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Gardnerella vaginalis and anaerobic bacteria in genital disease

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SUMMARY In a study of *Gardnerella vaginalis* and anaerobic bacteria in non-specific vaginitis (NSV) and other genital diseases 89 patients attending a genital medicine clinic had vaginal samples examined for conventional pathogens and for quantitative analysis of *G vaginalis* and aerobic and anaerobic bacterial flora. The overall incidence of *G vaginalis* was 20%; *G vaginalis* (mean concentration 7·0 $\log_{10}$/g of secretion) occurred predominantly in patients with NSV (57%) but also in sexual contacts of non-specific urethritis (NSU) (37·5%) and in patients with other conditions (11·8%). *G vaginalis* is therefore a relatively common isolate in patients with vaginal discharge.

The concentration of aerobic and anaerobic bacteria ranged from 4·9-11·0 $\log_{10}$/g of secretion with an anaerobe-to-aerobe ratio of 10 : 1. Anaerobic bacteria, particularly anaerobic Gram-positive cocci (mean concentrations 7·7 $\log_{10}$/g), were present in patients with NSV and in association with *G vaginalis,* but they also occurred in other clinical groups and with other pathogens, particularly *Trichomonas vaginalis.* Anaerobic bacteria may therefore play an important role in the pathogenesis of vaginal infections.

Introduction

Vaginal discharge is a common and distressing complaint in clinical practice. Known pathogens, such as *Candida* spp and *Trichomonas vaginalis,* are isolated from some patients, and of these the majority have so-called non-specific vaginitis (NSV). Rational treatment and management of patients with NSV has often been unsatisfactory because of the uncertainty surrounding its aetiology. Recently, there has been a resurgence of interest in the possible association of *Gardnerella vaginalis* with NSV.[1-3]

Since 1955, when Gardner and Dukes[4] first suggested that a small Gram-negative bacillus, *Haemophilus vaginalis* (now reclassified as *Gardnerella vaginalis*[5]) was associated with these symptoms, the pathogenic and taxonomic status of this organism has been the subject of much controversy. Some reports confirmed the original observations of a close association whereas others disputed any correlation between *G vaginalis* and NSV[6] and even suggested that *G vaginalis* is part of the normal vaginal flora.[7]

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Furthermore, the isolation of anaerobic bacteria in association with *G vaginalis*[1 3] and the response of patients to treatment with metronidazole [1-3] led to the suggestion that anaerobes may also play an important role in this syndrome, whereas other workers failed to isolate any anaerobes.[2] To determine whether *G vaginalis* alone or in combination with anaerobes are the cause of NSV it is important to establish their occurrence not only in patients with NSV[1 3] but also in those with other genital diseases. We therefore carried out a quantitative and qualitative study of the aerobic and anaerobic bacterial flora of vaginal secretions obtained from patients attending a genital medicine clinic from whom a variety of pathogens were isolated including *G vaginalis.*

Patients and methods

Eighty-nine patients attending the department of genital medicine, St Bartholomew's Hospital, London, had vaginal samples investigated routinely for the presence of known and potential pathogens, namely gonococci, *Candida,* trichomonads, ureaplasmas, and chlamydia, and also had vaginal samples collected for qualitative and quantitative analysis of the aerobic and anaerobic bacterial flora

TABLE 1 *Quantitative vaginal bacterial flora in genital disease. (Figures in brackets are the number of women with each diagnosis.)*

	Gonorrhoea (11)	*Trichomoniasis (17)*	*Candida infection (17)*	*Non-specific vaginitis (14)*	*Contact of non-specific urethritis (16)*	*Non-specific genital infection (7)*	*No venereal diseases (7)*
Total bacterial count							
Mean	8·2	8·4	7·8	8·2	8·2	8·0	7·5
Range	6·6-9·1	7·8-9·5	5·6-11·0	5·7-9·7	7·3-9·0	5·5-9·0	4·9-8·8
No of patients	11	17	11	14	16	7	7
Total aerobes							
Mean	7·7	7·3	7·0	7·2	7·3	7·0	6·2
Range	6·4-8·9	5·7-8·9	4·8-8·5	5·5-8·4	4·0-8·9	4·0-8·9	3·5-8·8
No of patients	11	17	17	14	16	7	7
Total anaerobes							
Mean	8·0	8·3	7·8	8·3	7·6	8·3	6·6
Range	6·2-9·1	7·4-9·5	5·5-11·0	5·4-9·7	5·7-8·8	7·2-8·9	4·9-8·5
No of patients	11	17	17	13	16	6	5
G vaginalis							
Mean	7·3	5·7	7·8	7·0	7·4	6·8	6·1
Range	6·2-7·6	5·6-5·7		5·1-8·4	5·6-8·7		
No of patients	2	2	1	8	6	1	1
Anaerobic cocci							
Mean	7·9	8·7	6·8	7·7	7·2	7·7	6·1
Range	6·2-8·8	6·2-9·1	3·2-8·6	5·1-9·0	3·7-8·8	5·1-8·9	3·0-8·5
No of patients	5	9	6	9	10	5	3
Bacteroides spp							
Mean	6·5	7·7	4·8	7·1	5·9	7·7	6·4
Range	5·1-7·9	7·0-8·1	4·0-5·6	5·5-8·7	4·9-7·8	7·0-8·6	5·8-7·0
No of patients	2	5	3	4	7	4	2

using a method described by us in detail.[8] Women who had taken antimicrobials in the preceding month were excluded from the study.

Specimens were processed in an anaerobic chamber using prereduced enriched culture media.[8] The minimum concentration of organisms that could be detected was 3·0 $\log_{10}$ cfu/g of secretion. Identification of organisms was carried out according to the methods of Cowan and Steel[9] for aerobic bacteria and Holdeman, Cato and Moore[10] for anaerobic bacteria. For technical reasons the results obtained for the chlamydia and ureaplasma cultures were invalid and will be excluded from this report.

G vaginalis was isolated on Brucella agar supplemented with 10% horse blood and differentiated from other oxidase negative and catalase-negative Gram-variable organisms by the production of a small β-haemolytic zone in human blood agar, by the hydrolysis of hippurate, and by the fermentation of galactose, starch, and maltose.[11 12]

Patients were grouped according to their disease syndrome and pathogens isolated—for example, gonococcal disease (GC), *Trichomonas vaginalis* (TV), *Candida,* non-specific vaginitis (NSV), non-specific genital infection (NSGI), non-specific urethritis contacts (NSU contact), and non-venereal disease (non-VD).

DEFINITIONS OF DIAGNOSES

In this study NSV was defined as the presence of an abnormal amount of vaginal discharge of unspecified colour, with or without inflammation of the vaginal mucosa (as judged by a single clinician), and existing in the absence of other pathogens and any contact with known sexually transmitted disease (STD). In addition, these patients did not have a gross excess of leucocytes on Gram-stained cervical smears or inflammatory changes affecting the epithelial cells on the Papanicolaou-stained cervical smears.

NSGI was defined as the presence of a gross excess of leucocytes in Gram-stained cervical smears, inflammatory changes affecting the epithelial cells in Papanicolaou cervical smears, and the absence of GC, TV, *Candida,* and any contact with a patient with any STD.

Patients classified as non-VD had no excess of vaginal discharge, no GC or *Candida,* none of the inflammatory changes noted in patients with NSGI, and no known contact with any STD.

Results

A summary of the incidence and the mean and range of concentration of the organisms isolated in the different clinical conditions is shown in table I. Aerobic bacteria were isolated from all 89 patients and anaerobic bacteria from all but four patients. The concentration of organisms in the different clinical conditions ranged from 4·9-11·0 $\log_{10}$ cfu/g of vaginal secretions. Anaerobic bacteria outnumbered aerobes by approximately 10 : 1 except in the contacts of NSU and the non-VD group, in whom the anaerobes were present at lower counts (table I). *G vaginalis* was isolated from eight of 14 (57%) patients with NSV and in six of 16 (37·5%) contacts of NSU (mean concentrations 7·0 and 7·4 $\log_{10}$ cfu/g respectively), *G vaginalis* was rarely isolated in cases of GC, TV, candidiasis, NSGI, and non-VD, where the overall incidence was 11·8%.

Anaerobic Gram-positive cocci were a common component of the vaginal flora in all conditions with high isolation rates from patients with NSGI (71%) and NSV (64%), contacts of NSU (62·5%), and patients with TV (53%). Bacteroides species were isolated from 27 patients and were not associated with any particular group (table I). The different species and the distribution of anaerobic Gram-positive cocci and bacteroides are shown in tables II and III. Anaerobic Gram-negative cocci were much

TABLE II *Strains of peptococci and peptostreptococci spp isolated from 47 patients*

Species	*No of strains isolated*	*Mean concentration ($\log_{10}$ cfu/g secretion)*
Pc magnus	11	7·9
Pc asaccharolyticus	13	8·3
Pc prevottii	2	7·8
Pc variabilis	3	5·9
Ps micros	7	7·8
Ps productus	3	6·0
Ps parvulus	2	7·0
Ps anaerobius	8	8·6
Unidentified	9	8·2
Total	58	

TABLE III *Strains of bacteroides spp isolated from 27 patients*

Species	*No of strains isolated*	*Mean concentration ($\log_{10}$ cfu/g secretion)*
B fragilis	3	7·6
B thetaiotaomicron	1	8·3
B vulgatus	1	7·9
B melaninogenicus		
ss *melaninogenicus*	6	6·4
ss *intermedius*	4	7·6
B bivius	11	7·3
B disiens	3	7·2
B assacharolyticus	4	7·9
Bacteroides spp (unidentified)	6	7·1

less commonly isolated and showed no correlation with any particular clinical condition. Anaerobic Gram-positive rods, especially lactobacilli, were also common. *Escherichia coli,* which was the only coliform organism isolated, was found in four patients, three of whom had NSV. A more detailed analysis of the bacterial findings will be reported elsewhere.

The association of anaerobic cocci with *G vaginalis* in the patients in the different groups is shown in the figure. The combination of both organisms in high concentration (>5·0 $\log_{10}$ cfu/g) of vaginal secretions occurred predominantly in patients with NSV (7/14) and in contacts of NSU (5/16), though two patients with TV and one in each group with candidiasis, GC, NSGI, and non-VD had a similar association. High counts of *G vaginalis* were found without anaerobic cocci in three patients (one GC, one NSV, and one contact of NSU). On the other hand, anaerobic Gram-positive cocci were present in high concentrations without *G vaginalis* in 29 patients, 26 of whom had counts greater than 5·0 $\log_{10}$ cfu/g of vaginal secretions. In those patients anaerobic Gram-positive cocci were associated with other pathogens (seven TV, five *Candida,* and four GC) and with various clinical conditions (four contacts of NSU, four with NSGI and two with NSV). Patients with *Candida* (11/17), non-VD (4/7), and TV (8/17) had neither *G vaginalis* nor anaerobic cocci and are therefore clustered in the lower left-hand corner of the figure.

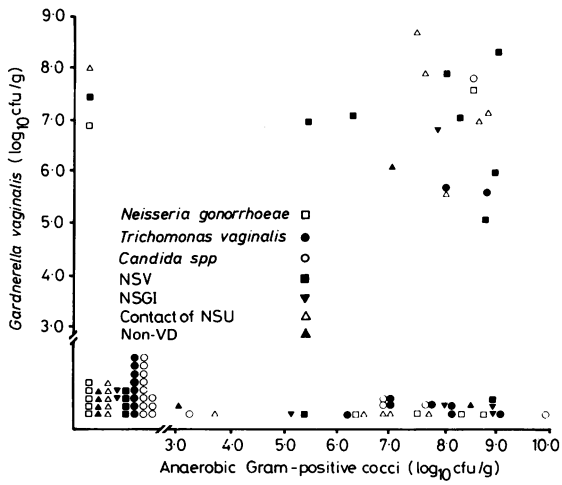

FIG *Incidence and concentration of G vaginalis and anaerobic Gram-positive cocci in the vaginal secretions of patients with genital disease*

Discussion

These results showed that *G vaginalis* was much more commonly isolated and in higher concentrations from patients with non-specific vaginitis than from the other clinical groups. Furthermore, anaerobic bacteria were present in high concentrations in patients with NSV and in association with *G vaginalis,* but they were not specific to this condition and occurred frequently in other clinical groups and in association with other pathogens. The predominant anaerobic bacteria isolated in association with *G vaginalis* were peptococci, whereas in contrast to previous reports[1 3] we found no correlation between bacteroides species and *G vaginalis.* Anaerobic Gram-positive cocci were also found together with other pathogens and in particular with *T vaginalis.* The isolation rate of anaerobic bacteria was similar to that previously reported from asymptomatic women.[7 13]

The controversy regarding the pathogenicity of *G vaginalis* still exists. Gardner and Dukes[4] found that 11 of 15 normal women volunteers who were inoculated with infected vaginal material developed clinical symptoms and had positive culture results for *H(G) vaginalis,* whereas only one of 13 volunteers inoculated with pure cultures of *H(G) vaginalis* developed a clinical infection.[14] This suggests perhaps that in the first experiment other organisms may have also been involved in producing the infection. Pheiffer *et al*[1] and Spiegel *et al*[3] isolated anaerobic bacteria in association with *G vaginalis* and suggested that certain anaerobes act with *G vaginalis* in causing vaginitis.[1 3] This is further supported by the efficacy of metronidazole treatment in these patients,[1-3] a drug which is highly active against anaerobic bacteria and only moderately active in vitro against the facultative anaerobe *G vaginalis,*[1-3] though the hydroxy metabolite of metronidazole may be more active in vitro against *G vaginalis* than the parent compound.[15]

The microbiological basis for the association of *G vaginitis* with anaerobes is not clear. The production of ammonia[16] and amines[1] by obligate anaerobes may raise the pH of the vaginal fluid thereby enabling *G vaginalis* to thrive.[3] But this is not specific to *G vaginalis;* anaerobes are also present with the other pathogens. Whether anaerobic bacteria merely provide a suitable milieu for the growth of pathogens or are actively involved in a mixed infection is not known; nor is it known whether changes in the numbers and types of anaerobic bacteria, in the absence of other vaginal pathogens, can cause symptoms as in our group with non-specific genital disease (NSGI), who may benefit from metronidazole treatment. It seems likely therefore

that anaerobic bacteria play a significant role in most vaginal infections as they do in other female pelvic infections.[17]

The higher incidence of *G vaginalis* in our patients who were contacts of NSU suggests that this organism may have been sexually transmitted. Earlier reports by Gardner and Dukes[4] showed that a high proportion of male sexual partners of women with NSV carried *G vaginalis* in their urethras, and Pheiffer *et al*[1] recovered *G vaginalis* from 79% of male sexual partners of infected women. Further prospective work in both partners is, however, needed to determine whether *G vaginalis* is sexually transmitted.

The isolation rate of *G vaginalis* from asymptomatic women and from patients with NSV is variable.[6 18] This may be due to differences in cultural techniques,[6 11 12] but it is also likely to be due to differences in the criteria used for defining NSV. Hence, it could be argued that *G vaginalis* vaginitis should be regarded as a separate clinical entity similar to candidosis or trichomoniasis thus enabling the search to continue for other aetiological agents in the culture-negative group.

In conclusion, *G vaginalis* is predominantly associated with non-specific vaginitis while anaerobic bacteria are not specific to this condition but are also associated with other pathogens and other conditions and may play an important role in the pathogenesis of these infections. Differential treatment regimens with quantitative microbiological analysis are required to assess their relative pathogenicity.

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