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***Chlamydia trachomatis* infection in African American women who exclusively have sex with women**

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Abstract

Little is known about whether *Chlamydia trachomatis* can be sexually transmitted between women or how often it occurs in women who have sex with women (WSW). We investigated *Chlamydia trachomatis* prevalence and serum *Chlamydia trachomatis*-specific antibody responses among African American WSW who reported a lifetime history of sex only with women (exclusive WSW) ($n = 21$) vs. an age-matched group of women reporting sex with women and men (WSWM) ($n = 42$). Participants completed a survey, underwent a pelvic examination in which a cervical swab was collected for *Chlamydia trachomatis* nucleic acid amplification testing (NAAT), and had serum tested for anti-*Chlamydia trachomatis* IgG1 and IgG3 antibodies using a *Chlamydia trachomatis* elementary body-based ELISA. No exclusive WSW had a positive *Chlamydia trachomatis* NAAT vs. 5 (11.9%) WSWM having a positive *Chlamydia trachomatis* NAAT ($p = 0.16$). Compared with WSWM, WSW were significantly less likely to be *Chlamydia trachomatis* seropositive (7 [33.3%] vs. 29 [69%], $p = 0.007$). Among *Chlamydia trachomatis* seropositive women, all were seropositive by IgG1, and the magnitude of *Chlamydia trachomatis*-specific IgG1 responses did not differ in *Chlamydia trachomatis*-seropositive WSW vs. WSWM. In conclusion, *Chlamydia trachomatis* seropositivity was relatively common in exclusive African American WSW, though significantly less common than in African American WSWM.

Keywords

Chlamydia; sexually transmitted infection; antibody; immunoglobulin; women; WSW; African American

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Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics approval

The study was approved by the University of Alabama at Birmingham Institutional Review Board (Protocol # F110609002) and the Jefferson County Department of Health.

Introduction

It is estimated that 5–20% of women engage in sexual activities with women during their lifetime.^{1,2} Despite this, little is known about whether *Chlamydia trachomatis* (CT) infection, the most prevalent bacterial sexually transmitted infection (STI), can be sexually transmitted between women or how often CT infection occurs in women who have sex with women (WSW). Establishing that CT can be transmitted from one woman to another and understanding how frequently this may occur could be useful information for educational purposes (with regard to the risk of pelvic inflammatory disease and subsequent complications including chronic pelvic pain and infertility) and informing CT screening practices among WSW.

Reports of CT transmission between WSW have largely been anecdotal.³ More recently, Singh et al. analysed data from 9358 family planning clinic visits with CT urogenital tests among women aged 15–24 years who reported sexual activity in the past year exclusively with women (WSW; $n = 5,714$) and with women and men (WSWM) ($n = 3,644$).⁴ Characteristics were compared with women who reported sexual activity exclusively with men only (WSM; $n = 585,258$). Interestingly, CT positivity among both WSW and WSWM was 7.1% compared with 5.3% among WSM; behavioural risks were more commonly reported by WSW and WSWM.

As the Singh et al.'s study⁴ implies, complicating evaluation of CT transmission between women is that a large proportion of WSW also have a lifetime history of sex with men and continue to do so,^{3–5} making it difficult to establish the source of their infection. In addition, because of the asymptomatic nature of most CT infections, they often go undetected and may be treated through receipt of antibiotics given for other reasons or resolve spontaneously.⁶ Thus, data on CT risk among WSW based on self-report and/or nucleic acid amplification (NAAT) testing for current genital CT infection may underestimate the true cumulative prevalence of CT; addition of CT serology would provide the means to better estimate prior CT infection. Also, the majority of data on CT prevalence among WSW have been collected in Caucasians,^{4,7–11} despite data from the Centers for Disease Control and Prevention revealing that the CT infection rate in African Americans is over six times the rate for Caucasians.¹² Hence, it is important to investigate this rate among African American WSW since such a disparity may exist. Finally, WSW may not be as concerned about STI risks from female sexual partners¹³ and may not routinely get tested for STIs, including CT.

Using data and specimens collected from a cross-sectional study evaluating STI risks and prevalence among African American WSW,¹⁴ we used a sensitive CT serological test, as well as results from genital CT NAAT and patient interview, to investigate CT seroprevalence and current genital CT infection in WSW without a lifetime history of sexual contact with men (exclusive WSW) and to compare differences in these CT measures to WSW who have sex with men (WSWM).

Methods

Study design and population

African American women aged 16 years or older who acknowledged a female sexual partner during the past year were recruited for a cross-sectional study evaluating STI risk behaviours and prevalence among WSW ($n = 163$; 78 WSW and 85 WSWM, based on report of past year sexual behaviour [oral, vaginal, and/or anal] with female and male partners).¹⁴ These women were recruited from the Jefferson County Department of Health (JCDH) STD Clinic in Birmingham, AL, USA and from the local community. From this cohort (for the purposes of this study), we identified all lifetime exclusive WSW ($n = 21$) and age-matched them in a 1:2 ratio to WSWM ($n = 42$). Women were excluded if they were pregnant or nursing. Informed consent was obtained from all participants in the study. Approval for the study was obtained from the University of Alabama at Birmingham (UAB) Institutional Review Board and from the JCDH.

Clinical data and sample collection

At enrolment, data on socio-demographic characteristics, sexual and STI history, and substance use were collected through a written, interviewer-administered survey. Participants received a pelvic exam in which a vaginal swab was collected for wet mount and *Trichomonas vaginalis* culture and a cervical swab was collected for CT and *Neisseria gonorrhoeae* NAAT. Serum was collected for syphilis and HIV screening, and additional sera were stored for future research.

CT NAAT and serology

Cervical swabs were tested for CT using the Gen-Probe Aptima Combo 2 (Gen-Probe, Inc., San Diego, CA) NAAT by the manufacturer's protocol. Serum was tested for anti-CT IgG1 and IgG3 antibodies using a CT elementary body (EB)-based enzyme-linked immunosorbent assay (ELISA) by reported methods¹⁵; IgG1 and IgG3 antibody subclasses were targeted based on our prior research revealing these subclasses comprised the predominant anti-CT serum antibody responses.¹⁵ Briefly, the antigen used for the ELISA was density-gradient purified, formalin-fixed CT EBs of serovars D/UW-3, F/IC-Cal-13, and J/UW-36 (provided by Dr. Richard Morrison, University of Arkansas for Medical Sciences, USA). 96-well Immulon 2 HB U-bottom plates (Thermo Scientific, Waltham, MA, USA) were coated with 2 µg EB/well. IgG1 and IgG3 responses against CT were detected using Sigma Fast p-nitrophenyl phosphate substrate (1 mg/ml) (Sigma Co., St. Louis, MO, USA) and the following alkaline phosphatase labelled anti-human Ig monoclonal anti-bodies: anti-IgG1 (pool of clones 4E3 [Southern Biotech, Birmingham, AL, USA] and HP6069 [Cal Biochem, La Jolla, CA, USA]) and anti-IgG3 (clone HP6050, Southern Biotech). The optical density (OD) of reactions was measured at 405 nm (OD₄₀₅) and cutoff values for positive IgG1 and IgG3 were 0.35 and 0.1, respectively. The reported serological responses (OD₄₀₅ values) represents the mean of triplicate determinations of 1/32 diluted serum.

Statistical analysis

Analyses were conducted using Stata (Stata Corp. Release 8.0, College Station, TX, USA). The primary outcome was the proportion (with 95% confidence intervals) of exclusive WSW vs. WSWM with laboratory evidence of CT infection, either a positive genital CT NAAT or being CT seropositive, the latter defined as having detectable serum IgG1 or IgG3 anti-CT antibody responses at or above the cut-off OD₄₀₅ value for the antibody subclass. Differences in CT positivity and associated participant characteristics in exclusive WSW vs. WSWM were assessed using Fisher's exact or Chi-squared tests as appropriate. Differences in the proportion positive by each IgG subclass and differences in the magnitude of IgG subclass responses (OD₄₀₅ values) between exclusive WSW vs. WSWM was assessed by Fisher's exact and Wilcoxon rank sum tests, respectively.

Results

Patient characteristics

The characteristics of the overall study cohort of 163 WSW have been previously reported.¹⁴ Table 1 shows the baseline patient characteristics of the subpopulation of 21 exclusive WSW and 42 WSWM evaluated in this study. Exclusive WSW more often completed a high school (HS) education/GED than WSWM (95.2% vs. 71.4%, $p = 0.045$). Exclusive WSW less often had current health insurance than WSWM (38.1% vs. 69.1%, $p = 0.019$). There were no significant differences between groups in terms of having a primary healthcare provider (PCP), a history of incarceration, use of marijuana or other illicit drugs, or a history of sex in exchange for money or drugs. Compared with WSWM, exclusive WSW were significantly less likely to report prior STIs (19.1% vs. 64.3%, $p = 0.001$), including prior CT infection, which was only reported by WSWM (0% vs. 33.3%, $p = 0.003$).

CT prevalence, seropositivity and antibody subclass responses among exclusive WSW vs. WSWM

Overall, 36 (57.1%) women had positive laboratory tests for CT: all 36 were CT seropositive and five tested CT-positive on a cervical swab CT NAAT. Of the 36 CT seropositive, all were IgG1 positive vs. 27 (75%) being IgG3 positive. All five participants with a CT-positive cervical swab NAAT were CT seropositive for both IgG1 and IgG3. Of the 14 WSWM who reported prior CT infection, 10 (71.4%) were CT seropositive; the four who were seronegative had weak anti-CT antibody responses (IgG1 OD <0.1 and IgG3 OD <0.05), suggesting the possibility of inaccuracy in their report of prior CT infection. CT test positivity was associated with education level, but not having a PCP, a history of incarceration, or use of marijuana or illicit drugs. All 13 participants (100%) who did not complete an HS education/GED tested CT positive vs. 23 of 50 (46%) of participants who completed HS/GED testing CT positive ($p < 0.001$).

No exclusive WSW had a positive CT NAAT vs. five (11.9%; 95% CI 1.7%–22.1%) WSWM having a positive CT NAAT ($p = 0.16$); two of these five WSWM had only had sex with women in the prior 12 months. Compared with WSWM, exclusive WSW were significantly less likely to be CT seropositive (7 [33.3%; 95% CI 11.3%–55.3%] vs. 29 [69%; 95% CI 54.5%–83.6%], $p = 0.007$). All CT seropositive exclusive WSW and WSWM

were seropositive by IgG1. Among the CT seropositive women, exclusive WSW were significantly less likely to be seropositive by IgG3 compared with WSWM (3 [42.9%] vs. 24 [82.8%], $p = 0.05$). Among the 36 women who were CT seropositive by IgG1, there was no significant difference in magnitude of the response between exclusive WSW and WSWM: median OD (SD) in the seven exclusive WSW vs. 29 WSWM was 1.16 (0.42–3.63) vs. 1.45 (0.44–>4) ($p = 0.37$) (Figure 1). Among the 15 CT seropositive by IgG3, the magnitude of the response was significantly higher in the three exclusive WSW vs. 24 WSWM (0.88 [0.74–1.02] vs. 0.28 [0.10–1.74]; $p = 0.03$); however, the magnitude of the IgG3 response was similar between these three exclusive WSW when compared with the subset of seropositive WSWM who also had a positive cervical CT NAAT (0.88 vs. 0.79).

Discussion

Our study revealed that one-third of African American women who exclusively only had sex with women in their lifetime were CT seropositive, not only suggesting that CT infection may be sexually transmitted between women but also suggesting that it may be relatively common in this population. The CT seroprevalence we demonstrated in exclusive WSW was comparable with the CT seroprevalence rate of 31% reported by Geisler et al. in a study of young adult women (39% African American, 60% Caucasian), presumably most being WSM, from the Birmingham, AL community who were tested with the same assay used in our study (CT EB ELISA).¹⁵ Similar to our prior study⁵ and to that of others,^{8,9} a lifetime history of sex with men among the population of WSW was common, limiting the available sample size of exclusive WSW for evaluation. Prior studies investigating CT infection among WSW, the majority of which evaluated Caucasian women who also had a lifetime history of sex with men, evaluated for current genital CT infection and found genital CT prevalence to be between 0% and 7%.^{4,7–11} In our study, while no exclusive WSW had genital CT detected, we did find that five (11.9%) of WSWM had genital CT detected; two of these five WSWM who were genital CT NAAT positive had only had sex with women, not men, in the prior 12 months, suggesting their genital CT infection may have been transmitted by another woman. Cumulatively, these findings suggest CT antibody testing may be a valuable tool in estimating the proportion of a WSW population that has had CT infection before, as CT infection frequency will be underestimated by only performing periodic genital CT screening. As our study focused on African American WSW, it is possible CT seroprevalence rates may differ in WSW of a different race/ethnicity.

Another key finding of our study was that exclusive WSW had a significantly lower CT seroprevalence rate compared with WSWM (33% vs. 69%). One possible reason for this is that CT may be less transmissible through sexual practices of exclusive WSW (i.e. receptive oral sex, digital vaginal sex, use of sex toys, etc.) vs. women participating in penile-vaginal sex with a man. It is also possible that WSWM may be more likely to be exposed to a core male sexual group with a high genital CT prevalence and are therefore more often exposed to CT than exclusive WSW. Interestingly, we found CT seropositivity was associated with less education, and that exclusive WSW more often completed HS/GED than WSWM; hence, it is possible that CT seroprevalence is lower in exclusive WSW because of safer sexual practices they may have been learned related to their education.

Finally, we evaluated antibody subclass responses against CT to gain further possible insight into timing of CT infection. It is believed that IgG3 is involved in early response to infection and then IgG1 emerges as a major effector in clearing infection.¹⁶ We previously found that the magnitude of IgG3 responses against CT began to decline within six months after treatment of CT infection.¹⁵ It may be that the presence of anti-CT IgG3 reflects more recent CT infection and absence of anti-CT IgG3 in those with anti-CT IgG1 reflects more remote/past infection. Our study found that of seven exclusive WSW who were CT seropositive by IgG1, only three (42.9%) were IgG3 positive, suggesting a little less than half had more recent CT infections. In contrast, of the 29 WSW CT seropositive by IgG1, 24 (82.8%) had IgG3 responses against CT suggesting that they had more recent infections. While we speculated that the magnitude of IgG1 responses against CT might be stronger for CT seropositive WSW because they may have had more CT infections/exposures than CT seropositive exclusive WSW, we found no difference in magnitude of IgG1 responses against CT between the groups. There appeared to be a stronger anti-CT IgG3 response in exclusive WSW, though this was based on only three exclusive WSW CT seropositive by IgG3 and there was no difference in IgG3 responses in these three women compared with WSW who had a positive genital CT NAAT.

Our study has limitations. Data were collected from a small sample of African American exclusive WSW and WSWM presenting to an STI clinic in Birmingham, AL, USA and may not be generalisable to other populations of sexual minority women. Survey data were obtained from participant self-report and may be limited by recall bias (as it relates to reported prior chlamydia, addition of CT serology helped to more accurately and objectively determine prior chlamydia). In addition, of the 21 lifetime exclusive WSW, five reported a lifetime history of sexual assault (four of five by a male only and one of five by both a male and a female; only one woman in this group was CT seropositive and she was only positive by IgG1, suggesting a more remote infection). It is thus possible that these respondents (and potentially others such as sex workers or women performing home donor insemination for pregnancy with high-risk men providing the semen) did not consider this when denying a lifetime history of sex with men. Unfortunately, we were unable to collect data on the timing of sexual abuse among women reporting this history or where the sexual abuse occurred (i.e. oral, vaginal, and/or anal sex), which could influence the likelihood of being positive by IgG1 or IgG3. In addition, we did not ask women if they would classify themselves as a past or current sex worker or whether they have performed home donor insemination for pregnancy. Future studies of CT seropositivity in lifetime exclusive WSW should include these questions. In addition, a limitation of commercially available CT antibody assays has been sensitivity and specificity. We previously demonstrated that our CT EB ELISA was highly sensitive (detecting more genital CT NAAT-proven infections than a commercial CT major outer membrane protein based ELISA [90% vs. 73%]) and appeared to be specific when including evaluation for cross reactivity when testing the same sera with *C. pneumoniae* EBs.¹⁵ While we previously demonstrated no change in the magnitude of the IgG1 responses at six months after CT infection,¹⁵ we do not know how long anti-CT IgG1 responses are sustained. Based on our ongoing research in an infertility cohort in whom we have readily detected anti-CT IgG1 (unpublished), we suspect IgG1 responses are sustained for years. Finally, it has been postulated that exchange of infected cervico-vaginal secretions

may be the mechanism of CT transmission among WSW⁴; additional data are needed to further explore associations between specific sexual behaviours and CT transmission among exclusive WSW.

Conclusion

Our study revealed that CT seropositivity was relatively common in exclusive African American WSW, though significantly less common than in African American WSWM. Future partner studies involving WSW in which both female sexual partners are CT seroconcordant could provide additional data on CT transmission among WSW.

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Figure 1.

Antibody subclass-specific responses detected by *C. trachomatis* elementary body ELISA in exclusive WSW and WSWM. Serum anti-*C. trachomatis* IgG1 and IgG3 responses in exclusive WSW ($n = 21$) and WSWM ($n = 42$) are shown as dot plot graphs of OD₄₀₅ values. Solid horizontal lines illustrate the median and interquartile range responses in subjects who are seropositive for the shown antibody subclass. The broken line (- -) indicates the cutoff OD value for a positive response for IgG1 (0.35) and IgG3 (0.1), and the number of subjects seronegative for the shown antibody subclass are listed below the broken line. The magnitude of the IgG1 response did not significantly differ between the 7 of 21 (33.3%) exclusive WSW and 29 of 42 (69%) WSWM who were seropositive by IgG1 (median OD 1.16 vs. 1.45). Although IgG3 seropositivity occurred less often in exclusive WSW (3 of 21 [14.3%]) than in WSWM (24 of 42 [57.1%]), the magnitude of the IgG3 response was stronger in IgG3 seropositive exclusive WSW vs. WSWM (median OD 0.88 vs. 0.28; $p = 0.03$).

Table 1.

Patient characteristics of exclusive WSW vs. WSWM.

Characteristic	Exclusive WSW (<i>n</i> = 21)	WSWM (<i>n</i> = 42)	Total (<i>n</i> = 63)
Median age (range)	22 (18–32)	22 (17–34)	22 (17–34)
Completed high school/GED ^a	20 (95.2%)	30 (71.4%)	50 (79.3%)
Currently has a PCP	6 (28.6%)	15 (35.7%)	21 (33.3%)
Currently has health insurance ^b	8 (38.1%)	29 (69.1%)	37 (58.7%)
Illicit drug use ever			
Marijuana	13 (61.9%)	30 (71.4%)	43 (68.3)
Other	1 (4.8%)	1 (2.4%)	2 (3.2%)
Jailed ever	3 (14.3%)	9 (21.4%)	12 (19.1%)
Sex in exchange for money or drugs			
With female partners	0 (0%)	2 (4.8%)	2 (3.2%)
With male partners	0 (0%)	3 (7.5%) ^e	3 (4.9%) ^e
Reported prior STIs ^c	4 (19.1%)	27 (64.3%)	31 (49.2%)
Reported prior chlamydia ^d	0 (0%)	14 (33.3%)	14 (22.2%)

^a *p* = 0.045^b *p* = 0.019^c *p* = 0.001^d *p* = 0.003^e Data missing for 2 patients.

Note: Exclusive WSW: women reporting a lifetime history of sex with women only; WSWM: women reporting a history of sex with women and men; GED: has passed a general education development test; PCP: primary healthcare provider; STIs: sexually transmitted infections.