

Anaerobes and *Gardnerella vaginalis* in non-specific vaginitis

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SUMMARY Clinical evidence of bacterial vaginosis was present in 25 (35%) of 72 patients attending a London venereology clinic and correlated significantly with abnormal organic acids in vaginal secretions (24/25), with *Gardnerella vaginalis* on culture (17/25), with complaints of vaginal malodour (15/25), and with a relative scarcity of white blood cells in vaginal secretions. Anaerobic vaginal flora were presumptively identified by gas-liquid chromatographic analysis of organic acids found in vaginal secretions. The clinical criteria used to diagnose bacterial vaginosis included the presence of at least three of the four following characteristics: (a) a vaginal pH ≥ 4.5 , vaginal secretions that (b) were homogeneous, (c) contained "clue" cells, and (d) released a "fishy" amine odour when mixed with 10% potassium hydroxide. Because 17 of the 25 patients with clinical bacterial vaginosis had both chromatographic bacterial vaginosis and *G vaginalis*, causative organisms were difficult to identify. None of the six patients who had *G vaginalis* but not chromatographic bacterial vaginosis had clinical bacterial vaginosis, but seven of the 10 women with chromatographic bacterial vaginosis but not *G vaginalis* had clinical bacterial vaginosis ($p < 0.02$, χ^2 with Yates's correction). This finding supports the recent suggestions that anaerobes are important in the production of clinical signs of bacterial vaginosis.

Introduction

Although vaginitis caused by yeasts or by *Trichomonas vaginalis* has been well described and documented, other forms continue to present diagnostic challenges to both doctors and researchers. These other forms have been lumped into the category commonly known as non-specific vaginitis (NSV). This designation, however, has not helped doctors attempting to treat an infection possibly caused by one or many micro-organisms.

To clarify the diagnostic confusion, Gardner and Dukes suggested 30 years ago that the vast majority of cases of NSV were caused by *Haemophilus vaginalis* (*Corynebacterium vaginale*),¹ which is currently known as *Gardnerella vaginalis*,² explaining that women with a grey, homogeneous, odorous discharge with a pH of 5.0 to 5.5 that yields no trichomonads probably have *H vaginalis* vaginitis.

More recently Pheifer *et al* noted that vaginal secretions from women with NSV that had those

clinical characteristics liberated an amine odour with, and often without, the addition of 10% potassium hydroxide.³ They also noted an increased concentration of anaerobes in these abnormal secretions. Chen *et al* showed that the distinctive odour was due to volatilisation of polyamines, particularly putrescine and cadaverine, produced by vaginal anaerobic bacteria.⁴

Practical diagnostic criteria for standard clinical use have been proposed by Amsel *et al*,⁵ who suggested that patients should be considered to have a specific kind of NSV if they have at least three of four characteristics: (a) a vaginal pH of more than 4.5 and vaginal secretions that (b) were homogeneous, (c) contained "clue" cells, and (d) released a "fishy" amine odour when mixed with 10% potassium hydroxide.

The term bacterial vaginosis has been proposed to define NSV of bacterial origin,⁶ and is used with that meaning in this report. For the purposes of this investigation it was assumed that bacterial vaginosis may occur with or without concurrent protozoan or fungal vaginal infections. Cases of trichomoniasis or candidosis are therefore not excluded or dealt with separately from those of bacterial vaginosis.

This study correlated the clinical signs of bacterial

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vaginosis with the presence of *G vaginalis* and vaginal anaerobic bacteria, which were presumptively identified by the pattern of organic acids noted on gas-liquid chromatography of vaginal secretions.⁷ It assessed what subjective symptoms of vaginitis, if any, were associated with the objective clinical signs of bacterial vaginosis, and studied the inflammatory response produced by bacterial vaginosis compared with other types of vaginitis.

Patients and methods

Seventy two women attending this clinic during May 1982 were examined for the presence of vaginal infections. The patients presented with a new problem, were not menstruating at the time of examination, and had not taken antibiotics during the month before their attendance. Any woman who fulfilled these criteria when the investigator was available was examined, and information on her medical background and current symptoms was recorded using a standardised questionnaire.

Each patient underwent a complete pelvic examination, during which the clinical findings were recorded on a standard form. Cervical samples for the isolation of *Neisseria gonorrhoeae*⁸ and vaginal samples for the isolation of *T vaginalis*⁹ and vaginal yeasts (grown on Sabouraud's agar)¹⁰ were collected from each patient. In addition, a swab from the posterior fornix was taken and sent to the laboratory in Amies modification of Stuart's transport medium for culture for *G vaginalis*. The vaginal pH was measured with pH indicator paper (Whatman Filter Paper, N/Range, 4/6; Scientific Supplies Co Ltd, London) applied direct to the vaginal walls by means of sponge forceps.

Vaginal secretions were collected by instilling 3 ml of sterile distilled water into the vagina, using a plastic syringe. A sterile cotton swab was then used to wipe discharge from the vaginal walls into a pool in the posterior fornix, where the discharge was mixed with the water (taking care to avoid cervical secretions). The mixture of water and vaginal discharge was then pipetted into a screw topped glass vial.

A drop from each of the vaginal secretions collected was examined microscopically to estimate the number of white blood cells and for the presence of *T vaginalis*, "clue" cells, a "fishy" odour on adding 10% postassium hydroxide, and fungal filaments or buds. Bacterial vaginosis was diagnosed using the criteria of Amsel *et al.*,⁵ except that a vaginal pH of ≥ 4.5 rather than >4.5 was used. After microscopical examination, the vaginal secretions were stored at -20°C .

Swabs from the posterior fornix were examined by standard microbiological methods in the microbiology laboratory of this hospital. Plates of purple agar base (Difco, East Molesey, Surrey, UK) containing bromocresol purple, naladixic acid, and colistin were inoculated with the swabs taken from the posterior fornix for identification of *G vaginalis*. After 50 μg metronidazole discs had been applied to their inoculated surfaces, plates were incubated at 37°C for 48 hours in anaerobic gas pac jars (BBL; Becton Dickinson and Co, Cockeysville, Maryland, USA). Gram stained smears of each swab were prepared and examined for the presence of *G vaginalis* (small pleomorphic, Gram variable bacilli, often in solid fields)² and for "clue" cells associated with *G vaginalis* (epithelial cells in varying degrees of disintegration, with indefinite outlines and granular looking cytoplasm).¹ Plates with characteristic colonial morphology that gave negative results for catalase and 1% potassium tellurite were reported as giving positive cultures.¹¹ (Although more sensitive methods have been reported, we were unable to carry the identification further routinely in this centre.) Inhibition of growth by metronidazole was noted.

A Pye Unicam Series 204 gas-liquid chromatograph equipped with a flame ionisation detector was used to analyse vaginal wash samples. The column was packed with Carbowax 20 mol/l TPA (tannic, polyphosphomolybolic, and amido acid) on AW.DMCS Chromosorb G, 80-100 mesh. A flame ionisation detector using air, hydrogen, and nitrogen in a 2.2 m (6 ft) column at 20 ml/minute was used. Samples were run at 145°C to extract volatile fatty acids and at 135°C to extract non-volatile fatty acids.

To extract volatile fatty acids from the vaginal secretions I pipetted 1 ml into a rubber stoppered tube and added three or four drops of 50% sulphuric acid followed by 1 ml of diethyl ether. The solution was mixed by gently inverting the tubes 20 times. The mixture was centrifuged sufficiently to break up the emulsion of ether and aqueous medium, and 1 μl of the top (ether) layer was injected into the column.

To extract non-volatile fatty acids from the vaginal secretions I pipetted 1 ml into a screw topped glass vial and added 2 ml of methanol and 0.4 ml of 50% sulphuric acid. The solution was heated in a water bath at 56°C for 30 minutes, after which 1 ml of water and 0.5 ml of chloroform were added. The mixture was centrifuged sufficiently to break up the emulsion of chloroform and aqueous medium, and 1 μl of the bottom (chloroform) layer was injected into the column.

A chromatographic diagnosis of bacterial vaginosis was made if propionate, isobutyrate, butyrate, or isovalerate was found or the ratio of succinate to lactate was ≥ 0.4 , or both.⁷

Data were analysed using the χ^2 test with Yates's correction (χ^2_c) as numbers were less than 100 ($n = 72$) and some values in 2×2 tables were less than 10.

Results

Of the 72 patients studied, 64 (89%) were white and 55 (76%) were aged 16-30. Most presented with complaints of vaginal discharge (24) or a suspected sexually transmitted disease (21), 10 complained chiefly of vulval irritation, while the remaining 17 women had other presenting problems. Seventeen patients (24%) were found to have yeast vaginitis, three harboured *T vaginalis*, and three *N gonorrhoeae*. Cervicitis due to infection with *Chlamydia trachomatis* was not assessed.

Table I shows that chromatographic diagnoses of bacterial vaginosis and the presence of *G vaginalis* on culture correlated with clinical diagnoses ($\chi^2_c = 45.9$ and 16.3 respectively; $p < 0.001$ for each). A diagnosis of bacterial vaginosis was suggested by chromatographic analysis of vaginal secretions in 29 (40%) patients; *G vaginalis* was isolated from 25 (35%). Chromatographic diagnoses also strongly correlated with the presence of *G vaginalis* on culture (19 out of 29 with chromatographic diagnoses versus 6 out of 43 with normal chromatography ($\chi^2_c = 18.1$; $p < 0.001$)).

TABLE I Correlation of clinical and laboratory (gas-liquid chromatographic) diagnoses of bacterial vaginosis and results of culture for *Gardnerella vaginalis*

	Clinical diagnoses	
	Positive (n = 25)	Negative (n = 47)
Chromatographic diagnoses:		
Positive	24	5
Negative	1	42
<i>G vaginalis</i> on culture:		
Present	17	8
Absent	8	39

Comparing clinical with chromatographic diagnoses and with results of culture for *G vaginalis*, $p < 0.001$ each (χ^2 test with Yates's correction).

Although 25 patients had clinical bacterial vaginosis, 17 (68%) of them had both chromatographic bacterial vaginosis and *G vaginalis*, which made it difficult to assess causative organisms. Table II shows that none of the six patients with *G vaginalis* but not chromatographic bacterial vaginosis had clinical bacterial vaginosis, although seven of the 10 patients with chromatographic bacterial vaginosis but not *G vaginalis* had clinical bacterial vaginosis ($\chi^2_c = 4.89$, $p < 0.02$).

TABLE II Correlation of clinically diagnosed bacterial vaginosis with positive cultures of *Gardnerella vaginalis* and with gas-liquid chromatographic analysis of organic acids in vaginal secretions

	Clinical diagnoses	
	Positive (n = 25)	Negative (n = 47)
<i>G vaginalis</i> present only	0	6
Chromatographic diagnoses		
positive only	7 (28%)	3
Both	17 (68%)	2
Neither	1 (4%)	36

Comparing positive chromatographic diagnoses only with *G vaginalis* present only, $p < 0.02$ (χ^2 test with Yates's correction).

Table III shows that "clue" cells seen on the wet preparation of vaginal secretions significantly correlated both with the isolation of *G vaginalis* and with abnormal chromatographs ($\chi^2_c = 15.60$ and 36.29 respectively, $p < 0.001$ for each). Hospital laboratory reports of "clue" cells seen in Gram stained smears of vaginal swabs also correlated significantly with the growth of *G vaginalis* on culture (18/25 versus 2/47) and with chromatographic bacterial vaginosis (19/29 versus 1/43) ($\chi^2_c = 34.03$ and 31.40 respectively, $p < 0.001$ for each). The finding of "clue" cells in wet preparations correlated significantly with "clue" cells in Gram stained smears ($\chi^2_c = 27.53$, $p < 0.001$).

I compared symptoms in patients with a single type of vaginitis, patients with more than one type of vaginitis, and patients who did not have vaginitis. The types of vaginitis showed no distinct patterns of symptoms. Table IV supports the relative absence of irritative symptoms in clinical bacterial vaginosis by showing microscopical findings of reduced numbers of white blood cells in the vaginal secretions of patients with *G vaginalis* or chromatographic

TABLE III Correlation of "clue" cells in wet preparations with those in Gram stained smears, with *Gardnerella vaginalis* on culture, and with chromatographic diagnoses of bacterial vaginosis

	Clue cells in normal saline	
	Present (n = 28)	Absent (n = 44)
Clue cells in Gram smears:		
Present	18	2
Absent	10	42
<i>G vaginalis</i> on culture:		
Present	18	7
Absent	10	37
Chromatographic diagnoses:		
Positive	24	5
Negative	4	39

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bacterial vaginosis only compared with the inflammatory response seen in the vaginal secretions of those with *T vaginalis*, *N gonorrhoeae*, or candidosis only.

TABLE IV Comparison of white blood count (WBC) with type of infection present on culture of vaginal secretions from 45 patients*

WBC/high power field	Culture negative (n = 25)	<i>Trichomonas</i> , <i>N gonorrhoeae</i> , or yeast only (n = 11)	<i>G vaginalis</i> or abnormal GLC only (n = 9)
None	5	1	2
1-10	12	2	4
10-50	7	5	2
>50	1	3	1

* *Chlamydia trachomatis* not assessed; 27 patients with mixed infections not included. GLC = gas-liquid chromatography.

Table V shows the presence of cervical or vaginal infections among women with clinical or chromatographic bacterial vaginosis. Most of the women with *G vaginalis* or *T vaginalis* were diagnosed clinically and on chromatographic grounds as having bacterial vaginosis. No appreciable correlations were found between the presence of clinical or chromatographic bacterial vaginosis and the isolation of *N gonorrhoeae*, *T vaginalis*, or vaginal yeasts, which supports the argument that bacterial vaginosis may occur with, as well as in the absence of, these infections.

TABLE V Prevalence of bacterial vaginosis in various groups of patients

Positive on culture (or GLC)	No (%) with bacterial vaginosis:	
	Clinically	On GLC
<i>N gonorrhoeae</i> on culture (n = 3)	1 (33)	1 (33)
<i>T vaginalis</i> on culture (n = 4)	3 (75)	3 (75)
Vaginal yeasts on culture (n = 17)	5 (29)	5 (29)
<i>G vaginalis</i> on culture (n = 25)	17 (68)	14 (56)
Bacterial vaginosis on GLC (n = 29)	24 (83)	29 (100)

*GLC = gas-liquid chromatography.

Raised acetate concentrations in vaginal secretions are probably related to carriage of *G vaginalis*. Table VI shows that the organic acid found most commonly on chromatographic analysis of vaginal washings from the 72 women studied was acetate (88%). Although only 41% of patients with acetate had clinical signs of bacterial vaginosis, 66% (31/47) of patients with clinical bacterial vaginosis had larger acetate peaks (>2 mm high), and 85% (40/47) of those without clinical bacterial vaginosis had small

acetate peaks (<2 mm high). Lactate peaks were found in 79% (57/72) of all patients, only one third of whom had clinical bacterial vaginosis. Succinate was found commonly in patients with and without clinical bacterial vaginosis. Of women with a ratio of succinate to lactate peaks of ≥ 0.4 , 92% (22/24) had clinical bacterial vaginosis. All those with propionate and butyrate, seven out of eight with isovalerate, and all four with succinate but without lactate had clinical bacterial vaginosis. Abnormal organic acids were present in vaginal secretions in 96% (24/25) of women with clinical bacterial vaginosis, as opposed to only 11% (5/47) of those without it (table I).

TABLE VI Prevalence of organic acids in vaginal washes from 72 patients

Type of acid	No (%) positive	No (%) with clinical bacterial vaginosis
Acetate (A)	63 (88)	26 (41)
A >2 mm	28 (39)	22 (79)
Lactate (L)	57 (79)	19 (33)
Succinate (S)	50 (69)	24 (48)
S/L ratio ≥ 0.4	24 (33)	22 (92)
Propionate	19 (26)	19 (100)
Butyrate	15 (21)	15 (100)
Isovalerate	8 (11)	7 (88)

Discussion

The word "non-specific" in its name suggests that the causes, diagnosis, and treatment of NSV are poorly understood. In this study, characteristic clinical signs⁵ and organic acids in vaginal secretions⁷ provided specific clinical and chromatographic diagnostic criteria.

Results of this study confirmed recent suggestions that anaerobes may be important in the production of bacterial vaginosis.³⁻⁷ Chromatography can be used to give a rapid laboratory diagnosis of bacterial vaginosis by measuring directly the characteristic organic acids in vaginal secretions produced by bacteria capable of causing bacterial vaginosis. This study confirmed the chromatographic criteria of Speigel *et al.*,⁷ which correlated significantly with both clinical diagnoses and with the presence of *G vaginalis*. Such findings support the notion that both *G vaginalis* and vaginal anaerobes are commonly associated with clinical signs of bacterial vaginosis (table I).

Twenty five (35%) patients had clinical bacterial vaginosis, but because 17 (68%) of them had both chromatographic bacterial vaginosis and *G vaginalis* it was difficult to assess causative organisms. None of the six patients with *G vaginalis* but without chromatographic bacterial vaginosis had clinical bacterial vaginosis, although seven of the 10 patients

with chromatographic bacterial vaginosis in the absence of *G vaginalis* had clinical bacterial vaginosis (although the numbers used are small, χ^2 with Yates's correction = 4.89, $p < 0.02$) (table II). This finding supports suggestions that anaerobic bacteria are important in the production of clinical signs of bacterial vaginosis.

The symptom correlating best with a clinical diagnosis of bacterial vaginosis was found to be vaginal malodour. Most clinic patients complained of excessive vaginal discharge, malodour, or vulval irritation. Symptoms of vaginitis, however, have been shown to be poor indicators of actual vaginal infections.¹² Objective clinical signs were therefore used to diagnose bacterial vaginosis. All women studied were examined for the presence of vaginal yeasts, *T vaginalis*, *N gonorrhoeae*, *G vaginalis*, and organic acids produced by vaginal bacteria. Cultures for *C trachomatis* were not available for all clinic patients included in the study, and infection with this micro-organism may have been responsible for some of the unexplained symptoms of abnormal vaginal discharge.¹³

"Clue" cells seen on both wet preparations and Gram stained smears were significantly associated with the isolation of *G vaginalis* on culture and with the presumptive diagnosis of vaginal anaerobic bacteria made by chromatography of vaginal secretions. This finding suggests that Gram staining of secretions is not necessary when looking for "clue" cells (table III).

The non-inflammatory nature of bacterial vaginosis was first described by Gardner and Dukes, who reported that white blood cells were not a prominent finding in vaginal secretions of women with *G vaginalis* infections.¹ When at least three of the four signs of bacterial vaginosis were present I found few white blood cells in vaginal secretions (table IV), a finding that applied to women with abnormal chromatographic patterns as well as to those with positive *G vaginalis* cultures.

The sensitivity of the gas-liquid chromatograph equipped with a flame ionisation detector in confirming clinical diagnoses of bacterial vaginosis was 96%; the specificity was 89%; and the predictive value was 83%. These results (table VI) were much better than those obtained in a previous study using a chromatograph equipped with a relatively less sensitive detector of thermal conductivity.¹⁴

Anaerobic bacteria are normally present in vaginal flora.¹⁵ The abnormal chromatographic patterns associated with bacterial vaginosis, however, suggest either the presence of new anaerobic bacteria or the overgrowth of existing species. *G vaginalis* existed alone in six patients in this study without apparent clinical effect on any of them. *G vaginalis* may simply

act as a marker indicating the presence of abnormal vaginal anaerobes or vaginal anaerobic overgrowth, or it may act by altering vaginal conditions to enhance anaerobic growth.⁴

Metronidazole 500 mg twice daily for seven days is currently the most effective treatment of bacterial vaginosis.^{3 7 16} Its therapeutic efficacy may be due to its inhibition of vaginal anaerobes, rather than of *G vaginalis*, which would further support the role of anaerobic bacteria in bacterial vaginosis. In vitro studies have shown *G vaginalis* to be comparatively insensitive to metronidazole,¹⁶ whose hydroxy metabolite, however, has an appreciable effect on *G vaginalis*.¹⁷

This investigation suggests the presence of anaerobes by showing abnormal organic acids in vaginal secretions in 96% (24/25) of clinical diagnoses of bacterial vaginosis. These findings support those of previous studies, which showed large numbers of anaerobic bacteria identified by species in women with clinical evidence of bacterial vaginosis.^{7 18}

Detecting organic acids in vaginal secretions is a relatively new laboratory technique for the presumptive identification of anaerobes in vaginal flora. Results of chromatographic analyses of organic acids are more rapidly available than those of microbial culture. This newer laboratory method may thus be helpful in the rapid diagnosis of bacterial vaginosis. Many doctors do not have ready access to facilities for culture of *G vaginalis* or to gas-liquid chromatography, in which case the four clinical characteristics can be assessed for diagnosis without the need for microbial culture or chromatography.

Bacterial vaginosis has been shown to be a discrete and easily diagnosed infection. Further studies are needed to investigate: the pathogenicity of *G vaginalis* and its interactions with other vaginal bacteria, particularly anaerobes; the carriage of anaerobic bacteria and *G vaginalis* in men; the sexual transmission of these organisms; and the treatment of infections with them in both sexes.

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