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# Prevalence of Chlamydial Genital Infection and Associated Risk Factors in Adolescent Females at an Urban Reproductive Health Care Center in Croatia

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**A B S T R A C T**

*The study was undertaken to determine the prevalence of chlamydial genital infection in sexually active, urban adolescent females 15–19 years; to identify behavioral, demographic, and clinical factors associated with chlamydial infections; and to develop criteria for potential screening strategies. 500 adolescent women, median age 17.7 years, who visited gynecological outpatient clinic in Children's Hospital Zagreb for different reasons were enrolled in this study. Gynecological exam, colposcopy, detection of chlamydial infection by the rapid direct immunoassay of endocervical swab (Clearview Chlamydia – Unipath), endocervical cytological examination – Papanicolaou smear, and questionnaire to obtain demographic, social, behavioral and presence of symptoms data were performed. Positive Chlamydia trachomatis test were found in 16.4% of participants, cytologic cervical abnormalities – cervical intraepithelial neoplasia (CIN I – CIN III) were found in 25.2% and cytological signs of Human papilloma virus were found in 11.4%. Stepwise multivariate logistic regression analysis identified five factors associated with infection: the age of menarche ≤13 years, ≥4 lifetime sexual partners, non-use of contraception (rare or never), cervical friability, and abnormal Papanicolaou test. Urban adolescent sexually active women are at high risk for chlamydial infection and other sexually transmitted diseases including HIV infection. Association between chlamydial genital infection and risk-taking sexual and contraceptive behavior was found. Routine Chlamydia trachomatis testing for this population is recommended as well as implementation of school based sexual health education because of their risk-taking sexual behavior.*

***Key words:*** *adolescents, Chlamydia trachomatis, sexually transmitted diseases*

## Introduction

Sexually transmitted infections (STIs) in adolescent population are an increasingly urgent public health concern, and illnesses related to *Chlamydia trachomatis* are among the most prevalent, damaging and costly[1]. As compared with other subpopulations, sexually active adolescent females between ages of 15 to 19 have the highest prevalence rates and are especially vulnerable to the adverse reproductive consequences of infection, including pelvic inflammatory disease (PID), infertility, ectopic pregnancy, and maternal and infant morbidity and mortality[2,3,4]. Laboratory and epidemiologic research have demonstrated that many bacterial sexually transmitted diseases (STDs), including chlamydial infections, facilitate HIV transmission[5,6]. Current data also indicate that cervical cancer is associated with *Chlamydia trachomatis* infection[7,8].

Many (70–80%) endocervical chlamydial infections escape attention because they are asymptomatic[2]. In as many as two-thirds to three-quarters of infected women, objective signs of cervical inflammation are absent. Physical signs, if present, are nonspecific and can be associated with variety of other sexually transmitted pathogens[9]. Consequently, screening of persons at high risk

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(encompassing sexually active adolescent women) is recommended[10]. Routine screening as well as treatment of those found positive, has been suggested as a preventive measure against the serious consequences of chlamydial infections, particularly PID[11]. However, screening programs are expensive and technically difficult, especially in countries with limited financial resources. Because of that, selective screening of women at high risk, based on demographic, behavioral, and clinical indicators, has theoretical and practical appeal[12].

This study was intended to determine the prevalence of chlamydial genital infection in urban adolescent women, to identify behavioral, demographic, clinical, and laboratory factors associated with chlamydial infection in exclusively adolescent sexually active women, and to determine predictors for chlamydial infection and criteria for screening strategies of asymptomatic adolescent sexually active females.

We hypothesized that demographic data, self reported sexual behavior, symptoms, clinical signs and laboratory findings would be helpful in distinguishing infected from uninfected adolescent women.

## Materials and methods

### *Study population*

Sexually active females, aged 15 to 19 years who were attending adolescent gynecological outpatient clinic and youth counseling center of the Department of Reproductive Health in Children's Hospital Zagreb in years 1999/02 for different reasons (routine annual examination, contraceptive method counseling, diagnosis and treatment of urogenital symptoms, reproductive tract infections (RTIs) / STDs, pregnancy detection, other problems) were consecutively enrolled. Patients who had taken antibiotics within the past 4 weeks, Papanicolaou (Pap) smears within 4 months, and patients who were pregnant were excluded from the study. There was 100 % acceptance rate among those offered the test.

The medical staff included 2 gynecologists and 3 nurse practitioners that were skilled in adolescent gynecological care.

Informed consent was required of all participants; the study protocol was approved by the Ethical and Scientific Committees of the Children's Hospital Zagreb.

### *Data collection and Laboratory methods*

Eligible patients were asked to complete a questionnaire that was self-administered, contained 30 items, and required approximately 10 minutes to complete. A trained nurse practitioner was available to help, if needed. Questions focused on current genitourinary symptoms and patient's medical and sexual history, including age of sexual debut, number of sexual partners, partner's symptoms, current contraceptive methods, prior pregnancies and STDs.

The gynecologists performed a speculum examination of the cervix, bimanual pelvic examination and colposcopy. An endocervical swab for *Chlamydia trachomatis* detection was obtained from all patients. The rapid direct immunoassay (Clearview Chlamydia-Unipath), which is a 30-minute single reagent immunoassay for the direct detection of chlamydia, was used because of satisfactory sensitivity and specificity when used in population with a high prevalence of chlamydia infection[13,14]. The swabs for testing by the Clearview assay were performed according to the manufacturer's instructions. The endocervical swabs were heated at 80 °C for 10 min in a heating block provided by the manufacturer. After cooling for 5 min at room temperature, the resulting specimen extract was tested on Clearview card. The formation of a blue line in the result window indicates the presence of chlamydial antigen in the extract. The line is formed due to the binding of chlamydial antigen to the blue latex and its immobilization by a zone of antibody located beneath the result window[15]. A confirmatory test was not performed because of financial reasons. An endocervical swab for chlamydia detection was followed by a cervical swab for Pap smear in order to detect cytological cervical abnormalities (CIN I-III) and cytological signs of HPV (the classic cytological manifestation of HPV genital infection is the presence of koilocytes). Samples for *Neisseria gonorrhoeae, Trichomonas vaginalis* and bacterial vaginosis were not collected during the study.

The gynecologists were asked to provide data on following: the presence of a cervical discharge, cervical erythema / edema / friability, ectopy, tenderness of uterus / adnexa. Azythromycin was administered orally, under supervision at the clinic, as a 1 g single dose to chlamydia positive patients and their partners[16]. Chlamydial eradication was evaluated 4 weeks after the treatment using Clearview Chlamydia test. The standardized data form was completed by gynecologists regarding findings on pelvic / cervical examination.

### *Statistical methods*

Associations between chlamydia positives and demographic / behavioral / clinical variables were studied by contingency tables and $\chi^2$ test. Independent risk factors of chlamydial infections were assessed using stepwise logistic regression, with variables entered into multivariate analysis on the basis of significance ($p<0.05$) in univariate analysis.

## Results

### *Characteristics of the study population*

Five hundred sexually active young women were enrolled in the study, with median age of 17.7 years (15 – 19). Reasons for clinic visits were routine annual pelvic examination (37.6 %), contraceptive counseling (20.0 %), suspected RTIs / STD (10.0 %), suspected pregnancy (7.6 %), and miscellaneous other problems, primarily menstrual, accounted for about 24.8% visits. Reasons for attendance at the clinic are presented in Table 1.

**TABLE 1**
REASONS FOR ATTENDANCE AT THE ADOLESCENT GYNECOLOGICAL CLINIC

| Variable | Positive CT Patients (n=82) | | Negative CT Patients (n=418) | |
|---|---|---|---|---|
| | N | (%) | N | (%) |
| Annual examination | 32 | (39.0) | 156 | (37.3) |
| Contraceptive counseling | 20 | (24.4) | 80 | (19.1) |
| Suspected STI/RTI | 6 | (7.3) | 44 | (10.5) |
| Suspected pregnancy | 5 | (6.1) | 33 | (7.9) |
| Other | 19 | (23.2) | 105 | (25.1) |

p=0.712, d.f. 4, CT – *Chlamydia trachomatis*, STI – Sexually transmitted infections, RTI – Reproductive tract infections

Eighty-two of endocervical smears (82/500) were chlamydia positive by rapid direct immunoassay (Clearview Chlamydia), and prevalence of *Chlamydia trachomatis* genital infection was 16.4%. Hundred and twenty-six Pap smears (126/500) were classified as cervical intraepithelial neoplasia (CIN I-III), thus cytological cervical abnormalities were detected in 25.2% subjects. Cytological signs of *Human papilloma virus* (HPV) were found in fifty-seven (57/500) patients (11.4%). Yeast forms (*Candida* spp) were identified in ninety-one (91/500) of microscopic examinations of vaginal discharge (18.2%). Colposcopy suspect findings were found in hundred-forty (140/500) of patients (28.0%). Prevalence of *Chlamydia trachomatis* genital infection, cytological signs of *Human papilloma virus* and yeast forms (*Candida* spp) are shown in Table 2.

**TABLE 2**
PREVALENCE OF GENITAL *CHLAMYDIA TRACHOMATIS, HUMAN PAPILLOMA VIRUS* AND *CANDIDA* SPP AMONG SEXUALLY ACTIVE ADOLESCENT FEMALES

| | | |
|---|---|---|
| *Chlamydia trachomatis* | 82/500 | 16.4 % |
| *Human papilloma virus** | 57/500 | 11.4 % |
| *Candida* spp** | 91/500 | 18.2 % |

*Suspected on cytological examination
**Microscopic examination

### *Risk factors*

Positive chlamydia results were associated with early age of menarche, ≤13 years (p=0.003). Other biological or demographic factors were not associated with chlamydial infection.

Sexual behavior of participants showed that subjects with positive chlamydia results were more likely than participants with negative results to report multiple lifetime sexual partners (p=0.005) and less likely to report consistent use of contraceptives (p=0.021). They also less likely reported use of condoms and consistent use of condoms, but these findings did not reach the level of statistical significance. Positive chlamydia results were not associated with early sexual debut. A greater proportion of positive chlamydia subjects reported multiple sexual partners in previous three months, and partner with previous STDs, although these findings did not reach the level of statistical significance. Positive chlamydia results were not associated with symptomatic sexual partner.

Symptoms reported by participants showed that a greater proportion of positive chlamydia subjects reported vaginal discharge, and bleeding or spotting during intercourse, metrorrhagia, and dysmenorrhea, but not to the level of statistical significance.

Clinical signs found by pelvic examination showed that subjects with positive chlamydia results were more likely to have signs of cervicitis (cervical erythema / edema / friability / cervical mucopurulent discharge) but only clinical sign of cervical friability reached the level of statistical significance (p=0.006). Chlamydial infections were associated with palpatory abdominal tenderness by bimanual pelvic examination (p=0.021).

Laboratory findings (cytology and colposcopy) showed that chlamydia infections were associated with the presence of cytological cervical abnormalities detected by Pap smears (CIN I-III) (p=0.046). Cytologic cervical dysplasiae were found in 25.2 % of adolescent females, and significantly greater proportion among *Chlamydia trachomatis* positive subjects (36.6% and 22.9%, respectively). Subjects with positive chlamydia results were more likely to have cytological signs of concurrent *Human papilloma virus* infection (15.9% and 10.5%, respectively), and suspect colposcopy features (34.1% and 26.8%, respectively), but these findings did not reach the level of statistical significance. Comparisons between positive and negative chlamydia groups are detailed in Table 3.

On the basis of significance level (p<0.05) in univariate analysis, selected variables were entered into multivariate analysis. The palpatory abdominal tenderness was not associated with infection in multivariate analysis. Factors shown by stepwise multivariate logistic regression to be independently associated with chlamydial infection were: the age of menarche ≤13 years, ≥4 lifetime sexual partners, non-use of contraception (rare or never), cervical friability, and abnormal Pap test (CIN I-III). Multivariate logistic regression analysis of factors associated with chlamydial infection is shown in Table 4.

## Discussion

The findings of our study confirm the overall high prevalence of infection with *Chlamydia trachomatis* found among adolescent women[17,18]. Numerous prevalence studies in various clinic populations have shown that sexually active adolescents have high rates of chlamydia infection. The National screening program in England reported chlamydia prevalence of 10.1% among women less than 25 years of age[19]. Women ages 16–19 years were 43.0% more likely to test positive for chlamydia than those 20–24 years old; 12.1% of 16–19 year olds and 8.8% of 20–24 years olds were infected[19]. The prevalence of

**TABLE 3**
DEMOGRAPHIC, BEHAVIORAL AND CLINICAL VARIABLES IN RELATION TO RAPID DIRECT IMMUNOASSAY TEST FOR *CHLAMYDIA TRACHOMATIS* (CT) RESULTS

| Variable | Positive CT Patients (n=82) | | Negative CT Patients (n=418) | | p* |
|---|---|---|---|---|---|
| | N | (%) | N | (%) | |
| Age (years) | | | | | |
| ≤ 16 | 18 | (22.0) | 111 | (26.6) | 0.496 |
| 17–18 | 54 | (65.8) | 246 | (58.9) | |
| > 18 | 10 | (12.2) | 61 | (14.6) | |
| Age at menarche (years) | | | | | |
| ≤ 13* | 72 | (87.8) | 306 | (73.2) | 0.003* |
| ≥ 14 | 10 | (12.2) | 112 | (26.8) | |
| Age at first intercourse (years) | | | | | |
| ≤ 15 | 26 | (31.7) | 115 | (27.5) | 0.348 |
| 16–17 | 51 | (62.2) | 258 | (61.7) | |
| ≥ 18 | 5 | (6.1) | 45 | (10.8) | |
| No. of sexual partners | | | | | |
| ≥ 4 lifetime sexual partners* | 20 | (24.4) | 50 | (12.2) | 0.005* |
| ≤ 3 lifetime sexual partners | 62 | (75.6) | 368 | (88.0) | |
| Contraceptive use | | | | | |
| Condom | 41 | (50.0) | 249 | (59.6) | 0.110 |
| Pills | 8 | (9.8) | 23 | (5.5) | 0.785 |
| None | 15 | (18.3) | 72 | (17.2) | 0.791 |
| Contraceptives (always/often)* | 42 | (51.2) | 271 | (64.8) | 0.021* |
| Condom (always/often) | 39 | (47.5) | 240 | (57.4) | 0.101 |
| History of | | | | | |
| Sexually transmitted diseases | 4 | (4.9) | 9 | (2.2) | 0.156 |
| Pregnancy | 2 | (2.4) | 7 | (1.7) | 0.101 |
| Partner | | | | | |
| With symptoms | 3 | (3.7) | 25 | (6.0) | 0.404 |
| With > 1 sexual partner | 21 | (25.6) | 132 | (31.6) | 0.894 |
| With previous STDs | 3 | (3.7) | 7 | (1.7) | 0.280 |
| New sexual partner | 14 | (17.1) | 75 | (17.9) | 0.851 |
| Symptoms | | | | | |
| Vaginal discharge | 56 | (68.3) | 249 | (59.6) | 0.139 |
| Bleeding/spotting (coital) | 9 | (11.0) | 31 | (7.4) | 0.277 |
| Metrorrhagia | 7 | (8.5) | 29 | (6.9) | 0.608 |
| Dysmenorrhea | 15 | (18.3) | 50 | (12.0) | 0.119 |
| Urinary frequency/dysuria | 15 | (18.3) | 82 | (19.6) | 0.780 |
| Abdominal pain | 17 | (20.7) | 107 | (25.6) | 0.351 |
| Signs | | | | | |
| Cervical erythema | 10 | (12.2) | 33 | (7.9) | 0.204 |
| Cervical edema | 3 | (3.7) | 7 | (1.7) | 0.241 |
| Cervical friability* | 32 | (39.0) | 100 | (23.9) | 0.006* |
| Cervical discharge | 20 | (24.3) | 98 | (23.4) | 0.854 |
| Ectopy | 57 | (69.5) | 306 | (73.2) | 0.497 |
| Adnexal tenderness | 6 | (7.3) | 15 | (3.6) | 0.124 |
| Abdominal tenderness* | 4 | (4.9) | 5 | (1.2) | 0.021* |
| Laboratory | | | | | |
| Wet prep: Yeast | 12 | (14.6) | 79 | (18.9) | 0.349 |
| PAP test (CIN I – CIN III)* | 30 | (36.6) | 96 | (22.9) | 0.046* |
| HPV cytological signs | 13 | (15.9) | 44 | (10.5) | 0.182 |
| Colposcopy: suspect features | 29 | (34.1) | 112 | (26.8) | 0.875 |

$\chi^2$ test *$p<0.05$

**TABLE 4**
LOGISTIC REGRESSION ANALYSIS OF FACTORS ASSOCIATED WITH *CHLAMYDIA TRACHOMATIS* (CT) INFECTION

| Variable | CT positives % | OR* | 95% CI* | p value |
|---|---|---|---|---|
| Age of menarche (years) | | | | |
| ≤13 | 19.0 | 2.70 | 1.33–5.80 | 0.005 |
| ≥14 | 8.2 | | | |
| Lifetime number of sexual partners | | | | |
| ≤3 | 14.4 | 0.47 | 0.25–0.86 | 0.015 |
| ≥4 | 28.6 | | | |
| Contraceptive use | | | | |
| Always/often | 13.4 | 0.58 | 0.35–0.96 | 0.032 |
| Rare/never | 21.4 | | | |
| Cervical friability | | | | |
| Yes | 24.2 | 2.06 | 1.22–3.47 | 0.006 |
| No | 15.9 | | | |
| Abnormal PAP | | | | |
| No | 13.9 | 0.51 | 0.30–0.87 | 0.014 |
| Yes (CIN I–III) | 23.8 | | | |

* OR – odds ratio; CI – confidence interval

genital chlamydia infection among young women in the United States appears to range from 1.5% to 39.1%[20]. The higher prevalence of infection is found in street youth and juvenile detention facilities[20]. In Slovenia, the prevalence of the disease among women aged 18–24 years were 4.1%[21]. There have been only several studies on the prevalence of chlamydial genital infection in adolescent population in Croatia. Previous studies in Croatia have shown prevalence for *Chlamydia trachomatis* genital infection from 13.4% to 18.0%[22–24].

Yet it is apparent from the current data that around the world, for a combination of reasons involving biology, psychology, ambient culture, and changing mores, sexually active adolescents have the highest rates of STDs of any age group[18]. Adolescents are probably at greater risk than older adults for acquiring STDs because of behavioral factors such as having multiple sex partners, engaging in unprotected intercourse, and choosing high-risk partners. Furthermore, chlamydia easily infects the immature cervix, making adolescent women more susceptible to infection than adult women[25,26]. Broader educational efforts to reduce risk taking sexual behavior among adolescents are needed[27,28].

Urban adolescent sexually active females are at high risk for chlamydial infections as well as for other STDs and abnormal Pap smears. This study supports previously described associations between chlamydial genital infection and risk-taking sexual and contraceptive behavior, including multiple sexual partners, sexual contact with multiple lifetime sexual partners, a new sexual partner, non-use or inconsistent use of condoms, abnormal cervical cytologic results, and HPV concurrent infection, with a lack of specific symptoms and clinical signs among infected women[8,29,30].

The findings of our study showed that combination of demographic data, self-reported sexual and contraceptive behavior, symptoms, and clinical signs could not distinguish *Chlamydia trachomatis* infected from uninfected adolescent women.

Chlamydial infections that are not treated can persist for a few months or more, leading to sustained transmission in population at risk. Chlamydial infections jeopardize the reproductive health of young women because they are frequently followed by PID, ectopic pregnancy, infertility, and chronic pelvic pain[31].

Responding to the problem of asymptomatic infection, routine screening is advised and should be immediately followed by treatment[32]. The efficacy of 1 g single oral dose of azithromycin appears to be a great advantage in terms of compliance particularly in adolescents[16].

Several limitation of this study should be mentioned. Firstly, Clearview Chlamydia test is a rapid immunoassay for the direct detection of *Chlamydia trachomatis* antigen. Previous studies that compared the Clearview to the other test methods for detection of *Chlamydia trachomatis* reported Clearview sensitivities that ranged from 50 to 95%[13,14,33,34] and specificity from 94 to 99% [13,14]. The Clearview test was used because of three reasons: (1) rapid »point of care« STD test allows treatment at the initial visit, what is important in population where many women will not return for treatment, and where the delay in treatment would result in significant STD transsmission[35], (2) satisfactory sensitivity (93.5%) and specificity (99%) when used in population with a high prevalence of chlamydia infection[13,14], (3) the test is cheap, the only additional cost was the test kit materials (£ 2.62 per patient)[15]. Although there are many tests available to detect chlamydial infections, most require laboratory facilities, and so are costly; and the results are

not usually available before the patient has left the clinic. However, the treatment rates could be poor when testing is centralized due to low return rates or delay in obtaining results. In some settings with high chlamydia prevalence population, as adolescents, rapidly available tests (that can be done at the physician's office / youth clinic and can provide a result while the patient is still there) may lead to more treatment of infected persons and prevent more complications when compared to traditionally laboratory based tests[35]. Although less sensitive than laboratory based tests, rapidly available tests can lead to more cases being treated when patient return rate for test result is low[36].

Second, our study is limited in the representativeness of the population sampled because only adolescents who were attending adolescent gynecological outpatient clinic and youth counseling center were included. We screened the part of population with higher risk for genital chlamydial infection. Previous studies have shown the higher prevalence of infection among young women attending STD clinic or family planning clinic[4,10,32]. These limitations may have influenced the prevalence of disease, but it is not clear that they would have influenced identification of risk factors associated with infections.

## Conclusions

The prevalence of *Chlamydia trachomatis* genital infections is high in the population of urban sexually active female adolescents. Available data confirm that other STDs, including chlamydia genital infections, facilitate HIV transmission and that early STD detection and treatment should be part of high quality, comprehensive HIV prevention strategy. This study supports previously described associations between chlamydial genital infection and risk-taking sexual and contraceptive behavior, with a lack of specific symptoms and clinical signs. Responding to the problem of asymptomatic infection, routine screening is advised. However, since the laboratory based tests are expensive, and resources may not be sufficient for routine testing of all patients, clinics and practitioners may select the least expensive screening tests, test only »high risk« clients, or empirically treat suspected infections (with or without treatment of contacts), but criteria for that should be determined according to the characteristics of »at risk« population. Until the criteria for selective screening / empirical therapy of asymptomatic women will be determined, routine chlamydial screening for urban high risk adolescents, followed by treatment, is recommended. We suggest routine (at least annual) screening of all sexually active women under 20 years of age attending gynecological health care clinics. Also, broader educational efforts to reduce risk-taking sexual behavior among adolescents are needed. Adolescents should be the most important target group for sexual health education and behavioral change in effective *Chlamydia trachomatis*, other STDs as well as HIV/AIDS preventive programs.

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## PROŠIRENOST *CHLAMYDIA TRACHOMATIS* GENITALNE INFEKCIJE U URBANIH ADOLESCENTICA I POVEZANOST S FAKTORIMA RIZIKA

### S A Ž E T A K

Studija je provedena u svrhu utvrđivanja proširenosti genitalne klamidijske infekcije u spolno aktivnih adolescentica dobi 15 do 19 godina iz urbane sredine, te povezanosti spolnog ponašanja, sociodemografskih i kliničkih faktora s klamidijskom infekcijom. U studiju je uključeno 500 adolescentica prosječne dobi 17,7 godina, pregledanih u Ambulanti za dječju i adolescentu ginekologiju, Klinike za dječje bolesti Zagreb. Učinjeni su ginekološki pregled, kolposkopija, endocervikalni bris za detekciju klamidijske infekcije brzim direktnim imunoesej testom (Clearview Chlamydia – Unipath), endocervikalni citološki bris – Papanicolaou test, a upitnikom su dobiveni podaci o spolnom ponašanju, sociodemografskim karakteristikama i prisutnosti simptoma. Pozitivna *Chlamydia trachomatis* nađena je u 16,4% ispitanica, cervikalne intraepitelijalne neoplazije (CIN I – CIN III) u 25,2% ispitanica i citološki znakovi *Humanog papiloma virusa* u 11,4% ispitanica. Multivarijantna logistička regresijska analiza identificirala je pet faktora povezanih s klamidijskom infekcijom: dob menarhe ≤13 godina, ≥4 spolna partnera, nekorištenje kontracepcije (rijetko ili nikad), fragilnost cerviksa i abnormalni Papanicolaou test. Spolno aktivne adolescentice iz urbane sredine pod visokim su rizikom za *Chlamydia trachomatis* genitalnu infekciju i druge spolno prenosive bolesti, uključivo i HIV infekciju. Potvrđena je povezanost genitalne klamidijske infekcije i rizičnog spolnog ponašanja. Preporučuje se rutinski *Chlamydia trachomatis* test spolno aktivnih adolescentica, kao i uvođenje zdravstvenog i spolnog odgoja u škole u svrhu prevencije rizičnog spolnog ponašanja.