

## The Vaginal Microbial Flora in Non-Specific Vaginitis

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The facultative and strictly anaerobic vaginal microbial flora was investigated in 40 women with non-specific vaginitis and in 40 control women seen in private gynaecological practice. *Gardnerella vaginalis*, anaerobic gram-negative bacilli, anaerobic gram-negative and gram-positive cocci were all associated with non-specific vaginitis ( $p < 0.001$ ), whereas lactobacilli occurred less frequently in non-specific vaginitis than in controls ( $p < 0.01$ ). The most common anaerobes were *Veillonella parvula*, *Bacteroides bivius*, *Bacteroides assaccharolyticus*, *Bacteroides capillosus* and *Peptococcus asaccharolyticus*. Anaerobic gram-negative curved rods were found in 11 % of cases of non-specific vaginitis. A characteristic pattern of short chain organic acids was found on gas liquid chromatographic analysis of vaginal secretions in non-specific vaginitis. A succinate/lactate peak ratio of 0.3 or more was found in 75 % of women with non-specific vaginitis ( $p < 0.001$ ). Clue cells, a positive amine test, a pH higher than 5.0, and the absence of lactobacilli on a Gram stained vaginal smear strongly correlated with non-specific vaginitis ( $p < 0.001$ ).

Since the original report of Gardner and Dukes on *Haemophilus vaginalis* (*Gardnerella vaginalis*) as a cause of bacterial non-specific vaginitis (NSV) (1), a pathogenic role for *Gardnerella vaginalis* has been disputed by some authors (2, 3). In other recent studies, however, a significant correlation was found between the isolation of *Gardnerella vaginalis* and manifestations of NSV (4, 5). In addition to an increased prevalence of *Gardnerella vaginalis*, vaginal fluid of women with NSV was found in one study to harbour higher concentrations of anaerobic organisms, particularly *Bacteroides* spp. and *Peptococcus* spp., as compared with women without vaginitis or with yeast vaginitis (6).

In the present study we investigated the facultative and anaerobic microbial flora of the vagina of 40 women with NSV and of 40 control women. Clinical and microscopic features and short chain organic acids in vaginal fluid were studied in these patients.

### Materials and Methods

**Patients.** Forty consecutively examined women with NSV and 40 consecutively examined women seeking contraceptive advice who did not complain of abnormal discharge or vaginal malodor were examined by either of two gynaecologists in private practice in Antwerp. Women who had received systemic antibiotics or vaginal medication in the preceding two weeks were not eligible for the study. At the time of the study, NSV was defined as an abnormal vaginal discharge in the absence of mucopurulent secretion from the endocervix, and of *Trichomonas vaginalis* and yeasts on a wet-mount microscopic examination. Women with a subsequent heavy growth of *Candida albicans* were also excluded from the analysis (two patients and two controls). The patients studied belonged to an urban middle class population in which 60 % of the women were employees. Patients and controls did not significantly differ in median age (26 and 27 years respectively), marital status, mean number of children (0.9 and 1.1 respectively), use of an intrauterine device (8 in each group), use of oral contraceptives (24 and 20 respectively), mean age at first sexual intercourse (18.8 and 20.2 years respectively), and number of sex partners in the previous year (1.1 in each group).

**Microbiological Investigation.** Specimens for microscopy and culture were collected from pooled secretions in the posterior fornix using a cotton swab. Smears were made for wet-mount examination (magnification  $\times 400$ ) and Gram stain. Vaginal specimens were inoculated immediately onto the following media: HBT-agar for *Gardnerella vaginalis* (7); Sabourraud

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agar for yeasts; anaerobic blood agar consisting of 2.84 % Schaedler broth (BBL), 1.5 % agar (Difco), 0.5 % yeast extract (Difco), 5 % sheep blood and 10 µg/ml vitamin K1; and vancomycin leaked blood agar consisting of 2.84 % Schaedler broth (BBL), 1.5 % agar (Difco), 5 % lysed sheep blood, 10 µg/ml vitamin K1, and vancomycin 7.5 µg/ml. An endocervical cotton swab was used to take a smear for Gram stain, and to inoculate modified Thayer Martin agar for *Neisseria gonorrhoeae*. An additional endocervical swab was placed into 2SP transport medium for *Chlamydia trachomatis*, which was cultured on cycloheximide treated McCoy cells. The anaerobic plates and HBT-agar were placed within 15 min into a GasPak anaerobic jar (BBL) at 36 °C, and examined three and seven days after inoculation. There was insufficient anaerobiosis in one case of NSV. This patient was excluded from further analysis. All growth was semiquantitatively assessed as 1+ to 4+ by standardized criteria. *Gardnerella vaginalis* was identified as described previously (8). Anaerobic gram-negative cocci and rods, and gram-positive cocci were identified by standard methods (9). The pH of vaginal fluid was measured with a Merck pH strip (range 3.8–5.4) on the top of a vaginal speculum. A drop of a 10 % KOH solution was placed on the vaginal fluid on a glass slide to detect 'fishy' odor substances (amine test) (4).

**Gas-Liquid Chromatography.** Pooled vaginal secretions were collected with a cotton swab which was placed in 1 ml sterile distilled water in a 2 ml plastic tube (Nunc). Specimens were prepared for gas-liquid chromatography by methods adapted from Spiegel et al. (6). Samples were acidified by addition of 0.1 ml of a 50 % aqueous solution of H<sub>2</sub>SO<sub>4</sub>. One ml of methanol was added, and the mixture was incubated in a 60 °C water bath for 30 min. Then 0.5 ml of water was added, and the non-volatile acids were extracted with 0.25 ml of chloroform. This solution was gently mixed and briefly centrifugated at 1000 g. A Perkin Elmer Sigma 3 chromatograph equipped with a flame ionization detector and a 2 m stainless steel column

with an internal diameter of 2.1 mm was used to analyze the samples. For detection of volatile and non-volatile organic acids the following operating conditions were used: column temperature 165 °C and 150 °C; detector temperature 250 °C; nitrogen carrier gas flow 30 ml/min; attenuation 0 and 2. Volatile acids were analyzed using headspace chromatography (10).

## Results

Among the controls, nine were found to have abnormal vaginal discharge and were analyzed separately. Table 1 shows the prevalence of *Gardnerella vaginalis*, lactobacilli, and anaerobes in the vagina of the 37 women studied with NSV and in the controls with and without discharge. Ninety-two percent (34/37) of the women with NSV harboured *Gardnerella vaginalis* in their vagina as compared to 37 % (7/38) of the controls ( $p < 0.001$ ). Of the nine controls with abnormal findings on examination, 78 % yielded *Gardnerella vaginalis* as compared to 24 % of the 29 women with normal findings on examination ( $p = 0.006$ , Fisher's exact test). All cultures except one from patients with NSV showed heavy growth of *Gardnerella vaginalis* compared to four of seven cultures from controls who had normal findings on examination ( $p = 0.025$ , Fisher's exact test).

*Lactobacillus* spp. were recovered less frequently and in lower concentrations from the 37 patients with NSV than from the 28 controls ( $p < 0.01$ ). In 15 of 34 patients with NSV,

**Table 1:** Microorganisms isolated from the vagina of women with non-specific vaginitis and controls. Number of cases with heavy growth in parenthesis.

Microorganism	Non-specific vaginitis (n = 37)	Controls	
		Discharge at examination (n = 9)	No abnormal vaginal discharge (n = 29)
<i>Gardnerella vaginalis</i>	34 <sup>a</sup> (33)	7 (6)	7 (4)
Lactobacilli	18 <sup>b</sup> (6)	5 (4)	27 (26)
Anaerobes	34 <sup>c</sup>	5	13
Anaerobic Gram-negative rods	32 <sup>d</sup> (25)	5 (4)	5 (3)
Anaerobic Gram-negative cocci	16 <sup>e</sup> (8)	3 (2)	1 (0)
Anaerobic gram-positive cocci	21 <sup>f</sup> (15)	5 (5)	4 (2)

<sup>a</sup>  $\chi^2 = 22.3$ ,  $p < 0.001$  for NSV versus all controls;

<sup>c</sup>  $\chi^2 = 15.4$ ,  $p < 0.001$ ;

<sup>e</sup>  $\chi^2 = 8.7$ ,  $p < 0.01$ ;

<sup>b</sup>  $\chi^2 = 9.1$ ,  $p < 0.01$ ;

<sup>d</sup>  $\chi^2 = 25.1$ ,  $p < 0.001$ ;

<sup>f</sup>  $\chi^2 = 7.2$ ,  $p < 0.01$ .

**Table 2:** Anaerobic species isolated from the vagina of women with non-specific vaginitis and controls.

Species	Non-specific vaginitis (n = 37)	Controls	
		Vaginal discharge (n = 9)	No vaginal discharge (n = 29)
<i>Bacteroides</i>			
<i>B. bivius</i>	12	2	1
<i>B. asaccharolyticus</i>	10	2	1
<i>B. disiens</i>	4	-	1
<i>B. melaninogenicus</i> ss. <i>intermedius</i>	5	2	1
<i>B. ureolyticus</i>	5	-	1
<i>B. oralis</i>	2	-	-
<i>B. vulgatus</i>	1	-	1
<i>B. capillosus</i>	8	1	-
<i>B. furcosus</i>	1	-	-
<i>B. ruminicola</i> ss. <i>brevis</i>	-	1	-
<i>B. amilophilus</i>	1	-	1
Unidentified	2	3	2
<i>Fusobacterium</i>			
<i>F. nucleatum</i>	5	2	1
<i>F. symbiosum</i>	2	-	1
<i>F. mortiferum</i>	2	-	-
Unidentified curved rods	4	1	-
Gram-negative cocci			
<i>Veillonella parvula</i>	14	3	1
<i>Acidaminococcus fermentans</i>	3	-	-
<i>Megasphaera elsdenii</i>	1	-	-
<i>Peptococcus</i>			
<i>P. asaccharolyticus</i>	7	2	-
<i>P. prevotii</i>	4	-	1
<i>P. magnus</i>	4	-	-
<i>P. productus</i>	1	-	-
Unidentified	1	-	-
<i>Peptostreptococcus</i>			
<i>P. anaerobius</i>	5	1	1
Unidentified	1	1	-
Unidentified gram-positive cocci	3	3	2

*Gardnerella vaginalis* was associated with lactobacilli as compared to six of seven controls with normal examination findings from whom *Gardnerella vaginalis* was isolated ( $p = 0.05$ , Fisher's exact test). The respective numbers for heavy growth of lactobacilli associated with *Gardnerella vaginalis* in the two latter groups were 3 out of 34 and 5 out of 7 ( $p = 0.002$ , Fisher's exact test).

Overall, anaerobes were isolated more frequently and in higher numbers from women with NSV than from controls. Anaerobic gram-negative bacilli were associated with NSV ( $p < 0.001$ ). The anaerobic species recovered from the vagina of women with NSV and con-

trols are listed in Table 2. Identification of gram-positive rods was not attempted. When the latter were excluded, an average of 2.9 and 1.1 anaerobic species were isolated from women with NSV and controls respectively. No single anaerobic species occurred in all or almost all cases of NSV, as was the case with *Gardnerella vaginalis*. The most frequently identified anaerobes were *Veillonella parvula*, *Bacteroides bivius*, *Bacteroides asaccharolyticus*, *Bacteroides capillosus* and *Peptococcus asaccharolyticus*. Anaerobic gram-negative curved rods resembling group-2 organisms of Durieux and Dublanchet (11) were found in 11% of 37 cases. Morphologically similar organisms were seen on a

**Table 3:** Volatile and non-volatile organic acids in vaginal fluid from 20 women with vaginitis and from 20 controls without vaginal discharge.

Organic acid	Non-specific vaginitis	Controls
Acetate	19 <sup>a</sup>	9
Propionate	14	—
Isobutyrate	5	1
Butyrate	13	—
Isovalerate	6	—
Pyruvate	3	—
Lactate	14	20
Oxalate	2	—
Succinate	20 <sup>b</sup>	6
Succinate/lactate peak heights ratio 0.3	15 <sup>c</sup>	2

<sup>a</sup>  $\chi^2 = 9.64$ ,  $p < 0.001$ ;

<sup>b</sup>  $\chi^2 = 18.6$ ,  $p < 0.001$ ;

<sup>c</sup>  $\chi^2 = 14.7$ ,  $p < 0.001$ .

Gram smear of one additional patient with NSV, but the organisms were never isolated.

*Bacteroides bivius*, *Bacteroides assacharolyticus*, *Bacteroides capillosus* and anaerobic curved rods nearly always occurred in high concentrations in the vaginal specimens of women with symptoms (including the controls with discharge). However, when present, *Gardnerella vaginalis* was always the predominant organism in these patients. Among women with NSV *Gardnerella vaginalis* was associated with anaerobic gram-negative rods in 31 of 34 cases as against 2 of 7 *Gardnerella vaginalis* positive

specimens from controls without vaginal discharge ( $p = 0.001$ , Fisher's exact test).

Table 3 lists the volatile and non-volatile organic acids detected in vaginal secretion by gas-liquid chromatography. Lactate was detected in 14 of 20 of the women with NSV, but in much lower concentrations than in the 20 controls without discharge (respective mean peak heights were 21 and 49 nm;  $p < 0.005$ , Student's t-test). In contrast, succinate peaks were higher in the NSV patients (respective mean peak heights were 64 and 7 nm;  $p < 0.005$ , Student's t-test). With the exception of lactate, all organic acids detected occurred more frequently in women with NSV than in controls. The succinate/lactate peak ratio was 0.3 or more in 75 % of the 20 women with NSV and in 10 % of the controls ( $p < 0.001$ ). No specific anaerobic species from the vagina yielded a chromatographic pattern equivalent to that produced by vaginal secretion of women with NSV. Among the anaerobes isolated it was often not possible to identify the species that might be responsible for the production of specific organic acids detected in vaginal fluid. Diagnostic findings in NSV are presented in Table 4. Clue cells, a positive amine test and a pH higher than 5.0 were all significantly associated with NSV. All women in whom clue cells were found harboured both *Gardnerella vaginalis* and anaerobic gram-negative bacilli. Lactobacilli were more often seen in vaginal smears of controls than of women with NSV ( $p < 0.001$ ), and 31 of 37 patients had less than one polymorphonuclear leucocyte per high power field ( $\times 1000$ ) on the smear.

**Table 4:** Diagnostic findings among women with non-specific vaginitis and controls.

Feature	Non-specific vaginitis (n = 37)	Controls	
		Discharge at examination (n = 9)	No vaginal discharge (n = 29)
Clue cells	30 <sup>a</sup>	4	—
Positive amine test	29 <sup>b</sup>	2	—
pH > 5.0	30 <sup>c</sup>	4	7
Lactobacilli on gram stained vaginal smear	7 <sup>d</sup>	5	26

<sup>a</sup>  $\chi^2 = 34.9$ ,  $p < 0.001$ ;

<sup>c</sup>  $\chi^2 = 18.5$ ,  $p < 0.001$ ;

<sup>b</sup>  $\chi^2 = 38.4$ ,  $p < 0.001$ ;

<sup>d</sup>  $\chi^2 = 27.0$ ,  $p < 0.001$ .

## Discussion

This study confirms the results of Spiegel et al. (6), who reported a significant association of both *Gardnerella vaginalis* and anaerobes, particularly *Bacteroides* spp. and *Peptococcus* spp., with NSV (6). Our results indicate that *Gardnerella vaginalis* plays an etiological role in NSV as well as being a member of the normal vaginal flora. The isolation of *Gardnerella vaginalis* in 92% of the women with NSV is in agreement with results in three previous reports (1, 4, 5). In agreement with the findings of Pfeifer and coworkers (4), we found that asymptomatic women with abnormal findings on vaginal examination also had a higher frequency of *Gardnerella vaginalis* and anaerobes than asymptomatic women with findings normal on vaginal examination. In contrast, lactobacilli were the predominant flora in women without signs or symptoms of vaginitis, but were infrequently found in large numbers in women with NSV. The presence of *Gardnerella vaginalis* in the absence of or with diminished growth of lactobacilli was strongly associated with NSV. The combination of these two findings seems to have a better predictive value for the diagnosis of NSV than heavy growth of *Gardnerella vaginalis* only (7). Anaerobes, like *Gardnerella vaginalis*, belong to the normal microbial flora of the vagina. Anaerobes, however, were found more frequently and in higher numbers in women with NSV than in controls. In several studies, *Bacteroides* spp. and *Peptococcus* spp. were isolated from the vagina of 5–50% of women examined (6, 12–16). Most of the authors did not provide clinical data and widely differing sampling and culturing methods were used. Goldacre et al. (17) reported an increased concentration of anaerobic gram-negative rods among a group of women with "troublesome vaginal discharge". *Bacteroides bivius*, *Bacteroides capillosus* and *Bacteroides disiens* are among the most common anaerobes isolated from the vagina (6, 16, 18). These organisms are recognized as important pathogens in gynaecological infections, including bacteraemia and pelvic abscess. In severe infections they are generally associated with other potentially pathogenic facultative bacteria (19, 20). The most frequently isolated

anaerobic species in this study was *Veillonella parvula*, which has not commonly been found in other series.

Spiegel et al. (6) were the first to apply direct gas liquid chromatography to the analysis of vaginal secretions. Like them, we found a characteristic chromatographic pattern associated with NSV. It was found possible to detect organic acids in suspensions of vaginal secretions collected on a swab. The collection of vaginal fluid with a pipette after washing the vagina with saline is more precise, but fairly cumbersome. In our study, higher lactate and lower succinate peaks were found in women with NSV than in that of Spiegel and coworkers (6). Thus we obtained a succinate/lactate peak ratio of  $\geq 0.3$  in women with NSV as compared to their ratio of  $\geq 0.4$ . Different equipment, different chromatographic techniques, and different methods of specimen collection might explain the difference to some extent. Gas liquid chromatography is a rapid and relatively simple method and the test result provides an additional objective criterium for the diagnosis of NSV.

The diagnostic findings listed in Table 4 all strongly correlated with NSV. The tests are cheap and easy to perform and provide objective positive criteria for the diagnosis of NSV instead of diagnosis by exclusion of other causes of vaginitis.

It has been suggested that a symbiotic relationship between *Gardnerella vaginalis* and certain anaerobic bacteria plays a role in the pathogenesis of NSV (6, 21, 22). The exact mechanism of this relationship is unknown. Further microbiological and physiological studies are necessary to explain how the vaginal flora is controlled and influenced, and why only a proportion of women who carry *Gardnerella vaginalis* in the vagina develop signs and symptoms of NSV.

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