

# Increasing Chlamydia Positivity in Women Screened in Family Planning Clinics: Do We Know Why?

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**Objective:** Following a 9-year 60% decline, chlamydia positivity increased 46% from 1997 through 2004 among young sexually active women screened in Region X family planning clinics. The objective of this analysis was to systematically examine the influences of risk factors, changing laboratory test methods, and interclinic variability on chlamydia positivity during this period.

**Study Design:** We analyzed data from 520,512 chlamydia tests from women aged 15 to 24 years screened in 125 family planning clinics. Multivariate logistic regression modeling was used to adjust the annual risk of chlamydia for the demographic, clinical, and sexual risk behavior characteristics associated with infection and for the increasing use of more sensitive laboratory test methods. A generalized linear mixed model was used to adjust for interclinic variability.

**Results:** We found a significant 5% annual increase in the risk of chlamydia even after adjusting for risk factors including laboratory test characteristics (odds ratio 1.05, 95% confidence interval: 1.04, 1.06). Variability among the clinics where screening occurred did not account for the increase.

**Conclusions:** Based on a review of all available data, we concluded that there was a true increase in chlamydia positivity over the 8-year period.

CHLAMYDIA TRACHOMATIS IS THE MOST common bacterial sexually transmitted infection (STI) in the United States, with an estimated 2.8 million new cases occurring each year.<sup>1</sup> Chlamydial infections are often asymptomatic, can persist for a prolonged period, and are an important preventable cause of reproductive sequelae in women, including pelvic inflammatory disease (PID), ectopic pregnancy, and infertility.<sup>2</sup> Screening for chlamydia has been shown to reduce the incidence of PID.<sup>3</sup>

In 1988, the first widespread screening and treatment program for chlamydia began in US Public Health Service Region X (Alaska, Idaho, Oregon, and Washington). The focus of the Region X Infertility Prevention Project is to screen all young sexually active women seen in the region's Title X family planning clinics.<sup>4</sup> During the first 9 years of the program, chlamydia positivity among women aged 15 to 24 years declined over 60%, from 10.3% in 1988 to 4.0% in 1996.<sup>5</sup> This decline corresponded with significant reductions in self-reported sexual risk behaviors.<sup>6</sup> Similar

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declines in chlamydia positivity were also seen in other areas of the United States where broad-based screening programs were instituted during the same time period.<sup>7–9</sup> However, there was a 46% increase in chlamydia positivity, from 3.9% in 1997 to 5.7% in 2004, among young women screened in Region X family planning clinics.<sup>5</sup> There have been many questions and much speculation about the reasons for the increases in positivity, including changes in laboratory test technology and screening higher-risk women; however, there have been no analyses systematically evaluating potential causes for these increases. The 3 objectives of our analysis were to examine: 1) demographic, clinical, and sexual behavioral risk characteristics associated with chlamydial infection and their influences on chlamydia positivity in women aged 15 to 24 years seen in Region X family planning clinics from 1997 through 2004; 2) the impact of changing laboratory test methods on the increases in positivity; and 3) the effect of interclinic variability on chlamydia positivity using a generalized linear mixed model.

## Materials and Methods

### Data Sources

We analyzed data from 520,512 chlamydia tests from women aged 15 to 24 years screened in 125 family planning clinics participating in the Region X Infertility Prevention Project from 1997 through 2004 (average 65,000 tests/yr). Women aged 24 years and younger were routinely screened for chlamydial infection at least annually as recommended by the Centers for Disease Control and Prevention and the US Preventive Service Task Force.<sup>10,11</sup>

All Region X family planning clinics used a common medical record form. Information collected included age; race; ethnicity; specimen collection date; clinical findings (ectopy, friable cervix, PID, cervicitis); self-reported sexual risk behaviors (having had a new sex partner in the past 60 days, having had multiple sex partners in the past 60 days, having had a symptomatic sex partner in the past 60 days, having had a sex partner who was diagnosed with chlamydia, and condom use during last sex); having had chlamydia in the past year; laboratory test type; and chlamydia test result. We included clinics that routinely screened for chlamydia during the entire 8-year period and had performed 50 or more chlamydia tests in at least 7 of the 8 years (about 90% of all chlamydia tests performed). We used data from the US Census to

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estimate the population density of the town or city where each family planning clinic was located and to examine the distribution of tests by clinic location (urban vs. rural).

### Laboratory Methods

Four state public health laboratories (Alaska, Idaho, Oregon, and Washington), a county health district laboratory (Spokane), and the University of Washington Chlamydia Laboratory performed chlamydia testing for the 125 family planning clinics. From 1997 through 2004, these laboratories switched to newer, more sensitive test methods, i.e., nucleic acid amplification tests (NAATs). Over the time period, non-NAATs included (in order of decreasing usage) enzyme immunoassays (MicroTrak II, Syva and Behring Diagnostic Products, Cupertino, CA); nucleic acid hybridization tests (Pace 2, Gen-Probe, San Diego, CA); nucleic acid hybridization assays (Hybrid Capture 2, Digene, Gaithersburg, MD); and cell cultures. The majority of NAATs used in Region X were ligase chain reaction tests (LCx, Abbott, Abbott Park, IL) and target capture transcription-mediated amplification assays (Aptima Combo 2, Gen-Probe, San Diego, CA).

### Statistical Analyses

Observed chlamydia positivity was calculated by dividing the number of positive tests by the total number of tests that were either positive or negative. We excluded tests with unsatisfactory or indeterminate results. For our first objective, among the variables collected, potential predictors of chlamydial infection were identified by odds ratios (OR) and 95% confidence intervals that were significant at  $P$  value  $<0.05$ . Stepwise logistic regression modeling was used to assess the risk of chlamydial infection for these predictors including demographic characteristics, clinical findings, self-reported sexual risk behaviors, type of laboratory test (NAAT vs. non-NAAT), and year of test. A  $P$  value  $<0.01$  was used for determining statistical significance. To assess the trend in risk of chlamydia by laboratory test type, we included an interaction term, year-laboratory test type, in the multivariate model.

Our second objective focused on changes in laboratory test methods and estimating the true disease prevalence. A switch to a more sensitive laboratory test method can result in an increase in observed chlamydia positivity even with no increase in true disease prevalence.<sup>12</sup> We used the sensitivity and specificity of each laboratory test method to calculate an adjusted positivity [test-specific adjusted positivity = (test-specific observed positivity + test specificity - 1)/(test sensitivity + test specificity - 1)].<sup>13</sup> We calculated the overall adjusted positivity as a weighted sum of the adjusted positivities for each test type. The sensitivity and specificity estimates used for the adjustment were culture (sensitivity 0.747, specificity 1.000); enzyme immunoassay tests with negative gray zone confirmation (sensitivity 0.810, specificity 0.996); other non-NAATs including Gen-Probe Pace 2 and Digene Hybrid Capture 2 assay (sensitivity 0.619, specificity 0.997); and, NAATs (sensitivity 0.855, specificity 0.997).<sup>14,15</sup> We compared the differences in trends for observed and adjusted chlamydia positivity.

Our third objective was to account for the variability in chlamydia positivity across the 125 family planning clinics. We used a generalized linear mixed model to examine the probability of a positive chlamydia test controlling for the random selection of the family planning clinics and the fixed effects of each of the identified risk factors. Specifically, the model assumed that total numbers of positive chlamydia tests at each level of a risk factor were independent binomial random variables, and the levels of the risk factor were linearly related to the logit of the probabilities of a positive chlamydia test. The model assigned all observations from

the same clinic the same adjustment to the intercept term, and these adjustments varied randomly from clinic to clinic. For the modeling we used the GLIMMIX procedure in Version 9.1 of SAS.<sup>16</sup>

## Results

### Who Was Screened for Chlamydia?

Of the 520,512 chlamydia tests performed from 1997 through 2004, 52% occurred in the state of Washington (Table 1). Twenty-two percent of the chlamydia tests were performed in women aged 15 to 17 years, 26% in women aged 18 to 19 years, and 52% in women aged 20 to 24 years. The majority of the tests occurred among non-Hispanic white women. Twenty-two percent of the tests were done in clinics located in small towns with populations  $<25,000$ ; 44% were done in clinics located in cities with 25,000 to under 100,000 residents, and the remaining 34% were done in clinics located in cities with populations of 100,000 or greater.

Twenty-six percent of tests were in women who reported having 1 or more sexual behavior risks in the past 60 days. Ninety-three percent of the tests were in women who had no clinical findings for chlamydial infection on physical examination. Three percent of the tests were among women who reported having had a previous chlamydial infection in the past year.

### Did the Demographic, Clinical, or Sexual Behavioral Risk Characteristics of the Women Screened Change Over Time?

The percent of women aged  $<18$  years decreased from 24% to 18% from 1997 through 2004; the percent of women aged 18 to 19 years remained stable, 25%; and the percent of women aged 20 to 24 years increased from 51% to 56% (Fig. 1). The race/ethnicity distribution remained stable over time. Although chlamydia screening volume increased 38% from 1997 through 2004, the distribution of tests performed across clinics located in different population densities, i.e., urban versus rural, did not change over time (data not shown). The proportion of women reporting sexual risk behaviors or who had a clinical finding on physical examination remained stable or decreased over the time period (Fig. 2). The proportion of women reporting having had a previous chlamydial infection in the past year increased from 2.6% in 1997 to 3.7% in 2004.

### Did the Change in Laboratory Test Methods Account for the Increase in Chlamydia Positivity?

There was an increase in the use of NAATs from 13% in 1997 to 60% in 2004 (Fig. 3). The observed chlamydia positivity increased to 46%. After adjusting chlamydia positivity to account for the use of more sensitive laboratory test methods and to better estimate true chlamydia prevalence, there continued to be an increase in positivity (65%) over the time period. This increase in adjusted positivity persisted when a range of laboratory test sensitivities and specificities for each laboratory test method were used (data not shown).

### Did Changes in Risk Factors Account for the Increase in Chlamydia Positivity?

Chlamydia positivity was associated with young age, nonwhite race, clinic city size  $>100,000$ , having 1 or more self-reported sexual behavioral risks, having had a sex partner with chlamydia, not using a condom at last sex, having 1 or more clinical findings, having had a positive chlamydia test in the past year, use of NAATs, and year (Table 2, Crude OR; model 1 adjusted ORs). Using multivariate logistic regression modeling to adjust for all the

TABLE 1. Characteristics of Women Aged 15–24 Yrs. and Chlamydia Positivity in Region X Family Planning Clinics, 1997–2004

Characteristic	No.	Percent	Chlamydia Positivity (%)
All women	520,512	100.0	5.1
State			
Alaska	11,773	2.3	4.4
Idaho	81,443	15.6	4.6
Oregon	157,751	30.3	3.9
Washington	269,545	51.8	6.0
Age group (yr)			
15–17	115,152	22.1	5.9
18–19	137,130	26.3	5.8
20–24	268,230	51.5	4.5
Race/ethnicity			
White, non-Hispanic	388,454	77.0	4.5
African American, non-Hispanic	24,017	4.8	10.1
American Indian/Alaskan native	5501	1.1	8.7
Asian/Pacific islander	20,691	4.1	6.8
Hispanic	65,590	13.0	5.9
Clinic city size, persons			
<10,000	49,810	9.6	4.0
10,000–24,999	64,857	12.5	4.6
25,000–49,999	91,172	17.5	5.1
50,000–99,999	138,563	26.6	5.4
100,000–499,999	98,390	18.9	5.7
≥500,000	77,720	14.9	5.2
New sex partner, past 60 d			
No	385,585	77.1	4.4
Yes	114,303	22.9	7.7
More than one sex partner, past 60 d			
No	450,068	90.2	4.7
Yes	48,891	9.8	9.4
Symptomatic sex partner, past 60 d			
No	458,083	97.6	4.6
Yes	11,138	2.4	18.8
One or more sexual behavioral risks, past 60 d*			
No	373,908	74.5	4.1
Yes	127,974	25.5	8.3
Sex partner with chlamydia			
No	497,787	98.7	4.9
Yes	6406	1.3	24.6
Condom use, last sex			
No	360,587	74.3	5.2
Yes	124,491	25.7	4.9
One or more clinical findings†			
No	433,280	92.9	4.4
Yes	33,195	7.1	13.4
Positive chlamydia test, past yr			
No	477,722	97.3	4.9
Yes	18,114	2.7	12.2

\*Includes having had a new sex partner, multiple sex partners, or a symptomatic sex partner in the past 60 d.

†Includes cervicitis, friable cervix, ectopy, and pelvic inflammatory disease.

above risk factors, there remained a significant 5% increase in the risk of chlamydial infection each year (OR 1.05, 95% confidence interval: 1.03, 1.05) (Table 2, model 1 adjusted ORs).

To assess the trend in risk of chlamydia by laboratory test method, we included an interaction term, year-laboratory test method, in the multivariate model. The year-laboratory test method interaction term was significant ( $P < 0.01$ ), indicating differences in the slopes of the trend lines for NAATs versus non-NAATs (data not shown). How-

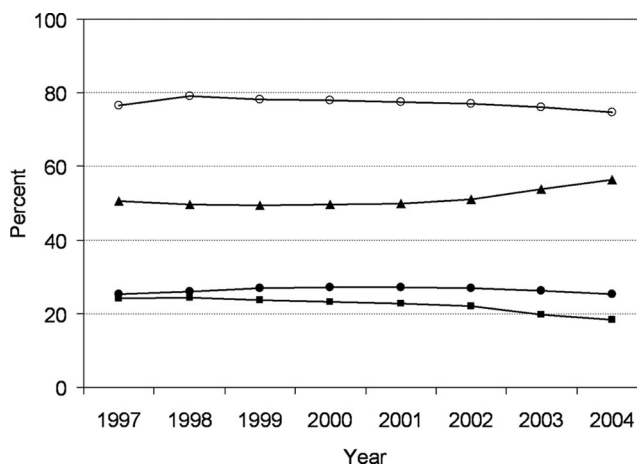


Fig. 1. Trends in characteristics of women aged 15 to 24 years screened for chlamydia, Region X family planning clinics, 1997–2004. —○— = race/ethnicity, white; —■— = 15–17 yrs; —●— = 18–19 yrs; —▲— = 20–24 yrs.

ever, there was an increase in the slopes of the trend lines for both NAATs and non-NAATs. The risk of chlamydia associated with NAATs increased 3%/yr, and the risk associated with non-NAATs increased 5% per year; thus, there was an increase in the risk of chlamydia over the time period regardless of the laboratory test method used.

*Did the Increases in Chlamydia Positivity Persist After Adjusting for Clinic?*

To account for the variability across the 125 family planning clinics, we used a generalized linear mixed model to assess the probability of a positive chlamydia test controlling for clinic as a random effect and each risk factor as a fixed effect (Table 2, model 2). The model would not converge when trying to include the 125 family planning clinics and all 10 risk factors in a single model. However, the models did converge when separate analyses were performed that

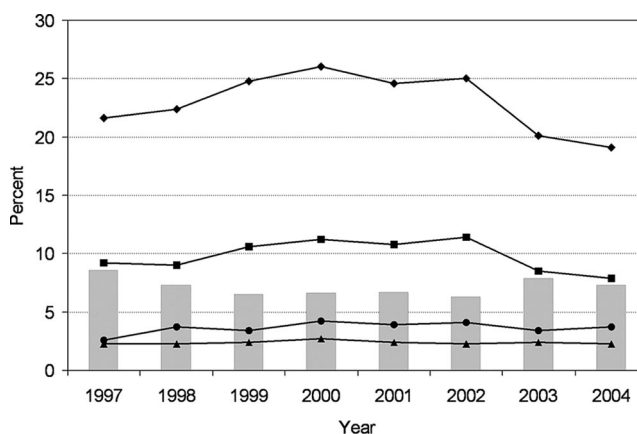


Fig. 2. Trends in self-reported sexual behavioral risks and clinical findings among women aged 15 to 24 years screened for chlamydia, Region X family planning clinics, 1997–2004. —■— = clinical findings (includes cervicitis, friable cervix, ectopy, and PID); —●— = multiple sex partners, past 60 days; —◆— = new sex partner, past 60 days; —●— = positive chlamydia test, past year; —▲— = symptomatic sex partner, past 60 days.

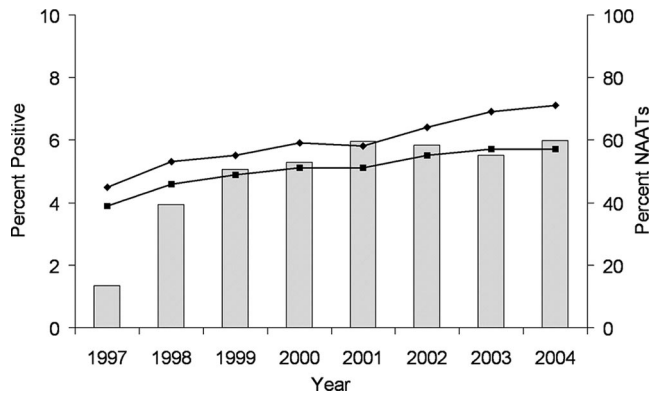


Fig. 3. Trends in observed and adjusted chlamydia positivity among women aged 15 to 24 years and percent of tests that were nucleic acid amplification tests (NAATs), Region X family planning clinics, 1997–2004. ■ = percent NAATs; —◆— = adjusted positivity; —■— = observed positivity.

included the 125 clinics as a random effect and a single risk factor as a fixed effect. The OR generated from these 10 generalized linear mixed models were similar to the adjusted OR from the multivariate logistic regression model that included all 10 of the risk factors (Table 2, model 1 and model 2). After adjusting for variability across the 125 clinics using the mixed model, there remained a significant 4% increased risk of chlamydial infection each year (OR 1.04, 95% confidence interval: 1.03, 1.04) (Table 2, model 2).

### Discussion

From 1997 through 2004 over half a million young sexually active women, of whom over 90% were asymptomatic, were

screened for chlamydia at 125 family planning clinics participating in the Region X Infertility Prevention Project. Over that time period, chlamydia positivity increased 46%. We systematically evaluated potential reasons for the increase, including changes in the characteristics of the women screened over time, the use of more sensitive laboratory testing methods, and the consistency of the increase across the 125 family planning clinics where the screening occurred. Based on the available data, we concluded that there was a true increase in chlamydia positivity over the 8-year period.

The proportion of women with demographic, clinical, or sexual behavioral risk characteristics associated with an increased risk of chlamydial infection remained stable or decreased over time. We found that the percent of women in the age group (15-to 19-year-old) at highest risk of chlamydial infection decreased. Overall, the proportion of women reporting any sexual risk behaviors also decreased. There was little change in the proportion of women who had an abnormal clinical finding on physical examination. The decline in the proportion of women with these risk factors should have resulted in a decrease, not an increase, in observed chlamydia positivity during this 8-year period. The proportion of women who reported a positive chlamydia test during the year before their clinic visit increased 42%. This may have been due to increasing efforts by some clinics to rescreen previously infected women. However, the proportionally large increase reflects a small absolute change—from 2.6% of testing in 1997 to 3.7% in 2004. This increase could not account for the overall increase in chlamydia positivity across the region. Thus, changes in the demographic, clinical, and sexual behavioral risk characteristics of the women screened could not account for the increase in positivity over time.

We adjusted chlamydia positivity to account for the use of more sensitive laboratory tests and to better estimate true chlamydia prevalence. Although statistical issues have been raised concerning the estimation of sensitivity and specificity for NAATs, the adjusted positivity increased over time even when a range of laboratory test

TABLE 2. Risk of Chlamydial Infection in Women Aged 15–24 Yrs. Seen in Region X Family Planning Clinics, 1997–2004

Characteristic	Crude		Model 1		Model 2	
	OR	95% CI	AOR	95% CI	AOR*	95% CI
Age group (yr)						
15–19	1.34	1.30–1.37	1.37	1.33–1.41	1.34	1.30–1.37
20–24	Reference	—	Reference	—	Reference	—
Race/ethnicity						
White	Reference	—	Reference	—	Reference	—
Nonwhite	1.62	1.58–1.67	1.59	1.54–1.64	1.56	1.51–1.60
Clinic city size, persons						
<25,000	Reference	—	Reference	—	Reference	—
25,000–99,999	1.22	1.18–1.27	1.15	1.10–1.20	1.27	1.11–1.45
≥100,000	1.27	1.22–1.31	1.18	1.14–1.23	1.27	1.09–1.48
One or more sexual behavioral risks <sup>†</sup>	2.12	2.06–2.17	1.95	1.89–2.00	2.11	2.06–2.17
Sex partner with chlamydia	6.35	5.99–6.74	3.87	3.60–4.16	6.17	5.82–6.55
No condom use, last sex	1.08	1.04–1.11	1.16	1.12–1.20	1.07	1.04–1.11
One or more clinical findings <sup>‡</sup>	3.33	3.21–3.45	2.92	2.81–3.04	3.45	3.32–3.57
Positive chlamydia test, past year	2.72	2.60–2.85	1.79	1.69–1.89	2.48	2.37–2.60
Chlamydia test type						
NAAT	Reference	—	Reference	—	Reference	—
Non-NAAT	0.62	0.61–0.64	0.73	0.71–0.76	0.67	0.64–0.69
Per year since 1997	1.05	1.04–1.06	1.05	1.03–1.05	1.04	1.03–1.04

\*Adjusted odds ratio based on generalized linear mixed model that included the risk factor as a fixed effect and clinic as a random effect.

<sup>†</sup>Includes having had a new sex partner; multiple sex partners or a symptomatic sex partner in the past 60 d.

<sup>‡</sup>Includes cervicitis, friable cervix, ectopy, and PID.

OR indicates odds ratio; CI, confidence interval; AOR, adjusted odds ratio.

sensitivities and specificities were used.<sup>17</sup> A multivariate logistic regression model was used to adjust for all of the demographic, clinical, sexual behavioral risk, and laboratory test characteristics associated with an increased risk of chlamydia. Even after taking into account of all those risk factors, there remained a significant 5% increase in the risk of chlamydia each year. We used a generalized linear mixed model to assess if differences in factors across the 125 individual clinics explained the increase in positivity. We concluded it was not likely that the variability among the clinics where screening occurred accounted for the annual increase in chlamydia positivity in the region.

Although we used all of the data available to systematically evaluate possible reasons for the increase in chlamydia positivity, we were unable to examine a broader set of individual and community-level factors that could have affected disease. Unmeasured individual characteristics, e.g., nonsexual risk behaviors or socioeconomic status, could not be evaluated as potential risk factors for chlamydia. In addition, we were unable to evaluate patients' sexual network characteristics, e.g., concurrency, and their influence on the risk of chlamydia during this time period. Previous research has suggested that partner selection over time, interrelationships among groups of individuals, and the disease and treatment status of network members affect the risk of chlamydia.<sup>18,19</sup>

Clinic screening coverage among young women, i.e., the proportion of women seen at the clinics and who received a chlamydia test during a calendar year, may impact chlamydia positivity. Previous studies found that screening coverage was between 50% and 60% in Region X.<sup>20,21</sup> Although trend data for screening coverage were not available, it is unlikely that there were dramatic changes in coverage that could have accounted for the increases in chlamydia positivity during this time period. Furthermore, it is unlikely that coverage reached 100% in the region. Low screening coverage could result in a number of undetected infections, contributing to a continuing reservoir of chlamydial infections.

A recent study of surveillance case report data in British Columbia, Canada, documented an increase in chlamydia case rates from 1998 through 2003.<sup>22</sup> The authors hypothesized that early treatment of chlamydial infection may increase a population's susceptibility to reinfection after the introduction of a chlamydia control program. However, this analysis did not consider sexual networks, screening coverage, or changes to more sensitive test methods and their effects on the increase in chlamydia case rates. A study that evaluated azithromycin treatment on trachoma infections in Vietnam also concluded that treatment might interrupt the duration of infection necessary for developing immunity, thus increasing the number of individuals susceptible to reinfection and adversely affecting the prevalence of disease over time.<sup>23</sup>

Increases in chlamydia prevalence that could not be explained by changes in laboratory test methods or changes in the demographic characteristics of the women screened have been documented in other areas of the United States and in Sweden.<sup>24,25</sup> Likewise, our findings suggest that there was a true increase in chlamydia positivity among young women screened in Region X family planning clinics from 1997 through 2004. However, our findings also suggest that future research on trends in chlamydia prevalence must focus on data beyond the traditional risk factor information currently collected. For example, simulation modeling has been used in the Netherlands to study the spread of chlamydia and to investigate the effects of several aspects of a screening program, namely sex and age selection for screening and partner referral, on the incidence and prevalence of the disease.<sup>26,27</sup> Adapting such a model to populations within the United States could be useful in assessing how varying levels of screening coverage, treatment rates, and partner referral might contribute to changes in

chlamydia prevalence over time. In addition, research is needed to understand how more detailed and comprehensive individual risk measures, social network characteristics, and broader community factors influence the trends in chlamydia prevalence. In the meantime, our analysis confirmed that younger sexually active women continue to be at a high risk for chlamydial infection. Continued screening of these women is critical to detect and treat infections and prevent future adverse reproductive sequelae.

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