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Bacterial Vaginosis and Vaginal Microorganisms in Idiopathic Premature Labor and Association with Pregnancy Outcome

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The vaginal microflora of 49 women in idiopathic preterm labor was compared with that of 38 term controls to determine whether the presence of bacterial vaginosis (BV) and/or specific microorganisms would influence the rate of preterm delivery. Demographic factors, pregnancy outcome, and reproductive history were also studied. BV, as defined by the presence of clue cells in a vaginal wet mount and characteristic microbial findings in a stained vaginal smear and vaginal culture, was more common in women with preterm labor and delivery than in controls ($P < 0.01$). The condition, diagnosed in 41% of women who had both preterm labor and delivery ($n = 22$) and in 11% each of women who had preterm labor but term delivery ($n = 27$) and controls, was associated with a 2.1-fold risk (95% confidence intervals, 1.2 to 3.7) for preterm birth prior to 37 weeks of gestation. BV was associated with low birth weight. Of 49 women with preterm labor, 67% (8 of 12) of women with BV were delivered of low-birth-weight neonates (<2,500 g) compared with 22% (8 of 37) of women without the condition ($P < 0.0005$). The presence of hydrogen peroxide-producing facultative *Lactobacillus* spp. was strongly negatively associated with both preterm delivery and BV. BV-associated microorganisms, i.e., *Mobiluncus*, *Prevotella*, and *Peptostreptococcus* species, *Porphyromonas asaccharolytica*, *Fusobacterium nucleatum*, *Mycoplasma hominis*, and high numbers of *Gardnerella vaginalis* were significantly associated with preterm delivery; all species also strongly associated with BV ($P = 0.0001$ for each comparison). *Mobiluncus curtisii* and *Fusobacterium nucleatum* were recovered exclusively from women with preterm delivery. Our study clearly indicates that BV and its associated organisms are correlated with idiopathic premature delivery.

One of the most important objectives of perinatology is to diminish preterm delivery (PTD) and to predict preterm contractions which will go to delivery. Only approximately 7% of all deliveries are premature, but they account for more than 80% of perinatal morbidity (42, 62). The frequency of preterm births has not significantly decreased over the last 30 years despite the widespread use of potent tocolytic agents.

To date, causes of prematurity can be found in fewer than half of all cases (1). Factors historically found to correlate with increased risk of adverse pregnancy outcome include young (<17 years) and advanced (>35 years) maternal age, low socioeconomic status, black race, single marital status, low weight prior to pregnancy, a history of adverse pregnancy outcome, three or more abortions, antepartum hemorrhage, history of previous sexually transmitted diseases, antenatal urinary tract infection, cigarette smoking during pregnancy, fetal gender, and multiple gestation (1, 7, 27, 28, 47). However, only low socioeconomic status, antepartum hemorrhage, and a history of adverse pregnancy outcome have consistently been reported as risk factors, with a history of adverse pregnancy outcome being the most significant with a two- to sixfold risk of recurrence of preterm birth (1, 27, 40, 47). In addition, different studies have indicated that abnormal pregnancy outcomes, i.e., preterm labor (PTL), PTD, and preterm rupture of membranes, may be related to ascending genital microflora. Specific organisms such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and group B streptococci have all been correlated with one or more of such abnormal outcomes of pregnancy (2, 16, 17, 19, 40, 48, 49, 51).

Recently, bacterial vaginosis (BV), a common vaginal syndrome in women of reproductive age, has been associated with increased risks for prematurity and premature rupture of membranes (15, 16, 17, 40, 47). The controversial hypothesis that the cervicovaginal microflora may initiate preterm birth has now gained support from accumulated data suggesting a causal association between the presence of such flora and adverse pregnancy outcome. However, this hypothesis has been advanced mainly by a few clinical centers in the United States. The purpose of the present study was to examine the validity of the hypothesis in Sweden, where all deliveries take place in community hospitals, and therefore the study will reflect the condition of the whole population. The aim of the present study was to characterize the vaginal microflora of Swedish women in PTL to determine whether the presence or absence of specific vaginal microorganisms and/or BV would influence the rate of onset of PTD. Demographic factors, pregnancy outcome, and reproductive history characteristics were also studied.

MATERIALS AND METHODS

Study population. Women consecutively admitted for PTL at the Department of Obstetrics and Gynaecology, East Hospital, Gothenburg, Sweden, were enrolled during a 6-month period in 1991. Each subject gave informed consent. The women were at a gestational age of 23 to 35 completed weeks on the basis of the last menstrual period combined with ultrasonographic data.

Of a total of 120 women in PTL, we excluded from further analysis 63 patients with recognizable causes of prematurity,

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including diabetes mellitus (two patients), preeclampsia (11 patients), abruption placentae (8 patients), placenta previa (1 patient), premature rupture of the membranes (defined as rupture of the membranes before onset of painful labor; 25 patients), more than one fetus (5 patients), intrauterine growth retardation (5 patients), known congenital malformation (1 patient), cervical cerclage (2 patients), and Rh immunization (3 patients). Another eight women were admitted at night and were not approached by study personnel. Thus, the final study population (cases) consisted of 49 women. None of the women had been treated with antibiotics within 4 weeks of enrollment.

Study design. Epidemiological and pregnancy outcome data were collected. Epidemiological data included demographic characteristics (socioeconomic status, age, weight, parity, marital status, sexual activities, and physically demanding leisure activities), reproductive history (abortions and previous preterm gestations of <37 weeks), and medical complications (urinary tract infections, alcohol abuse, cigarette smoking, and previous genital and sexually transmitted diseases).

Apart from PTL, pregnancy outcome characteristics included requirements for tocolytic therapy, gestational age at delivery, and birth weight. Labor was defined by two or more painful regular contractions every 10 min for at least 2 h. PTD was subdivided into delivery occurring between 34 and <37 weeks gestation (generally associated with a good neonatal outcome) and delivery occurring prior to 34 weeks gestation (associated with an increased perinatal morbidity and mortality).

Control patients. Control patients consisted of 38 women in labor at term (≥37 weeks of gestation), admitted to the same hospital during the study period, who met none of the exclusion criteria and had no complications of pregnancy (preterm rupture of membranes, preterm contractions, or vaginal bleeding).

Sampling. Specimens were obtained during speculum examination at the time the patient was admitted for being in labor. With dacron swabs, samples were collected from the urethra and the endocervix for isolation of *C. trachomatis*; the swabs were transported to the laboratory in sucrose-phosphate transport media. With cotton-tipped, charcoal-treated swabs, specimens were taken from the endocervix for isolation of *N. gonorrhoeae* and from the posterior vaginal fornix for isolation of other aerobic, facultative, and anaerobic bacteria and yeast species. These specimens were transported to the laboratory in a modified Stuart medium. For microscopic evaluation of the vaginal flora, a specimen from the posterior fornix was collected with a cotton-tipped swab, which was rolled over a glass slide, air dried, and fixed with methanol. This smear was sent to the laboratory.

Culture. The following culture media were used: for isolation of anaerobes, prereduced blood agar (Oxoid) with 4% defibrinated horse blood, hemin (5.0 mg/liter), and vitamin K_1 (0.5 mg/liter); for *Mobiluncus* spp., prereduced Columbia agar (Oxoid) with 2.5% horse serum, nalidixic acid (15 mg/liter), and tinidazole (1.0 mg/liter) (21); for *Gardnerella vaginalis*, human blood bilayer medium with Tween 80 (70); for lactobacilli, Rogosa agar (Oxoid); for *N. gonorrhoeae*, gonococcal agar; for yeast species, Sabouraud agar. *M. hominis* and *U. urealyticum* were cultured and identified with the Mycoscreen (International Mycoplasma, Toulon, France), including a modified Sheppard A7 medium (65). In addition, all specimens were inoculated onto hematin and blood agar for isolation of aerobic and facultative species. Each agar plate was streaked into four zones, with decontamination of the loop after inoculating each area. Specimens for isolation and identification of *C. trachomatis* were stored at 4°C until inoculation onto cycloheximide-treated McCoy cells as previously described (55). All specimens were cultured within 18 h.

Media used for the isolation of anaerobes were incubated in an anaerobic cabinet in an atmosphere of 80% N_2–10% CO_2–10% H_2 at 37°C, Sabouraud agar was incubated in air at 30°C, and all other media were incubated under 10% CO_2 at 37°C. After incubation for 48 h (mycoplasmas, 72 h), the plates were inspected under a stereomicroscope every second day for 10 days.

The growth of the different species was semiquantitated as follows: 1+, ≤10 colonies in the primary streak area; 2+, >10 colonies in the primary and <10 colonies in the secondary streak areas; 3+, >10 colonies in the secondary and <10 colonies in the tertiary streak areas; 4+, >10 colonies in the tertiary streak area.

Anaerobic bacteria were identified as described previously (22, 68). Identification of aerobic and facultative bacteria was made by standard methods routinely used in our laboratory. *G. vaginalis* and *Mobiluncus* spp. were identified as described previously (25, 70).

Determination of hydrogen peroxide production. The H_2O_2 production of isolated lactobacillus strains was determined by the method previously described by Eschenbach and coworkers (14).

Microscopy. A wet mount of vaginal secretion from the posterior fornix was diluted with normal saline and examined by phase-contrast microscopy (×400 magnification) for identification of clue cells, bacterial morphology, trichomonads, and bacilli with corkscrew motility which were deemed to be *Mobiluncus* spp.

For the identification of yeast cells and pseudohyphae, a wet smear diluted with 10% potassium hydroxide was used.

In the laboratory, the vaginal smears were stained with methylene blue and examined by light microscopy (×1,000 magnification) for the presence of clue cells and morphotypes of lactobacilli, *Gardnerella* spp., *Mobiluncus* spp., and other bacteria (23). In addition, the number of polymorphonuclear leukocytes (PMN) in the smears was determined. The number of PMN was quantitated as follows: 1+, ≤5; 2+, >5 to 20; 3+, >20 to 40; 4+, >40, in any of five random oil-immersion fields (×1,000 magnification). After this first examination, all smears were washed in 96% ethanol, Gram stained, and studied for Gram stain characteristics.

The smears were evaluated and registered before the primary examination of the cultures by one of the investigators (E.H.), who remained blinded to patient symptoms, clinical findings, and outcomes throughout the study.

Diagnosis of BV. A diagnosis of BV was defined by a Gram-stained smear evaluated as indicative of BV by the method of Spiegel et al. (67), in addition to the presence of clue cells in wet mount and a stained vaginal smear and a vaginal culture revealing none or <2+ facultative lactobacilli and a profuse mixed flora consisting of mainly anaerobes [*Prevotella* (*Bacteroides*) spp., *Porphyromonas* spp., butyrate-producing peptostreptococci, *Mobiluncus* spp., and/or fusobacteria] together with *G. vaginalis*.

Statistical analysis. The tests of two proportions were done with the Fisher exact tests. The *P* values were from two-tailed tests; a *P* value of ≤0.05 was considered to indicate significance. An unpaired Student's *t* test was used to compare gestational age and birth weight between groups. Relative risks and their 95% confidence intervals (CI) were calculated according to Gardner and Altman (15a).

TABLE 1. Characteristics of women in PTL and term labor

Characteristic	Result for PTL cases (n = 49)			Result for term labor controls (n = 38)
	PTD (n = 22)		TD (n = 27)	
	<34 weeks (n = 15)	34–36 weeks (n = 7)		
Demographic				
Maternal age (yr)	30.7 ± 5.5	29.3 ± 6.0	26.7 ± 5.7	28.0 ± 4.3
No. (%) <20 yr old	0	0	5 (19)	0
No. (%) >35 yr old	4 (27)	2 (29)	1	2 (5)
No. (%) of smokers	7 (47)	3 (43)	10 (37)	8 (21)
Reproductive history				
No. (%) nulliparous	5 (33)	4 (57)	15 (55)	21 (55)
No. (%) primigravida	3 (20)	3 (43)	13 (48)	13 (34)
No. (%) with previous PTD	5 (33)	0	2 (7)	0
No. (%) with ≥3 spontaneous or induced abortions	3 (20)	0	1	0
Pregnancy outcome				
Time from PTL to delivery (days)[a]	4.8 ± 6.1	19 ± 12.8	41.1 ± 23.2	
Neonate mean birth weight (g)	1,641 ± 399	2,572 ± 345	3,574 ± 425	3,703 ± 367
No. (%) of low-birth-weight neonates (<2,500 g)	14 (93)	2 (29)	0	0
No. (%) of very-low-birth-weight neonates (<1,500 g)	5 (33)	0	0	0

[a] Mean number of days from onset of PTL to delivery.

RESULTS

Population characteristics. Of the 49 women experiencing PTL, PTD occurred for 22 and term delivery (TD) occurred for 27 women (see Table 1). Of the 22 PTD women, 15 delivered at a gestational age of <34 weeks (mean, 29.9 ± 2.4 weeks; range, 25 to 33 weeks), and 7 women delivered between 34 and 36 weeks of gestation (mean, 34.9 ± 2.1 weeks; see Table 1).

There was a significant difference in the presence of BV in PTD women compared with that in both TD women and controls (see Table 3). Of the nine PTD women with BV, six delivered at a gestational age of <34 weeks (mean, 29.7 ± 3.5 weeks; range, 26 to 33 weeks) and three delivered after 34 completed weeks (mean, 34.2 ± 0.3 weeks). BV was significantly associated with a 2.10-fold risk (95% CI, 1.2 to 3.7) for preterm birth prior to 37 weeks of gestation but not with delivery prior to 34 weeks (see Table 4). If only the preterm or full-term onset of labor is considered (not PTD or full-term delivery), there was no significant difference in the presence of BV in PTL women compared with that in controls (12 of 49 [24%] versus 4 of 38 [11%], $P = 0.162$).

Demographic characteristics, reproductive history, and pregnancy outcome of cases and controls are shown in Tables 1 and 2; in Table 2, the women are subdivided into those with and those without BV. The numbers of nulliparous women and primigravidas were similar among the groups. There was no difference in mean maternal age between the PTL women and the controls. However, 6 (27%) of the 22 PTD women, 4 of whom delivered prior to 34 weeks, were >35 years old, compared with 1 TD woman ($P < 0.05$) and 2 controls ($P < 0.01$; Table 1). The only >35-year-old woman with BV delivered prior to 34 weeks of gestation (Table 2). No woman was younger than 19 years, and one was >40 years old. Women in the three groups were comparable in terms of socioeconomic status, physically demanding leisure activities, and sexual activities; all had a normal weight prior to pregnancy.

Forty-five percent of PTD women were cigarette smokers compared with 37 and 21% of TD women and controls, respectively (not significant; Table 1). Of the 49 PTL women, 20 (41%) were smokers compared with 8 of 38 controls (21%, $P = 0.0001$). Smoking habits were similar among women with or without BV (Table 2), and smoking was not associated with any special microbial findings.

A significantly higher frequency of previous PTDs was found in the PTD group than in the controls (23% versus 0%, $P < 0.01$) but not compared with the TD group (23% versus 7%, $P = 0.060$; Table 1). None of the women with BV had a history of a previous PTD (Table 2).

Three or more spontaneous and/or induced abortions were more common among PTD women than the women of the other two groups (i.e., TD women and controls), although this was not statistically significant (Table 1). One PTD woman with BV had a history of three abortions (Table 2). The frequency of one or two spontaneous and/or induced abortions did not differ significantly among the different groups (data not shown).

Previous history of sexually transmitted diseases was similar in the three groups (Table 2). Although some women reported being treated for bad-smelling vaginal discharge, it was not possible to get any reliable data concerning earlier episodes of BV since this expression was unknown for more than half of the women.

During the present pregnancy, two TD women had been treated for chlamydial cervicitis 4 or more weeks prior to enrollment. None of five women with candidiasis was symptomatic, and none was treated for the condition. Only one PTD woman had a urinary tract infection, which was verified by culture taken at the time of PTL.

Two women had had vaginal bleeding at some occasion during the pregnancy, and 10 PTD women and two TD women had some bleeding associated with contractions (Table 2).

Pregnancy outcome. All but four PTL women received parenteral or oral tocolytic therapy (Table 2). The mean number of days from onset of PTL to delivery is shown in Table 1.

The 22 infants delivered preterm had a mean birth weight of 1,937 ± 505 g. The 15 children born before 34 completed weeks of gestation had a mean weight of 1,641 ± 359 g

TABLE 2. Characteristics of women in PTL and term labor[a]

Characteristic	Result for PTL cases (n = 49)						Result for term labor controls (n = 38)	
	PTD (n = 22)				TD (n = 27)			
	<34 weeks (n = 15)		34–36 weeks (n = 7)					
	BV (n = 6)	Non-BV (n = 9)	BV (n =3)	Non-BV (n = 4)	BV (n = 3)	Non-BV (n = 24)	BV (n = 4)	Non-BV (n = 34)
Demographic								
Maternal age (yr)	30.2 ± 2.1	30.0 ± 3.4	29.1 ± 3.2	28.3 ± 4.8	27.2 ± 3.3	26.0 ± 4.4	27.9 ± 3.3	28.3 ± 4.8
No. <20 years	0	0	0	0	0	5	0	0
No. (%) >35 years	1	3	0	2	0	1	0	2 (6)
No. (%) married	6 (100)	9 (100)	3 (100)	4 (100)	3 (100)	21 (88)	4 (100)	34 (100)
No. (%) of smokers	3 (50)	4 (44)	1	2 (50)	3 (100)	7 (29)	0	8 (24)
No. (%) of alcohol users								
Daily	0	2 (22)	0	0	0	0	0	0
Occasionally	1	1	0	1	1	3 (13)	1	2 (6)
Reproductive history								
No. (%) nulliparous	2 (33)	3 (33)	2 (67)	2 (50)	1	14 (58)	2 (50)	19 (56)
No. (%) primigravida	1	2 (22)	1	2 (50)	2 (67)	11 (46)	1	12 (35)
No. (%) with previous PTD	0	5 (56)	0	0	0	2 (8)	0	0
No. (%) with ≥3 spontaneous or induced abortions	1	2 (22)	0	0	0	1	0	0
No. (%) with previous STD[b]	1	0	1	0	0	3 (13)	0	2 (6)
No. (%) with vaginal bleeding								
During the pregnancy	1	0	0	0	0	1	0	0
Associated with the contractions	2 (33)	4 (44)	2 (67)	2 (50)	1	1	0	0
Pregnancy outcome								
No. (%) receiving tocolytic therapy	4 (67)	8 (89)	2 (67)	4 (100)	3 (100)	24 (100)	0	0
Neonate mean birth weight (g)	1,565 ± 309	1,691 ± 382	2,223 ± 318	2,834 ± 171	3,498 ± 283	3,511 ± 425	3,714 ± 253	3,709 ± 534
No. (%) of low-birth-weight neonates (<2,500 g)	6 (100)	8 (100)	2 (67)	0	0	0	0	0
No. (%) of very-low-birth-weight neonates (<1,500 g)	3 (50)	2 (22)	0	0	0	0	0	0
No. (%) of infants with serious diagnoses[c]	6 (100)	9 (100)	1	0	0	0	0	0

[a] The women are subdivided into those with (BV) and those without (non-BV) BV.
[b] STD, sexually transmitted diseases (chlamydial cervicitis, except for one case of trichomoniasis in the TD group).
[c] Diagnoses of a more serious character included septicemia in two cases, pulmonary distress, and idiopathic respiratory distress syndromes.

(range, 920 to 2,220 g). The 7 children born between 34 and 36 weeks had a mean weight of 2,572 ± 345 g (range, 1,955 to 3,060 g; $P = 0.0001$; Table 1).

Of the 49 women with PTL, 67% (8 of 12) of women with BV were delivered of low-birth-weight neonates (<2,500 g) compared with 22% (8 of 37) of women without the condition (Table 2; $P < 0.0005$). Among the 22 PTD women, the 9 women with BV had neonates (three girls and six boys) with significantly lower mean birth weights than the 13 women without the condition (four girls and nine boys; 1,765 ± 389 g versus 2,242 ± 587 g, $P < 0.05$). In the group with delivery between 34 and 36 weeks of gestation, the corresponding values for women with BV and those without were 2,223 ± 318 g versus 2,834 ± 171 g ($P < 0.05$; Table 2); the two low-birth-weight neonates (<2,500 g; one girl and one boy) in this group were born to mothers with BV (Table 2). In the group with delivery prior to 34 weeks, the six women with BV also had neonates (one girl and five boys) with lower mean birth weights (1,565 ± 309 g versus 1,691 ± 382 g) and an increased proportion of neonates with very low (<1,500 g) birth weights (3 of 6 [50%] versus 2 of 9 [22%]) compared with the nine women without the condition (two girls and seven boys; Table 2). These differences are not statistically significant.

The mean weight of the infants in the TD group was 3,574 ± 425 g (range, 2,625 to 4,315 g), and that in the control group was 3,703 ± 367 g (range, 3,225 to 4,940 g; Table 1). In the latter two groups, there was no difference in birth weight between neonates born to mothers with or without BV (Table 2). There was no significant difference in mean number of gestation weeks between women with and without BV within the different groups (Table 2).

Sixteen (73%) of the 22 preterm-born infants had diagnoses of a serious character (Table 2). All children were alive 2 months postpartum.

Microbiology. (i) Sexually transmitted agents. None of the women had positive cultures for *C. trachomatis* or *N. gonorrhoeae* or positive microscopy for trichomonads. One TD woman had genital herpes during the pregnancy but not in relation to partus.

(ii) Miscellaneous organisms. Bacterial isolates that occurred in fewer than 20% of the women and whose distribution did not differ significantly among the different groups (*Enterococcus* spp., viridans group streptococci, coagulase-

TABLE 3. BV and vaginal occurrence of microorganisms in women in PTL and term labor

BV or organism	No. (%) of women in which BV or organism occurred			P		
	PTL cases (n = 49)					
	PTD (n = 22)	TD (n = 27)	Term labor controls (n = 38)	PTD vs TD	PTD vs controls	TD vs controls
BV	9 (41)	3 (11)	4 (11)	<0.05	<0.01	NS[a]
Fusobacterium nucleatum	8 (36)	0	0	0.001	0.001	NS
Mobiluncus curtisii	8 (36)	0	0	0.001	0.001	NS
Mobiluncus mulieris	6 (27)	1	0	<0.05	<0.01	NS
Porphyromonas asaccharolytica	8 (36)	1	1	<0.005	0.001	NS
Prevotella bivia	10 (45)	1	2 (5)	0.001	0.0001	NS
Prevotella disiens	11 (50)	5 (19)	3 (8)	<0.05	<0.001	NS
Prevotella melaninogenica	6 (27)	1	2 (5)	<0.05	<0.05	NS
Peptostreptococcus anaerobius	12 (55)	5 (19)	7 (18)	<0.05	<0.01	NS
Peptostreptococcus asaccharolyticus	12 (55)	6 (22)	8 (21)	<0.05	0.01	NS
Gardnerella vaginalis	14 (64)	19 (70)	21 (55)	NS	NS	NS
Gardnerella vaginalis >2+[b]	11 (50)	0	0	0.0001	0.0001	NS
Mycoplasma hominis	10 (45)	7 (26)	3 (8)	NS	<0.005	NS
Lactobacillus spp.						
H_2O_2[c]	4 (18)	21 (78)	34 (89)	0.0001	0.0001	NS
Non-H_2O_2[d]	12 (55)	16 (59)	12 (32)	NS	NS	<0.05

[a] NS, not significant.
[b] *G. vaginalis* recovered in 3+ or 4+ amounts.
[c] Hydrogen peroxide-producing facultative lactobacilli.
[d] Non-hydrogen peroxide-producing facultative lactobacilli.

negative staphylococci, *Corynebacterium* spp., *Bifidobacterium* spp., and anaerobic *Lactobacillus* spp.) were not tabulated. Isolates that occurred only in individual women in the groups (*Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Serratia marcescens*, *Campylobacter fetus* subsp. *fetus*, other *Campylobacter* spp., *Haemophilus* spp., and group B and G streptococci) were also not tabulated. None of the women had positive cultures for *Staphylococcus aureus* or *Listeria monocytogenes*.

The occurrences of the different vaginal microorganisms and BV in the women with PTL and in the controls are shown in Table 3. Except for *G. vaginalis*, these organisms consistently occurred in at least 2+ amounts but mostly in 3+ or 4+ amounts. The relative risk of PTD in women with BV at the onset of labor is shown in Table 4. In Table 5, the occurrence of the different microorganisms is reported with the women subdivided into those with and those without BV.

(iii) BV-associated organisms. With the exception of *G. vaginalis*, all BV-associated organisms were significantly more common in PTD women than in controls (Table 2). With an additional exception of *M. hominis*, this was also true in a comparison of PTD and TD women (Table 3). In the study population, each of the BV-associated organisms shown in Table 5 was significantly more common in women with BV than in those without the condition (P = 0.0001 for each comparison).

TABLE 4. Relative risk of PTD in women with BV at onset of labor

Outcome	No. of women with outcome		Relative risk[a]	95% CI
	With BV (n = 12)	Without BV (n = 37)		
Preterm birth at 34 to 37 weeks of gestation (n = 22)	9	13	2.10	1.2–3.7
Preterm birth at <34 weeks of gestation (n = 15)	6	9	2.05	0.9–4.6

[a] For details, see Materials and Methods.

The occurrence of *G. vaginalis* was similar in women in the PTD, TD, and control groups. However, cultures revealing large numbers of the organism (3+ or 4+ amounts) were significantly more common in PTD women than in either TD women (P = 0.0001) or controls (P = 0.0001; Table 3) as well as in PTL women compared with controls (11 of 49 [22%] versus 0 of 38, P < 0.001; Table 3). All women with BV, regardless of their delivery group, harbored *G. vaginalis* in contrast to 33 to 67% of women without BV (Table 5). Thus, among the study population, *G. vaginalis*, irrespective of the level quantitated, was significantly more common in women with BV than in those without BV (100% versus 57%, P = 0.0001).

The two *Mobiluncus* species and *Prevotella melaninogenica* were the only organisms exclusively recovered from women with BV; *Mobiluncus curtisii* was recovered only from PTD women (Table 5).

Prevotella disiens, *Prevotella bivia*, *Porphyromonas asaccharolytica*, and *Fusobacterium nucleatum* occurred only in women with BV or abundant mixed flora consisting mainly of non-BV-associated organisms, lacking or with low numbers of H_2O_2-producing lactobacilli. *F. nucleatum* was recovered only from PTD women (Table 5).

Bacteroides ureolyticus and *Prevotella intermedia* occurred in two PTD women each, all with BV.

Mycoplasma hominis was significantly more common among PTD women than among controls (P < 0.005; Table 3) as well as among PTL women than among controls (17 of 49 [35%] versus 3 of 38 [8%], P = 0.0001). The organism was more common in women with BV than in women without the condition: in PTL women, it was present in 9 of 12 (75%) versus 8 of 37 (22%), P = 0.0001; in PTD women, it was

TABLE 5. Vaginal occurrence of microorganisms in women in PTL and term labor[a]

Organism	No. (%) of women in which organism occurred							
	PTL cases (n = 49)						Term labor controls (n = 38)	
	PTD (n = 22)				TD (n = 27)			
	<34 weeks (n = 15)		34–36 weeks (n = 7)					
	BV (n = 6)	Non-BV (n = 9)	BV (n = 3)	Non-BV (n = 4)	BV (n = 3)	Non-BV (n = 24)	BV (n = 4)	Non-BV (n = 34)
BV associated								
Fusobacterium nucleatum	5 (83)	2 (22)	1	0	0	0	0	0
Mobiluncus curtisii	5 (83)	0	3 (100)	0	0	0	0	0
Mobiluncus mulieris	4 (67)	0	2 (67)	0	1	0	0	0
Porphyromonas asaccharolytica	4 (67)	1	3 (100)	0	1	0	1	0
Prevotella bivia	6 (100)	1	3 (100)	0	1	0	2 (50)	0
Prevotella disiens	5 (83)	2 (22)	3 (100)	1	2 (67)	3 (13)	3 (75)	0
Prevotella melaninogenica	5 (83)	0	1	0	1	0	2 (50)	0
Peptostreptococcus anaerobius	6 (100)	1	3 (100)	2 (50)	3 (100)	2 (8)	4 (100)	3 (9)
Peptostreptococcus asaccharolyticus	6 (100)	2 (22)	2 (67)	2 (50)	3 (100)	3 (13)	3 (75)	5 (15)
Gardnerella vaginalis	6 (100)	3 (33)	3 (100)	2 (50)	3 (100)	16 (67)	4 (100)	17 (50)
Mycoplasma hominis	5 (83)	2 (22)	2 (67)	1	2 (67)	5 (21)	2 (50)	1
Others								
Lactobacillus spp.								
H_2O_2[b]	0	2 (22)	0	2 (50)	0	21 (88)	1	33 (97)
Non-H_2O_2[c]	1	7 (78)	0	4 (100)	1	15 (63)	1	11 (33)
Escherichia coli[d]	1	3 (33)	1	2 (50)	0	3 (13)	0	4 (12)
Ureaplasma urealyticum[d]	2 (33)	4 (44)	1	2 (50)	1	11 (46)	1	16 (47)
Candida albicans[d]	0	1	0	2 (50)	1	7 (29)	0	4 (12)

[a] The women are subdivided into those with and without BV (BV and non-BV, respectively).
[b] Hydrogen peroxide-producing facultative lactobacilli.
[c] Non-hydrogen peroxide-producing facultative lactobacilli.
[d] No significant difference in the recovery rates among the different groups or among women with (BV) or without (non-BV) BV.

present in 7 of 9 (78%) versus 3 of 13 (23%), $P = 0.01$; in TD women, it was present in 67% versus 21%, not significant; and in controls, it was present in 2 of 4 (50%) versus 1 of 34 (3%), $P < 0.05$ (Table 5).

(iv) Lactobacilli. There was a significant difference in the recovery rates of H_2O_2-producing facultative *Lactobacillus* spp. from the vaginas of PTD women and those from TD women and controls ($P = 0.0001$ for each comparison; Table 3). Lactobacilli were recovered from only 18% of PTD women and from 78 and 89% of TD women and controls, respectively. Among the 49 PTL women, H_2O_2-producing lactobacilli were significantly less common in women with BV (0 of 12) than in women without BV (25 of 37; $P = 0.0001$). A similar observation was made in the control group: H_2O_2-producing lactobacilli occurred in 1 of 4 (25%) women with BV versus 33 of 34 (97%) without BV ($P = 0.0001$) (Table 5). The women in the different groups who were lacking H_2O_2-producing lactobacilli had either BV, profuse mixed flora consisting of at least five different, mainly non-BV-associated species, or candidiasis.

Non-H_2O_2-producing *Lactobacillus* spp. were more common, but not significantly so, in PTD and TD women than in controls (Table 3). These lactobacilli were significantly more common in women without BV than in those with the condition: in PTL women, they were found in 26 of 37 (85%) versus 2 of 12 (11%), $P = 0.0001$; in controls, they were found in 11 of 34 (32%) versus 1 of 4 (25%), $P = 0.0001$ (Table 5).

None of the anaerobic *Lactobacillus* strains isolated produced H_2O_2.

(v) Yeast species. *Candida albicans*, the only yeast species isolated, was more frequently recovered from TD women than from the PTD women or controls (Table 5). These differences were not significant. The organism occurred in only one woman with BV. Candidiasis was diagnosed in five women, four in the TD group and one in the PTD group: they all had 4+ pure cultures of *C. albicans*. Yeast cells, pseudohyphae, and 4+ PMN were seen in the vaginal smears.

Overall, only 3 (14%) of the 22 PTD women had normal, clean lactobacillus smears and vaginal cultures dominated by facultative H_2O_2-producing lactobacilli compared with 20 of 27 TD women (74%; $P = 0.0001$) and 32 of 38 controls (84%; $P = 0.0001$).

No PMN were detected in the vaginal smears from 8 of the 12 PTL women with BV (67%), while 4 had 1+ amounts. Except for five PTD women with 2+ amounts, PMN occurred in at least 3+ amounts in all other PTD women and controls.

DISCUSSION

The etiology of PTL is multiple and poorly understood. Some obstetrical conditions such as polyhydramniosis, preeclampsia, and malformations are more or less obvious, while in many cases the pathophysiological mechanisms still are unknown. To date, causes of prematurity can be found in fewer than half of all cases (1). During the last three decades, the prevention and treatment of certain intercurrent pregnancy-induced diseases such as diabetes mellitus and erythroblastosis fetalis have decreased the perinatal morbidity and mortality. In addition, because of more advanced medical facilities, the survival frequency of premature neonates

with very low birth weights has increased considerably. However, such medical progress is in contrast to the almost nonexistent decrease in PTL and PTD. This is especially true for so-called idiopathic PTL (10). The accurate identification of a patient at risk for PTD is of immense importance for prevention and treatment. Efforts to distinguish special risk groups and identify early diagnostic signs of threatening PTD have been mainly without effect. Current nonspecific and nonsensitive identification methods have included obstetrical history, demographic factors, and premonitory symptoms (38). The recent demonstration of fetal fibronectin in cervicovaginal secretions of pregnant women during the second and third trimesters has been identified as a reliable predictor of PTD (35). The concentration of fetal fibronectin is measured with a sensitive immunoassay with the monoclonal antibody FDC-6 (41).

The presence of certain genital microfloras has been associated with increased risks for prematurity. Martin et al. (39) reported the first cohort study to document a relationship between cervical occurrence of *C. trachomatis* and prematurity, a relationship supported in several recent studies (2, 17, 40) but not in others (18, 19). Untreated *N. gonorrhoeae* has been associated with PTD and low birth weight: its causative role is suggested by reduced rates of PTD after appropriate treatment (13, 64). In their review on infection and PTL, Romero and Mazor concluded that no convincing evidence supports an association between *T. vaginalis* and prematurity (58). In the present study, none of the women had positive cultures for *C. trachomatis* or *N. gonorrhoeae* at enrollment. Neither were any trichomonads detected by microscopy.

A significant association between enteropharyngeal bacteria, i.e., *Escherichia coli*, *Klebsiella* spp., *S. aureus*, and *Haemophilus* spp., and PTL has been reported (44). This is in contrast to the findings in our study in which the occurrences of such organisms were similar among the different groups of women. Neither were such bacteria associated with the presence of BV.

Recently, BV has been associated with adverse pregnancy outcome (15–17, 40). BV is the most common vaginal disorder seen in women of reproductive age in primary health care (26). The main complaint of women with this condition is a malodorous vaginal discharge. However, many women with demonstrable signs have no symptoms. In the absence of coexisting infections, the inflammatory reactions of the vaginal epithelium are absent or not prominent in BV. The condition is diagnosed in one of five pregnant women (17, 31, 49). All bacterial species associated with BV occur among the endogenous flora of the intestinal tract (23).

The presence of BV in middle and late gestation has been associated with PTL, preterm rupture of membranes, and PTD (16, 17, 40, 44, 47). Also, BV in early pregnancy has been associated with increased risks for such adverse pregnancy outcomes in one study (31) but not in another (49). However, different criteria for the diagnosis of BV were used in these studies, i.e., clinical criteria (49), Gram stain criteria (40), abnormal gas-liquid chromatography pattern (14, 16, 17), and the presence of *G. vaginalis* and *Bacteroides* spp. in a vaginal culture (31, 44). Also, the study designs differed markedly, e.g., regarding enrollment of cases, study population, exclusion criteria, time of enrollment, specimens taken, and time span from diagnosis of BV to delivery as well as information given on the outcome of pregnancy. However, in spite of all these differences, almost all studies have shown an association between BV and prematurity, supporting the hypothesis that the microflora involved in BV is a risk factor for prematurity. BV was also associated with infection of the chorioamnion (17, 21). Thus, the condition appears to predispose for an ascending infection of the chorioamnion and amniotic fluid, which eventually may lead to PTD. In the present study, BV was significantly associated with PTD ($P < 0.01$) but not with PTL. The condition was associated with a 2.1-fold increase of the risk of PTD prior to 37 weeks of gestation.

Regarding factors previously found to correlate with an increased risk of adverse pregnancy outcome, we found advanced maternal age (>35 years) significantly associated with PTD. PTD, TD, and control women, regardless of whether they had BV, were comparable regarding marital and socioeconomic status, physically demanding leisure activities, and history of previous sexually transmitted diseases.

Maternal cigarette smoking has been associated with PTD and preterm rupture of membranes in some studies (7, 66, 73) but not in others (47, 53). In an epidemiologic study, a 2.1-fold increase of the risk of preterm birth was found among women who smoked cigarettes throughout pregnancy compared with nonsmokers (73). However, cigarette smoking and coffee consumption were the only factors measured in relationship to occurrence of adverse pregnancy outcome (73). In the present study, cigarette smoking was significantly associated with PTL but not with PTD.

A history of prior PTD has been associated with a two- to sixfold risk of recurrence of PTD (1, 40, 47). Also in the present study, a significantly higher frequency of previous PTDs was found among PTD women than among controls ($P < 0.01$). None of the women with BV had a history of previous PTD, thus eliminating the single best historical predictor of PTD in these women.

A history of three or more spontaneous and/or induced abortions has been correlated with an increased risk of PTL (47). In this study, only a few PTL women, one with BV, had such a history.

With the exception of group B streptococci (51), the role of urinary tract infection and asymptomatic bacteriuria in PTD is not well established. McGregor and coworkers reported frequent urinary tract infection and antepartum treatment for urinary infection significantly related to development of PTL (47). In this study, only one woman had a urinary tract infection, caused by *Proteus mirabilis*, and she delivered preterm.

BV has been associated with low birth weight. Gravett et al. reported that women with BV had neonates with lower mean birth weights (2,960 ± 847 g versus 3,184 ± 758 g, $P < 0.01$) and an increased proportion of neonates with birth weights of <2,500 g (24% versus 15%, $P < 0.05$) compared with women without the condition; no information on fetal gender was given (17). In agreement, we also found BV associated with low birth weight; of the 49 PTL women, 67% of women with BV were delivered of low-birth-weight neonates (<2,500 g) compared with 22% of women without the condition ($P < 0.001$). Despite the fact that 2 PTD women without BV were delivered of neonates with the lowest birth weights in the study, 970 and 920 g, respectively, the 9 PTD women with BV had neonates with lower mean birth weights (1,765 ± 389 g versus 2,242 ± 587 g, $P < 0.05$) than the 13 women without the condition. The numbers of boys versus girls delivered were similar in the different groups. The mean numbers of gestation weeks were similar for women with and without BV within the different groups.

In a cohort study, McGregor et al. found that women carrying female fetuses appeared to have an increased risk of

PTL compared with women carrying male fetuses, with the highest probability for women with BV (males, relative risk of 4.6; females, relative risk of 11.3) (47). However, no information as to whether the women were delivered of preterm or full-term infants was given. In the present study, nearly 70% (15 of 22) of the PTD neonates (≤36 weeks) were boys, regardless of whether their mothers had BV (6 of 9 [67%] versus 9 of 13 [69%]).

PMN most probably play an important defensive role at the mucosal surface. In vaginal smears from pregnant women, the numbers of PMN most often are higher than those of nonpregnant women. In this study, most women had high numbers, i.e., ≥3+ levels, of PMN. Interestingly, PTL women with BV had no or only low numbers, i.e., 1+ levels, of PMN, while controls with BV all had ≥3+ levels.

It has been reported that pregnant women with vaginal colonization by facultative lactobacilli producing H_2O_2 were less likely to have BV, symptomatic candidiasis, or vaginal occurrence of the BV-associated organisms *G. vaginalis*, *Bacteroides* species, peptostreptococci, *M. hominis*, or viridans streptococci (25). These findings are in agreement with those in the present study; the women who were lacking H_2O_2-producing lactobacilli had either BV, profuse mixed vaginal floras consisting of at least five different, mainly non-BV-associated species, or candidiasis. The recovery of H_2O_2-producing facultative *Lactobacillus* spp. was highly negatively associated with both PTD and BV. Also, non-H_2O_2-producing *Lactobacillus* spp. were negatively associated with BV. Thus, normal, clean lactobacillus smears and vaginal cultures dominated by facultative H_2O_2-producing *Lactobacillus* species were strongly negatively associated with PTD.

In the present study, we found that all of the BV-associated bacteria and *G. vaginalis* only when present in high numbers were significantly associated with PTD. In addition, their presence was strongly associated with BV, irrespective of the levels of the bacteria.

Even when semiquantitative techniques have been used, growth of *G. vaginalis* has been shown to be nonspecific for the detection of BV (29). The recovery of *G. vaginalis* was also not associated with PTL or PTD (47). Gravett et al. reported similar recovery rates of *G. vaginalis* among women in PTL and among controls (16). In agreement, we found no difference in the presence of *G. vaginalis* in women in the PTD, TD, and control groups. However, the occurrence of high levels of the organism was strongly associated with both PTL and PTD.

Mobiluncus spp. are highly specific for cases of BV, in which they frequently occur (26). McGregor et al. reported that for women with BV who also had *Mobiluncus* morphotypes identified by Gram stain, the relative risk of PTL was even higher than for women with BV without the organisms (relative risk, 3.8; 95% CI, 1.32 to 11.50) (47). In the present study, the two *Mobiluncus* species were recovered exclusively from BV-positive women with PTL; *M. curtisii* was recovered only from those with PTD.

The presence of vaginal *Bacteroides* spp. has been associated with an increased hazard of PTD. High concentrations ($>10^4$ CFU/ml) of *P. bivia* were associated with a 2.0-fold (95% CI, 1.4 to 2.9) risk of PTD, and *Bacteroides fragilis* was associated with a 1.7-fold (95% CI, 1.0 to 2.8) risk of preterm birth (31). However, no information on the presence of BV was given. Women with vaginal occurrence of *Bacteroides* spp. at their first prenatal visit had a 40% increased PTD rate compared with those without such organisms (49). In addition, *Bacteroides* spp. (with the identification of *Bacteroides fragilis* and other *Bacteroides* species) were associated with a 1.5-fold (95% CI, 1.2 to 1.9) risk of PTL (44). In the present study, we found *P. bivia*, *P. disiens*, *P. melaninogenica*, and *P. asaccharolytica* (all previously belonging to the genus *Bacteroides*) to be associated with PTD cases as well as strongly associated with cases of BV, in which they occurred in high numbers. None of the women harbored *B. fragilis*, which in our experience is a rare isolate in fertile women without underlying malignancy.

Fusobacterium species occur among the endogenous flora of the oral cavity and the intestinal tract in humans and animals. The well-known clinical importance of *F. nucleatum* in human infections is regularly reported. Still, the virulence mechanisms of the organism remain largely unknown. Unlike previous findings, new chemotaxonomic methods have revealed that *F. nucleatum* strains could be placed in three broad groups, within which evidence of further subgroups is apparent (34). In our study, *F. nucleatum* was recovered only from PTD women with BV or women with disturbed abundant flora lacking H_2O_2-producing lactobacilli.

The role of genital mycoplasmas in prematurity remains controversial. Our study confirms the findings of others that genital colonization by *U. urealyticum* is not associated with increased risk of PTD (19, 39, 47, 69). *U. urealyticum* occurred in almost 45% of all women, and its presence was not associated with any other special microbial findings or BV. Maternal cervicovaginal colonization by *M. hominis* has been inconsistently associated with PTD. Some investigators have found a positive association (33, 40, 47, 52) not confirmed by others (19, 48, 69). In our study, *M. hominis* was significantly associated with both PTL and PTD. However, the organism was also significantly associated with cases of BV, in which it occurred in high numbers.

Rabe et al. reported the prevalence of viridans group streptococci and their association with BV in pregnant women (54). Of 482 women, 147 (30%) harbored such organisms; *Streptococcus acidominimus* and *Streptococcus morbillorum* were significantly more common among women with BV than among those without BV, whereas the distributions of other species were similar in the two groups of women. Viridans streptococci were not associated with BV by multivariate analysis (29). In the present study, none of the species of viridans group streptococci was significantly associated with BV or PTD.

Several cervicovaginal microorganisms produce proteases, sialidase (neuraminidase), and mucinase, which may facilitate their passage across the cervical barriers to the lower uterine segment, as well as collagenases, which may focally contribute to a weakness of chorioamnion (45, 46). However, the mechanisms whereby microorganisms may initiate PTL and PTD have not been established. One theory invokes the ability of some bacteria to release high levels of phospholipase A_2 (4, 5). Phospholipase A_2 releases the arachidonic acid bound to fetal chorioamnion and maternal decidua tissue with the resultant increase in prostaglandin E_2 production (6, 32, 50), which in turn stimulates myometrial contractions and effects cervical changes. Although the precise role of prostaglandins is not yet defined, it is likely that these agents play a fundamental role in term labor (9). However, for PTL, evidence supporting such a role is less firm and our understanding of what clinical importance a local increase of prostaglandin concentration may have is limited (8, 36, 37, 60). Relatively fulminant cervicovaginal infections may exist without causing uterine contractions. Quantitative as well as qualitative aspects of the cervicovag-

inal microflora may be important (45). Bennett et al. showed that the addition of purified phospholipase A_2 to amnion cells resulted in changes in arachidonic metabolism identical to those caused by the addition of bacterial products (6). Although the provoking bacterial products have not been defined, lipopolysaccharides, one of the major components of the outer membrane of gram-negative bacteria, and lipoteichoic acid, bound to the membrane of many gram-positive bacteria, have been found to stimulate prostaglandin production by amnion and decidual cells (8, 57, 59). However, the effects of microbial products on prostaglandin production by intrauterine tissues are highly dose dependent (36, 50); the concentration of lipopolysaccharide required to elicit prostaglandin formation by amnion cells is rarely present in the amniotic cavity (61). Other suggested mechanisms behind infection-associated PTL include the production of cytokines, e.g., tumor necrosis factor and interleukin-1, which are endogeneous host products secreted by stimulated monocytes or macrophages. Both interleukin-1 and tumor necrosis factor stimulate prostaglandin production by human amnion and decidual cells (8, 56, 59). Higher levels of interleukin-6 were found in the amniotic fluid of women in PTL with intra-amniotic infection than in women in PTL without such infection (63).

Obviously, the question arises whether treatment with antibiotics should be presented in cases of threatening idiopathic PTL when signs of cervicovaginal infection are present. Recent trials allow no definite conclusion regarding the efficacy of antibiotics in prolonging pregnancy in such cases. However, there is one trial implying that erythromycin therapy against genital mycoplasmas improved the pregnancy outcome, i.e., it increased birth weight (43). Yet, another one found no improvement regarding low birth weight, PTL or PTD, or preterm rupture of membranes with erythromycin treatment (52); the results in both studies were compared with those of a placebo. At present, the knowledge and diagnostic methods are not sufficient for recommending antibiotic therapy in routine clinical practice. However, randomized trials involving selected groups of different cervicovaginal infection(s) and/or undesirable microbial colonization associated with PTL need to be conducted.

Today, metronidazole is generally accepted as the drug of choice for treatment of BV. Since metronidazole is contraindicated in the first trimester because of a possible teratogenic effect, amoxicillin, although less effective than metronidazole, has been suggested as an alternative treatment of the condition in women during this time period of the pregnancy (11). Also, cefadroxil might be an alternative therapy against BV in pregnant women (71). Erythromycin is not effective against BV. Oral erythromycin treatment was effective in only 4 of 13 cases of BV, a result interpreted as reduced efficiency of the drug in the acidic milieu in the vagina (12). Treatment failure (persistence of three or more clinical signs of BV) occurred in 81% (13 of 16) of patients given erythromycin compared with 17% (3 of 18) of women treated with metronidazole ($P < 0.001$) (72). Intermittent vaginal treatment with a lactate gel, which lowers the vaginal pH, thus restoring the normal acidity in the vagina without any systemic effect, has been reported to be effective against BV in nonpregnant women in a controlled study (3) as well as in pregnant women in an open trial (24). The encouraging therapeutic results in these studies indicate the need for further controlled studies of pregnant women. That therapy effective against BV early in pregnancy may serve as a prophylactic measure beneficial to pregnancy outcome is an exciting possibility which remains to be explored. Controlled trials with antibiotics or alternatives versus placebo are necessary to further clarify a correlation between the condition and PTD and low birth weight.

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