JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1977, p. 482-488

Vol. 6, No. 5
Printed in U.S.A.

Bacteriology of the Urethra in Normal Men and Men with Nongonococcal Urethritis

WILLIAM R. BOWIE,[1,2]* HELEN M. POLLOCK,[3] PATRICIA S. FORSYTH,[3] JOHN F. FLOYD,[3] E. RUSSELL ALEXANDER,[4] SAN-PIN WANG,[4] AND KING K. HOLMES[1,2]

Department of Medicine, U.S. Public Health Service Hospital,[1] and the Departments of Medicine,[2] Laboratory Medicine,[3] and Pathobiology and Epidemiology,[4] University of Washington, Seattle, Washington 98114

Received for publication 11 April 1977

Sixty-nine Caucasian males without a previous history of urethritis and who developed nongonococcal urethritis (NGU) and 39 similar men without urethritis (NU) were cultured from the urethra for *Chlamydia trachomatis, Mycoplasma hominis, Ureaplasma urealyticum,* aerobes, and anaerobes. *C. trachomatis* infection was proven by culture or serology in 26 (38%) of the NGU group and 1 (3%) of the NU group; the *C. trachomatis*-negative NGU group had significantly more *U. urealyticum* (81%) than the *C. trachomatis*-positive NGU group (42%) or the NU group (59%). Aerobes were isolated from all but two men with *C. trachomatis*-negative NGU. Anaerobes were isolated from significantly more NU men (91%) than from men with NGU (66%). The aerobic and anaerobic flora of the two NGU groups were similar. The NU group had significantly more aerobic lactobacilli, *Haemophilus vaginalis,* alpha-hemolytic streptococci (not *Streptococcus faecalis*), and anaerobes, predominantly *Bacteroides* species. This study has provided information about the prevalence and the variety of the aerobic and anaerobic microbiological flora of the anterior urethra of sexually active males. It does not implicate any bacteria other than *C. trachomatis* and *U. urealyticum* as potential causes of NGU.

Chlamydia trachomatis has been consistently isolated from 30 to 50% of men with nongonococcal urethritis (NGU) (9, 17, 26, 28, 29), 20 to 30% of men with gonorrhea (10, 27-29), and less than 5% of men with no evidence of urethritis (2, 3, 17, 26, 28, 29). Men with gonorrhea and *C. trachomatis,* if treated with a regimen that eradicates *Neisseria gonorrhoeae* but not *C. trachomatis,* will usually develop postgonococcal urethritis (17, 27). Serology utilizing the microimmunofluorescence (IFA) test to detect humoral antibody to *C. trachomatis* supports the conclusion that *C. trachomatis* is a cause of NGU (3, 17).

The cause of the majority of cases of NGU, in which the presence of *C. trachomatis* cannot be diagnosed by isolation or serology, is not certain. Several studies have supported a role for *Ureaplasma urealyticum* (T-mycoplasma) in *C. trachomatis*-negative NGU (2, 3). Other organisms, especially *Haemophilus vaginalis* (8, 21) and some strains of corynebacteria (12), have been suggested as possible causes. However, because information about the composition of the normal urethral flora in sexually active young males is limited, it is difficult to ascertain the importance of such organisms. Several groups have studied the aerobic urethral flora of males with and/or without urethritis, but the results have not shown a consistent correlation between urethritis and organisms other than *C. trachomatis* and *U. urealyticum* (1, 2, 13, 23-25, 33, 36). The anaerobic flora of the anterior urethra has been studied infrequently, and no consistent picture of the normal or abnormal anaerobic urethral flora has emerged from these studies (1, 13, 18, 24, 25). No group has utilized contemporary anaerobic methodology to simultaneously study anaerobes in conjunction with cultures for aerobes, *C. trachomatis, Mycoplasma hominis,* and *U. urealyticum.* This study was undertaken to: (i) determine the aerobic and anaerobic flora of the anterior urethra of sexually active males with and without urethritis and (ii) attempt to delineate etiological agents contributing to *C. trachomatis*-negative NGU by comparing the microbiological flora of the urethra of *C. trachomatis*-positive and *C. trachomatis*-negative NGU patients and men without urethritis.

(This paper was presented in part at the 15th Interscience Conference on Antimicrobial Agents and Chemotherapy, 24-26 September 1975, Washington, D.C.)

MATERIALS AND METHODS

Study population. Caucasian males attending the Seattle-King County Venereal Disease Clinic with complaints of discharge and/or dysuria and who had a demonstrable urethral exudate were included in this study if they were under 36 years old, had not previously had NGU, gonorrhea, or symptoms suggestive of urethritis, had not taken antimicrobials in the preceding 3 months, had three or fewer sex partners in the preceding 3 months, had symptoms for less than 1 month, were not allergic to sulfonamides, had negative Gram stain and culture of urethral exudate for *N. gonorrhoeae,* and had pyuria. Men attending the same clinic who fulfilled the same criteria, except that they had no symptoms and signs of urethritis or pyuria, formed a "no urethritis" (NU) comparison group. Written consent was obtained from all men in the study.

Initial evaluation. Urethral exudates from urethritis patients were examined by Gram stain and cultured on Thayer-Martin medium to exclude gonorrhea. A calcium alginate urethrogenital swab was then inserted into the urethra 1 to 2 cm beyond the fossa navicularis and was placed into transport medium for isolation of *C. trachomatis.* Men with no urethritis underwent urethral culture and Gram stain to exclude gonococcal infection. All men were asked to return 1 to 3 days later, without having voided overnight.

Second visit. The penis was stripped from posterior to anterior three to four times, and then both the NU men and the NGU patients underwent the following studies. A urethral specimen was obtained for isolation of *C. trachomatis,* a second swab was inserted 1 cm further to obtain a urethral swab for Gram stain, and a third swab was then introduced 1 cm further and was used to directly inoculate bacteriological plates and broth. The first 15 ml of overnight urine was collected (first-voided urine), and the patient voided most of the remainder of the urine. A prostatic massage was then performed, and the patient voided again. The first 15 ml was collected (postprostatic massage urine). A 0.01-ml portion of each urine specimen was directly inoculated onto plated agar media. Two-milliliter portions were placed into a prereduced transporter (Anaport, Scott Laboratories) and into enriched peptone broth (Becton-Dickinson and Co.). A 3-ml portion of the first-voided urine was placed in a sterile tube, refrigerated for 2 to 3 h, and then cultured for *M. hominis* and *U. urealyticum.*

Approximately 7 ml of each urine specimen was centrifuged at 500 × *g* for 10 min. The supernatant was discarded, and the sediment was resuspended in 0.5 ml of residual urine and examined by high-dry microscopy (×400) for trichomonads and leukocytes. The presence of 20 or more polymorphonuclear leukocytes in at least two of five random fields was considered to represent pyuria.

Bacteriological isolation methods. Transport medium for *C. trachomatis* isolation was frozen at −70°C until cultures were performed, usually within 2 to 3 weeks. *C. trachomatis* isolation was performed as previously described (35). *M. hominis* and *U. urealyticum* isolation was performed, using A-6 agar as described by Shepard and Hubbard (31) and broth medium as previously described (17).

Urethral swabs were first inoculated directly onto chocolate agar plates (CAP) and blood agar base plates (BAP) containing 5% sheep blood that had been held in a GasPak anaerobic jar overnight and then were placed into prereduced chopped meat-glucose broth (Scott Laboratories). First-voided and postprostatic massage urines were directly inoculated onto CAP and BAP. The prereduced transporter was maintained at 37°C and then taken to the laboratory within 3 h for culturing in chopped meat-glucose broth and onto BAP and blood agar base containing 5% laked sheep blood and menadione (10 μg/ml) (LK). These plates had been held in a reduced GasPak jar. CAP were immediately placed in a candle jar, and BAP and LK were immediately returned to a GasPak anaerobic jar. All inoculated media were incubated at 37°C.

Bacteriological identification. Isolates identified as *H. vaginalis* were small gram-negative bacilli showing "diphtheroid" arrangements on Gram stain and producing small, entire, raised, grayish colonies on CAP after 48 h of incubation (19). All isolates grew poorly or not at all on BAP, grew well on CAP, and were catalase and oxidase negative. All fermented glucose and maltose in peptone-water base with 10% added fetal calf serum, 1% carbohydrate, and Andrade indicator. None fermented lactose, and 25% fermented sucrose. The glucose fermentation fatty acid end product was predominantly acetic acid by gas chromatographic analysis on a Hewlett-Packard instrument (22). Forty percent of the isolates also produced a small amount of lactic acid.

Other aerobes were identified by standard techniques (20). Anaerobes were identified by using prereduced media (Scott Laboratories) according to the schema of the Virginia Polytechnic Institute (16).

The quantity of organisms was recorded semiquantitatively: subculture—if present only on subculture; 1-3—equivalent to 100 to 300 colonies/ml of urine specimen; few—400 to 900/ml; <1+—1,000 to 5,000/ml; 1+—1,500 to 5,000/ml; 2+—5,000 to 10,000/ml; 3+—10,000 to 50,000/ml; and 4+—>50,000/ml.

***C. trachomatis* serology.** Immunofluorescence studies for humoral antibody to *C. trachomatis* were performed as previously described (3, 34).

Statistical analysis. The proportions of groups affected by selected variables were compared by using the Fisher exact test (7) with $n \leq 60$ and chi-square analysis with Yates correction (4) with $n > 60$. Mean and standard deviation of series of data were compared by Student's t test (4).

RESULTS

Sixty-nine men with NGU and 39 NU men were studied. *C. trachomatis* was isolated from 1 (3%) of the NU group and 23 (33%) of the NGU patients (3). Three *C. trachomatis* culture-negative men in the NGU group were considered to have *C. trachomatis*-positive NGU on the basis of serum IFA titers which demonstrated seroconversion, a fourfold rise, or immunoglobu-

lin M IFA to *C. trachomatis*. The remaining 43 NGU patients were considered to have *C. trachomatis*-negative NGU (3). The two NGU groups and the NU group were similar in age, age at time of first intercourse, and years of education. The NU group had a higher number of total sex contacts in their lifetime, with a median of 8.0 versus 4.9 for the NGU group ($P < 0.05$).

Isolation of mycoplasmas. The results of the cultures for *M. hominis* and *U. urealyticum* are shown in Table 1. *M. hominis* were isolated from 19 to 22% of all groups. *U. urealyticum* was isolated significantly more frequently from men with *C. trachomatis*-negative NGU than from either those with *C. trachomatis*-positive NGU ($P < 0.005$) or those without urethritis ($P < 0.05$). The isolation rates for the *C. trachomatis*-positive NGU group and the NU group were not significantly different.

Isolation of other aerobic bacteria. The isolation rates of other aerobic organisms are shown in Table 2. An organism was considered to be present in an individual if it was isolated from any specimen. The mean number ± 1 standard deviation of different aerobic species (excluding *C. trachomatis* and mycoplasma) isolated from each patient was 2.2 ± 0.86 for the *C. trachomatis*-positive NGU group, 2.1 ± 0.86 for the *C. trachomatis*-negative NGU group, and 3.5 ± 0.75 for the NU group ($P < 0.001$ versus *C. trachomatis*-positive NGU; $P < 0.001$ versus *C. trachomatis*-negative NGU). There were no differences in isolation rates of individual species between men with *C. trachomatis*-positive and *C. trachomatis*-negative NGU. However, compared with each of these two NGU groups, the NU group had significantly higher isolation rates of lactobacilli, alpha-hemolytic streptococci (not enterococci), and *H. vaginalis*. Aerobic gram-negative rods were rarely isolated, and there were no isolates of *Staphylococcus aureus*. Because of reports that novobiocin-resistant, coagulase-negative staphylococci cause lower urinary tract infections in women (30), 62 coagulase-negative staphylococci that were isolated from men with NGU were tested for novobiocin resistance. All were sensitive to a 5-μg novobiocin disk.

In the majority of patients, if an organism was isolated from one of the three specimens, it was isolated from at least one other specimen. When the isolation results for the first five organisms listed in Table 2 were combined, organisms that were isolated from one specimen were isolated from all three specimens in 54% of patients. When one of these organisms was isolated from any specimen, the organism was isolated significantly less frequently from the prostatic massage urine (72%) than from the endourethral swab (81%) ($P < 0.025$) or the first-voided urine (84%) ($P < 0.01$). In some patients an aerobic organism other than chlamydia or a mycoplasma was isolated from only one specimen. This was the endourethral swab in 11%, the first-voided urine in 7%, and the postprostatic massage urine in 4%. The median number per milliliter of first-voided urine of each of the first five aerobic organisms in Table 2 was 100 to 300/ml for the NGU group and 1,000 to 5,000/ml for the NU

TABLE 1. *Percentage of patients with mycoplasmas isolated from first-voided urine*

Organism	Chlamydia-positive NGU ($n = 26$)	Chlamydia-negative NGU ($n = 43$)	No urethritis ($n = 39$)
Mycoplasma hominis	19	19	22
Ureaplasma urealyticum	42 ($P<0.005$)	81 ($P<0.05$)	59

TABLE 2. *Percentage of individuals from whom aerobic bacteria were isolated from the urethra*

Organism	*C. trachomatis*-positive NGU ($n = 26$)	*C. trachomatis*-negative NGU ($n = 43$)	No urethritis ($n = 33$)	NGU vs NU (P)
Staphylococcus epidermidis	96	88	88	NS[a]
Corynebacterium species	77	63	58	NS
Lactobacilli	27	37	82	<0.0001
Haemophilus vaginalis	8	2	58	<0.0001
Alpha-hemolytic streptococci	4	7	36	<0.001
Acinetobacter species	8	0	6	NS
Escherichia coli	0	0	3	NS
Haemophilus influenzae	0	0	3	NS
Haemophilus parainfluenzae	4	0	3	NS
Other gram-positive cocci	0	0	12[b]	NS
No aerobes	0	5	0	NS

[a] NS, $P > 0.05$.

[b] Two nonhemolytic streptococci, one non-group A beta-hemolytic streptococcus, and one *Streptococcus faecalis*.

group. Four NGU patients colonized with aerobic lactobacilli, two with diphtheroids, and one with *Staphylococcus epidermidis* had 5,000 to 10,000 colonies/ml. Seven NU patients with *H. vaginalis*, three with lactobacilli, two with *S. epidermidis*, two with alpha-hemolytic streptococci, and one with diphtheroids had 5,000 to 10,000 organisms per ml of first-voided urine. One NU patient had >50,000 *H. vaginalis* colonies/ml.

A relationship could not be shown between the isolation rate of any of the organisms shown in Table 2 and the total number of sexual contacts the individuals had had in their lifetime.

Anaerobic bacterial isolates are listed in Table 3. There were significantly fewer men in the NU group without anaerobes (9%) than in the *C. trachomatis*-positive NGU group (36%) ($P < 0.05$) or the *C. trachomatis*-negative NGU group (32%) ($P < 0.05$). The increased rate of isolation of anaerobes from the NU group was primarily due to an increased isolation rate of gram-negative rods, predominantly *Bacteroides* species. *Bacteroides* were isolated from 55% of the NU group, 27% of the *C. trachomatis*-positive NGU group ($P < 0.20$), and 20% of the *C. trachomatis*-negative NGU group ($P < 0.01$). The most common species were *B. corrodens* and *B. melaninogenicus*. *B. fragilis* was isolated from only two NU men. Three *Bacteroides* isolates could not be identified as to species by analysis of metabolic end products on gas-liquid chromatography, and eight died in subculture before gas-liquid chromatographic analysis. There were no significant differences between groups in the rate of isolation of gram-positive rods. None of the gram-positive rods that were seen on Gram stain, or were isolated but died on subculture, had the morphology of *Clostridium* species or diphtheroids. Trichomonads and fungi were not seen on examination of any urine sediment. Fungi were neither seen on Gram stain nor isolated.

DISCUSSION

Aerobic and anaerobic bacteria were isolated from almost all sexually active NU men and from most men with NGU. Except for the increased isolation rate of *U. urealyticum* from men with *C. trachomatis*-negative NGU, there were no significant differences between *C. trachomatis*-positive and *C. trachomatis*-negative cases of NGU in rate of isolation of aerobes or anaerobes. However, aerobic lactobacilli, *H. vaginalis*, alpha-hemolytic streptococci (not enterococci), and *Bacteroides* were isolated significantly more frequently from the NU group than from the NGU group.

Other groups have examined the urethral flora of males with and/or without urethritis (1, 6, 12, 23–25, 33, 36). The data from most of these studies have been condensed and summarized in Table 4. Except for the present study, no group reported differences between NGU patients and NU men.

TABLE 3. *Percentage of individuals from whom anaerobic bacteria were isolated from the urethra*

Organism	*C. trachomatis*-Positive NGU ($n = 22$)	*C. trachomatis*-negative NGU ($n = 37$)	No urethritis ($n = 33$)	NGU vs NU (P)
Gram-negative rods				
Total	27	24	58	<0.005
Bacteroides corrodens	14	5	27	<0.05
B. melaninogenicus	18	11	21	NS[a]
B. fragilis	0	0	6	NS
B. capillosus	0	0	3	NS
B. biacutus	0	0	3	NS
Bacteroides species (other)	9	11	15[b]	NS
Fusobacterium species	0	3	6	NS
Gram-negative cocci	0	0	0	NS
Gram-positive rods				
Total	45	41	27	NS
Lactobacilli	27	16	24	NS
Propionibacterium species	9	11	9	NS
Eubacterium species	5	8	0	NS
Other[c]	9	11	0	NS
Gram-positive cocci[d]	50	59	73	NS
No anaerobes	36	32	9	<0.025

[a] NS, $P < 0.05$.

[b] Gas-liquid chromatographic fatty acid end products of three isolates were obtained, but did not fit the other species.

[c] Died in subculture prior to final identification.

[d] Anaerobic gram-positive cocci were not identified as to species.

TABLE 4. *Percentage of individuals from whom aerobic bacteria were isolated by urethral culture*

Organism	Literature citation										
	1[a]	36	25	23	13		33		6	3	
	45 NGU[b]	19 NGU	91 Mixed[c]	27 NGU	35 NU	36 NGU	28 NU	59 Mixed[d]	35 Mixed[e]	33 NU	69 NGU
No aerobes	4	0	74	7	0	6	85	90	0	0	3
Gram-positive cocci	37										
Staphylococcus epidermidis		89	7	11	82	75	0	0	100	88	91
S. aureus			0	30	0	0	4	2	0	0	0
Viridans streptococci		5	2	0	23	31	0	0	9	36	6
Streptococcus faecalis		0	1	0			4	5	0	3	0
Gram-positive rods	34										
Corynebacterium species	25	53	5	22	26	47	0	0	84	58	68
NSU corynebacteria (13)	0	0	0	0	89	75	0	0	0	0	0
Lactobacilli	0	0	0	0	0	0	0	0	0	82	33
Gram negative rods (except *Haemophilus vaginalis*)	24	21	9	19	17	8	7	3	0	15	3
H. vaginalis	0	0	0	26	0	0	0	0	0	58	4

[a] Percentage of total isolates rather than percentage with each organism.
[b] Number of subjects and group.
[c] 10 women and 81 NGU men; results combined in original paper.
[d] Unstated number of men with gonorrhea included; results combined in original paper with men having NGU.
[e] 6 NU, 14 NGU, 15 gonorrhea; results combined in original paper.

Sompolinsky et al. (33) and Morrison (25) had unusually low isolation rates for aerobes. In the other studies at least one aerobe was isolated from 93% or more of the individuals; Mehta et al. isolated few *S. epidermidis* but many *S. aureus* (23). Helmholz in the U.S. (15) reported isolation of *S. aureus*. This has not been found in the more recent studies. Whittington simply called isolates staphylococci (36).

The study by Davis et al. (6) had relatively high isolation rates of *Corynebacterium* species, but there was not a significant difference between men with and without NGU. The corynebacteria are a heterogeneous and poorly characterized group, and it is possible that some subgroup of this genus could be associated with NGU. The rate of recovery of so-called NGU corynebacteria by Furness et al. (12, 13) was the same for normals as for men with NGU. *H. vaginalis* has been proposed as a cause of some cases of NGU (8, 21), but results of these studies do not suggest that *H. vaginalis* is associated with NGU. Many of the gram-negative rods recovered by Ambrose and Taylor (1) were *Pseudomonas aeruginosa,* but the fossa navicularis was washed with aqueous zephirin before the urethra was cultured in that study.

In the present study, at least one species of anaerobe was isolated from 66% of the men with NGU and 91% of the NU men. These results agree with those of Finegold et al. (11), who suggested that anaerobes are part of the normal urethral flora. They cultured the first-voided urine from 17 males with suspected urinary tract infection but without evidence of urethritis. One patient who was possibly on antimicrobials had a sterile urine. The other 16 yielded at least one aerobe. Eight also yielded a total of 17 anaerobic or capnophilic strains.

Several groups (1, 18, 25) have either failed to isolate anaerobes from men with and/or without urethritis or else have isolated them from only a small fraction. In contrast to other studies, Hafiz et al. (14) reported a striking correlation between NGU and urethral infection with an anaerobe that was identified as *Clostridum difficile.* Whereas strains of *C. difficile* described in *Bergey's Manual* (32) and by Cowan and Steel (5) did not reduce nitrates or produce acid from sucrose, Hafiz's strains did. The biochemical methodologies are not comparable, because Hafiz et al. used phenol red indicator to measure acid production, rather than a pH meter or Andrade indicator to detect a drop in pH to 5.5 or less (5, 32). In addition, urethral discharges of NGU cases were compared with voided urines of a control group. In our study, no gram-positive rods with the morphology of *Clostridium* species were seen on Gram strain or isolated in anaerobic culture, although the methods should have been adequate for the isolation of these organisms (10).

Cultures for viruses were not obtained in this study. A previous study from Seattle has shown that *Herpesvirus hominis,* cytomegalovirus, and other viruses are rarely isolated from the urethra of men with NGU, gonorrhea, or no urethritis (17). Although about 30% of men with primary

genital herpes and about 10% with recurrent herpes have dysuria and/or a urethral discharge (H. Adams, presented at the 15th Interscience Conference on Antimicrobial Agents and Chemotherapy, 24–26 September 1975, Washington, D.C.), the diagnosis is usually obvious because of external penile herpetic lesions. Consequently, we did not culture for viruses in this study. Similarly, at least in Seattle, cultures and examination of the urethral discharge and urine for trichomonads and fungi are usually negative (17); therefore, we did not specifically culture for these organisms.

The decreased isolation of aerobes and anaerobes from men with NGU cannot readily be explained. Although fewer controls than men with NGU harbored *U. urealyticum*, many controls simultaneously yielded *U. urealyticum* and large numbers of other organisms on culture. Within the NGU group, the number of aerobic or anaerobic isolates was similar when *C. trachomatis*-positive men were compared with *C. trachomatis*-negative men and when *U. urealyticum*-positive men were compared with *U. urealyticum*-negative men. This suggests that either these men have a preexisting alteration in the total urethral flora, which may have predisposed them to developing NGU, or else some other factor, such as competition with some other organism or the presence of urethral exudate containing leukocytes, inhibitory substances, altered pH, or antibody, decreased the number of organisms in vivo or decreased their ability to grow in culture.

Except for an association of some NGU cases with *U. urealyticum*, which is discussed elsewhere (3), these studied have failed to show a significant association between any other organism and *C. trachomatis*-negative NGU. The possibility that some particular strains of corynebacteria, coagulase-negative staphylococci, or anaerobic gram-positive organisms could be associated with NGU has not been excluded since our isolates were not subgrouped biochemically or by other methods. It remains possible that a fastidious variant form of *N. gonorrhoeae* or of some other bacteria not identified in these studies is responsible for some cases.

ACKNOWLEDGMENTS

This research was supported by Public Health Service research grants AI-12192 (from the National Institute of Allergy and Infectious Diseases) and EY-00219 (from the National Eye Institute) and by a fellowship from the Medical Research Council of Canada to W.R.B.

LITERATURE CITED

1. **Ambrose, L. S. S., and W. W. Taylor.** 1953. A study of the etiology, epidemiology, and therapeusis of nongonococcal urethritis. Am. J. Syph. Gonorrhea and Vener. Dis. **37**:501–513.
2. **Bowie, W. R., E. R. Alexander, J. F. Floyd, J. Holmes, Y. Miller, and K. K. Holmes.** 1976. Differential response of chlamydial and ureaplasma associated urethritis to sulfafurazole (sulfisoxazole) and aminocyclitols. Lancet **ii**:1276–1278.
3. **Bowie, W. R., S.-P. Wang, E. R. Alexander, J. Floyd, P. S. Forsyth, H. M. Pollock, J.-S. Lin, T. M. Buchanan, and K. K. Holmes.** 1977. Etiology of nongonococcal urethritis: evidence for *Chlamydia trachomatis* and *Ureaplasma urealyticum*. J. Clin. Invest. **59**:735–742.
4. **Colton, T.** 1974. Statistics in medicine, 1st ed., p. 127–131, 174–177. Little, Brown and Co., Boston.
5. **Cowan, S. T., and K. J. Steel.** 1974. Manual for the identification of medical bacteria, p. 67. Cambridge University Press, Cambridge.
6. **Davis, G., B. Smithurst, A. Talbot, E. Townsend, and A. Parry.** 1973. Microbiological studies on non-gonococcal urethritis in Brisbane. Med. J. Aust. **2**:268–271.
7. **Diem, K. (ed.).** 1962. Scientific tables, 6th ed., p. 113–123. Geigy Pharmaceuticals, Ardsley, N.Y.
8. **Dunkelberg, W. E., and S. C. Woolvin.** 1963. *Haemophilus vaginalis* relative to gonorrhea and male urethritis. Mil. Med. **128**:1098–1101.
9. **Dunlop, E. M. C., J. D. Vaughan-Jackson, S. Darougar, and B. R. Jones.** 1972. Chlamydial infection. Incidence in "nonspecific" urethritis. Br. J. Vener. Dis. **48**:425–428.
10. **Eschenbach, D. A., T. M. Buchanan, H. M. Pollock, P. S. Forsyth, E. R. Alexander, J.-S. Lin, S.-P. Wang, B. B. Wentworth, W. M. McCormack, and K. K. Holmes.** 1975. Polymicrobial etiology of acute pelvic inflammatory disease. N. Engl. J. Med. **293**:116–171.
11. **Finegold, S. M., L. G. Miller, S. L. Merrill, and D. J. Posnick.** 1965. Significance of anaerobic and capnophilic bacteria isolated from the urinary tract, p. 159–178. *In* H. E. Kass (ed.), Progress in pyelonephritis. Davis and Co., Philadelphia.
12. **Furness, G., H. Kamat, Z. Kaminski, and J. J. Seebode.** 1971. Isolation of corynebacteria from non-specific urethritis. J. Urol. **106**:557–561.
13. **Furness, G., M. H. Kamat, Z. Kaminski, and J. J. Seebode.** 1973. An investigation of the relationship of nonspecific urethritis corynebacteria to the other microorganisms found in the urogenital tract by means of a modified chocolate agar medium. Invest. Urol. **10**:378–391.
14. **Hafiz, S., M. G. McEntegart, R. S. Morton, and S. A. Waitkins.** 1975. *Clostridium difficile* in the urogenital tract of males and females. Lancet **i**:420–421.
15. **Helmholz, H. F.** 1950. Determination of the bacterial content of the urethra: a new method, with results of a study of 82 men. J. Urol. **64**:158–166.
16. **Holdeman, L. V., and W. E. C. Moore (ed.).** 1972. Anaerobe laboratory manual. Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, Va.
17. **Holmes, K. K., H. H. Handsfield, S.-P. Wang, B. B. Wentworth, M. Turck, J. B. Anderson, and E. R. Alexander.** 1975. Etiology of nongonococcal urethritis. N. Engl. J. Med. **292**:1199–1205.
18. **Justesen, T., M. L. Nielson, and T. Hattel.** 1973. Anaerobic infections in chronic prostatitis and chronic urethritis. Med. Microbiol. Immunol. **158**:237–248.
19. **Lapage, S. P.** 1974. *Haemophilus vaginalis*, p. 368–370. *In* R. E. Buchanan and N. E. Gibbons, (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
20. **Lennette, E. H., E. H. Spaulding, and J. P. Truant. (ed.).** 1974. Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
21. **Leopold, S.** 1953. Heretofore undescribed organism iso-

lated from the genitourinary system. U.S. Armed Forces Med. J. 4:263–266.
22. **Malone, B. H., M. Schreiber, N. J. Schneider, and L. V. Holdeman.** 1975. Obligatory anaerobic strains of *Corynebacteria vaginale* (*Haemophilus vaginalis*). J. Clin. Microbiol. 2:272–275.
23. **Mehta, U. S., V. S. Rana, and V. P. Vaishnav.** 1967. A bacteriological study of acute urethritis. Indian J. Pathol. Bacteriol. 10:170–176.
24. **Melen, B., and B. Linnros.** 1952. Pleuropneumonia-like orgnisms in cases of non-gonococcal urethritis in man. Acta Derm. Venereol. 32:77–85.
25. **Morrison, A. I.** 1963. Investigation of non-gonococcal urethritis by anaerobic culture. Br. J. Vener. Dis. 39:118–120.
26. **Oriel, J. D., P. Reeve, P. Powis, A. Miller, and C. S. Nicol.** 1972. Chlamydia infection: isolation of chlamydia from patients with nonspecific genital infection. Br. J. Vener. Dis. 48:429–436.
27. **Oriel, J. D., P. Reeve, B. J. Thomas, and C. S. Nicol.** 1975. Infection with chlamydia group A in men with urethritis due to *Neisseria gonorrhoeae*. J. Infect. Dis. 131:376–382.
28. **Richmond, S. J., A. L. Hilton, and S. K. R. Clarke.** 1972. Chlamydial infection: role of chlamydia subgroup A in nongonococcal and postgonococcal urethritis. Br. J. Vener. Dis. 43:437–444.
29. **Schachter, J., L. Hanna, E. C. Hill, S. Massad, C. W. Sheppard, J. E. Conte, Jr., S. N. Cohen, and K. F. Meyer.** 1975. Are chlamydial infections the most prevalent venereal disease? J. Am. Med. Assoc. 231:1252–1255.
30. **Sellin, M., D. I. Cooke, W. A. Gillespie, D. G. H. Sylvester, and J. D. Anderson.** 1975. Micrococcal urinary tract infections in young women. Lancet ii:570–572.
31. **Shepard, M. C., and D. R. Hubbard.** 1970. Identification of "T" mycoplasmas in primary agar cultures by means of a direct test for urease. Ann. N.Y. Acad. Sci. 174:809–819.
32. **Smith, L. D. S., and G. Hobbs.** 1974. *Clostridium*, p. 559. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
33. **Sompolinsky, D., Z. Harari, F. Solomon, E. Caspi, D. Krakowski, and E. Henig.** 1973. A contribution to the microbiology of urethritis. Isr. J. Med. Sci. 9:438–446.
34. **Wang, S.-P., J. T. Grayston, E. R. Alexander, and K. K. Holmes.** 1975. Simplified microimmunofluorescence test with trachoma lymphogranuloma venereum (*Chlamydia trachomatis*) antigens for use as a screening test for antibody. J. Clin. Microbiol. 1:250–255.
35. **Wentworth, B. B., and E. R. Alexander.** 1974. Isolation of *Chlamydia trachomatis* by use of 5-iodo-2-deoxyuridine-treated cells. Appl. Microbiol. 27:912–916.
36. **Whittington, M. J.** 1962. Possible virus aetiology of nonspecific urethritis. Br. J. Vener. Dis. 38:200–208.