JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1990, p. 2035–2039
0095-1137/90/092035-05$02.00/0

Vol. 28, No. 9

Reservoir of Four Organisms Associated with Bacterial Vaginosis Suggests Lack of Sexual Transmission

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Received 26 February 1990/Accepted 19 May 1990

This study consisted of a search for the possible reservoir and mode of spread of the four bacterial vaginosis-associated organisms *Mobiluncus mulieris, Mobiluncus curtisii, Mycoplasma hominis*, and *Gardnerella vaginalis*. Their occurrence in rectal, oral, and pharyngeal specimens from women with and without bacterial vaginosis, their male sexual consorts, four homosexual men, and children (altogether, 374 people) was studied. Genital samples were also obtained from all adults. All four organisms were isolated from the rectums of 45 to 62% of women with bacterial vaginosis and 10 to 14% of women without bacterial vaginosis. They also occurred in the rectums of males and children. *M. hominis* was recovered from the oropharynxes of 12 adults whose sexual consorts had genital occurrences of the organism. *Mobiluncus* spp. occurred only in the vaginas of women with bacterial vaginosis (97%). The organisms were only infrequently recovered from genital samples from 135 males. Organisms were recovered from the urethras and/or coronal sulci of 10 of 44 male consorts of women with bacterial vaginosis. However, after 2 weeks of condom use during sexual intercourse, only *M. hominis* remained in the urethra of one man. These findings suggest that the organisms associated with bacterial vaginosis are not spread sexually but colonize the vagina from an endogenous intestinal tract site. The pathophysiological mechanisms leading to bacterial vaginosis in a subpopulation of all women are still unknown.

The microbial spectrum of the vaginal flora of women with bacterial vaginosis (BV) has been studied in detail during recent years (1, 3, 13, 24, 27). Particular attention has been devoted to *Gardnerella vaginalis* and, more recently, to certain anaerobes, especially *Mobiluncus mulieris* and *Mobiluncus curtisii*, as well as *Mycoplasma hominis*. However, the exact etiology of bacterial vaginosis is still unknown. *G. vaginalis* and *M. hominis* can occur as endogenous organisms in the vaginal flora in a population of healthy women but are numerically increased in women with BV, in whom they are present in most cases. In contrast, *Mobiluncus* spp. are rarely found in healthy women, but are isolated from the vaginas of women with BV in up to 90% of cases (13). *G. vaginalis*, *M. hominis*, and *Mobiluncus* spp. may all occasionally ascend to the upper genital tract of females and have been isolated from cases of endometritis and salpingitis (18, 30; E. Holst, Abstr. 4th Eur. Congr. Clin. Microbiol. 1989, 357, p. 148). Likewise, all these organisms may invade the urinary tract (4, 19; E. Holst, unpublished data). They have also been isolated from abscess contents and wound infections (7, 23, 25; E. Holst, Ph.D. thesis, University of Uppsala, Uppsala, Sweden, 1987). In addition, both *G. vaginalis* and *M. hominis* have been isolated from blood specimens (6, 22).

The pathophysiological mechanisms that lead to BV in subpopulations of women are still unknown. This study consisted of a search for the possible reservoir as well as modes of transfer of four BV-associated microorganisms, i.e., *G. vaginalis*, *M. hominis*, *M. mulieris*, and *M. curtisii*. Their occurrences in the rectum, mouth, and oropharynx were studied in healthy women with a normal lactobacillus-dominated vaginal flora, women with BV, their male partners, as well as children. Genital specimens from the adults were also studied.

MATERIALS AND METHODS

Study population. The study encompassed six groups of people: (i) 148 women with clinically proven BV, i.e., at least three of the following criteria were fulfilled: a vaginal pH of >4.7, a positive amine test, the presence of "clue cells" in a vaginal smear, and a characteristic, homogeneous, adherent vaginal discharge; all these women had had several episodes of recurrent BV during the years before they entered the study; (ii) 69 healthy women with a normal lactobacillus-dominated vaginal flora; with the exception of two women, who had a vaginal pH of 4.8, none of these women fulfilled any of the four criteria for BV; (iii) 82 male partners of 82 of the women with BV; (iv) 49 male partners of 49 of the healthy women; (v) 4 homosexual men (two monogamous couples), (vi) 22 children (age, 2 to 11 years; 12 females; 10 males); none of the children had had any sexual experiences. All children and males were healthy. All males were uncircumcised. With the exception of the homosexual men, all persons denied having had anal intercourse. No person studied had received any antimicrobial treatment within the previous month.

Specimens were taken from the rectums, mouths, and oropharynxes of all 374 people. Vaginal specimens (posterior fornix) were obtained from all women. Specimens were also taken from the urethras, coronal sulci, and seminal fluid of the males. With the exception of 44 males (see below) and their sexual partners, no person had had sexual intercourse for at least 72 h prior to sampling. Urethral, rectal, and coronal sulcus samples were again obtained from these 44 males after 2 weeks of condom use during sexual intercourse. These men were consorts of women with BV who harbored all four organisms in their vaginas concomitantly. All 44 couples had had sexual intercourse less than 20 h prior to the first sampling.

TABLE 1. Recovery of *M. mulieris*, *M. curtisii*, *G. vaginalis*, and *M. hominis* from rectal specimens from different study groups

No. of subjects	Study group	No. (%) of subjects			
		M. mulieris	*M. curtisii*	*G. vaginalis*	*M. hominis*
148	Women with BV	83 (56)	92 (62)	66 (45)	80 (54)
69	Healthy women	8 (12)	10 (14)	7 (10)	8 (12)
82	Male partners of women with BV	7 (9)	9 (11)	4 (5)	6 (7)
49	Male partners of healthy women	3 (6)	5 (10)	2 (4)	4 (8)
4	Homosexual men	1	1	0	3
22	Children	1 (5)	2 (9)	2 (9)	2 (9)

Seminal fluid was collected in sterile glass tubes and transferred to rubber-stoppered tubes filled with CO_2 and to chopped meat broth (9). The rectum was externally cleansed prior to sampling. Rectal specimens were obtained by blindly passing a swab 3 cm into the anal canal, using lateral pressure to avoid sampling of feces. All swab specimens were collected with cotton-tipped, charcoal-treated swabs and transported to the laboratory in modified Stuart medium. All samples were cultured within 2 to 12 hours.

For the recovery of *Mobiluncus* spp., Columbia agar with 2.5% horse serum and nalidixic acid (15 mg/liter) and tinidazole (1.0 mg/liter) was used (10). Human blood bilayer agar with Tween 80 (28) was used for *G. vaginalis*, and modified Shephard medium (17) was used for *M. hominis*.

The growth of each of the four organisms in the different specimens was quantitated 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 areas.

M. mulieris, *M. curtisii*, *M. hominis*, and *G. vaginalis*, were identified as described previously (12, 17, 28).

RESULTS

Rectal specimens. The recovery rates of *M. mulieris*, *M. curtisii*, *G. vaginalis*, and *M. hominis* in rectal specimens from the 374 people in the six study groups are given in Table 1. Both for women with BV and healthy women, there were no significant proportional differences in rates of isolation of the four study organisms from the rectum. All organisms were, however, isolated less frequently from the healthy women, in whom the isolation rates were approximately the same as those in the males and the children (Table 1). Women with BV harbored at least two of the four organisms in their rectums concomitantly. A total of 47 of the 148 women harbored both *Mobiluncus* spp., 36 women harbored only *M. mulieris*, 45 women harbored *M. curtisii*, and 20 (14%) women harbored neither of the *Mobiluncus* species. Of the individuals in the other five study groups, only one healthy woman and one male partner of a healthy woman harbored two of the organisms in the rectum concomitantly; both had *M. curtisii* and *M. hominis*.

Among the children, one boy (age 2 years) and one girl (age 4 years) harbored *M. curtisii* in the rectum. Three girls (ages, 5, 8, and 9 years) had *M. mulieris*, *M. hominis*, and *G. vaginalis*, respectively. Two boys (ages, 3 and 10 years) harbored *G. vaginalis* and *M. hominis*, respectively.

Vaginal specimens. The recovery rates of the four organisms from vaginal specimens are given in Table 2. In healthy women, *G. vaginalis* and *M. hominis* were found in sparse numbers in 29 of 69 (42%) and 6 of 69 (9%) women, respectively. Only one healthy woman harbored both these organisms in the vagina. Two of the seven women with *G. vaginalis* in the rectum also harbored the organism in their vaginas. The corresponding figures for *M. hominis* were two of eight women.

Of the 148 women with BV, 111 harbored both *Mobiluncus* spp. in the vaginas concomitantly, 19 had only *M. mulieris*, 13 had only *M. curtisii*, and 5 had neither organism. In two of five women in this last group, a *Mobiluncus* sp. was recovered from the rectum. Of 36 women with vaginal cultures negative for *M. hominis*, 18 harbored the organism in their rectums.

Male genital specimens. The occurrences of *Mobiluncus* spp., *G. vaginalis*, and *M. hominis* in male genital samples are given in Table 3. One of the four organisms was recovered from 13 of 82 partners of women with BV. Only one man harbored more than one of them (*M. mulieris* and *M. hominis*). *Mobiluncus* spp. were never recovered from the male consorts of healthy women; one man harbored *G. vaginalis* and one man harbored *M. hominis*. All four homosexual men harbored *M. hominis*, and one homosexual man harbored *M. curtisii*.

All organisms recovered from seminal fluid were also found in the urethra. *M. curtisii* and *G. vaginalis* were more often found in samples from the coronal sulcus than in those from the urethra in one and two men, respectively.

Two partners of women with BV who had either *G. vaginalis* or *M. hominis* in the rectum harbored the respective organisms in the urethra and seminal fluid. *G. vaginalis* was also recovered from the coronal sulcus in these two men. Of the remaining men, only the four homosexual men had concomitant genital and rectal occurrences of the organisms: *M. hominis* in three men and *M. curtisii* in one man.

Effect of condom use. The isolation rates of the four microorganisms in 44 of the 82 male partners of women with BV are given in Table 4. None of the organisms was recovered from 10 of 44 men at the time of the initial

TABLE 2. Recovery of the four BV-associated organisms from the vaginas of 217 women

No. of subjects	Study group	No. (%) of subjects			
		M. mulieris	*M. curtisii*	*G. vaginalis*	*M. hominis*
148	Women with BV	130 (88)	124 (83)	146 (99)	112 (76)
69	Healthy women	0	0	29 (42)	6 (9)

TABLE 3. Recovery of the four BV-associated organisms from the urethras, coronal sulci, and seminal fluid of 135 males

Organism	No. of subjects[a]								
	Partners of women with BV (n = 82)			Partners of healthy women (n = 49)			Homosexual men (n = 4)		
	u	cs	sf	u	cs	sf	u	cs	sf
M. mulieris	1	0	1	0	0	0	0	0	0
M. curtisii	2	3	1	0	0	0	1	1	1
G. vaginalis	4	7	3	0	1	0	0	0	0
M. hominis	3	1	2	2	0	2	3	1	2

[a] u, Urethra; cs, coronal sulcus; sf, seminal fluid.

sampling. At the time of the second sampling, after 2 weeks of condom use during sexual intercourse, only 1 of 44 men still harbored *M. hominis* in the urethra. Similar isolation rates were found in the initial and second rectal specimens (Table 4).

Oropharyngeal specimens. Samples from the oropharynx were culture positive for *M. hominis* in 12 people: in two women with BV (with concomitant vaginal and rectal occurrences of this organism) as well as in their partners, in six males (whose partners all had *M. hominis* in their vaginas), and in two of the four homosexual men (whose partners had *M. hominis* in the urethra and the rectum, respectively). All remaining pharyngeal and oral specimens were culture negative for the four organisms studied.

Semiquantitation of numbers of colonies of the four organisms recovered from the different specimens. *M. mulieris*, *M. curtisii*, *G. vaginalis*, and *M. hominis* were isolated in 3+ or 4+ amounts from 39 of 83 (47%), 48 of 92 (52%), 19 of 66 (29%), and 46 of 80 (58%) rectal specimens from women with BV, respectively. With the exception of *M. mulieris* and *G. vaginalis*, which were present in 1+ amounts in two and three rectal specimens, respectively, all four organisms were isolated in 2+ amounts from the remaining specimens. The four organisms were isolated in 1+ amounts from 63 of 85 (74%) and in 2+ amounts from 22 of 85 (26%) rectal specimens from healthy women, males, and children.

M. mulieris, *M. curtisii*, *G. vaginalis*, and *M. hominis* were isolated in 3+ or 4+ amounts from 57 of 130 (44%), 79 of 124 (64%), 132 of 146 (90%), and 91 of 112 (81%), vaginal samples from women with BV, respectively. The remaining specimens all yielded 2+ amounts of the organisms.

G. vaginalis and *M. hominis* were isolated in 1+ or 2+ amounts from vaginal specimens from healthy women. The four organisms were isolated in 1+ or 2+ amounts from male genital specimens. The only exceptions were *M. hominis* and *G. vaginalis*, which were present in 3+ amounts in two specimens each from the coronal sulci of three partners of women with BV and one homosexual man. *M. hominis* was isolated in 2+ or 3+ amounts from oropharyngeal specimens.

TABLE 4. Recovery of the four BV-associated organisms from the urethras, coronal sulci, and rectums of 44 male partners of women with BV[a]

Organism	No. of subjects					
	Urethra		Coronal sulcus		Rectum	
	I[b]	II[c]	I	II	I	II
M. mulieris	1	0	0	0	3	3
M. curtisii	2	0	3	0	4	5
G. vaginalis	2	0	5	0	1	1
M. hominis	2	1	1	0	3	3

[a] All women harbored all four organisms concomitantly in their vaginas.
[b] I, Initial specimens, which were obtained less than 20 h after intercourse.
[c] II, Second specimens, which were obtained 2 weeks after the initial specimen, after condoms were used during sexual intercourse.

DISCUSSION

It is well documented that some other species associated with BV, e.g., certain *Bacteroides* spp. and *Peptostreptococcus* spp., are part of the normal intestinal flora. Results of the present study support the hypothesis that the reservoirs of *Mobiluncus* spp., *G. vaginalis*, and *M. hominis* are also in the intestinal tract. All four organisms could be isolated from the rectums of women, men, and children. The difference in isolation rates between women with BV, on the one hand, and healthy women, on the other, might be due to the occurrence of the microorganisms in higher numbers in the rectums of women who develop this condition compared with those in the rectums of healthy subjects. If the culture media used are not selective enough, strains are likely to be picked up only from individuals in whom the organisms are present in high numbers. The medium used for the isolation of *Mobiluncus* spp. in this study suppressed growth of most anaerobes but not that of streptococci, enterococci, and most other microaerophilic and faculative gram-positive species. Also, the human blood bilayer agar medium with Tween 80 used for *G. vaginalis* detection is only partially selective and does not suppress growth of most gram-positive species.

Hallén et al. (8) found *Mobiluncus* spp. in rectal samples from 23 (53%) of 43 women with BV and in only 2 of 24 women without this condition. In an earlier study (13), *Mobiluncus* spp., *G. vaginalis*, and *M. hominis* were isolated from the rectums of 7, 8, and 11 of 34 women with BV, respectively, but not from 67 women with vaginal discharges with other etiologies. Cultures of rectal specimens from 22 male partners of the 34 women with BV yielded *G. vaginalis* and *M. curtisii* in two and one case, respectively. Dawson and co-workers (2) could not isolate *G. vaginalis* from the rectums of any of 57 men attending a sexually transmitted disease clinic. The observation in this study that all four species studied could also be isolated from rectal samples from male partners of healthy women, homosexual men, and children suggests that they can all be part of the normal intestinal flora, although they probably occur in low numbers. They would then be very difficult to detect because of the complexity of that flora. Thus, the situation might be comparable to that of some other intestinal tract microbes, e.g., *Clostridium difficile*. None of the 374 individuals in this study harbored *Mobiluncus* spp. or *G. vaginalis* in their mouths or throats. *M. hominis* was found in the oropharynx of 12 adults. It was notable that the organism was recovered only from individuals whose partners had a genital occurrence of *M. hominis*. This might indicate a genital-oral transmission of this organism. *M. hominis* can be recovered from the pharynx in newborns (16). Babies delivered by cesarean section yield fewer isolates than do those born vaginally, because it is presumed that colonization takes place in the birth canal. The findings in throat specimens in this study conform with those made in an earlier study (13), in which *M. hominis* was found in 2 of 23 male partners of women with BV, and neither *Mobiluncus* spp. nor *G. vaginalis* could be recovered from this site.

Earlier studies concerning male sexual consorts of women with BV generally have been limited to cultures for *G. vaginalis*, while the cooccurrence of anaerobic bacteria has been poorly evaluated. The rates of isolation of *G. vaginalis* in urethral samples of such consorts, all of whom were asymptomatic, have ranged from 30 to 95% (1, 5, 21). In some studies on the prevalence of *G. vaginalis* among men, it has also been discussed what genital sample should be taken for the optimal recovery of the organism. Thus, Kinghorn et al. (15) recovered the organism from 14 of 192 consecutive men attending a clinic for sexually transmitted diseases. Those investigators found that the isolation rate was significantly higher from preputial samples than it was from urethral samples. In contrast, 11.4% of 430 men also attending a sexually transmitted disease clinic were found to harbor the organism in the urethra, whereas all preputial samples were culture negative (2). *G. vaginalis* was found in 22 seminal fluid specimens obtained from 58 men attending an infertility clinic, and anaerobes (bacteroides or fusobacteria) were found in 20 men (14). The occurrence of *G. vaginalis*, *Mobiluncus* spp., and other anaerobes in urethral samples from 309 men attending a sexually transmitted disease clinic has been studied (11). *G. vaginalis* was recovered from 14 men (4.5%) and *M. curtisii* was recovered from 10 men (3.2%). With the exception of *Bacteroides ureolyticus*, which occurred in 58 men, only sparse growth of anaerobes (and only species considered as part of the normal urethral flora, mainly *Peptostreptococcus magnus*) was detected (11). In the present study, none of the three genital specimens alone was sufficient for the optimal recovery of all of the four organisms studied. Thus, *G. vaginalis* and *M. curtisii* were isolated more often from the coronal sulcus than from the urethra, while the opposite was true with *M. hominis*. In no case was any of the four organisms recovered from seminal fluid without also being found in the urethra. It has been shown previously (26) that for the optimal recovery of *M. hominis*, specimens should be taken from both the urethra and the coronal sulcus.

Nitroimidazoles are the drugs of choice for the treatment of women with BV, but the recurrence rate is high. It has been claimed that BV is a condition that can spread sexually and that the recurrence of symptoms may be a reinfection by sexual consorts. The isolation of the four BV-associated organisms from men raises again the question of whether they generally colonize men and whether men provide a reservoir which may lead to reinfection of women. Of 82 men studied here, all of whom were consorts of women with BV, one of the four organisms was recovered from the urethra, coronal sulcus, or both of 12 of them and two of the four organisms were recovered from 1 of them. Of these 13 men, 10 belonged to the group of 44 men who all had had intercourse with their partners less than 20 h before samples were obtained. However, after 2 weeks of use of condoms during sexual intercourse, only one man still harbored *M. hominis* in the urethra, while the other men were all culture negative for the four organisms. These findings indicate that, in contrast to the rectal colonization, *Mobiluncus* spp. and *G. vaginalis*, in general, do not permanently colonize the genital tracts of healthy males, but exist transiently and are merely the result of continued passive acquisition from their female partners. The necessity of treating the male partners of women with recurrent BV is controversial. In an international, multicenter, randomized, double-blind trial with long-term follow-up, it was found that simultaneous treatment with metronidazole of sexual partners of women with BV did not decrease the rate of recurrence (20). Vejtorp et al. (29) came to the same conclusion after they treated such male consorts. However, in that study the last follow-up examination was made 5 weeks after the completion of treatment.

The infrequent recovery of *G. vaginalis*, both *Mobiluncus* spp., as well as *M. hominis* from the genital tracts of male sexual partners of women with BV conforms with previous observations in such men (11, 13). This indicates that sexual transfer, in its commonly accepted sense, is not an important mode of transmission of these organisms. The spread of these organisms to the vagina from the rectum seems more likely. The reason that the vaginal flora changes in a subpopulation of all women is still unknown. This and the exact (if any) etiology of BV remain to be established.

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