ELSEVIER

MICROBIOLOGIC AEROBIC STUDIES ON NORMAL MALE URETHRA

DEBORAH MONTAGNINI SPAINE, ELSA M. MAMIZUKA, AGNALDO PEREIRA CEDENHO, AND MIGUEL SROUGI

ABSTRACT

Objectives. To carefully collect samples from the external urethral orifice, navicular fossa, and penile urethra and perform a semiquantitative evaluation and identification of gram-positive and gram-negative bacteria present in the normal male urethra.

Methods. Thirty uncircumcised male patients 18 to 40 years old without any inflammatory and/or infectious urethral processes were enrolled in this study. Samples were collected from the external urethral orifice, navicular fossa, and penile urethra with sterile alginate swabs that were immediately transferred to tubes containing buffered phosphate solution. Inoculation was done by spreading 0.01 mL of the buffered solutions on sheep blood agar plates and MacConkey agar plates; the plates were then incubated at 36.5°C for 24 hours. After this period, the quantification and identification of each type of colony was performed.

Results. Among the 30 patients studied, 12 (40%) had bacteria isolated from the three segments, 10 (33.3%) had bacterial colonization in two segments, and 8 (26.7%) had colonization in only one segment (external urethral orifice). *Staphylococcus* coagulase-negative species, group viridans alpha-hemolytic streptococci, *Corynebacterium* species, and *Enterococcus* species were the bacteria more frequently isolated from these three segments.

Conclusions. From the findings in this study, it was clear that the bacterial urethral flora was abundant, not evenly distributed, concentrated in the external urethral orifice and navicular fossa, and basically consisted of gram-positive aerobic bacteria. UROLOGY **56:** 207–210, 2000. © 2000, Elsevier Science Inc.

The male urethra represents the ultimate route of the urogenital tract and normally is the passage for urine and secretions from the testes, epididymis, vas deferens, seminal vesicles, prostate, and bulbourethral and paraurethral glands. Because of the transitional mucosal epithelium surface of the terminal portion of the male urethra, colonization of the urogenital tract by several microorganisms is possible after birth. Many factors control this phenomenon, including keratinization and mucus in the epithelial surface, bacterial adherence, microbial interaction, antimicrobial substances, phagocytosis, and humoral and cellular immunities.[1] In brief, the process of bacterial adherence starts with chemoattractant stimuli that lead bacteria to bind to epithelial tissue. At this point, the bacteria must find a suitable environment for the expression of their virulence potential, which will ultimately enhance their attachment to the surface. If this adherence does not induce a local inflammatory reaction, normal or autochthonous flora will be settled, and this may play a significant role in protecting the urethra against infection by urogenital pathogens.[2,3]

Considering that male urethra colonization has often been mentioned[4,5] but never completely described, our interest in this research was to determine whether the different segments of the normal male urethra contained similar bacteria or were sterile. Thus, the present study sought to collect without contamination aerobic gram-positive and gram-negative bacteria found in the distal segment of the male urethra and perform a semiquantitative evaluation and identification of these bacteria.

This study was presented at the 94th Annual Meeting of the American Urological Association, Dallas, Texas, May 1 to 6, 1999.

From the Disciplina de Urologia, Escola Paulista de Medicina, Universidade Federal de São Paulo and Departamento de Análises Clínicas, Faculdade de Ciências, Farmacêuticas, Universidade de São Paulo, São Paulo, Brasil

Reprint requests: Deborah M. Spaine, M.D., Laboratório de Urologia, Universidade Federal de São Paulo, Rua Botucatu, 740 04023-062, São Paulo, Brasil

Submitted: November 8, 1999, accepted (with revisions): March 23, 2000

© 2000, ELSEVIER SCIENCE INC.
ALL RIGHTS RESERVED

0090-4295/00/$20.00
PII S0090-4295(00)00615-4

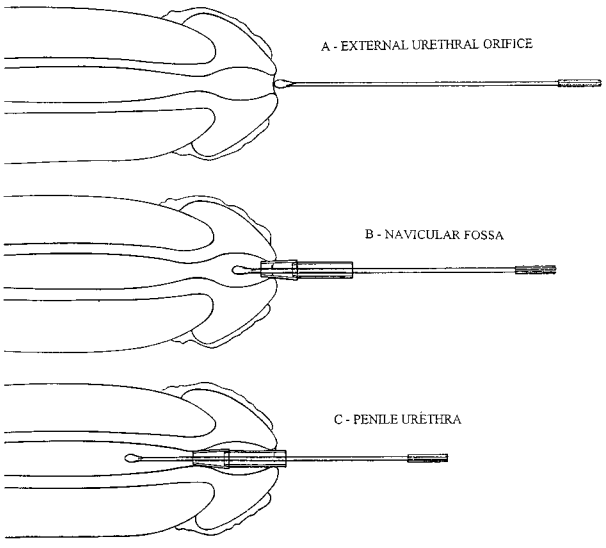

FIGURE 1. *(A-C) Instruments and different segments of the distal urethra.*

TABLE I. ***Frequency and bacterial types isolated from the three urethral segments of 30 patients evaluated***

	External Orifice (%)	Navicular Fossa (%)	Penile Urethra (%)
Staphylococcus coagulase-negative	90	37	17
Streptococcus viridans	50	23	23
Corynebacterium species	40	20	3.3
Enterococcus species	20	10	7
Staphylococcus aureus	3.3	0	0
Streptococcus pyogenes	3.3	3.3	0
Enterobacter species	3.3	0	0
Streptococcus agalactiae	3.3	0	0
Micrococcus species	3.3	0	0

MATERIAL AND METHODS

Thirty uncircumcised male patients between 18 and 40 years old from a university-based urology division without any previous history of genitourinary tract problems were enrolled in this study. To rule out sexually transmitted diseases and to confirm the absence of any inflammatory and infectious processes, preliminary laboratory tests were performed. To be included in the study, the patients were required to have less than 4 polymorphonuclear leukocytes in the Gram-stained urethral smear under oil immersion field,[6,7] less than 10,000 leukocytes/mL in the first-voided urine sample,[8] and negative results for *Trichomonas vaginalis, Chlamydia trachomatis, Gardnerella vaginalis,* fungi, *Neisseria gonorrhoeae, Ureaplasma urealyticum,* and *Mycoplasma hominis.* All patients were told to abstain from sexual activity 7 days before having the test performed and to keep an interval of at least 6 hours between the last voided urine and the collection of the samples.[9] Those who had received any antibiotics within the preceding 4 weeks were excluded from the study.

This protocol was carefully explained to all patients, and informed consent was obtained from them according to the university ethics committee.

Sample Collection

Samples were collected from three different parts of the distal urethra: the external urethral orifice, navicular fossa, and penile urethra.

To collect a sample from the external urethral orifice, the first sterile alginate swab (Mini-tip Culturette, Becton Dickinson), previously damped in a buffered sterile phosphate solution (pH 7.2), was inserted and rotated around the orifice for 30 seconds (Fig. 1A).

To collect a navicular fossa sample and at the same time prevent possible contamination from the outside, a sterile polyethylene catheter 0.5 cm in diameter and 1.0 cm long was initially introduced and held in position through the external orifice. Thus, with the orifice kept permanently open and with a another sterile swab, a sample from the navicular fossa was collected 1.5 cm from the external orifice, applying the same procedure described for the external urethral orifice (Fig. 1B).

To collect a sample from the penile urethra, a sterile polyethylene catheter 0.5 cm in diameter and 2.5 cm long was passed through the external urethral orifice all the way down to the initial portion of the penile urethra. Holding the catheter in place, a third sterile swab was introduced into the catheter and slid down to collect a sample 3 cm from the external orifice. Once that was done, the swab was withdrawn carefully and the catheter discarded (Fig. 1C).

Sample Preparation

After collection, each swab was transferred to a tube containing 3 mL of a buffered sterile phosphate solution (pH 7.2). The tube was agitated for 1 minute on a vortex mixer (500 rpm) and then the swab was discarded and the tube incubated at 36.5°C for 1 hour. After this period, 0.01 mL of the solution that contained the sample from the external orifice was transferred to a 5% sheep blood agar plate and another 0.01 mL of the same solution was transferred to a MacConkey agar plate. The inoculate was spread homogeneously on the entire surface of the medium, and the plates were incubated at 36.5°C for 24 hours.

Semiquantitative Count and Identification of the Detected Bacterial Colonies

Because 3 mL of a buffered phosphate solution was used in each tube for every sample collected and 0.01 mL was inoculated per agar plate, the number of each type of colony grown on the agar plates multiplied by 300 represented the total number of each bacteria found in the different urethral regions. After quantification of the colonies, a Gram stain was prepared from each type of colony. The genera *Staphylococcus, Micrococcus* and *Streptococcus* were differentiated on the basis of the catalase test, glucose fermentation-oxidation, and susceptibility to furazolidone (100 μg) and bacitracin (0.04 U).[10,11] *Staphylococcus* species were identified through coagulase, novobiocin (5 mg) susceptibility, and the Micro Scan kit (Baxter Diagnostics).[10,11] *Streptococcus* species were identified using biochemical tests, including susceptibility to bacitracin

TABLE II. ***Species and mean number of colonies isolated from the three urethral segments of 30 patients studied***

	External Orifice	Navicular Fossa	Penile Urethra
Streptococcus viridans	28,560 (15/30)	10,500 (8/30)	3,000 (7/30)
Enterococcus species	24,350 (6/30)	1,900 (3/30)	750 (2/30)
Corynebacterium species	14,325 (12/30)	3,800 (6/30)	0
Staphylococcus coagulase-negative	9,428 (28/30)	1,950 (11/30)	720 (5/30)

(0.04 U) and optochin (5 mg), sodium hippurate hydrolysis, CAMP test, growth tolerance in 6.5% sodium chloride, bile-esculin reaction, and the type of hemolysis produced on 5% sheep blood agar.[10,11]

All the gram-negative bacilli species were initially identified by the typical morphologic features of the colony in MacConkey agar and biochemical tests (carbohydrate use, orthonitrophenyl galactosidase (ONPG) activity, indole production, methyl-red test, Voges-Proskauer test, citrate use, urease production, decarboxylation of lysine, ornithine, and arginine, phenylalanine deaminase production, hydrogen sulfide production, and motility).[12]

RESULTS

The frequency and the various types of bacteria detected in the external urethral orifice, navicular fossa, and penile urethra are shown in Table I. According to these data, *Staphylococcus* coagulase-negative, group viridans alpha-hemolytic streptococci, *Corynebacterium* species and *Enterococcus* species were more frequently isolated from these segments; urethral colonization by gram-negative bacteria was very rare.

Table II presents the mean number of colonies of the four predominant bacterial types found in the distal portion of the male urethra.

According to the data obtained in this study, every patient had the external orifice colonized by at least one type of bacterium, and 12 patients (40%) had bacteria isolated from the three segments investigated. Ten patients (35%) presented with bacterial colonization in only two segments (external urethral orifice and navicular fossa). Finally, 8 patients (25%) had bacterial colonization only in the urethral orifice.

Staphylococcus haemolyticus, S. auriculares, S. hominis, S. epidermidis, and *S. simulans* were the species of *Staphylococcus* coagulase-negative identified by the Micro Scan Kit. The distribution of group viridans alpha-hemolytic streptococci was *Streptococcus salivarius, S. sanguis* II, *S. mutans, S. mitis, S. intermedius, S. constellatus,* and *S. morbillorum.*

COMMENT

In addition to an extensive mucosal epithelium surface in the external urethral orifice, the male urethra has direct communication with the external environment, offering favorable conditions for bacterial colonization. However, many questions are still unanswered about how this colonization takes place, what are the most commonly found bacteria, and what factors can affect its composition. With these questions in mind, this study was designed to collect and appropriately quantify the main bacterial strains living in the very distal end of the male urethra. For that, a polyethylene catheter was specially developed to ensure appropriate specimen collection and prevent bacterial contamination by the other lower portions of the urethra through which the swab had to pass.

By following the inclusion criteria (absence of inflammatory and/or infectious urethral processes), nine bacterial species were isolated in the urethral orifice, five species in the navicular fossa, and four in the penile urethra. Thus, we were able to demonstrate that the external urethral orifice was not only the site of greatest bacterial concentration, but also the area at which the greatest variety of bacterial species was found. Contrasting with these findings, the other urethral sites studied rarely presented with more than one species. Thus, we concluded that the external orifice is the most colonized area and as we go further proximally in the urethra, the number and variety of bacteria species are smaller. The navicular fossa had no bacteria isolated in 8 patients (27%), and the penile urethra was sterile in 18 patients (60%).

The results obtained in this study demonstrated that the bacterial urethral flora is abundant, not evenly distributed, and concentrated in the external urethral orifice and navicular fossa. The bacteria also basically constituted gram-positive aerobic bacteria. All the bacterial types described in Table I were considered usual inhabitants in the normal male genitourinary tract.

It is also worth noting that the microorganisms identified in this study represent the minimal estimates of colonization for each urethral segment because we used only a 0.01-mL aliquot from the prepared buffered solution.

CONCLUSIONS

The external urethral orifice was the male urethra segment with the greatest bacterial concentra-

tion and also the greatest variety of bacterial species. The bacterial urethral flora was abundant, not evenly distributed, and basically consisted of gram-positive aerobic bacteria. *Staphylococcus* coagulase-negative species, group viridans alpha-hemolytic streptococci, *Corynebacterium* species, and *Enterococcus* species were the bacteria more frequently isolated from the three segments investigated.

REFERENCES

1. Cohen MS, Weber RD, and Mardh PA: Genitourinary mucosal defenses, in Holmes KK, Mardh PA, Sparling PF, *et al* (Eds): *Sexually Transmitted Diseases*. New York, McGraw-Hill, 1990, pp 117–127.

2. Mardh PA, Colleen S, and Hovelius B: Attachment of bacteria to exfoliated cells from the urogenital tract. Invest Urol **16:** 322–326, 1979.

3. Marrie TJ, Lam J, and Costerton JW: Bacterial adhesion to uroepithelial cells: a morphologic study. J Infect Dis **142:** 239–246, 1980.

4. Bowie WR, Pollock HM, Forsyth PS, *et al:* Bacteriology of the urethra in normal men and men with nongonococcal urethritis. J Clin Microbiol **6:** 482–488, 1977.

5. Fari A: Étude bactériologique des uréthrites. J Urol (Paris) **89:** 39–48, 1983.

6. Swartz SL, Kraus SJ, Herrmann KL, *et al:* Diagnosis and etiology of nongonococcal urethritis. J Infect Dis **138:** 445–454, 1978.

7. Arya OP, Mallinson H, Andrews BE, *et al:* Diagnosis of urethritis: role of polymorphonuclear leukocyte counts in Gram-stained urethral smears. Sex Transm Dis **11:** 10–17, 1984.

8. Desai K, and Robson HG: Comparison of the Gram-stained urethral smear and first-voided urine sediment in the diagnosis of nongonococcal urethritis. Sex Transm Dis **9:** 21–25, 1982.

9. Simmons PD: Evaluation of the early morning smear investigation. Br J Med Dis **54:** 128–129, 1978.

10. Balows A, Hausler WJ Jr, Herrmann KL, *et al* (Eds): *Manual of Clinical Microbiology,* 5th ed. Washington, DC, American Society of Microbiology, 1991.

11. Koneman EW, Allen SD, Dowell VR Jr, *et al: Color Atlas and Textbook of Diagnostic Microbiology,* 3rd ed. Philadelphia, JB Lippincott, 1988, pp 311–353.

12. Krieg RN, and Holt JG: *Bergey's Manual of Systematic Bacteriology*. Baltimore, Williams & Wilkins, 1984, vol 1, pp 408–422.