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Prevalence of rectal *Trichomonas vaginalis* and *Mycoplasma genitalium* in male patients at the San Francisco STD clinic, 2005-2006

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Sexually transmitted infections (STI), such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, Herpes Simplex Virus (HSV), and *Treponema pallidum*, are common causes of proctitis among gay men and other men who have sex with men (MSM) [1, 2]. Inflammatory proctitis caused by an STI may increase the susceptibility and infectivity of HIV[3, 4]. In many cases of proctitis, however, no etiological organism is detected[1]. The advent of nucleic acid amplification techniques (NAATs) presents an opportunity to detect organisms previously difficult to isolate from the rectum. Two such organisms, *Trichomonas vaginalis* and *Mycoplasma genitalium*, have been implicated in male urethritis, female cervicitis and endometrial infection[5-11].

M. genitalium is a small bacterium that was difficult to identify until the development of the polymerase chain reaction (PCR) technique in the 1991[12, 13]. Though studies have detected *M. genitalium* from the urethra of MSM[14, 15], only one study investigated *M. genitalium* in the rectum by PCR[16], yet a correlation with rectal symptoms was not reported. Recently, a comparison of multitarget real-time PCR and a transcription-mediated amplification (TMA) research assay found both assays to be highly accurate in the detection of *M. genitalium* from male urine and female vaginal swabs[17].

T. vaginalis is a common curable STI worldwide, causing an estimated 174 million new cases annually[18]. Conventional methods for detection include culture or microscopic visualization on vaginal wet preparation; both require live organisms for accuracy and have modest sensitivity. The development of NAATs has increased case detection[19]: in a recent study that investigated accuracy of the *T. vaginalis* culture, PCR and TMA, both TMA and PCR detected significantly more *T. vaginalis* infections than culture[20].

Identifying the etiological causes of proctitis is important in order to deliver appropriate treatment and decrease the risk for HIV transmission. This study explored the rectal prevalence of *T. vaginalis* and *M. genitalium* in a population of MSM in San Francisco, and examined their role in symptomatic and asymptomatic rectal infection.

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This was a cross-sectional pilot study of 500 consecutive rectal specimens collected at the San Francisco municipal STD clinic from November 11, 2005 to January 4th, 2006. As per current standard of care, all MSM who reported receptive anal sex within six months prior to their clinic visit were screened for *N. gonorrhoeae* and *C. trachomatis* by TMA (Aptima Combo2, Gen-Probe, San Diego, USA), validated by the San Francisco Department of Public Health Laboratory for rectal swabs[21]. MSM with rectal symptoms (i.e., rectal pruritus, pain, tenesmus, bleeding or discharge) were evaluated by anoscopy and tested for *N. gonorrhoeae* and *C. trachomatis* by TMA and HSV by PCR[22]. Rectal discharge was evaluated by Gram stain on-site and the diagnosis of proctitis was made by the presence of one or more polymorphonuclear neutrophils per high-powered field. HIV positive patients are not offered HIV testing; therefore HIV status was determined either by patient report or the result of HIV testing records at the San Francisco municipal STD clinic. During the time period of the study, initial reactive enzyme immunoassays were tested in duplicate (Vironostika HIV-1 Microelisa, bioMerieux Inc., Durham, NC) and confirmed by Fluorognost HIV-1 IFA (Sanochemia Pharmazeutika, Vienna, Austria). All MSM were routinely screened for syphilis by Venereal Disease Research Laboratory test.

All *C. trachomatis* and *N. gonorrhoeae* TMA swab specimens were routinely sent to the San Francisco Department of Public Laboratory for testing. For the purpose of this study, aliquots of the remnant rectal specimens were de-identified and sent to the Johns Hopkins University International Sexually Transmitted Diseases Research Laboratory for batched testing by research TMA assays for *M. genitalium* and *T. vaginalis* (analyte-specific reagent, Gen-Probe, San Diego, USA)[17, 20]. The cutoff for a positive reaction was 40,000 relative light units for the *M. genitalium* assays and 60,000 relative light units for the *T. vaginalis* assays. We used the initial positive tests for our analysis; however, since neither the *M. genitalium* nor *T. vaginalis* assays have been validated previously for the rectal site, positive tests with sufficient specimen were repeat tested once by TMA and again by a research PCR[17, 20]. Non-identifying, patient information from electronic records was reviewed for the analysis. The University of California, San Francisco's and Johns Hopkins' human subjects committees approved this project as exempt for human subject consideration.

Frequencies and logistic regression were performed by STATA (Version 9). Age and selected patient characteristics with a p-value less than 0.10 in the univariate analysis were included in the multivariate model. The strength of statistical association was determined by proximity to an alpha level of 0.05.

We detected 27 (5.4%) positive rectal specimens for *M. genitalium*, 3 (0.6%) for *T. vaginalis*, 50 (10%) for *C. trachomatis*, and 57 (11.4%) for *N. gonorrhoeae*. Forty specimens (8.0%) were from patients with rectal symptoms, and 26 specimens (5.2%) were from patients whom were diagnosed with proctitis.

Of the 27 positive rectal specimens for *M. genitalium*, 23 of 24 (95.8%) available specimens were repeatedly positive by TMA, and 17 of 25 (68.0%) tested positive by PCR. Three specimens from the asymptomatic patients were initially positive for *T. vaginalis*; however, only one of three specimens was repeatedly positive by TMA and all three specimens were negative by research PCR.

Results from the univariate and multivariate analysis of factors associated with *M. genitalium* are displayed in the Table. In the univariate analysis, positive HIV status (OR 3.1, 95% CI 1.4-6.9) and *C. trachomatis* (OR 3.5, 95% CI 1.4-8.7) co-infection were strongly associated with *M. genitalium* infection. Rectal symptoms (OR 2.8, 95% CI 1.0-8.0) and proctitis (2.4, 95% CI 0.7-8.7) were weakly associated with *M. genitalium* infection. In the multivariate analysis, only positive HIV status remained strongly associated

with *M. genitalium* infection. After controlling for confounding, a strong association emerge association between younger age and *M. genitalium* (test for trend, $p=0.051$).

Specimens included 26 from patients who were diagnosed with clinical proctitis. Fifteen (58%) of 26 specimens had an organism identified (Figure). Of note, no specimens from patients with proctitis tested positive for *T. vaginalis*. Three specimens (12%) from patients with proctitis tested positive for *M. genitalium*. Of these three patients, two were co-infected with *C. trachomatis* and one was co-infected with Herpes Simplex Virus, Type 1. Because all three positive specimens for *M. genitalium* in patients with proctitis had other co-infections, *M. genitalium* identification did not increase the number of cases of proctitis with an identified pathogen.

The advent of molecular amplification testing continues to advance our understanding of STIs and their clinical syndromes. We tested 500 rectal specimens collected from MSM and found a *M. genitalium* prevalence of 5%. Rectal *M. genitalium* was strongly associated with HIV status, and weakly associated with rectal symptoms or clinical proctitis. The association between positive HIV status and *M. genitalium* may be explained by the frequency of exposure resulting from increased unprotected anal sex in MSM who are HIV-infected in San Francisco[23]. HIV-infected patients not using condoms for receptive anal sex would be at higher risk of *M. genitalium* exposure and infection. Though this pilot study shows a weak association between *M. genitalium* and rectal symptoms or clinical proctitis, a study with a higher number of events may reveal a stronger association for this trend.

We also evaluated the role of *M. genitalium* and *T. vaginalis* in the etiology of clinical proctitis. In a study of a similar population in San Francisco, Klausner and colleagues found no etiological organism was identified in 45% of clinical proctitis cases[1]. That proportion of unknown etiology was similar to the proportion of unknown etiology in our study. According to our study, neither *M. genitalium* nor *T. vaginalis* explained any more cases of symptomatic proctitis than *C. trachomatis*, *N. gonorrhoeae*, HSV, or syphilis.

Only one of 500 samples repeatedly tested positive for *T. vaginalis* by TMA, and this case tested negative by PCR. Though the TMA may be more sensitive than the PCR, a prevalence of 0.2% in this high risk STI sample is low. It is unlikely that *T. vaginalis* colonizes the rectum. The lack of detection of rectal *T. vaginalis* in our large sample of specimens from a high risk population supports the reported concept of site specificity [24].

Though evaluating the sensitivity and specificity *M. genitalium* and *T. vaginalis* TMA assays in rectal specimens was not an objective of this study, prior validation studies have not been done. The small number of *T. vaginalis* specimens in our study continues to preclude us from this analysis. For samples with sufficient specimen, 95.8% of initial *M. genitalium* positives were confirmed with a second assay. Those samples that were TMA positive and PCR negative may represent an increase in sensitivity of TMA, or TMA false positives.

In conclusion, though it is likely that *M. genitalium* infects the rectum, it is unclear if it contributes to clinical syndromes. However, asymptomatic infections could be an important reservoir for continued spread of STIs, as well as increased HIV transmission. More research should be undertaken to better understand rectal infection by *M. genitalium*, as well as other inflammatory causes of proctitis.

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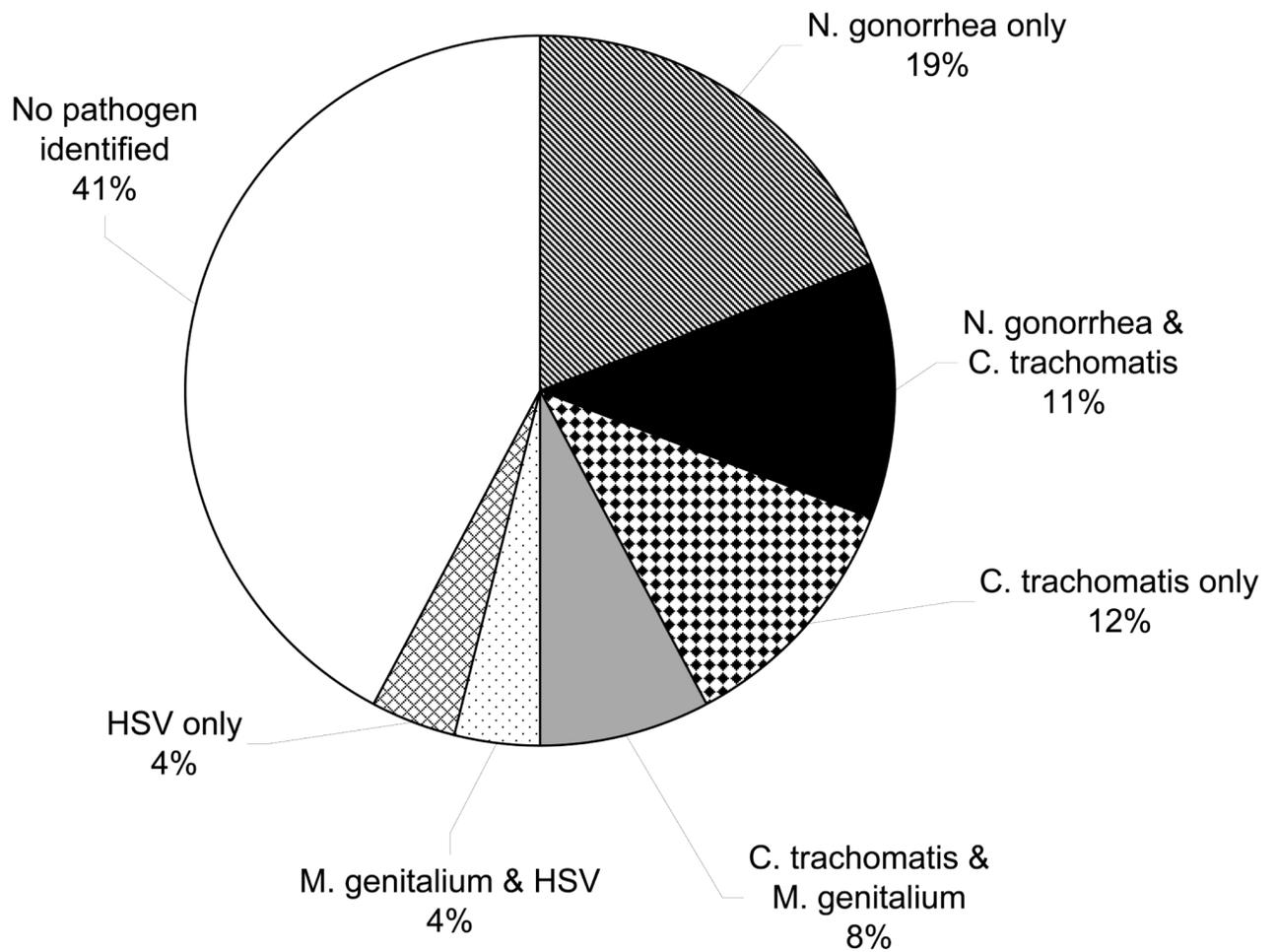


Figure.

Frequency of diagnosis of sexually transmitted infections in men who have sex with men with proctitis (n = 26 San Francisco municipal STD clinic, 2005-2006)

Table

Unadjusted and adjusted odds ratios for rectal specimens positive for *M. genitalium*, San Francisco municipal STD clinic, 2005-2006.

Variable	Prevalent <i>M. genitalium</i> Infection n/N (%)	Unadjusted		Adjusted ^a	
		OR (95% CI)	p-value ^b	OR (95% CI)	p-value ^b
Total N = 500	27/500 (5.4%)				
Age			p= .356		p= .147
< 20 years	3/18 (16.7%)	3.9 (1.0-15.3)		6.8 (1.5-30.5)	
20 to 24 years	4/69 (5.8%)	1.2 (0.4-3.9)		1.7(0.5-5.8)	
25 to 34 years	8/167 (4.8%)	1.0 (0.4-2.5)		1.1 (0.4-2.8)	
> 35 years	12/246 (4.9%)	1		1	
Race			p= .610		p= .390
White	12/269 (4.5%)	1		1	
Black	2/38 (5.3%)	1.2 (0.3-5.5)		0.9 (0.7-4.3)	
Latino	7/125 (5.6%)	1.3 (0.5-3.3)		1.0 (0.4-2.7)	
API/ Native American	6/68 (8.8%)	2.1 (0.7-5.7)		2.6 (0.9-7.7)	
HIV Status			p= .005		p= .010
Uninfected	13/359 (3.6%)	1		1	
Infected	14/133 (10.5%)	3.1 (1.4-6.9)		3.2 (1.3-7.8)	
Rectal symptoms ^d			p= .070		p= .353
No	22/460 (4.8%)	1		1	
Yes	5/40 (12.5%)	2.8 (1.0-8.0)		1.7 (0.6-5.3)	
Proctitis ^e			p= .209		p= .699
No	24/474 (5.1%)	1		1	
Yes	3/26 (11.5%)	2.4 (0.7-8.7)		0.66 (0.1-5.3)	
<i>C. trachomatis</i> , rectal			p= .014		p= .115
Negative	20/450 (4.4%)	1		1	
Positive	7/50 (14.0%)	3.5 (1.4-8.7)		2.3 (0.9-6.1)	
<i>N. gonorrhoea</i> , rectal			p= .580		p= .947
Negative	23/443 (5.2%)	1		1	

Variable	Prevalent	Unadjusted		Adjusted ^a	
	<i>M. genitalium</i>				
	Infection				
	n/N (%)	OR (95% CI)	p-value ^b	OR (95% CI)	p-value ^b
Positive	4/57 (7.0%)	1.4 (0.5-4.1)		1.0 (.32-3.4)	
<i>T. vaginalis</i> , rectal					
Negative	27/497 (5.4%)				
Positive	0/3	No observations		No observations	
Herpes Simplex Virus,					
Type 1 or 2 ^f					
Negative	1/26 (3.9%)	1			
Positive	1/5 (20%)	6.3 (0.3-121.3)			
Early syphilis					
No	26/489 (5.3%)	1	p= .615		p= .846
Yes	1/11 (9.1%)	1.78 (0.2-14.4)		1.3 (0.1-10.9)	

^aAll factors were adjusted for age, HIV status, rectal symptoms, and rectal chlamydia

^bLikelihood ratio test

^cTest for trend

^dRectal symptoms were defined as a history of rectal pain, bleeding, discharge or tenesmus.

^eProctitis was diagnosed as the presence of one or more neutrophils under high powered field and oil-immersion.

^fHSV 1 and 2 specimens for PCR were collected for symptomatic patients only. There were not enough events to enter HSV 1 and 2 into the multivariate model.