Screening for *Chlamydia trachomatis* Infection in College Women with a Polymerase Chain Reaction Assay

Robert L. Cook, Kirsten St. George, Maryann Lassak, Nhung Tran, John P. Anhalt, and Charles R. Rinaldo

This study sought to determine factors associated with chlamydial infection in a low-prevalence college health setting and to determine the testing characteristics of a polymerase chain reaction (PCR) assay for chlamydial infection (AMPLICOR chlamydia test; Roche Diagnostic Systems, Indianapolis) in this population. Young women (*n* = 1,149) at a university student health clinic underwent testing for cervical chlamydial infection by PCR assay and culture; the characteristics of women with and without chlamydial infection were compared. Chlamydial infection was diagnosed for 26 students (2.3%). The sensitivity and specificity of PCR assay and culture were 85% and 100% and 54% and 100%, respectively. Students with chlamydial infection were more likely to be 20 years of age or younger, have symptoms, report prior chlamydial infection or gonorrhea, report exposure to a sexually transmitted disease (STD), be black, or have cervical signs during examination; however, none of these were significant predictors for asymptomatic women. PCR assay detected significantly more cervical infections than did culture in this college student population. These data are consistent with recommendations for testing college women with symptoms, STD exposure, or age of younger than 25 years.

*Chlamydia trachomatis* infection is the most commonly reported sexually transmitted disease (STD) in the United States [1]. In young women, chlamydia is a primary cause of pelvic inflammatory disease, which can lead to ectopic pregnancy, infertility, and other adverse health outcomes [2, 3]. An additional and important consequence of chlamydial infections is that they appear to increase two- to fourfold the risk of HIV transmission through sexual intercourse [4, 5].

Most genital chlamydial infections in women are asymptomatic; therefore, screening has been recommended for those at risk. Several tests are available for chlamydia screening including culture, direct fluorescent antibody staining, immunologic detection of antigen (EIAs), analysis with DNA probes, and DNA amplification tests such as PCR and ligase chain reaction assays [6]. Until recently, culture was considered the accepted standard for chlamydia testing because of its 100% specificity. This standard has been reconsidered because the newer nonculture tests have detected cases of chlamydial infection missed by culture and have improved sensitivities, although direct fluorescent antibody staining and EIA occasionally have false-positive results [6]. Nearly all previous evaluations of chlamydia testing characteristics have included high-risk populations. However, diagnostic tests may have different sensitivities and specificities for clinically distinct populations [7], and there is evidence to suggest that chlamydia tests may be less sensitive for populations in low-prevalence settings or asymptomatic populations [8–11]. To our knowledge, no previous study has reported the testing characteristics of PCR analysis for a college health population, among whom the average prevalence of chlamydial infection in 1997 was estimated to be between 2% and 3% [12].

Health care planners must also decide whom to screen for chlamydial infection. Screening recommendations include selective screening based on patient history and physical examination findings [13–15], universal screening of all women younger than a certain age [16–18], and combinations of universal screening for younger women and selective screening for older women [2]. Risk factors for chlamydial infection may be unique in low- and high-prevalence settings [19], and we could identify no recent study that specifically examined factors associated with chlamydial infection in students in a low-prevalence college health setting.

The objectives of this study were to identify specific clinical or historical factors associated with chlamydial infection that might be used to help develop screening criteria for college
women and to determine the testing characteristics of a PCR assay for chlamydia and chlamydia culture for students in a low-prevalence college health care setting.

Methods

The study population included consecutive women seen in the gynecology section of an urban university health center between March 1995 and April 1996. All students who were having a routine or acute gynecologic examination were invited to participate in the study; clinicians estimated that >80% of those invited agreed to participate. Clinicians recorded appropriate historical information and clinical findings in the student’s health record. Each student also completed a brief form about her current contraceptive use. During the examination, the clinician obtained two cervical swabs from each participant, one for chlamydia culture and the other for PCR analysis for chlamydia. The order of the swabs was reversed halfway through the study.

Chlamydia culture and PCR testing for chlamydia were performed in the same laboratory. Swabs for chlamydia culture were collected into M4 transport media. Supernatant media was decanted from shell vial cultures of BGMK cells (Viromed, Minneapolis), and 1 mL of M4 transport media was inoculated per vial. Inoculated vials were centrifuged at 1,000 g for 1 hour at 37°C, the inoculum was decanted, and the cultures were overlaid with 1 mL of chlamydia isolation medium (Bartels, Issaquah, WA) per vial. After 48 hours of incubation at 37°C, cultures were fixed with methanol, and inclusion bodies were visualized by immunofluorescent staining of the major outer membrane protein with the Syva MicroTrak C. trachomatis culture confirmation test (Behring Diagnostics, Cupertino, CA). Stained cultures were observed under a fluorescence microscope at a magnification of ×250 for typical green fluorescent inclusion bodies.

PCR testing for chlamydia was performed with the AMPLICOR chlamydia test (Roche Diagnostic Systems, Indianapolis), which amplifies on the cryptic plasmid of C. trachomatis. PCR analysis was performed according to the manufacturer’s instructions. Briefly, swab specimens were collected into PCR collection tubes (Roche Diagnostic Systems). Specimen diluent was added to samples, and 50 µL of diluted samples together with 50 µL of Master Mix (Roche Diagnostic Systems) was transferred to PCR reaction tubes. Positive and negative controls supplied in the kit, as well as positive and negative samples generated in the laboratory, were included in every test batch. Amplifications were performed on 9600 Thermal Cyclers (Perkin-Elmer, Norwalk, CT) according to the manufacturer’s instructions.

Amplified samples were denatured, and a portion was transferred to hybridization plates. After hybridization at 37°C for 1 hour, reaction wells were incubated with avidin–horseradish peroxidase conjugate followed by substrate. Color reactions were stopped with sulfuric acid, and the results were read on a Titertek Multiskan spectrophotometer (ICN Pharmaceuticals, Costa Mesa, CA) at 450 nm. Absorbances of <0.2, 0.2–0.5, and >0.5 were considered negative, equivocal, and positive, respectively. Equivocal samples were tested again in duplicate, and results of the three tests (one original and two repeats) were analyzed. If two of the three test results showed an absorbance of >0.25, the sample was considered positive.

If the result was negative by culture but positive by PCR analysis, the test sample was sent to a different laboratory (Roche Diagnostic Systems) for confirmatory testing with PCR analysis at a different site on the genome, the major outer membrane protein gene. If the sample was positive by culture but negative by PCR analysis, the AMPLICOR chlamydia test was repeated in the same laboratory.

Chlamydial infection was diagnosed if the culture was positive, regardless of the results of PCR analysis. If the culture was negative but PCR analysis was positive, chlamydial infection was diagnosed only if the confirmatory PCR test at the second site was also positive. By using the expanded case definition, the sensitivity, specificity, positive predictive value, and negative predictive value were determined for culture and PCR tests.

Three students without chlamydial infection who enrolled in the study consecutively after each case were chosen as controls. Thus, controls came from the same base population, and their tests were performed under the same laboratory conditions. Three controls were chosen to obtain sufficient power (β = 0.8) to detect odds ratios for chlamydial infection in the 3.0 to 4.0 range at an α level of .05; additional controls would not have significantly improved the statistical power. One control was selected twice; only the data from her first visit were included in the analysis.

Clinical data were obtained by retrospective chart abstraction by a trained research assistant who was not informed of the study purpose. University records provided the age and race of the student if these data were not clearly labeled in the chart. Information about the student’s current symptoms, sexual risk behaviors, history of any STD, history of gonorrhea or chlamydial infection, exposure to a partner with STD, and clinical examination findings were abstracted directly from clinical notes in the charts. Use of condoms over the previous year (always, sometimes, or never) was recorded from information the student had provided at study enrollment.

The percentages of cases and controls with each clinical and historical characteristic were determined. Univariate analyses were conducted first with the total sample and then for asymptomatic women and their controls by means of the χ² or Fisher’s exact test with use of Stata statistical software (Stata Corporation, College Station, TX). Odds ratios and 95% confidence intervals were determined for each factor with use of an α level of .05 (two-sided). Each factor that was found to be statistically significant in the univariate analysis was then included in a multivariate conditional logistic regression model for matched case-control groups.

Results

Twenty-six of the 1,149 students were diagnosed with chlamydial infection (overall prevalence, 2.3%). Four cases were
Table 1. Univariate associations of chlamydial infection with demographic, behavioral, and clinical factors for female college students from 1995 to 1996.

<table>
<thead>
<tr>
<th>Factor</th>
<th>No. (%) of subjects</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases (n = 26)</td>
<td>Controls (n = 77)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 y or older</td>
<td>4 (15)</td>
<td>21 (27)</td>
<td>Referent</td>
</tr>
<tr>
<td>21–24 y</td>
<td>8 (31)</td>
<td>36 (47)</td>
<td>1.2 (0.3–4.1)</td>
</tr>
<tr>
<td>20 y or younger</td>
<td>14 (54)</td>
<td>20 (26)</td>
<td>3.7 (1.1–12.4)</td>
</tr>
<tr>
<td>Black</td>
<td>9 (35)</td>
<td>3 (4)</td>
<td>13.0 (3.4–49.4)</td>
</tr>
<tr>
<td>Any prior STD</td>
<td>7 (27)</td>
<td>12 (16)</td>
<td>2.0 (0.7–5.6)</td>
</tr>
<tr>
<td>Prior chlamydial infection or gonorrhea</td>
<td>4 (15)</td>
<td>3 (4)</td>
<td>4.5 (1.0–19.4)</td>
</tr>
<tr>
<td>Partner with STD</td>
<td>3 (12)</td>
<td>1 (1)</td>
<td>9.9 (1.3 to &gt;100)</td>
</tr>
<tr>
<td>Always uses condoms*</td>
<td>5 (26)</td>
<td>21 (30)</td>
<td>0.8 (0.3–2.5)</td>
</tr>
<tr>
<td>Current symptoms²</td>
<td>14 (54)</td>
<td>21 (27)</td>
<td>3.1 (1.2–7.7)</td>
</tr>
<tr>
<td>Clinical finding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal discharge³</td>
<td>10 (38)</td>
<td>17 (22)</td>
<td>2.5 (1.0–6.6)</td>
</tr>
<tr>
<td>Cervical friability or discharge</td>
<td>5 (19)</td>
<td>4 (5)</td>
<td>4.4 (1.1–16.4)</td>
</tr>
</tbody>
</table>

NOTE. STD = sexually transmitted disease.
* Data missing for 15 participants (seven cases and eight controls).
† Including discharge, itching, and dyspareunia.
‡ Data missing for two participants (one case and one control).

Table 2. Multivariate associations of chlamydial infection with demographic, behavioral, and clinical factors for female college students from 1995 to 1996.

<table>
<thead>
<tr>
<th>Factor</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of 20 y or younger</td>
<td>3.5 (1.0–12.8)</td>
<td>.05</td>
</tr>
<tr>
<td>Black</td>
<td>28.1 (2.9–208)</td>
<td>.004</td>
</tr>
<tr>
<td>Partner with STD</td>
<td>33.3 (1.3–872)</td>
<td>.03</td>
</tr>
</tbody>
</table>

NOTE. STD = sexually transmitted disease.

Diagnosed by culture alone; 10, by culture and PCR analysis; and 12, by PCR analysis alone. Of the four students whose infections were diagnosed by culture alone, two had PCR analysis results that were positive on repeated testing. The repeated test results were not included in the analysis. All specimens that were initially PCR analysis–positive and culture-negative were confirmed to be positive by the alternative PCR method. The sensitivity, specificity, positive predictive value, and negative predictive value for PCR analysis and culture were 85%, 100%, 100%, and 99.6% and 54%, 100%, 100%, and 99%, respectively.

Clinical and historical features of the 26 cases and the 77 controls were compared (table 1). Age of 20 years or younger, black race, a partner with STD, current symptoms, and clinical signs of cervical friability or discharge were each significantly associated with chlamydial infection (P < .05). Of these factors, only age of 20 years or younger, black race, and exposure to STD were statistically significant predictors of chlamydial infection in logistic regression analysis, and the 95% confidence intervals for these predictors were very wide (table 2).

When the analysis was restricted to women without symptoms, none of the risk factors were significantly associated with chlamydial infection (table 3). Of the asymptomatic women with chlamydial infection, 42% were younger than 21 years of age, and 75% were younger than 25 years of age. A screening strategy that tested all women with symptoms or who were exposed to STD and that tested asymptomatic women younger than 25 years of age would have identified 92% of women infected with chlamydia in this sample.

Discussion

Screening young women for chlamydial infection has been widely recommended because it reduces the risk of pelvic inflammatory disease [20] and may reduce the risk of HIV transmission [21]. However, there are conflicting conclusions about the best strategy for selecting women to screen for chlamydial infection [2, 14, 16–18]. Several recent analyses concluded that age alone may be the best predictor of infection in settings where the prevalence is 9% to 20% [16, 17] or 3% to 6% [18].

In this college health setting, the overall prevalence of chlamydial infection was 2.3%, and the prevalence among asymptomatic women was <2%. This prevalence is lower than infection rates in college health settings reported in the 1980s [22, 23] but is consistent with the average prevalence in 36 U.S. college health clinics participating in a more recent survey from the American College Health Association [12]. College students account for one-quarter of the U.S. population aged 18–24 years and represent a population with numerous risk factors for chlamydial infection. Approximately 75% of female...
Table 3. Univariate associations of chlamydial infection with demographic, behavioral, and clinical factors for asymptomatic female college students from 1995 to 1996.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Cases (n = 12)</th>
<th>Controls (n = 26)</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 y or older</td>
<td>3 (25)</td>
<td>12 (46)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21–24 y</td>
<td>4 (33)</td>
<td>9 (35)</td>
<td>1.8 (0.3–9.0)</td>
<td>&gt;.2</td>
</tr>
<tr>
<td>20 y or younger</td>
<td>5 (42)</td>
<td>5 (19)</td>
<td>2.2 (0.4–11.8)</td>
<td>&gt;.2</td>
</tr>
<tr>
<td>Black</td>
<td>2 (17)</td>
<td>1 (4)</td>
<td>5.0 (0.6 to &gt;100)</td>
<td>&gt;.2</td>
</tr>
<tr>
<td>Any prior STD</td>
<td>3 (25)</td>
<td>2 (8)</td>
<td>4.0 (0.7–23.5)</td>
<td>&gt;.2</td>
</tr>
<tr>
<td>Prior chlamydial infection or gonorrhea</td>
<td>2 (17)</td>
<td>0</td>
<td>...* (1.2 to &gt;100)</td>
<td>.09</td>
</tr>
<tr>
<td>Partner with STD</td>
<td>2 (17)</td>
<td>0</td>
<td>...* (1.2 to &gt;100)</td>
<td>.09</td>
</tr>
<tr>
<td>Always uses condoms(^2)</td>
<td>4 (40)</td>
<td>6 (26)</td>
<td>1.8 (0.4–8.6)</td>
<td>&gt;.2</td>
</tr>
<tr>
<td>Clinical finding</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal discharge(^3)</td>
<td>3 (27)</td>
<td>4 (15)</td>
<td>2.1 (0.4–10.4)</td>
<td>&gt;.2</td>
</tr>
<tr>
<td>Cervical friability or discharge</td>
<td>2 (17)</td>
<td>2 (8)</td>
<td>2.4 (0.4–15.7)</td>
<td>&gt;.2</td>
</tr>
</tbody>
</table>

NOTE. STD = sexually transmitted disease.
* Unable to determine OR with no controls.
\(^{2}\) Data missing for five students (two cases and three controls).
\(^{3}\) Data missing for one case.

college students aged 18–24 years report sexual activity at least once, 25% have six or more lifetime sexual partners [24, 25], and <50% use condoms [24–26]. The only factors that were predictive of chlamydial infection in the logistic regression analysis were age of 20 years or younger, black race, and a partner with STD, and the 95% confidence intervals for these estimates were very wide (table 2). Surprisingly, there was no decrease in the prevalence of chlamydial infection among students who reported always using condoms, although this finding is consistent with recent results from studies in other settings [16, 17]. Chlamydial infection has been found to be more common in blacks in some populations [14, 18, 23, 27] but not in others [13, 28]. There is no known biological reason that race should affect rates of chlamydial infection; nevertheless, membership in a specific ethnic group strongly influences the potential pool from which one chooses sex partners and will increase the likelihood of contracting chlamydial infection once the disease is introduced into that population [29].

This study found that PCR testing for chlamydia detected significantly more infections than did chlamydia culture in this female college student population. Several tests are available to screen for chlamydial infection. Tests with greater sensitivity will detect more infections, and tests with greater specificity will have fewer false-positive results. In this study, we were unable to compare PCR analysis with other nonculture chlamydia tests such as EIA, direct fluorescent antibody staining, and DNA probe analysis. Other studies have found that these nonculture tests are more sensitive than culture but generally less sensitive and specific than PCR analysis [6]. The sensitivity of culture in this study (54%; 95% CI, 33%–73%) was lower but not significantly different from the 56%–85% sensitivity found in other studies that compared culture with PCR analysis [6, 8]. However, one could postulate that the chlamydia infectious burden was lower for some women in this low-prevalence college health setting, thereby resulting in a lower sensitivity of culture.

The sensitivity of PCR analysis that was calculated for this population (85%; 95% CI, 65%–95%) was also slightly lower but not significantly different from that found for other populations in low-prevalence settings where sensitivities of PCR analysis ranged from 91% to 95% [30, 31]. The presence of PCR inhibitors in some samples could have interfered with the assay [32] and may account for our finding that two culture-positive cases were initially negative by PCR assay but later positive by repeated testing. Newer versions of commercial PCR assays include an internal control to detect the presence of inhibitors.

Several limitations of the study deserve mention. Most of the clinical and historical data were collected by retrospective chart review, thus resulting in missing data on specific sexual risk behaviors for 80% of the subjects. It is probable that some women who had never had sexual intercourse were tested as part of the study, and no data from males were collected. The method used to confirm discrepant test results has been criticized by some investigators because all samples are not tested with the third method [33], yet any error is likely to be small. Finally, the study did not have sufficient power to detect small differences in risk factors for asymptomatic women because of the small number of cases of asymptomatic chlamydial infection.

The excellent testing characteristics of PCR analysis raise the question of whether this type of test should be more widely
used in college health services. Even though DNA amplification tests such as PCR analysis often cost more than other testing methods, they may be the best overall choice for a population in a low-prevalence setting because of their high specificity, which is especially important for these populations because it will lower the percentage of false-positive results. DNA amplification tests offer the additional advantage of non-invasive testing of urine samples or self-collected vaginal swab specimens, which provide new opportunities for screening young women who are not having a gynecologic examination [34].

The issue of whether college health programs should screen all women, selectively screen based on age alone, or selectively screen based on age and other risk factors remains uncertain. Universal screening of all women will likely identify all infections in a population but is the most expensive testing strategy. In settings like some college health services where the prevalence among asymptomatic women is low, cost pressures lead to attempts to restrict screening to only the highest risk groups. Selective screening will save money allocated to testing but may miss some chlamydial infections. Age-based screening is generally more cost-effective when the disease prevalence is low and can be cost-saving as long as the age-based criteria will identify most of the infections [35]. Additional cost-effectiveness analyses may be useful to assist with screening decisions when the prevalence is very low (e.g., <2%). Until additional data are available, a screening strategy consistent with recent recommendations to test women with symptoms, those who have been exposed to STD, and all asymptomatic young women over 25 years of age appears to be sound and would have identified 92% of the cases of chlamydial infection in this population.

Acknowledgments

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References