Comparison of *Chlamydia trachomatis* Seroprevalence and Risk Factors for Infection Among Women who have Sex with Women and Women who have Sex with Men in the 2013-2016 cycles of the National Health and Nutrition Examination Survey, United States

Keely Paris

A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Public Health

University of Washington

2023

Committee:

Lisa E. Manhart

Christine M. Khosropour

Jennifer E. Balkus

Program Authorized to Offer Degree:

Epidemiology

University of Washington

Abstract

Comparison of *Chlamydia trachomatis* Seroprevalence and Risk Factors for Infection Among Women who have Sex with Women and Women who have Sex with Men in the 2013-2016 cycles of the National Health and Nutrition Examination Survey, United States

Keely Paris

Chair of the Supervisory Committee:
Lisa E. Manhart
Department of Epidemiology

Objectives: Little is known about the lifetime prevalence and risk factors for sexually transmitted infections (STIs) among cisgender women who have sex with women (WSW), despite this subpopulation being at risk for STIs. Using data from a large, nationally-representative survey, we estimated the seroprevalence of *Chlamydia trachomatis* (CT) among WSW and compared it to seroprevalence in women who have sex with men (WSM). We also identified factors that may be associated with an increased or decreased risk of ever having had CT infection.

Methods: Publicly available CT serological data from sexually experienced women aged 18-39 participating in the 2013-2014 and 2015-2016 cycles of the National Health and Nutrition Examination Survey (NHANES) were used to estimate weighted seroprevalence in WSW and WSM. WSW were defined as women who reported ever having had sex with a woman in their lifetime, while WSM reported having had sex with a man but never having had sex with a

woman in their lifetime. Seropositivity was determined by the detection of Pgp3 antibodies to CT using an ELISA assay. We used Poisson regression with robust standard errors to identify factors associated with CT seropositivity in each group, and to estimate the association between WSW and CT seroprevalence, adjusted for factors associated with CT seropositivity in both groups.

Results: WSW were younger, had a younger age at sexual debut, and more lifetime male sex partners than WSM. The weighted CT seroprevalence in WSM (n=1207) was 28.6% (95% CI: 24.4, 32.9) and 38.4% (95% CI: 30.0, 46.9) in WSW (n=218). After adjusting for age, race/ethnicity, income-poverty ratio, age at sexual debut, number of lifetime male sex partners, having chlamydia in the past year, and sexual identity, there was no significant difference in CT seroprevalence between WSM and WSW (aPR=1.22 [95% CI: 0.98, 1.53], p=0.07). A non-Hispanic Black or an "other" race/ethnicity, a higher lifetime number of male sex partners, and a history of CT in the last year was associated with higher CT seroprevalence in both WSW and WSM. Among WSM, individuals reporting a Hispanic race/ethnicity, an income below the income/poverty ratio, and a younger age at sexual debut also had higher CT seroprevalence. Sexual identity was not associated with seroprevalence in WSM, but among WSW, individuals who identified as bisexual had lower CT seroprevalence than WSW who identified as straight.

Conclusion: WSW had comparable CT seroprevalence with WSM after adjusting for sociodemographic characteristics, sexual behavior characteristics, and recent CT infection, indicating that the risk for CT infection may be comparable in these two groups. Given their younger age at sexual debut and likelihood of having male as well as female partners, providers should follow the same CT screening guidelines for WSW as they do for WSM.

INTRODUCTION

In 2019, over 1.8 million cases of *Chlamydia trachomatis* (CT) were reported in the United States, making it the most commonly reported sexually transmitted infection (STI) in the country.[1] Sixty-one percent of these infections occurred in 15-24 year olds.[2] Much of the prior research on STIs like CT has been conducted in populations at high risk for infection, including adolescents/young adults and cisgender men who have sex with men (MSM); this leaves other populations, specifically other sexual and gender minorities, understudied.[3,4] In particular, few studies have assessed STIs in cisgender women who have sex with women (WSW), due in part to beliefs that they have a lower likelihood of CT or *Neisseria gonorrhoeae* (NG) infections.[3]

In individuals assigned female at birth, untreated CT infections can lead to serious complications, including pelvic inflammatory disease (PID), which in turn may lead to ectopic pregnancy, tubal factor infertility, and chronic pelvic pain.[2] Despite the potential for severe sequelae, CT infection is often asymptomatic; therefore, the U.S. Preventive Services Task Force (USPSTF) currently recommends annual screening for all sexually active women younger than 25.[2,5,6] In addition to screening this age group, the USPSTF recommends screening women 25 years of age or older with an increased risk of infection, such as those with new or multiple sex partners, or a sex partner with an STI.[6] Cisgender WSW are often missed by CT screening efforts because of perceptions that they are at lower likelihood of exposure to infection compared to cisgender women who have sex with men (WSM).[3,7] However, a study conducted in family planning clinics from 1997 to 2005 found a comparable prevalence of CT infection between women who had sex with men exclusively in the past year (5.3%), women who had sex with men and women in the past year (7.1%), and women who had sex with women exclusively in the

past year (7.1%).[8] Additionally, two cross-sectional studies found a self-reported lifetime prevalence of any STIs in WSW of approximately 20%.[4,9]

Research has also demonstrated that WSW may engage in similar behaviors to WSM that increase the likelihood of exposure to and/or acquisition of STIs. This includes engaging in transactional sex, substance use, sex with males, and low adherence to safer sex practices such as condom/barrier method use and not changing the condom on shared sex toys between use.[4,7,10] Prior research has shown that between 87% to 97% of WSW have ever had a male sexual partner.[3,11] Cisgender WSW who also have male partners could be at higher risk for certain STIs (including chlamydia, gonorrhea, syphilis, and HIV) than WSW who do not have male partners given the increased contact between infected fluids and mucosal membranes in the vagina that enhances the efficiency of transmission during penile-vaginal sex. STI transmission has also been documented between female sex partners regardless of history with male sex partners.[3]

Still, comparatively little research has been done on the lifetime risk of CT infection among WSW, particularly using serology in a representative sample of the population. Serologic assays detect the presence of anti-CT antibody, indicating a prior CT infection, and can provide an estimate of the prevalence of lifetime CT infection in the population.[12] Antibody responses to plasmid gene product 3 (Pgp3) are a reliable measure of a past or prevalent CT infection, as Pgp3 antigens are highly conserved across CT isolates, with the corresponding immunoglobulin G (IgG) antibodies able to persist for over 10 years in cisgender women.[12,13]

The National Health and Nutrition Examination Survey (NHANES) provides a unique opportunity to examine CT seroprevalence in WSW and compare it to WSM. NHANES performed serology on serum specimens from women aged 14-39 years old for the presence of Pgp3 antibodies to *C. trachomatis* in the 2013-2014 and 2015-2016 cycles. Although two prior

studies have used chlamydial serologic assay results from NHANES to examine population-level seroprevalence in women,[14,15] neither study reported any detail about the WSW population and their unique risk and protective factors for lifetime exposure to infection. By using population-based data from a nationally-representative sample, we can gain a more in-depth understanding of factors associated with seroprevalent CT in WSW and how these compare to WSM, allowing us to identify potential health disparities and potential opportunities for action.

METHODS

Study Design

NHANES is a cross-sectional survey in which participants are selected using a complex, multistage probability sampling method to create a representative sample of the non-institutionalized, civilian population in the US. Data collection by NHANES involves 3 parts: an in-home, face-to-face health interview by a trained interviewer using a computer assisted personal interviewing system (CAPI); a standardized physical examination in a mobile examination center (MEC); and a private self-administered interview using an audio computer-assisted self-interviewing (ACASI) system in the MEC. The in-home interview includes questions about participant socioeconomic status indicators, demographics, diet, and certain health-related questions. Sensitive health questions about sexual behavior are asked in the MEC in private interview rooms using an ACASI system. Physicians, dentists, and health technicians in the MEC examine participants during the physical examination section, and biological specimens (including urine, blood, and vaginal/penile swabs) are collected. Specimens are stored in −80°C freezers until assaying. We conducted a secondary analysis using data from the 2013-2014 and 2015-2016 cycles of NHANES.

Measures

NHANES 2013-2016 did not ask about birth sex or gender identity; NHANES only asked "Is [name of household member] male or female?" in the screener module. Therefore, we use the terms *male* or *men* and *female* or *women* to represent the response option available to the respondent.

Ever having sex with a woman was determined using the question "*Have you ever had any kind of sex with a woman? By sex, we mean contact with another woman's vagina or genitals*". If the participant answered "*Yes*" they were considered to be WSW. WSW could have had both female and male partners, as long as they reported a history of having sex with a woman during their lifetime. In contrast, WSM were those that answered "*No*" to ever having had sex with a woman and "*Yes*" to ever having had sex with a man. History of sex with men was determined using the derived variable "ever had sex with a man", which derived a binary outcome using answers from 3 questions: "*Have you ever had vaginal sex with a man?*", "*Have you ever performed oral sex on a man?*", and "*Have you ever had anal sex with a man?*". Women who reported never having had any kind of sex with a man or a woman were excluded from all analyses.

Prior infection with CT was determined by the detection of Pgp3 antibodies to *C. trachomatis* using an ELISA assay. Presence of Pgp3 antibodies demonstrates either a prior infection with CT, or a prevalent infection for which sufficient time has passed for an antibody response to be present. A positive result was determined by a normalized absorbance value greater than 0.303 at 450 nm, the cutoff for which was established by performing the assay on

Pgp3 coated plates and using a receiver operating characteristic (ROC) panel to identify the cutpoint.[16]

We assessed factors that have been previously investigated or associated with an increased or decreased risk of CT or other STIs among cisgender women, including sociodemographic characteristics and sexual behaviors.[3–5,8,9,14,15,17–19] We evaluated age (18-24, 25-29, 30-34, 35-39), race/ethnicity (NHANES response options were: non-Hispanic [NH] White, NH Black, NH Asian, Hispanic [including Mexican American and participants of other Hispanic ethnicities], and "other" race/ethnicity [including multiracial and all other racial/ethnic categories]), educational attainment (< high school; high school graduate or GED recipient; completed some college or received an associate's degree; ≥college graduate), and marital status (married or living with partner; divorced, separated, or widowed; and never married). Marital status was not asked of 18- or 19-year-old individuals. We also evaluated income-poverty ratio (below the low-income threshold [<130% of the federal poverty level for participants in the 2013-2014 cycle/<185% of the federal poverty level in the 2015-2016 cycle] and above the low-income threshold), and insurance status (insured or uninsured). Sexual behavior characteristics included: age at sexual debut (≤ 13, 14-15, 16-17, or ≥18); lifetime number of sex partners, male (0-1, 2-4, ≥5) and female (0, 1, 2-4, ≥ 5); frequency of condomless sex in the past year (never, less than half the time, about half the time, more than half the time, and always); self-reported history of a non-CT STI diagnosis (ever being diagnosed with HPV, genital warts, and/or genital herpes, and/or being diagnosed with gonorrhea in the past year [yes or no for each]); history of CT within the past year (being told by a doctor that they had CT in the past year and/or testing positive on a urine nucleic-acid amplification test [NAAT] for CT [yes or no] at the time of the NHANES interview); and self-described sexual identity (straight, lesbian or gay, bisexual, and

other). Although the language in the response options for self-described sexual identity changed slightly between cycles, these changes did not substantively alter participants' understanding of the answer choices and we used the categorization from the 2015-2016 cycle. In the regression models, age and age at sexual debut were assessed in one-year increments and condom use was categorized as consistent (never having condomless sex), and inconsistent (having condomless sex less than half the time, about half the time, more than half the time, or always).

Data Analysis

We calculated the weighted seroprevalence of CT infection among all individuals stratified by gender of sex partner (WSM and WSW), using the 2013-2014 and 2015-2016 MEC sample weights and dividing the 2-year sample weights by two based on the NHANES guidelines for combining two cycles. Within both strata of gender of sex partner, we estimated the weighted seroprevalence by sociodemographic and sexual behavior characteristics. We used univariate Poisson regression and Wald-based 95% confidence intervals to identify factors that were significantly associated with having sex partners that were women (WSW) relative to only having partners that were men, and to estimate the prevalence ratio (PR) and Wald-based 95% confidence interval (CI) for the association of each factor with CT seropositivity stratified by gender of sex partner.

We first developed one multivariable model for WSM and another for WSW. In each model, we assessed age and condom use *a priori*, and assessed any factors associated with CT seropositivity in the univariate model at a p-value of ≤ 0.10. We retained any factors that were either significantly associated ($p<0.05$) with seropositivity or changed other estimates included in the model by 10% or more. In a final multivariable regression model estimating the association

of gender of sex partner and CT seroprevalence, we adjusted for factors associated with CT seropositivity in either sex-partner specific multivariable regression model. All analyses were done using R statistical software.

RESULTS

For the 2013-2014 and 2015-2016 NHANES cycles, there were a total of 19,357 participants of all ages who took part in the MEC examinations and questionnaires, 9,850 of whom were females. Of these, the sexual behavior questionnaire was administered to 5,172 females aged 18-69 who were able to complete the questions (i.e., not mentally impaired and could understand English, Spanish, Korean, Vietnamese, or Chinese). CT serology was performed on 1,724 females aged 18-39 who had documented chlamydia NAAT tests.

Our study population included females who were sexually experienced and had responded to the question asking if they had ever had sex with a woman. We excluded 101 females who reported never having any type of sex. We further excluded 198 females who either refused to answer or were missing data for the question "*Have you ever had any kind of sex with a woman?*". Our final sample, based on NHANES eligibility criteria and our exclusion criteria, was 1,425 sexually experienced females aged 18-39 who provided a valid serum sample and answered the questions about ever having had sex with a woman and ever having had sex with a man.

Characteristics of WSW compared to WSM

Of the 1,425 individuals included in our analyses, 1,207 (83.6%) reported exclusively male sex partners (WSM) and 218 (16.4%) reported ever having had sex with a woman (WSW).

Seven WSW reported never having had sex with a man during their lifetime. The overall population was majority NH White, had completed at least some of a college education, were insured, and above the low-income threshold (Table 1).

WSW had a significantly lower mean age than WSM (27.7 vs 28.99), and 18- to 24-year-olds were proportionally the largest age strata for WSM (28.2%) and WSW (33.6%). A higher proportion of NH White individuals were WSW whereas a higher proportion of NH Asian or Hispanic individuals were WSM. Similarly, a higher proportion of women who reported an age at sexual debut ≤ 13 years old were WSW than those with debut at ≥18 years old; a higher proportion of individuals with ≥5 lifetime male sex partners were WSW than those with 0 or 1 male partner; and a higher proportion of those who had CT within the past year were WSW, than those who had not had CT. The majority of WSM self-identified as straight (97.1%) whereas most WSW self-identified either as bisexual (46.8%) or straight (43.0%). Other characteristics significantly associated with reporting a history of sex with a woman included: below the income-poverty ratio; completing a high school education/GED or some college/an associate degree; and never marrying or being divorced/widowed/separated. Reporting a history of HSV, HPV, genital warts, or gonorrhea; reporting condomless sex more than half the time but not always; and self-identifying their sexuality as anything other than straight were also associated with reporting a history of sex with women (Table 1).

Population Seroprevalence

The weighted CT seroprevalence in our overall study population was 30.2% (95% CI: 25.7, 34.8), as previously reported.[14] The weighted population-level seroprevalence of CT among WSW was 38.4% (95% CI: 30.0, 46.9) and 28.6% (95% CI: 24.4, 32.9) among WSM.

Comparison of seroprevalence in WSM and WSW

The unadjusted CT seroprevalence among WSW was significantly higher than among WSM (PR=1.34 [95% CI: 1.12, 1.60], p=0.002). However, after adjusting for age, race, income-poverty ratio, age at sexual debut, number of lifetime male sex partners, history of CT in the past year, and sexual identity, there was no significant difference in CT seroprevalence between WSM and WSW (aPR=1.22 [95% CI: 0.98, 1.53], p=0.07).

Characteristics Associated with CT Seropositivity in WSW

In univariable analyses, CT seroprevalence was higher in NH Black WSW (68.0% [95% CI: 54.2, 81.7]) compared to NH White WSW (30.0% [95% CI: 18.5, 41.4) (Table 2), but similar in Hispanic and "other" race/ethnicities. Higher CT seroprevalence was associated with ≥5 lifetime male sex partners, having CT within the last year, and identifying as bisexual. No other sociodemographic or sexual behavior characteristics were associated with seropositivity in WSW. In multivariable analyses (Table 3), NH Black (aPR=2.28 [95% CI: 1.40, 3.71]) WSW and WSW with another race/ethnicity (aPR=2.06 [95% CI: 1.06, 3.99]) had higher seroprevalence than NH White WSW (Table 3). WSW with ≥5 lifetime male sex partners were approximately five times more likely to be seropositive than WSW with ≤1 male sex partners (aPR=5.16 [95% CI: 2.29, 11.59]). WSW who had CT in the past year had a seroprevalence approximately twice as high as those without a diagnosis (aPR=1.97 [95% CI: 1.13, 3.46]). WSW who identified as bisexual were almost half as likely to be seropositive as WSW who identified as straight (aPR=0.57 [95% CI: 0.34, 0.97]).

Characteristics associated with CT Seropositivity in WSM

In univariable analyses among WSM, CT seroprevalence was significantly higher in NH Black (62.1% [95% CI: 53.9, 70.4]) and Hispanic women (32.3% [95% CI: 27.8, 36.6]) compared to NH White women (21.6% [95% CI: 16.6, 26.6]). Other characteristics associated with seropositivity in WSM included: being less than a college graduate; never married or divorced, separated, or widowed; and being below the low-income threshold (Table 2). With regards to sexual behaviors, a younger age at sexual debut; a higher lifetime number of male partners; and a history of CT within the past year were associated with CT seropositivity in WSM (Table 2). There was no association between age and CT seropositivity. In multivariable analyses (Table 3), NH Black (aPR=2.23 [95% CI: 1.73, 2.89]) and Hispanic WSM (aPR=1.52 [95% CI: 1.15, 2.02]) had higher CT-seropositivity than NH White WSM (Table 3). WSM below the low-income threshold also had a significantly higher seroprevalence (aPR=1.29 [95% CI: 1.01, 1.65]) than WSM above the low-income threshold. Older age at sexual debut was associated with lower seroprevalence relative to WSM who were younger at sexual debut (aPR=0.89 per year [95% CI: 0.86, 0.93)]. WSM that reported ≥5 male sex partners in their lifetime were nearly two and a half times more likely to be seropositive than with ≤1 male sex partners (aPR=2.29 [95% CI: 1.44, 3.64]). WSM with a history of CT in the past year were almost twice as likely to be seropositive (aPR=1.87 [95% CI: 1.36, 2.57]).

DISCUSSION

In this nationally-representative, population-based survey, the seroprevalence among sexually experienced females aged 18-39 was 30.2%, similar to what has been previously reported in this population.[14] The seroprevalence of CT infection was 38.4% in WSW and 28.6%

in WSM. Although CT seropositivity in WSW was higher than in WSM in unadjusted analyses, this association was no longer significant after adjusting for characteristics associated with CT. In WSW, seroprevalence was higher in those with a non-Hispanic Black or another race/ethnicity, a higher lifetime number of male sex partners, a history of CT in the past year, and a sexual identity of "straight". Among WSM, CT seropositivity was higher in women reporting NH Black or Hispanic race/ethnicities relative to those with a NH White race/ethnicity. Similarly, WSM above the income-poverty ratio had a lower CT seroprevalence compared to those below the income-poverty ratio. In addition, seroprevalence was higher among WSM who were younger when they became sexually active, had more male sex partners, and had CT in the past year.

WSW in our study population were younger, less likely to have completed a college education, and more likely to have never been married than WSM. This is consistent with previous reports of women who have sex with both men and women, reflecting the large number of women who have sex partners of both genders in our data relative to women who exclusively have sex with women.[11,20] WSW also had a significantly younger age at sexual debut and significantly more male sex partners in their lifetime than WSM, both of which have been associated with WSW (including those who also have sex with men) in prior research.[11,17,20,21] In a British national survey, 62.3% of women who had ever had sex with men and women reported having ten or more lifetime male sex partners, compared to 19.7% of WSM.[20] In addition, a study using NHANES data found that 29.6% of women who had ever had sex with a woman but only 12.0% of women who had only had sex with men had an age at sexual debut at 14 years old or younger.[11] Almost half of WSW in our population reported their sexual orientation as straight, demonstrating the discordance between sexual behavior and sexual identity that has been

characterized in other literature.[11,22] Sexual identity has a complex and indirect relationship with sexual experience, and many women who have had sexual experiences with other women identify as heterosexual or straight; a study in Australia reported that only 31% of women who reported ever having sexual experiences with women and ever feeling attraction towards women identified as bisexual or lesbian.[22]

Though few studies have been done on the lifetime prevalence of CT infection or STIs in WSW, our estimates are higher than previous studies based on self-reported diagnoses with any STI, which estimated a lifetime prevalence around 20%.[4,9] In line with our findings, a study using data from the same cycles of NHANES reported a weighted CT seroprevalence of approximately 40% among women who have ever had sex with women, though the study authors sought to examine the association between high chlamydial seropositivity and upper genital tract sequelae, and did not examine specific risk factors of CT infection among WSW.[15] Among WSW in our study population, race/ethnicity was the only sociodemographic factor associated with seropositivity, with NH Black women having an adjusted seroprevalence over twice as high as NH White women. Prior research has reported a high prevalence of both acute and lifetime CT among Black WSW who reported recent sex with women, demonstrating the unique disparities in sexual healthcare delivery as well as stressors like institutionalized racism, sexism, and homophobia faced by this subpopulation.[17,19] These disparities can manifest in unequal access to quality screening and preventive health services, decreased access to affordable health insurance plans, and stigma from healthcare providers that can increase the disease burden of STIs in Black WSW.[4,23] Though an earlier study found that those with who were living with a partner or dating had a lower lifetime history of STIs, we did not observe a lower CT seroprevalence in WSW who were married/living with a partner.[4] CT seroprevalence was also

five times higher in those with ≥5 lifetime male sex partners, but was not associated with number of female sex partners, suggesting that the lifetime number of male partners has a greater influence on CT seropositivity. Prior studies have demonstrated that a higher average number of lifetime male partners is an important predictor of seroprevalence in women overall.[14,18]

Additionally, though women with a younger age at sexual debut were significantly more likely to be WSW, age at sexual debut was not associated with seropositivity in this subpopulation. This finding is contrary to prior studies on CT seroprevalence in the overall population of women, which have found that women who are younger at sexual debut have a higher seroprevalence than those who are older at sexual debut.[14,15,18] We hypothesize that the relationship between age at sexual debut and seropositivity in WSW was instead captured by the association between lifetime number of male sex partners and seropositivity, as women with a younger age at sexual debut were sexually active longer and may have had more male sex partners. An earlier study also found that WSW describing their sexual orientation as lesbian had a lower prevalence of lifetime STI than bisexual WSW, but the seroprevalence in self-described bisexual WSW in our population was significantly lower than straight WSW, while seropositivity in WSW who identified as lesbian was no different than WSW who identified as straight.[4] Bisexual WSW in our study were less likely than straight WSW to be CT seropositive, which may be explained by the discordance between sexual behavior and sexual identity that has been observed in other studies, as bisexual and straight WSW have similar lifetime sexual behavior but only differ by identity.[11,22]

The characteristics that we identified as associated with CT seropositivity among WSM have previously been associated with increased CT seropositivity in women regardless of gender of sex partner, including a non-White or Hispanic race/ethnicity, low income, a lower age at

sexual debut, a higher lifetime number of male sex partners, and a history of CT in the past year.[14,15,18] Contrary to a prior study that observed seroprevalence increasing with age in 16-44 year old women using double-antigen sandwich ELISAs, we did not observe a significant increase in seropositivity as age increased among either WSM or WSW.[18] This could be due to the limited age range in our population, or because the sensitivity of the CT Pgp3 ELISA wanes slightly with time since most recent infection, and CT incidence is highest in women under 25.[1,24] The CT Pgp3 ELISA may have been slightly more sensitive in younger women who had a more recent infection, and may have failed to pick up prior infections in some older women for whom over a decade had passed since their most recent infection. Seroprevalence was not higher in those who reported exclusively condomless sex in the past year, likely because condom use was reported for the past 12 months, while seroprevalence is a long-term indicator of infection.

Despite higher CT seroprevalence in WSW than in WSM in unadjusted analyses, there was no significant difference in seropositivity between WSM and WSW after adjustment. Common factors associated with CT seropositivity in both groups (high lifetime number of male sex partners, a NH Black race/ethnicity, and a history of CT in the past year) may partly explain this. Clinicians should focus on the characteristics associated with CT infection in all women, taking care to ask about recent sexual history with partners of any gender. STI prevention strategies and messaging for WSW should be multilevel and address the variety of sexual practices and identities in this group, while also training clinicians in providing sexual healthcare free of the stigma that might otherwise discourage WSW from seeking care.[4]

Our study was subject to several limitations. There were only seven eligible women who reported having had sex with women exclusively in the 2013-2014 and 2015-2016 cycles of NHANES, which required us to combine WSW and WSMW into one category. While this

provided adequate power for our analyses, the lack of specificity prevented us from examining any differences in risk or protective factors against infection between women who have sex only with other women and women who have sex with both men and women. The lack of distinction between gender and sex assigned at birth in the survey design may also lead to misclassification of the exposure, as transgender or gender non-conforming individuals may not be asked relevant sexual behavior or reproductive health questions due to sex-specific skip patterns. This misclassification has been present in other national surveys and could bias our overall results and limit generalizability.[25] Also of note is the phrasing of the sexual orientation question in the 2013-2014 cycle which conflates sexual identity with sexual attraction. This may result in minor misclassification of responses that could underestimate the effect of sexual orientation on seroprevalence.[26] In addition, our analyses were limited to non-institutionalized, civilian women between the ages of 18 and 39, though it would have been preferable to include adolescents and women above 40 to improve generalizability. Bias could also be present if the population that consented to a medical examination and provided serum samples fundamentally differed from the general population. Furthermore, the cross-sectional design of this study prevents us from making conclusions related to causality. Finally, by defining WSW as ever having had sex with a woman, we cannot differentiate our findings by sex of current sexual partners, and we are not able to determine if the seropositive individuals were infected by male or female sex partners. However, our adjusted analyses demonstrated that a high number of male partners but not a high number of female partners was associated with CT seroprevalence, suggesting that a substantial part of CT risk was due to male partners.

The primary strengths of this study are the CT Pgp3 ELISA's ability to determine lifetime infection, and the large, nationally-representative survey design of NHANES. CT Pgp3

antibodies have been shown to persist for over ten years in a majority of cisgender women, making them a reliable marker of both past infections and prevalent infections that have generated an antibody response.[12,13] Most prior studies on the lifetime prevalence of CT in WSW have used self-reported diagnoses, whereas our study was strengthened by its use of clinical markers for long-term infection, which are more sensitive and less biased than self-reported data.[4,19] Furthermore, our use of population-based data allows for more generalizable, less biased estimates for seroprevalence than clinic-based data or convenience surveys that have previously been used for WSW.[4,17,19]

In summary, WSW had a substantial burden of lifetime CT infection, but this was not significantly higher than WSM after adjusting for sociodemographic and sexual behavior characteristics. This study further elucidated previous reports of lifetime CT infection and sexual behaviors in WSW, and provides important information on STI morbidity in an understudied sexual minority group. It also emphasizes considerable disparities by race/ethnicity and highlights the need to address the disparities that occur at the intersection of race/ethnicity and gender of sex partner. Future research should consider the heterogeneity in women described as "WSW", taking care to differentiate between women who ever had sex with a woman, women who exclusively have sex with women, and women who have sex with men and women in order to fully understand differences in sexual behavior and risk for CT infection. Though women with female sex partners have historically been considered to be at lower risk for certain bacterial STIs like CT, women who have recently had exclusively female partners may have a different STI risk profile compared to women who had a female partner in the more distant past, highlighting the need to differentiate not only by gender of sex partner but also by the recency of those partners. Nevertheless, overall, our findings demonstrate that many WSW's risk of CT is

comparable to WSM and young WSW should receive annual CT screening as frequently as do young WSM. Health care providers should ensure that they are delivering prevention messages to WSW as well as to WSM, tailoring them to incorporate the factors that are associated with increased risk of CT infection in each group.

References

1. CDC. *Sexually Transmitted Disease Surveillance 2019*. Department of Health and Human Services; 2021. https://www.cdc.gov/std/statistics/2019/std-surveillance-2019.pdf

2. *Detailed STD Facts - Chlamydia*. Centers for Disease Control and Prevention Accessed January 19, 2023. https://www.cdc.gov/std/chlamydia/stdfact-chlamydia-detailed.htm

3. Gorgos LM, Marrazzo JM. Sexually Transmitted Infections Among Women Who Have Sex With Women. *Clin Infect Dis*. 2011;53(suppl_3):S84-S91. doi:10.1093/cid/cir697

4. Logie CH, Navia D, Loutfy MR. Correlates of a lifetime history of sexually transmitted infections among women who have sex with women in Toronto, Canada: results from a cross-sectional internet-based survey. *Sex Transm Infect*. 2015;91(4):278-283. doi:10.1136/sextrans-2014-051745

5. Torrone E, Papp J, Weinstock H. Prevalence of Chlamydia trachomatis Genital Infection Among Persons Aged 14–39 Years — United States, 2007–2012. *Morb Mortal Wkly Rep*. 2014;63(38):834-838.

6. US Preventive Services Task Force. Screening for Chlamydia and Gonorrhea: US Preventive Services Task Force Recommendation Statement. *JAMA*. 2021;326(10):949-956. doi:10.1001/jama.2021.14081

7. Dolan KA, Davis PW. Nuances and shifts in lesbian women's constructions of STI and HIV vulnerability. *Soc Sci Med*. 2003;57(1):25-38. doi:10.1016/S0277-9536(02)00305-2

8. Singh D, Fine DN, Marrazzo JM. Chlamydia trachomatis Infection Among Women Reporting Sexual Activity With Women Screened in Family Planning Clinics in the Pacific Northwest, 1997 to 2005. *Am J Public Health*. 2011;101(7):1284-1290. doi:10.2105/AJPH.2009.169631

9. Bauer GR, Welles SL. Beyond Assumptions of Negligible Risk: Sexually Transmitted Diseases and Women Who Have Sex With Women. *Am J Public Health*. 2001;91(8):1282-1286.

10. Pinto VM, Tancredi MV, Neto AT, Buchalla CM. Sexually transmitted disease/HIV risk behaviour among women who have sex with women. *AIDS*. 2005;19:S64. doi:10.1097/01.aids.0000191493.43865.2a

11. Xu F, Sternberg MR, Markowitz LE. Women Who Have Sex With Women in The United States: Prevalence, Sexual Behavior and Prevalence of Herpes Simplex Virus Type 2 Infection—Results From National Health and Nutrition Examination Survey 2001–2006. *Sex Transm Dis*. 2010;37(7):407. doi:10.1097/OLQ.0b013e3181db2e18

12. Wills GS, Horner PJ, Reynolds R, et al. Pgp3 Antibody Enzyme-Linked Immunosorbent Assay, a Sensitive and Specific Assay for Seroepidemiological Analysis of Chlamydia trachomatis Infection. *Clin Vaccine Immunol CVI*. 2009;16(6):835-843. doi:10.1128/CVI.00021-09

13. Horner PJ, Wills GS, Righarts A, et al. Chlamydia trachomatis Pgp3 Antibody Persists and Correlates with Self-Reported Infection and Behavioural Risks in a Blinded Cohort Study. *PLOS ONE*. 2016;11(3):e0151497. doi:10.1371/journal.pone.0151497

14. Petersen MR, Patel EU, Grabowski MK, Gaydos CA, Quinn TC, Tobian AAR. Seroprevalence of Chlamydia trachomatis Among Female Adults in the United States: The National Health and Nutrition Examination Surveys. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 2020;73(3):e629-e637. doi:10.1093/cid/ciaa1879

15. Anyalechi GE, Hong J, Danavall DC, et al. High Plasmid Gene Protein 3 (Pgp3) Chlamydia trachomatis Seropositivity, Pelvic Inflammatory Disease, and Infertility Among Women, National Health and Nutrition Examination Survey, United States, 2013–2016. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 2021;73(8):1507-1516. doi:10.1093/cid/ciab506

16. Centers for Disease Control and Prevention. *National Health and Nutrition Examination Survey Laboratory Protocol*. National Center for Health Statistics; 2015. https://wwwn.cdc.gov/nchs/data/nhanes/2013-2014/labmethods/CHLMDA_H_MET_CHLMYDIA.pdf

17. Rahman N, Ghanem KG, Gilliams E, Page K, Tuddenham S. Factors Associated with Sexually Transmitted Infection Diagnosis in Women Who have Sex with Women, Women Who Have Sex with Men and Women Who Have Sex with Both. *Sex Transm Infect*. 2021;97(6):423-428. doi:10.1136/sextrans-2020-054561

18. Woodhall SC, Wills GS, Horner PJ, et al. Chlamydia trachomatis Pgp3 Antibody Population Seroprevalence before and during an Era of Widespread Opportunistic Chlamydia Screening in England (1994-2012). *PLOS ONE*. 2017;12(1):e0152810. doi:10.1371/journal.pone.0152810

19. Muzny CA, Sunesara IR, Martin DH, Mena LA. Sexually Transmitted Infections and Risk Behaviors Among African American Women Who Have Sex With Women: Does Sex With Men Make a Difference? *Sex Transm Dis*. 2011;38(12):1118. doi:10.1097/OLQ.0b013e31822e6179

20. Mercer CH, Bailey JV, Johnson AM, et al. Women Who Report Having Sex With Women: British National Probability Data on Prevalence, Sexual Behaviors, and Health Outcomes. *Am J Public Health*. 2007;97(6):1126-1133. doi:10.2105/AJPH.2006.086439

21. Burkill S, Waterhouse P. Sources of Sex Information Used by Young British Women Who Have Sex with Women (WSW) and Women Who Have Sex Exclusively with Men (WSEM): Evidence from the National Survey of Sexual Attitudes and Lifestyles. *Sex Res Soc Policy*. 2019;16(1):22-30. doi:10.1007/s13178-018-0327-z

22. Smith AMA, Rissel CE, Richters J, Grulich AE, de Visser RO. Sex in Australia: Sexual identity, sexual attraction and sexual experience among a representative sample of adults. *Aust N Z J Public Health*. 2003;27(2):138-145. doi:10.1111/j.1467-842X.2003.tb00801.x

23. Prather C, Fuller TR, Jeffries WL, et al. Racism, African American Women, and Their Sexual and Reproductive Health: A Review of Historical and Contemporary Evidence and Implications for Health Equity. *Health Equity*. 2018;2(1):249-259. doi:10.1089/heq.2017.0045

24. Horner PJ, Wills GS, Reynolds R, et al. Effect of time since exposure to Chlamydia trachomatis on chlamydia antibody detection in women: a cross-sectional study. *Sex Transm Infect*. 2013;89(5):398-403. doi:10.1136/sextrans-2011-050386

25. Tordoff D, Andrasik M, Hajat A. Misclassification of Sex Assigned at Birth in the Behavioral Risk Factor Surveillance System and Transgender Reproductive Health: A Quantitative Bias Analysis. *Epidemiology*. 2019;30(5):669. doi:10.1097/EDE.0000000000001046

26. Rothwell CJ, Madans JH, Cynamon ML. Sexual Orientation in the 2013 National Health Interview Survey: A Quality Assessment. 2014;(169).

Table 1: Sociodemographic Characteristics and Sexual Behaviors of Sexually Experienced Women Aged 18 to 39 Years in NHANES 2013-2016 Cycles, by Gender of Sex Partner

	Overall	**Women who have had sex with men (WSM) only**	**Women who have ever had sex with a woman (WSW)**	**Univariable association with having female sex partners (WSW relative to WSM)**	
Characteristic	**N (%)**	**N[1] (weighted %)**	**N[1] (weighted %)**	**Univariable prevalence ratio (95% CI)**	**P-value***
Total	-	1207 (83.6%)	218 (16.4%)	-	-
Age group, years					
Mean (SE)		28.99 (±0.27)	27.7 (±0.55)	-	**0.05**[+]
18-24	458 (29.1)	382 (28.2%)	76 (33.6%)	1.40 (0.84, 2.34)	0.19
25-29	316 (24.5)	260 (24.1%)	56 (26.5%)	1.32 (0.81, 2.14)	0.26
30-34	323 (22.9)	273 (23.4%)	50 (20.4%)	1.08 (0.63, 1.86)	0.77
35-39	328 (23.6)	292 (24.4%)	36 (19.4%)	1.0 (ref)	-
Race/ethnicity					
Non-Hispanic White	506 (58.3)	413 (57.2%)	93 (64.0%)	1.0 (ref)	-
Non-Hispanic, Black	259 (11.0)	205 (10.3%)	54 (14.3%)	1.18 (0.85, 1.65)	0.30
Non-Hispanic Asian	138 (5.0)	127 (5.4%)	11 (2.8%) [a]	**0.50 (0.25, 1.01)**	**0.053**
Hispanic[2]	442 (21.0)	398 (22.4%)	44 (13.8%)	**0.60 (0.41, 0.88)**	**0.01**

	Overall	Women who have had sex with men (WSM) only	Women who have ever had sex with a woman (WSW)	Univariable association with having female sex partners (WSW relative to WSM)	
Characteristic	**N (%)**	**N[1] (weighted %)**	**N[1] (weighted %)**	**Univariable prevalence ratio (95% CI)**	**P-value***
Other[2]	80 (4.8)	64 (4.7%)	16 (5.2%)	0.99 (0.54, 1.82)	0.97
Education level					
Less than high school	242 (12.5)	210 (12.8%)	32 (10.7%)	1.47 (0.98, 2.21)	0.06
High school graduate/GED	310 (19.0)	257 (17.6%)	53 (25.9%)	**2.34 (1.42, 3.84)**	**0.002**
Some college/Associates degree	534 (38.9)	436 (37.5%)	98 (46.0%)	**2.02 (1.37, 2.99)**	**0.001**
College graduate or above	339 (29.6)	304 (32%)	35 (17.3%)	1.0 (ref)	-
Marital status (among over 20)[3]					
Married/living with partner	721 (60.2)	637 (62.6%)	84 (48.4%)	1.0 (ref)	-
Divorced/separated/widowed	113 (8.1)	87 (7.6%)	26 (10.5%)	**1.62 (1.15, 2.28)**	**0.01**
Never married	433 (31.7)	344 (29.9%)	89 (41.1%)	**1.61 (1.12, 2.32)**	**0.01**
Income-poverty ratio[4]					
Below low-income threshold	610 (36.2)	499 (34.4%)	111 (45.1%)	**1.45 (1.06, 1.99)**	**0.048**

	Overall	Women who have had sex with men (WSM) only	Women who have ever had sex with a woman (WSW)	Univariable association with having female sex partners (WSW relative to WSM)	
Characteristic	**N (%)**	**N[1] (weighted %)**	**N[1] (weighted %)**	**Univariable prevalence ratio (95% CI)**	**P-value***
Above low-income threshold	742 (63.8)	643 (65.6%)	99 (54.9%)	1.0 (ref)	-
Insurance status					
Insured	1078 (80.3)	921 (81.3%)	157 (75.3%)	1.0 (ref)	-
Uninsured	345 (19.7)	284 (18.7%)	61 (24.7%)	1.33 (0.96, 1.86)	0.09
Age at sexual debut, in years[5]					
Mean (SE)		17.5 (±0.13)	15.6 (±0.17)	-	**<0.0001[+]**
≤13	61 (3.7)	38 (2.6%)	23 (10.1%)	**4.83 (3.35, 6.94)**	**<0.0001**
14-15	322 (22.3)	257 (20.5%)	65 (32.3%)	**2.60 (1.86, 3.65)**	**<0.0001**
16-17	453 (34.2)	384 (33.9%)	69 (35.6%)	**1.87 (1.29, 2.73)**	**0.002**
≥18	543 (39.8)	503 (43.1%)	40 (22.1%)	1.0 (ref)	-
# lifetime male sex partners					
Mean (SE)		6.4 (±0.3)	15.6 (±1.37)	-	**<0.0001**
0-1	312 (20.2)	294 (22.7%)	18 (6.9%)	1.0 (ref)	-

	Overall	Women who have had sex with men (WSM) only	Women who have ever had sex with a woman (WSW)	Univariable association with having female sex partners (WSW relative to WSM)	
Characteristic	**N (%)**	**N[1] (weighted %)**	**N[1] (weighted %)**	**Univariable prevalence ratio (95% CI)**	**P-value***
2-4	417 (27.5)	388 (30.3%)	29 (13.3%)	1.41 (0.75, 2.65)	0.27
≥5	694 (52.3)	524 (47.0%)	170 (79.8%)	**4.45 (2.42, 8.15)**	**<0.0001**
# lifetime female sex partners					
Mean (SE)		-	3.09 (±0.33)	-	-
0	1207 (83.6)	1207 (100%)	0 (0%)	-	-
1	79 (6.3)	-	79 (38.5%)	-	-
2-4	106 (7.5)	-	106 (46.0%)	-	-
≥5	33 (2.5)	-	33 (15.6%)	-	-
Frequency of condomless sex in last year[6]					
Never	273 (17.8)	241 (18.6%)	32 (13.7%)	1.0 (ref)	-
Less than half the time	234 (15.9)	204 (16.1%)	30 (14.4%)	1.18 (0.72, 1.94)	0.50
About half the time	97 (6.6)	82 (6.7%)	15 (5.8%)	1.15 (0.54, 2.43)	0.71
More than half the time	155 (12.4)	122 (11.4%)	33 (17.7%)	**1.85 (1.13, 3.05)**	**0.02**

	Overall	Women who have had sex with men (WSM) only	Women who have ever had sex with a woman (WSW)	Univariable association with having female sex partners (WSW relative to WSM)	
Characteristic	**N (%)**	**N[1] (weighted %)**	**N[1] (weighted %)**	**Univariable prevalence ratio (95% CI)**	**P-value***
Always	561 (47.3)	476 (47.1%)	85 (48.4%)	1.33 (0.85, 2.08)	0.20
History of HSV, genital warts, HPV, or gonorrhea[7]					
Yes	206 (17.3)	158 (15.8%)	48 (25.2%)	**1.61 (1.24, 2.08)**	**0.001**
No	1217 (82.7)	1047 (84.2%)	170 (74.8%)	1.0 (ref)	-
Chlamydia in the past year[8]					
Yes	68 (3.8%)	53 (3.3%)	15 (5.9%)	**1.62 (1.12, 2.33)**	**0.01**
No	1355 (96.2%)	1152 (96.7%)	203 (94.1%)	1.0 (ref)	-
Sexual identity[9]					
Straight	1226 (88.2)	1140 (97.1%)	86 (43.0%)	1.0 (ref)	-
Lesbian or gay	25 (1.5)	0 (0%)	25 (8.9%)	**12.48 (10.02, 15.54)**	**<0.0001**
Bisexual	128 (9.9)	32 (2.7%)	96 (46.8%)	**9.64 (7.56, 12.31)**	**<0.0001**
Other	11 (0.4)	b	b	b	**b**

[1] Count data is unweighted, but all proportions are weighted according to NHANES medical analytic guidelines using medical examination center (MEC) weights.

*P-values derived using univariate Poisson regression unless otherwise specified
[+]P-value derived from weighted t-test
[a] Relative standard error >30% but <40%; interpret with caution.
[2] "Hispanic" includes Mexican American and participants of other Hispanic ethnicity; "Other" includes multirace, and all other racial/ethnic categories.
[3] Not asked of participants 18-19 years old.
[4] Income threshold for low-income was 130% FPL in the 2013-2014 cycle and 185% FPL in the 2015-2016 cycle. “Below low-income threshold” corresponds to being below 130% FPL for participants in the 2013-2014 cycle and being below 185% FPL in the 2015-2016 cycle.
[5]Zero participants reported an age of sexual debut below 13 years old.
[6] Information on past year condom use was only asked to individuals who reported vaginal or anal sex in the past year.
[7] History of HSV, HPV, or gonorrhea defined as ever being told by a doctor that they had HSV, genital warts, or HPV, or being told by a doctor within the last year that they had gonorrhea.
[8] “Yes” includes those who were either told by a doctor within the last year that they had chlamydia, or tested positive on a urine CT NAAT at the time of examination
[8] Between the 2013-2014 and 2015-2016 cycles, NHANES changed the possible answers for the sexual identity question: “Do you think of yourself as . . .”. In 2013-2014, the answer options were: “Heterosexual or straight (attracted to men)”, “Homosexual or lesbian (attracted to women)”, “Bisexual (attracted to men and women)”, “Something else”, “Not sure”, “Refused”, and “Don’t know”. In 2015-2016, the answer options were: “Lesbian or gay”, “Straight, that is, not lesbian or gay”, “Bisexual”, “Something else”, “Not sure”, “Refused”, and “Don’t know”. For our analyses, those who answered “heterosexual” or “straight” were classified as straight, those who answered “homosexual” or “lesbian or gay” were lesbian or gay, those who answered “bisexual” were bisexual, and those who answered “something else” were classified as other.
[b] Relative standard error >50%; estimate is suppressed.

Table 2. Univariate Analysis of Factors Associated with CT Seropositivity in Sexually Experienced Women Aged 18 to 39 Years in NHANES 2013-2016 Cycles (n=1425), by Gender of Sex Partner

	CT Seropositivity							
	Women who have had sex with men (WSM) only (n=1207)				**Women who have ever had sex with a woman (WSW) (n=218)**			
Characteristic	**N**	**Weighted Seroprevalence (95% CI)**	**Prevalence Ratio (95% CI)**	**P value***	**N**	**Weighted Seroprevalence (95% CI)**	**Prevalence Ratio (95% CI)**	**P value***
Age group, years								
18-24	382	30.5 (24.9, 36.1)	0.97 (0.75, 1.25)	0.78	76	29.6 (16.2, 43.0)	0.72 (0.41, 1.25)	0.23
25-29	260	24.6 (17.9, 31.3)	0.78 (0.53, 1.15)	0.20	56	43.5 (31.0, 56.0)	1.06 (0.62, 1.79)	0.83
30-34	273	27.6 (21.2, 34.0)	0.87 (0.66, 1.15)	0.32	50	43.8 (25.8, 61.9)	1.06 (0.62, 1.84)	0.82
35-39	292	31.6 (23.9, 39.3)	1.0 (ref)	-	36	41.2 (25.4, 57.0)	1.0 (ref)	-
Race/ethnicity								
Non-Hispanic, White	413	21.6 (16.6, 26.6)	1.0 (ref)	-	93	30.0 (18.5, 41.4)	1.0 (ref)	-
Non-Hispanic, Black	205	62.1 (53.9, 70.4)	**2.87 (2.22, 3.72)**	**<0.005**	54	68.0 (54.2, 81.7)	**2.27 (1.42, 3.61)**	**0.001**
Non-Hispanic, Asian	127	15.7 (10.0, 21.4)	0.73 (0.46, 1.14)	0.16	11	[c]	[c]	[c]
Hispanic[1]	398	32.3 (27.8, 36.6)	**1.49 (1.15, 1.92)**	**0.003**	44	44.1 (29.4, 58.9)	1.47 (0.86, 2.53)	0.15

	CT Seropositivity							
	Women who have had sex with men (WSM) only (n=1207)				Women who have ever had sex with a woman (WSW) (n=218)			
Characteristic	**N**	**Weighted Seroprevalence (95% CI)**	**Prevalence Ratio (95% CI)**	**P value***	**N**	**Weighted Seroprevalence (95% CI)**	**Prevalence Ratio (95% CI)**	**P value***
Other[1]	64	38.6 (19.2, 58.0)	**1.78 (1.05, 3.02)**	**0.03**	16	57.6 (22.1, 93.1)[a]	1.92 (0.93, 3.98)[a]	0.08[a]
Education level								
Less than high school	210	38.6 (30.4, 46.8)	**2.03 (1.45, 2.85)**	**<0.001**	32	38.3 (17.7, 58.8)	1.41 (0.51, 3.92)	0.50
High school graduate/GED	257	35.7 (28.3, 43.0)	**1.88 (1.37, 2.59)**	**<0.001**	53	41.7 (26.3, 57.2)	1.54 (0.70, 3.36)	0.27
Some college/Associates degree	436	30.2 (24.6, 35.8)	**1.59 (1.20, 2.12)**	**0.003**	98	40.9 (30.2, 51.5)	1.50 (0.77, 2.94)	0.22
College graduate or above	304	19.0 (14.0, 24.0)	1.0 (ref)	-	35	27.2 (9.8, 44.5)[a]	1.0 (ref)	-
Marital status (among over 20)[2]								
Married/living with partner	637	23.9 (19.6, 28.2)	1.0 (ref)	-	84	32.2 (20.4, 43.9)	1.0 (ref)	-
Divorced/separated/ widowed	87	47.2 (32.3, 62.2)	**1.98 (1.41, 2.78)**	**<0.001**	26	48.8 (27.1, 70.6)	1.52 (0.89, 2.59)	0.12

	CT Seropositivity							
	Women who have had sex with men (WSM) only (n=1207)				Women who have ever had sex with a woman (WSW) (n=218)			
Characteristic	**N**	**Weighted Seroprevalence (95% CI)**	**Prevalence Ratio (95% CI)**	**P value***	**N**	**Weighted Seroprevalence (95% CI)**	**Prevalence Ratio (95% CI)**	**P value***
Never married	344	33.7 (28.4, 38.9)	**1.41 (1.14, 1.73)**	**0.002**	89	44.4 (34.8, 54.0)	1.38 (0.91, 2.09)	0.12
Income-poverty ratio[3]								
Below low-income threshold	499	37.9 (32.0, 43.8)	**1.67 (1.31, 2.14)**	**<0.001**	111	41.2 (29.2, 53.2)	1.13 (0.76, 1.69)	0.53
Above low-income threshold	643	22.6 (18.0, 27.2)	1.0 (ref)	-	99	36.4 (25.6, 47.2)	1.0 (ref)	-
Insurance status								
Insured	921	27.5 (22.6, 32.4)	1.0 (ref)	-	157	35.7 (24.8, 46.5)	1.0 (ref)	-
Uninsured	284	33.8 (27.2, 40.4)	1.23 (0.94, 1.61)	0.13	61	46.9 (33.8, 59.9)	1.31 (0.83, 2.07)	0.23
Age at sexual debut, in years[4]								
≤ 13	38	51.1 (37.6, 64.5)	**3.32 (2.22, 4.95)**	**<0.0001**	23	46.8 (23.8, 69.8)	1.77 (0.79, 3.98)	0.16
14-15	257	43.1 (36.8, 49.3)	**2.80 (2.11, 3.70)**	**<0.0001**	65	33.0 (21.1, 45.9)	1.25 (0.72, 2.17)	0.41
16-17	384	32.7 (26.3, 39.0)	**2.12 (1.61, 2.80)**	**<0.0001**	69	47.5 (32.7, 62.2)	1.80 (0.89, 3.66)	0.10

	CT Seropositivity							
	Women who have had sex with men (WSM) only (n=1207)				Women who have ever had sex with a woman (WSW) (n=218)			
Characteristic	**N**	**Weighted Seroprevalence (95% CI)**	**Prevalence Ratio (95% CI)**	**P value***	**N**	**Weighted Seroprevalence (95% CI)**	**Prevalence Ratio (95% CI)**	**P value***
≥18	503	15.4 (11.1, 19.7)	1.0 (ref)	-	40	26.4 (10.5, 42.3)[a]	1.0 (ref)	-
# lifetime male sex partners								
0-1	294	10.0 (6.0, 14.1)	1.0 (ref)	-	18	7.7 (4.1, 11.4)	1.0 (ref)	-
2-4	388	23.8 (18.2, 29.4)	**2.37 (1.48, 3.78)**	**0.001**	29	30.9 (11.2, 50.6)	**3.99 (1.69, 9.45)**	**0.003**
5+	524	40.7 (34.4, 47.0)	**4.05 (2.61, 6.30)**	**<0.0001**	170	42.1 (32.4, 51.7)	**5.44 (3.15, 9.40)**	**<0.0001**
# lifetime female sex partners								
0	1207	28.6 (24.4, 32.9)	-	-	0	-	-	-
1	0	-	-	-	79	40.6 (29.0, 52.3)	1.0 (ref)	-
2-4	0	-	-	-	106	39.6 (28.1, 51.1)	0.98 (0.65, 1.46)	0.90
≥5	0	-	-	-	33	29.6 (12.4, 46.8)	0.73 (0.38, 1.38)	0.32
Frequency of condomless sex in								

	CT Seropositivity							
	Women who have had sex with men (WSM) only (n=1207)				Women who have ever had sex with a woman (WSW) (n=218)			
Characteristic	**N**	**Weighted Seroprevalence (95% CI)**	**Prevalence Ratio (95% CI)**	**P value***	**N**	**Weighted Seroprevalence (95% CI)**	**Prevalence Ratio (95% CI)**	**P value***
last year[5]								
Never	241	27.2 (19.9, 34.5)	1.0 (ref)	-	32	43.4 (22.8, 64.0)	1.0 (ref)	-
Less than half the time	204	33.4 (26.9, 39.9)	1.23 (0.87, 1.72)	0.23	30	46.7 (26.0, 67.4)	1.08 (0.54, 2.15)	0.83
About half the time	82	34.7 (21.7, 47.7)	1.28 (0.76, 2.14)	0.34	15	22.2 (2.4, 41.9)[b]	0.51 (0.18, 1.42)[b]	0.19[b]
More than half the time	122	35.9 (27.0, 44.9)	1.32 (0.95, 1.83)	0.09	33	43.2 (22.6, 63.9)	1.00 (0.47, 2.11)	0.99
Always	476	25.7 (20.4, 30.9)	0.94 (0.70, 1.28)	0.70	85	37.0 (27.3, 46.7)	0.85 (0.50, 1.46)	0.54
Prior history of HSV, genital warts, HPV, or gonorrhea[6]								
Yes	158	30.7 (23.6, 37.8)	1.09 (0.87, 1.35)	0.44	48	46.5 (28.8, 64.2)	1.30 (0.80, 2.13)	0.28
No	1047	28.2 (23.9, 32.5)	1.0 (ref)	-	170	35.7 (26.0, 45.5)	1.0 (ref)	-
Chlamydia in the past year[7]								

	CT Seropositivity							
	Women who have had sex with men (WSM) only (n=1207)				Women who have ever had sex with a woman (WSW) (n=218)			
Characteristic	**N**	**Weighted Seroprevalence (95% CI)**	**Prevalence Ratio (95% CI)**	**P value***	**N**	**Weighted Seroprevalence (95% CI)**	**Prevalence Ratio (95% CI)**	**P value***
Yes	53	76.9 (61.1, 92.7)	**2.85 (2.26, 3.60)**	**<0.0001**	15	68.3 (38.5, 98.1)	**1.87 (1.23, 2.85)**	**0.005**
No	1152	27.0 (22.8, 31.1)	1.0 (ref)	-	203	36.5 (28.5, 44.6)	1.0 (ref)	-
Sexual identity[8]								
Straight	1140	29.1 (24.7, 33.4)	1.0 (ref)	-	86	48.1 (37.7, 58.5)	1.0 (ref)	-
Lesbian or gay	0	-	-	-	25	41.5 (18.0, 65.0)	0.86 (0.43, 1.72)	0.66
Bisexual	32	17.5 (3.3, 31.8)[b]	0.60 (0.26, 1.42)[b]	0.24[b]	96	28.1 (15.7, 40.5)	**0.58 (0.37, 0.93)**	**0.03**
Other	5	[c]	[c]	[c]	6	44.3 (2.8, 85.9)[b]	0.92 (0.32, 2.65)[b]	0.87[b]

* P-values derived using univariate Poisson regression unless otherwise specified

[a] Relative standard error >30% but <40%; interpret with caution.

[b] Relative standard error >40% but <50%; interpret with caution.

[c] Relative standard error >50% or cell size <5, suppressed according to NCHS guidelines

[1] "Hispanic" includes Mexican American and participants of other Hispanic ethnicity; "Other" includes multirace, and all other racial/ethnic categories.

[2] Not asked of participants 18-19 years old.

[3] Income threshold for low-income was 130% FPL in the 2013-2014 cycle and 185% FPL in the 2015-2016 cycle. “Below low-income threshold” corresponds to being below 130% FPL for participants in the 2013-2014 cycle and being below 185% FPL in the 2015-2016 cycle.

[4] Zero participants reported an age of sexual debut below 13 years old.

[5] Information on past year condom use was only asked to individuals who reported vaginal or anal sex in the past year.

[6] History of HSV, HPV, or gonorrhea defined as ever being told by a doctor that they had HSV, genital warts, or HPV, or being told by a doctor within the last year that they had gonorrhea.
[7] "Yes" includes those who were either told by a doctor within the last year that they had chlamydia, or tested positive on a urine CT NAAT at the time of examination
[8] Between the 2013-2014 and 2015-2016 cycles, NHANES changed the possible answers for the sexual identity question: "Do you think of yourself as . . .". In 2013-2014, the answer options were: "Heterosexual or straight (attracted to men)", "Homosexual or lesbian (attracted to women)", "Bisexual (attracted to men and women)", "Something else", "Not sure", "Refused", and "Don't know". In 2015-2016, the answer options were: "Lesbian or gay", "Straight, that is, not lesbian or gay", "Bisexual", "Something else", "Not sure", "Refused", and "Don't know". For our analyses, those who answered "heterosexual" or "straight" were classified as straight, those who answered "homosexual" or "lesbian or gay" were lesbian or gay, those who answered "bisexual" were bisexual, and those who answered "something else" were classified as other.

Table 3. Multivariate analysis of factors associated with CT seropositivity among sexually experienced women aged 18 to 39 years in NHANES 2013-2016 cycles, by gender of sex partner, adjusted for factors significantly associated with seroprevalence for each gender of sex partner group in the univariate model

	Women who have had sex with men (WSM) only (n=1207)			**Women who have ever had sex with a woman (WSW) (n=218)**		
Characteristic	**N**	**Adjusted Prevalence Ratio (95% CI)**	**P-value***	**N**	**Adjusted Prevalence Ratio (95% CI)**	**P-value***
Age (per year)	1207	1.01 (0.99, 1.03)	0.23	218	1.01 (0.97, 1.05)	0.43
Race/ethnicity						
Non-Hispanic, White	413	1.0 (ref)	-	93	1.0 (ref)	-
Non-Hispanic, Black	205	**2.23 (1.73, 2.89)**	**<0.0001**	54	**2.16 (1.40, 3.34)**	**0.002**
Non-Hispanic, Asian	127	1.23 (0.80, 1.89)	0.33	11	[a]	[a]
Hispanic[1]	398	**1.52 (1.15, 2.02)**	**0.005**	44	1.55 (0.93, 2.59)	0.09
Other[1]	64	1.52 (0.93, 2.47)	0.09	16	**2.06 (1.06, 3.99)[b]**	**0.03[b]**
Income-poverty ratio[2]						
Below low-income threshold	499	**1.29 (1.01, 1.65)**	**0.04**	111	-	-
Above low-income	643	1.0 (ref)	-	99	-	-

	Women who have had sex with men (WSM) only (n=1207)			Women who have ever had sex with a woman (WSW) (n=218)		
Characteristic	**N**	**Adjusted Prevalence Ratio (95% CI)**	**P-value***	**N**	**Adjusted Prevalence Ratio (95% CI)**	**P-value***
threshold						
Age at sexual debut (per year)		**0.89 (0.86, 0.93)**	**<0.0001**		-	-
# lifetime male sex partners						
0-1	294	1.0 (ref)	-	18	1.0 (ref)	-
2-4	388	**1.59 (1.00, 2.53)**	**0.05**	29	**3.83 (1.52, 9.63)[b]**	**0.007[b]**
≥5	524	**2.29 (1.44, 3.64)**	**0.001**	170	**5.16 (2.29, 11.59)**	**0.0005**
Chlamydia in the past year[3]						
Yes	53	**1.87 (1.36, 2.57)**	**<0.001**	15	**1.97 (1.13, 3.46)**	**0.02**
No	1152	1.0 (ref)	-	203	1.0 (ref)	-
Sexual identity[4]						
Straight	1140	-	-	86	1.0 (ref)	-
Lesbian or gay	0	-	-	25	0.99 (0.53, 1.85)	0.97

	Women who have had sex with men (WSM) only (n=1207)			Women who have ever had sex with a woman (WSW) (n=218)		
Characteristic	**N**	**Adjusted Prevalence Ratio (95% CI)**	**P-value***	**N**	**Adjusted Prevalence Ratio (95% CI)**	**P-value***
Bisexual	32	-	-	96	**0.57 (0.34, 0.97)**	**0.04**
Other	5	-	-	6	0.81 (0.34, 1.89)[c]	0.60[c]

[*] P-values derived using multivariate Poisson regression unless otherwise specified

[a] Relative standard error >50%, suppressed according to NCHS guidelines

[1] "Hispanic" includes Mexican American and participants of other Hispanic ethnicity; "Other" includes multirace, and all other racial/ethnic categories. Prior to constructing these categories for the final analysis, we will first explore the seroprevalence in all race/ethnicity categories to verify that the 5-category variable is the appropriate way to represent race/ethnicity.

[2] Income threshold for low-income was 130% FPL in the 2013-2014 cycle and 185% FPL in the 2015-2016 cycle.

[b] Relative standard error >30% but <40%; interpret with caution.

[3] "Yes" includes those who were either told by a doctor within the last year that they had chlamydia, or tested positive on a urine CT NAAT at the time of examination

[4] Between the 2013-2014 and 2015-2016 cycles, NHANES changed the possible answers for the sexual identity question: "Do you think of yourself as . . .". In 2013-2014, the answer options were: "Heterosexual or straight (attracted to men)", "Homosexual or lesbian (attracted to women)", "Bisexual (attracted to men and women)", "Something else", "Not sure", "Refused", and "Don't know". In 2015-2016, the answer options were: "Lesbian or gay", "Straight, that is, not lesbian or gay", "Bisexual", "Something else", "Not sure", "Refused", and "Don't know". For our analyses, those who answered "heterosexual" or "straight" were classified as straight, those who answered "homosexual" or "lesbian or gay" were lesbian or gay, those who answered "bisexual" were bisexual, and those who answered "something else" were classified as other.

[c] Relative standard error >40% but <50%; interpret with caution.