Anaerobes in genitourinary infections in men

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SUMMARY Urethral and sub-preputial swabs from 150 men were examined. There was a strong association between the isolation of anaerobic bacteria, particularly *Bacteroides* spp, and a clinical diagnosis of balanoposthitis, non-specific urethritis (NSU), or both. Aerobic bacteria formed the predominant flora in 28 healthy controls whereas anaerobes were predominant in specimens from 79 patients with balanoposthitis, from 24 with NSU, and from 19 with both. *Bacteroides* spp were the commonest isolates in all patient groups; *B asaccharolyticus, B melaninogenicus* ss *intermedius, B ureolyticus,* and *B bivius* were the most common species. The results obtained with the two swabs were identical except that *Gardnerella vaginalis* was isolated from the urethral swab only in five patients.

Introduction

In recent years there has been increasing interest in anaerobic bacteria and growing awareness of their role in disease. Improvements in techniques for isolating and identifying anaerobic bacteria[1-4] have resulted in an increased isolation of anaerobes from clinical specimens and the recognition that particular species are associated with different types of infection. Anaerobic infections in man are caused most commonly by non-clostridial anaerobes, of which *Bacteroides* spp are the most important. Anaerobic cocci have a smaller but still important role.[5-7]

Bacteroides spp are obligate parasites that form a major component of the normal flora in man and also cause severe sepsis.[8 9] In the female genital tract *Bacteroides* spp form part of the normal vaginal flora[10-13] and are implicated in infections that include superficial ulcers and abscesses of the genitalia and deep infections such as endometritis, puerperal or postabortal uterine sepsis, and pelvic abscesses.[8 9 14] There is also evidence that *Bacteroides* spp may be associated with *Gardnerella vaginalis* in the pathogenesis of non-specific vaginitis.[15] There is much less information about the role of anaerobes in the male genitourinary tract. Gram-negative anaerobic bacilli have been isolated from penile ulcerations and prostatic abscesses and from

infections adjacent to the genitourinary tract—for example, perianal and perirectal abscesses.[16-19]

Balanoposthitis and non-specific urethritis (NSU) are common conditions in patients attending departments of genitourinary medicine. They are distressing for the patients but are often treated inadequately because the cause is not well established. We report the results of our studies of the bacterial flora of the male urethra and genitalia in healthy control subjects and in patients with urethritis or balanoposthitis or both, with particular reference to the occurrence of anaerobic bacteria.

Patients and methods

STUDY POPULATION

A total of 150 men who attended the department of genitourinary medicine, Royal Infirmary, Sheffield, was studied. None of the patients had taken any antibiotic during the two weeks before examination and they had not passed urine for at least three hours. There were 28 control subjects with no clinical signs of genital tract disease; 79 patients with balanitis, posthitis, or balanoposthitis alone; 24 with non-specific urethritis (NSU) alone; and 19 with both balanoposthitis and NSU. The diagnosis of NSU was made by finding ≥10 pus cells per microscopic field (magnification × 600) on a Gram-stained smear of urethral exudate from men for whom microscopic and cultural investigations for *Neisseria gonorrhoeae* and *Trichomonas vaginalis* were negative. In addition, men with NSU had the typical findings of anterior with or without posterior urethritis in the two-glass urine test. The diagnosis of balanoposthitis

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was based on clinical examination; affected patients were subsequently divided into those with erosive and those with non-erosive lesions. Table I shows the demographic data, past histories of sexually transmitted diseases, and recent sexual histories of the men in each group.

SPECIMEN COLLECTION

Two specimens, one preputial and one urethral, were collected from each patient and control with plain cotton wool swabs. These were immediately broken off into screw capped vials of Amies' medium with charcoal[20] and sent to the anaerobe laboratory, department of medical microbiology, where they were processed within two hours of collection.

LABORATORY INVESTIGATIONS

Each swab was agitated, thoroughly squeezed out in about 0·6 ml of proteose peptone No 3 (Difco), and then placed in 20 ml of reinforced clostridial medium (RCM) (Oxoid) with paracresol as enrichment for *Clostridium difficile*.[21] Two drops (0·04 ml) of the proteose peptone suspension were seeded on to each of six plates: two blood agar plates (blood agar base No 2 (Oxoid) with 7% defibrinated horse blood (Oxoid)); Sabouraud's agar (Oxoid); Rogosa agar (Oxoid); CCF (cycloserine, cefoxitin, fructose, and egg yolk) agar[22]; and BM (bacteroides medium) agar with kanamycin 75μg/ml (BMK).[23] A 5 μg metronidazole disc was placed in the inoculum well of each BMK plate for presumptive recognition of anaerobes. The Sabouraud's agar and the liquid RCM were incubated aerobically at 37°C. One blood agar plate was incubated in air plus 5-10% CO_2 at 37°C. The other four plates were incubated anaerobically in BTL (Baird and Tatlock Ltd) or Whitley (Don Whitley Scientific) anaerobic jars with 5 g catalyst sachets in an atmosphere of H_2 90% and CO_2 10% (British Oxygen Company) according to the method of Collee et al.[24] The jars were incubated for 48 hours before initial examination. Representative colonies of each colonial type suspected of being an anaerobe were subcultured on to two plates of the same medium as for primary isolation; one was incubated aerobically and the other anaerobically. Primary plates were reincubated for 48 hours and checked for additional colonial types, including pigmented colonies, and then incubated for another three days (total seven days) and examined with particular reference to the appearance of pitting. Aerobic cultures were examined after 24 hours.

IDENTIFICATION

Aerobes, facultative organisms, and non-sporing Gram-positive anaerobes were identified by the methods of Cowan.[25] Clostridia were identified by the methods of Willis,[9] by colonial growth on CCF agar and RCM, and by gas-liquid chromatography (GLC) if indicated. Gram-negative anaerobic bacilli sensitive to metronidazole were identified by their microscopic and colonial morphology, pigment production, haemolysis on blood agar, and by a combined set of tolerance, antibiotic resistance, biochemical, and fermentation tests.[4 26] GLC was performed on representative pigmented *Bacteroides* strains to supplement other tests for identifying *B asaccharolyticus*.

Results

In both healthy controls and disease group patients, identical results were obtained from the paired sub-preputial and urethral swabs in terms of the number, species, and relative proportions of anaerobic and aerobic species with the sole exception of *Gardnerella vaginalis*, which was isolated from the urethral swab only in five patients.

Anaerobic bacteria were isolated from only six (21%) of the healthy normal controls but from most of the patients in all three disease groups; they were found in 60 (76%) men with balanoposthitis alone, in 16 (67%) men with NSU alone, and in 18 (95%) with both diseases ($x^2 = 25·9$, $10·8$, and $24·3$, $p<0·001$ for each comparison). In patients from whom anaerobes were isolated the anaerobic species formed the predominant microbial flora, and *Bacteroides*

TABLE I *Demographic details of patient and control groups*

Subject group	Age		%Caucasian	%Circumcised	No of men with past histories of:			No of men with the indicated number of sexual partners in the previous month		
	Mean	Range			No STD	NSU	Other STD	0	1	>1
Control (n = 28)	30·8	(21-73)	82	18	14	13	7	6	17	5
Balanoposthitis (n = 79)	29·8	(18-66)	94	2·5	47	24	31	9	63	7
NSU (n = 24)	28·3	(20-43)	88	4	6	16	9	3	18	3
Both diseases (n = 19)	27·5	(17-34)	84	0	7	9	12	2	12	5

NSU = non-specific urethritis; STD = sexually transmitted disease

spp were the commonest isolates; they were found in 84 (89%) of the 94 patients and four (67%) of the six healthy controls with anaerobes (table II). Of the 152 strains of anaerobic bacteria isolated, there were 115 (76%) strains of *Bacteroides* spp, 33 (22%) strains of anaerobic cocci, three (2%) of *Clostridium* spp, and one (0·6%) bifidobacterium. Among the bacteroides isolates the saccharolytic melaninogenicus/oralis group and the asaccharolytic group were equally represented. *B asaccharolyticus* was the commonest species followed by *B melaninogenicus* ss *intermedius, B ureolyticus,* and *B bivius;* other *Bacteroides* spp were isolated less frequently (table III).

B ureolyticus was isolated from five (36%) of 14 men with erosive balanoposthitis compared with eight (10%) of 82 men with non-erosive balanoposthitis ($\chi^2 = 4·9$, p = 0·05). The isolation rate of the combination of *B asaccharolyticus* and *B ureolyticus* was also higher in patients with erosive balanoposthitis (four of 14, 29%) than in those with

TABLE II *Isolation of anaerobes from 150 men attending the genitourinary clinic*

| Subject group | No of subjects | No (%) of subjects with: | |
		Anaerobes	Bacteroides spp
Controls	28	6 (21)	4 (14)
Patients (total)	122	94 (77)*	84 (69)*
Balanoposthitis alone	79	60 (76)*	54 (68)*
NSU alone	24	16 (67)*	14 (58)*
Both diseases	19	18 (95)*	16 (84)*

*p<0·001
NSU = non-specific urethritis

non-erosive balanoposthitis (four of 82, 5%) ($\chi^2 = 6·0$, p<0·05). Anaerobic cocci were rarely isolated in the absence of concomitant *Bacteroides* species. They were isolated from three (11%) controls, 20 (26%) men with balanoposthitis alone, six (25%) men with NSU alone, and four (21%) men with balanoposthitis and NSU. These differences were not significant.

Aerobic bacteria were the predominant microbial flora in all the healthy controls. Coagulase-negative staphylococci and coryneforms were the common species. Neither *G vaginalis* nor *Candida albicans* was found in these subjects and anaerobic bacteria, where found, were present only in small numbers. Aerobic organisms were also isolated frequently from patients in the disease groups (113 out of 122, 93%) but in smaller numbers than the anaerobes. *C albicans* was isolated from 25 men with either balanoposthitis or NSU, in 18 (72%) of whom it was found in association with anaerobic bacteria. *G vaginalis* was found in five men with balanoposthitis, one of whom also had NSU (table IV).

Comparison of the isolation rates of anaerobes from patient and control groups showed that the association between the isolation of anaerobes and a clinical diagnosis of balanoposthitis or NSU or both was highly significant ($\chi^2 = 25·9$, 10·8, and 24·3, p<0·001 for each comparison) and that the *Bacteroides* spp were the significant anaerobic bacteria (p<0·001). The association between anaerobic cocci and balanoposthitis or NSU was not significant, because *Bacteroides* spp were isolated from 24 of the 30 patients with anaerobic cocci.

TABLE III *Isolation rate of anaerobes in three groups of patients and controls (150 subjects)*

| Species | Total No of isolates | No of isolates from subject groups: | | | |
		Control (n = 28)	Balanoposthitis (n = 79)	NSU (n = 24)	Both diseases (n = 19)
All anaerobes	152	8	95	24	25
Bacteroides spp	115	5	72	18	20
Asaccharolytic group	58	2	36	9	11
B asaccharolyticus	42	2	26	6	8
B ureolyticus	16	0	10	3	3
Melaninogenicus/oralis group	57	3	36	9	9
B melaninogenicus					
ss *melaninogenicus*	8	1	5	0	2
ss *intermedius*	17	0	14	2	1
ss *levi*	2	0	1	0	1
B bivius	16	1	9	4	2
B disiens	7	1	4	1	1
B oralis	7	0	3	2	2
Anaerobic cocci	33	3	20	6	4
Gram-positive	27	2	16	5	4
Veillonella spp	6	1	4	1	0
Clostridium spp	3	0	2	0	1
Cl difficile	2	0	2	0	0
Cl ramosum	1	0	0	0	1
Bifidobacteria	1	0	1	0	0

NSU = non-specific urethritis

TABLE IV *Isolation rate of aerobes in three groups of patients and controls (150 subjects)*

		No of isolates from subject groups:			
Species	Total No of isolates	Control (n = 28)	Balanoposthitis (n = 79)	NSU (n = 24)	Both diseases (n = 19)
All aerobes	300	66	156	41	37
Coagulase-negative staphylococci	141	28	72	22	19
Coryneforms	120	28	57	18	17
Staphylococcus aureus	4	0	3	1	0
α-haemolytic streptococci	16	8	8	0	0
β-haemolytic streptococci	4	1	3	0	0
Proteus spp	6	1	5	0	0
Lactobacilli	4	0	4	0	0
Gardnerella vaginalis	5	0	4	0	1
Candida albicans	25	0	16	5	4

NSU = non-specific urethritis

Similarly, anaerobes were isolated from most of the patients with *C albicans*. The association between anaerobes and balanoposthitis or NSU was significant irrespective of the presence or absence of *C albicans*.

Discussion

Anaerobic bacteria were identified as the predominant microbial flora in specimens from a large proportion of patients with balanoposthitis, NSU, or both but were found in small numbers or not at all in controls. Some other workers have failed to show such an association between clinical diagnosis and the isolation of anaerobes. The successful isolation of *Bacteroides* spp, in particular, from patients in the present study may be explained by the utmost care taken in specimen collection, in measures to minimise delays in transport to the laboratory, and in seeding culture media. Moreover, prolonged incubation periods of up to seven days increased the isolation rates for all *Bacteroides* spp by 11% compared with a 48-hour incubation period. As with our previous findings,[27] this increase was even greater (25%) for *B ureolyticus*. In the present study *Bacteroides* spp were the significant component of the association between anaerobic bacteria and balanoposthitis and NSU. *B asaccharolyticus* was the species isolated most commonly and other studies have shown an association between this species and ulcerative or gangrenous lesions of the perineum and genitalia.[14] Fontaine *et al*[28] recently studied anaerobic bacteria in men with urethritis; although the overall isolation rate from men with NSU (89%) was similar to that seen in our study, they isolated anaerobes from a higher proportion of their controls (80%). We cannot readily explain this difference, although we did take great care to select controls not only without evidence of NSU but also without balanoposthitis or

other genitourinary infections. The patients who attend genitourinary clinics have very varied histories. If valid comparisons are to be made the control and patient groups should match in age, ethnic origin, number of sexual partners, and past history of STD. It is difficult to obtain perfectly matched populations but the demographic details of the four groups that we investigated showed only very minor differences. Fontaine *et al* did, however, find a significant difference in isolation rates of Gram-negative anaerobic bacilli between diseased men and controls which our findings corroborate.

Our studies showed a clear association between the presence of *Bacteroides* spp in large numbers and the clinical diagnoses of balanoposthitis and NSU, but there was no significant association with anaerobic cocci. *B ureolyticus* was also implicated in the more severe erosive form of balanoposthitis. This correlates with our previous finding of an association between *B ureolyticus* and necrotic or ulcerative genital lesions. These results do not prove a causative relationship but do merit further studies to correlate the isolation of *Bacteroides* spp with other factors such as sexual activity and previous history of STD.

Anaerobes were isolated from a large proportion of men with balanoposthitis associated with *C albicans*. This suggests that other primary genital pathogens may cause initial damage to the host defence mechanisms and allow subsequent proliferation of anaerobic bacteria which may have a secondary pathogenic role. This is recognised in infections of the female genital tract; in pelvic inflammatory disease primary pathogens such as gonococci or *Chlamydia trachomatis* may damage cervical defence mechanisms and allow anaerobic bacteria to reach the fallopian tubes where they may cause a tubo-ovarian abscess. If anaerobic bacteria have a similar role in genital tract infections in men, particularly in NSU, this might explain the frequent occurrence of relapse or persistent evidence of

infection although the original causative pathogen has been eradicated. We restricted our studies of urethritis to patients with NSU because the aetiology of this condition remains incompletely understood and was of particular interest to us. *C trachomatis* and genital mycoplasmas have been implicated in a variable proportion of cases. The availability of cultures for chlamydia is restricted in our clinic and they could not be performed routinely in this study. There were insufficient data to comment on the prevalence of anaerobes in chlamydia-positive and chlamydia-negative cases. Prospective studies of the anaerobic urethral flora in patients with urethritis caused by other established pathogens are now under way to clarify this problem.

Further laboratory and clinical studies are necessary to elucidate the pathogenicity of *Bacteroides* spp, of which we have studied two, in infections of the male genital tract. It is important to determine whether they are primary or secondary pathogens, whether they are sexually transmissible, whether the sub-preputial sac is a site for their carriage, and if specific treatment is necessary particularly in relapsing or recurrent NSU.

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