 0.01. Differences in mean fatty acid concentrations were not observed in comparisons between the posttherapy and control populations. However, 31% of women in the posttherapy group did not have detectable lactic acid in vaginal washings, whereas this was the case for only 3% of women in the control group ($P < 0.01$).

Assay of polyamine composition (Table 3) revealed putrescine and cadaverine in 74 and 77% of washings and at mean concentrations of 175 and 511 nmol/ml, respectively. Following therapy, the prevalence of putrescine did not decrease ($P = 0.19$), but the mean concentration, when detected, was three times lower than that during acute BV ($P = 0.01$). In contrast, both the prevalence ($P = 0.01$) and the mean concentration ($P = 0.02$) of cadaverine were lower in the posttherapy group. Comparison of the amine composition in the posttherapy group with that in the control group failed to detect differences in cadaverine prevalence and its mean concentration. The prevalence of putrescine in the posttherapy group was, however, twice that in the control group ($P < 0.01$), although the mean concentration, when detected, was not different.

In order to evaluate individual factors that could be used

TABLE 3. Comparison of biochemical assay results during 31 episodes of acute BV and following metronidazole therapy

Biochemical assay	Acute BV (n = 31)		Posttherapy (n = 26)		Controls (n = 31)	
	Mean	%	Mean	%	Mean	%
Fatty acids[a]						
Lactic acid	0.7	20	1.2	69	0.7	97
Succinic acid	0.5	40	0.1	4	0.1	10
Acetic acid	0.4	81	0.1	33	0.3	14
Succinate/lactate ratio	1.41		0.01		0.01	
Profile code ≥3[b]	3.4	61	1.4	15	1.2	13
Polyamines[c]						
Putrescine	175	74	61	58	158	23
Cadaverine	511	77	126	46	123	26

[a] Units for fatty acid means are micrograms per milliliter.
[b] Units for profile code means are the code number.
[c] Units for polyamine means are nanomoles per milliliter.

TABLE 4. Comparison of clinical and side-room laboratory factors after metronidazole therapy in patients with an early recurrence of BV (≤35 days) versus those with a late recurrence of BV (>35 days)

Abnormal code	Code (range)	Early (n = 9)		Late (n = 10)	
		Mean code	% Abnormal	Mean code	% Abnormal
Subjective discharge	≥1[a] (0–3)	1.1	78	0.4	40
Vaginal secretions	≥1 (0–3)	0.1	13	0	0
Genital malodor	≥1 (0–3)	0	0	0	0
KOH amine whiff test	≥1 (0–3)	0	0	0.1	13
pH of secretions (≥4.5)	≥2 (0–3)	2.0	11	1.5	0
Wet mount flora	≥3 (0–5)	2.8	56	1.4	22
Clue cells ≥20%	≥2 (0–3)	0.7	22	0.3	30
Motile rods	≥1 (0–3)	0.1	11	0.1	10
PMNs[b]	≥1 (0–3)	1.0	33	0.1	11
Gram stain flora	≥3 (0–5)	3.0	56	1.4	11
Curved gram-negative rods	≥1 (0–3)	0.2	22	0.1	11
Overall clinical code	≥7 (0–10)	3.6		1.5	

[a] Code considered abnormal.
[b] PMNs, Polymorphonuclear leukocyte/epithelial cell ratio of >1.

to predict the early recurrence of BV, the 26 asymptomatic, successfully treated episodes of BV were further separated into two groups: those that recurred with syptomatic BV at or within 35 days of the end of therapy (mean recurrence interval) and those that remained asymptomatic for more than 35 days after the end of therapy. All women had a recurrence of BV within 1 year after the end of therapy. Clinical, side-room laboratory, microbiological culture, and biochemical data were compared between nine women who had a recurrence of BV and 10 women who remained asymptomatic. Because of the small numbers involved, no statistical analysis was performed.

Women with early recurrence complained of an abnormal discharge at the end of therapy more frequently than women with late recurrence did (Table 4). The severity of the discharge was, however, greater in the early recurrence group compared with that in the late recurrence group, at mean codes of 1.1 and 0.4, respectively. The prevalence of an abnormal pH and the mean pH code were not different between the two groups. Abnormal Gram-stained smears of vaginal secretions were observed more frequently among women with early recurrence (56%) than among women with late recurrence (11%). Abnormal wet mount smears were observed in 56% of the early recurrence group and 22% of the late recurrence group. The mean code for severity of abnormality was higher for both Gram-stained and wet smears from women in the early recurrence group (3.0 and 2.8, respectively) than for smears from women in the late recurrence group (1.4 and 1.4, respectively).

A higher prevalence of total abnormalities and a higher severity of each abnormality in the women who had a recurrence of BV early compared with women who had a recurrence >35 days after the end of therapy were reflected in a higher mean clinical code for the early recurrence group compared with that for the late recurrence group (3.6 and 1.5, respectively).

DISCUSSION

Despite the considerable progress reported in understanding the epidemiology, pathophysiology, and treatment of BV (5, 7, 9, 12, 25, 27, 28), scant information is available regarding recurrent BV. Most therapeutic trials evaluate treatment outcome at the completion of treatment day 7 or, at best, 28 to 35 days following the termination of therapy, and longer follow-up is rare (3, 4, 10, 21, 22). Early recurrence rates at 30 to 35 days posttherapy range from 11 to 40% (3, 4, 22). Moreover, Hillier and Holmes (16) reported that 80% of patients with acute BV can be expected to have a recurrence within 9 months after metronidazole therapy.

Possible explanations for recurrence include reinfection, either endogenous or by a male partner who is colonized with BV-associated microorganisms, or relapse of infection because of the persistence of BV-associated microorganisms which are inhibited and reduced in number but not eliminated by therapy. Under the latter circumstances, relapse may also be facilitated by the failure to reestablish the normal, and perhaps protective, lactobacillus-dominant flora following therapy (12). It is also conceivable that relapse may result from the persistence of another, yet unidentified critical microbial agent or host factor that creates susceptibility to infection. The role of antimicrobial resistance of the multiple pathogens involved in BV is also undetermined. Until the pathogenesis of recurrent BV is more clearly understood, effective strategies for therapy are unlikely to emerge.

Thus, while recurrence of symptomatic BV is common, the gravity of the problem has largely been unappreciated and ignored. Moreover, we were impressed by a group of inveterate sufferers with recurrent bouts of symptomatic BV who constitute an enormous therapeutic dilemma. At the time of a first follow-up visit, women are predictably asymptomatic. Nevertheless, within 1 to 3 months the majority of women can be expected to return with identical symptoms and to respond once more to repeat medication. This observation was confirmed in our study, in which patients were predictably asymptomatic immediately following the cessation of 7 days of metronidazole therapy and no longer met the composite clinical criteria required for a diagnosis of BV.

The ideal method for elucidating the pathogenesis of recurrent BV would have been to study a large number of women with BV in whom BV did and did not recur after therapy and compare posttreatment cultures and laboratory data for each group. Unfortunately, because of the nature of the University Clinic, virtually all patients were referred because of recurrent, chronic, or recalcitrant disease. Ac-

cordingly, we chose to study longitudinally and repetitively a small group of women with recurrent disease in an attempt to identify whether they had a relapse or were reinfected. In this context, the control group that we used was of reference value only, and no statistical comparison was performed. Nevertheless, the data obtained from the small group of women with frequently recurring BV provides valuable new information and strongly points to relapse rather than reinfection as being the explanation for early recurrences. It is apparent, however, that the results derived from this small study are biased toward the recalcitrant nature of BV in these patients and may not reflect the mechanism of recurrence in women experiencing their first episode of recurrence.

The clinical, microbiological, GLC, and HPLC data obtained in the study population described here are largely similar to those for women with BV presented previously (7, 8, 11, 31). During episodes of symptomatic acute BV, microbiological data were virtually identical to those presented in textbooks and provided no new information into the pathogenesis of recurrent BV. Similarly, comparison of microbiological findings in successive episodes in the same individual revealed a similar spectrum of microorganisms with each recurrence, although patients differed among themselves with regard to which of the anaerobic species increased. The short-term symptomatic response to metronidazole in the study group was 90% and was similar to that observed in numerous therapeutic studies (4, 10, 21, 22).

It was the posttherapy vaginal laboratory and microbiological studies that revealed the most useful information. We excluded patient episodes from evaluation if patients partially or completely failed to improve clinically on metronidazole. All the patients studied posttherapy were asymptomatic and no longer satisfied the composite diagnosis for BV. In some conventional clinical studies, these women might have been considered "cured" (15). Nevertheless, residual abnormalities were still apparent in more than 70% of the women. In fact, only 23% of the women had complete absence of any of the major diagnostic criteria. Almost one-third of the asymptomatic women had a grossly abnormal wet mount or Gram stain or the presence of >20% clue cells. Although the vaginal pH was considerably improved, it remained marginally elevated at 4.5 to 4.7 in almost two-thirds of patients; infrequently, it was elevated above 4.7. Similarly, biochemical parameters measured by GLC, although reflecting overall improvement with increased concentrations of lactic acid and reduced concentrations of succinic and acetic acids, nevertheless continued to reflect significant abnormalities when they were compared with the biochemical parameters for healthy controls. Putrescine and cadaverine were also found significantly more frequently in "cured" women than in the control group.

All of the residual abnormalities presented above reflect an improved but still fundamentally abnormal vaginal ecosystem. Microbiological studies revealed that the demonstrable residual abnormalities were more subtle than expected. *G. vaginalis* was present in less than 20% of women, although when it was present this species was still present in high numbers. A frequent finding was that of a persistent increase in total obligate anaerobic bacterial species in terms of both prevalence and persistently high numbers. This was more apparent with regard to anaerobic gram-negative rods. Notably, facultative aerobes were also more frequent in the study group, as were *Corynebacterium* species, although the numbers were relatively low. One might have predicted that, given the frequency with which persistently abnormal Gram stains and saline preparations were observed, lactobacillus population numbers would remain reduced in comparison with the normal population numbers. Somewhat inexplicably, however, *Lactobacillus* species numbers closely resembled those found in the control group. Nevertheless, this should not infer that the normal dominant *Lactobacillus* species flora was established since individual *Lactobacillus* species were not reported and we also did not measure the hydrogen peroxide-producing capacity of lactobacillus isolates (12). In addition, as mentioned above, lactic acid was absent from 30% of the patients' vaginal secretions ($P < 0.01$).

The overall summary is that following a course of metronidazole, asymptomatic, "cured" women continue to manifest significant abnormalities in their vaginal flora. The residual abnormalities could be scored and quantified and varied from one patient to the next. The most frequently found residual abnormalities were the mildly elevated vaginal pH (4.5 to 4.6), the persistent presence of clue cells, and abnormal wet mount and Gram stain reflecting a persistent coccobacillary flora and continued dominance of the anaerobic flora without the return of lactobacilli.

The significance of the residual abnormalities was further reflected by the correlation between the quantitative overall severity code and the time to recurrence of symptomatic BV. Thus, the more severe the persistent disruption, the higher the clinical code and likelihood of early recurrence. All patients in the study had a recurrence of BV; this fact, together with the small patient numbers involved, precluded the possibility of comparing women who did and did not have recurrences.

The high frequency of residual abnormalities following successful therapy, together with the correlation between the severity of abnormalities and time to recurrence, strongly suggest that recurrent BV is due to relapse rather than reinfection. It is difficult to conceive of women having recurrences on a frequent, often monthly, basis purely because of the reintroduction of the BV-associated organisms into the vagina. This does not negate the possibility that *G. vaginalis* or *Mobiluncus* species is sexually transmitted. It does indicate, however, that it takes more than just the introduction of *G. vaginalis* to cause the tremendous upheaval in vaginal microbiology that accompanies BV. Relapse appears to reflect a failure of the complete normalization of the vaginal ecosystem without indicating resistance to metronidazole or the specific persistence of any single bacterial species or physical condition. We were also unable to corroborate the hypothesis that relapse directly correlates with failure to reacquire lactobacilli in large numbers; however, the lactobacillus concept needs additional study.

The therapeutic implications of this study remain conjectural. Would more prolonged therapy with metronidazole (14 or 21 days) result in a lower recurrence rate with fewer residual vaginal microbial abnormalities, especially those pertaining to anaerobes? Is 2% clindamycin therapy similarly associated with a high recurrence rate? Should patients with recurrent BV receive maintenance therapy? Should this include antianaerobic therapy or methods to maintain a normal pH? Numerous studies have indicated that treatment of male sexual partners fails to reduce the incidence of recurrence of BV (16, 29). Perhaps it would be reasonable to evaluate all asymptomatic patients immediately following metronidazole or clindamycin therapy and, if a high predictive score was present, continue therapy for a few more days. The need for clinical studies in this group of patients who are difficult to treat is pressing, especially with the

recently described association between BV and upper genital tract disease (14, 17).

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