

Bacterial vaginosis in women attending STD clinic: diagnostic criteria and prevalence of *Mobiluncus* spp

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SUMMARY The diagnostic criteria of bacterial vaginosis (BV) and the prevalence of *Mobiluncus* spp as detected by monoclonal antibodies were investigated in all new women patients attending the sexually transmitted disease (STD) clinic in Uppsala during a four month period. Of 455 patients, 164 fulfilled the generally accepted criteria for BV, but in 57 of them simultaneous infection with a recognised pathogen was diagnosed. BV was thus the only clinical diagnosis in 107 (24%) of the women. The sniff test and clue cells in the wet smear were the two criteria most relevant for the diagnosis of BV. The sniff test was positive in 95% (156) of the 164 patients with BV and negative in all other cases. The corresponding figure for the clue cells was 98% (160 of 164), but clue cells were also detected in 19 patients without BV. Though 99% (162) of women with BV had a vaginal pH of more than 4.5, so did 83 women without BV. Only 59% (96) of women fulfilling the criteria of BV had a characteristic discharge.

Mobiluncus spp were present in 20% (90) of the 455 women and in 50% (53) of the 107 women with BV only. Of the 90 *Mobiluncus* spp isolates, *M. curtisii* comprised 44% (40), *M. mulieris* 34% (31), and both strains together 21% (19). *Mobiluncus* spp were detected with monoclonal antibodies in 35 women who had no motile curved rods on wet smear microscopy. Furthermore, *Mobiluncus* spp were often detected in women infected with recognised pathogens, as well as in a few women without signs of genital infection.

Bacterial vaginosis (BV) is a common diagnosis in women complaining of vaginal discharge. Criteria for the syndrome have been defined by Eschenbach *et al.*¹ The aetiology is not established, but studies show that the vaginal microbial flora in women with BV is altered from lactobacilli to mixed anaerobes.^{2,3} Among the different anaerobic bacteria occurring in samples from women with BV, the genus *Mobiluncus* (containing the two species *M. mulieris* and *M. curtisii*) has attracted specific interest,⁴ as it has been isolated almost exclusively from women with BV.^{5,6} In contrast, the first organism associated with BV, *Gardnerella vaginalis*, has been isolated from about 50% of clinically healthy women.^{7,8} The position of *Mobi-*

luncus spp in the ecology or even aetiology of BV has been difficult to establish, partly because the commonly used culture methods are very labour intensive and have been reported to give suboptimal isolation rates.^{9,10}

We have described the production of monoclonal antibodies against protein antigens occurring in *M. curtisii* and *M. mulieris*.¹¹ These monoclonal antibodies have been used successfully for the direct detection of *Mobiluncus* spp in a small series of clinical specimens of vaginal discharge using immunofluorescence.¹¹ Monoclonal antibodies to *Mobiluncus* spp were used in the study published here to define the prevalence of *Mobiluncus* spp in women attending a sexually transmitted disease (STD) clinic. The design of the study allowed us to evaluate the clinical criteria used for the diagnosis BV as well as to investigate the occurrence of *Candida* spp, *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, and *Chlamydia trachomatis* in patients fulfilling the criteria for BV.

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Patients and methods

PATIENTS

From November 1984 to February 1985 all new women attending the STD clinic at the University Hospital in Uppsala were included in the study. The clinic is the only STD department in an area serving about 150 000 people, about 20 000 of whom are university students. As far as the local police authorities know there is no prostitution in the city, and no woman in the study identified herself as a prostitute. A total of 455 women aged 16 to 51 (mean age 25) was studied, 187 (41%) of whom had one sexual partner during the previous year and 13 (3%) had had six partners or more.

METHODS

After a standardised interview all patients had a routine gynaecological examination. To detect *Ngonorrhoeae*, *Ctrachomatis*, *Tvaginialis* and *Candida* spp routine laboratory methods were used. *Ngonorrhoeae* was cultivated on chocolate agar and GC base agar (Difco, England) supplemented with 1% Isovitalex (BBL, Scotland).¹¹ Chlamydiae were isolated by a modification of the method of Ripa and Mårdh.¹² *Tvaginialis* was isolated on Diamond's medium.¹³ Fungal cultivation was processed as outlined in the *Manual of clinical microbiology*,¹⁴ and yeasts were identified as *C albicans* by the demonstration of germ tube production three hours after incubation in serum at 35°C. The preparation and use of monoclonal antibodies for detecting *Mobiluncus* spp in clinical samples has been described previously.¹⁵

Smears were taken from the cervix with a cotton tipped swab and from the urethra using a 1 µl inoculating loop (NUNC, Denmark), and examined by direct microscopy. Urethritis was defined as more than 10 polymorphonuclear leucocytes (PMNL) per field (× 1000) in the stained smear from the urethra.¹⁶ Cervicitis was defined as mucopus in the cervical os and more than 10 PMNL per field (× 1000) in the stained smear from the cervix.¹⁷ Wet smears and smears to be stained by monoclonal antibodies to *Mobiluncus* spp were taken from the vaginal fornices with a 10 µl inoculating loop (NUNC, Denmark). Wet smears were examined by light microscopy (× 400) for the presence of yeast cells, *Tvaginialis*, epithelial cells and clue cells, PMNL, lactobacilli, and motile curved rods. A wet smear was considered to show inflammation if the number of PMNL exceeded the number of epithelial cells. The other criteria looked for were recorded as showing positive or negative results.

The sniff test (amine test) was performed with 10% potassium hydroxide, and results were recorded as positive or negative. The vaginal pH was measured

using pH paper graded in steps of 0.2 (Merck). For a diagnosis of BV the patient should fulfill at least three of the following criteria: vaginal pH of more than 4.5, sniff test positive, clue cells in the wet smear, or characteristic vaginal discharge on examination.¹

A woman was considered to have no signs of genital infection if there was no urethritis, cervicitis, or condylomata, the wet smear contained fewer PMNL than epithelial cells, and none of the specific pathogens listed above was identified.

Results

DIAGNOSIS OF BV

Table 1 shows the clinical diagnoses of all 455 women (based on history, physical findings, and results of isolation of microbial pathogens) in relation to the fulfilment of criteria for BV (in the presence or absence of other diagnoses) and the detection of *Mobiluncus* spp using immunofluorescence on direct vaginal smears. Apart from the 107 women with a clinical diagnosis of BV only, other micro-organisms were identified in 57 patients who also fulfilled the criteria for BV. Most of the women with gonorrhoea and trichomoniasis and more than half of those with chlamydial infections fulfilled the criteria for BV. Eight women with a clinical diagnosis of BV had cervicitis, 14 urethritis, and 31 had an inflammatory wet smear.

Table 2 shows the relevance of the individual criteria in the scoring system for diagnosing BV in the 164 patients with BV, 59% of whom had the characteristic vaginal discharge. Table 2 also shows the number of patients with BV as a proportion of those who fulfilled one criterion for BV. Only 66% (162) of the 245 women with a vaginal pH of more than 4.5 and 79% (96) of the 121 with a characteristic discharge had BV.

Table 1 Clinical diagnoses of 455 women in relation to fulfilment of criteria for bacterial vaginosis (BV) (in the absence and presence of other diagnoses) and detection of *Mobiluncus* spp using monoclonal antibodies

Clinical diagnosis*	No (%)	No (%) fulfilling criteria for BV	No (%) with <i>Mobiluncus</i> spp
Candidal vaginitis	148 (33)	12 (8)	14 (10)
Bacterial vaginosis	107 (24)	107 (100)	53 (50)
Chlamydial infection	65 (14)	37 (57)	14 (22)
Gonorrhoea	7 (2)	5 (71)	3 (43)
Trichomoniasis	9 (2)	7 (78)	3 (33)
Condyloma	21 (5)	0	0
Non-specific urethritis	21 (5)	14 (67)	3 (14)
Non-specific cervicitis	19 (4)	8 (42)	3 (16)
Other diagnoses	25 (5)	0	0
Healthy	64 (14)	0	3 (5)

*Some patients had more than one diagnosis.

Table 2 Number of women with bacterial vaginosis (BV) satisfying one criterion for BV out of 455 women

Clinical criterion	No (%)	No (%) fulfilling criterion for BV
Vaginal pH > 4.5	245 (54)	162 (66)
Clue cells present	179 (39)	160 (89)
Sniff-text positive	156 (34)	156 (100)
Characteristic discharge	121 (27)	96 (79)
Lactobacilli absent from wet smear	164 (36)	150 (91)

OCCURRENCE OF *MOBILUNCUS* SPP IN PATIENTS WITH BV

Mobiluncus spp were present in 20% (90) of the 455 women (table 1) compared with 50% (53) of the 107 women with a final diagnosis of BV only; *M mulieris* in 17, *M curtisii* in 20, and both *Mobiluncus* species concomitantly in 16.

In addition to the information on the relation between wet smear and immunofluorescence, to be commented on later, table 3 shows that of the 164 women with BV, including the 57 with a clinical diagnosis based on the finding of a recognised pathogen, 73 (45%) harboured *Mobiluncus* spp. Of the 90 *Mobiluncus* spp found in all 455 women, *M curtisii* was found in 40 (44%), *M mulieris* in 31 (34%), and the two species together in 19 (21%).

To evaluate the usefulness of wet smear detection of motile curved rods for the presumptive identification of vaginal *Mobiluncus* spp, results on wet smear microscopy were compared with the detection of *Mobiluncus* spp in vaginal smears by immunofluorescence (table 3). Wet smear microscopy and immunofluorescence gave positive results in 51%, and immunofluorescence in another 33%. Table 3 also shows that motile curved rods were seen in the wet smear in 12 cases where immunofluorescence did not detect *Mobiluncus* spp. When additional slides from these 12 patients were examined further by immunofluorescence and Gram staining, two possible false negative results were found, as Gram negative curved rods were seen in stained smears but no *Mobiluncus* spp were detected by immunofluorescence. No curved rods or *Mobiluncus* spp could be seen in smears from the other 10 patients.

Table 3 Findings of *Mobiluncus* spp by immunofluorescence and of motile curved rods by wet smear microscopy in 455 women and in the 164 with bacterial vaginosis (BV)

<i>Mobiluncus</i> spp	Rods in total study population (n = 455)		Rods in women fulfilling criteria for BV (n = 164)	
	Present	Absent	Present	Absent
<i>M mulieris</i> only	19	12	19	6
<i>M curtisii</i> only	23	17	23	9
Both species	13	6	13	3
Neither species	12		12	

Seventeen women without BV had *Mobiluncus* spp on testing with monoclonal antibodies, but were wet smear negative for motile curved rods: eight had *M curtisii*, six *M mulieris* and three harboured both strains. One of these 17 patients had gonorrhoea, 11 had candidal vaginitis (all with lactobacilli in the vaginal wet smear and one with concomitant chlamydial infection), two women had cervicitis, and three were considered to be healthy.

Discussion

This study of 455 consecutive women was undertaken at the STD department of the University Hospital, Uppsala. There were a few promiscuous women in the study, but most of the women had had one or two sexual partners during the previous year, which is comparable to what is known in general about Swedish women of these age groups (Lewin B, unpublished observation).

The criteria for diagnosing BV are generally accepted,¹⁵ but the usefulness of these criteria is not uniform. The amine test and the presence of clue cells in the wet smear are both reliable indicators of BV (table 2). Almost all women with BV had a vaginal pH of more than 4.5, but in this study this was found in many women without BV. These criteria are objective and reproducible. The fourth criterion, the characteristic quality of the vaginal discharge, is subjective. Not surprisingly, therefore, it is neither sensitive nor specific, and seems to be less useful for clinical purposes. Absence of lactobacilli is part of the definition of BV proposed at the BV symposium in Stockholm 1984 and, as can be seen from table 2, this can be used as another wet smear criterion of BV together with clue cells.

In this study 164 women fulfilled the conventional criteria for BV. Evidently the criteria for BV do not exclude other infections,¹⁸ but reports on the occurrence of other microbes in patients with BV are scarce, and most authors seem to exclude infections with recognised pathogens from a diagnosis BV. In this study 8% of patients with candidal vaginitis and 57% of those with chlamydial infections had BV (table 1). Most patients with gonorrhoea and trichomoniasis also had BV. From a clinical point of view this is a problem, because there is no clear distinction between a diagnosis of BV based on the accepted criteria and a clinical diagnosis of BV, which excludes infections with recognised pathogens.

As suggested by the name, BV is not considered to be a true infection.¹⁹ Of the 107 women in this study with a clinical diagnosis of BV, however, eight (8%) had cervicitis, 14 (13%) urethritis, and 31 (29%) had an inflammatory wet smear. These inflammatory reactions were not linked to the presence of *Mobi-*

luncus spp. We did not culture for *Mycoplasma* species in this study, though mycoplasmas have been reported in connection with BV.²⁰ Whether these inflammatory reactions are caused by mycoplasmas or some other pathogen, or whether some cases of BV might be inflammatory, has to be studied further.

In this study 90 women had *Mobiluncus* spp. Of these, 55 (61%) were identified as having motile curved rods in their wet smears. From a clinical point of view it seems reasonable to assume that the presence of motile rods in the wet smear indicates *Mobiluncus* spp. In this study there were 12 cases in which the wet smear was positive without findings by immunofluorescence. Two of these were possible false negatives on immunofluorescence, as Gram negative curved rods were found on examination of additional slides. Some other organism with a similar motion may exist in the wet smear, but so far this has not been reported. The anaerobe similar to *Bacteroides ureolyticus* reported by Fontaine *et al.*, which is associated with non-gonococcal urethritis, is said to have a twisting motility.²¹ The prevalence of that microbe in vaginosis was not studied.

In the women studied, the prevalence of *Mobiluncus* spp was 20%, and in those with clinical BV it was 50% (table 1). The prevalence of motile curved rods in wet smears was 22% in all the women and 50% in those with BV (table 3). These figures are compatible with what has been reported by others,^{22 23} and it thus seems that *Mobiluncus* spp are not present in all patients with BV. *M. curtisii* is the most common species and is usually found alone. We also found more *M. mulieris* and more cases where both strains occurred simultaneously than reported before.⁶ The detection of *Mobiluncus* spp using monoclonal antibodies probably gives a more representative view of the relative proportions of *M. curtisii* and *M. mulieris* compared with culture, with its very time consuming selection of colonies⁹ and suboptimal isolation rates.^{6 22}

Mobiluncus spp are most often found in vaginas with a disturbed ecology, primarily of women with BV. In this study, however, we found *Mobiluncus* spp in women with different clinical infections and also in three healthy women. We have recently reported the presence of *Mobiluncus* spp in the rectums of women with *Mobiluncus* associated BV (paper read at 2nd world congress on STD, Paris, 1986). One hypothesis is that *Mobiluncus* spp, like *Candida* spp, contaminate the vagina from the rectum, which would explain finding *Mobiluncus* spp in women with all kinds of genital infections as well as in healthy women.

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