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Expansion of Comprehensive Screening of Male Sexually Transmitted Infection Clinic Attendees with *Mycoplasma genitalium* and *Trichomonas vaginalis* Molecular Assessment: a Retrospective Analysis

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ABSTRACT Of 1,493 encounters of males at a sexually transmitted infection (STI) clinic in a community with a high prevalence of STI, *Chlamydia trachomatis* was detected in 8.7% and *Neisseria gonorrhoeae* was detected in 6.6%. Additional *Trichomonas vaginalis* and *Mycoplasma genitalium* screening found 17.4% and 23.9% of the encounters, respectively, to be positive for STI. STI agents were detected in 13.7% of urine specimens; addition of pharyngeal and rectal collections to the analysis resulted in detection of STI agents in 19.0% and 23.9% of encounters, respectively. A total of 101 (23.8%) encounters of identified STI involved sole detection of *M. genitalium*. Expansion of the STI analyte panel (including *M. genitalium*) and additional specimen source sampling within a comprehensive STI screening program increase identification of male STI carriers.

KEYWORDS *Mycoplasma genitalium*, transcription-mediated amplification, *Trichomonas vaginalis*

Extraurogenital screening for agents of sexually transmitted infection (STI) has been advocated in a number of clinical and public health scenarios (1). Studies have reported increased rates of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* detection from pharyngeal and rectal specimens via nucleic acid amplification testing compared to rates found with culture (2–7). Moreover, differences in clinical and *in vitro* detection rates yielded by transcription-mediated amplification (TMA) versus DNA amplification modalities have been demonstrated (4–9), likely owing to target capture-based removal of endogenous inhibitors (10, 11). Detection of *Trichomonas vaginalis* RNA from pharyngeal specimens (12) and *Mycoplasma genitalium* RNA from urine specimens (13) of male STI clinic attendees has recently been reported on the basis of commercial TMA assays. The purpose of this investigation was to assess both the capacity of TMA for detection of *M. genitalium* from pharyngeal and rectal specimens and the potential for multispecimen analysis in the overall identification of male carriers of STI agents.

(Results of this work were presented in part at ASM Microbe, Boston, MA, 16 to 20 June 2016.)

With an Institutional Review Board-approved protocol, screening practices for male attendees of a Milwaukee, WI, STI clinic were audited from March 2014 through December 2015. The high-STI-prevalence nature of this community was noted previously (14). Aliquots of first-void urine were dispensed into Aptima urine specimen transport tubes (Hologic, San Diego, CA). Pharyngeal and rectal swab specimens were obtained using the Aptima unisex swab specimen collection kit (Hologic).

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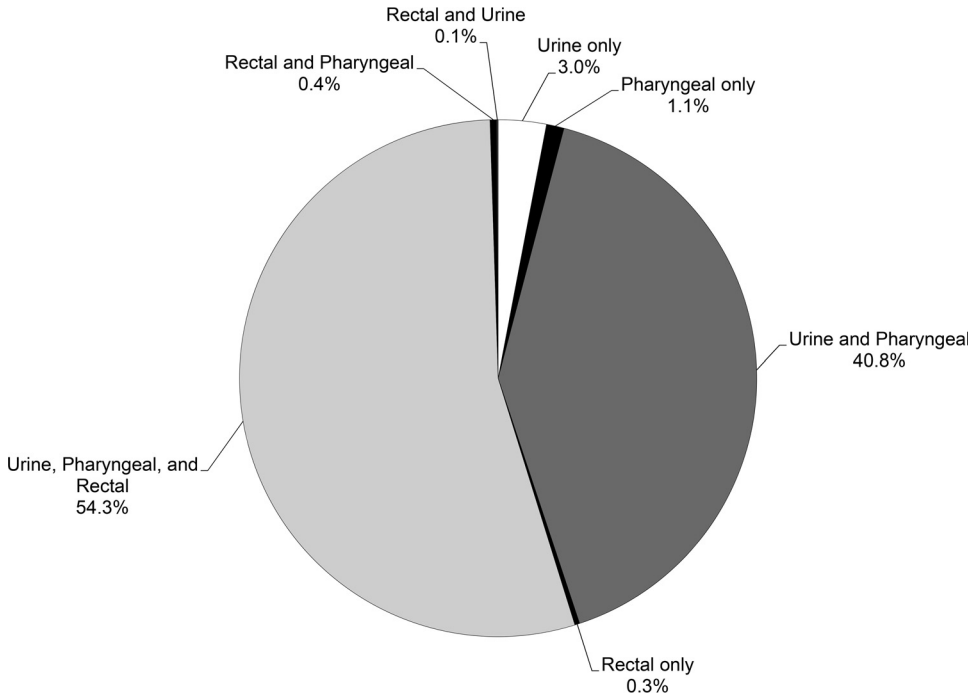

FIG 1 Screening of 1,493 men from a Milwaukee, WI, STI clinic, delineated by permutations of urine, pharyngeal, and rectal specimen collection.

C. trachomatis- and *N. gonorrhoeae*-specific analyses from all collections were performed with the Aptima Combo 2 assay (Hologic), including laboratory-validated assessments of pharyngeal and rectal specimens. *T. vaginalis* detection occurred via an Aptima Trichomonas vaginalis assay (Hologic) that was laboratory validated for analysis of male urine and pharyngeal specimens. Detection of *M. genitalium* occurred via TMA-based analyte-specific reagent (ASR) (Hologic). All assays were performed on Tigris DTS (Hologic). *M. genitalium* ASR relative light unit output of ≥50,000 signified a positive result. The significance test of proportions was used to determine whether changes in detection rates were significant.

Collection of one and two specimens was used to manage 4.4% and 41.3% of patient encounters, respectively (Fig. 1). The two most commonly detected STI agents from pharyngeal collections were *N. gonorrhoeae* (3.3%) and *T. vaginalis* (1.5%) (Fig. 2). *M. genitalium* was detected from 0.9% of pharyngeal specimens. Detection rates of *M. genitalium* (5.8%), *C. trachomatis* (6.0%), and *N. gonorrhoeae* (6.4%) from rectal specimens were generally similar to those of *M. genitalium* (6.6%) and *C. trachomatis* (5.1%) from urine specimens (Fig. 2). Analysis of rectal specimens for *T. vaginalis* was not attempted because previous data demonstrated 0% prevalence in this population from this specimen source (12).

Traditional molecular STI assays have targeted *C. trachomatis* and *N. gonorrhoeae*. TMA-based detection of other STI agents has been documented from male urogenital (*T. vaginalis*, *M. genitalium*) and pharyngeal (*T. vaginalis*) specimens (12, 13, 15, 16). *C. trachomatis* and *N. gonorrhoeae* screening from both urine and extraurogenital sources in this study resulted in 228 instances of STI (15.3% incidence rate) (Fig. 3). Addition of *T. vaginalis* and *M. genitalium* analytes into the comprehensive screen increased incidence rates to 17.4% ($P = 0.11$ versus *C. trachomatis* and *N. gonorrhoeae* screening) and 23.9% ($P < 0.0002$ versus combined *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis* screening), respectively.

A total of 1,493 clinic encounters resulted in the collection of 1,465 urine specimens, 1,443 pharyngeal specimens, and 823 rectal specimens. This numeric rank formulated the basis of the sequential order of the analysis presented in Fig. 4. Screening only urine for all four analytes resulted in 205 (13.7%) instances of STI diagnosis (Fig. 4). This value increased

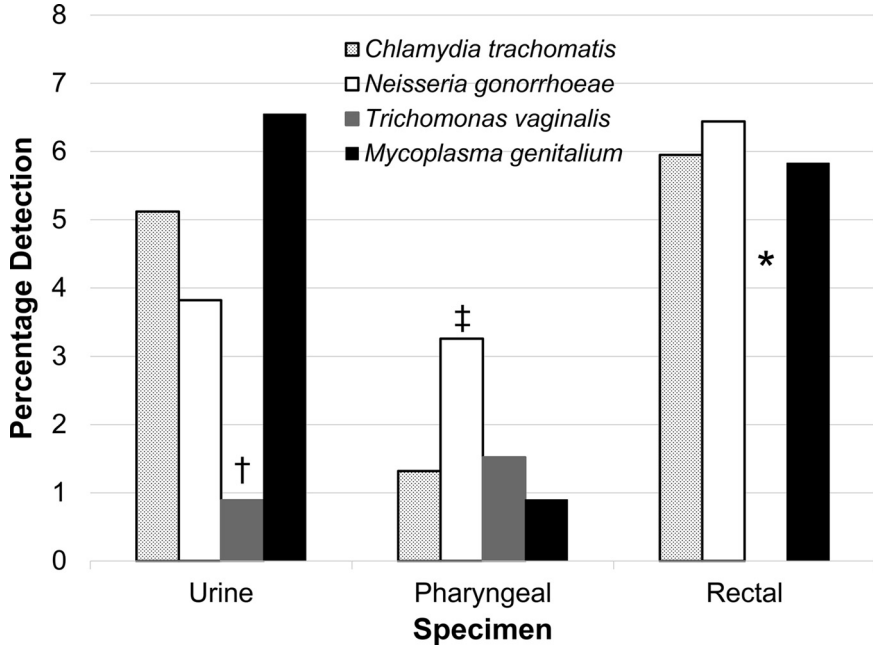

FIG 2 Transcription-mediated amplification detection rates of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, and *Mycoplasma genitalium* in 1,465 urine, 1,443 pharyngeal, and 823 rectal specimens. †, $P < 0.0002$ versus detection of other etiologies; ‡, $P \leq 0.002$; *, not tested.

to 284 patient encounters by addition of pharyngeal screening ($P < 0.0002$). Addition of rectal screening accounted for the remainder of the 357 instances of STI diagnosis in this cohort ($P = 0.001$ versus combined urine and pharyngeal screening). Of the 205 instances of STI diagnosis via urine screening, sole *M. genitalium* detection was observed in 73 encounters (4.9% of all clinic encounters) (Fig. 4); addition of pharyngeal and rectal

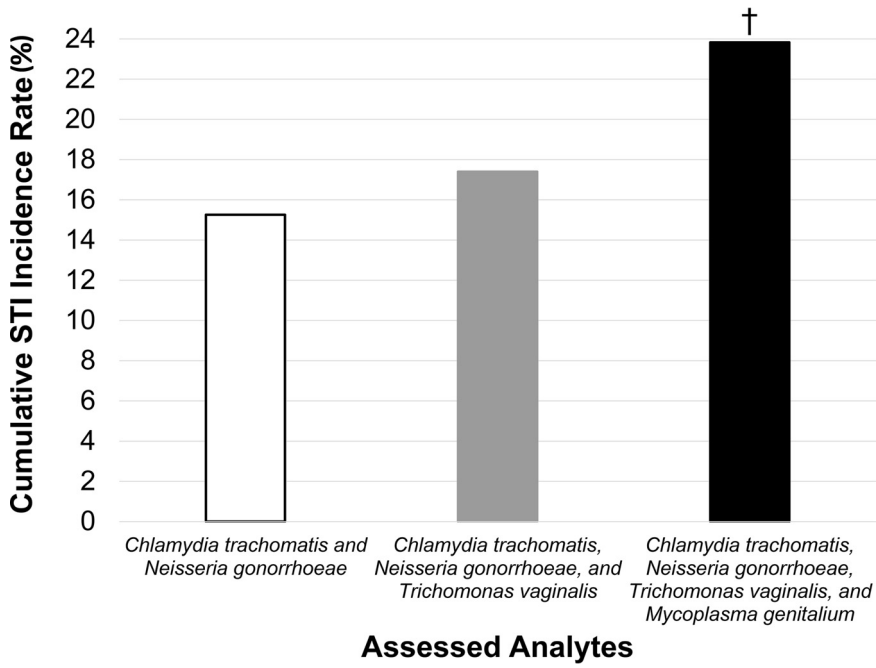

FIG 3 Cumulative STI incidence rate, as a function of additional transcription-mediated amplification assays. †, $P < 0.0002$ versus combined *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* screening.

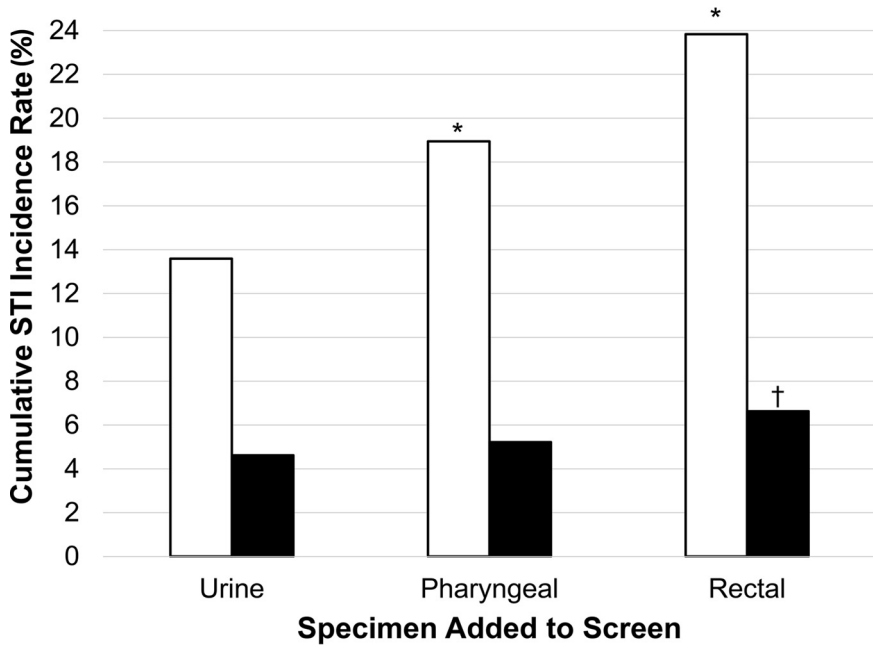

FIG 4 Cumulative STI (open bars) and *Mycoplasma genitalium* (solid bars) incidence rates, as function of additional specimen source analysis. *, $P \leq 0.001$ versus single- or double-collection paradigms; †, $P = 0.02$ versus urine *M. genitalium* screening.

screening increased sole *M. genitalium* detection rates to 5.3% and 6.8% ($P = 0.03$), respectively, of all clinic visits. In total, sole detection of *M. genitalium* contributed to 23.8% of encounters that yielded at least one STI.

Among Centers for Disease Control and Prevention recommendations for STI management of sexually active men who have sex with men (MSM) is an annual test for rectal infection with *C. trachomatis* and *N. gonorrhoeae* in those who have experienced receptive anal intercourse during the previous 12 months (1). Pharyngeal testing for *N. gonorrhoeae* should be performed in MSM who have received oral intercourse during that interval. Although pharyngeal *C. trachomatis* screening is not a recommendation, studies have described a propensity of pharyngeal *C. trachomatis* carriage to result in disease transmission to genital sites (17, 18). More-frequent screening may be indicated per additional sexual risk factors.

Perhaps as a result of these updated guidelines, the percentage of encounters resulting in multiple specimen collections in this study (95.6%) exceeded that from one previous survey of this STI clinic population (64.2%) (12). Not only did multisource sampling and multianalyte testing in an STI clinic population provide general benefit, a rather high-risk, susceptible population may also be appreciated when analyzing the demographics characterized by extraurogenital *M. genitalium* carriage. The median ages of attendees with detectable pharyngeal and rectal *M. genitalium* were 24.0 and 24.5 years, respectively. Men with detectable pharyngeal and rectal *M. genitalium* averaged 10.4 and 7.2 sexual partners, respectively, over the previous 12 months. Moreover, 93.3% and 81.8% of men with detectable rectal and pharyngeal *M. genitalium*, respectively, engaged in homosexual practices.

M. genitalium detection in pharyngeal and rectal specimens was confirmed by positive results generated by repeat *M. genitalium* ASR from 92.3% and 91.5% of specimens, respectively. Previous data revealed that repeat TMA analysis performed the same as alternative target testing for confirmation of STI agent detection (19, 20). In general, specificity of *M. genitalium* ASR within a female acute and subacute population was demonstrated by 98.8% concordance between results from *M. genitalium* ASR and alternative target testing (21).

In conclusion, procurement of rectal, pharyngeal, and urine specimens increases overall identification of male STI carrier status. Moreover, the advent of commercial *M. genitalium* ASR may result in a substantial percentage of sole *M. genitalium* detection within this demographic. Additional studies are warranted to determine financial and disease transmission impacts relative to routine incorporation of this assay into a comprehensive STI screening algorithm.

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