JOURNAL OF CLINICAL MICROBIOLOGY, May 2011, p. 1990–1992 Vol. 49, No. 5
0095-1137/11/$12.00 doi:10.1128/JCM.00129-11

Utility of Urine, Vaginal, Cervical, and Rectal Specimens for Detection of *Mycoplasma genitalium* in Women▽†

Rebecca A. Lillis,[1]* M. Jacques Nsuami,[1] Leann Myers,[2] and David H. Martin[1]

Section of Infectious Diseases, Louisiana State University Health Sciences Center, New Orleans, Louisiana,[1] and Department of Biostatistics and Bioinformatics, Tulane School of Public Health and Tropical Medicine, New Orleans, Louisiana[2]

Received 21 January 2011/Accepted 7 March 2011

This study assessed the utility of urine, vaginal, cervical, and rectal specimens for the detection of *Mycoplasma genitalium* in women by using our laboratory-developed PCR assay. The relative sensitivity was 85.7% for the vaginal swab specimen, 74.3% for the endocervical swab specimen, 61.4% for the urine specimen, and 24.3% for the rectal swab specimen.

The diagnosis of sexually transmitted diseases (STDs) is increasingly being made using laboratory specimens that patients can collect themselves, such as urine in men and women and vaginal swabs in women. These self-collected specimens have proven useful for reliably detecting *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in both men and women by using nucleic acid amplification tests (NAATs) (2, 7).

Mycoplasma genitalium is strongly associated with nongonococcal urethritis in men, and its clinical significance in women and the determination of its role as a sexually transmitted agent are receiving growing attention (5, 8). Because there is currently no approved and commercially available diagnostic test for the detection of *M. genitalium*, clinical studies of *M. genitalium* infections use local laboratory-developed PCR tests to diagnose infection (11). Regardless of which NAAT is used, the determination of optimal specimen types for the detection of the organism under different circumstances is important.

As part of a study of the prevalence and risk factors for *M. genitalium* among women, conducted in STD clinic patients in New Orleans, we assessed the utility of urine, vaginal, cervical, and rectal specimens for the detection of *M. genitalium* in women by using a previously described PCR assay developed in our laboratory (9, 10).

Women age 18 or older who attended the New Orleans STD clinic for any reason between 28 May 2003 and 26 February 2004 were approached for inclusion in the study and were enrolled after completing the informed consent process. The study was approved by the institutional review board of the Louisiana State University Health Sciences Center. Pregnant women, women with a history of hysterectomy, and those who reported using antibiotics in the past 3 months were excluded from participation.

After obtaining informed consent, a complete sexual behavior, STD, obstetric, and gynecologic history was obtained from each study participant and recorded in a standardized form, along with the patient's demographic information. To detect *M. genitalium*, four laboratory specimens were obtained in the following order. A first-void urine specimen was collected prior to performance of a pelvic examination and kept at 4°C before transport to the laboratory. Following insertion of a nonlubricated speculum into the vagina, a vaginal swab was obtained from the posterior fornix. After cleaning the face of the cervix, an endocervical swab was obtained. A rectal swab was obtained last. Each of the three swabs was placed in a dry transport tube and held at 4°C prior to transport to the laboratory at the end of the day.

Following DNA purification, an *M. genitalium*-specific PCR was performed as described in detail by Mena et al. (9, 10), except that instead of Southern blotting, a dot blot assay was used as the *M. genitalium*-specific amplicon detection method. Details of the dot blot method are available from the authors on request. If any one of the specimens was positive, all four specimens were retested using the remaining DNA lysate for the cervical, vaginal, and rectal specimens and a frozen unprocessed urine sample.

M. genitalium infection was defined conservatively as any two initial PCR tests positive or any initial PCR test positive at one site only, which was confirmed by a repeat test using the same specimen.

We determined specimen-relative sensitivity by dividing the number of infected women for each specimen or combination of specimens by the total number of infected women. A Fisher's exact test was used for statistical comparison.

During the 9-month study period, first-void urine, vaginal, cervical, and rectal swabs for *M. genitalium* PCR testing were obtained from 400 women. Rectal swab results were missing for two women. Women were between 18 and 54 years old (mean ± standard deviation, 25.6 ± 6.8 years; median, 23.5 years), and 94.8% were African American. Almost all (99.0%; $n = 396$ women) reported vaginal sex, 41.5% ($n = 166$ women) performed oral sex, 68.3% ($n = 273$ women) received oral sex, and 11.0% ($n = 44$ women) reported anal sex.

Overall, 70 women (17.5%) met our definition of *M. genitalium* infection. Among *M. genitalium*-infected women, five tested positive on initial and repeat testing of all four specimens, 15 tested positive on initial and repeat testing of three specimens, nine tested positive on initial and repeat testing of

* Corresponding author. Mailing address: Louisiana State University School of Medicine, Section of Infectious Diseases, 1542 Tulane Ave., New Orleans, LA 70112. Phone: (504) 599-1457. Fax: (504) 568-6752. E-mail: rlilli@lsuhsc.edu.

† Supplemental material for this article may be found at http://jcm.asm.org/.

▽ Published ahead of print on 16 March 2011.

TABLE 1. Relative sensitivities of individual specimens and combinations of urogenital specimens for PCR detection of *M. genitalium* in all infected women ($n = 70$)

Specimen	No. (%) of specimens in which *M. genitalium* was detected	95% confidence interval
Individual specimens		
Vaginal swab	60 (85.7)	74.8, 92.6
Cervical swab	52 (74.3)	62.2, 83.7
Urine	43 (61.4)	49.0, 72.6
Rectal swab	17 (24.3)	15.2, 36.3
Combinations of urogenital specimens		
Vaginal swab and/or cervical swab	67 (95.7)	87.2, 98.9
Vaginal swab and/or urine	65 (92.9)	83.4, 97.3
Cervical swab and/or urine	61 (87.1)	76.5, 93.6
Vaginal swab and/or cervical swab and/or urine	69 (98.6)	91.2, 99.9

two specimens, and one patient each tested positive on initial and repeat testing of each of the four specimen types (see Table S1 in the supplemental material). There were an additional 22 women who had one initial test positive but who did not meet our definition of an *M. genitalium*-infected patient.

The prevalence of urogenital infection was 16.3% (65/400), and the prevalence of rectal infection was 4.3% (17/398). The prevalence of rectal infection was 6.8% (3/44) among women who reported having anal sex and 4.0% (14/354) among women who did not report anal sex ($P = 0.42$, Fisher's exact test). Only one patient was infected exclusively at the rectal site (see Table S1 in the supplemental material). She reported having anal sex, in addition to giving and receiving oral sex and having vaginal sex.

The relative sensitivity of each specimen and combinations of urogenital specimens are presented in Table 1. The vaginal swab specimen was the individual specimen that had the highest relative sensitivity for detecting *M. genitalium* (85.7%), followed by the endocervical swab specimen (74.3%), urine specimen (61.4%), and the rectal swab specimen (24.3%). The best combination of any two urogenital specimens for detection of *M. genitalium* was the vaginal and/or cervical swab, with a relative sensitivity of 95.7%.

Our study is the first to compare the utility of urine, cervical, vaginal, and rectal specimens for the detection of *M. genitalium* by NAATs in women, although one published study has compared vaginal, cervical, and urine specimens (13) and another has compared cervical and urine specimens for *M. genitalium* detection (6).

Rectal specimens were obtained, because we hypothesized that there could be a gut reservoir for *M. genitalium*, as *M. genitalium* DNA has been detected more frequently in homosexual than in heterosexual men (4) and more frequently in rectal than in urine specimens of homosexual men (1, 12). Per our definition of an *M. genitalium*-infected patient, 17 women were classified as having a rectal *M. genitalium* infection. Overall, anal sex was reported by 11% of women in this cohort. Although rectal specimens tested positive less commonly than urogenital specimens, there was one woman who engaged in anal sex and whose only *M. genitalium*-positive result was from her rectal specimen, suggesting that *M. genitalium* might be acquired through receptive anal sex in heterosexual women. However, rectal specimens contributed little to the determination of the infected patient status in this study, as only 5/70 women would not have been defined as infected had we not obtained and tested rectal swabs.

The single best specimen for the detection of *M. genitalium* infection in this study was the vaginal swab specimen, followed in order of decreasing relative sensitivity by the endocervical swab specimen, urine specimen, and the rectal swab specimen. The addition of an endocervical swab result to that of the vaginal swab result increased the relative sensitivity of *M. genitalium* detection to 95.7%. A weakness of our study is the absence of an established gold standard assay for the detection of the organism of interest, in this case, *M. genitalium*. Thus, we are able to determine only a relative sensitivity within the context of our own assay rather than absolute sensitivity and specificity. However, our results are very similar to those of Wroblewski et al., who determined relative sensitivities of vaginal, endocervical, and urine specimens not only by their laboratory's PCR assay but also by the Gen-Probe research-only transcription-mediated amplification assay (13). The implications of these findings are several. First, for women having pelvic examinations, a single swab inserted initially into the endocervical canal and then dipped into the vaginal secretion pool may be the optimal single specimen for *M. genitalium* detection (3). Second, if it is determined that widespread screening programs for *M. genitalium* are necessary for public health reasons, self-collected vaginal swabs probably would be the best approach. Considerable research has shown that self-collected vaginal swabs are equal to clinician-collected vaginal swabs for diagnosis of *C. trachomatis* and *N. gonorrhoeae*, and there is no reason to doubt that the same would be true for *M. genitalium*. Third, while self-collected urine is a good specimen for *C. trachomatis* and *N. gonorrhoeae* screening in women, the relative sensitivity of this specimen for MG is relatively poor. Clearly, further study will be necessary to test the hypotheses put forward here.

This work was supported by the Louisiana Board of Regents grant HEF (2001-2004)-04.

We thank Mary Welch for excellent technical assistance.

REFERENCES

1. **Bradshaw, C. S., et al.** 2009. *Mycoplasma genitalium* in men who have sex with men at male-only saunas. Sex. Transm. Infect. **85:**432–435.
2. **Buimer, M., et al.** 1996. Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by ligase chain reaction-based assays with clinical specimens from various sites: implications for diagnostic testing and screening. J. Clin. Microbiol. **34:**2395–2400.
3. **Edberg, A., et al.** 2009. Endocervical swabs transported in first void urine as combined specimens in the detection of *Mycoplasma genitalium* by real-time PCR. J. Med. Microbiol. **58:**117–120.
4. **Hooton, T. M., et al.** 1988. Prevalence of *Mycoplasma genitalium* determined by DNA probe in men with urethritis. Lancet **1:**266–268.
5. **Jensen, J. S.** 2004. *Mycoplasma genitalium*: the aetiological agent of urethritis and other sexually transmitted diseases. J. Eur. Acad. Dermatol. Venereol. **18:**1–11.
6. **Jensen, J. S., E. Björnelius, B. Dohn, and P. Lidbrink.** 2004. Comparison of first void urine and urogenital swab specimens for detection of *Mycoplasma genitalium* and *Chlamydia trachomatis* by polymerase chain reaction in patients attending a sexually transmitted disease clinic. Sex. Transm. Dis. **31:** 499–507.
7. **Knox, J., et al.** 2002. Evaluation of self-collected samples in contrast to practitioner-collected samples for detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* by polymerase chain reaction among women living in remote areas. Sex. Transm. Dis. **29:**647–654.
8. **Manhart, L. E., K. K. Holmes, J. P. Hughes, L. S. Houston, and P. A. Totten.**

2007. *Mycoplasma genitalium* among young adults in the United States: an emerging sexually transmitted infection. Am. J. Public Health **97:**1118–1125.
9. **Mena, L. A., T. F. Mroczkowski, M. Nsuami, and D. H. Martin.** 2009. A randomized comparison of azithromycin and doxycycline for the treatment of *Mycoplasma genitalium*-positive urethritis in men. Clin. Infect. Dis. **48:** 1649–1654.
10. **Mena, L., X. Wang, T. F. Mroczkowski, and D. H. Martin.** 2002. *Mycoplasma genitalium* infections in asymptomatic men and men with urethritis attending a sexually transmitted disease clinic in New Orleans. Clin. Infect. Dis. **35:** 1167–1173.
11. **Palmer, H. M., C. B. Gilroy, P. M. Furr, and D. Taylor-Robinson.** 1991. Development and evaluation of the polymerase chain reaction to detect *Mycoplasma genitalium*. FEMS Microbiol. Lett. **61:**199–203.
12. **Soni, S., et al.** 2010. The prevalence of urethral and rectal *Mycoplasma genitalium* and its associations in men who have sex with men attending a genitourinary medicine clinic. Sex. Transm. Infect. **86:**21–24.
13. **Wroblewski, J. K. H., L. E. Manhart, K. A. Dickey, M. K. Hudspeth, and P. A. Totten.** 2006. Comparison of transcription-mediated amplification and PCR assay results for various genital specimen types for detection of *Mycoplasma genitalium*. J. Clin. Microbiol. **44:**3306–3312.