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Mycoplasma Recovery from the Male Genitourinary Tract: Voided Urine Versus the Urethral Swab

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A study of *Mycoplasma* recovery from the genitourinary tract was made on a group of 50 males attending a venereal disease clinic. The purpose of the investigation was to compare recovery rates of mycoplasma from two types of clinical specimens—the urethral swab and voided urine. The total number of positive cultures did not differ significantly when either the swab or urine was used; *Mycoplasma hominis* type 1 was the only taxonomic species isolated, either alone or mixed with T-strain mycoplasma. Recovery rates of the two types from both the swab and the urine did not differ significantly. Age did not relate to the presence of mycoplasma in the group studied.

In attempting to recover mycoplasma from the male genitourinary (GU) tract, urethral scrapings and urethral swabs are usually employed in the United States. Some discomfort or anxiety, or both, on the part of the patient is experienced with these procedures. This is especially true in testing patients attending a venereal disease clinic.

The use of voided urine for the recovery of mycoplasma from the GU tract has not been adequately investigated in the United States. In England, Csonka, Williams, and Corse (3) studied isolation rates by employing urethral swabs and the voided urine specimens and found both techniques to compare satisfactorily. Black and Rasmussen (1), however, found these rates to differ significantly in recovering one species of *Mycoplasma* from the male urethra in Denmark.

It should be noted that mycoplasma recoveries have been found to be influenced somewhat by geographical boundaries in the past. Dienes and Madoff (4) reported the isolation and recognition of *Mycoplasma hominis* type 2 as a common species of the human genital tract in the United States. Out of numerous isolates in Europe, however, workers have not been able to recover *M. hominis* type 2 from this anatomic site, suggesting the absence of this species in that continent. One could also surmise that this might be a reflection of differences in techniques used in the different areas.

For the success of the English investigators to have more meaning, corroborative studies must be conducted in other geographical areas. This was the primary consideration when the present study was initiated.

If recovery rates of mycoplasma from the male GU tract prove to be consistently comparable employing both techniques cited above, presently used procedures may be discontinued in favor of the voided urine specimen, which is easier to obtain.

MATERIALS AND METHODS

Media. The PPLO agar and broth media employed were those described by Ford (D. K. Ford, *personal communication*). After reconstituting the PPLO agar (Difco) with distilled water the *p*H was adjusted to 5.5—anticipating a rise when the horse serum (*p*H 7.9) was added. The agar was autoclaved for 15 min and cooled to approximately 56 C, and an 80-ml portion was supplemented with 10 ml of agamma horse serum (Hyland Laboratories, Los Angeles, Calif.), 10 ml of yeast extract (Fleischmann's Active Dry Yeast, Standard Brands, N.Y.), 0.05 ml of a sterile 10% solution of urea, and 1,000 units of potassium penicillin G per ml. The final *p*H was 6.75. The agar was dispensed in 5-ml portions in plastic petri dishes (60 by 15 mm; Falcon Products, Inc.) and stored in a closed container at 4 C.

Reconstituted PPLO broth (Difco) was supplemented with the same nutrients as noted above. An 0.002-ml volume of a 1% solution of phenol red was added to detect the urease activity of T-strain mycoplasma. The final *p*H was 6.65; the broth was dispensed in 5-ml portions in screw-capped test tubes and stored at −20 C.

Population studied. Subjects tested consisted of males attending a venereal disease clinic, because of symptoms of, or exposure to, venereal disease. Ages ranged from 14 to 44 years; all individuals were from a similar ethnic background and of similar economic status. Of the 50 subjects tested, 43 revealed symptoms of gonorrhea, namely, dysuria and urethral discharge.

TABLE 1. *Species and prevalence of mycoplasma isolated from the genitourinary tract*

Specimen	*M. hominis*	T-strain	*M. hominis* and T-strain	Total no. of isolates	No growth in 5 days
Urethral swab	18 (51%)	5 (14%)	12 (34%)	35 (70%)	15
Voided urine	15 (52%)	7 (24%)	7 (24%)	29 (58%)	21

Of the remaining 7 patients, 5 reported to the clinic because of venereal contact with an infected female, and two because of a penile rash.

Specimen collection. A 50-ml sample of voided urine was collected in a sterile 50-ml screw-capped test tube from each patient. A cotton-tipped wire swab (such as that used to secure nasopharyngeal specimens) was introduced approximately 1 inch (2.54 cm) into the urethra and rotated gently. After carefully swabbing the entire surface of a PPLO agar dish, the swab was placed in a screw-capped test tube containing 5 ml of PPLO broth.

A 50-ml volume of urine was centrifuged at 1,000 $\times$ *g* at 0 C for 15 min; the supernatant fraction was decanted, leaving approximately 0.5 ml of urine in the tube. A 0.1-ml amount of the mixed sediment was added to a PPLO agar dish and spread across the entire surface with a glass rod. The same volume was added to a screw-capped test tube containing 5 ml of PPLO broth. Culturing of specimens was begun within 3 hr after collection.

Identification of isolates. Large colony *Mycoplasma* were identified by the growth inhibition technique (2). T-strains were identified by morphology, utilization of urea (5), and failure of the colonies to increase in size with prolonged incubation periods.

RESULTS

The majority of mycoplasma recoveries were realized in patients between the ages of 14 and 29, but it should be noted that this age group comprised 90% of the population tested. It was concluded that the prevalence of mycoplasma in the population studied did not relate to age.

The predominance of *M. hominis* type 1 in the male GU tract, when mycoplasma was recovered, was conspicuous (Table 1). This species was isolated either alone or mixed with T-strain mycoplasma from 85% of the cultures when the urethral swabs were employed. When the urine specimens were tested, 76% of the cultures revealed these results. Two isolates of T-strain mycoplasma alone were obtained from urine when the swab specimens were negative, but this mycoplasma was isolated five times, together with *M. hominis*, from urethral swabs when the urine specimens were negative.

Large colony *Mycoplasma* measured 75 to 150 μm in diameter; T-strains reached a maximum of 30 μm, with the smallest colony measuring 10 μm in diameter.

DISCUSSION

The use of voided urine for the recovery of mycoplasma from the GU tract is not a popular technique in the United States, probably owing to inadequate information regarding this procedure. Isolation rates using urine specimens and urethral swabs compared favorably in a study conducted in England, but the two techniques differed somewhat in an investigation performed in Denmark. Thus, existing data suggest that mycoplasma recoveries from the GU tract might be influenced by geographical location. However, this could be a function of differences in laboratory techniques employed in the two countries. *M. hominis* type 2, once considered a common genital strain in the United States, was never reported among isolates by European workers. This species, which is antigenically indistinguishable from *M. arthritidis*, a murine mycoplasma, has not been recovered from the human genitalia in this country in recent years. *M. hominis* type 2 was not isolated in the present investigation.

Our study did not indicate a relationship between mycoplasma infection and age. Recovery rates utilizing both techniques were compared statistically by using the Chi square (χ^2) test with 1 degree of freedom at the 5% level of significance. Total recoveries using the swab method did not differ significantly from recoveries from urine ($\chi^2_{.95} = 1.56$). Isolation rates of *M. hominis* type 1 alone, or these two species as a mixed infection from the swab and urine, did not differ significantly. However, a more critical analysis of the data would suggest that the urethral culture is still preferable to the urine culture for the isolation of mycoplasma from the GU tract.

Repeated cultures of urine in an attempt to increase the recovery of mycoplasma from those patients whose urethral swab cultures were positive and urine specimen cultures negative would be of interest. Unfortunately, all patients were treated with oxytetracycline after their first visit (a large number of hypersensitivity reactions due to potassium penicillin G, which was formerly employed in this clinic, prompted the use of oxytetracycline). Follow-ups after treatment were very difficult in this clinic.

Thus, the present investigation supports the

findings of Csonka and his co-workers in England, but the results reported from Denmark by Black and Rasmussen point out the need for further studies to be made in this area of mycoplasmology.

It is worthy of note that Black and Rasmussen added a 1:1,500 concentration of thallium acetate to their medium. This concentration of thallium acetate is toxic to T-strains (5), and one could surmise that a higher percentage of this type was isolated from the urethral scrapings than from the urines due to the possible protection afforded the organisms by their association with epithelial cells. Parasitized epithelial cells from the urethra have been observed and found to contain either intracytoplasmic coccoid bodies or elongated elements attributed to T-strain colonization (5). The results of Black and Rasmussen would have been more meaningful if the thallium acetate had not been used, or if it had been employed in a lower concentration. Taylor-Robinson and Addey (6) successfully recovered T-strains from urethral swabs in the presence of a 1:4,000 thallium acetate concentration. This chemical was not employed in the study conducted by Csonka and his associates, nor was it used in the present investigation.

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