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Extragenital Screening Is Essential for Comprehensive Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in the Pediatric Population

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ABSTRACT *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are the two most common causes of sexually transmitted disease in the United States. Studies in adults, mostly in men who have sex with men, have shown that the prevalence of *C. trachomatis* and *N. gonorrhoeae* infections is much higher in extragenital sources compared to urogenital sources. A similar large sample of data on the burden of *C. trachomatis* and *N. gonorrhoeae* infections by anatomic site is lacking in children. We retrospectively analyzed data from 655 patients tested for *C. trachomatis* (887 specimens) and *N. gonorrhoeae* (890 specimens) at the Children's Hospital of Philadelphia. We restricted the analysis to include patients between 2 and 17 years of age that had all three sources (urine, oropharynx, and rectum) collected at the same visit. The final data set included specimens from all three sources from 148 and 154 patients for *C. trachomatis* and *N. gonorrhoeae*, respectively. Specimens were tested for *C. trachomatis* and *N. gonorrhoeae* using a Gen-Probe Aptima Combo 2 assay. The burden of *C. trachomatis* and *N. gonorrhoeae* infection was significantly higher in the 14- to 17-year age group (24.7%, $P = 0.041$; 25.8%; $P = 0.001$) compared to the 10- to 13-year (5.9%; 5.6%), 6- to 9-year (4.6%; 4.6%), and 2- to 5-year (8.3%; 0%) age groups, respectively. The positivity rate for *C. trachomatis* was highest for rectal (16.2%), followed by urine (5.4%) and oropharyngeal (0.7%) sites. The positivity rate for *N. gonorrhoeae* was highest for rectal sites (10.4%), followed by oropharyngeal (9.7%) and urine (1.9%) sites. The source with highest diagnostic yield is rectum for *C. trachomatis* and rectum and oropharynx for *N. gonorrhoeae*. Hence, extragenital screening is critical for the comprehensive detection of *C. trachomatis* and *N. gonorrhoeae* in the pediatric population.

KEYWORDS children, *Chlamydia trachomatis*, extragenital infection, *Neisseria gonorrhoeae*, pediatric, screening

Chlamydia trachomatis and *Neisseria gonorrhoeae* are the two most common causes of sexually transmitted diseases (STDs) in the United States (https://www.cdc.gov/std/stats17/natoverview.htm). In 2017, a total of 1,708,569 *C. trachomatis* infections were reported to the U.S. Centers for Disease Control (CDC; https://www.cdc.gov/std/stats17/chlamydia.htm).

Chlamydial STDs became nationally notifiable in 1995, and since then there has been a steady increase in the rate of reported infections in the United States. The infection rate increased from 367.5 cases per 100,000 population in 2007 to 528.8 cases per 100,000 population in 2017, a net 43.9% increase. The burden of *C. trachomatis* infection within the general U.S. population is highest among women of child-bearing age. This is particularly important because most of the infections can be asymptomatic, but if left untreated they can cause a myriad of medical issues, including pelvic

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inflammatory disease, infertility, ectopic pregnancy, and facilitate the transmission of HIV (1). Hence, it is imperative that proper diagnostic tools are in place to identify and treat such infections.

Gonorrhea is the second most common reported STD in the United States (https://www.cdc.gov/std/stats17/gonorrhea.htm). In 2017 a total of 555,608 cases of gonococcal infections were reported to the CDC. In the past 7 years, there has been an increase in the prevalence of *N. gonorrhoeae* infections in the United States from 100.2 cases per 100,000 population in 2010 to 171.9 cases per 100,000 population in 2017, a net 71.6% increase (2). The burden of gonococcal infection is highest among adolescents and young adults, mainly black men. Complications of gonococcal infection includes urethritis, dysuria, epididymitis, prostatitis, proctitis, arthritis, and conjunctivitis (3). The increase in the burden of gonococcal infection is particularly concerning, since this gonococci have been shown to readily develop resistance to antimicrobials (4, 5). The CDC regards drug-resistant *N. gonorrhoeae* as an urgent public health concern (4). A comprehensive testing strategy for *N. gonorrhoeae* is needed so that all infections can be identified and so appropriate treatment is initiated promptly to prevent further cycles of transmission. Current diagnostic methods have limitations, such as low sensitivity for culture or low specificity for the nucleic acid amplification test (NAAT) (3, 6, 7). NAAT-based assays have superior sensitivity compared to routine bacterial culture for *C. trachomatis* and *N. gonorrhoeae*, including in extragenital sources (3, 6). This could be particularly important in detecting infections in children and adolescents that are victims of sexual assault. According to the National Intimate Partner and Sexual Violence Survey in 2011, there were 1.8 cases of sexual abuse per 1,000 children and adolescent in the United States (https://www.acf.hhs.gov/opre/research/project/national-incidence-study-of-child-abuse-and-neglect-nis-4-2004-2009). Detection of STI in children under the age of consent (13 years in the state of Pennsylvania) would be evidence of crime in the state of Pennsylvania (https://www.pcar.org/laws-policy/age-consent). In the United States, current NAAT-based diagnostic assays are not approved for extragenital sources; therefore, diagnostic laboratories have to validate the assays for clinical use (7).

Studies in adults, primarily studies performed with men who have sex with men (MSM), have shown that the burden of *C. trachomatis* STDs and gonorrhea is disproportionately higher in the oropharynx and rectum compared to urogenital sites such as urine (3, 8, 9). Because of sampling convenience, urine testing alone for *N. gonorrhoeae* and *C. trachomatis* is common (3). Therefore, a substantial proportion of infections will be missed if only just one source is tested, primarily urine. Based on these data, current CDC recommendations include annual screening for *C. trachomatis* and *N. gonorrhoeae* at extragenital sources based on risk factors (oral or anal exposure) (3). Current recommendations on sexually transmitted infection (STI) screening in children exist only for survivors of sexual assault (10). For adolescents, this includes NAAT-based testing on extragenital sources with a history of sexual contact at those anatomic sites. For sexually abused children, NAAT can be performed on urine specimens but is not recommended for extragenital testing since some assays have a lower specificity for *N. gonorrhoeae*, primarily in the oropharynx.

Data on the relative yield of sampling the oropharynx, rectum, and urine for *C. trachomatis* and *N. gonorrhoeae* laboratory testing are sparse in pediatric populations, mainly due to limited testing of these sources and probably also due to relatively low suspicion of extragenital infection in this patient population (10, 11). A recent study in sexually abused children showed that *C. trachomatis* and *N. gonorrhoeae* infections were detected at extragenital sites in the absence of a history of extragenital sexual contact, highlighting the importance of comprehensive screening practices, especially in children being evaluated for sexual assault (12). A limitation of that study is that it only included patients that potentially had a high-risk exposure and did not determine discordances in *C. trachomatis* and *N. gonorrhoeae* positivity by anatomic site.

In this study, we present data from a large population of children tested for STDs that

shows that *N. gonorrhoeae* and *C. trachomatis* extragenital infections are common and that a large fraction of these infections will be missed if only urine testing is performed.

MATERIALS AND METHODS

Study population. We retrospectively analyzed data from 655 patients from whom 887 and 890 specimens, respectively, were collected for *C. trachomatis* and *N. gonorrhoeae* testing at the Infectious Disease Diagnostics Laboratory (IDDL) at the Children's Hospital of Philadelphia (CHOP) over a 5-year period from 1 October 2012 to 27 October 2017. We then restricted the analysis to include patients that were between 2 and 17 years old and had all three anatomic sites (urine, oropharynx, and rectum) sampled at the same visit. The final analysis included all three types of specimen from 148 patients for *C. trachomatis* and 154 patients for *N. gonorrhoeae*. The median age was 15 years (interquartile range, 7 to 17 years), and 40.9% of the patients were female. We also analyzed the overall prevalence, regardless of the number of sites tested (one or more) at the same visit for *C. trachomatis* and *N. gonorrhoeae* in this population.

The clinical sites that sent samples for *C. trachomatis* and *N. gonorrhoeae* testing were almost exclusively (96%) outpatient locations and included CHOP's emergency department (32% of patients), the adolescent family planning clinic (17.6%), and the primary care service (9.7%).

Laboratory testing methods. Specimens submitted to the IDDL were tested with a target amplification nucleic acid probe on the Aptima Combo 2 (AC2) assay for *C. trachomatis* and *N. gonorrhoeae* (Hologic, Sunnyvale, CA) (7). The nucleic acid amplification test (NAAT) is the method of choice for detecting *C. trachomatis* and *N. gonorrhoeae* in clinical specimens, including those obtained from extragenital sites (13, 14). Hologic's AC2 assay is U.S. Food and Drug Administration (FDA) approved for male urine and endocervical, vaginal, and urethral swabs (7). This assay has FDA approval for testing in all ages, including pediatric samples; however, the performance of this assay has not been evaluated in children younger than 14 years of age (7). The analytic sensitivity of the AC2 assay is one inclusion-forming unit (IFU)/assay for *C. trachomatis* and 50 cells/assay for *N. gonorrhoeae* (7). According to the manufacturer, there is no cross-reactivity of the *C. trachomatis* and *N. gonorrhoeae* targets with other 154 bacterial, viral, parasitic, and fungal isolates tested, including nongonococcal *Neisseria* spp. However, cross-reactivity of *N. gonorrhoeae* with other commensal *Neisseria* spp., such as *Neisseria meningitidis* and *Neisseria sicca*, that reside in extragenital sites has been reported with an AC2 assay, albeit at a very low frequency (15). Bachmann et al. showed that NAAT analyses of oropharyngeal samples on three different platforms (Aptima Combo 2 assay, BD ProbeTec ET, and Roche Cobas Amplicor) were more sensitive than culture (91.9 to 100% versus 65.4%) for the diagnosis of oropharyngeal *N. gonorrhoeae* (6). The specificity of all three NAATs was inferior to that of culture; however, the specificity of the AC2 assay (96.2%) was better than the other two NAATs (BD, 94.2%; Roche, 71.8%) and close to that of culture (99%). None of the positive oropharyngeal samples in this study were confirmed by a second method (culture or an alternate NAAT target); hence, we cannot completely exclude the possibility of false-positive *N. gonorrhoeae* samples.

We did an in-house validation of the oropharyngeal swab for *C. trachomatis* and *N. gonorrhoeae* by testing 64 previously characterized positive and negative samples. Limit-of-detection (LoD) experiments were performed by serial dilution of commercial control material in a negative oropharyngeal swab matrix. The LoD for oropharyngeal swabs was 0.025 IFU for *C. trachomatis* and 3.2 cells for *N. gonorrhoeae* per reaction. Our validation data did not show any false-positive *N. gonorrhoeae* in oropharyngeal sources. The positive and negative agreement for both *C. trachomatis* and *N. gonorrhoeae* was 100%; hence, the assay was deemed acceptable for *C. trachomatis* and *N. gonorrhoeae* testing in the oropharynx. Similar validation studies were performed for rectal swab specimens with acceptable performance.

Statistical analyses. Chi-squared tests were used to assess differences in the results by age and sex. A binomial exact test was used to estimate the prevalence of *C. trachomatis* and *N. gonorrhoeae* infections and the 95% confidence interval (CI) by age and anatomic site. The binomial exact test was also used to estimate the percentage of *C. trachomatis* and *N. gonorrhoeae* infection missed and the 95% CI by different screening sites (urine only, rectum only, and oropharynx only). Data were analyzed using STATA version 13.0 (STATA Corp., College Station, TX).

RESULTS

Among patients with specimens available from all three anatomic sources (urine, oropharynx, and rectum), the burden of *C. trachomatis* infection was significantly higher (24.7%; $P = 0.041$) for the 14- to 17-year-old group than for the 10- to 13-year-old (5.9%), the 6- to 9-year-old (4.6%), and the 2- to 5-year-old (8.3%) groups (Table 1). Likewise, the prevalence of gonorrhea was significantly higher in 14 to 17 year olds (25.8%; $P = 0.001$) than in 10 to 13 year olds (5.6%), 6 to 9 year olds (4.6%), and 2 to 5 year olds (0%). In summary, 14 to 17 year olds are more likely to have *C. trachomatis* and *N. gonorrhoeae* infections, a finding that is most consistent with the increased sexual activity in adolescence.

There were a total of 148 patients that had specimens submitted from all three anatomic sites for *C. trachomatis* testing, with 25 patients (16.9%) testing positive from at least one site (Fig. 1 and Table 1). The anatomic sources of the majority of positive samples were the rectum (16.2%), followed by urine (5.4%) and the oropharynx (0.7%). Of the 444 samples tested from all sites for *C. trachomatis* from the 148 patients, 33 samples

TABLE 1 Prevalence of *C. trachomatis* and *N. gonorrhoeae* infections by age among patients sampled at all three anatomic sites (urine, oropharynx, and rectum) during the same visit

Age range (yr)	% (95% CI), no. positive/total no. of samples	
	C. trachomatis	*N. gonorrhoeae*
2–5	8.3 (1.02–27.0), 2/24	0 (0–13.71), 0/25
6–9	4.5 (0.11–22.84), 1/22	4.5 (0.11–22.84), 1/22
10–13	5.9 (0.14–28.7), 1/17	5.5 (0.14–27.29), 1/18
14–17	24.7 (16.0–35.3), 21/85	25.8 (17.14–36.21), 23/89
Overall positivity by patient	16.9, 25/148	16.2, 25/154

were positive (7.4%) (Table 2). For *N. gonorrhoeae*, there were 154 patients with specimens submitted for testing from all three sites, with 16.2% of the patients having a positive test (Fig. 1 and Table 2). By sampling site, the positivity rate was highest for rectal sites (10.3%), followed by oropharyngeal (9.7%) and urine (1.9%) sites. Overall, 7.3% of all 462 samples tested from all sites for *N. gonorrhoeae* from the 154 patients were positive.

We then looked at the potential percentage of chlamydial and gonococcal infections that would be missed by screening just one anatomic site. Among patients with all three anatomic sources tested for *C. trachomatis* infection, 96, 68 and 4% would have been missed by testing only the oropharynx, urine and rectum alone, respectively (Fig. 2). Likewise, for *N. gonorrhoeae* infections, 88, 40, and 36%, respectively, would have been missed by testing only the oropharynx, urine, and rectum alone (Fig. 2).

Patients that had all three anatomic sites tested for *C. trachomatis* and *N. gonorrhoeae* likely represented a high-risk population, with three-site testing performed possibly because of elicited or suspected risk factors. Therefore, we also looked at the overall prevalence of *C. trachomatis* and *N. gonorrhoeae* in children of the same age range regardless of the number of sites tested (one or more) at the same visit, including children who had all three sites sampled at the same visit. A total of 887 specimens were tested for *C. trachomatis*, with a positivity rate of 6.9%. The positivity rate was highest for rectal samples (10.2%), followed by urine (5.6%) and then the oropharynx (1.1%). Likewise, for *N. gonorrhoeae*, a total of 890 specimens were available for testing, with 5.7% testing positive. The positivity rate was highest for the oropharynx (9.8%), followed by rectal samples (6.2%) and urine (2.8%).

DISCUSSION

We show that the prevalence of *C. trachomatis* and *N. gonorrhoeae* infection is significantly higher in 14 to 17 year olds than in younger children, which most likely

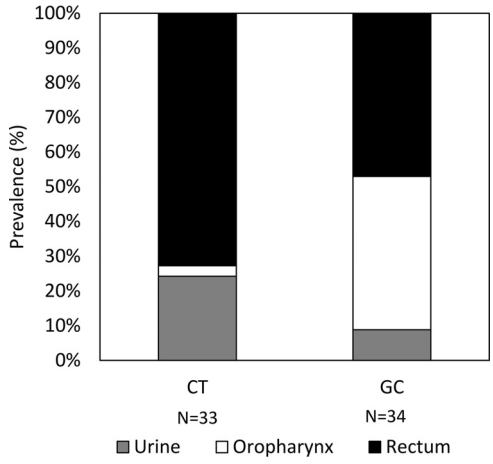

FIG 1 Distribution of *C. trachomatis* and *N. gonorrhoeae* infections by anatomic sites. CT, *C. trachomatis*; GC, *N. gonorrhoeae*.

TABLE 2 Prevalence of *C. trachomatis* and *N. gonorrhoeae* infections by anatomic sites among patients where samples from all three anatomic sites were available for testing

Site	% (95% CI), no. positive/total no. of samples	
	C. trachomatis	*N. gonorrhoeae*
Oropharynx	0.7 (0.02–3.7), 1/148	9.7 (5.5–15.5), 15/154
Rectum	16.2 (10.7–23.2), 24/148	10.3 (6.05–16.32), 16/154
Urine	5.4 (2.4–10.4), 8/148	1.9 (0.40–5.58), 3/154
Overall positivity by sample	7.43, 33/444	7.35, 34/462

is due to greater sexual activity in adolescence. This observation is supported by findings from a recent study of children and adolescents that showed increasing seropositivity for *C. trachomatis* with age, likely reflecting increasing sexual behavior (16).

Studies in adults show that the distribution of chlamydial and gonococcal infection is heterogeneous by anatomic site and can be mostly attributed to the site of sexual contact (3, 8, 9, 17–19). In the present study, the positivity rate for *C. trachomatis* was 16.9% in children between 2 and 17 years of age that were tested at all three anatomic sites; this rate is higher than the rates reported for the adult MSM population (10 to 13.3%) (17, 19). This is probably due to potential high-risk exposure in these children that prompted testing of all three anatomic sources compared to routine voluntary testing done in adult MSM population through STD clinics. Our study shows a discordance in positivity for *C. trachomatis* by anatomic site, with the highest positivity rates for rectal specimens compared to urine and oropharyngeal specimens. This is similar to the findings in the adult MSM population, where the highest prevalence of *C. trachomatis* has been shown to be the rectum (7.4 to 23%), followed by urine (2.3 to 5.2%) and the oropharynx (1.4 to 1.9%) (9, 18–20). Likewise, for gonorrhea, the overall prevalence was 16.2%, which is about the same as the overall prevalence of *N. gonorrhoeae* in the high-risk adult MSM population (16.7%) (19). Among patients with gonorrhea, there was also a discordance in infection rate by anatomic site, with the highest positivity rate for rectal and oropharyngeal specimens and the lowest positivity rate for urine specimens. These findings are similar to what is observed in the adult MSM population, with the rectum (3.6 to 24%) or oropharynx (5 to 9.2%) being the most common sites of infection, and urine being the least common site (0.4 to 6.0%) (3, 9, 18–20). There are a limited number of studies examining the prevalence of rectal *C. trachomatis* infection and gonorrhea in the adult female population. Such studies show a high rate of extragenital infection with *C. trachomatis* (3.7 to 13%) and *N. gonorrhoeae* (2.4 to 6%) in women with high-risk exposure (21, 22). Hence, our data on the high prevalence of *C. trachomatis* and *N. gonorrhoeae* in extragenital sources are in agreement with the published literature for the adult MSM population and adult women. This study in children also shows that detection of most chlamydial infections would have been

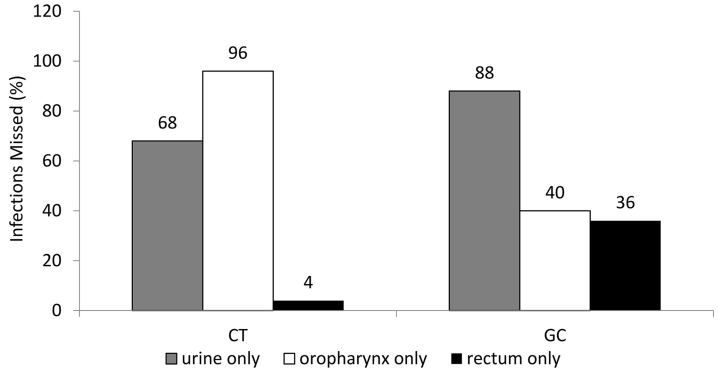

FIG 2 *C. trachomatis* and *N. gonorrhoeae* infections missed by different screening practices. CT, *C. trachomatis*; GC, *N. gonorrhoeae*.

missed by testing just the oropharynx or urine, with the fewest cases being missed by testing rectal specimens. Likewise, for gonococcal infections, most cases would have been missed by testing urine alone compared to the significantly lower number of cases missed by testing oropharyngeal and rectal sources.

There were no patients positive for *C. trachomatis* or *N. gonorrhoeae* in all three anatomic sites. It is important to note that most oropharyngeal infections with *C. trachomatis* and *N. gonorrhoeae* are asymptomatic; hence, in the absence of screening, such silent infections may not be diagnosed and thus remain untreated (20). This is important since the individual could serve as a reservoir for ongoing transmission cycles, especially with increasing reports of oral-genital sexual practice among adolescents (23, 24). A recent study in children evaluated for sexual victimization showed that *C. trachomatis* and *N. gonorrhoeae* can be detected in anatomic sources where no prior sexual contact was reported (12). A comprehensive screening practice for *C. trachomatis* and *N. gonorrhoeae* with a history or suspicion of recent sexual contact could aid in diagnosis of STI.

Extensive sampling to identify cases of STI is imperative in the pediatric population since the diagnosis of STIs after the neonatal period in children under the age of consent (13 years in the state of Pennsylvania) can be considered a matter of sexual assault (https://www.pcar.org/laws-policy/age-consent). In fact, data from an evidence-based systematic review showed that in children younger than 12 years of age, most of the cases of *C. trachomatis* (75 to 94%) and *N. gonorrhoeae* (36 to 85%) infections were linked to sexual assault (25). A recent study in children and adolescents being evaluated for sexual abuse shows that *C. trachomatis* infection and gonorrhea were detected in extragenital sites (oropharynx and rectum), even when sexual contact with the abuser's genitals was not reported (12). A recent study showed that appropriate STI testing, including for *C. trachomatis* and *N. gonorrhoeae* in extragenital sites was done only for 5% of adolescents presenting to the emergency department with oropharyngeal or anorectal chief complaints (26). This is concerning, particularly due to the high burden of extragenital infection compared to that of urogenital sites.

One of the main limitations of this study is generalizability, since we restricted our main analysis to include patients with specimens collected from all three anatomic sources collected at the same time. This probably represents a high-risk population, as pediatricians are most likely to collect sample from all three sources when there is history of oral and anal exposure. In fact, CHOP's emergency department pathway for the evaluation of children for sexual abuse recommends *C. trachomatis* and *N. gonorrhoeae* testing on any anatomic site with possible exposure (https://www.chop.edu/clinical-pathway/sexual-abuse-concerns-clinical-pathway-indications-sti-screening). Other limitations include a relatively small sample size, even though we reviewed 5 years of data, since the collection of all three specimen types is uncommon, even in adults.

In conclusion, our study shows that the burden of *C. trachomatis* and *N. gonorrhoeae* infection is highest in the 14- to 17-year age group compared to other pediatric age groups and that extragenital site sampling, especially of the rectum, increases diagnostic yield. Our study highlights the significance of comprehensive screening practices, including genital and extragenital sites for detection of *C. trachomatis* and *N. gonorrhoeae* in the pediatric population. We are hoping that the data presented by our study will encourage physicians to test more anatomic sources when there is history or suspicion of sexual contact. The lack of FDA-cleared assays that include extragenital sites makes such testing more difficult, and these results will hopefully encourage diagnostic assay manufacturers to pursue FDA clearance for *C. trachomatis* and *N. gonorrhoeae* testing in extragenital sites.

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