

Microbial Flora Present in the Anterior
Urethra of Venereal Disease Patients

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OBJECTIVE: It is the purpose of this study to determine the microorganisms present in the anterior urethra of males attending a Venereal Disease Clinic.

BACKGROUND: Microorganisms isolated from patients with urethritis may be clearly pathogenic or they may be organisms which are commonly associated with normal flora of the skin. Gram positive cocci have frequently been implicated in non-specific urethritis (NSU) and urinary tract infections (1, 2, 3). Anaerobic organisms have been reported by various investigators as pathogens of the genito-urinary tract (4,5).

DESCRIPTION: The patient population consisted of 72 men attending the Venereal Disease Clinic of the Royal Thai Army Hospital, Bangkok, Thailand. Patient's ages ranged from 17-24 years (average 22.8). The patients were separated into two groups. Group I consisted of all patients presenting with symptoms of urethritis. Group II were those patients with venereal disease other than urethritis. Both groups were sexually active, and of the same age and socioeconomic position. Specimens for culture were obtained from the anterior urethra of all patients using a calcium alginate naso-pharyngeal swab. The swab was roll-streaked on various plated media immediately after it was obtained. A duplicate specimen was obtained from the anterior urethra for the culture of anaerobic organisms. All clinical specimens were inoculated into three basic media: modified Thayer-Martin (TM) media (6), 5% sheep blood agar plates (BAP), and anaerobic broth media. The anaerobic media consisted of a pre-reduced broth (Basal Medium-PY-Peptone Yeast) which was used as a transport medium until the sample could be transferred to chopped meat media (7). The cultures were transferred to the laboratory at the close of the clinic (normally less than two hours after collection) where they were streaked for isolation.

The plate media were incubated at 35°C under increased CO₂ tension (candle extinction jar). The samples for anaerobic culture were removed from the basal media by piercing the rubber stopper with a sterile needle and aspirating the fluid with a syringe. The specimen was then inoculated into chopped meat broth media under a stream of CO₂ gas rendered free of trace amounts of oxygen by passing through a heated copper oven (Sargent Welch Scientific Co.). The tubes were sealed with rubber stoppers and shrinkable cellulose sealing bands (Pharmaceutical Laboratory, Perry Point, MD.). Simultaneously a 0.5 ml portion of the basal media was inoculated onto duplicate BAP's and streaked for isolation. The plates were incubated in an oxygen free atmosphere using Gas-Paks (Baltimore Biological Laboratories, Cockeysville, Maryland) at 37°C for 48 hours. When colonies developed they were subcultured to biochemicals for identification. The chopped meat broth cultures were observed for a maximum of two weeks and then discarded if bacterial growth was not detected by Gram stain. If growth developed they were subcultured to BAP's and incubated anaerobically at 37°C for 24-48 hours. Colonies isolated on BAP's were further subcultured to biochemicals for identification. All biochemical subcultures were observed for a maximum of 14 days before discarding.

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All aerobic plates were examined after 24 hours of incubation for the growth of colonies with morphology resembling that of *Neisseria sp.* In some cultures it was necessary to incubate the plates an additional 24 hours before growth was adequate for evaluation. Suspect colonies were identified as *N. gonorrhoeae* on the basis of morphology, oxidase reaction, Gram stain, and sugar fermentations. Organisms which were inhibited on TM media were evaluated using BAP's. Subsequent identification employed tubed biochemical media. Significance of differences between groups was determined by Chi-square testing employing Yates correction (8).

PROGRESS: There were no significant differences in the recovery of Gram positive cocci between the two groups. Anaerobic organisms were found only slightly less frequently than the Gram positive cocci. They were isolated 10% more often in patients with urethritis as compared to those with other venereal diseases (Table 1). This difference is not significant.

Neisseria gonorrhoeae was found in 40.5% of the patients in Group I. In Group II patients, 5.7% were found to harbor *N. gonorrhoeae*, thus representing the asymptomatic carrier (Table 1).

Two patients were found to have *Staphylococcus aureus* and three had fecal organisms. None of these occurred alone, but were found concomitantly with other Gram positive cocci and anaerobes. There were no significant differences in the isolation of anaerobes and Gram positive cocci in patients with gonococcal urethritis and non-gonococcal urethritis (Table 2).

Table 1. Microorganisms Found in the Anterior Urethra of Urethritis Patients Compared with Those Having Venereal Disease Other Than Urethritis

Organisms Found	V.D. Other Than Urethritis (35 Patients)	Urethritis (37 Patients)
Gram-positive Cocci	29 (83%)	32 (87%)
Anaerobic Organisms	21 (60%)	26 (70%)
Anaerobes and Gram-positive Cocci	18 (51%)	22 (59%)
<i>Neisseria gonorrhoeae</i>	2 (5.7%)	15 (41%)

Table 2. Microorganisms Found in the Anterior Urethra of Gonococcal (GC) Urethritis and Non-gonococcal Urethritis Patients

Organisms Found	G.C. Urethritis (15 Patients)	Non-G.C. Urethritis (22 Patients)
Gram-positive Cocci	4 (27%)	5 (23%)
Anaerobic Organisms	1 (6.7%)	2 (9.1%)
Anaerobes and Gram-positive Cocci	9 (60%)	12 (55%)
<i>Neisseria gonorrhoeae</i>	1 (6.7%)	0
Fecal Organisms	0	3 (14%)

DISCUSSION: Non-specific urethritis is frequently a complaint of males attending military health clinics. The cause of this condition is currently unknown. Gram positive cocci, diphtheroid bacilli, chlamydia, and the T-strain of mycoplasma have all been alleged to be causative agents (6). Gram positive cocci, have been found by some observers to be the cause of NSU. However, we found these organisms present with nearly identical frequencies in both of our study groups. It may be inferred from this observation that while the Gram positive cocci may be pathogenic they are frequently present as opportunist commensals. The susceptibility of the individual may in some way lead to the disease state, but this is unclear at this time.

Anaerobes were found in 60% of our nonurethritis patients and 70% of those with urethritis. In another sample population (unpublished observations) consisting of 17 normal healthy males, we found 15 (88%) harboring anaerobes in the urethra. Anaerobes have been found in the urethra of both symptomatic and asymptomatic males attending a venereal disease clinic. These findings confirm the presence of anaerobes in the male anterior urethra. Swenson, et al, found anaerobic bacteria causative in 80% of female genital tract infections (9). These organisms have also been found in normal vaginal secretions (10). Therefore, they should be considered as part of the normal microbial flora in both the male and female genital tract. They may serve as sources of genito-urinary infections in susceptible sexually active males.

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