

Bacterial vaginosis: prevalence in outpatients, association with some micro-organisms and laboratory indices

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SUMMARY Seven hundred and ninety three women were investigated, aged between 16 and 78 years, to evaluate the prevalence of bacterial vaginosis (BV) and some associated micro-organisms, and to discuss the significance of laboratory indices correlated to this pathology. BV was diagnosed on the basis of four distinct criteria: a positive result of the test for amines with 10% KOH (odour-test), the presence of clue cells on fresh microscopic examination, a pH > 4.5 and direct Gram stain positive (the presence of more than 40 Gram negative or Gram variable coccobacilli per microscopic field by 1000 magnifications under oil immersion). The total prevalence of BV was 20.5% (163); similar percentages were found in both fertile and pregnant women, whereas a lower percentage (12.7%) was found in menopausal women. *Gardnerella vaginalis* was present in 235 (29.6%) of the 793 women, in 144 (88.3%) of the 163 with BV and in 91 (14.4%) of the 630 women without BV. *Mobiluncus species* was present in 8.2% (65) of the total population, in 38.6% (63) of the women with BV and only in two (0.3%) of the women without BV. In the women with BV lower percentages were found for *Trichomonas vaginalis*, yeasts, *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. The absence of a definite relationship between BV and cultural isolation of *G vaginalis* is confirmed whereas the role played by *Mobiluncus spp* still has to be clarified. It is concluded that it is not necessary to screen with all four laboratory indices. Two positive indices from a panel of three (excluding pH > 4.5 and direct Gram stain positive in the same panel) allows the correct diagnosis of BV in almost all cases.

Bacterial vaginosis (BV), a syndrome of probably mixed bacterial aetiology without signs of inflammation of the vaginal mucosa (also known as non-specific vaginitis), has been studied over a long period to establish the aetiological role of a particular micro-organism, *Gardnerella vaginalis*. Gardner and Dukes^{1,2} were the first to establish a connection between the micro-organism and the disorder over 30 years ago.

Although *G vaginalis* has been biochemically identified in eight biotypes,³ there are still no definite findings and no clear pathogenicity for any of these in relation to BV. The association, however, remains between the syndrome and the micro-organism (once called *Haemophilus vaginalis* or *Corynebacterium vaginalis*), and is confirmed in a number of studies.⁴⁻⁸

In BV, *G vaginalis* is usually associated with bacteria which are mostly anaerobic,^{5,6,9-15} which suggests that this association on a symbiotic basis is the cause of the infection.^{6,9,10}

The lack of a precise aetiological reference and ignorance of the infective mechanism have created and continue to create confusion and uncertainty. In fact, studies are still commonly found with reference to isolation of *G vaginalis* rather than to the pathology itself,¹⁶⁻¹⁹ or studies in which patient selection is only made on the basis of symptoms,¹⁸⁻¹⁹ despite the fact that well-defined diagnostic criteria for BV have been established which do not take either the symptoms or a specific micro-organism into consideration.^{7,20}

These criteria, classified by Amsel *et al*⁷ on the basis of previous observations made by other authors,^{5,9,21} are based on determination of the pH of the secretion (> 4.5), on the presence of a characteristic fishy smell produced by the reaction with 10% KOH, on the observation of clue cells on microscopic examination and on the physical characteristics of the vaginal

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secretion (homogeneity, consistency and colour).

This parameter has, however, shown notably subjective elements which have led to its replacement by the direct Gram stain of the secretion itself.⁸ Other methods have also been found^{9,10} which are useful in diagnosing BV, but which are not practicable in normal laboratories.

From the epidemiologic point of view, BV has not been studied in depth and the data available at present are rather scarce. The aim of our study was to evaluate the prevalence of BV outpatients who underwent microbiological study of their vaginal secretion, regardless of their age and their physiological condition. The role of some micro-organisms and laboratory indices associated with the pathology are also discussed.

Patients and methods

PATIENTS

Seven hundred and ninety three consecutive female outpatients between 16 and 78 years of age, who were referred to our laboratory by their family doctor, were examined over a period of three years (January 1985–December 1987). None of these women had undergone any kind of vaginal and/or cervical screening before coming to our laboratory. Of 793 patients, 680 were at a fertile age, 32 were pregnant, 71 were in menopause and 10 had undergone hysterectomy. Almost all the patients were symptomatic and complained of one or more of the following symptoms: leucorrhoea, pruritis, burning, dyspareunia, pelvic pain, secretion with unpleasant odour; a small minority complained of urological symptoms or were sent by the urologist because their partners had urethritis or other inflammatory diseases of the urogenital tract.

A card was filled in for each patient with her personal details, anamnesis, symptoms and laboratory reports.

LABORATORY METHODS

The secretion was taken from the posterior vaginal fornix with a cotton swab, and was placed directly on Agar Sabouraud culture to look for yeasts, on human blood agar with the addition of gentamycin sulphate, nalidixic acid and anfotericine B (*Gardnerella vaginalis* selective supplement-OXOID) for isolation of *G vaginalis* and on sheep blood agar without inhibitors. The latter was used as free growth culture. The plates of human agar blood and sheep blood agar were incubated for 48 hours at 37°C in candle jar, whereas the Sabouraud agar plate was incubated for 24–48 hours at 37°C (in air).

The yeasts which produced germinating tubes after 3 hours' incubation in human serum were identified as *Candida albicans* while the other yeasts were identified

using the Mycotube system (Roche diagnostic). All the colonies which, after 48 hours' incubation on human blood agar, showed a diffused ring of beta-haemolysis, were presumably identified as *G vaginalis*. They were negative catalase, did not produce haemolysis on sheep blood agar and on Gram testing showed up as small gram negative or gram variable²² rods (or coccobacilli). A swab with vaginal secretion was placed in a sterile test tube containing 0.5 ml of saline so as to obtain a turbid solution, while a smear was prepared from another swab, which was then stained using the Gram method.

The pH was measured using Merck indicator strips. A drop of the secretion-saline mixture was used for the fresh microscopic examination (400 ×) to look for clue cells, *Trichomonas vaginalis* and yeasts. Clue cells were identified as those vaginal epithelial cells with edges darkened by the presence of numerous small bacteria adhering to the surface.

Two drops of the same mixture of secretion and saline were put on a slide and after the addition of 1–2 drops of 10% KOH the presence or absence of amines was determined (odour test); the result was considered positive if there was a characteristic fishy smell immediately after the addition of the KOH.^{5,9}

By means of microscopic examination after Gram staining (1000 ×), the morphological characteristics of the bacteria were evaluated: the Gram positive rods, which were rather large and even pleiomorphous, were classified as belonging to the lactobaccilar flora, whilst the comma-shaped bacteria, which were either Gram negative or Gram variable, were presumptively identified as mobiluncus species.

The various morphological types present on the smear were counted and the results were expressed per microscopic field (pmf) as absent, rare (< 1 pmf), 1+ (1–10 pmf), 2+ (11–40 pmf), 3+ (41–80 pmf) and 4+ (> 80 pmf). The results were recorded after taking the average number of bacteria (for each morphological type) found in five microscopic fields, using the areas of the smear with higher concentration to facilitate counting.

Secretions from the endocervix were collected on two alginate coated swabs and investigated for *N gonorrhoeae* and *C trachomatis*. When testing for *N gonorrhoeae* the material was inoculated on Tayer Martin Agar immediately after the sample was taken and the medium was incubated in candle jar for 24–48 hours at 37°C. Biochemical identification was made using the Neisseria system (Pasteur Institute). *C trachomatis* was looked for using the direct immunofluorescent method with monoclonal antibodies (Microtrak Syra–Bracco).

DEFINITION OF BV

The criteria used in this study to define BV can be

considered a variation of those put forward by Amsel *et al.*⁷

All the patients who, irrespective of the presence or absence of traditional vaginal pathogens (*Trichomonas vaginalis* and *Mycetes*) and cervical pathogens (*N gonorrhoeae* and *C trachomatis*), were found to have three out of four of the following laboratory indices simultaneously were considered to have BV:

- A - Microscopic examination of the vaginal secretion after Gram staining showed a mixed bacterial flora with a number of gram negative or gram variable coccobacilli > 2+ (direct Gram stain positive);
- B - pH > 4.5;
- C - Positive odour test (with 10% KOH);
- D - Presence of clue cells during the fresh microscopic examination (400 ×).

STATISTICAL METHODS

The chi square test with Yates' correction and the exact test of Fisher²³ were used to make the comparison between proportions, whereas calculation of the predictive values was made by applying Bayes' formula.²⁴

Results

Out of 793 women investigated, 163 were found to have BV (20.5%). In the groups of fertile and pregnant women, BV was found in 20.9% (142/680) and 21.9% (7/32) respectively, whereas in the group of women in menopause, the frequency was found to be 12.7% (9/71). These differences are not statistically significant. Of the 10 women who had undergone hysterectomy, five (50%) had BV.

To check if there were differences in distribution of BV due to age in the group of fertile women, the patients were divided into six age groups, each with a span of five years, except for the last one (16-20, 21-25, 26-30, 31-35, 36-40, > 40). Frequency of BV found was 22.9% (11/48), 19.5% (36/185), 17.5% (31/177), 22.4% (26/116), 21.4% (15/70) and 27.4%

Table 1 Association of micro-organisms isolated with bacterial vaginosis (BV)

Micro-organism	Women with BV (n = 163)		Women without BV (n = 630)	
	No	%	No	%
<i>Gardnerella vaginalis</i>	144	88.3	91	14.4
<i>Mobiluncus species</i> *	63	38.6	2	0.3
<i>Trichomonas vaginalis</i>	19	11.6	44	7.0
Yeasts†	9	5.5	124	19.7
<i>Chlamydia trachomatis</i>	8/151	5.3	5/600	0.8
<i>Neisseria gonorrhoeae</i>	1/151	0.7	2/600	0.3

*Identification on direct Gram strain smear (1000 ×).

†*Candida albicans* 112 strains, *Candida species* 21 strains.

Table 2 Frequency of findings of combinations of the laboratory indices* used to diagnose the 163 cases of bacterial vaginosis

Combinations	No	%
A + B + C + D	108	66.3
A + B + C (clue cells absent)	18	11.0
A + B + D (negative odour test)	28	17.2
A + C + D (pH < 4.5)	9	5.5
B + C + D (direct Gram stain negative)	0	0.0

*A = direct Gram stain positive. B = pH > 4.5. C = Positive odour test. D = Clue cells present.

(23/84) respectively. The difference between the various age groups is not statistically significant.

Out of all the patients investigated, *G vaginalis* was found in 235 (29.6%), yeasts in 133 (16.8%), *Trichomonas vaginalis* in 63 (7.9%) and *Mobiluncus species* in 65 (8.2%).

At cervical level, out of 751 women *C trachomatis* was found in 13 (1.7%) and *N gonorrhoeae* in three (0.4).

The association between the single micro-organisms and BV is shown in table 1.

G vaginalis and *Mobiluncus spp* were found simultaneously in 36.8% (60) of the 163 women with BV. In these women, when *Mobiluncus spp* was found with Gram staining, a positive culture for *G vaginalis* followed; this occurred in 95.2% (60/63) of the cases.

Table 2 shows the percentages of combinations of the laboratory indices used to define BV: the most frequent finding (66.3%) was the one including all four, whereas the least frequent was where the pH was lower than or equal to 4.5.

The combination of indices B + C + D, which foresees a microscopic examination with a number of gram negative or gram variable coccobacilli < 2+ (direct Gram stain negative), was never found.

Table 3 shows how patients not affected by BV very

Table 3 Frequency of finding two laboratory indices only in 630 patients classified as not affected by bacterial vaginosis (BV) according to the criteria used

Combination of two laboratory indices	No	%
A + B	47	7.46
A + C	1†	0.16
A + D	2	0.32
B + C	1	0.16
B + D	1	0.16
C + D	0	0.00

*Criteria for the diagnosis of BV: simultaneous finding of 3 out of 4 of the following laboratory indices: A) direct Gram stain positive, B) pH > 4.5, C) positive odour test, D) clue cells present.

†Patient having had hysterectomy with presence of *Mobiluncus species*, doubtful presence of clue cells and with pH = 4.5.

rarely have association of two laboratory indices (except the A + B combination).

In this study two symptoms reported by the patients at the time of swab-taking were also taken into consideration: leucorrhoea and the unpleasant smell of the secretion. 76.1% (124/163) women with BV and 63% (397/630) without BV ($p < 0.01$) complained of leucorrhoea, whilst 14.7% (24/163) of the women with BV and only 1.3% (8/630) of those without BV ($p < 0.001$) complained of discharge with an unpleasant smell.

Discussion

A positive direct Gram stain of vaginal secretion, pH > 4.5, positive odour test and the presence of clue cells are the laboratory indices used by us at present for diagnosis of BV. This diagnosis does not take into account the symptoms, because of their poor specificity or sensitivity and because of the high percentage of women with asymptomatic BV.^{7,10} The data given in literature available at present, regarding the prevalence of the pathology, are not very extensive and often cannot be compared, in particular owing to the different methodological approach used in patient selection. However, BV is probably the most common vaginal infection in countries with high socio-economic development. Amsel *et al*⁷ reported a prevalence of about 20% of women attending a gynaecological clinic. Gravett *et al*²⁵ indirectly diagnosed BV using liquid-gas chromatography in 19% of 534 cases of pregnant women.

Using similar criteria to ours, Hallén *et al*²⁶ and Eschenbach *et al*²⁷ find a frequency of 36% (164/455) and 33% (210/640) respectively in women attending an STD clinic.

A prevalence of 20.5% found in the population examined in this study therefore reflects the situation found elsewhere fairly accurately. The woman's age and physiological state do not seem to influence the onset of the pathology.

With regard to the isolation of *G vaginalis* in the secretions of the population investigated, there is a higher percentage than reported by other authors^{16-18,28,29} and this may be linked both to the different techniques used to isolate and identify the micro-organism, and to the different make-up of the population.

Different and more selective examination techniques³⁰ determine the increase in frequency of isolation of *G vaginalis*, above all in women without BV.^{7,30}

There remains the problem of the relationship between the occurrence of the micro-organism and the presence of the pathology under examination. Statistically we reached the conclusion that, whereas absence of *G vaginalis* almost certainly excludes BV

(negative predictive value = 96.6%), the presence of the micro-organism is only predictive for the illness in 61.3% of cases. These values may be different in other laboratories since they are influenced both by the prevalence of BV and by the procedures used to cultivate and identify *G vaginalis*.

However, our data confirms the findings of other authors,⁷ but it does not support those who claim¹⁷⁻¹⁸ that routine culture examination is necessary to isolate *G vaginalis* in the study of genital infections.

Systematic and widespread testing for *G vaginalis* can be useful but is not indispensable, and also proves costly both financially and in terms of work involved, since it does not represent the decisive index for diagnosis of BV.

Since data regarding the presence of *G vaginalis* as a predictive index of a future onset of BV are also lacking, routine culture of the bacterium would only have an epidemiologic value.

In agreement with other authors,³¹ it is therefore necessary to reaffirm the concept of the non-existence of a definite identity between BV and *G vaginalis*. Among the anaerobic bacteria most frequently associated with BV, apart from those belonging to the bacteroides and peptococcus genera, the "gram negative" vibrios are of particular interest today, placed systematically under the mobiluncus genus with the two species *M mulieris* and *M curtisii*.³²

The role of these micro-organisms in determining BV is not known and the percentage of findings varies from 9.0% to 68.0% depending on the authors^{4,11-12,15,33-35} and the identification techniques used.

On the basis of our data (table 1), in agreement with Spiegel *et al*¹⁵ and with Roberts *et al*,³⁵ the presence of mobiluncus spp in the vaginal secretion represents a high probability BV indicator (positive predictive value = 96.9%), even if the absence of the micro-organism does not exclude it (negative predictive value = 86.3%). In the women with BV it seems that even the genital mycoplasmas are found with greater frequency and/or in greater concentrations compared with the women without BV.^{5,25} Our preliminary data (unpublished) also show that there is an association between these micro-organisms and BV: this is more evident for *M hominis* (*M hominis* vs BV $p < 0.001$; *U. urealyticum* vs BV $p < 0.05$). However, the significance of their presence remains unknown and further studies are necessary to understand the role they play. Whether they represent an active component in determining BV or whether they are simple commensals or even whether they are an overriding infection is still to be clarified.

Microbiologically, the problem remains complex with many uncertainties. We do, however, agree with the opinion expressed by Hillier and Eschenbach,³¹ about the need to re-examine the history of BV.

Another factor of some importance, above all for the therapeutical implications which it may bring about, is the association between BV and the classical vaginal and cervical pathogens (table 1). The literature does not give many data about these mixed forms of infection and they therefore merit more detailed study. It is, however, advisable for a systematic search for these pathogens to be carried out in all laboratories.

Passing on to the reference indices, the importance of the clue cells, associated in various studies with BV, is noted.^{2,5,7-8,37}

We think that it is important to emphasize that this research should be carried out on fresh samples and not after Gram staining.

In the cases of BV, the pH of the secretion is usually over 4.5. However, it is greater than 4.5 even in a notable percentage of women without BV so, rather than a true index of the syndrome, it should be considered a sign of abnormality. As can be deduced from table 3, the odour test was only rarely positive in the women without BV, confirming the data given in other studies,^{7,38} which classify it as an indicator test for the pathology. The direct Gram stain represents the other important index. Finding mixed bacterial flora consisting mainly of small gram negative or gram variable rods, each time associated with gram positive cocci and/or curved rods (*Mobiluncus spp*) and/or other gram negative bacteria, with simultaneous absence of lactobacillar flora, decidedly points to the diagnosis of BV. This microscopic picture is in sharp contrast to that seen in "normal" women where the smear is dominated by lactobacillar flora.

The fact that the B + C + D combination (table 2) was never found alone, but always associated with index A shows that the latter is the most sensitive laboratory index. Apart from this, in the area of the pathology in question, it supplies more information than culture growth for *G vaginalis*. In our opinion this research can be omitted since there is also the possibility in these cases of presumptive identification on a microscopic basis.⁸

The simultaneous finding of the four laboratory indices only occurs in 66% of cases. On the other hand, percentages varying from 5.5% to 17% show the association of three indices (table 2). From re-examination of the data, we have also noted that the positive outcome of two out of four (excluding the A + B combination as deduced from table 3), is very rare in women without BV and this could mean that testing for all four laboratory indices is superfluous. In short, the use of a protocol which foresees the association of the odour test, the clue cell test and direct Gram stain or odour test, clue cell testing and measurement of the pH, allow the laboratory and/or physician to diagnose BV just as efficiently and rapidly. At least two of these must be found to be simultaneously positive.

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