Genitourin Med 1986;62:256-63

Quantitative studies of vaginal bacteria

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SUMMARY A quantitative method of culture, based on a weighed sample and with results expressed as colony forming units (cfu)/g was assessed and used to investigate the vaginal flora of normal women and that of women with vaginal disease. Samples were collected by means of disposable plastic loops into modified proteose peptone water transport medium in preweighed bottles. Counts expressed as cfu/g of secretion were consistent, whereas counts expressed as cfu/ml were inconsistent. Results obtained with specimens manipulated on the open bench were the same as those from duplicate samples processed in an anaerobic chamber.

The normal vaginal flora was predominantly aerobic — lactobacilli, coryneforms, and coagulase negative staphylococci — with counts of $\geqslant 10^8$ cfu/g for lactobacilli. These were also present in patients with candidosis, but the flora in patients with trichomoniasis, bacterial vaginosis, gonorrhoea, or chlamydial infection was predominantly anaerobic. The commonest anaerobes were *Bacteroides* spp, particularly *B bivius;* they were found in 55% of controls but at counts of 10^2 cfu/g lower than in the patients, most of whom had high counts of anaerobes ($>10^8$ cfu/g). The isolation rate of *Gardnerella vaginalis* was not appreciably greater from patients with bacterial vaginosis, and the quantitative cultures on controls and patients who were *G vaginalis* positive were the same ($\simeq 10^7$ cfu/g).

Quantitative studies show greater differences than qualitative cultures between normal controls and patients with vaginal infections, indicating that some symptoms and signs of such infections may be related to quantitative polymicrobial changes.

Introduction

Vaginal discharge is a common condition that has several causes. A purulent discharge (vaginitis) may be caused by vaginal infection with *Trichomonas vaginalis* or *Candida albicans*, or may be associated with cervicitis caused by *Neisseria gonorrhoeae* or *Chlamydia trachomatis*. In the condition now known as bacterial vaginosis[1] (formerly known as non-specific vaginitis or anaerobic vaginosis[2]) the discharge is offensive but not purulent and is characterised by the presence of large numbers of *Gardnerella vaginalis*, anaerobic bacteria (mostly *Bacteroides* spp of the melaninogenicus-oralis group), and microaerophilic curved rods of the genus *Mobiluncus*.[1 3-6] The role of these bacteria in the pathogenesis of bacterial vaginosis is not clear. Most workers agree that *G vaginalis* is characteristically present in large numbers and is evident in Gram stained smears and in appropriate cultures, but opinions differ about its presence in the normal vaginal flora. Several groups of workers have isolated *G vaginalis* from more than 90% of women with a clinical diagnosis of bacterial vaginosis but from few women with normal vaginas,[7 8] but McCormack *et al*[9] found almost equal isolation rates (33%) from patients and controls. The different reports of qualitative studies may reflect quantitative differences in the *G vaginalis* population between the groups and the sensitivity of the cultural methods.

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Accepted for publication 16 December 1985

Non-sporing anaerobic bacteria are implicated in infections of the female genital tract,[10] including superficial abscesses, vaginal infections, salpingitis, and pelvic abscesses, but again their contribution to the normal vaginal flora is debatable. Different groups have reported isolation rates for non-sporing anaerobes from women with normal vaginas of 4%[6 11] to >65%.[12-14] These differences, in part, reflect different standards of anaerobic culture — those who have used more sophisticated methods have reported the higher rates — and may again be related to the numbers of anaerobes present and the sensitivity of the cultural methods.

Most studies on vaginal flora have been based on qualitative methods. It is now important to define the relative contribution of the different microbial components in normal flora, in patients with specific infections by known pathogens, and in those with conditions that may have a polymicrobial aetiology. This has led some investigators to use quantitative techniques based on either the volume or the weight of the sample of vaginal secretion analysed. Various methods have been devised to determine the sizes of samples of vaginal secretions on which viable counts may be based. Bartlett *et al*[15] used paired swabs sequentially inserted into the posterior vagina to collect vaginal secretions; one swab was used to determine the weight of the specimen and the other was expressed in Cary-Blair transport medium for aerobic and anaerobic viable counts. Onderdonk *et al*[16] and Levison *et al*[17] combined the weight based swab method with the use of calibrated loops to collect standard volumes of secretions for viable counts. Calibrated "cervical slime suckers" were used by Lindner *et al*[18] to collect measured volumes of secretions from the endocervix and posterior fornix. Wilks *et al*,[18] however, found that none of these methods was satisfactory. They advocated a weight based method, in which the sample was collected by a bacteriological loop and smeared on the sides of a preweighed tube of modified Cary-Blair transport medium that was then reweighed to calculate the weight of the sample.

In this study, methods based on weight and volume of sample were compared for quantifying the vaginal microbial flora. Two transport media and two methods of anaerobic culture were also compared, and the most reliable method was applied to study the vaginal flora of asymptomatic women and of women with candidosis, trichomoniasis, bacterial vaginosis, gonorrhoea, or chlamydial infection.

Patients and methods

STUDY POPULATION

Quantitative bacteriological studies were carried out on 100 women attending the department of genitourinary medicine, Royal Hallamshire Hospital, Sheffield. They were divided into seven groups on the basis of their confirmed diagnoses: group I comprised 22 healthy asymptomatic controls with negative laboratory investigations for specific pathogens; group II (n=16) had vaginal candidosis; group III (n=12) had *T vaginalis* vaginitis; group IV comprised 22 women with bacterial vaginosis diagnosed on the basis of a thin homogeneous discharge ("flour paste appearance") with a distinct fishy odour and confirmed by the presence of clue cells and Gram variable bacilli in a Gram stained film and a positive amine test[3]; group V patients (n=12) had gonorrhoea; group VI patients (n=10) had *C trachomatis* infection; and the six patients allocated to group VII had positive cultures for both *N gonorrhoeae* and *C trachomatis*.

CULTURE METHODS

Quantitative cultures were made on a set of eight plates of selective and non-selective media. Samples of vaginal secretions were collected into transport media and a series of ten fold dilutions was made (0·1 ml in 0·9 ml) in the same medium. Initially, dilutions of 10^3 - 10^7 were plated on the test media but subsequently this was restricted to dilutions of 10^4 - 10^6 when these had been found to be suitable; 0·1 ml volumes were spread with glass or metal spreaders on the following media: blood agar (two plates), bacteroides medium (BM) agar and BM agar with kanamycin 75 mg/l (BMK),[19] cycloserine-cefoxitin-fructose agar (CCFA), Rogosa agar, Sabouraud's agar, and MacConkey's agar. One blood agar plate was incubated in air and 5% carbon dioxide and the Sabouraud's and MacConkey's agars in air at 37°C. These plates were examined after 24-48 hours. The other plates were incubated anaerobically at 37°C and examined after 48 hours.

Colony types were counted with a Gallenkamp colony counter and representative colonies were subcultured for identification by the methods of Cowan[20] for aerobes and facultative organisms, Duerden *et al*[21] and Rotimi *et al*[22] for non-sporing anaerobes, and Willis[23] for clostridia.

PILOT STUDIES

A series of pilot studies was performed to select the best method for quantitative studies of the vaginal flora.

Comparison of transport media

Two transport media were tested in parallel for duplicate specimens from 18 women. The media were modified proteose peptone (Proteose Peptone No 3 (Difco) 15 g/l and sodium bicarbonate 1 g/l in distilled water, (pH 7·2) with cysteine hydrochloride 0·75 g/l added after autoclaving) and VMG II medium,[24] each dispensed in 1 ml volumes in 7 ml screw capped bottles. The bottles were then weighed accurately (0·0001 g; Analytical Balance, Sartorius). Secretions

from the posterior fornix were collected from 18 women with calibrated 10 μl disposable bacteriological loops (Nunc), which were immediately broken off into the bottles of transport media; two loopfuls were introduced into each bottle. The samples were processed within 30 minutes. The secretions were dislodged from the loops and homogenised in the transport media by vortex mixing (Rotamixer) for one minute. The loops were then removed aseptically and the bottle reweighed to calculate the weight of secretions sampled. A first viable count was performed immediately and a second after the bottles had been left at room temperature for one hour.

Comparison of sample sizes by weight and volume
The results for the samples taken from the 18 women were expressed as colony forming units (cfu)/g based on the weighed samples and as cfu/ml, on the assumption that each loopful was 0·01 ml, to determine the most reliable method of measuring the sizes of samples.

Comparison of aerobic and anaerobic processing
Samples were collected from 10 women into modified proteose peptone water and weighed as above. The bottles were then introduced into an anaerobic chamber (Mark I, Don Whitley Scientific, Shipley, West Yorkshire) where the specimens were divided into two portions. Viable counts were performed on each portion: for the first portion all manipulations were done in the anaerobic chamber and the anaerobic plates were incubated in the chamber in an atmosphere of nitrogen 80%, hydrogen 10%, and carbon dioxide 10%; the second portion was removed from the chamber and processed on the open bench, and the anaerobic plates were incubated in an anaerobic jar with an atmosphere of hydrogen 90% and carbon dioxide 10%, as described previously.[25] The counts obtained by the two methods were compared after the plates had been incubated at 37°C for 48 hours.

SURVEY OF VAGINAL BACTERIA
Based on the results of the pilot studies, modified proteose peptone water was selected as the transport medium for the main survey, specimen sizes were measured by weighing, and all manipulations for viable counts were done on the open bench. Anaerobic plates were incubated in the anaerobic chamber for convenience. Samples were collected from 72 women and processed within 30 minutes.

The results obtained in the pilot studies and in the survey were combined for the comparison of the bacterial flora in the seven subject groups.

The χ^2 test was used to compare the isolation rates of the different bacteria in the controls and six patient groups.

Results

A total of 100 women was studied: 22 asymptomatic controls with no signs of vaginal disorder (group I) and 78 patients with genitourinary infections (groups II to VII). Table I shows their demographic data: there were no differences between the control and the patient groups.

TABLE I *Demographic details of study population*

Demographic character	*Controls (n = 22)*	*Patients (n = 78)*	*All subjects (n = 100)*
Mean age (range)	23·8 (19-46)	25·1 (17-50)	24·3 (17-49)
Marital status:			
Single	15	55	70
Divorced	1	6	7
Married	6	17	23
Past history of:			
Candidosis	2	6	8
Trichomoniasis	0	2	2
Non-specific vaginosis	2	7	9
Chlamydial infection	1	4	5
Gonorrhoea	0	4	4
Others (warts)	1	5	6
None	16	60	76
Contraceptives:			
Oral	13	46	59
Intrauterine device	1	4	5
Sheath	0	7	7
None	8	21	29

PILOT STUDIES
Comparison of transport media
There were no differences between the results obtained with the two transport media from duplicate specimens from 18 women. In each case the same number of species was isolated from each medium and the total (aerobic and anaerobic) viable counts (expressed as cfu/g) were the same (table II). There were no differences between the results obtained with specimens processed within 30 minutes of collection and those obtained after specimens had been held at room temperature for one hour. Proteose peptone water was selected for subsequent studies because it contained fewer ingredients, was easier to prepare, and was cheaper.

Comparison of sample sizes by weight and volume
The weight of vaginal secretions collected with the calibrated loops varied between different loopfuls in the same patient and between different subjects. In duplicate specimens from the same subject (proteose peptone and VMG II samples) viable counts expressed as cfu/g were consistent, whereas counts

TABLE II *Weights of 10µl loopfuls and total viable counts (colony forming units (cfu)) of vaginal secretions from 18 women*

	Proteose peptone water specimen			*VMG II specimen*		
Case No.	*Weight (g)**	*cfu/ml*	*cfu/g*	*Weight (g)†*	*cfu/ml*	*cfu/g*
1	0·0116	$1·2 \times 10^7$	$1·0 \times 10^7$	0·0520	$5·2 \times 10^7$	$1·0 \times 10^7$
2	0·0086	$8·0 \times 10^8$	$9·2 \times 10^8$	0·0648	$5·9 \times 10^9$	$9·2 \times 10^8$
3	0·0682	$7·8 \times 10^8$	$1·1 \times 10^8$	0·0184	$2·0 \times 10^8$	$1·1 \times 10^8$
4	0·0107	$5·1 \times 10^6$	$4·8 \times 10^6$	0·0080	$3·7 \times 10^6$	$4·7 \times 10^6$
5	0·0098	$4·5 \times 10^8$	$4·6 \times 10^8$	0·0194	$8·9 \times 10^8$	$4·6 \times 10^8$
6	0·0377	$1·7 \times 10^9$	$4·5 \times 10^8$	0·0126	$5·5 \times 10^8$	$4·4 \times 10^8$
7	0·0044	$2·4 \times 10^8$	$4·5 \times 10^8$	0·0252	$1·3 \times 10^9$	$5·4 \times 10^8$
8	0·0614	$6·3 \times 10^8$	$1·0 \times 10^8$	0·0370	$3·7 \times 10^8$	$1·0 \times 10^8$
9	0·0618	$3·4 \times 10^8$	$5·5 \times 10^7$	0·0128	$7·0 \times 10^7$	$5·5 \times 10^7$
10	0·0050	$4·0 \times 10^5$	$8·0 \times 10^5$	0·0483	$3·8 \times 10^6$	$8·0 \times 10^5$
11	0·0594	$7·2 \times 10^7$	$1·2 \times 10^7$	0·0089	$9·7 \times 10^5$	$1·1 \times 10^7$
12	0·0090	$7·0 \times 10^8$	$7·8 \times 10^8$	0·0061	$4·7 \times 10^6$	$7·8 \times 10^8$
13	0·0120	$4·3 \times 10^7$	$3·6 \times 10^7$	0·0190	$6·7 \times 10^7$	$3·5 \times 10^7$
14	0·0812	$2·8 \times 10^9$	$3·4 \times 10^8$	0·0888	$3·1 \times 10^9$	$3·5 \times 10^8$
15	0·0646	$2·0 \times 10^9$	$3·0 \times 10^8$	0·0090	$2·7 \times 10^8$	$3·0 \times 10^8$
16	0·0494	$3·8 \times 10^8$	$7·7 \times 10^7$	0·0144	$1·1 \times 10^8$	$7·7 \times 10^7$
17	0·0125	$2·0 \times 10^7$	$1·6 \times 10^7$	0·0067	$1·0 \times 10^7$	$1·6 \times 10^7$
18	0·0289	$1·5 \times 10^8$	$5·2 \times 10^7$	0·0400	$2·0 \times 10^8$	$5·1 \times 10^7$

* Coefficient of variation for 18 weights = 78·9;
† Coefficient of variation for 18 weights = 82·9;
Coefficient of variation for all (36) weights = 81·5.

expressed as cfu/ml varied widely (table II). To confirm this finding six loopfuls of secretions collected from one subject were added to separate 1 ml volumes of proteose peptone water. All were processed in parallel. Table III shows the weights of the six samples and the total viable counts expressed as cfu/g and cfu/ml. Measurements of sample size by weight were chosen for subsequent studies.

Comparison of aerobic and anaerobic processing

There were no differences between the viable counts (cfu/g) obtained with specimens processed on the open bench and the anaerobic plates incubated in anaerobic jars and the duplicate specimens processed in the anaerobic chamber. In subsequent experiments specimens were processed on the open bench and anaerobic plates were incubated in the anaerobic chamber for convenience.

TABLE III *Comparison of weights and total viable counts (colony forming units (cfu)) of six 0·01 ml loopfuls of vaginal secretions collected from one subject*

Sample weight (g)	*Total viable count*	
	cfu/g	*cfu/ml*
0·0293	$5·8 \times 10^7$	$1·7 \times 10^8$
0·0790	$5·8 \times 10^7$	$5·5 \times 10^8$
0·0061	$5·7 \times 10^7$	$3·5 \times 10^7$
0·0109	$5·8 \times 10^7$	$6·3 \times 10^7$
0·0502	$5·8 \times 10^7$	$2·9 \times 10^8$
0·0310	$5·8 \times 10^7$	$7·7 \times 10^8$

QUANTITATIVE SURVEY OF VAGINAL BACTERIA

Aerobic and facultative species

Table IV shows the numbers and percentages of subjects in each group from whom aerobic and facultative bacteria were isolated. They were isolated from most controls and most patients with candidosis, trichomoniasis, or bacterial vaginosis, but from only a minority of patients with gonorrhoea or chlamydial infections. Lactobacilli, coryneforms, and coagulase negative staphylococci were the most common species isolated from controls (group I) and from patients with candidosis (group II). Lactobacilli were less common in patients with trichomoniasis (group III), or bacterial vaginosis (group IV), and were not isolated from other patient groups. *G vaginalis* was isolated from 40% of patients with trichomoniasis (group III) or bacterial vaginosis (group IV), but was also isolated from 18% of controls (group I), and a small proportion of patients with other diagnoses.

Table V shows the mean viable counts of the aerobic and facultative species in those subjects from whom they were isolated. The total aerobic counts were in the range $\log_{10}$ 5·0 - 8·8 cfu/g for controls and all patient groups. The mean viable counts of lactobacilli in all groups from which they were isolated were $\log_{10} > 8·0$

TABLE IV *Rate of isolation of aerobic and facultative species from vaginal secretions*

Bacteria	No (percentage in groups) of subjects from whom bacteria were isolated						
	*I (n = 22)	II (n = 16)	III (n = 12)	IV (n = 22)	V (n = 22)	VI (n = 10)	VII (n = 6)
All aerobes	18(82)	11(69)	7(58)	16(73)	4(33)	4(40)	1(17)
Gardnerella vaginalis	4(18)	4(25)	5(42)	10(45)	1 (8)	1(10)	1(17)
Lactobacilli	15(68)	11(69)	2(17)	2 (9)	0	0	0
Coryneforms	15(68)	9(56)	7(58)	8(36)	3(25)	3(30)	0
Coagulase negative staphylococci	13(59)	8(50)	5(42)	6(27)	2(17)	1(10)	0
α – haemolytic streptococci	5(23)	1 (6)	2(17)	2 (9)	0	0	0
β – haemolytic streptococci	4(18)	2(13)	0	0	0	1(10)	0
Streptococcus faecalis	1 (5)	0	0	1 (5)	1 (8)	0	1(17)
Escherichia coli	0	0	1 (8)	0	1 (8)	0	0

*I normal controls; II candidosis; III trichomoniasis; IV bacterial vaginosis; V chlamydial infection; VI gonorrhoea; VII chlamydial and gonorrhoeal infection.

cfu/g; coryneforms and coagulase negative staphylococci were present in smaller numbers ($\log_{10}$ 5·0 - 6·1 cfu/g). In the small number of specimens from which *Streptococcus faecalis* was isolated it was present in large numbers ($\log_{10} > 8{\cdot}0$ cfu/g). Though *G vaginalis* was isolated more commonly from patients with bacterial vaginosis (group IV) or trichomoniasis (group III), the viable counts of this species were the same ($\log_{10}$ 6·8 - 7·2 cfu/g) in all specimens from which it was isolated (from controls or patients of all groups).

Anaerobic species

Table VI gives the numbers and percentages of subjects in each group from whom anaerobic bacteria were isolated. Anaerobes were isolated from a little more than half the controls (55%), from only 31% of patients with candidosis, but from most patients in the other five groups. The commonest anaerobes isolated from all groups were *Bacteroides* spp: most belonged to the melaninogenicus-oralis group, with *B bivius* the most common species. There were fewer isolates of asaccharolytic *Bacteroides* spp, but most of these were from patients with bacterial vaginosis (group IV) or chlamydial infection (groups V and VII). Gram positive anaerobic cocci were isolated from all groups but most commonly from patients with bacterial vaginosis, gonorrhoea, or chlamydial infection (groups IV to VII).

Table VII shows the mean viable counts of anaerobes in those subjects from whom they were isolated. The total anaerobe counts in controls were similar to those of aerobic and facultative bacteria, in the range $\log_{10}$ 4·8 - 8·1 cfu/g; if bifidobacteria are disregarded other anaerobe counts were $\log_{10} < 7{\cdot}0$ cfu/g. In patients with candidosis anaerobe counts

TABLE V *Mean viable counts (colony forming units (cfu)) of aerobic and facultative bacteria from vaginal secretions*

Bacteria	$\log_{10}$ mean cfu/g in subject group						
	*I (n = 22)	II (n = 16)	III (n = 12)	IV (n = 22)	V (n = 12)	VI (n = 10)	VII (n = 6)
Gardenerella vaginalis	7·1	6·9	6·8	7·2	7·2	7·1	7·2
Lactobacilli	8·8	8·6	8·0	8·1	0	0	0
Coryneforms	6·0	6·0	5·4	5·9	5·0	5·1	0
Coagulase negative staphylococci	5·6	5·5	6·0	6·1	5·8	6·0	0
α – haemolytic streptococci	6·6	6·0	7·1	6·4	0	0	0
β – haemolytic streptococci	7·2	7·0	0	0	0	7·4	0
Streptococcus faecalis	8·4	0	0	8·5	8·3	0	8·5
Escherichia coli	0	0	6·9	0	6·5	0	0

*I normal controls; II candidosis; III trichomoniasis; IV bacterial vaginosis; V chlamydial infection; VI gonorrhoea; VII chlamydial and gonorrhoeal infection.

TABLE VI *Rate of isolation of anaerobic species from vaginal secretions*

Bacteria	No (percentage in groups) of subjects from whom bacteria were isolated						
	*I (n = 22)	II (n = 16)	III (n = 12)	IV (n = 22)	V (n = 12)	VI (n = 10)	VII (n = 6)
All anaerobes	12(55)	5(31)	8(67)	16(75)	10(83)	8(80)	6(100)
All *Bacteroides* spp	9(41)	4(25)	6(50)	15(68)	8(67)	7(70)	6(100)
B bivius	7(32)	3(19)	4(33)	9(41)	7(58)	5(50)	4 (67)
B disiens	3(14)	0	0	3(14)	3(25)	1(10)	2 (33)
B oralis	2 (9)	0	0	3(14)	0	1(10)	1 (17)
B ruminicola	0	0	1 (8)	0	0	0	0
B intermedius	4(18)	1 (6)	1 (8)	4(18)	2(17)	1(10)	1 (17)
B asaccharolyticus	2 (9)	0	0	6(27)	2(17)	0	1 (17)
B ureolyticus	0	1 (6)	0	1 (5)	1 (8)	0	0
Fusobacterium nucleatum	0	1 (6)	0	1 (5)	0	0	0
Anaerobic cocci	6(27)	1 (6)	3(25)	8(36)	6(50)	4(40)	3 (50)
Bifidobacteria	2 (9)	0	0	2 (9)	0	0	0
Clostridium perfringens	0	0	0	1 (5)	1 (8)	0	0
Eubacteria	0	0	1 (8)	0	0	0	1 (17)

*I normal controls; II candidosis; III trichomoniasis; IV bacterial vaginosis; V chlamydial infection; VI gonorrhoea; VII chlamydial and gonorrhoeal infection.

TABLE VII *Mean viable counts (colony forming units (cfu)) of anaerobic bacteria from vaginal secretions*

Bacteria	Log_{10} mean cfu/g in subject group						
	*I (n = 22)	II (n = 16)	III (n = 12)	IV (n = 22)	V (n = 12)	VI (n = 10)	VII (n = 6)
Bacteroides bivius	6·1	6·3	8·4	8·9	9·3	9·6	9·8
Bacteroides disiens	5·8	0	0	6·8	7·9	8·3	8·0
Bacteroides oralis	6·0	0	0	7·0	0	8·2	8·0
Bacteroides ruminicola	0	0	7·4	0	0	0	0
Bacteroides intermedius	4·8	5·9	6·3	7·7	8·0	8·1	8·0
Bacteroides asaccharolyticus	5·0	0	0	6·8	7·4	0	7·3
Bacteroides ureolyticus	0	6·8	0	7·6	7·8	0	0
Fusobacterium nucleatum	0	6·9	0	8·6	0	0	0
Anaerobic cocci	6·7	6·9	7·3	7·6	8·4	8·3	8·6
Bifidobacteria	8·1	0	0	8·0	0	0	0
Clostridium perfringens	0	0	0	7·1	7·0	0	0
Eubacteria	0	0	8·1	0	0	0	8·0

*I normal controls; II candidosis; III trichomoniasis; IV bacterial vaginosis; V chlamydial infection; VI gonorrhoea; VII chlamydial and gonorrhoeal infection.

were similar to those in controls, but in all other patient groups the anaerobe viable counts were greater, $\log_{10} > 8{\cdot}0$ cfu/g in patients with trichomoniasis (group III) and $\log_{10} > 8{\cdot}9$ cfu/g in other groups (group IV to VII). The species with high counts in specimens from the patient groups were generally the same as those that were present at lower counts in controls.

Comparison between groups

The χ^2 test was used to compare the rates of isolation of particular species from different patient groups and from controls. Lactobacilli were isolated significantly less often from groups III ($p<0{\cdot}01$) and IV ($p<0{\cdot}001$) and not at all from groups V to VII. There were no significant differences between the rates of isolation of *G vaginalis*, even in patients with bacterial vaginosis (group IV) ($\chi^2 = 3{\cdot}7$; $0{\cdot}05 < p < 0{\cdot}01$).

Of the anaerobes, *Bacteroides* spp were isolated significantly more often from group VII only ($p < 0{\cdot}05$). Their isolation from group IV (bacterial

vaginosis) did not reach significance ($\chi^2 = 3{\cdot}3$; $0{\cdot}05<p<0{\cdot}1$). *G vaginalis*, however, was isolated from 10 of the 22 patients in this group, and *Bacteroides* spp were isolated from eight (80%) of these; this combination was significantly more common in this group ($p<0{\cdot}05$).

There were no differences in either the types of isolate or in their viable counts between subjects who were using oral contraceptives and those who were not. There were insufficient numbers to compare groups using other contraceptives.

Discussion

The pilot studies showed that measuring samples of the vaginal secretion by volume was not reliable and that viable counts expressed as cfu/ml could be misleading. On the contrary, there were consistent results when the sizes of the samples of secretions were determined by weighing and the viable counts were expressed as cfu/g of secretion. The use of a simple transport medium such as modified proteose peptone water and the manipulation of the specimens on the open bench before incubation in either an anaerobic chamber or an anaerobic jar gave satisfactory results; it did not seem to be necessary to do all the manipulations in an anaerobic environment.

The contribution of various species of bacteria to bacterial vaginosis (non-specific vaginitis) and other infections of the female genital tract is the subject of some controversy. It is now thought that many of these infections are polymicrobial and that the non-sporing anaerobes are an important component. As many of these bacteria are present in the normal vagina the pathogenesis of these infections may be related to quantitative changes in the vaginal flora. Recent studies have shown the importance of quantitative sampling and the inadequacy of simple qualitative sampling to elicit the relative contribution of the different bacteria in such mixed infections.[4 9 18 26] In this study we used a quantitative culture method to investigate the relative contributions of different species of bacteria and to compare these with the qualitative isolation rates of the species in normal controls and in mixed patient groups.

Our baseline studies of normal controls showed, as expected, that the predominant flora was aerobic (lactobacilli, coryneforms, and staphylococci). *G vaginalis* was found in 18% of these women, a similar figure to the 13% found by Taylor *et al.*[6] Anaerobes were isolated from 55% of these normal controls, which is similar to the isolation rates of $\simeq$ 65% reported by Gorbach *et al*,[12] Bartlett *et al*,[15] Sanders *et al*,[13] and Duerden,[14] but much higher than the 8·6% of Neary *et al*,[27] and particularly the 4% of Lindner *et al*[11] and Taylor *et al*[6] in their quantitative studies.

In the six patient groups, as well as the isolation of specific pathogens *(Cand albicans, T vaginalis, N gonorrhoeae* and *C trachomatis)*, there were some qualitative differences in the general vaginal flora, particularly the absence of lactobacilli from patients with gonorrhoea and chlamydial infection. Quantitative studies, however, showed much greater differences between these groups – and some similarities.

Although there was an increase in the isolation rate of *G vaginalis* from women with bacterial vaginosis (45%) compared with that from women with normal vaginas, it was only marginally significant. In contrast, Taylor *et al*[6] isolated *G vaginalis* from 65% of women with bacterial vaginosis. Some quantitative studies found that although qualitative isolation rates may be similar, the concentration of *G vaginalis* increased from $<10^4$ cfu/ml in women with normal vaginas to $>10^7$ in women with bacterial vaginosis[4]: Taylor *et al*[6] recovered 41 isolates of *G vaginalis* from 82 vaginal samples at a mean count of 10^{10} cfu/ml. Using our weight based sampling method, however, we did not find any difference between the counts of *G vaginalis* from patients with bacterial vaginosis and those from normal women; when *G vaginalis* was present the mean viable counts were $\simeq 10^7$ cfu/g in each group. The varied diagnostic and sampling criteria for bacterial vaginosis impedes comparison of patient groups in different studies.

Quantitative differences were most apparent with anaerobes. When anarobes were present in normal controls the mean count was $<10^7$ cfu/g, whereas in all patient groups — except those with candidosis — the mean counts were $> 10^8$ cfu/g and mostly 10^9 cfu/g. This indicates that there is an aerobic vaginal environment in candidosis but a predominantly anaerobic one in the other conditions studied. *T vaginalis* is itself an anaerobic protozoan parasite, and it is not surprising that the bacteria associated with it are also anaerobic. The findings in bacterial vaginosis support the hypothesis that anaerobes, particularly *Bacteroides* spp, have an important role in the condition, perhaps more important than that of *G vaginalis*. In patients with bacterial vaginosis eight of 10 from whom *G vaginalis* was isolated also had anaerobes. Similarly, eight (67%) of the 12 *G vaginalis* negative patients had positive cultures for anaerobes, and whereas the numbers of *G vaginalis* in positive specimens were unchanged, the number of *Bacteroides* increased 100-fold.

C trachomatis and *N gonorrhoeae* cause cervicitis, vaginal discharge, and have been implicated in pelvic inflammatory disease. Interestingly, the highest concentrations of anaerobes in our study population were found in patients with either or both of these pathogens. This may indicate that the primary pathogens damage the epithelial lining of the vagina and change the local environment, allowing anaerobes to colonise and mul-

tiply more easily, and that the anaerobes may then help exacerbate the infection or delay cure, even after the primary pathogen has been treated. We found similar proportions of anaerobes in cervical cultures from patients with acute salpingitis, in which *N gonorrhoeae* and chlamydiae were the commonest primary pathogens (GR Kinghorn, S Hafiz, BI Duerden, unpublished observations).

As has been found in other studies, *B bivius* was the most common anaerobic isolate (qualitatively and quantitatively) in controls and patient groups. It seems to have the greatest potential for colonisation and multiplication in the vagina, and a high count of this organism may be a general indicator of a group of genital diseases.

Generally, quantitative studies have shown greater differences between the bacterial flora in normal subjects and in patients with various vaginal infections than may be evident from qualitative studies. The use of a method based on sample weight overcomes some problems of reproducibility that may have led to confusion in studies based on sample volume. The clinical signs and symptoms in conditions characterised by vaginal discharge may partly be the result of changes in the balance of species in the vaginal flora.

We thank the staff of the department of genitourinary medicine, Royal Hallamshire Hospital for collecting the specimens and providing the clinical data and Mrs Hazel Storer for typing the manuscript.

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