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Clinical Laboratory Assessment of *Mycoplasma genitalium* Transcription-Mediated Amplification Using Primary Female Urogenital Specimens

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Following analysis of primary cervix, vagina, and first-void female urine specimens for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* via commercial transcription-mediated amplification (TMA), residual material was subjected to *Mycoplasma genitalium* research-use-only TMA. Representation within a 2,478-specimen retrospective study set was established by comparison to a 6-month audit of clinical *C. trachomatis* TMA (12,999 specimens) on the basis of the *C. trachomatis* detection rate, specimen source distribution, clinic location, and age. *M. genitalium* was detected in 282 (11.4%) patients. This rate was higher than those seen with *T. vaginalis* (9.0%; *P* = 0.005), *C. trachomatis* (6.2%), and *N. gonorrhoeae* (1.4%). Positive *M. genitalium* results were confirmed by repeat testing or alternative-target TMA at a rate of 98.7%. The mean age of the *M. genitalium*-infected females (24.7 years) was lower than that of the *T. vaginalis*-infected females (mean, 30.1 years; *P* < 0.0001) and higher than that of the *C. trachomatis*-infected females (mean, 23.8 years; *P* = 0.003). Of 566 patient encounters positive for at least one sexually transmitted infection (STI), 35.9% exhibited sole detection of *M. genitalium* (*P* ≤ 0.0004 versus sole detection of other STI agents) and 26.1% were solely positive for *T. vaginalis* (*P* < 0.0002 versus *C. trachomatis*). The *M. genitalium* and *T. vaginalis* detection rates among 755 patients at urban emergency departments were 14.6% and 13.0%, respectively (*P* = 0.37). A 10.0% *M. genitalium* detection rate from other facilities exceeded that of *T. vaginalis* (7.2%; *P* = 0.004). Incorporation of *M. genitalium* TMA into comprehensive testing programs would detect *M. genitalium* in a significant proportion of females, particularly those in outpatient obstetrics and gynecology (OB/GYN) settings.

The sexually transmitted infection (STI) agent *Mycoplasma genitalium* has historically had a role of pathogenicity in male nongonococcal urethritis (1). Recent evidence has implicated the bacterium in clinically significant disease in females (2, 3). Additional studies suggest that *M. genitalium* infection promotes HIV acquisition (4–6) and virus shedding (7, 8). Moreover, in a recent meta-analysis, Lis et al. (9) reported significant associations between *M. genitalium* infection and cervicitis, pelvic inflammatory disease, preterm birth, and spontaneous abortion.

Until recently, a lack of reliable testing options has curtailed laboratory diagnosis of *M. genitalium* infection. Culture and serologic modalities have been limited by sensitivity and/or cross-reactivity with other mycoplasmas (1) and are becoming supplanted by molecular diagnostics, largely on a research basis. PCR-based assays have correlated *M. genitalium* DNA burden with clinical condition (10, 11) and treatment efficacy (12) in males. Quantitative molecular analysis has sought to study progression of genital disease in females (13). In the realm of laboratory diagnosis, initial studies of target capture-based transcription-mediated amplification (TMA) in females reported 87.8% (14) and 96.9% (15) sensitivity values from vaginal specimens. PCR sensitivity values from the same cohorts were 92.9% and 93.8%, respectively.

We recently demonstrated the utility of TMA-based *M. genitalium* detection in male specimens originating from both STI and community outpatient clinics (16). In contrast, previous studies of *M. genitalium* TMA in females have largely focused on high-risk demographics (14, 15, 17–19). Assessment of *M. genitalium* detection rates in a communitywide setting would be beneficial. We

now provide findings of the importance of TMA-based *M. genitalium* evaluation of females in a metropolitan health care system.

(Results of this work were previously presented, in part, at the 115th General Meeting of the American Society for Microbiology, New Orleans, LA, 30 May to 2 June 2015.)

MATERIALS AND METHODS

Setting. In addition to seven acute care facilities, Wheaton Franciscan Laboratory serves an approximately 125-clinic metropolitan outpatient physician group in a three-county region of southeastern Wisconsin. A portion of this service area extends beyond the Milwaukee-Waukesha-West Allis metropolitan statistical area (MSA). Analyses of data from U.S. MSAs document a long-standing trend of high STI prevalence in the Milwaukee-Waukesha-West Allis MSA. Aggregation of these data revealed an average chlamydia rate of 678.0 per 100,000 population over a 10-year interval (20). This value averaged the second highest in the United States and represented a 77.3% increase over the average national cumulative MSA rate of 382.3 per 100,000 population. The same MSA generated an

TABLE 1 Distribution of specimen source submissions and *C. trachomatis* detection rates during a 6-month audit of routine *C. trachomatis* screening, with comparison to representative distributions of specimens collected for retrospective *M. genitalium* TMA analysis

Specimen source	% source distribution within specimens analyzed in:			% *C. trachomatis* detection from specimens analyzed in:		
	C. trachomatis routine screening	*M. genitalium* TMA assessment	*P* value	*C. trachomatis* routine screening	*M. genitalium* TMA assessment	*P* value
Cervix	77.7	76.5	0.20	6.2	6.2	0.99
Urine	17.5	17.9	0.65	6.3	6.1	0.85
Vagina	4.8	5.6	0.10	6.9	6.5	0.87
Total				6.3	6.2	0.91

average annual rank of 2.56 for gonorrhea rate among United States MSAs (264.0 per 100,000 population; 112.6% higher than the national MSA rate of 124.2 per 100,000 population).

Specimen submission for routine screening. Approximately 2-ml aliquots of first-void female urine were added to Aptima urine specimen transport tubes per the Aptima Combo 2 Assay (Hologic, Incorporated, San Diego, CA) package insert protocol following specimen procurement (21). Aliquots were stored at 2 to 30°C and tested within 30 days of primary collection. Primary genital swab specimens were obtained using an Aptima unisex swab specimen collection kit (21), stored at 2 to 30°C, and tested within 30 days of collection.

The Aptima Combo 2 Assay was utilized for the detection of *N. gonorrhoeae*-specific 16S rRNA and *Chlamydia trachomatis*-specific 23S rRNA (22) from all urine aliquots. The samples were additionally subjected to Aptima Trichomonas vaginalis (Hologic) testing for detection of organism-specific 18S rRNA (23).

Retrospective specimen collection for *M. genitalium* TMA analysis. A 6-month laboratory information system-based audit of female STI ordering practices was conducted for the purpose of assembling a representative study set for *M. genitalium* TMA. Assessed parameters included specimen source (urine, cervical specimen, or vaginal specimen), *C. trachomatis* detection rate, geographic location, and patient age. This study was governed by the Wheaton Franciscan Healthcare Institutional Review Board.

Molecular detection of *M. genitalium*. Residual clinical material was subjected to a research-use-only TMA-based assay for detection of *M. genitalium*-specific 16S rRNA (referred to as *M. genitalium* TMA; Hologic). Reagents were prepared by using Aptima general-purpose reagents spiked with *M. genitalium*-specific oligonucleotides. A 50-μl aliquot of target capture oligonucleotide was spiked into Aptima target capture reagent; 50-μl aliquots of T7 and non-T7 oligonucleotides were spiked into reconstituted Aptima reconstitution reagent; and a 50-μl aliquot of acridinium ester-labeled hybridization oligonucleotide was spiked into Aptima hybridization buffer. All assays were performed on an automated TIGRIS DTS platform (Hologic).

Interpretation of results. *M. genitalium* TMA relative-light-unit values of ≥50,000 derived from urogenital specimen testing were interpreted as representing positive results (14, 17). A subset of positive results, as each residual specimen allowed, was confirmed by repeat testing. An additional subset of positive specimens was forwarded for a TMA-based alternative-target confirmatory assay (Hologic).

Statistical analysis. The STI phenotype was defined as a *M. genitalium*, *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis* distribution within a given health care encounter that yielded detection of at least one STI agent. The significance test of proportions determined if differences in either the rates of positive screening results or the STI phenotypes were significant. The *t* test for independent samples determined if differences in mean patient age associated with positive results were significant in the comparisons between STI etiologies. The alpha level was set at 0.05 before the investigations commenced, and all *P* values are two tailed.

RESULTS

Collection of specimens for *M. genitalium* TMA assessment. Retrospectively collected specimens ($n = 2,478$) were compared to data gathered from a 6-month audit of routine screening practices for *C. trachomatis*. Proportional specimen source distributions did not differ between routine *C. trachomatis* screening practices and specimens gathered for *M. genitalium* TMA ($P \geq 0.10$; Table 1). In addition, *C. trachomatis* detection rates showed no difference from those seen in the collection for *M. genitalium* TMA ($P = 0.91$). Detection rates stratified by specimen source were also similar ($P \geq 0.85$; Table 1). Among the urogenital specimens submitted for routine *C. trachomatis* screening, 18.6% and 48.0% were derived from females aged ≤20 and 21 to 30 years, respectively. Analogous proportions collected for *M. genitalium* TMA were 19.0% and 47.0% ($P = 0.60$ and 0.38, respectively; data not illustrated). Comparative data for successive age in decades exhibited no differences ($P \geq 0.07$).

Twenty health care locations accounted for 89.2% of all routine *C. trachomatis* submissions (Table 2). No differences in proportional specimen distributions between routine laboratory *C. trachomatis* screening and the *M. genitalium* TMA collection ($P = 0.11$) or stratified by individual health care location ($P \geq 0.28$) were noted. These entities were also the basis for arbitrary categorization of health care locations as outpatient obstetrics and gynecology (OB/GYN), inpatient OB/GYN, suburban family care, urban family care, and emergency room (ER)/urgent care. Moreover, 242 (9.8%) specimens in the *M. genitalium* TMA collection originated from 34 additional health care locations (Table 2).

Detection of *M. genitalium* RNA. The overall detection rate of *M. genitalium* (11.4%) exceeded those of the other STI agents ($P \leq 0.005$). *T. vaginalis* also demonstrated increased detection (9.0% rate) versus *C. trachomatis* and *N. gonorrhoeae* ($P \leq 0.0003$). Detection rates for *C. trachomatis* and *N. gonorrhoeae* were 6.2% and 1.4%, respectively. A total of 208 specimens with detectable *M. genitalium* contained enough residual material to allow for repeat analysis; 207 (99.5%) yielded a positive result. In addition, a subset of 242 specimens was subjected to alternative-target TMA analysis (Table 3). A 98.8% concordance of results was observed.

Female urogenital specimens with detectable *M. genitalium* RNA. The rates of detection of *M. genitalium* from 1,896 cervical, 444 urine, and 138 vaginal specimens were 11.5%, 10.1%, and 13.8%, respectively ($P \geq 0.23$; Fig. 1). In similar fashion, the *C. trachomatis* and *N. gonorrhoeae* detection rates, stratified by specimen source, revealed no differences ($P \geq 0.85$ and 0.18, respec-

TABLE 2 Distribution of specimen submissions among the 20 most frequently visited health care locations during a 6-month audit of routine *C. trachomatis* screening and comparison to representative distributions of specimens collected for retrospective *M. genitalium* TMA analysis

Health care location	% total specimen submissions for *C. trachomatis* screening[a]	% total specimen collections for *M. genitalium* TMA assessment[b]	*P* value
Outpatient OB/GYN 1	16.69	16.38	0.71
Outpatient OB/GYN 2	9.12	9.60	0.44
Outpatient OB/GYN 3	4.14	4.08	0.89
Outpatient OB/GYN 4	3.21	3.39	0.64
Outpatient OB/GYN 5	3.02	3.39	0.33
Outpatient OB/GYN 6	1.96	1.86	0.73
Outpatient OB/GYN 7	1.54	1.45	0.75
Outpatient OB/GYN 8	1.52	1.49	0.91
Inpatient OB/GYN	1.46	1.69	0.38
Suburban family care 1	3.62	3.55	0.86
Suburban family care 2	1.58	1.53	0.87
Urban family care 1	5.36	5.37	0.99
Urban family care 2	3.21	3.55	0.38
Urban family care 3	2.93	2.70	0.54
ER/urgent care 1	11.95	12.47	0.46
ER/urgent care 2	4.85	4.96	0.80
ER/urgent care 3	5.12	5.00	0.82
ER/urgent care 4	4.06	3.79	0.53
ER/urgent care 5	2.66	2.54	0.73
ER/urgent care 6	1.15	1.41	0.28
Top 20 locations	89.2	90.2	0.11
34 other health care locations	10.8	9.8	0.11

[a] Total submissions, 12,999.
[b] Total collections, 2,478.

tively). The *T. vaginalis* detection rate from urine (11.3%) exceeded the rate from cervical specimens (8.2%; $P < 0.04$) and was similar to the detection rate (11.6%) from vaginal specimens ($P = 0.91$).

The detection rate of *M. genitalium* in females aged ≤20 years was 16.8%. This rate decreased for each successive age decade (Fig. 2), leading to a 4.6% value in a subset of 22 females aged >60 years. *M. genitalium* was detected in females 14 years to 65 years of age, with a mean age of 24.7 years (data not illustrated). This mean was similar to the mean age of *N. gonorrhoeae* detection (23.5 years; $P = 0.30$) but different from those for *C. trachomatis* detection (22.8 years; $P = 0.003$) and *T. vaginalis* detection (30.1 years; $P < 0.0001$).

M. genitalium detection delineated by health care setting. Other than a 9.0% rate of *C. trachomatis* detection from the ER/urgent care setting, *C. trachomatis* and *N. gonorrhoeae* detection rates did not exceed 5.4% for a given health care setting (data not illustrated). Two health care locations, ER/urgent care and outpatient OB/GYN, accounted for 72.1% of specimens analyzed by *M. genitalium* TMA. Specimens from outpatient OB/GYN were more likely to contain detectable *M. genitalium* than *T. vaginalis* ($P = 0.001$; Table 4). In contrast, specimens from ER/urgent care had similarly high rates of *M. genitalium* and *T. vaginalis* detection (14.6% and 13.0%, respectively; $P = 0.37$).

Codetection of other STI agents with _M. genitalium_. *M. genitalium* was a component of 49.8% of the female STI phenotypes

TABLE 3 Tandem performance of *M. genitalium* TMA and alternative-target TMA assays on a subset of 242 primary female urogenital specimens

Alternative-target TMA result	No. of *M. genitalium* TMA results	
	Positive	Negative
Positive	27	1
Negative	2	212

and the sole component of 35.9% of the phenotypes in this study (Table 5). Sole detection of *T. vaginalis* was observed with 26.1% of the phenotypes; *T. vaginalis* was a constituent of 39.2% of the STI phenotypes ($P ≤ 0.0004$ compared to *M. genitalium*). These values were greater than the analogous data for *C. trachomatis* and *N. gonorrhoeae*.

In general, equal abundances of *M. genitalium*- and *T. vaginalis*-based STI phenotypes existed among the inpatient OB/GYN, family care, and ER/urgent care settings ($P ≥ 0.26$; Table 5). However, 41.3% of the outpatient OB/GYN phenotypes consisted of sole *M. genitalium* detection, while sole detection of *T. vaginalis* comprised 21.8% of the phenotypes ($P < 0.0002$).

Of the 282 specimens with detectable *M. genitalium*, only 28.0% revealed detection of an additional STI agent (data not illustrated). Between 28.6% and 36.6% of inpatient OB/GYN, family care, and ER/urgent care specimens with detectable *M. genitalium* involved codetection with another STI agent. In contrast, only 19.8% of the outpatient OB/GYN specimens testing positive for *M. genitalium* exhibited an additional STI agent.

DISCUSSION

Evidence for the clinical significance of *M. genitalium* in female reproductive tract disease is increasing. Lis et al. (9) demonstrated a significant association of *M. genitalium* incidence and increased risk of cervicitis with a pooled odds ratio (OR) of 1.65. A pooled OR of 2.53 (adjusted for coinfection) was calculated for an association with pelvic inflammatory disease. When studies utilizing serodiagnosis were excluded, the OR increased to 2.73. Additional meta-analyses associated *M. genitalium* infection with increased risk of preterm birth (pooled OR of 2.33, accounting for coinfection) and spontaneous abortion (pooled OR of 1.82). These data point out a clinical need for effective laboratory diagnostics specific to this STI agent.

Our data show elevated rates of *M. genitalium* detection in females, with significantly greater rates than those for *Trichomonas vaginalis*—a pathogen previously demonstrated to be present in abundance in the high-prevalence Milwaukee STI community (24, 25). Of 282 detections of *M. genitalium*, 237 were subjected to confirmation by repeat testing or alternative-target TMA; 98.7% of such tests yielded a positive result. Previous literature has espoused the value of repeat testing in the confirmation of positive TMA results (26). Furthermore, in the course of determining the specificity of *M. genitalium* TMA, 213 TMA-negative specimens were subjected to alternative-target TMA. Only one specimen (0.5%) yielded a positive result by alternative-target TMA. This rate of discordant results was less than the 1.6% to 4.6% values reported for *T. vaginalis* alternative-target testing (24, 27, 28). The parasitic species *Trichomonas tenax* has substantial genetic homology with *T. vaginalis* (29), which hypothetically could contribute to decreased specificity of *T. vaginalis* alternative-target TMA,
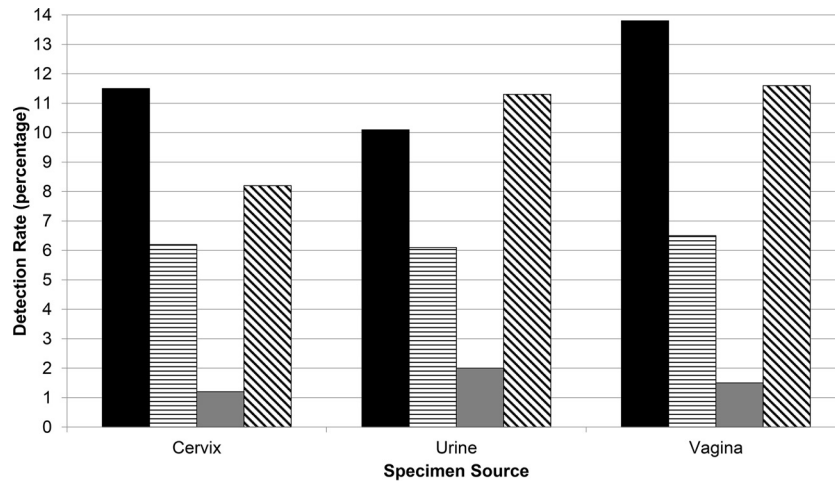
FIG 1 Cervical, first-void urine, and vaginal specimen detection rates for *M. genitalium* (solid bars), *C. trachomatis* (horizontally hatched bars), *N. gonorrhoeae* (gray bars), and *T. vaginalis* (diagonally hatched bars) from 2,478 female patients.

particularly in cases of pharyngeal *T. vaginalis* carriage (28). The high specificity for *M. genitalium* alternative-target TMA implies lack of cross-reactivity with other mycoplasmas and clearly verifies the increased *M. genitalium* detection in this population.

Previous studies of *M. genitalium* TMA have focused largely on high-risk demographics. Huppert et al. (17) reported a 22.4% detection rate from 331 female attendees of an adolescent health center/emergency department. Furthermore, Gaydos et al. (18) documented an approximate 19% detection rate among female STI clinic patients. A major strength of our large-scale study is the extrapolation of *M. genitalium* TMA to primary clinical practice in a broad geographic area. Data from Tables 1 and 2 confirm the representative nature of the study set. Past reports from our laboratory have summarized diagnostic assay performance within high-prevalence STI cohorts (16, 20). Subsequent to these reports, our laboratory has extended its testing scope to locales outside the Milwaukee-Waukesha-West Allis MSA. While this has resulted in decreases of STI etiology detection by the laboratory (e.g., the *C.*

trachomatis detection rate at [non-MSA] ER/urgent care 3 was 8.9%, while the analogous detection rate at [MSA] ER/urgent care 1 was 13.3%; data not illustrated), it has also provided a diverse population basis for practical assessment of commercial *M. genitalium* TMA.

Past surveillance efforts in general European and U.S. female populations using molecular diagnostics (30, 31) have reported *M. genitalium* detection rates of 1.0% to 2.3%. A meta-analysis published by McGowin and Anderson-Smits (32) calculated a detection rate of 2.0% from low-risk female populations. In a South American OB/GYN cohort of 1,338 women, Hitti et al. (33) documented a 3.1% cervical *M. genitalium* detection rate by TMA. This report is interesting for a number of reasons. First, it documents *M. genitalium* detection in an OB/GYN population and associates its presence with preterm birth and younger maternal age. It also reveals a strong association with *C. trachomatis* detection and a marginal association with *T. vaginalis* detection. It should be noted that those authors utilized culture for laboratory
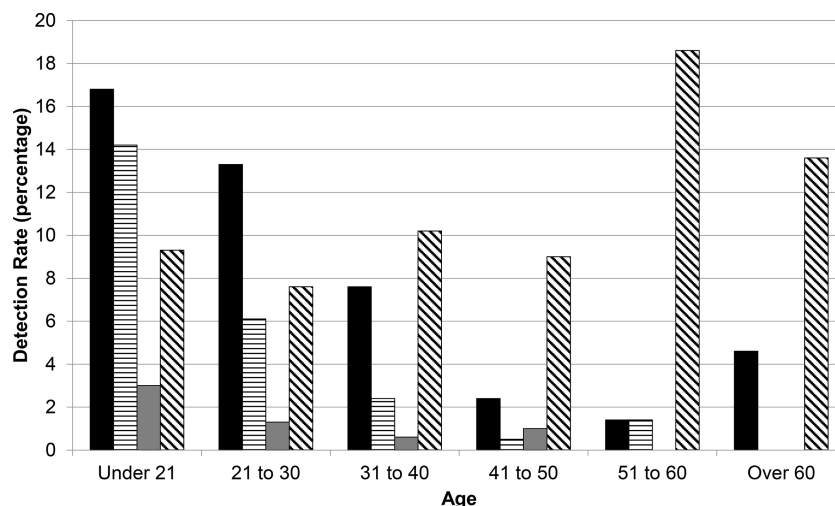

FIG 2 *M. genitalium* (solid bars), *C. trachomatis* (horizontally hatched bars), *N. gonorrhoeae* (gray bars), and *T. vaginalis* (diagonally hatched bars) detection rates, delineated by age, from 2,478 female urogenital specimens.

TABLE 4 *M. genitalium* and *T. vaginalis* detection rates for females seeking health care in ER/urgent care and non-ER/urgent care settings

Health care setting	*n*	No. (%) of specimens with detection of:		*P* value
		M. genitalium	*T. vaginalis*	
Outpatient OB/GYN	1,032	106 (10.3)	66 (6.4)	0.001
Inpatient OB/GYN	46	7 (15.2)	5 (10.9)	0.54
Suburban family care	261	18 (6.9)	14 (5.4)	0.47
Urban family care	384	41 (10.7)	39 (10.2)	0.81
ER/urgent care	755	110 (14.6)	98 (13.0)	0.37

diagnosis of trichomoniasis, a modality shown to be far less sensitive than molecular diagnostics (34). Our outpatient OB/GYN population was characterized by an increased rate of *M. genitalium* in general and by a significantly increased rate of *M. genitalium* detection in comparison to *T. vaginalis* detection (*P* = 0.001; Table 4).

STI phenotyping essentially predicts the likelihood of a given etiology for STI diagnosis during a health care encounter. In contrast to past reports showing a strong association between *M. genitalium* detection and the concomitant presence of *C. trachomatis* (17, 33), only 4.2% of total STI phenotypes in our study involved codetection of *M. genitalium* and *C. trachomatis* (Table 5). Among the STI phenotypes, 7.1% consisted of *M. genitalium* and *T. vaginalis* codetection. Moreover, while *M. genitalium* was a constituent of nearly half of the STI phenotypes, 39.2% and 27.2% of all phenotypes had some component of *T. vaginalis* and *C. trachomatis*, respectively. Detection rate and STI phenotype data reveal significant differences with respect to *M. genitalium* and *T. vaginalis* in the outpatient OB/GYN setting. These clinics were more likely to produce a significant increase in *M. genitalium* detection (Table 4) as well as STI phenotypes specific to the organism (Table 5). The other four arbitrary health care classifications did not experience this phenomenon. Napierala et al. (16) previously described a *M. genitalium*/*T. vaginalis* dichotomy within a 2,750-male cohort in which the STI phenotypes of STI clinic attendees and patients seeking outpatient clinic care consisted predominately of *M. genitalium* and *T. vaginalis*, respectively. The differential distribution of these two STI agents, which apparently may exist to a degree in both genders, warrants further investigation.

With respect to an optimal specimen source for TMA-based detection of *M. genitalium* on a single-specimen basis, Wroblewski et al. (14) discussed the relative sensitivities of vaginal and cervical specimens, which were 84% and 60%, respectively. The relative sensitivity of first-void urine specimens was 58% and was thought to be the result of increased susceptibility of a cell wall-devoid microbe to lysis in urine. Using an infected-patient standard, Mobley et al. (19) reported TMA-based *M. genitalium* sensitivities of 72.6% and 58.9% from vaginal and cervical specimens, respectively. Our data reveal equivalent *M. genitalium* detection rates from cervical specimens (11.5%), vaginal specimens (13.8%), and first-void urine (10.1%; *P* ≥ 0.23). However, our data are limited by the fact that not all three specimen sources were submitted from a given patient during routine clinical practice. Therefore, comparison of performance characteristics by specimen source could not be accomplished in this retrospective assessment. Of further interest, previous studies in our female patient population demonstrated increased *T. vaginalis* detection from first-void urine specimens (25, 35). We also show increased *T. vaginalis* detection from first-void urine compared to cervical specimens (*P* = 0.04; Fig. 1). These data trended higher than the combined cervical specimen/vaginal specimen data (*P* = 0.06). Some concern lies in how the increased utility of the first-void urine specimen for *T. vaginalis* could coexist with the aforementioned *M. genitalium* data in the context of (single) specimen source recommendations for a four-agent STI screen. Additional large-scale communitywide studies may be necessary to determine the true utility of urine for *M. genitalium* TMA, particularly when such specimens would be assayed for the organism in routine fashion without prolonged specimen storage.

In summary, increased detection rates of *M. genitalium* in a communitywide setting over those of other STI agents, including *T. vaginalis*, suggest that *M. genitalium* TMA can provide a benefit to multiple demographics and multiple clinical practice specialties. In addition, the setting of the outpatient OB/GYN is itself an important area for *M. genitalium* screening. *M. genitalium* TMA has recently become commercially available in an analyte-specific reagent (ASR) format. Prior to FDA clearance in 2011, *T. vaginalis* TMA was also commercially available as an ASR. Napierala et al. (35) demonstrated progressively increased utilization of that assay over a 3-year interval. Should an analogous pattern be observed

TABLE 5 Sexually transmitted infection phenotypes determined by TMA-based assays specific for *M. genitalium*, *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis*, delineated by category of health care setting

STI phenotype[a,b]				No. (%) of specimens with indicated phenotype(s)[b]					
M. genitalium	*C. trachomatis*	*N. gonorrhoeae*	*T. vaginalis*	Outpatient OB/GYN	Inpatient OB/GYN	Suburban family care	Urban family care	ER/urgent care	Total[c]
+	−	−	−	85 (41.3)	5 (41.7)	12 (29.3)	26 (33.8)	75 (32.6)	203 (35.9)
+	+	−	−	3 (1.5)	2 (16.7)	3 (7.3)	2 (2.6)	14 (6.1)	24 (4.2)
+	−	−	+	14 (6.8)	0 (0.0)	1 (2.4)	11 (14.3)	14 (6.1)	40 (7.1)
−	+	−	−	43 (20.9)	0 (0.0)	8 (19.5)	9 (11.7)	30 (13.0)	90 (15.9)
−	+	+	−	1 (0.5)	0 (0.0)	1 (2.4)	0 (0.0)	4 (1.7)	6 (1.1)
−	+	−	+	7 (3.4)	0 (0.0)	0 (0.0)	2 (2.6)	14 (6.1)	23 (4.1)
−	−	+	−	4 (1.9)	0 (0.0)	1 (2.4)	0 (0.0)	8 (3.5)	13 (2.3)
−	−	−	+	45 (21.8)	5 (41.7)	13 (31.7)	25 (32.5)	60 (26.1)	148 (26.1)

[a] +, positive nucleic acid amplification test result; −, negative nucleic acid amplification test result; STI, sexually transmitted infection.
[b] Phenotypes that were observed in ≥1.5% of patients from at least two health care settings are included. No significant difference was noted within the excluded comparisons.
[c] Phenotypes that were observed in <1.0% of encounters are not included in this category. *M. genitalium* was a constituent of 49.8% of all phenotypes, *C. trachomatis* of 27.2%, *N. gonorrhoeae* of 6.0%, and *T. vaginalis* of 39.2%.

with *M. genitalium* ASR, potential benefits of a more-comprehensive STI screen may be realized in the realm of laboratory diagnosis and public health.

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