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Detection of rectal *Chlamydia trachomatis* in heterosexual men who report cunnilingus

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Abstract

Background.—Rectal infection with *Chlamydia trachomatis* (CT) is frequent in women who deny receptive anal sex and is thought to arise from autoinoculation of the rectum from vaginal secretions. An alternate hypothesis is that oral sex inoculates and establishes gastrointestinal tract infection. Distinguishing these hypotheses is difficult in women. In men, autoinoculation is unlikely and heterosexual men frequently perform oral sex, but rarely participate in receptive anal exposure behaviors.

Methods.—We enrolled high-risk men with and without nongonococcal urethritis (NGU) who presented to a sexually transmitted infection clinic in Indianapolis, Indiana. Urine and rectal swabs were collected and tested for urogenital and rectal CT, *Neisseria gonorrhoeae* (NG), and *Mycoplasma genitalium* (MG). Men completed surveys concerning symptoms, sexual orientation, and detailed recent and lifetime oral and anal sexual behaviors.

Results.—Rectal CT was detected in 2/84 (2.4%) heterosexual men who reported cunnilingus, but no lifetime receptive anal behaviors. All of the men who denied receptive anal behaviors were negative for rectal NG and MG. In homosexual and bisexual men, rectal CT prevalence was high (9.7%) and rectal NG (4.8%) and MG (4.8%) were also detected.

Conclusions.—We detected rectal CT infections in heterosexual men who reported cunnilingus but denied receptive anal behaviors. Oral sex may be a risk factor for rectal CT infection via oral inoculation of the gastrointestinal tract.

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Summary.—We observed a low incidence of rectal *C. trachomatis* infection in heterosexual men.

Keywords

Chlamydia trachomatis; rectal infection; *Mycoplasma genitalium*; *Neisseria gonorrhoeae*; heterosexual; homosexual; bisexual; oral transmission; fecal-oral

INTRODUCTION

Chlamydia trachomatis (CT) is frequently detected in rectal specimens from asymptomatic sexually-active women who deny receptive anal intercourse (RAI) (1–9). Although the natural history of rectal CT infection is unclear, it is thought that the presence of CT in rectal specimens usually reflects shedding of infectious CT organisms from an established rectal focus. Consistent with this interpretation, intra-rectal inoculation of mice and non-human primates with *Chlamydia* establishes long-lasting asymptomatic infection and concomitant shedding of infectious chlamydiae from the rectum (10).

Studies primarily of men who have sex with men (MSM) have determined that receptive anal intercourse (RAI) is an important risk factor for rectal CT, as well as *Neisseria gonorrhoeae* (NG) and *Mycoplasma genitalium* (MG) infection. However, RAI does not explain rectal CT prevalence in women, since rates are similar in high-risk women who do and who do not report RAI (3, 7, 8, 11). Proximity of the urogenital tract and anorectum causes an increased risk for urinary tract infections in women compared to men (12, 13). Thus, autoinoculation of the rectum with infected vaginal secretions could also be a source of rectal CT in women who deny RAI. However, a significant proportion of women with rectal CT do not have concurrent urogenital CT (11), which suggests that autoinoculation alone may not fully explain rectal CT rates in women who deny RAI.

An alternate explanation for the detection of rectal CT in individuals who deny RAI has been termed the “oral hypothesis” (10, 14, 15). This hypothesis proposes that oral sex (cunnilingus, fellatio, or anilingus) efficiently inoculates the gastrointestinal tract with CT and that rectal CT detection may indicate shed CT organisms from one or more established gastrointestinal foci. The oral hypothesis is consistent with the known biology of other closely related *Chlamydia* species for which the fecal-oral route is a primary mode of transmission and the gastrointestinal tract is the site of productive infection (10). In humans, it is unknown if oral inoculation can establish CT gastrointestinal infection or rectal CT shedding. Although most women at risk for CT infection report fellatio (16), the potential contributions of oral exposure and autoinoculation cannot be easily differentiated in women.

Heterosexual men might be a better model for evaluating the oral hypothesis because men commonly engage in cunnilingus, but not RAI or other sexual practices that could directly inoculate the rectum. Rectal autoinoculation by urethral CT also seems less likely in men than in women due to the increased distance between the rectum and urethra. Only a few studies of rectal CT have included heterosexual men. One study of 169 heterosexual men failed to detect any case of oropharyngeal or rectal CT, but only 11 of these men had urogenital CT (4). A retrospective study detected CT in rectal swabs from 3 heterosexual men, but these men reported rectal exposures so direct inoculation could not be excluded.

(17). A prospective study detected rectal CT in 1.3% (N=4) of heterosexual male swingers compared to 8.9% of MSM (1). However, specific sexual behaviors were not defined in this study. Another study of male swingers reported that oral and vaginal sex was very common (both >95%) and 61% engaged in anal sex (18). Thus, prior studies of rectal CT in men have not collected sufficient behavioral data to identify all of the potential routes of CT inoculation, especially anal behaviors that could result in direct CT inoculation (anal intercourse, anilingus, and use of sex toys/fingers). Also, reported sexual orientation alone is insufficient to exclude anal behaviors as prior studies have shown that reported sexual orientation may not accurately reflect sexual behaviors (19, 20).

To address limitations of prior studies of rectal CT in men and evaluate the oral hypothesis, we enrolled men with and without NGU presenting to a sexually transmitted diseases (STD) clinic in Indianapolis, Indiana, and obtained urine and rectal swabs for CT testing. The men completed detailed surveys reporting their sexual orientation and recent and lifetime sexual behavior practices. As an additional control for reported anal exposures, we also tested rectal swabs for NG and MG.

MATERIALS AND METHODS

Study population and procedures

As part of an ongoing 5-year study of NGU, the Idiopathic Urethritis Men's Project (IUMP), we recruited males 18 years of age with and without urethritis who presented to the Marion County Public Health Department (MCPHD) Bell Flower STD Clinic in Indianapolis, Indiana. Cases were men with NGU, defined as clinician-observed signs and or patient-reported symptoms of urethritis and 5 polymorphonuclear cells (PMN) per high-power field by urethral Gram stain smear. Controls were asymptomatic men with no signs or symptoms of urethritis. Men whose urethral smears showed Gram negative diplococci were presumed to have NG infection and were excluded from the study. Interested participants provided written consent and were then enrolled. Men were interviewed and a physical exam performed. Each participant also completed an extensive self-administered computer-assisted survey, which included detailed questions regarding demographics, prior STD history, sexual orientation, and specific recent (i.e., within the last year) and lifetime (i.e., ever engaged in) sexual behaviors. A first-catch urine and rectal swab were obtained for CT, NG, and MG testing. Men diagnosed with NGU were treated with azithromycin (1 gm orally directly observed), advised to refer all sexual partners for treatment, and abstain from sexual activity for 1 week. The study was approved by the Indiana University/Purdue University-Indianapolis Institutional Review Board and by MCPHD.

Pathogen testing and Calculation of CT loads

Pathogen-specific nucleic acid amplification and CT load testing was performed by the Indiana University School of Medicine Infectious Diseases Laboratory. CT and NG testing was performed as previously reported (21). MG testing was performed using an in-house quantitative PCR assay, as previously described (22). Rectal swab CT load quantitation was derived by comparison to a serial dilution of a known concentration of CT elementary bodies.

Statistical analysis

All analyses were performed using SAS® version 9.4 statistical software. For demographic and medical characteristics, descriptive statistics (medians, ranges, frequencies, and percentages) were computed to assess variables' distributional properties and to describe the sample. Comparisons of the baseline demographics and STI test results among groups defined by sexual behaviors, self-reported sexual identity, and acceptance of the rectal swab were performed using the Kruskal-Wallis tests for continuous measures, while Chi-squared tests, or Fisher's exact tests if the expected value of cells was below 5, were used for categorical variables.

RESULTS

Cohort description

One hundred ninety-seven men who enrolled in the IUMP study provided a rectal swab and are included in this analysis (Figure 1). The majority of the men who declined to provide a rectal swab self-identified as heterosexual (N=108/243, 44.4%). The majority of MSM (N=62, 96.9%) agreed to provide a rectal swab, with only 3.1% declining. The demographics and clinical characteristics of the self-identified heterosexual men who provided a rectal swab and declined to provide a rectal swab were similar. The reported anal exposures differed in the two groups. The men who provided rectal swabs reported receiving anilingus and receptive anal play more frequently (Table S1). Characteristics of the study participants are described in Table 1. The median age was 28 (range 18–68), 46.7% were African American, and 48.2% complained of symptoms of urethritis. One hundred thirty-five (68.5%) identified as heterosexual, 17.8% identified as homosexual, and 13.7% identified as pansexual, bisexual, or asexual. Consistent with the IUMP study design, all the men diagnosed with NGU reported urethral symptoms (Table 1). Abdominal and rectal symptoms were reported in 1% (N = 2) and 1.5% (N = 3) of men, respectively.

Reported sexual behaviors in heterosexual men

Cunnilingus was the second most frequent lifetime behavior (after vaginal intercourse) in the self-identified heterosexual men (N=135) with 82% reporting this behavior in the last year and 93.2% reporting lifetime behavior (Table 2). A significant proportion of these men also reported performing anilingus on a female partner in the last year (23.1%) or lifetime (38.1%). In the self-identified heterosexual men, anilingus on a female partner was only reported by men who also reported cunnilingus with female partners. Two (1.5%) of the self-identified heterosexual men reported lifetime RAI. A larger proportion reported non-RAI lifetime receptive anal behaviors with female partners including anal play (20.9%) or receiving anilingus (23.1%) that could potentially inoculate the rectum. Overall, 84/135 (62.2%) of the self-identified heterosexual men reported both lifetime cunnilingus and no receptive anal behaviors.

Prevalence of Urethral CT and Rectal CT, NG, and MG

Overall, 37 (18.8%) men had urethral CT, and 21 (10.7%) men had urethral MG (Table 3). No men had urethral NG, consistent with the IUMP study design.

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Rectal CT was detected in 8/197 men (4.1%) and none of these men reported anorectal symptoms. Six of the anorectal CT cases were in men who were behaviorally bisexual or homosexual. However, among the group of 84 self-identified heterosexual men who reported cunnilingus but no receptive anal exposures in their lifetimes, 2 (2.4%) had rectal CT. One of these two men reported cunnilingus within the prior 60 days, while the other reported cunnilingus and anilingus with a woman more than a year previously.

Rectal MG was detected in 3/197 (1.5%) men. All of these men were behaviorally bisexual or homosexual, with all reporting lifetime anal exposures (included RAI), and the majority reporting these behaviors within the last year. Although men with urethral NG were excluded, consistent with the IUMP design, 3/197 (1.5%) men had rectal NG. Similar to the men with rectal MG, all these men reported receptive anal behaviors within the last year. When limited to MSM, prevalence of rectal NG was 4.8% (3/62) and MG was 4.8% (3/62).

In two men, CT was detected in both the urethra and rectum. One of these men self-identified as bisexual and reported receptive anal behaviors. The other was one of the two heterosexual men who denied any receptive anal behaviors.

Calculation of CT loads

To minimize the possibility that rectal CT results were due to false-positives, we repeated CT testing of the CT-positive rectal swabs and quantified the CT loads (Figure 2). All of the positive rectal CT tests were confirmed CT-positive by re-testing. In the heterosexual men, one of the CT loads was higher and the other lower than the median of the 6 CT positive swabs from the self-identified homosexual and bisexual men. The CIs were similar between the two groups.

DISCUSSION

In this study, we report detecting rectal CT in 2/84 (2.4%) behaviorally heterosexual men who denied ever engaging in receptive anal behaviors which could directly inoculate the rectum with CT, but who engaged in cunnilingus. This suggests that oral CT inoculation may have occurred in these men, given that the only mucosal sites of exposure were the urethra and oropharynx. In our study, all of the rectal CT cases were asymptomatic, which is consistent with prior studies, which reported that asymptomatic rectal CT is common (23).

To our knowledge, our study is the first that supports the hypothesis that fecal-oral transmission of CT occurs in humans. Two other lines of evidence suggest that CT can transit the stomach and infect the gastrointestinal tract and/or rectum in humans. An *in vitro* study demonstrated that CT can survive incubation at low pH, comparable to gastric environments (24). More importantly, CT DNA has been detected in the appendix and colon (25) and CT inclusions were also visualized in the appendix and rectal tissues, confirming that CT can infect these tissues. It is unclear if transit of CT to the appendix and rectal tissues occurred through the fecal-oral route or ascended proximally from rectal inoculation.

Prior studies of rectal CT in heterosexual men had limitations, but support the findings of our study. A review of 5 studies of the prevalence of rectal CT infections in heterosexual

men found the median rate was 0.9% (range 0.0–9.1%), which is comparable with our results (23). In individuals who deny receptive anal sex, the rectal CT prevalence appears to be strikingly lower in men (approximately 1.5% in our study) than in women (13.4%) (2). This could reflect a more important role for autoinoculation in acquisition of rectal CT in women compared to men. Indeed, the rate of concordant urogenital and rectal CT infection has been reported to be much higher in women (73%) (2), compared to the 3.7% concordance rate in the self-identified heterosexual men in our study (1/27). Another possibility is that the different prevalence of rectal CT in men and women reflects the different risks of oral inoculation posed by fellatio and cunnilingus. Fellatio exposes the oral mucosa directly to epithelial cells of the urethral meatus and to ejaculate. In contrast, cunnilingus primarily exposes the oropharynx to the external genitalia and superficial vaginal surfaces which are not sites of CT infection. Therefore, we posit that the number of infectious CT elementary bodies ingested by individuals performing cunnilingus may be relatively low, compared to women who perform fellatio.

We cannot exclude that some heterosexual men in our study inaccurately reported their sexual behaviors, but we attempted to minimize this using multiple approaches. First, sexual behaviors were not an exclusion criterion (i.e., no secondary gain was afforded by denying anal sex) and data were collected by computer-assisted self-reporting to minimize bias from sexual behaviors stigma. Nonetheless, due to desirability bias, some of heterosexual men may not have been comfortable reporting receptive anal behaviors even in the setting of an anonymous interview. Second, we compared the demographic and clinical characteristics of the self-identified heterosexual men who provided and declined to provide rectal swabs and found no significant differences between the groups. However, selection bias is possible given that anal exposures were more commonly reported from men who provided rectal swabs. Third, we also tested all men for rectal NG and MG, which have not been documented to establish rectal infection via the fecal-oral route. NG and MG were each detected in 3 homosexual and bisexual men (4.8%) who reported RAI. Importantly, no men who denied lifetime anal sex, including the two men who had rectal CT, had rectal NG or MG detected, which supports their reported behaviors.

In our study, we also quantified the rectal swab CT load to test if our findings could be explained by low level nucleic acid contamination or false-positive tests. Interestingly, compared to rectal CT loads from men who engaged in RAI, and likely had direct rectal inoculation of CT, the median CT load was slightly higher in the two men without RAI. Further, the false positive rate of CT nucleic amplification tests is less than <1% (26) and all of the rectal CT-positive tests were confirmed to be true-positives by re-extracting the original specimens and repeat CT testing.

Our study has strengths and limitations. A major strength is our use of a detailed sexual behavior survey to assess anal exposure behaviors (including anilingus, toys, fingers, etc.) in heterosexual men. Also, we asked about lifetime, in addition to recent, sexual behaviors to minimize confounding by prolonged (>1 year) rectal CT infections or inaccurate reporting of recent anal exposures. Another strength is that our study was controlled by comparison to homosexual men and other pathogens that are introduced into the rectum by RAI (NG and MG). Limitations of our study are that this is a single-site study of men presenting to an

STD clinic for evaluation and our findings may not be generalizable to other populations. Also, we used a nucleic acid amplification test to detect rectal CT infections, which cannot differentiate established infections from contamination with non-viable organisms (e.g., residual undigested CT DNA passing through the gastrointestinal tract). Our study design excluded men with NGU who had Gram stain PMN counts <5 (eg, 2–4 PMNs per high-powered field), which may be a limitation.

In summary, our results support the hypothesis that rectal CT infections can be acquired by oral sex, but suggest that this is relatively uncommon in heterosexual men. Further research is needed to assess the oral route for gastrointestinal infection in both women and men and to define if the infectious inoculum differs between different oral behaviors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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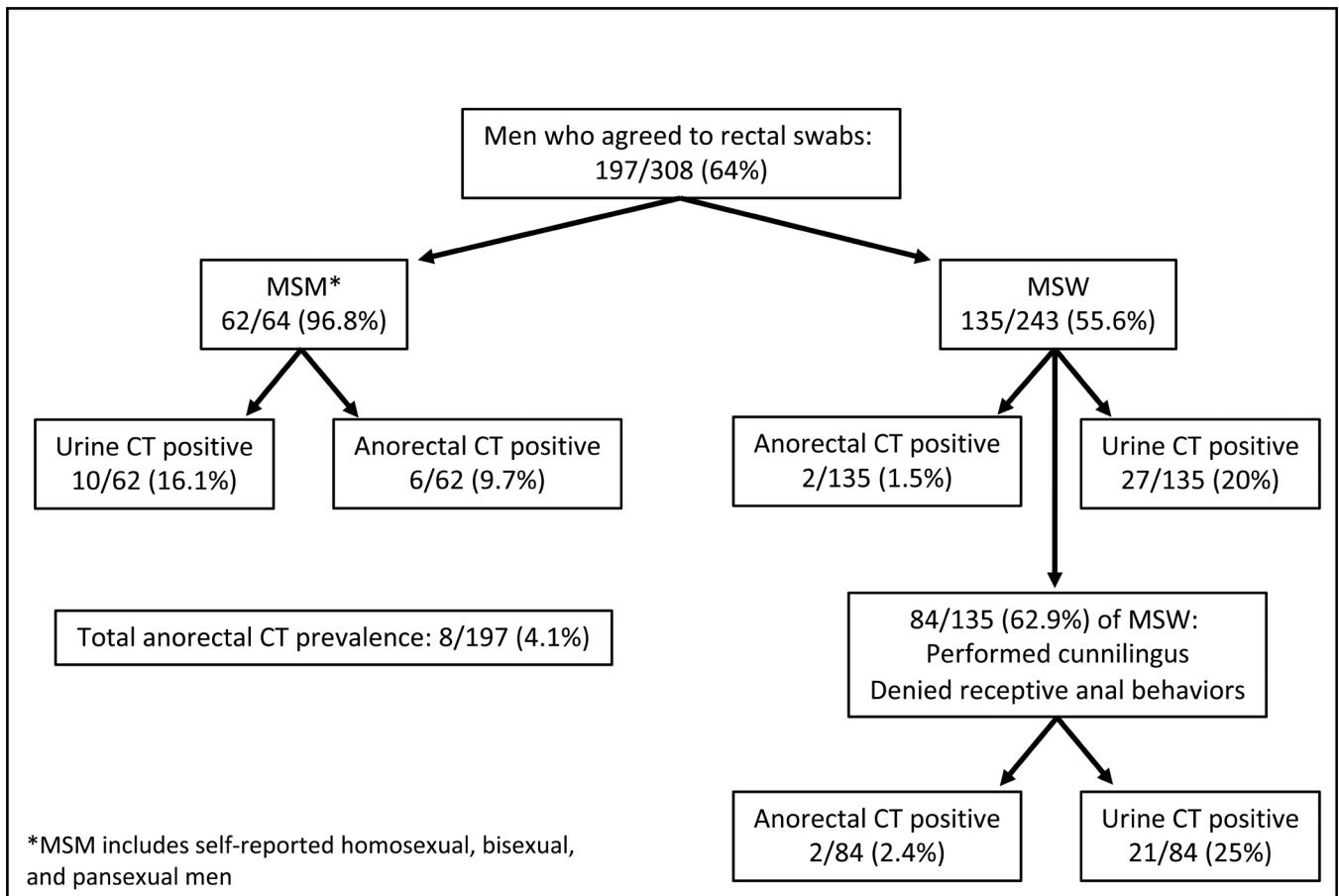


Figure 1.
Anorectal Testing by Self-Reported Sexual Orientation.

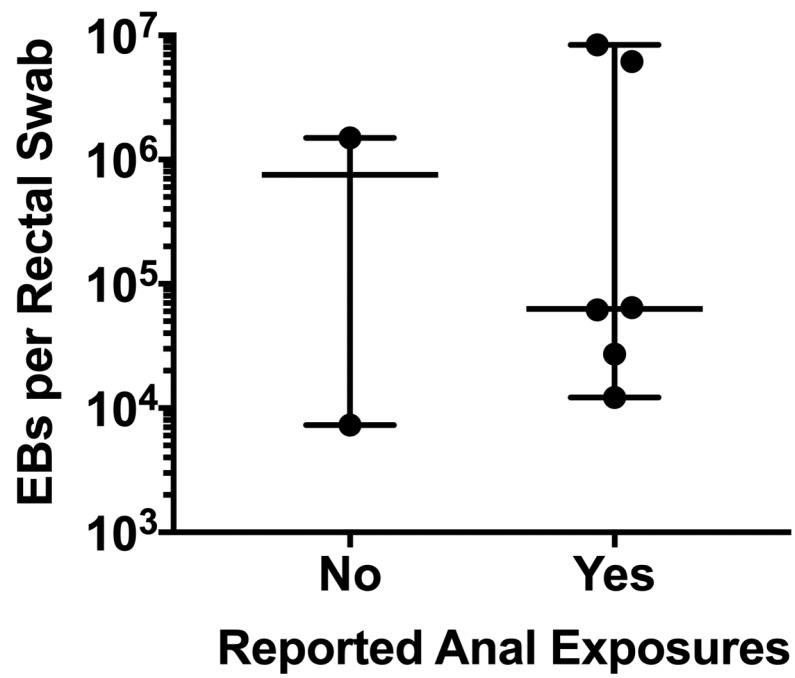


Figure 2.

CT load in rectal swabs from men with and without reported anal exposures. The horizontal line denotes the median and the outer lines denote the 95% CI. The Y-axis is \log_{10} .

Table 1.

Baseline demographic and clinical characteristics of the study population

Characteristic	NGU (N=95)	Asymptomatic (N=102)	Total (N=197)	p-value
Age (years) median (range); IQR	28 (18–60); 24–37	27 (18–68); 23–37	28 (18–68); 23–37	0.59
Race				<0.0001
African American	59 (62.1%)	33 (32.4%)	92 (46.7%)	
Caucasian	22 (23.2%)	55 (53.9%)	77 (39.1%)	
Other *(including Hispanic)	14 (14.7%)	14 (13.7%)	28 (14.2%)	
Age at first sex (years) median (range); IQR	15 (5–24); 14–17	16 (4–26); 14–18	16 (4–26); 14–17	0.0189
Number lifetime partners median (range); IQR	13.5 (1–300); 6.75–30	12 (0–400); 6–30	13 (0–400); 6.5–30	0.9438
Number partners in last year median (range); IQR	4 (1–100); 2.25–6	3 (0–20); 2–5	3 (0–100); 2–5	0.0105
Number partners in last 2 months median (range); IQR	2 (0–20); 1–3	2 (0–9); 1–2	2 (0–20); 1–3	0.0160
Sexual orientation, self-reported				0.0624
Asexual	0 (0%)	0 (0%)		
Bisexual	9 (9.5%)	15 (14.7%)	24 (12.2%)	
Homosexual (MSM)	11 (11.6%)	24 (23.5%)	35 (17.8%)	
Heterosexual (MSW)	73 (76.8%)	62 (60.8%)	135 (68.5%)	
Other *	2 (2.1%)	1 (1%)	3 (1.5%)	
Prior STI history, self-reported				
<i>Chlamydia trachomatis</i>	45/91 (49.5%)	31/97 (32%)	76/188 (40.4%)	0.0175
NGU	34/88 (38.6%)	12/97 (12.4%)	46/185 (24.9%)	<0.0001
Reason for presenting to clinic				<0.0001
Genital symptoms	77 (81.1%)	3 (2.9)	80 (40.6%)	
Worried about STI	13 (13.7%)	22 (21.6%)	35 (17.8%)	
Contact to STI	2 (2.1%)	6 (5.9%)	8 (4.1%)	
Had STI (testing or treatment)	---	---	---	
Screening for STI	1 (1.1%)	53 (52%)	54 (27.4%)	
General check-up and physical exam	2 (2.1%)	14 (13.7%)	16 (8.1%)	
Other	---	4 (3.9%)	4 (2%)	
Symptoms Reported				
Urethral	95/95 (100%)	---	95 (48.2%)	
Discharge	85 (89.5%)		85 (43.1%)	
Dysuria	29 (30.5%)		29 (14.7%)	
Burning tingling	39 (41.1%)		39 (19.8%)	
More than one	56 (58.9%)		56 (28.4%)	
Abdominal	---	2 (2%)	2 (1%)	
Pain		0 (0%)	0 (0%)	
Diarrhea		2 (2%)	2 (1%)	
Rectal (itching/irritation)	3 (3.2%)	---	3 (1.5%)	

* Men reporting 'other' for their self-reported sexual orientation stated that they were pansexual and reported sexual activity with both male and female partners.

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Site-specific sexual behaviors by self-reported sexual orientation

Table 2.

Sexual Behaviors	Self-Reported Sexual Orientation						Total (N=197)	
	Bisexual/Other* (N=27)		Homosexual (N=35)		Heterosexual (N=135)			
	In last year	Lifetime	In last year	Lifetime	In last year	Lifetime		
Oral exposures								
Performed cunnilingus	12/26 (46.2%)	22/26 (84.6%)	0/35 (0%)	11/35 (31.4%)	109/133 (82%)	124/133 (92.3%)	121/204 (59.3%)	
Performed fellatio	21/27 (77.8%)	24/27 (88.9%)	32/35 (91.4%)	34/35 (97.1%)	4/130 (1.5%)	4/130 (30.8%)	55/192 (28.6%)	
Performed anilingus	13/26 (50%)	18/26 (69.2%)	124/35 (68.6%)	26/35 (74.5%)	32/134 (23.9%)	51/134 (38.1%)	69/192 (35.9%)	
Female partner	5/26 (1%)	8/26 (30.8%)	0/35 (0%)	0/35 (0%)	31/134 (23.1%)	51/133 (38.1%)	36/195 (18.5%)	
Male partner	9/25 (36%)	13/25 (52%)	24/35 (68.6%)	26/35 (74.3%)	2/129 (1.6%)	2/129 (1.6%)	35/189 (18.5%)	
Urethral exposures								
Vaginal intercourse	14/26 (53.8%)	25/26 (96.2%)	0/35 (0%)	11/35 (31.4%)	125/133 (94%)	130/133 (97.7%)	139/194 (71.6%)	
Insertive anal intercourse	18/26 (69.2%)	24/26 (92.3%)	29/35 (82.9%)	32/35 (91.4%)	48/134 (35.8%)	81/134 (60.4%)	95/191 (49.7%)	
Female partner	7/26 (26.9%)	12/26 (46.2%)	0/34 (0%)	0/34 (0%)	47/134 (35.1%)	80/134 (59.7%)	54/194 (27.8%)	
Male partner	13/25 (52%)	17/25 (68%)	29/35 (82.9%)	32/35 (91.4%)	2/130 (1.5%)	3/130 (2.3%)	44/190 (23.2%)	
Received fellatio	27/27 (100%)	27/27 (100%)	33/35 (94.3%)	35/35 (100%)	122/134 (91%)	129/134 (96.3%)	182/196 (92.9%)	
Female partner	14/25 (56%)	24/25 (96%)	0/35 (0%)	11/35 (31.4%)	122/134 (91%)	129/134 (96.3%)	136/194 (70.1%)	
Male partner	22/27 (81.5%)	26/27 (96.3%)	33/35 (94.3%)	35/35 (100%)	3/130 (2.3%)	6/130 (4.6%)	58/192 (30.2%)	
Anal exposures								
Received anilingus	13/26 (50%)	17/26 (65.4%)	28/35 (80%)	31/35 (88.6%)	15/134 (11.2%)	31/134 (23.1%)	56/188 (29.8%)	
Female partner	3/25 (12%)	5/25 (20%)	0/35 (0%)	1/35 (2.9%)	15/133 (11.3%)	31/133 (23.3%)	18/193 (9.3%)	
Male partner	12/25 (48%)	15/25 (60%)	28/35 (80%)	31/35 (88.6%)	2/128 (1.6%)	2/128 (1.6%)	42/188 (22.3%)	
Receptive anal intercourse	12/26 (46.2%)	17/26 (65.4%)	25/35 (71.4%)	34/35 (97.1%)	2/130 (1.5%)	2/130 (1.5%)	39/191 (20.4%)	
Receptive anal play (sex toy or fingers)	12/26 (46.2%)	17/26 (65.4%)	23/35 (65.7%)	31/35 (88.6%)	15/134 (11.2%)	28/134 (20.9%)	51/195 (26.2%)	
Female partner	6/26 (24%)	12/26 (46.2%)	28/35 (5.7%)	4/35 (11.4%)	15/132 (11.4%)	28/133 (21.1%)	23/193 (11.9%)	
Male partner	11/25 (44%)	13/25 (52%)	23/35 (65.7%)	31/35 (88.6%)	2/130 (1.5%)	3/130 (2.3%)	36/187 (19.3%)	

Table 3.

Prevalence of Urogenital and Rectal CT infections stratified by anal sex behaviors

Testing	Reported anal exposures*		No reported history of anal exposures		Total (N=197)
	In last year (N=68)	Lifetime (N=96)	In last year (N=129)	Lifetime (N=101)	
Urine					
CT +	8 (11.8%)	10 (10.4%)	29 (22.5%)	27 (26.7%)	37 (18.8%)
MG +	2 (2.9%)	9 (9.5%)	19 (15.2%)	12 (12.2%)	21 (10.8%)
Rectal swab					
CT +	6 (8.8%)	6 (6.3%)	2 (1.6%)	2 (2%)	8 (4.1%)
MG +	2 (2.9%)	3 (3.1%)	1 (0.8%)	0 (0%)	3 (1.5%)
NG +	3 (4.4%)	3 (3.1%)	0 (0%)	0 (0%)	3 (1.5%)

* Anal exposures include receptive anilingus, receptive anal play (fingers, sex toys), or receptive anal intercourse