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

Priyanka Uprety¹,², Ana María Cárdenas¹,²

¹Infectious Disease Diagnostics Laboratory, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA

²Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

Running head: Extragenital CT/GC Infection in Children

Address correspondence to Ana María Cárdenas, amcardenas@gmail.com

Present address: 3401 Civic Center Blvd, Philadelphia, PA 19104
Background: *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) are the two most common causes of sexually transmitted disease in the US. Studies in adults, mostly in MSM, have shown that the prevalence of CT/GC infections is much higher in extragenital sources compared to urogenital sources. Similar large sample size data on the burden of CT/GC infections by anatomic site is lacking in children.

Methods: We retrospectively analyzed data from 655 patients tested for CT (887 specimens) and GC (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 dataset included specimens from all three sources from 148 and 154 patients for CT and GC, respectively. Specimens were tested for CT/GC using the Gen-Probe Aptima Combo 2 Assay.

Results: The burden of CT and GC infection was significantly higher in the 14-17-year age group (24.7%; \( p = 0.041 \) and 25.8%; \( p = 0.001 \)) compared to the 10-13 year (5.9%, 5.6%), 6-9 year (4.6%, 4.6%) and 2-5 year (8.3%, 0%) age groups, respectively. The positivity rate for CT was highest for rectal (16.2%) followed by urine (5.4%) and oropharyngeal (0.7%) sites. The positivity rate for GC was highest for rectal sites (10.4%), followed by oropharyngeal (9.7%) and urine (1.9%).

Conclusions: The source with highest diagnostic yield is rectum for CT and rectum and oropharynx for GC. Hence, extragenital screening is critical for the comprehensive detection of CT and GC in the pediatric population.

INTRODUCTION
Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (GC) are the two most common causes of sexually transmitted diseases (STDs) in the United States (US) (https://www.cdc.gov/std/stats17/natoverview.htm). In 2017, a total of 1,708,569 CT infections were reported to the US Centers of Disease Control (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 US. 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 CT infection within the general US 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 can cause a myriad of medical issues including pelvic 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 US (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 GC infections in the US 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 include urethritis, dysuria, epididymitis, prostatitis, proctitis, arthritis and conjunctivitis (3). The increase of the burden of gonococcal infection is particularly concerning,
since this organism has been shown to readily develop resistance to antimicrobials (4, 5). CDC regards drug-resistant GC as an urgent public health (4). A comprehensive testing strategy for GC is needed so that all infections can be identified and appropriate treatment initiated promptly to prevent further cycles of transmission. Current diagnostic methods have limitations, with low sensitivity for culture or low specificity for nucleic acid amplification test (NAAT) (3, 6, 7). NAAT-based assays have superior sensitivity compared to routine bacterial culture for CT/GC, 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 1000 children and adolescent in the US (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 US, 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 done in the men who have sex with men (MSM), have shown that the burden of CT 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 GC and CT 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 CT and GC at extragenital sources.
based on risk factors (oral or anal exposure) (3). Current recommendation on STI screening in children exist only for survivors of sexual assault (10). For adolescents, this includes NAAT-based testing on extragenital sources with history of sexual contact at those anatomic sites. For children of sexual assault, NAAT is acceptable for urine specimens, but not recommended for extragenital testing as some assays have lower specificity for GC, primarily in the oropharynx.

Data on the relative yield of sampling the oropharynx, rectum and urine for CT and GC laboratory testing is sparse in pediatric populations, mainly due to limited testing of these sources and probably due to relatively low suspicion of extragenital infection in this patient population (10, 11). A recent study in sexually abused children showed that CT and GC 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 recent study is that it only included patients that potentially had a high-risk exposure and did not determine discordances in CT/GC positivity by anatomic site.

In this study, we present data from a large population of children tested for STDs that shows that GC and CT 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 were collected for CT and GC testing, respectively at the Infectious Disease Diagnostics Laboratory (IDDL) at the Children’s Hospital of Philadelphia (CHOP) over a 5-year period from 10/1/2012 to 10/27/2017. 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 CT and 154 patients for GC. The median age was 15 years (IQR: 7, 17) and 40.9% of the patients were female. We also analyzed the overall prevalence, regardless of the number of sites tested (1 or more) at the same visit for CT and GC in this population.

The clinical sites that sent samples for CT and GC 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 Assay (AC2) for CT/GC (Hologic, Sunnyvale, CA) (7). Nucleic acid amplification tests (NAAT) is the method of choice for detection of CT and GC in clinical specimens, including in extragenital sites (13, 14). Hologic’s AC2 assay is FDA-approved for male urine, endocervical, vaginal and urethral swab (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 less than 14 years of age (7). The analytic sensitivity of the Aptima Combo 2 assay is 1 inclusion-forming unit/assay for CT and 50 cells/assay for GC (7). Per the
manufacturer, there is no cross-reactivity of the CT and GC targets with other 154 bacterial, viral, parasitic and fungal isolates tested, including non-gonococcal Neisseria spp. However, cross-reactivity of GC with other commensal Neisseria spp. that reside in extragenital sites like Neisseria meningitidis and Neisseria sicca has been reported with APTIMA Combo 2 assay, albeit at very low frequency (15). Bachmann et al. showed that NAAT testing on oropharyngeal samples on three different platforms (Aptima Combo 2 Assay, BD ProbeTec ET, Roche Cobas Amplicor) was more sensitive than culture (91.9-100% vs. 65.4%) for the diagnosis of oropharyngeal GC (6). Specificity of all 3 NAATs was inferior to that of culture, however specificity of Aptima Combo 2 assay (96.2%), was better than the other two NAAT (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 alternate NAAT target), hence we cannot completely exclude the possibility of false-positive GC samples. We did an in-house validation of the oropharyngeal swab for CT/GC by testing 64 previously characterized positive and negative samples. Limit of detection (LoD) experiments were done by serial dilution of commercial control material) in a negative oropharyngeal swab matrix. The LoD for oropharyngeal swab was 0.025 inclusion forming unit (IFU) for CT and 3.2 cells for GC per reaction. Our validation data did not show any false positive GC in oropharyngeal sources. The positive and negative agreement for both CT/GC was 100%, hence the assay was deemed acceptable for CT/GC testing in the oropharynx. Similar validation studies were done for rectal swab specimens with acceptable performance.

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

RESULTS
Among patients with specimens available from all three anatomic sources (urine, oropharynx and rectum), the burden of CT infection was significantly higher (24.7%; p=0.041) for the 14 to 17-year-old group, compared to the 10 to 13 (5.9%), 6 to 9 (4.6%) and 2 and 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), compared to the 10 to 13 (5.6%), 6 to 9 (4.6%) and 2 to 5 year-olds (0%). In summary, 14 to 17 year olds are more likely to have CT/GC infection, most consistent with increased sexual activity in adolescence.

There were a total of 148 patients that had specimens submitted from all three anatomic sites for CT testing, with 25 patients (16.9%) testing positive from at least one site (Figure 1, 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%). Thirty-three of the 444 samples tested from all sites for CT from the 148 patients were positive (7.4%) (Table 2). For GC, there were 154 patients with specimens submitted for testing from all three sites with 16.2% of the patients having a positive test (Figure 1, 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 GC 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 CT infection, 96, 68 and 4% would have been missed by testing only the oropharynx, urine and rectum alone, respectively (Figure 2). Likewise, for GC infection, 88, 40 and 36% would have been missed by testing only the oropharynx, urine and rectum alone, respectively (Figure 2).

Patients that had all three anatomic sites tested for CT and GC 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 CT/GC in children of the same age range regardless of the number of sites tested (one or more) at the same visit, including the children who had all three sites sampled at the same visit. A total of 887 specimens were tested for CT, with a positivity rate of 6.9%. The positivity rate was highest for rectal samples (10.2%), followed by urine (5.6%) then the oropharynx (1.1%). Likewise, for GC, there were a total of 890 specimens 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 CT and GC infection is significantly higher in 14-17 year-olds, compared to younger children, which most likely is due to greater sexual activity in adolescence. This observation is supported by findings from a recent study on children and
adolescents that showed increasing seropositivity for CT with age, likely representing 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 this study in children between 2 and 17 years of age that were tested for CT in all three anatomic sites, the positivity rate for CT was 16.9%, which is higher than the rates of 10-13.3% reported in the adult MSM population (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 CT by anatomic site with highest positivity rates for rectal specimens, compared to urine and oropharyngeal specimens. This is similar to the findings in adult MSM population where the highest prevalence of CT has been shown to be the rectum (7.4-23%), followed by urine (2.3-5.2%) and the oropharynx (1.4-1.9%) (9, 18-20). Likewise, for gonorrhea, the overall prevalence was 16.2%, which is about the same as the overall prevalence of GC 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 with what is observed in adult MSM population with the rectum (3.6-24%) or oropharynx (5.9.2%) being the most common sites of infection, and urine being the least common site (0.4-6.0%) (3, 9, 18-20). There are a limited number of studies looking at the prevalence of rectal CT infection and gonorrhea in the adult female population. Such studies show a high rate of extragenital infection with CT (3.7-13%) and GC (2.4-6%) in
women with high-risk exposure (21, 22). Hence, our data on high prevalence of CT and GC 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 missed by testing just the oropharynx or urine, with the fewest cases being missed by testing rectal specimens. Likewise, for gonococcal infections, most of the cases would have been missed by testing urine alone, compared to significantly lower number of cases missed by oropharyngeal and rectal sources.

There were no patients positive for CT or GC in all three anatomic sites. It is important to note that most oropharyngeal infections with CT/GC are asymptomatic, hence in absence of screening, such silent infections may not be diagnosed and 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 CSA showed that CT/GC can be detected in anatomic sources with no prior sexual contact (12). A comprehensive screening practice for CT/GC with a history or suspicion of recent sexual contact could aid in diagnosis of STI.

Extensive sampling to identify cases of sexually transmitted infections (STI) is imperative in the pediatric population as the diagnosis of STI’s 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 less than 12 years of age, most of the cases of CT (75-94%) and GC (36-85%) infections were linked to sexual assault (25). A recent study in children and adolescents being evaluated for sexual abuse shows that CT 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 CT/GC 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 high burden of extragenital infection compared to that of urogenital sites.

One of the main limitations of this study is generalizability, as 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 evaluation of children with sexual abuse recommends CT/GC 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 collection of all three-specimen types is uncommon, even in adults.

In conclusion, our study shows that the burden of CT/GC infection is highest in the 14-17-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 CT/GC 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 CT/GC testing in extragenital sites.

NOTES

Disclaimer. None

Conflict of Interest. All authors: No potential conflicts.

Financial Support: None

REFERENCES


### TABLES

Table 1: Prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections by age among patients with all 3 anatomic sites (urine, oropharynx and rectum) sampled at the same visit.

<table>
<thead>
<tr>
<th>Age Category (Years)</th>
<th>% CT (95% CI) # positive/ total</th>
<th>% GC (95% CI) # positive/ total</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections by anatomic sites (urine, oropharynx or rectum) among patients where samples from all three anatomic sites were available for testing.

<table>
<thead>
<tr>
<th>Anatomic Site</th>
<th>% CT (95% CI)</th>
<th>% GC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># positive/ total</td>
<td># positive/ total</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>0.7 (0.02-3.7) 1/148</td>
<td>9.7 (5.5-15.5) 15/154</td>
</tr>
<tr>
<td>Rectum</td>
<td>16.2 (10.7-23.2) 24/148</td>
<td>10.3 (6.05, 16.32) 16/154</td>
</tr>
<tr>
<td>Urine</td>
<td>5.4 (2.4-10.4) 8/148</td>
<td>1.9 (0.40, 5.58) 3/154</td>
</tr>
<tr>
<td>Overall positivity by sample</td>
<td>7.43 (3.4-10.1) 8/444</td>
<td>7.35 (3.4-10.1) 34/462</td>
</tr>
</tbody>
</table>

**Abbreviations:** CT, *Chlamydia trachomatis*; GC, *Neisseria gonorrhoeae*; CI, Confidence Interval

**FIGURES**

Figure 1: Distribution of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections by anatomic sites
Figure 2: *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Infections Missed by Different Screening Practices

Abbreviations: CT, *Chlamydia trachomatis*; GC, *Neisseria gonorrhoeae*
Abbreviations: CT, Chlamydia trachomatis; GC, Neisseria gonorrhoeae

Downloaded from http://jcm.asm.org/ on February 13, 2021 by guest