

# Isolation and identification of anaerobic organisms from the male and female urogenital tracts

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**SUMMARY** Since *Bacteroides* spp may play an important role together with *Gardnerella vaginalis* in the pathogenesis of non-specific vaginitis and balanoposthitis, anaerobic organisms were isolated and identified from 34 female and 20 male patients attending a department of genitourinary medicine, and the results compared with the clinical findings and the presence of other routinely isolated pathogens. Twenty-three different organisms were recovered from 13 of the 20 men; of these organisms, 20 were of the *Bacteroides* spp. Most isolates were of the *B melaninogenicus/oralis* group, which forms part of the commensal vaginal flora in women.

A definite association between the presence of anaerobes and other pathogens needs to be firmly established, as this obviously causes important complications in treating these conditions.

## Introduction

Very little is known about the normal commensal flora of the male urethra or of the way it may be modified by the presence of pathogenic organisms.

The Gram negative anaerobic bacilli that form part of the commensal flora of the female genital tract have been well documented by Duerden.<sup>1</sup> *Bacteroides* spp were isolated from the cervix and posterior fornix of the vagina in 65% of women, the most common species found being of the *B melaninogenicus/oralis* group. The study group consisted of normal healthy women attending a family planning clinic; women with vaginal pathology were not included.

In a study by Hafiz *et al*<sup>2</sup> of the Gram positive anaerobic bacillus, *Clostridium difficile*, in the urogenital tract of men and women higher isolation rates of this organism were found in patients attending a department of genitourinary medicine than in control groups attending family planning or urology clinics. Hall and O'Toole<sup>3</sup> first isolated strains of *Cl difficile* from meconium and faeces of infants, which suggested that material from the adult vagina should be examined for these organisms. When vaginal specimens from women attending a department of genitourinary medicine were compared with those from a group attending a family

planning clinic *Cl difficile* was identified in 71% and 18% respectively.<sup>2</sup> *Cl difficile* was isolated from urethral swabs from all the men with non-specific urethritis, but no positive cultures were obtained from men attending the urology clinic. Thus, *Cl difficile* seemed to be an opportunist, infecting the urogenital tract already damaged by a recognised pathogen, or it might have been the primary pathogen in cases of non-specific urethritis.

The causative organisms of non-specific vaginitis (NSV) and urethritis (NSU) are open to controversy. *Gardnerella vaginalis* was isolated from 8% of women attending a family planning clinic, and of these 17% had positive cultures for *Cl difficile* from high vaginal specimens.<sup>4</sup> In the presence of *G vaginalis* abnormalities of vaginal discharge—particularly an offensive odour—were common. The culture rates (6%) of *G vaginalis* in this study differed appreciably from those of McCormack *et al*,<sup>5</sup> who isolated the organism from 32% of vaginal swabs from female undergraduates and of Rodgers *et al*, who found an isolation rate of 60% in women attending a sexually transmitted disease clinic.<sup>6</sup>

Spiegel *et al*<sup>7</sup> assessed the role of anaerobic bacteria in NSV. Previous studies had found increased numbers of *G vaginalis* and an increased concentration of anaerobic bacteria in the vaginal fluid of women with NSV. The efficacy of metronidazole in the treatment of this condition also suggests that anaerobes may have an important role in the syndrome. *G vaginalis* was cultured from 17% of the women with NSV and *Bacteroides* spp from 19%. Again, most isolates of *Bacteroides* spp were of

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Accepted for publication 29 November 1982

the *B. melaninogenicus/oralis* group, as were most isolates found in normal healthy women.

The present study was undertaken to determine the carriage rate of bacteroides and clostridia in the urogenital tract of men and women attending the department of genitourinary medicine in Sheffield, to identify the strains isolated, and to correlate these findings with the patients' symptoms, clinical signs, and the presence of other routinely isolated pathogens.

### Patients and methods

#### COLLECTION OF SPECIMENS

Cervical swabs were collected from 34 women attending the department of genitourinary medicine, Sheffield, under direct vision during examination with a Cusco's speculum and before bimanual examination. The swabs were broken off immediately into Amies' transport medium with charcoal. Specimens were not included from women receiving antibiotic treatment. Urethral swabs were collected from 20 men and transported as above. Samples were processed on the same day that they were collected.

The anaerobic procedure of Collee *et al*<sup>8</sup> was followed; specimens were incubated at 37°C in an atmosphere of 90% H<sub>2</sub> and 10% CO<sub>2</sub>.

#### ISOLATION OF BACTEROIDES

Each swab was used to seed a plate of prereduced BM agar which had been made selective for *Bacteroides* spp by the addition of kanamycin 75 µg/ml and vancomycin 2.5 µg/ml.<sup>9</sup> After incubation for at least 48 hours the plates were examined; smears of representative colonies were Gram stained and appropriate individual colonies subcultured on to BM agar with a metronidazole disc. An aerobic control plate of the particular colony type was also set up.

#### ISOLATION OF CLOSTRIDIA

Each swab was used to seed three plates: blood agar; reinforced clostridial agar, and CCFA, a selective media for *Cl. difficile*.<sup>10</sup> After incubation for 48 hours the plates were examined and smears from isolated colonies Gram stained. Each appropriate colony was subcultured on to a blood agar plate for anaerobic incubation and for aerobic control cultures.

After the four plates had been seeded the swab was placed in a universal container filled with RCM broth plus 0.2% paracresol as a selective medium for *Cl. difficile*.<sup>11</sup> These were incubated anaerobically at 37°C and examined for evidence of growth. The broths were subcultured on to selective media after several weeks.

#### IDENTIFICATION OF ISOLATES

The strains of *Bacteroides* were identified by the methods of Duerden.<sup>9</sup> This included colonial and cell morphology, pigment production, haemolysis, antibiotic disc resistance tests with discs containing neomycin 1000 µg, kanamycin 1000 µg, penicillin 2 units, and rifampicin 15 µg, tolerance tests with taurocholate, deoxycholate, Victoria blue 4R, and ethyl violet, biochemical tests for the production of indole, digestion of gelatin, and hydrolysis of aesculin, and fermentation tests with glucose, lactose, sucrose, rhamnose, trehalose, mannitol, and xylose.

The strains of clostridia were identified on the basis of colonial and cell morphology, fermentation of glucose, maltose, lactose, sucrose, salicin, and mannitol, gelatin digestion, and neutralisation of lecithinase by appropriate antitoxin.<sup>12</sup> The identity of the strains was confirmed by gas-liquid chromatographic analysis.

### Results

Fifty-four specimens were examined, from which 48 strains of clostridia or bacteroides were isolated for further study; 23 of these strains were isolated from 20 urethral swabs from men and 25 from 34 cervical specimens from women.

#### WOMEN

Anaerobes were isolated from 50% of the female patients. The isolation rate for symptomatic and asymptomatic women was 61% and 27% respectively. Overall isolation rate of *G. vaginalis* was 41%-48% from symptomatic and 27% from asymptomatic women. Both organisms were found in 26% of patients, 35% in symptomatic and 9% in asymptomatic women (table I).

Twelve women had a diagnosis of NSV; of these chlamydiae were isolated from 50% and anaerobes

TABLE I Isolation of anaerobes and *G. vaginalis* from patients attending a genitourinary medicine clinic

Organisms isolated	No of patients:		Total No
	With symptoms*	Without symptoms†	
Women:			
Anaerobes	14	3	17
<i>G. vaginalis</i>	11	3	14
Anaerobes and <i>G. vaginalis</i>	8	1	9
Men:			
Anaerobes	8	5	13

\*23 women and 15 men

†11 women and 5 men

also from 50% (table II). Both chlamydiae and anaerobes were isolated from 25% of these women. *G vaginalis* was isolated from 42% of the women with NSV and *G vaginalis* and anaerobes from 25%.

Of 25 species of anaerobes isolated from 17 specimens, 24 were isolates of *Bacteroides* spp (table III). *B fragilis* does not usually form part of the normal vaginal flora. Three women had *B fragilis* isolated; two of these women had symptoms and two harboured *B oralis* and *G vaginalis*.

A total of 21 species of anaerobes were isolated from 23 symptomatic women and four species from 11 asymptomatic women (table IV). Among the symptomatic women, 14 isolates were of the *B melaninogenicus/oralis* group, two were *B fragilis*, and five *B asaccharolyticus*. In the asymptomatic group there were two isolates of the *B melaninogenicus/oralis* group, one isolate of *B fragilis*, and one of *Cl perfringens*.

TABLE II Isolation of chlamydiae, *G vaginalis*, and anaerobes from patients with NSV

Organism isolated	No of women with NSV
Chlamydiae	6
Anaerobes	6
<i>G vaginalis</i>	5
<i>G vaginalis</i> and anaerobes	3
Chlamydiae and anaerobes	3

TABLE III Species of *Bacteroides* isolated

	No isolated
<i>Bacteroides</i> spp	24
<i>B melaninogenicus/oralis</i>	16
<i>B asaccharolyticus</i>	5
<i>B fragilis</i> *	3

\*One also had an isolate of *Cl perfringens*

TABLE IV Relation between species isolated and presence of symptoms in women

Organism isolated	No (%) of symptomatic women	No (%) of asymptomatic women
<i>B melaninogenicus/oralis</i>	14 (67)	2 (50)
<i>B fragilis</i>	2 (9.5)	1 (25)
<i>B asaccharolyticus</i>	5 (25)	
<i>Cl perfringens</i>		1 (25)

#### MEN

The overall isolation rate of anaerobes from the male patients was 65% (table I). Anaerobes were isolated from 53% of symptomatic and from 100% of asymptomatic men. Many of these men had more than one species isolated.

A total of 14 had a diagnosis of NSU. Nine of these men had anaerobic organisms isolated, four also had *N gonorrhoeae*. Fourteen isolates of *Bacteroides* spp were recovered from these men, most (9/14) being of the *B melaninogenicus/oralis* group (table V). There were also two isolates of clostridia from these men.

Six isolates of *Bacteroides* spp were recovered from the six men who did not have a diagnosis of NSU. Three of these isolates were of the *B melaninogenicus/oralis* group. There was one isolate of *Cl sporogenes* from this group of men.

TABLE V Relation between species of anaerobes isolated and diagnosis of NSU in men

Organism isolated	No of men with NSU	No of men without NSU	Total
<i>Bacteroides melaninogenicus/oralis</i>	9	3	12
<i>fragilis</i>	2	2	4
<i>asaccharolyticus</i>	2		2
<i>corrodens</i>	1		1
<i>fusobacterium necrogenes</i>		1	1
<i>Clostridia tetanomorphum</i>	1		1
<i>sporogenes</i>	1	1	2

#### Discussion

These studies have confirmed that a range of anaerobic bacteria can be isolated from the female genital tract and also from the male urethra.

Duerden<sup>1</sup> isolated *Bacteroides* spp from 65% of asymptomatic women attending a family planning clinic. In the present study *Bacteroides* spp were isolated from only three of 11 asymptomatic women—that is, <30%. This discrepancy may be due to the relatively small sample, especially when a large variation in the number of anaerobic organisms isolated is dependent on the phase in the menstrual cycle. Moreover, sampling techniques were different; only samples from the external os were taken in this study. There may also have been some inherent differences between the two study populations.

Anaerobic organisms were isolated from 14 of the 23 symptomatic women. *G vaginalis* was isolated from about 50% of the symptomatic women but only from about 25% of those without symptoms. These figures compare favourably with those of McCormack *et al*<sup>5</sup> and Rodgers *et al*<sup>6</sup>.

Symptomatic women also had a considerably higher isolation rate of both *G vaginalis* and anaerobes (about 30%) than those women without symptoms (9%).

Among the 12 women who had a diagnosis of NSV, anaerobes were isolated from 50% and *G*

*vaginalis* from 42%. Anaerobes together with *G vaginalis* were present in 25% of these women; a further 25% had anaerobes and *Chlamydia trachomatis* isolated. Most isolates of *Bacteroides* spp were of the *B melaninogenicus/oralis* and *B asaccharolytic* groups which comprise most of the normal vaginal flora. When *B fragilis* was found, however, the incidence of associated symptoms or other pathogens was no higher. This generally supports the findings of Spiegel *et al.*,<sup>7</sup> who reported a greater prevalence of anaerobes and *G vaginalis* in women with NSV.

In men anaerobic organisms were isolated from eight of 15 symptomatic and from all five asymptomatic patients. A total of 14 men had a diagnosis of NSU; anaerobes were isolated from nine of these. The species of anaerobes found was similar to those in the women. A total of 16 anaerobic isolates were obtained from 14 men with NSU and seven isolates from six men without.

With this sample size it is not possible to make any clear distinction between these two groups; however, when symptoms are taken into account anaerobes were isolated from only slightly more than 50% of symptomatic men whereas they were present in all the asymptomatic men. This might suggest that the presence of symptoms is associated with a disturbance of the normal anaerobic flora. Whether or not this is the case, it appears that anaerobes occur commonly in the male urethra in many circumstances, both with and without either other sexually transmitted pathogens or symptoms. Their specific role either as a commensal or copathogen requires further investigation.

Though several species of Gram positive anaerobic bacilli were isolated from both the male and female patients, *Cl difficile* was not isolated. The criteria for identification used by Hafiz *et al*<sup>2</sup> were insufficiently precise. Organisms which formed a characteristic pattern in RCM broth with paracresol were found to

be diphtheroids in Gram stained smears and others grew aerobically on media selective for enteric organisms. These organisms did not grow on the specific isolation medium (CCFA) used for *Cl difficile*.

I thank Difco Laboratories for their financial support, Dr B I Duerden for his supervision and encouragement, and Dr G R Kinghorn for his cooperation in collecting the specimens. I also thank Julia Faulkner for her technical advice and Hazel Bland for typing this report.

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