

Microbiological, epidemiological and clinical correlates of vaginal colonisation by *Mobiluncus* species

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Abstract

The microbiological and epidemiological correlates of vaginal colonisation by *Mobiluncus* species were examined among randomly selected women attending a sexually transmitted disease (STD) clinic. Women positive for *Trichomonas vaginalis* were excluded. *Mobiluncus* spp. were detected by Gram stained vaginal smear in 21% of 633 STD clinic patients, including 53% of those with and 4% of those without bacterial vaginosis (BV), as diagnosed by clinical criteria. *Gardnerella vaginalis* and *Mycoplasma hominis* detected by vaginal culture and *Mobiluncus* detected by vaginal Gram stain were each independently associated with BV after adjusting by logistic regression for the presence of sexually transmitted disease pathogens, gravidity, parity and number of lifetime sexual partners ($p < 0.001$ for each organism). Bacterial vaginosis was negatively correlated with isolation of lactobacilli, yeast and herpes simplex virus. After adjusting for presence or absence of BV, women with *Mobiluncus* were more likely to harbour *G vaginalis* (odds ratio 5.6, 95% confidence interval 1.6-19.5), *M hominis* (OR 3.7, 95% CI 2.0-7.0) and *Neisseria gonorrhoeae* (OR 2.9, 95% CI 1.4-6.0) and less likely to harbour vaginal yeast (OR 0.4, 95% CI 0.2-1.0); were more likely to be black (OR 2.7, 95% CI

1.5-4.6), and to have been pregnant (OR 1.8, 95% CI 1.1-3.1); but after the adjustment for BV, vaginal colonisation by *Mobiluncus* was not associated with symptoms of odour, abdominal pain, menstrual irregularities, or with adnexal tenderness. In summary, *Mobiluncus*, *Gardnerella vaginalis* and *Mycoplasma hominis* were independently associated with a clinical diagnosis of bacterial vaginosis, and *Mobiluncus* was further associated with the presence of BV-associated microorganisms (*M hominis* and *G vaginalis*), *N gonorrhoeae*, black race, and gravidity.

Introduction

There are currently two known species of the genus *Mobiluncus*: *M curtisii* and *M mulieris*.¹ These organisms are extremely fastidious, requiring fresh moist media and extended anaerobic incubation for isolation. Because of the difficulty in isolating these organisms by culture, most researchers attempting to define the prevalence of this organism have used direct techniques such as Gram stain, wet smear, direct staining with monoclonal antibodies or DNA probes. For example, curved rods were detected in Gram stained smears of vaginal fluid by Spiegel *et al*² in 51% of 61 women with bacterial vaginosis (that is, nonspecific vaginitis), and by Cristiano *et al*³ in 39% of 163 women with bacterial vaginosis and 0.3% of women without bacterial vaginosis. Hallen *et al*⁴ detected *Mobiluncus* spp. with monoclonal antibodies in 20% of 455 women attending a sexually transmitted disease clinic, including 50% of the 107 women with BV, while Roberts *et al*⁵ detected *Mobiluncus* by Gram stain and DNA probe in 33% of 92 consecutive female STD clinic patients, including 68% of women with BV. *Mobiluncus* has been less highly associated with BV among pregnant women. Krohn⁶ reported finding *Mobiluncus* morphotypes by Gram stain in 28% of 122 pregnant women with BV compared with 3% of 471 pregnant women without clinical signs of BV.

Many questions remain concerning the ecology of *Mobiluncus* spp, including the factors responsible for

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vaginal colonisation and the clinical manifestations of vaginal colonisation with these organisms. Further, bacterial vaginosis, whether defined by the clinical criteria of Amsel,⁷ by Gram stain⁸ or simply by the presence of clue cells, has been associated with several symptoms, signs and laboratory findings, as well as with adnexal tenderness.⁹ It remains to be determined whether any of the several microorganisms associated with bacterial vaginosis including *Mobiluncus* spp, are associated with, or perhaps responsible for, particular symptoms, signs or laboratory abnormalities.

Thomason *et al*¹⁰ found that women whose vaginal fluid contained motile curved rods detected by wet mount had the clinical signs of bacterial vaginosis, and a ten-fold increased prevalence of vaginal colonisation by anaerobic Gram negative bacteria, as compared with women whose wet mount examination did not show curved rods. No studies to date have examined the associations of *Mobiluncus* spp with other vaginal or cervical pathogens. Thus it is uncertain whether the association of *Mobiluncus* with bacterial vaginosis is independent of its association with other microorganisms thought to be involved in this syndrome, or whether vaginal colonisation by *Mobiluncus* requires the presence of certain other organisms. Finally, the possible role of factors other than the presence of other organisms in predisposing to *Mobiluncus* colonisation warrants further study. Holst *et al*¹¹ have suggested that rectal colonisation may predispose to vaginal colonisation. Other risk factors have not yet been implicated.

In this study, the frequency of several vaginal and cervical pathogens was obtained among 633 consecutive randomly selected patients attending a Sexually Transmitted Disease (STD) clinic in Seattle. The prevalence of *Mobiluncus* by Gram stain and the association of this organism with bacterial vaginosis, with various demographic and behavioural risk factors, and with other genital microorganisms are described.

Materials and methods

Study population

Women presenting to the Harborview Medical Center, Seattle King County Department of Public Health Sexually Transmitted Disease (STD) Clinic were eligible for study if they were seeking care for the first time or for a new problem, were at least 16 years old, not pregnant, and had not received any antibiotics in the prior two weeks. The study clinician selected a sample of patients from among those waiting to be seen, using a table of random numbers. Women who were positive for *T vaginalis* by wet mount or culture were excluded, leaving 633 eligible for study.

Patient examination

A standardised medical, gynaecological and sexual history was taken. The vulva, vagina and cervix were examined by colposcopy and the characteristics of any discharge were noted. The pH of the vaginal fluid was determined with pH indicator sticks (MCB Reagents, Gibbstown, JJ). A second smear of vaginal fluid was made with normal saline for observation by light microscopy for clue cells. Release of a "fishy" odour from vaginal fluid was tested by addition of 10% KOH. Women were diagnosed as having BV clinically if they had three of the four following criteria: homogeneous vaginal discharge, presence of clue cells in vaginal fluid, fishy odour after mixing vaginal fluid with 10% potassium hydroxide (KOH), and a vaginal fluid pH ≥ 4.7 .⁹ Abdominal and bimanual pelvic examinations were also performed.

Detection of *Mobiluncus* by Gram stain

Vaginal Gram stains were evaluated by one of the authors for the presence of curved, Gram negative to Gram variable rods. The evaluator had no knowledge of the clinical findings of the patients. The ability of the evaluator to identify correctly *Mobiluncus* morphotypes in vaginal smears was confirmed by performing detailed vaginal cultures and direct DNA hybridisation assays on a subset of 103 consecutive vaginal specimens using methods previously described.⁵ Of these 103 specimens, *Mobiluncus* was detected by culture or DNA hybridisation with the whole chromosomal DNA probe in 26 of 31 cases that were positive by Gram stain, for a sensitivity of 84%. The remaining 72 specimens were negative by culture, DNA hybridisation assay and Gram stain, giving a specificity and positive predictive value of 100% for detection by Gram stain.

Detection of other microorganisms

Vaginal or endocervical swab specimens for isolation of *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Mycoplasma hominis*, *Gardnerella vaginalis*, *Lactobacillus* and *Candida albicans* were inoculated immediately onto the appropriate growth media as previously described.¹² Endocervical specimens for *Chlamydia trachomatis* and herpes simplex virus (HSV) cultures were transported in tissue culture medium with antibiotics and stored at 4°C until inoculated (within 6 hours for HSV and cytomegalovirus and within 24 hours for *C trachomatis*).

Statistical methods

Associations between *Mobiluncus* or BV and the presence of other pathogens or demographic variables were first identified by univariate analyses. These associations were measured using the odds ratio (OR) with the corresponding P value based on the chi square test of whether the OR was equal to one (indicating no association).¹³ Approximate 95%

Table 1 Association of selected organisms with bacterial vaginosis among a cohort of 633 women

Organism	Bacterial vaginosis		Univariate		Multivariate*	
	Present = 205	Absent n = 428	OR (95% CI)	p	OR (95% CI)	p
<i>G. vaginalis</i>	97%	58%	24.3 (10.2, 62.1)	<0.001	11.9 (4.4, 31.8)	<0.001
<i>M. hominis</i>	82%	27%	12.2 (7.9, 18.9)	<0.001	5.7 (3.3, 9.9)	<0.001
<i>Lactobacillus</i>	67%	87%	0.3 (0.2, 0.5)	<0.001	0.5 (0.3, 0.8)	0.02
<i>Mobiluncus</i>	53%	4%	29.7 (16.3, 54.7)	<0.001	13.8 (7.0, 27.4)	<0.001
<i>U. urealyticum</i>	94%	84%	2.9 (1.5, 5.7)	<0.001	0.6 (0.2, 0.6)	0.4
Yeast	12%	33%	0.3 (0.2, 0.5)	<0.001	0.4 (0.2, 0.8)	0.01
<i>C. trachomatis</i>	14%	15%	1.0 (0.6, 1.6)	0.85	0.6 (0.3, 1.2)	0.19
<i>N. gonorrhoeae</i>	16%	7%	2.5 (1.5, 4.4)	<0.001	0.9 (0.4, 2.0)	0.81
Herpes simplex virus	5%	11%	0.4 (0.2, 0.9)	<0.02	0.3 (0.1, 0.7)	0.01
Cytomegalovirus	10%	5%	1.3 (1.0, 1.8)	0.06	1.2 (0.5, 2.8)	0.68

*All organisms included in the model so that analysis of each in relation to bacterial vaginosis was adjusted for the presence of the other microorganisms.

confidence intervals for the true ORs were constructed utilising the log OR and its standard error.¹³ Adjustment for BV in a stratified analysis was done using the Mantel-Haenszel procedure to provide an estimate of the pooled OR.¹³ Multiple logistic regression analyses were used to adjust associations between *Mobiluncus* or BV (dependent variables) and demographic factors or other vaginal pathogens (independent variables).¹⁴ Odds ratios, adjusted for the independent variables, were estimated from this model by exponentiating the resulting coefficients. Approximate 95% confidence intervals were constructed using these coefficients and their standard errors.

Results

What microorganisms are independently associated with bacterial vaginosis?

As summarised in table 1, *Mobiluncus* was detected by Gram stain in 20% of women attending the STD clinic, including 110 (53%) of those with and 16 (4%) of those without a clinical diagnosis of bacterial vaginosis (unadjusted odds ratio 30, 95% confidence interval 16–55). Likewise, *G. vaginalis*, *M. hominis*, *U. urealyticum*, and *N. gonorrhoeae* were positively associated with a diagnosis of BV by univariate

analysis while facultative lactobacilli, yeast and HSV were negatively associated.

Vaginal colonisation with *Mobiluncus* was associated with positive vaginal cultures for *G. vaginalis*, *M. hominis* and *N. gonorrhoeae* (table 2). After adjusting for the presence or absence of bacterial vaginosis, vaginal colonisation by *Mobiluncus* was found to be associated with isolation of *G. vaginalis* (OR 5.6, 95% CI 1.6, 19.5), *M. hominis* (OR 3.7, 95% CI 2.0, 7.0) and *N. gonorrhoeae* (OR 2.9, 95% CI 1.4, 6.0) and negatively associated with isolation of yeast (OR 0.4, 95% CI 0.2, 1.0) (table 2).

In view of the multiple associations between these infections, a logistic regression analysis was used to determine which of these organisms were independently associated with BV. The association between each organism and BV was adjusted for coinfection by each of the other organisms. As shown in table 1, *Mobiluncus*, *G. vaginalis*, and *M. hominis* remained strongly and independently associated with BV, and facultative lactobacilli, yeast and HSV were negatively associated after adjustment in the multivariate analysis.

To assess the possibility that each of these associations is secondary to some other unidentified microbial pathogen or nonmicrobial correlate of BV, the relationship of *Mobiluncus*, *G. vaginalis*, *M.*

Table 2 Microbiological correlates of *Mobiluncus*, adjusted for bacterial vaginosis

Organism	Bacterial vaginosis		No bacterial vaginosis		OR (95% CI)*	p
	<i>Mobiluncus</i> + n = 109	<i>Mobiluncus</i> – n = 95	<i>Mobiluncus</i> + n = 16	<i>Mobiluncus</i> – n = 410		
<i>G. vaginalis</i>	99%	95%	87%	57%	5.6 (1.6, 19.5)	0.005
<i>Lactobacillus</i>	73%	61%	63%	88%	1.1 (0.6, 1.8)	0.67
<i>Mycoplasma hominis</i>	89%	74%	69%	26%	3.7 (2.0, 7.0)	<0.001
<i>Ureaplasma urealyticum</i>	95%	93%	88%	84%	1.4 (0.6, 3.5)	0.63
Yeast	10%	15%	6%	24%	0.4 (0.2, 1.0)	0.04
<i>C. trachomatis</i>	16%	12%	25%	14%	1.6 (0.8, 3.2)	0.20
<i>N. gonorrhoeae</i>	23%	9%	19%	10%	2.9 (1.4, 6.0)	0.004
Herpes simplex virus	6%	4%	19%	11%	1.6 (0.7, 4.3)	0.44
Cytomegalovirus	10%	10%	0%	5%	0.8 (0.4, 2.3)	0.86

*Comparison between *Mobiluncus* present versus *Mobiluncus* absent, adjusted for bacterial vaginosis

Table 3 Association of *Mobiluncus* with epidemiological characteristics, after adjusting for the presence or absence of bacterial vaginosis

	Bacterial vaginosis		No bacterial vaginosis		OR (95% CI)	p
	<i>Mobiluncus</i> Present n = 109	<i>Mobiluncus</i> Absent n = 95	<i>Mobiluncus</i> Present n = 16	<i>Mobiluncus</i> Absent n = 410		
Demographic factors						
Mean age, SD	25.1, 6.2	24.3, 6.2	23.6, 7.0	23.7, 5.6		0.32
Married	9%	3%	6%	8%	2.1 (0.7, 6.5)	0.25
Black Race	36%	14%	25%	13%	2.7 (1.5, 4.6)	<0.001
Unemployed	50%	40%	56%	30%	1.8 (1.1, 2.9)	0.03
Years education, SD	12.4, 1.7	12.6, 2.1	13.1, 2.5	13.0, 2.3		0.62
Low socioeconomic status	60%	46%	40%	40%	1.5 (0.9, 2.6)	0.15
Income ≤US\$3600	58%	47%	60%	44%	1.6 (1.1, 2.5)	0.03
Obstetric and gynaecological factors						
Gravidity ≥ 1	76%	63%	63%	53%	1.8 (1.1, 3.1)	0.04
Parity ≥ 1	46%	34%	31%	20%	1.7 (1.0, 2.8)	0.06
Douche (ever)	76%	75%	69%	65%	1.1 (0.6, 1.9)	0.82
Douche for odour	12%	8%	0%	4%	1.3 (0.5, 3.8)	0.81
Sexual history						
Age first intercourse, SD	15.6, 2.7	15.0, 2.7	15.4, 3.1	16.5, 2.6	—	0.11
New partner in past 30 days	38%	34%	13%	27%	1.0 (0.6–1.8)	1.0
≥15 Lifetime partners	42%	45%	44%	33%	1.0 (0.6, 1.7)	1.0
Uncircumcised partner						
Past 30 days	14%	15%	13%	9%	1.1 (0.5, 2.4)	0.93
Ever	51%	44%	25%	36%	1.1 (0.7–1.9)	0.77
Rectal intercourse						
Past 30 days	8%	9%	6%	9%	0.8 (0.3, 1.9)	0.70
Ever	29%	42%	50%	35%	0.8 (0.5, 1.3)	0.40
Current contraception						
None	44%	34%	44%	27%	0.63 (0.38–1.03)	0.08
Oral contraceptive	26%	30%	25%	39%		
Barrier	13%	20%	19%	22%		
Antibiotic past 30 days						
	2%	0%	6%	12%	0.31 (0.06–2.1)	0.33

hominis, lactobacilli and HSV to BV was further adjusted for variables found to be associated both with BV and with the individual microorganisms, where $p < 0.1$ or $OR > 2$, or < 0.6 for both associations. The following variables which are all potential correlates of sexual exposure and behaviour were assessed for such associations: age, marital status, race, employment status, years of education, family income, gravidity, parity, history of douching, history of tampon use, age of first intercourse, new partner in the past 30 days, number of lifetime partners, history of sexual intercourse with an uncircumcised partner (ever and within one month), history of rectal intercourse (ever and within one month), current contraceptive method, and antibiotic use in the past month. All of these variables with the exception of age, marital status, tampon use, family income and rectal intercourse were associated with BV and at least one other infection. This presumably reflects the relative homogeneity of the STD clinic population with respect to these variables.

After further adjustment for these potential confounders as well as for coinfections, the following microorganisms remained positively associated with a diagnosis of bacterial vaginosis: *G vaginalis* (OR 16.8, 95% CI 5.3, 53.4), *M hominis* (OR 6.4, 95% CI 3.4, 11.8) and *Mobiluncus* (OR 12.4, 95% CI 5.7,

26.7), while the following microorganisms remained negatively associated with BV: facultative lactobacilli (OR 4.7, 95% CI 0.24, 0.91), yeast (OR 0.42, 95% CI 0.21, 0.86) and HSV infection (OR 0.25, 95% CI, 0.09, 0.68).

Is Mobiluncus colonisation correlated with clinical symptoms or signs?

As noted above, *Mobiluncus* was strongly associated with the clinical criteria for BV. However, among women with BV, those colonised with *Mobiluncus* were no more likely than those not colonised to complain of odour (52% vs 59%), prolonged menstrual bleeding (14% vs 14%), increased menstrual bleeding (13% vs 13%), abdominal pain (43% vs 37%), or to have signs of yellow discharge (55% vs 55%) or adnexal tenderness (4% vs 4%). Likewise those women with *Mobiluncus* were as likely as those without to have more than 30 polymorphonuclear leukocytes per high power field in the vaginal wet mount or Gram stained endocervical smear.

Are there risks factors or risk markers for vaginal colonisation by Mobiluncus?

After adjusting for BV, colonisation with *Mobiluncus* was associated with black race and lack of employment and low income, but not with educational status (table 3). Of the gynaecological

and obstetric factors investigated, gravidity and parity were associated with colonisation by *Mobiluncus* after stratifying for BV. Among women with BV, colonisation with *Mobiluncus* was associated with past infection with *N gonorrhoeae* (41% vs 27%, $p = 0.06$) but not with past infection with any other STD pathogen or syndrome.

Women harbouring *Mobiluncus* tended toward an earlier age at first intercourse, but were no more likely to have had a new sexual partner in the past month, to have had sexual intercourse with an uncircumcised partner, or to have had rectal intercourse. Contraceptive use was less common among those women colonised with *Mobiluncus*, and these women were less likely to use barrier or oral contraceptives, although these relationships were not statistically significant.

Discussion

The vaginal microflora of women with bacterial vaginosis is characterised by decreased prevalence and concentration of lactobacilli and an increased prevalence of *Gardnerella*, anaerobic rods and cocci^{11 15 16} and *Mycoplasma hominis*.^{11 17} Krohn *et al*⁹ reported that *G vaginalis*, *M hominis* and *Bacteroides* species were independently associated with a clinical diagnosis of BV among 593 pregnant women. Martius *et al*¹² in a case-control study of women in preterm labour, showed that *Mobiluncus* spp, *G vaginalis*, *Bacteroides melaninogenicus* and *M hominis* were independently associated with BV as diagnosed by vaginal Gram smear. Like Krohn and Martius, we found that *Lactobacillus* were significantly less often recovered from women with BV after adjusting for other microorganisms. The current study also suggests that lower genital tract yeast and herpes simplex virus are negatively associated with BV, and that cervical *N gonorrhoeae* is positively associated with BV. Cristiano *et al*⁸ have reported that vaginal yeasts are negatively associated with bacterial vaginosis and that *C trachomatis* was positively associated with bacterial vaginosis (5% vs 1%, OR 6.7, 95% CI 2.5, 18). We did not find a relationship between bacterial vaginosis and cervical *C trachomatis*.

Thomason *et al*¹⁰ found that women with motile curved rods on vaginal wet mount were more likely to be colonised with *Gardnerella*, *Bacteroides* and *Peptostreptococcus*, but only 40 women were studied and sexually transmitted disease pathogens were not recovered. We did not analyse the association of *Mobiluncus* with other anaerobes in this study.

We found that while *Mobiluncus* was strongly associated with clinical criteria for BV there was no indication that vaginal *Mobiluncus* was associated with particular symptoms or signs among women with BV. Larsson and Bergman¹⁸ reported that

thirteen women with intermenstrual bleeding or spotting of 1 to 12 months duration were positive for *Mobiluncus* by vaginal wet mount. After treatment with metronidazole and the disappearance of *Mobiluncus*, the irregular bleeding episodes stopped. In the present study, we found no association of *Mobiluncus* with increased or prolonged menstrual bleeding or increased intermenstrual bleeding.

The association of *Mobiluncus* with black race, unemployment, and low income suggests the need to assess further the role of behaviour or risk factors among low socioeconomic status women as risk factors for colonisation by *Mobiluncus*. The association of *Mobiluncus* with present and past infection by *N gonorrhoeae* after adjusting for BV suggest a common epidemiology for these two organisms. Others have reported that *Mobiluncus* can be recovered from up to 53% of the rectal specimens from women with bacterial vaginosis.¹⁹ We were unable to detect an association between *Mobiluncus* and recent or past rectal intercourse, or recent or past vaginal intercourse with an uncircumcised partner.

In this study *Mobiluncus* was detected by examining a Gram stained vaginal smear for characteristic curved rod morphotypes. While direct vaginal Gram stain is a relatively insensitive method for detection of any microorganism, most attempts to isolate *Mobiluncus* from the vagina suggest culture remains insensitive. Hjelm²⁰ isolated the organism from 10 of 17 vaginal specimens in which motile rods were seen by wet mount. Spiegel *et al*² isolated the organism from only 6 of 31 vaginal specimens whose Gram stains showed curved rods, while Thomason *et al*¹⁰ isolated motile curved rods from 5 of 21 women who had motile curved forms in the wet smears of their vaginal fluid. Pahlson *et al*²¹ were able to recover *Mobiluncus* from 70% of the vaginal samples positive for *Mobiluncus* by direct monoclonal antibody staining, even after using an elaborate dilution technique for culture. Methods employing cold enrichment,²² selective media or alkaline treatment²³ have not proved optimal for *Mobiluncus*. Thus, in this study we chose to detect *Mobiluncus* by examination of Gram stained vaginal smears, a method we found to be reproducible and specific, despite the limits in sensitivity.

In summary, *Mobiluncus*, *G vaginalis* and *M hominis* are independently associated with bacterial vaginosis. Among women with BV, *Mobiluncus* is not associated with unique clinical manifestations. This cross-sectional study suggests a possible relationship of low SES for vaginal colonisation by *Mobiluncus*, a relationship that warrants further study.

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